Investigation of Genetic and Clinical Risk Factors for the Prediction of Early Endocrine Therapy Side Effects

Michael James Hale

Centre for Cancer Prevention
Wolfson Institute of Preventive Medicine
School of Medicine and Dentistry
Queen Mary University of London

Submitted in partial fulfillment of the requirements
of the Degree of Doctor of Philosophy

June 2021

To all those who have helped me
on this learning adventure

Statement of Originality

I, Michael Hale, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university. The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without the prior written consent of the author.

Signature:

Date: 25^{th} June 2021

Details of collaborations and publications

This thesis was undertaken at the Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, by the thesis author (Michael Hale) under the supervision of Dr Ivana Sestak and Professor Jack Cuzick. Portions of the work detailed in this thesis have been presented in national and international scholarly publications, as follows (journal publications highlighted in bold):

- Chapter 5: Results from sections 5.3.2 investigating single SNP associations with hot flushes and section 5.3.4 using the LASSO model for prediction of hot flushes were presented at the European Breast Cancer Conference (Virtual) October 2^{nd} - 3^{rd} 2020.

- Chapter 5: Results from section 5.3.3 investigating SNPs associated with vaginal discharge were presented as a poster at the UK Interdisciplinary Breast Cancer Conference Birmingham January 2020.

- Chapter 8: The analysis of early reported side effects in pre and postmenopausal women on the IBIS-I trial and their association with breast cancer incidence was published as an article in **The Breast**.
  Hale, M.J., Howell, A., Dowsett, M., Cuzick, J., Sestak, I. (2020) Tamoxifen related side effects and their impact on breast cancer incidence: a retrospective analysis of the randomised IBIS-I trial. *The Breast*, 54:216-221.

Funding

This work was supported by Cancer Research UK [grant number: EMSG1J5R].

I have received funding from the British Association of Cancer Researchers to attend the UK Interdisciplinary Breast Cancer Conference and funding from the School of Medicine and Dentistry to attend the European Breast Cancer Conference.

Acknowledgements

First and foremost, I owe a huge amount to my primary supervisor Dr Ivana Sestak. From the first day of my PhD to the last you have been there to support and guide me through and I am eternally grateful for your encouragement and patience and everything that you have done for me and for the PhD.

Professor Jack Cuzick, Dr Adam Brentnall, Dr Caroline Reuter, and to the members of my progression panels: Professor Stephen Duffy, Dr Minouk Schoemaker and Professor Mitch Dowsett. I thank you for taking the time to read and comment on my research. The discussions and advice you have given me during the course of my PhD have been invaluable. It has been a privilege to work with you all and to learn and benefit from your immense knowledge and wisdom.

Thank you also to everyone involved in the IBIS-I and the IBIS-II trials. A particular thank you to the women enrolled on these trials who allowed their data to be analysed, without whom none of this would be possible. Furthermore, I would like to thank Cancer Research UK for supporting me financially with the PhD studentship. They do fantastic work, and it is a source of great honour and pride to have played a part in their invaluable work.

Thank you to all the PhD students and colleagues I have worked with over the last three years. Thank you for the lunches, beers, and for your friendship. You have made working at the Wolfson fun and enjoyable and for that I thank you immensely.

During my PhD it has been humbling to learn how much I depend on other people, both physically and emotionally and I am very fortunate to have had my friends and family by my side over this period. To Mum, Dad, Naomi and Ivy-Rose, thank you for everything, most of all your unwavering love, patience and support over the years. I could not have done this without you and I am very lucky to have such a wonderful family. To my soon to be extended family the Johnsons, several of whom have been unlucky enough to sit through countless presentation rehearsals and who have read large portions of my thesis, thank you for welcoming me to your family.

Finally, to Kelly-Marie. Your love, keen eye for a spelling or grammar mistake, and constant support and motivation has meant I really couldn't have done this without you. I look forward to the future with you and know with your support anything is possible.

Abstract

Breast cancer is the most common women's cancer worldwide. Prevention trials show that endocrine therapy can significantly reduce the incidence of breast cancer, though benefits must be weighed against side effects. Concerns about side effects are cited as a major reason behind poor uptake and adherence to endocrine therapy. However, research focussed on the risk factors of side effects or their impact on long-term breast cancer outcomes is limited. Hypotheses of this thesis are that there are multiple diverse risk factors for side effects; that risk factors can be used to predict side effect incidence; and that early reported side effects are a sign of endocrine therapy efficacy.

Hypotheses were tested through studies of clinical baseline factors, genetic risk factors and sex hormones to explore associations with side effects in women from the IBIS-I and IBIS-II trials. Statistical algorithms combining risk factors were subsequently used to develop side effect prediction models and risk scores. Lastly, a study assessed the effect of reporting side effects on breast cancer incidence during long-term follow-up of IBIS-I and IBIS-II.

Markers including menopausal status, BMI, age, reproductive factors, and sex hormones were found to affect the risk of side effects. However, no significant genetic markers were identified. Prediction of side effects using a side effect risk score derived from a logistic regression model, containing clinical and sex hormone factors was possible, but performance was poor. Further investigation of risk factors is required before accurate prediction is possible. Contrary to the initial hypothesis, reporting hot flushes in women randomised to tamoxifen and reporting arthralgia in women randomised to anastrozole was associated with increased risk of breast cancer, particularly oestrogen receptor-positive breast cancer in postmenopausal women.

Overall, risk factors were identified that impact a woman's risk of side effects. The negative impact of side effects on breast cancer outcomes was also established.

Contents

1 Introduction and literature review **31**

1.1 Introduction to breast cancer 32

1.1.1 Incidence 32

1.1.2 Mortality rates 35

1.1.3 Risk factors of breast cancer 37

1.1.3.1 Family history 37

1.1.3.2 Genetic factors 38

1.1.3.3 Lifestyle and reproductive factors 41

1.1.3.4 Mammographic density 44

1.1.3.5 Mammographic density and breast cancer risk factors . 46

1.1.4 Prevention 50

1.1.5 Oestrogens and the oestrogen receptor 51

1.2 Endocrine therapy for prevention of breast cancer 53

1.2.1 Selective oestrogen receptor modulators (SERMs) 58

1.2.1.1 Tamoxifen 58

1.2.1.2 Tamoxifen prevention trials 61

1.2.1.3 Additional SERM prevention trials 63

1.2.2 Aromatase inhibitors (AIs) 64

1.2.2.1 Aromatase inhibitor prevention trials 66

1.2.2.2 Adjuvant AI trials 68

1.3 Prevalence of side effects of endocrine therapy in breast cancer prevention trials 70

1.4 Risk factors for endocrine therapy side effects 77

1.4.1 Gyanecological symptoms 77

1.4.1.1 Risk factors for gynaecological symptoms 78

1.4.2 Hot flushes 81

1.4.2.1 Risk factors for hot flushes 82

1.4.3 Musculoskeletal events 90

1.4.3.1 Risk factors of musculoskeletal events 90

1.5 Adherence to endocrine therapy 94

1.6 Models for predicting side effect risk 96

2 Aims, hypotheses and thesis scope **98**

2.1 Hypothesis 98

2.2 Objectives and scope 99

3 General methods **103**

3.1 International Breast Cancer Intervention Study I (IBIS-I) 103

3.2 International Breast Cancer Intervention Study II Prevention Trial (IBIS-II) 107

3.3 Randomised controlled trials 111

3.4 Statistical methods 112

3.4.1 Logistic regression 112

3.4.2 Testing for heterogeneity . . . 115
3.4.3 Genomic analysis . . . 116
3.4.3.1 Quality control . . . 118
3.4.3.2 Removal of duplicated samples . . . 118
3.4.3.3 Per-individual quality control . . . 118
3.4.3.4 Per-marker quality control . . . 119
3.4.3.5 Individual and multi loci analysis with logistic regression 120
3.4.4 Significance levels in genomic analysis . . . 123
3.4.5 Variable selection: Least Absolute Shrinkage and Selection Operator (LASSO) . . . 124
3.4.6 Model performance classification . . . 127
3.4.7 Survival analysis . . . 129
3.4.7.1 Survival and hazard . . . 130
3.4.7.2 Kaplan-Meier survival estimation . . . 130
3.4.7.3 The hazard ratio and Cox regression . . . 131
3.4.8 Statistical software . . . 134
3.4.9 Authors contribution . . . 134

4 Association of baseline factors with endocrine therapy side effects 135

4.1 Introduction . . . 135
4.2 Methodology . . . 140
4.2.1 Study population . . . 140
4.2.2 Study measures . . . 140

4.2.3 Statistical methods 141

4.3 Results 144

4.3.1 Analysis of IBIS-I risk factors 148

4.3.1.1 Analysis of the effect of menopausal status on the incidence of side effects after 6-months of follow up 148

4.3.2 Analysis of IBIS-I risk factors for endocrine side effects in premenopausal women 151

4.3.2.1 Premenopausal women randomised to tamoxifen 151

4.3.2.2 Premenopausal women randomised to placebo 151

4.3.3 Analysis of IBIS-I risk factors for endocrine side effects in postmenopausal women 157

4.3.3.1 Postmenopausal women randomised to tamoxifen . . . 157

4.3.3.2 Postmenopausal women randomised to placebo 158

4.3.3.3 Interaction analysis of risk factors and allocated treatment 159

4.3.4 Analysis of endocrine side effect risk factors in the IBIS-II study 164

4.3.4.1 Arthralgia events in women randomised to anastrozole . 164

4.3.4.2 Arthralgia events in women randomised to placebo . . . 164

4.3.4.3 Hot flushes in women randomised to anastrozole 168

4.3.4.4 Hot flushes in women randomised to placebo 168

4.3.4.5 Gynaecological symptoms in women randomised to anastrozole 172

4.3.4.6 Gynaecological symptoms in women randomised to placebo 172

4.3.4.7 Interaction analysis between risk factor and allocated treatment 175

4.4 Discussion . . . 176

4.5 Conclusions . . . 183

5 The relationship between genetic factors and side effects in women randomised to tamoxifen 185

5.1 Introduction . . . 185

5.2 Methodology . . . 191

5.2.1 Participants . . . 191

5.2.2 Genotyping assay methods . . . 191

5.2.3 Study design . . . 191

5.2.4 Analysis methods . . . 192

5.2.4.1 Quality control . . . 192

5.2.4.2 Statistical analysis of individual loci . . . 195

5.2.4.3 Multi-loci analysis . . . 195

5.2.4.4 Polygenic risk score . . . 196

5.2.4.5 Statistical software . . . 197

5.2.4.6 Author contributions . . . 197

5.3 Results . . . 198

5.3.1 Study demographics . . . 198

5.3.2 Candidate gene study results . . . 199

5.3.3 GWAS results . . . 203

5.3.4 Multi-loci results . . . 209

5.4 Discussion . . . 213

5.5 Conclusions 223

6 Investigation of baseline sex hormone concentrations and early side effects incidence in women from the IBIS-I and IBIS-II studies 224

6.1 Introduction 224

6.2 Methodology 232

6.2.1 Study population 232

6.2.2 Sample size calculation 233

6.2.3 Exclusion of oestradiol from analysis 234

6.2.3.1 Suitability of ELISA for analysis of sex hormones . . . 234

6.2.4 Enzyme linked immunosorbent assay (ELISA) protocols 235

6.2.4.1 General principles of ELISA 235

6.2.4.2 Sample preparation and measurement 236

6.2.4.3 Direct ELISA assay 236

6.2.4.4 Sandwich ELISA assay 237

6.2.5 Sex hormone assay procedures 237

6.2.5.1 Testosterone ELISA protocol 238

6.2.5.2 Dehydroepiandrosterone sulphate ELISA protocol . . . 239

6.2.5.3 Sex Hormone Binding Globulin ELISA protocol 240

6.3 Statistical principles and considerations 244

6.3.1 Calibration curves and determination of sample hormone concentrations 244

6.3.1.1 Testosterone 244

6.3.1.2 Bioavailable testosterone (bioT) 245

6.3.1.3 SHBG 245

6.3.1.4 DHEA-S 246

6.3.2 Coefficient of variability (CV or %CV) 246

6.3.3 Analysis of sex hormone association with side effects and baseline factors 248

6.4 Results 250

6.4.1 Baseline characteristics 250

6.4.2 IBIS-I results 253

6.4.2.1 Correlations between hormones 253

6.4.2.2 Sex hormone concentrations in the tamoxifen and placebo arms of IBIS-I 254

6.4.2.3 IBIS-I: sex hormone and SHBG associations with side effect outcomes 256

6.4.2.4 Analysis of sex hormones by quintile 258

6.4.2.5 IBIS-I: sex hormone association with side effect risk factors 262

6.4.3 IBIS-II results 267

6.4.3.1 Correlations between sex hormones 267

6.4.3.2 Sex hormone concentrations in the anastrozole and placebo arms of IBIS-II 268

6.4.3.3 IBIS-II: Sex hormone and SHBG associations with side effect outcomes 270

6.4.3.4 Sex hormone analysis by quintile 272

6.4.3.5 IBIS-II: sex hormone association with side effect risk factors 276

6.5 Discussion 281

6.6 Conclusions 290

7 Prediction models for early reported side effects of endocrine therapy 291

7.1 Introduction 291

7.2 Methodology 296

7.2.1 Study populations 296

7.2.2 Statistical methods 297

7.2.2.1 Data preparation 297

7.2.2.2 Variable selection 298

7.2.2.3 Missing data 298

7.2.2.4 Addition of genomic information to prediction models . 299

7.2.2.5 Addition of baseline sex hormone concentrations to prediction models 300

7.2.2.6 Dataset partition and 10-fold cross-validation 301

7.2.2.7 Prediction models 302

7.2.2.8 Classifier performance 302

7.2.2.9 Development of a side effect risk (SER) score 303

7.2.2.10 Outcomes 304

7.3 Results 305

7.3.1 Study demographics 305

7.3.2 Statistically significant risk factors of side effects 307

7.3.3 Prediction of side effects in IBIS-I premenopausal women 309

7.3.3.1 Model development for premenopausal women using cross-validation . . . 309

7.3.3.2 Validation of trained premenopausal models . . . 310

7.3.3.3 Addition of genomic data to baseline prediction models 311

7.3.3.4 Side effect risk score in premenopausal women randomised to tamoxifen . . . 312

7.3.4 Prediction of side effects in IBIS-I postmenopausal women . . . 314

7.3.4.1 Model development for postmenopausal women using cross-validation . . . 314

7.3.4.2 Validation of trained postmenopausal models . . . 315

7.3.4.3 Addition of genomic information to the IBIS-I postmenopausal women prediction model . . . 316

7.3.4.4 Addition of baseline sex hormone concentrations to the IBIS-I postmenopausal women prediction model . . . 317

7.3.4.5 Side effect risk score in postmenopausal women randomised to tamoxifen . . . 318

7.3.5 IBIS-II . . . 320

7.3.5.1 Model development for postmenopausal women randomised to anastrozole using cross-validation . . . 320

7.3.5.2 Validation of trained postmenopausal models . . . 321

7.3.5.3 Addition of sex hormone information to IBIS-II prediction models . . . 322

7.3.5.4 Side effect risk score for postmenopausal women randomised to anastrozole . . . 323

7.4 Discussion . . . 325

7.5 Conclusions . . . 330

8 Endocrine therapy related side effects and their impact on breast cancer incidence in the IBIS-I and IBIS-II studies 331

8.1 Introduction . . . 331

8.2 Methodology . . . 335

8.2.1 Study population . . . 335

8.2.2 Statistical analysis . . . 336

8.3 Results . . . 338

8.3.1 Baseline characteristics . . . 338

8.3.2 IBIS-I . . . 341

8.3.2.1 Side effects and breast cancer outcomes in women randomised to tamoxifen during the IBIS-I study . . . 341

8.3.2.2 Impact of side effects on breast cancer incidence in pre and postmenopausal women randomised to tamoxifen in the IBIS-I trial . . . 344

8.3.2.3 Impact of side effects on ER-positive breast cancers in women randomised to tamoxifen . . . 346

8.3.2.4 Influence of time since menopause on breast cancer outcomes in women on the IBIS-I study . . . 348

8.3.2.5 Side effects and breast cancer outcomes in the IBIS-I placebo group . . . 348

8.3.3 IBIS-II . . . 351

8.3.3.1 IBIS-II analysis of side effects and breast cancer incidence in women randomised to anastrozole . . . 351

8.3.3.2 Impact of side effects on ER-positive breast cancer in women randomised to anastrozole . . . 353

8.3.3.3 Influence of time since menopause on breast cancer outcomes in women on the IBIS-II study 355

8.3.3.4 Side effects and breast cancer outcomes in the IBIS-II placebo group 357

8.4 Discussion 359

8.5 Conclusions 366

9 Conclusions and further work **368**

9.1 Conclusions and findings 368

9.2 Further research: identifying risk factors for side effects 374

9.2.1 Investigation of genetic features 374

9.2.2 Continuation of sex hormone study 375

9.2.3 Mammographic density and the association with menopausal-like side effects during tamoxifen therapy for breast cancer risk reduction 376

9.2.4 Development and evaluation of side effect prediction models . . . 377

9.2.5 Implications of using data from RCTs and the need for real-world evidence 377

9.3 Impact of research 378

Bibliography 381

A Appendix 1 **450**

B Appendix 2 **452**

List of Figures

1.1 Age specific new breast cancer cases incidence rates in the UK in 2013-2015. Breast cancer incidence increases up until the ages of 65-69 before then decreasing (Cancer Research UK, 2019a). 33

1.2 Global breast cancer incidence in women in 2018. Values are estimated age-standardized rate (ASR) per 100,000 women. (International Agency for Research on Cancer, 2018) 34

1.3 Age-standardised mortality rates per 100,000 population for UK women between 1971 and 2016. (Cancer Research UK, 2019a). 36

1.4 The structure of ERα and ERβ, consisting of five domains. A/B domain contains the AF-1 region for the transcriptional activation of oestrogen-responsive genes. C domain for binding of the ER to the ERE. D domain is a hinge region. Ligand binding occurs in the E domain and the F domain contains the AF-2 region that allows association with co-regulatory proteins required for the activation or cessation of transcription. AF-1/2 = Activation Function Domain 1/2, DBD = DNA-Binding Domain, LBD = Ligand Binding Domain, NTD = N-Terminal Domain. 53

1.5 Primary and secondary metabolism of tamoxifen and its metabolites. The major metabolic routes involve conversion to N-desmethyl-tamoxifen followed by conversion to endoxifen. (Adapted from (Lyon et al., 2012). 59

3.1 The basic framework of a confusion matrix 127

4.1 Analysis profile with the number of women remaining in each treatment arm after removal of women with missing side effect data 140

5.1 Quality control procedure prior to sample analysis 193

5.2 Manhattan plot of all SNPs passing QC. Blue line represents significance level of 1×10^{-5}. SNPs highlighted in green are SNPs which met a statistical significance level of 5×10^{-5} for hot flushes, vaginal discharge, vaginal dryness, irregular bleeding or any gynaecological symptom. . . . 203

5.3 Linkage disequilibrium plot of all genotyped positions on Chromosome 19. LD patterns from Phase III of the 1000 genomes project – CEU population was used to determine linkage. Panel below the LD plot identifies genes and gene positions. Genotyped SNPs are plotted as squares, with the colour indicating the degree of pairwise LD between SNPs. Red indicates strong pairwise LD and reducing LD is displayed by the intensity, white indicates no LD. 207

6.1 Sex steroid hormone biosynthesis and metabolism pathway 227

6.2 Illustration of direct ELISA method used for measurement of testosterone and DHEA-S concentrations (MBL Life Science, 2017). 237

6.3 Illustration of the procedure for sandwich ELISA. This procedure was used for the determination of SHBG concentrations (MBL Life Science, 2017). 237

6.4 Correlations between hormones and SHBG. Panel A: Women randomised to Tamoxifen. Panel B: Women randomised to placebo. Abbreviations: DHEA-S = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin; T = Testosterone; bioT = bioavailable testosterone; ln = ln-transformed. 254

6.5 Adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG by quintile and side effects in women randomised to tamoxifen. Sex hormones adjusted for BMI, HRT use, and prior hysterectomy. CI = Confidence Intervals, DHEA-S = Dehydroepiandrosterone-sulphate, N = Number, SHBG = Sex Hormone Binding Globulin. 260

6.6 Adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG by quintile and side effects in women randomised to placebo. Sex hormones adjusted for BMI, HRT use, and prior hysterectomy. CI = Confidence Intervals, DHEA-S = Dehydroepiandrosterone-sulphate, N = Number, SHBG = Sex Hormone Binding Globulin. 261

6.7 Correlations between hormones and SHBG. Panel A: Women randomised to Anastrozole. Panel B: Women randomised to placebo. Abbreviations: DHEA-S = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin; T = Testosterone; bioT = bioavailable testosterone; ln = ln-transformed. 267

6.8 Adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG by quintile and side effects in women randomised to anastrozole. Sex hormones adjusted for BMI, HRT use, smoking history, and hysterectomy. CI = Confidence Intervals, DHEA-S = Dehydroepiandrosterone-sulphate, N = Number, SHBG = Sex Hormone Binding Globulin. 274

6.9 Adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG by quintile and side effects in women randomised to placebo. Sex hormones adjusted for BMI, HRT use, smoking history, and hysterectomy. CI = Confidence Intervals, DHEA-S = Dehydroepiandrosterone-sulphate, N = Number, SHBG = Sex Hormone Binding Globulin. 275

7.1 Consort diagram displaying study numbers for the IBIS-I and IBIS-II analysis and number of women removed from each analysis 297

7.2 Depiction of the ten-fold cross-validation procedure used for model development (Delen et al., 2005) 301

7.3 Side effect risk score for premenopausal women with and without side effects. Bars are percentage of the affected or unaffected population at each score. Solid vertical lines are the 1^{st} and 3^{rd} quartiles, and the dotted line is the median 313

7.4 Side effect risk score for postmenopausal women with and without side effects. Bars are percentage of the affected or unaffected population at each score. Solid vertical lines are the 1^{st} and 3^{rd} quartiles, and the dotted line is the median 319

7.5 Side effect risk score for postmenopausal women with and without side effects. Bars are percentage of the affected or unaffected population at each score. Solid vertical lines are the 1^{st} and 3^{rd} quartiles, and the dotted line is the median 324

8.1 Analysis profile with the number of women remaining in each treatment arm after removal of women with missing side effect data and those who had breast cancer or died in the first six months of trial. 336

8.2 Kaplan-Meier graphs for breast cancer incidence in the tamoxifen arm with and without side effects. A – Hot flushes, B – Vaginal discharge, C – Vaginal dryness. 342

8.3 Kaplan-Meier graphs for breast cancer incidence in the tamoxifen arm with and without side effects in postmenopausal women. A – Hot flushes, B – Vaginal discharge, C – Vaginal dryness 345

8.4 Kaplan-Meier graphs for breast cancer incidence in the IBIS-II anastrozole arm with and without side effects in postmenopausal women. A –Arthralgia, B – Hot flushes, C – Gynaecological symptoms. 352

8.5 Kaplan-Meier graphs for ER-positive breast cancer incidence in the IBIS-II anastrozole arm with and without side effects in postmenopausal women. A –Arthralgia, B – Hot flushes, C – Gynaecological symptoms. 354

9.1 Proposed interaction of endocrine therapy, sex hormones and mammographic density in relation to vasomotor incidence in women taking tamoxifen for the prevention of breast cancer 376

B.1 IBIS-I six month follow up CRF form 453

B.2 IBIS-I final follow up CRF form 454

B.3 IBIS-II Entry CRF form 455

B.4 IBIS-II six month follow up CRF form (continued on following page) . . 456

B.5 IBIS-II six month follow up CRF form continued 457

B.6 IBIS-II final follow up CRF form (Page 1 of 4) 458

B.7 IBIS-II final follow up CRF form (Page 2 of 4) 459

B.8 IBIS-II final follow up CRF form (Page 3 of 4) 460

B.9 IBIS-II final follow up CRF form (Page 4 of 4) 461

B.10 IBIS-II notification of death CRF form 461

List of Tables

1.1 Summary of breast cancer prevention trials using SERMs or AIs 54

1.2 Summary of the data on side effects from all the prevention trials performed with tamoxifen compared to placebo 72

1.3 Summary of side effect incidence from the prevention trials performed with anastrozole compared to placebo 74

3.1 Demographic of women enrolled on IBIS-I according to randomised treatment 105

3.2 Demographic of women enrolled on IBIS-II according to randomised treatment 110

3.3 Genotypic distribution between cases and controls. Wild type – zero copies of the variant allele, heterozygous – one copy each of the variant and wild type allele, and homozygous – two copies of the variant allele (Adapted from Sasieni 1997). 120

3.4 Allelic distribution between cases and controls (Adapted from Sasieni 1997). 121

4.1 Demographics of women included in this analysis from IBIS-I according to randomised treatment 145

4.2 Demographics of women included in this analysis from IBIS-II according to randomised treatment allocation 147

4.3 Odds ratios and 95% confidence intervals for the impact of menopausal status on major side effect incidence reported at the 6-month follow up during the IBIS-I trial 150

4.4 Odds ratios and 95% confidence intervals for hot flushes and gynaecological symptoms for premenopausal women in IBIS-I randomised to tamoxifen. Multivariate models include adjustment for other univariately significant risk factors 153

4.5 Odds ratios and 95% confidence intervals for hot flushes and gynaecological symptoms for premenopausal women in IBIS-I randomised to placebo. Multivariate models include adjustment for other univariately significant risk factors 155

4.6 Odds ratios and 95% confidence intervals for hot flushes for postmenopausal women in IBIS-I randomised to tamoxifen with reported side effects. Multivariate models include adjustment for other univariately significant risk factors 160

4.7 Odds ratios and 95% confidence intervals for hot flushes for postmenopausal women in IBIS-I randomised to placebo with reported side effects. Multivariate models include adjustment for other univariately significant risk factors 162

4.8 Odds ratios and 95% confidence intervals for arthralgia in postmenopausal women in IBIS-II randomised to anastrozole with reported side effects in the first 6 months. Multivariate analysis includes all univariately significant risk factors 166

4.9 Odds ratios and 95% confidence intervals for arthralgia in postmenopausal women in IBIS-II randomised to placebo with reported side effects in the first 6 months. Multivariate analysis includes all univariately significant risk factors 167

4.10 Odds ratios and 95% confidence intervals for hot flushes in postmenopausal women in IBIS-II randomised to anastrozole with reported side effects in the 6 months. Multivariate analysis includes all risk factors which were significant in the univariate analysis 170

4.11 Odds ratios and 95% confidence intervals for hot flushes in postmenopausal women in IBIS-II randomised to placebo with reported side effects in the 6 months. Multivariate analysis includes all risk factors which were significant in the univariate analysis 171

4.12 Odds ratios and 95% confidence intervals for gynaecological symptoms in postmenopausal women in IBIS-II randomised to anastrozole with reported side effects in the first 6 months. Multivariate analysis includes all risk factors which were significant in the univariate analysis 173

4.13 Odds ratios and 95% confidence intervals for gynaecological symptoms in postmenopausal women in IBIS-II randomised to placebo with reported side effects in the first 6 months. Multivariate analysis includes all risk factors which were significant in the univariate analysis 174

5.1 Candidate genes selected for the candidate gene analysis. Genes are grouped by function and may appear more than once if they belong to more than one functional pathway 192

5.2 Table of markers failing per marker quality control on each chromosome. HWE – Hardy-Weinberg Equilibrium, MAF – Minor Allele Frequency . 194

5.3 Demographic of women remaining in analysis (N = 310) after removal of failed individuals with respect to randomised treatment 198

5.4 Table of per allele OR and 95% confidence intervals for all SNPs with P $< 8.3\times$x10^{-4} for an association with a side effect without and after adjustment for covariates. After adjustment for covariates the p-value for the test and the FDR are provided. For those where no SNPs were P $< 8.3\times$x10^{-4} the top five results are displayed. 201

5.5 Table of all statistically significant SNPs at 5x10^{-5} level. CI – Confidence Interval, OR – Odds Ratio, SNP – Single Nucleotide Polymorphism. *Gene loci in bold contain the SNP, gene loci non-bold are the nearest gene to the SNP of interest. 206

5.6 Summary of selected variables for models defining side effects. Min – is the value of lambda.min and 1SE the lambda value at one standard error from the minimum lambda 210

6.1 Baseline hormonal concentrations of women with and without hot flushes (Arizanović et al., 2018) 233

6.2 Table of calibrators for the calibration curve to determine testosterone concentrations during each run 238

6.3 Table of calibrators for the calibration curve to determine testosterone concentrations during each run 239

6.4 SHBG calibration standards and volumes of each solution required to make each standard 242

6.5 Coefficient of variance for intra- and inter-assay variation of testosterone, SHBG and DHEA-S ELISA kits 248

6.6 Baseline characteristics of all participants from the IBIS-I and IBIS-II trials included in the sex hormone analysis 251

6.7 Means and standard deviations (SD) of ln-transformed circulating concentrations of sex hormones and SHBG in postmenopausal women by randomisation and case control status. P-values are Kruskal-Wallis test with Dunn's post-hoc adjustment for multiple comparisons P-values . . 255

6.8 Unadjusted and adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG as a continuous variable and side effects in all women and those randomised to tamoxifen or placebo in the IBIS-I trial 257

6.9 Means, 95% confidence intervals and Kruskal-Wallis chi-squared p-values for variance of sex hormone and sex hormone binding globulin concentrations and age, BMI, HRT smoking and reproductive factors 264

6.10 Means and standard deviations (SD) of ln-transformed circulating concentrations of sex hormones and SHBG in postmenopausal women by randomisation and case control status. P-values are Kruskal-Wallis test with Dunn's post-hoc adjustment for multiple comparisons P-values . . 269

6.11 Unadjusted and adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG as a continuous variable and side effects in all women and those randomised to anastrozole or placebo in the IBIS-II trial. 271

6.12 Means, 95% confidence intervals and Kruskal-Wallis chi-squared p-values for variance of sex hormone and sex hormone binding globulin concentrations and age, BMI, HRT smoking and reproductive factors 279

7.1 Baseline characteristics of all participants in each of the analysis groups defined by trial on which a woman was enrolled and menopausal status within the IBIS-I trial 306

7.2 A summary of statistically significant baseline risk factors for pre and postmenopausal women randomised to tamoxifen in IBIS-I or anastrozole in IBIS-II trials. All OR are from multivariate models, only risk factors which were statistically significant in full multivariate models are shown and included in these final models. For full model results see chapter 4 tables 4-4, 4-6, 4-8, 4-10, 4-12. 308

7.3 Predictive performance expressed as the area under the ROC curve, sensitivity, and specificity of the logistic regression and LASSO models for the prediction of side effects, in the 10-fold cross-validation training and validation sets for premenopausal women randomised to tamoxifen in IBIS-I 310

7.4 Predictive performance expressed as the area under the ROC curve, sensitivity, and specificity of the logistic regression and LASSO models for the prediction of side effects, in the 10-fold cross-validation training and validation sets for postmenopausal women randomised to tamoxifen in IBIS-I 315

7.5 Predictive performance expressed as the area under the ROC curve, sensitivity, and specificity of the logistic regression and LASSO models for the prediction of side effects, in the 10-fold cross-validation training and validation sets for postmenopausal women randomised to anastrozole in IBIS-II 321

8.1 Baseline characteristics for the women eligible for analysis as part of IBIS-I or IBIS-II 340

8.2 Hazard ratios, 95% confidence intervals and P-values for breast cancer incidence within treatment arm comparison according to side effects for overall, premenopausal and postmenopausal women 343

8.3 Hazard ratios, 95% confidence intervals and P-values for ER-positive breast cancer incidence within treatment arm comparison according to side effects for overall, premenopausal and postmenopausal women . . . 347

8.4 Hazard ratios, 95% confidence intervals and P-values for breast cancer incidence within treatment arm comparison according to side effects for overall, premenopausal and postmenopausal women in IBIS-I 350

8.5 Hazard ratios and 95% confidence intervals for breast cancer incidence given the presence of side effects compared to no reported side effects at 6-month depending on type of breast cancer and time since menopause in women from IBIS-II 356

8.6 Hazard ratios, 95% confidence intervals and P-values for breast cancer incidence within the placebo group of IBIS-II 358

A.1 Author's contribution to each chapter 451

List of abbreviations

ABC	Avidin-Biotin-Peroxidase Complex
AI	Aromatase Inhibitor
AIA	Aromatase inhibitor Induced Arthralgia
AIC	Akaike Information Criterion
ATAC	Arimidex, Tamoxifen Alone or in Combination
AUC	Area Under the Curve
BIC	Bayesian Information Criterion
BIG	Breast International Group
BMI	Body mass index
CI	Confidence Interval
(%) CV	(Percentage) Coefficient of Variance
CYP	Cytochrome P450 enzyme
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone- Sulphate
DNA	Deoxyribonucleic acid
DVT	Deep Vein Thrombosis
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme Linked Immunosorbent Assay
ERα	Estrogen Receptor Alpha
ERβ	Estrogen Receptor Beta
E2	17β - Oestradiol
FPR	False Positive Rate
GC-MS	Gas Chromatography – Mass Spectrometry
GWAS	Genome Wide Association Study
HER2	Human Epidermal growth factor Receptor 2
HF	Hot Flush

HRP	Horse Radish Peroxidase
HRT	Hormone Replacement Therapy
HWE	Hardy-Weinberg Equilibrium
IBIS-I	First International Breast Cancer Intervention Study
IBIS-II	Second International Breast Cancer Intervention Study
IES	Intergroup Exemestane Study
IL	Interleukin
LASSO	Least Absolute Shrinkage and Selection Operator
LCIS	Lobular Carcinoma In Situ
LC-MS	Liquid Chromatography – Mass Spectrometry
LC-MS/MS	Liquid Chromatography – Tandem Mass Spectrometry
LD	Linkage Disequilibrium
MAF	Minor Allele Frequency
MMP	Matrix Metalloproteinase
MORE	Multiple Outcomes for Raloxifene Evaluation
NSABP	National Surgical Breast and Bowel Project
OD	Optical Density
OR	Odds ratio
PBS	Phosphate Buffered Saline
PE	Pulmonary Embolism
PEARL	Postmenopausal Evaluation and Risk-Reduction with Lasofoxifen
PR	Progesterone receptor
PRS	Polygenic risk score
QC	Quality Control
ROS	Reactive Oxygen Species
RR	Relative Risk
RUTH	Raloxifene Use for The Heart
SERM	Selective oestrogen receptor modulator
SHBG	Sex Hormone Binding Globulin
SNP	Single nucleotide polymorphism
STAR	Study of Tamoxifen and Raloxifene
TMB	3,3',5,5'-tetramethylbenzidine
TPR	True Positive Rate
TSG	Tumour Suppressor Gene
VVA	Vulvovaginal Atrophy

Chapter 1: Introduction and literature review

Side effect

A secondary, typically undesirable effect of a drug or medical treatment.

'many anti-cancer drugs now in use have toxic side effects'

When discussing side effect occurrence as a result of a drug or medical treatment, it would be easy to assume that they are in some way a simple by-product of an intervention targeting a different biological site or mechanism. This is often not the case and many side effects result from disruption of the same biological site or mechanism the intervention targets. This has led to speculation that side effects could be used as a predictor of whether an intervention will ultimately be successful.

However, as with many diseases, side effects can be remarkably complex and display wide variation across a population that otherwise may seem very similar. It is therefore necessary to investigate possible underlying causes for side effects in order to understand (i) how likely a patient is to have a given side effect and (ii) whether these side effects could be used to show the benefit of the intervention. If the latter of the associations can be established, then the definition of side effects could be changed to include their potential use as key markers of therapeutic success.

The aim of this thesis is to establish whether markers can be identified to predict the likelihood of side effects in women receiving endocrine therapy for prevention of breast cancer. A further aim is to establish whether a model can be created from these markers to predict the potential of developing side effects as a result of a medical intervention. Additionally, can side effects be predictive of outcomes after endocrine therapy for breast cancer prevention.

This introductory chapter, presents an overview of breast cancer, explores the prevention trials and sets out the biological markers known to impact the incidence of breast cancer and treatment related side effects. It reviews the work to date assessing the impact of these markers on side effects, and addresses the use of models for prediction of breast cancer risk.

1.1 Introduction to breast cancer

1.1.1 Incidence

One of the major health problems facing society today is breast cancer. In women, breast cancer remains the most common cancer diagnosed worldwide. Worldwide in 2018, over two million new breast cancer cases and over 620,000 deaths were recorded in men and women; and cases are rapidly increasing (Bray et al., 2018). In the UK, over 55,000 new diagnoses are made in women, and while survival rates are improving, approximately 11,400 women die each year (Cancer Research UK, 2020). While 99% of all breast cancers are diagnosed in women, about 1% are diagnosed in males (Cancer Research UK, 2020). In 2017, about 390 cases were diagnosed in men and 80 deaths were reported (Cancer Research UK, 2020).

Between 1995 and 2015 the incidence of breast cancer in women increased from 139 cases per 100,000 to 170 cases per 100,000, an increase of 22.3% (Cancer Research UK, 2019a). However, for males, breast cancer incidence rates have remained stable over the same time period (Cancer Research UK, 2020). Unfortunately, there is no single, simple answer for this increase. Instead, it is probably the result of a number of factors - some of which are controllable, while other are not modifiable or avoidable.

Breast cancer rates have risen especially quickly among women aged 65-69 likely as a result of inclusion of women in this age group in the national breast screening programme (Cancer Research UK, 2020). The rate of breast cancer screening in England increased from 64% of eligible women to 77% between 2002 and 2009 after which time screening rates remained consistent until 2012 (Cancer Research UK, 2020). Since 2012 there has been a gradual decrease in breast cancer screening coverage to 75% in 2019; however, coverage in women aged 65-69 has remained high at 76% coverage (Cancer Research UK, 2020). An increase in the number of women attending screening probably lead to an increased number of cancers being detected as screening programmes are meant to detect early-stage cancers. Early-stage cancers are easier to treat successfully so, rates are expected to rise when a new group of women are invited for screening(Cancer Research UK, 2020).

However, screening is not without controversy. Critics propose that screening identifies

cancers which may never progress to cause any health problems and that the impact on breast cancer mortality is minimal but risks over-diagnosis (Bleyer & Welch, 2012; Heath, 2014).

Age is important as people now live longer than ever, and this is a major risk factor for many cancers including breast cancer. Increasing age correlates strongly with breast cancer incidence, with about 25% of new cases occurring in people older than 75 (Cancer Research UK, 2020). Whilst the figure of 1 in 8 women is a lifetime risk of breast cancer, this risk changes throughout a woman's lifetime with risk increasing sharply around the time of menopause. In women, incidence rises steadily between 25 and 29 and more sharply between 35-39. The highest increase for new cases in males is 60-64. The highest rates of breast cancer, for both males and females occur in the 85-89 age group (Cancer Research UK, 2020) (Figure 1.1).

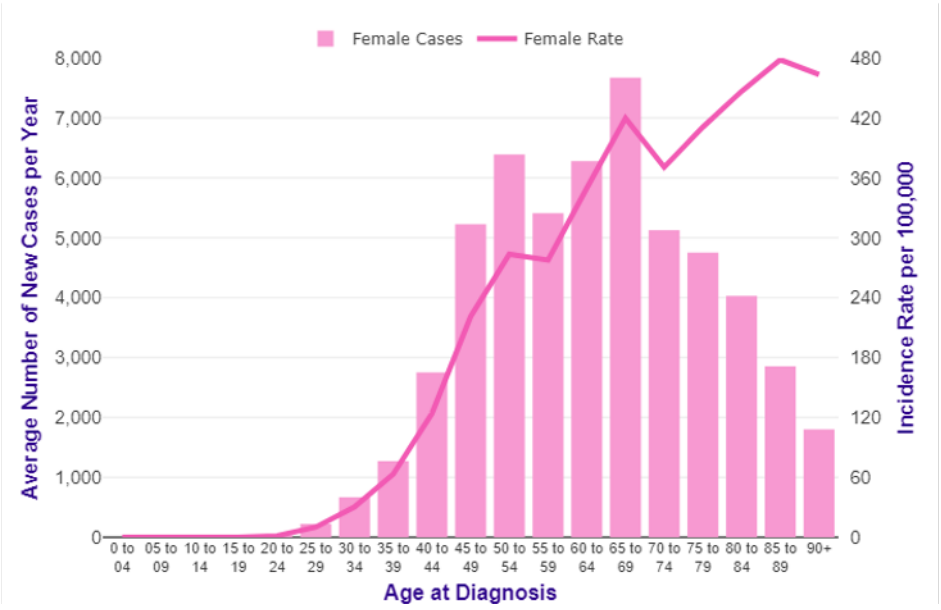


Figure 1.1: Age specific new breast cancer cases incidence rates in the UK in 2013-2015. Breast cancer incidence increases up until the ages of 65-69 before then decreasing (Cancer Research UK, 2019a).

Several lifestyle factors can affect the risk of breast cancer. Alcohol consumption, and body weight, particularly in women who are postmenopausal, have been associated with an increase in breast cancer risk due to increases in oestrogen concentrations. In contrast, exercise may reduce the risk of breast cancer by up to 40%. Whilst the mechanism behind this change is not fully understood, evidence points to hormone concentrations as the major factor in this reduction. In addition, having children protects against breast cancer. Women who have their first child at an earlier age are

less likely to have breast cancer. Their risk is further decreased as they have more children and if they spend longer breastfeeding (Ma et al., 2006).

Hormone replacement therapy (HRT) is another factor which can increase the risk of breast cancer. Although it is unclear how much HRT is associated with recent trends. Between 1992 and 2001, the rate of HRT use increased rapidly with around 25% of women aged 45-69 using HRT (Cancer Research UK, 2020). However, in the early 2000s some developed countries such as the United States and the United Kingdom reported a decrease in the rate of breast cancer incidence, which is likely due in part to the decrease in use of HRT in postmenopausal women as a result of the release of the Women's Health Initiative (WHI) report on HRT (Ravdin et al., 2007; Writing Group for the Women's Health Initiative Investigators, 2002).

Incidence of breast cancer varies by geographic location with knowledge of the relationship between geographic regions and breast cancer incidence steadily increasing. Through-out the world rates are highest in western countries and are lower across Africa and Asia (Figure 1.2). A study on Japan migrants to the US suggest that the reasons for lower incidence are environmental and not genetic (Ziegler et al., 1993).

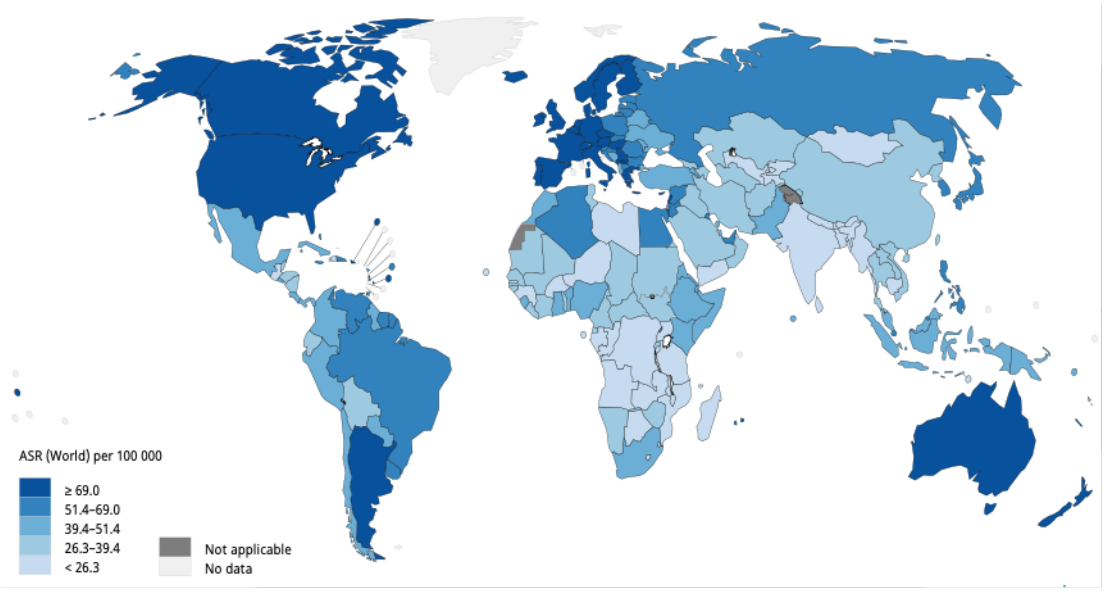


Figure 1.2: Global breast cancer incidence in women in 2018. Values are estimated age-standardized rate (ASR) per 100,000 women. (International Agency for Research on Cancer, 2018)

However, the most rapid increase has been occurring in regions which previously have had a relatively low breast cancer burden such as South America, Africa and Asia (Bray et al., 2004). This likely reflects the trend of postponing childbirth until later in

life and a general increase in the rate of obesity.

However, the coverage of cancer registries varies considerably through-out the world. The proportion of the population covered by cancer registries varies by continent with those of Africa (2%), Asia (6%) and central and South America (8%) notably lower than the coverage in Europe (42%), Oceania (78%) and North America (95%) (Bray et al., 2015). The lower coverage in particular regions must be taken into consideration when reviewing cancer incidence worldwide.

1.1.2 Mortality rates

Despite improvements in detection and survival rates, breast cancer remains the second largest cause of cancer related deaths in women in the UK, Western Europe and North Americas (Cancer Research UK, 2019a).

Although breast cancer incidence in the UK has been rising, mortality has been steadily declining over the last 20 years (Figure 1.3). Almost two in three women with breast cancer now survive the disease for more than 20 years compared to less than 50% in the 1990s, and more than 75% survive for 10 years or more (Cancer Research UK, 2019b). The start of the breast cancer screening programme in the UK in 1988 for women aged between 50 and 64 may partly explain the declining mortality trend. Although resulting in higher incidence rates due to earlier detection, the screening programme has had a positive effect on overall survival rate with women being diagnosed and treated quicker than previously.

In the UK there is an increase in breast cancer incidence and a decrease in breast cancer mortality which is generally the case in more-developed regions. However, there are still some countries, such as Japan, which are experiencing increases in incidence and mortality rates (Cancer Research UK, 2019b).

Whilst incidence in the UK has dropped, incidence of breast cancer in Japan has been rising since 1960 (Taira et al., 2016). Over the same period Japan has a lower breast cancer mortality rate than the UK, 20.4 per 100,000 compared to 27.9 per 100,000 in the UK (Cancer Research UK, 2020; Taira et al., 2016). The mortality rate in UK females since 1960 has decreased by more than a third (35%) compared to an increase in mortality in Japanese women (Kawamura & Sobue, 2005; Saika & Sobue, 2009;

Taira et al., 2016). These increases in mortality are not due to poorer survival but as a result of increased incidence (Taira et al., 2016).

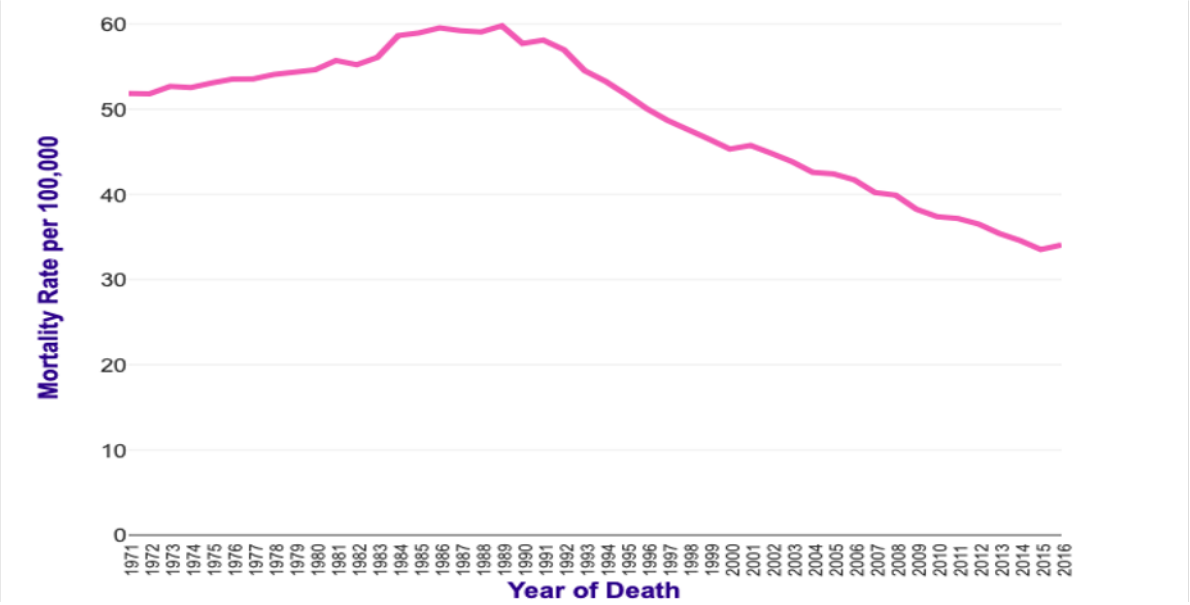


Figure 1.3: Age-standardised mortality rates per 100,000 population for UK women between 1971 and 2016. (Cancer Research UK, 2019a).

One explanation for this trend may be an increased exposure to the westernised diet, characterised by a higher fat content than the traditional diet, and the subsequent development of obesity (Iwasaki et al., 2007; Suzuki et al., 2011). Additionally, exposure to radiation, family history of breast cancer and HRT use also display convincing evidence for breast cancer increases in Japanese women (Taira et al., 2016). Other possible explanations include an increase in habitual alcohol consumption among middle-aged females (Suzuki et al., 2010). Whilst an increase in tobacco smoking may also increase breast cancer incidence (Taira et al., 2016).

The decrease in mortality observed in the UK is partly due to the introduction of the screening programme. However, screening is not the only intervention that has led to a decrease in mortality. Since the early 1980s when mortality was at its highest rate treatment options have changed dramatically. Chemotherapy continues to improve with shorter courses proving to be as effective as longer courses; however, side effects of chemotherapy still remain a concern. Endocrine therapy, such as tamoxifen or aromatase inhibitors, used for prevention of new and recurrent breast cancers have also had a major impact on reducing breast cancer incidence. Additionally, advances have occurred in the treatment of human epidermal growth factor receptor 2

(HER2)-enriched tumours, with the development of monoclonal antibodies and kinase inhibitors. Despite advances, these treatments come at a huge financial cost and with important side effects (Zurrida & Veronesi, 2015).

1.1.3 Risk factors of breast cancer

Comparison of low-risk populations migrating into populations at higher-risk has shown that as generations progress the cancer risk becomes closer to that of the higher-risk population (Ziegler et al., 1993). This suggests that these previously low-risk populations are changing their habits to become more like the high-risk populations. Risk factors of breast cancer are varied, from age, reproduction (nulliparity and late age of first birth), inherited genetic predisposition, mammographic density, anthropometric factors (obesity), exogenous hormone intake and alcohol intake (Bray et al., 2018; Breast Cancer Now, 2018). Some of these risk factors are avoidable and some are modifiable meaning that for some breast cancer is preventable. Currently, about 23% of breast cancer in the UK is deemed preventable with obesity, alcohol and radiation the leading causes behind these preventable cases (Cancer Research UK, 2020). Although breast cancer can be due to inherited factors, studies indicate that approximately 5% of all breast cancer and up to 25% of familial breast cancer cases have genetic causes due to inheritance of high-penetrance *BRCA1* or *BRCA2* mutations (Antoniou & Easton, 2006; Skol et al., 2016). This suggests that the majority of breast cancers can be traced to factors linked to environment and lifestyle (Bray et al., 2018).

1.1.3.1 Family history

The genetic predisposition of cancers is based on observations of multiple cancers within a family and cancer risk increasing in individuals who suffer from certain syndromes, such as Li-Fraumeni syndrome (Malkin, 2011). Clusters of cancers within families have been attributed to combinations of shared genes and shared environments and lifestyles. Studies have determined that risk of breast cancer increases two-fold if a close relative has suffered from breast cancer (Collaborative Group on Hormonal Factors in Breast Cancer, 2001). Categorisation of family history has ranged from simple presence or absence of breast cancer in the family to more specific definitions based on the number, type and age of diagnosis of family members with breast cancer

(Colditz et al., 1996; Egan et al., 1998; Pharoah et al., 2000). However, factors such as the number of female relatives, and the number of years they have lived have a significant bearing on familial risk. Women who have many relatives who have reached older ages would be expected, to have more reported breast cancer cases than those who have fewer younger relatives (Brewer et al., 2017). Therefore, taking age-structure and size of the family could improve risk stratification. Brewer et al (2017) developed a familial history score (FHS), which divides the number of observed cancers, including those in male relatives, by the expected number of cancers based on the age structure of the family and national incidence rates, providing a standardised incidence ratio. Application of the FHS to assess breast cancer risk found that, when combined with age of diagnosis in relatives, the FHS gives a much stronger predictor of risk than other models based solely on the number of relatives with breast cancer (Brewer et al., 2017).

1.1.3.2 Genetic factors

A history of breast cancer within the family is a strong risk factor, particularly when *BRCA1* and *BRCA2* genes are mutated. Three bands of genetic mutations have been established for breast cancer: high, moderate and low penetrance mutations (Mavaddat et al., 2010). Variant penetrance is considered high, moderate, or low according to lifetime risk of breast cancer: high (>50%), moderate (20% to 50%), and low (<20%).

High penetrance mutations, such as *BRCA1* or *BRCA2*, are rare but are associated with high breast cancer risk. The overall prevalence of *BRCA1* or *BRCA2* mutations differs depending on the population under study. In the general population prevalence is normally very low, approximately 0.2–0.3%, but is increased to approximately 3% in all breast cancer carriers, rising further to 6% in those who develop breast cancer before age 40 (Lippi et al., 2017). In women with a positive familial history of breast cancer, prevalence can be as high as 20% (Lippi et al., 2017, Nelson et al., 2019). Inherited genetic mutation account for 5-10% of all breast cancer cases and mutations in *BRCA1* and *BRCA2* significantly increase an individual's risk of breast cancer particularly in those with a family history of breast cancer (Easton et al., 1993; Friedman et al., 1994; Lippi et al., 2017; Miki et al., 1994; Wooster et al., 1994). The 5-year prospective follow-up of a prospective cohort study by Kuchenbaeker et al. (2017) which included 6,036 *BRCA1* and 3,820 *BRCA2* female carriers, demonstrated that the overall breast cancer risk for women carrying *BRCA1* mutations who had a positive familial history

was 73%, and 65% in women with *BRCA2* oncogenic mutations and positive familial history for breast cancer (Kuchenbaecker et al., 2017).

Moderate penetrance mutations, like *ATM*, *CHEK2* and *PALB2*, tend to be protein shortening variants of DNA repair genes and have been shown to increase susceptibility to breast cancer (Easton et al., 2015). Moderate penetrance mutations are found in approximately 1-2% of the general population but frequency may increase to up to 5% in breast cancer patients and those with a family history (Shiovitz & Korde, 2015). Carriers of moderate penetrance mutations have an approximately 2 to 4-fold increased risk of developing breast cancer compared with the general population although the risk may be higher in women with a family history of breast cancer (Shiovitz & Korde, 2015). Studies in the UK population have estimated that combined, moderately penetrant gene mutations account for <3% of familial breast cancer after accounting for BRCA mutation status in women with a personal or family history of breast cancer (Shiovitz & Korde, 2015).

Low penetrance mutations are common, but only carry a small increase in risk individually. However, given the prevalence of these mutations they often occur in combination, and increase breast cancer risk in a polygenic fashion. Low penetrance genetic mutations, such as single nucleotide polymorphisms (SNPs), are often distributed throughout the genome and are commonly used to evaluate genetic variability. The difference between a polymorphism and a mutation is that a mutation is any change away from the normal DNA sequence commonly found in the population and hence the mutation is rare or abnormal. Whereas, a polymorphism is a DNA sequence variation which is common in the population. Variations are most often found in the DNA between genes. Some SNPs act as biological markers, aiding location of genes that are associated with disease. SNPs occurring within a gene or in a regulatory region near a gene, may play a more direct role in disease by affecting the gene's function; however, most SNPs have no effect on health or development.

SNPs found in a gene coding region can be either synonymous or nonsynonymous. Synonymous SNPs do not affect the protein sequence, while nonsynonymous SNPs change the amino acid sequence of a protein and can be further subcategorised as missense or nonsense. Missense SNPs result in an amino acid change in the protein which can lead to its malfunction and underpins the disease. Nonsense SNPs are a change in base that leads to a premature stop codon creating a truncated and incomplete protein. SNPs

found in non-coding regions can impact gene splicing, transcription factor binding, message RNA degradation or the sequence of noncoding RNA. Gene expression which is affected by SNPs found in a noncoding region is referred to as an expression SNP and can be located upstream or downstream from the gene.

Genetic changes have been identified which are important in the study of human health. SNPs have been found which affect an individual's response to drugs, and underlying risk of developing diseases (Weinshilboum & Wang, 2017). SNPs have also been used to track the inheritance of disease genes within families. The focus of future studies will be the identification of SNPs associated with complex diseases, such as heart disease and cancer, to further improve treatments.

Candidates for low penetrance genes have been selected on the basis that gene mutations lead to alterations in the protein affecting a pathway involved in carcinogenesis. The main focus being on such biological pathways as detoxification of carcinogens, oestrogen metabolism and DNA damage repair (Weber & Nathanson, 2000). Additionally, numerous genome-wide association studies (GWAS) have been used to identify over 300 novel SNP loci with an association with breast cancer, these SNPs had not previously been linked with a pathway to carcinogenesis (Mavaddat et al., 2019).

Over 100 different breast cancer GWAS have been performed, leading to the identification of over 182 variants associated with breast cancer risk at a genome wide ($P<5\times10^{-8}$) level of significance (GWAS Catalog, 2018). Each of these variants are associated with small effect sizes and therefore, there has been a need for larger GWAS data sets to detect true genetic associations (Amos et al., 2017). The establishment of the Breast Cancer Association Consortium and The Collaborative Oncological Gene Environment Study have made large scale GWAS possible with the main aim of better understanding the genetic influence on multiple cancers. Both these large consortia have been utilised for confirming the breast cancer risk of previously reported breast cancer associated loci (Michailidou et al., 2013, 2015). However, a recent study suggested that combining SNPs to form polygenic risk scores (PRS) may explain up to 18% of the risk (Michailidou et al., 2017).

Polygenic risk scores (PRS) have been used to identify a larger fraction of the population at risk of breast cancer but have been underutilised in the prevention setting. If PRS can be applied to the identification of women who are at low risk of side effects,

PRS could further increase the target population for prevention (Khera et al., 2018). Several studies have proposed SNP panels as a new source for breast cancer risk. The first by Cuzick et al. (2017) investigated 88 SNPs in a nested case-control study of women enrolled in IBIS-I (Cuzick et al., 2017). The second by Evans et al. (2017) used PRS of 18 SNPs coupled to known mutations to *BRCA1/2* to assess whether the 18 SNPs provided further important information regarding risk of breast cancer (Evans et al., 2017).

1.1.3.3 Lifestyle and reproductive factors

In addition to genetic factors, some modifiable lifestyle and environmental factors have been associated with breast cancer risk, such as previous breast disease; age at menarche; parity, particularly age at first pregnancy; and the use of exogenous hormones, such as HRT or oral contraceptives (Kelsey et al., 1993; Pike et al., 1993).

Most of the risk factors described above increase the lifetime exposure to oestrogens in an individual. It is well known that lifetime oestrogen exposure is responsible for increased breast cancer risk, which is supported by clinical studies (Dall & Britt, 2017; Travis & Key, 2003).

Age at menarche, as with age of menopause, is associated with breast cancer risk. However, the association is reversed as older age at menarche reduces breast cancer risk whereas older age of menopause increases breast cancer risk (Dall & Britt, 2017). Sisti et al. (2015) found that women have a 5% increased risk of breast cancer for each year younger at onset of menarche (Sisti et al., 2015). While the collaborative group on hormonal factors in breast cancer found that women who experience menopause before the age of 45 halve their risk of breast cancer compared to those who go through menopause after the age of 55 (Collaborative Group on Hormonal Factors in Breast Cancer, 2012).

In addition to age at menarche being a risk factor, there is also evidence that breast cancer risk is positively associated with longer duration between breast development and menarche i.e. with the pubertal tempo (Bodicoat et al., 2014). Increased breast cancer risk suggests that development of breast ducts may be a time when risk of carcinogenesis is heightened and greater attention needs to be given to how breast

cancer risk varies through-out puberty and at each of the different stages of puberty (Bodicoat et al., 2014).

In contrast, age at first pregnancy and increased parity have a protective effect from breast cancer, with a risk reduction of 7% per pregnancy (Ma et al., 2006). Parity has been shown to reduce the risk of breast cancer by 25% over women who are nulliparous. However, this protective effect is not immediate, with studies showing an increase in risk during and after pregnancy before disappearing leading to reduced risk, and is limited only to ER-positive disease (Kelsey et al., 1993; Ma et al., 2006, 2010; Sivaraman & Medina, 2002). Comparisons of uniparous and nulliparous women show that uniparous women are at higher risk of breast cancer in the 10 years following delivery, with the most notable increases seen in those who are 30 or older at time of delivery (Dall & Britt, 2017). Furthermore, a precise characterization of the factors responsible for the effect of pregnancy will be helped by the observation that each early age pregnancy, women below 30 at childbirth, offers cumulative protection against breast cancer. The likely mechanism behind why an early pregnancy is protective is due to differentiation of breast cells during pregnancy which is protective of breast cancer. This shortens the window during which breast tissue is the most sensitive to carcinogenicity, between menarche and first birth, and therefore reduces breast cancer risk.

Exogenous oestrogens may also have implications for breast cancer risk. The most common exposure to exogenous oestrogens comes in the form of oral contraceptives and HRT (Collaborative Group on Hormonal Factors in Breast Cancer, 1997). Women who use HRT have an increased risk of developing the disease and risk increases with longer HRT use (Collaborative Group on Hormonal Factors in Breast Cancer, 2019). There was significant excess risk for oestrogen-progestagen therapy (RR = 1.60 (95% CI 1.52 - 1.69)) and a smaller but significant increase of 17% (RR = 1.17 (1.10 - 1.26)) for oestrogen only HRT in the first 1-4 years of use. For women using HRT for 5-14 years, breast cancer risk increases two-fold (RR = 2.08 (2.02 - 2.15)) for oestrogen-progestagen and a 33% increase was observed (RR = 1.33 (1.28 - 1.37)) for oestrogen only HRT. After cessation of HRT increased risk persists for more than 10 years with the magnitude of the risk dependent on prior duration of use. However, little excess risk exists if HRT was used for less than a year (Collaborative Group on Hormonal Factors in Breast Cancer, 2019).

In the UK, for women aged between 45 and 64 the prevalence of HRT use to relieve

symptoms of the menopause increased through-out the 1990s from 18.6% in 1992 to 27.7% in 1998 (Bromley et al., 2004). However, the negative consequences on breast cancer risk only became apparent when epidemiological data from the Women's Health Initiative study in 2002 showed a correlation between combined oestrogen-progesterone HRT use and an increase in breast cancer development (Chlebowski et al., 2003; Writing Group for the Women's Health Initiative Investigators, 2002). The study also showed that oestrogen-only HRT decreased the risk of breast cancer. However, this is a topic of debate as another similar study has shown the opposite (Million Women Study Collaborators, 2003). A meta-analysis conducted for HRT studies has shown that there is a positive correlation between all types of HRT, except vaginal oestrogens, and breast cancer with risks increasing with duration of use (Collaborative Group on Hormonal Factors in Breast Cancer, 2019). The collaborative group on hormonal factors in breast cancer analysis also found that oestrogen-progesterone risks were greater than for oestrogen only (Collaborative Group on Hormonal Factors in Breast Cancer, 2019).

The link between the use of oral contraceptive and breast cancer remains in question. However, there is evidence to suggest that women using oral contraceptives have a higher risk of breast cancer than those who are not, but risk is only increased whilst women are actively taking oral contraceptives. (Collaborative Group on Hormonal Factors in Breast Cancer, 1996).

In addition to the factors discussed above, various lifestyle factors can have a significant impact on breast cancer risk. Postmenopausal women who are overweight have an increased risk of breast cancer of 10-20% compared to those with a body mass index (BMI) of < 25 kg/m^2 (Reeves et al., 2007). This is likely due to adipose tissue being a key site for conversion of testosterone to oestrogen. Adjusting relative risk calculations to include oestradiol concentrations reduces the risk to a non-statistically significant 2% association between BMI and increased breast cancer risk (Key et al., 2003). However, this is not the case in premenopausal women where obesity has been found to be inversely associated with breast cancer (The Premenopausal Breast Cancer Collaborative Group, 2018). An inverse linear association exists between BMI and breast cancer risk and is observed even in those who are not overweight. The association for BMI in women who were aged 18-24 was stronger than that seen in women aged 45-54 years (HR per 5 kg/m^2 difference 0.77 vs 0.88). Associations between ER-positive disease is stronger for all ages per 5 kg/m^2 increase in BMI compared to ER-negative dis-

ease. However, association of increased BMI was still observed for ER-negative disease (The Premenopausal Breast Cancer Collaborative Group, 2018). The hypothesis behind increased adiposity being protective of both ER-positive and ER-negative cancers suggests that both non-hormonal and hormonal pathways could be involved.

While non-hormonal pathways exist, such as those involving inflammatory mediators, evidence that the inverse association is predominantly seen in ER-positive breast cancers rather than ER-negative breast cancers, suggests a hormonal mechanism is more likely (The Premenopausal Breast Cancer Collaborative Group, 2018). Hypotheses focus on evidence that obese women with abnormal ovulatory cycles have reduced oestrogen exposures (Pike et al., 1993). Approximately 5% of total oestradiol synthesis in premenopausal women occurs via aromatisation of androgens in fatty tissues. In women with high BMI this can trigger a negative-feedback loop which alters normal ovarian function leading to an abnormal ovulatory cycle (Dowsett & Folkerd, 2015). It has also been reported that premenopausal women with high BMI had lower oestradiol, total testosterone, SHBG, and progesterone concentrations, but greater free testosterone compared to premenopausal women with lower BMI (Tworoger et al., 2006).

Diet may also increase the risk of breast cancer, with those who have a higher fat intake having a 13% increased risk over those who are on a low-fat diet (World Cancer Research Fund, 2017). Increased alcohol consumption also increases the risk of breast cancer with every 10g (1 unit) of alcohol a day corresponding to an increased relative risk of 12% (World Cancer Research Fund, 2017).

1.1.3.4 Mammographic density

The relative amounts of fat, connective tissue, and epithelial tissue determines the breasts appearance on a mammogram. Fatty areas appear dark and radiological lucent, whereas connective and epithelial tissue appear as areas of high radiologic density. Mammographic density (MD) is the radiodense area. MD can be expressed as a percentage or as an area-based density. Percentage MD, quantifies the percent of the breast area observed on a mammogram that is radiodense or white (Ursin & Qureshi, 2009). Whereas area-based density is an absolute measure that quantifies the amount of fibroglandular tissue in mm^2 or cm^2. Non-dense breast area and total breast area are two additional measures related to MD that can also be used in the prediction of

breast cancer risk (Abdolell et al., 2016).

MD has been found to be one of the strongest independent predictors of breast cancer risk with increased risk associated with higher MD (Boyd et al., 1998; Oza & Boyd, 1993). Women with the highest density have a 4-6 fold increased risk of breast cancer compared to women with less dense breasts (Torres-Mejía et al., 2005; Ziv et al., 2004).

The most common method of MD measurement is by visual assessment, in which experts assign mammograms to one of four classes, where the top two classes are termed as "dense" (Sickles et al., 2013). Alternatively, mammograms can be classified into one of six Boyd categories depending on the proportion of visual density (Boyd et al., 1995). However, these methods can suffer from variability both between mammogram reviewer and in how one reviewer rates mammograms. The broad assessment of using MD in this manner has not substantially increased the predictive power of breast cancer risk models. This is possibly due to categorical classification assuming a homogeneous risk within each group resulting in inaccuracies when estimating outcomes. Therefore, a more precise measure of MD, provided on a continuous scale, may offer improved prediction power over broader categorical measures of MD.

A visual assessment of percentage density can be recorded as a visual analogue scale, thereby providing a continuous measure of breast density. Additionally, the availability of semi- and fully automated quantitative mode of assessment, such as Cumulus or Volpara, can also provide reliable and continuous measures of percentage density and absolute density. Continuous scales of MD can be included more easily than categorical measures in cancer risk models (Astley et al., 2018; Pettersson et al., 2011).

Semi-automated thresholding methods, such as Cumulus, were developed to reduce the variability seen in visually assessed mammograms and provide a reproducible measurement of MD (Boyd et al., 1995). However, trained observers are still required, meaning that these methods are still open to some reviewer variability. In addition, visual analogue scales and Cumulus are all relative, area-based methods, so breast positioning and patient weight may affect the estimates produced via these methods (Boyd et al., 1995). Therefore adjustment for BMI should be performed for better interpretation of these percentage MD outcomes (Boyd et al., 2006).

Fully-automated volumetric measures of mammography, such as Volpara or CumulusV, are now a standard through-out the UK (Astley et al., 2018). Volumetric measures

of mammographic density are made by using measured breast thickness and x-ray attenuation of raw or "for processing" full-field digital mammography image to estimate the dense and non-density tissue volume for each pixel. The sum of the dense pixel volume gives the volumetric density of the breast (Brandt et al., 2019). The use of fully automated density assessment allows for risk stratification in screening and for development of the most appropriate screening frequency and method for an individual whilst saving time and providing high reliability and accuracy.

The biological mechanisms for why MD is a risk factor for breast cancer are not clearly understood. However, the fact that density is increased by HRT use and reduced by tamoxifen, suggests that it is a marker of stimulation of breast tissue by oestrogens (Cuzick et al., 2004; McTiernan et al., 2005; Ursin et al., 2004). Several studies have reported that dense breasts contain more epithelial hyperplasia than less dense breasts (Hawes et al., 2006; Khan et al., 2007). However, these findings are not consistent (Boyd et al., 1992). Other features of dense breasts include stromal fibrosis, higher levels of collagen and altered expression of stromal proteins (Alowami et al., 2003; Guo et al., 2001; Oza & Boyd, 1993). The role of stromal proteins and collagen in the initiation of cancer in epithelial cells is not clear; however, it is known that stromal-epithelial interactions are important in breast carcinogenesis (Barcellos-Hoff & Medina, 2005). Therefore, although not completely understood MD is associated with markers enabling epithelial growth and has a biological basis for its role in breast cancer development.

1.1.3.5 Mammographic density and breast cancer risk factors

MD is associated with genetic factors and with many other breast cancer risk factors including age, BMI and reproductive factors (Boyd et al., 1998; Oza & Boyd, 1993).

Studies of twins suggests that a large percentage of MD variation is due to inherited genetic factors (Stone et al., 2006; Ursin et al., 2009). Studies have shown a 60% correlation between percentage mammographic density (%MD) in monozygotic twins compared with 30% in dizygotic twins (Boyd et al., 2002a). A number of epidemiological studies have been performed to determine which genes are important using a candidate gene approach. However, a review by Kelemen et al. (2008) suggests that this approach has had little success (Kelemen et al., 2008). Common genetic variants identified by GWAS which play a modest role in breast cancer risk have thus far not

had any significant association with MD (Tamimi et al., 2008). Newer studies have focussed on the role of genes associated with oestrogen metabolism, such as *HSD17B1*, *CYP1B1* and *COMT*, together with hormone metabolism and insulin growth factor genes as candidates for influencing MD (Brand et al., 2018; Diorio et al., 2008; Dumas & Diorio, 2010; Keller et al., 2015; Tamimi et al., 2008). Brand et al. (2018) conducted a genetic association study and found three novel loci, one for percent volume (*HABP2*) and two for absolute volume (*INHBB*, *LINC01483*). One locus has been previously linked to ER-negative breast cancer (*INHBB*). While *HABP2* and *LINC01483* represent new loci for breast cancer susceptibility (Brand et al., 2018).

Inherited genes with known association to breast cancer include *BRCA1* and *BRCA2* mutations; and evidence suggests both of these mutations have a weak association with MD. However, another study found no difference in MD between *BRCA1* or *BRCA2* mutation carriers compared to non-carriers (Mitchell et al., 2006). There is some disagreement regarding the strength of the association between MD and SNPs identified in GWAS or candidate gene studies. Therefore, the relationship between SNPs, MD and breast cancer risk remains an active area of research.

Other breast cancer risk factors such as age at menarche, parity and menopause have shown inconsistent results for association with MD. Numerous studies have found no evidence of an association between age at menarche and MD (Gapstur et al., 2003; Hart et al., 2015; Modugno et al., 2006; Nguyen et al., 2013). In contrast, one study has reported a positive association between %MD and increasing age at menarche in both pre and perimenopausal women in unadjusted models, but the trend was not statistically significant after adjustment for age, race, study site, body BMI, and smoking (Butler et al., 2008). MD is inversely associated with parity as MD reduces at first full-term pregnancy and reduces further in a linear manner with subsequent pregnancies (Boyd et al., 2007). The reduction in MD is thought to be as a result of lower circulating concentration of reproductive hormones post-pregnancy. In contrast, MD, as with breast cancer risk increases with age at first birth (Gram et al., 1995; Titus-Ernstoff et al., 2006). The association between MD and breast cancer risk is not limited to pre-menopausal women, since postmenopausal women with higher MD also have a higher risk of breast cancer (Byrne et al., 1995). MD reduces through-out menopause (Boyd et al., 2002b). It is hypothesised that these reductions are as a result of decreased circulating sex hormones post menopause and decrease in the lobular structures of the

breast; which also increases the risk of breast cancer (Ghosh et al., 2010).

In contrast to breast cancer risk MD decreases with age (Burton et al., 2017; Krishnan et al., 2017; Ursin & Qureshi, 2009). This change in MD reflects the reduction in glandular tissue and accompanying increase in fat which occur with increasing age. This relationship may seem contradictory given that both age and MD are positively associated with breast cancer risk but are inversely associated with each other.

To understand why this scenario exists, it is important to consider how breast tissue ages rather than assuming the calendar age for breast tissue. A model proposed by Pike et al.(1983) considered breast tissue aging as the lifetime exposure to circulating hormones, such as oestrogen, and growth factors, such as insulin-like growth factor (IGF-1), which stimulate epithelial and stromal cell division (Oza & Boyd, 1993; Pike et al., 1983; Spicer et al., 1994). Pike's model postulates that the rate of breast tissue aging is fastest at menarche, but is slower during pregnancy, and then slows further in the perimenopausal period, and is at its lowest at the end of menopause (Pike et al., 1983). This model suggests that an earlier age at menarche, nulliparity, later age at first birth and later age at menopause increase cumulative exposure to hormones. These risk factors for increasing hormone exposure for breast tissue are also known to be risk factors for breast cancer. Hence hormonal exposures throughout a woman's lifetime are likely the link between MD, age and breast cancer risk.

In addition to age, BMI is a major confounder of MD. Percent MD is inversely associated with BMI as women who have higher BMI are also more likely to have greater quantities of non-dense fatty tissue and greater total breast tissue (Baglietto et al., 2014; Soguel et al., 2017). Taken together these features lead to lower percentage MD. That MD and obesity are both positively associated with breast cancer risk complicates this relationship (McCormack & Dos Santos Silva, 2006; Reeves et al., 2007). It is therefore important that when considering %MD as a risk factor for breast cancer that any estimates are adjusted for BMI to avoid underestimating risk. Failure to adjust for BMI (or age) would lead to confounded estimates of breast cancer risk (Boyd et al., 2006).

It has been widely demonstrated in clinical trials that treatment with a SERM can reduce breast density (Chow et al., 2000; Cuzick et al., 2011). The associations between exogenous hormones and MD suggest that endogenous oestrogens may influence breast

density. Indeed, there is evidence to show that circulating oestrogens do increase MD (Bertrand et al., 2018; Oza & Boyd, 1993; Spicer et al., 1994).

However, the relationship between circulating sex hormones and MD in postmenopausal women is more inconsistent. Studies show that there is little to no association between MD and serum oestrogen concentration in postmenopausal women (McCormack et al., 2009; Tamimi et al., 2005, 2007). However, this may be due to oestrogen concentrations declining with age and the difficulties obtaining accurate oestrogen concentrations. This is not the case in premenopausal women, whose MD seems to have a strong association with circulating oestrogens (Tamimi et al., 2005, 2007). In postmenopausal women, no association between circulating androgens have been observed before or after adjustment for BMI with the exception of free testosterone which was inversely associated with MD prior to adjustment for BMI. (Tamimi et al., 2005).

Other studies have attempted to relate breast density with exposure to mitogens, such as prolactin, and insulin-like growth factors (IGF) (Walker et al., 2009). In postmenopausal women, studies have shown positive association between prolactin and MD; however, no statistically significant associations with IGF-I have been observed (Greendale et al., 2007). High levels of serum IGF-I in premenopausal women have shown significantly positive associations with MD but only weak association with concentrations of prolactin (Diorio et al., 2005; Walker et al., 2009).

In addition to endogenous hormones, exogenous hormones have been associated with MD. Use of HRT after the menopause and the impact on MD has be the focus of numerous studies. Two trials in the USA, the Postmenopausal Estrogen and Progestin Interventions trial and the Women's Health Initiative (WHI) trial found a 5% and 6% increase in MD respectively in women taking HRT for a year (McTiernan et al., 2005; Ursin et al., 2004). There is large individual variation in how these treatments affect MD part of this variation can be explained by changes in oestrogen concentrations suggesting that understanding how women metabolise oestrogens is important in determining the role of hormones on MD and understanding individual variation (Ursin et al., 2004).

The effects of HRT on MD only occur short-term, as MD decreases approximately 4 weeks after ending HRT (Harvey et al., 1997). The risk of breast cancer also decreases, to a level of a non-HRT user, within a few years of stopping HRT (Beral et al., 2011).

In addition to an increase in MD in response to HRT, MD can be lowered through the use of tamoxifen. Cuzick et al. (2004) investigated the impact of tamoxifen, compared to placebo on MD in healthy women at high-risk of breast cancer (Cuzick et al., 2004). Results showed that tamoxifen use (7.9%, 95%CI (6.9% - 8.9%)) led to a greater reduction in MD compared to placebo (3.5%, 95%CI (2.7% - 4.3%)) during the first 18 months of treatment. The tamoxifen-associated reduction was observed regardless of body weight, parity and menopausal status although there was a statistically significant interaction with age (Cuzick et al., 2004). This suggests that changes in MD could be a biomarker for the efficacy of tamoxifen for prevention. AIs, such as anastrozole or exemestane, can also be used for the prevention of breast cancer (Cuzick et al., 2014; Goss et al., 2011). However, it is currently unknown whether AIs have any effect on MD and on whether MD could be used as a biomarker of AI efficacy.

1.1.4 Prevention

The majority of primary risk factors are not modifiable as they stem from chronic exposure to endogenous hormones. However, there are strategies for prevention, which over a longer duration, may prove beneficial (Bray et al., 2018).

Increasingly, countries are employing primary prevention strategies, including lifestyle changes. However, use of other strategies such as hormonal therapies remains low (Smith et al., 2016). Lifestyle changes to moderate alcohol intake, maintain a healthy body weight, and regular physical activity could prevent up to 23% of new breast cancer diagnoses (Colditz & Bohlke, 2014; Dartois et al., 2016). Hormone therapy options can reduce the impact of exogenous hormones on breast cancer risk. The use of endocrine therapy for women at high risk of breast cancer has been highly effective. Both selective oestrogen-receptor modulators (SERMs) and aromatase inhibitors (AIs) have been shown to decrease the risk of breast cancer incidence in women at high-risk, decrease the risk of recurrence and improve survival time in those women with breast cancer (Cuzick et al., 2015, 2020; Goss et al., 2011; Key et al., 2001; Powles et al., 2007; Veronesi et al., 2007). While the benefits of SERMS and AIs are clearly shown in clinical trials, these trials also highlight the issue of side effects. Each of these aspects are discussed in more depth in sections 1.2.

There are multiple ways to target the reduction in population risk of breast cancer by

25%. One example could be to target the entire population and reducing the risk by 25% across the whole population. Alternatively, if a high risk (three-fold higher than average) group of about 20% of the population were targeted and their risk reduced by 42%, this would give the same 25% reduction in breast cancer risk across the population. It is worth noting, however, that while targeting higher risk groups will mean that the intervention is more cost-effective, and the balance of favourable and unfavourable effects will improve, the public health impact can diminish if one is overly selective. Thus, for example, targeting *BRCA* mutation carriers might be very cost-effective but would only influence a small minority of breast cancers.

Intervention for prevention is usually targeted at higher risk populations, as targeting the entire population is not useful with only those at high risk having a benefit of the preventive therapy. For those at lower risk, the risk reduction would not be as large as the high-risk population. However, interventions have adverse effects which must be taken into account and only given to those whose risk of the event is high enough to outweigh the negative adverse effects. The risks involved in giving endocrine therapy to a whole population could be unacceptable if the number of severe adverse events as a result of side effects increases beyond the benefit seen in the population. The alternative plan is to target the high-risk population, this would result in fewer adverse events while decreasing the number of breast cancer events, the combination of which reduces the number of life-threatening events (Gail, 2009). The most effective way of improving this latter strategy is to understand which women are at high-risk and which are at low risk of side effects to ensure the best risk benefit ratio.

1.1.5 Oestrogens and the oestrogen receptor

Epidemiological data shows that women with breast cancer exhibit a higher blood serum and urinary levels of oestrogen when compared to those without breast cancer (Dorgan et al., 1996; Tamimi et al., 2007; Toniolo et al., 1995).

Oestrogens, including the subtypes oestrone, 17β-oestradiol, and oestriol are steroid hormones which promote the proliferation of normal and cancerous cells. Oestrogens are the primary sex hormone in females and are therefore involved in numerous physiological processes, such as the menstrual cycle, breast acini formation and maintenance of endometrial tissues and bones (Maggi et al., 2004; Sims et al., 2002). Epidemiolog-

ical data show high levels of oestrogens can have an adverse effect on health via their involvement in breast and ovarian cancer development. Additionally, high concentrations of oestrogens also increase risk of blood clots and strokes (Henderson & Lobo, 2012; Vinogradova et al., 2019).

The synthesis of oestrogen is controlled by follicle stimulating hormone and luteinising hormone, both of which are excreted from the anterior pituitary gland. These hormones stimulate the ovaries, corpus luteum and adrenal cortex to synthesise oestrogen. In premenopausal women the major source of oestrogen is the ovaries; however, this is not the case in postmenopausal women whose ovaries no longer produce oestrogens. For postmenopausal women the major source of oestrogens is the conversion of testosterone to oestrogen, catalysed by aromatase (CYP19), occurring in the adrenals and adipose tissues, such as those found in the breast (Simpson, 2003). Breast adipose tissue has emerged as a key site for aromatisation with tissue concentrations in healthy breasts having a 4-6 fold higher oestrogen concentration than that found in serum (Simpson, 2003).

Only 5% of total oestrogens are biologically active; the rest is bound to plasma proteins, such as sex hormone binding globulin (SHBG) and albumin, rendering them inactive. Oestrogenic action is mediated via the nuclear receptors ERα and ERβ. Although ERα and ERβ are encoded for by separate genes (*ESR1* on chromosomes 6 and *ESR2* on chromosome 14 respectively), they share significant sequence homology. They are composed of five domains denominated A-F (Figure 1.4).

The structure of ERα and ERβ are very similar in certain regions, the DNA-binding domain of ERα and ERβ are 97% similar (Parker et al., 1993). However, the homology between other regions of the receptors varies widely, hence the transcription of some genes is exclusive to one or other of the two receptors. Intriguingly, one ligand can be both an agonist and antagonist depending on which of the two ER forms it binds to. Despite significant sequence homology between ERα and ERβ, the receptors are distributed differently in diverse body tissues and possibly mediate different effects. ERβ expression is also often down-regulated in cancer, whereas ERα expression is often increased in cancer patients (Holst et al., 2007).

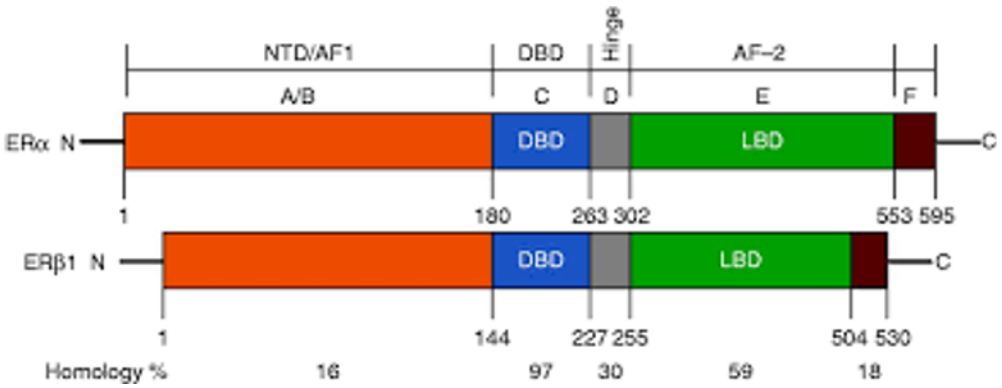


Figure 1.4: The structure of ERα and ERβ, consisting of five domains. A/B domain contains the AF-1 region for the transcriptional activation of oestrogen-responsive genes. C domain for binding of the ER to the ERE. D domain is a hinge region. Ligand binding occurs in the E domain and the F domain contains the AF-2 region that allows association with co-regulatory proteins required for the activation or cessation of transcription. AF-1/2 = Activation Function Domain 1/2, DBD = DNA-Binding Domain, LBD = Ligand Binding Domain, NTD = N-Terminal Domain.

Oestrogens can freely diffuse across a cell membrane allowing binding to ERs in the cell nucleus. The receptor-ligand complex then acts as a potent transcription factor controlling the expression of numerous genes, including those encoding for progesterone receptors and growth regulating proteins (Mourits et al., 2001). These growth regulation proteins are secreted by the tumour cell to stimulate growth of surrounding cells while other factors stimulate the growth of surrounding structural tissues such as fibroblasts and endothelial cells. Fibroblasts and endothelial cells then complete the signalling cycle by releasing growth factors and other proteolytic enzymes, which promote invasion and metastasis (Mourits et al., 2001).

The action of oestrogens is therefore a key target in breast cancer prevention. Endocrine therapies like SERMs, target the oestrogen mechanisms through blocking oestrogen receptors, or by inhibiting production of oestrogen, like aromatase inhibitors.

1.2 Endocrine therapy for prevention of breast cancer

Endocrine therapy represents one of the major forms of breast cancer prevention strategies as well as for treatment of ER-positive breast cancers. The main classes of endocrine therapies used are SERMs and AIs (Ali et al., 2016). Each of these endocrine therapies work through different mechanisms. A summary of breast cancer prevention trials using both SERMs and AIs is shown in Table 1.1.

Table 1.1: Summary of breast cancer prevention trials using SERMs or AIs

Trial name	IBIS-I	Royal Marsden Hospital Trial	NSABP P-1 Trial
Type of trial	Prevention	Prevention	Prevention
Study design	Double-blind randomised trial	Double blind randomised trial	Double blind randomised trial
Type of intervention	Placebo or Tamoxifen 20mg/ day for 5 years	Placebo or tamoxifen 20mg/day for up to 8 years	Placebo or Tamoxifen 20mg/ day for 5 years
Methods			
Type of population	Healthy women aged 35-70 at high risk of breast cancer	Women 30-70	Healthy women aged 35- or older at high risk of breast cancer
Outcome measures (primary)	Frequency of breast cancer including DCIS	Breast cancer occurrence	Prevention of invasive breast cancer
Outcome measures (secondary)	Reported side-effects and other events	Clinical, toxicity and compliance	Frequency of myocardial infractions and bone fractures
Study characteristics			
Total number enrolled	7,154	2,471	13,388
Experimental groups/ numbers	Placebo = 3,575 Tamoxifen = 3,579	Placebo= 1,233 Tamoxifen = 1,238	Placebo = 6,707 Tamoxifen = 6,681
Follow-up length	Every six month for 5 years treatment, annually thereafter	Every 6 months for 8 years	Follow up for 7 years
Median follow-up length at last publication	192 months (16 Years)	156 months (13 Years)	84 months (7 years)

Continued on next page

Table 1.1 – continued from previous page

Trial name	RUTH	GENERATIONS	Italian Tamoxifen Prevention Trial
Type of trial	Prevention	Prevention	Prevention
Study design	Double blind randomised trial	Double blind randomised trial	Double blind randomised trial
Type of intervention	Placebo or Raloxifene 60 mg/ day for 5 years	Placebo or Arzoxifene 20 mg/day for 3 years	Placebo or Tamoxifen 20 mg/day for 5 years
Methods			
Type of population	Postmenopausal women aged 55 or older with established or increased risk of CHD	Postmenopausal women aged 60-85 with low bone mass or osteoporosis	Healthy women aged 35-70
Outcome measures (primary)	Coronary events, invasive breast cancer	New vertebral fractures and invasive breast cancer	Occurrence and deaths from Breast cancer
Outcome measures (secondary)	Fracture, myocardial or non-coronary arterial revascularisation, leg amputation, and hospitalisation for any cause.	Coronary heart disease, stroke, cognitive function, days of disability, all fractures and other adverse events	Reported Side-effects and other events
Study characteristics			
Total number enrolled	10,101	9,354	5,406
Experimental groups/ numbers	Placebo = 5,057 Raloxifene = 5,044	Placebo = 4,678 Arzoxifene = 4,676	Placebo = 2,706 Tamoxifen = 2,700
Follow-up length	Twice yearly clinical visits	At months 3, 6, 12, 24, and 36	10 year follow up. Twice yearly clinical visits
Median follow-up at last publication	67 months	36 months	131 months (11 Years)

Continued on next page

Table 1.1 – continued from previous page

Trial name	MORE/CORE	STAR
Type of trial	Prevention	Prevention
Study design	Double blind randomised trial	Double blind randomised trial
Type of intervention	Placebo or Raloxifene at 60 mg/day or 120 mg/day for 3 years for MORE additional 4 year for CORE at 60 mg/day	Tamoxifen 20 mg/day or Raloxifene 60 mg/day for 5 years
	Methods	
Type of population	Women aged under 80 with osteoporosis who were 2 years postmenopausal	Postmenopausal, aged 35 years, with a minimum Gail model 5-year breast cancer risk of 1.66%
Outcome measures (primary)	MORE: Risk of fracture in postmenopausal women with osteoporosis. CORE: Incidence of invasive breast cancer	Incidence of invasive breast cancer
Outcome measures (secondary)	MORE: Invasive breast cancer. CORE: Adverse events including vaginal bleeding, endometrial hyperplasia, endometrial cancer, deep vein thrombosis, pulmonary embolism, or retinal vein thrombosis	Non-invasive breast cancer, endometrial and other cancers, and vascular-related events, ischemic heart disease, stroke, and osteoporotic fractures
	Study characteristics	
Total number enrolled	MORE = 7,705 CORE = 5,213	19,747 randomised 19,490 included in last follow-up report
Experimental groups/ numbers	MORE: Raloxifene = 5,129, Placebo = 2,576 CORE : Raloxifene = 3,510 Placebo = 1,703	Tamoxifen = 9,736 Raloxifene = 9,754
Follow-up length	MORE: Every 6 months for 3 years CORE: Baseline then annually	Every 6 months for 5 years treatment, Annually thereafter
Median follow-up at last publication	MORE = 40 months CORE = 96 months	81 months

Continued on next page

Table 1.1 – continued from previous page

Trial name	IBIS-II	MAP.3
Type of trial	Prevention	Prevention
Study design	Double blind randomised trial	Double blind randomised trial
Type of intervention	Placebo or Anastrozole 1 mg/ day for 5 years	Placebo or 25mg/day Exemestane for 5 years
Methods		
Type of population	Healthy postmenopausal women aged 45 or older	Healthy women aged 35- or older at high risk of breast cancer
Outcome measures (primary)	Histologically confirmed breast cancer	Incidence of invasive breast cancer
Outcome measures (secondary)	Reported Side-effects and other events	Any breast cancer, serious adverse events and side effects
Study characteristics		
Total number enrolled	3,864	4,560
Experimental groups/ numbers	Placebo = 1,944 Anastrozole = 1,920	Placebo = 2,275 Exemestane = 2,285
Follow-up length	6 months, 12 months then annually for 5 years	6 months, 12 months then annually for 5 years
Median follow-up at last publication	131 months (11 Years)	35 months (3 Years)

1.2.1 Selective oestrogen receptor modulators (SERMs)

1.2.1.1 Tamoxifen

SERMs, such as tamoxifen and raloxifene, compete with oestrogen at the receptor and can displace oestrogen. This inhibits function and disrupts the homeostasis of systems and pathways that require oestrogen. Binding to the ER does not alter the shape of the receptor, as the binding of oestrogen does, and does not recruit the necessary co-activators for oestrogenic signalling, which inhibits the proliferation of breast cells (Shang & Brown, 2002).

Tamoxifen has been used in breast cancer treatment for both pre and postmenopausal women for over 40 years. When tamoxifen is prescribed for five years to women post-surgery, it approximately halves the recurrence rates and reduces breast cancer mortality by a third ((EBCTCG) Early Breast Cancer Trialists' Collaborative Group et al., 2011). In the prevention setting, tamoxifen has shown to reduce breast cancer incidence by 31% overall. However, when considering ER positive cancers only, the reduction increases to approximately 50%. Thus far tamoxifen shows no significant impact in mortality when compared to the placebo group although very long follow-up will be needed for this (Cuzick et al., 2015). More discussions about prevention trials using SERMs will be discussed in section 1.2.2.

Tamoxifen has a complex pharmacology. Tamoxifen itself is a weak antioestrogen, which requires extensive metabolism to produce a range of metabolites, some of which, such as endoxifen, demonstrate more potent antioestrogenic activity (Jordan, 2007; Wu et al., 2009). In addition to its antioestrogenic properties, tamoxifen can act as an ER agonist depending on the tissue and whether particular coactivators or corepressors are present (Shang & Brown, 2002).

Tamoxifen undergoes extensive primary and secondary metabolism to endoxifen in the liver catalysed by cytochrome P450 enzymes via one of two pathways: N-desmethylation and 4-hydroxylation (Figure 1.5).

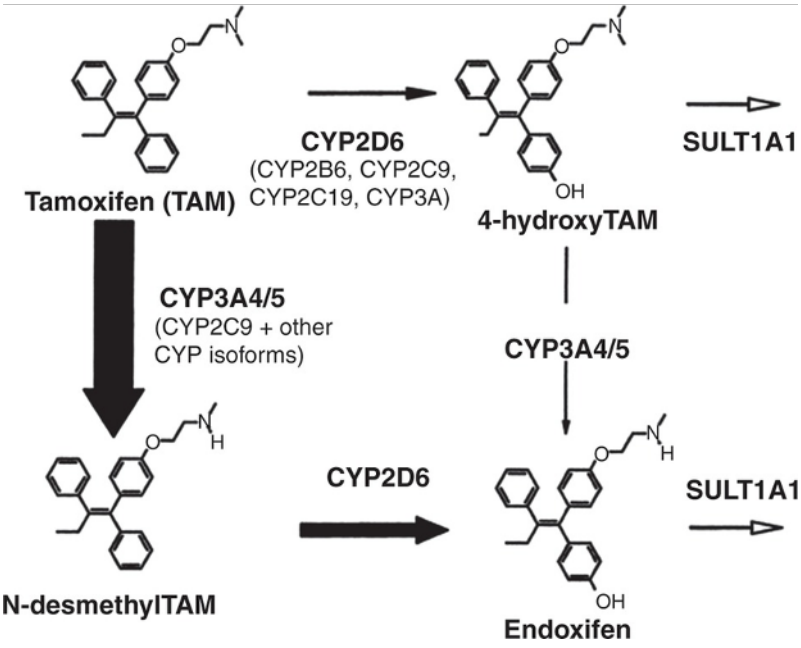


Figure 1.5: Primary and secondary metabolism of tamoxifen and its metabolites. The major metabolic routes involve conversion to N-desmethyl-tamoxifen followed by conversion to endoxifen. (Adapted from (Lyon et al., 2012).

Over 90% of tamoxifen metabolism occurs through demethylation to N-desmethyl-tamoxifen catalysed by CYP3A4/5 followed by conversion to endoxifen catalysed by CYP2D6 (Desta et al., 2004). A secondary pathway also exists via hydroxylation of tamoxifen to 4-hydroxy-tamoxifen, controlled by CYP2D6, CYP3A4 and CYP2C19, which is then converted to endoxifen by CYP3A4/5 (Sanchez-Spitman et al., 2017).

The hydroxylation step in Figure 1.5, converting tamoxifen to 4-hydroxy-tamoxifen or N-desmethyl-tamoxifen to endoxifen, is considered to be an activation step as both endoxifen and 4-hydroxy-tamoxifen exhibit a near 100-fold greater antioestrogenic action than tamoxifen itself with maximum inhibition occurring in the range of 100-1000 nM (Lim et al., 2005; Wu et al., 2009). However, while variation in tamoxifen concentrations is well known, 4-hydroxy-tamoxifen concentrations are normally low, and variation is not well understood. This is in contrast to endoxifen which generally has a plasma concentration 10-fold higher than 4-hydroxy-tamoxifen and displays large variation (Desta et al., 2004; Ingle et al., 1999; Stearns et al., 2003).

While tamoxifen is considered anti-oestrogenic in breast tissues, it is considered mildly oestrogenic in endometrium (Hu et al., 2015). In some circumstances these agonist effects could be beneficial, as they may help to prevent bone demineralisation and

subsequent bone loss in postmenopausal women. However, there is clear evidence that long-term use of tamoxifen can increase the risk of other cancers such as endometrial cancer ((EBCTCG), 1998). Tamoxifen use is also linked to a variety of side effects most commonly hot flushes and gynaecological symptoms. Additionally, the agonistic effects of tamoxifen may also be contribute to the development of tamoxifen resistant traits (DeFriend et al., 1994). Although the precise mechanisms behind resistance to tamoxifen remain unclear with several mechanisms proposed leading to anti-oestrogen resistance (Chang, 2012).

The site-specific effects of tamoxifen and the effects seen in pre and postmenopausal women are complex, ensuring that determination of the biological effect observed in individuals is very difficult to predict. Both ERα and ERβ are expressed in different levels in different tissues and have different ligand binding properties (Paige et al., 1999). These may be key reasons why tamoxifen has such different actions in different tissues.

Tamoxifen has also shown to have both agonistic and antagonistic effects within the same tissue, such as breast tissue. Within breast tissue, tamoxifen induces progesterone receptors (PRs) (agonist effect) and at the same time inhibits breast cancer growth (antagonist effect). These findings suggest that tamoxifen is cell specific rather than tissue specific with regards to its mode of action. The cell specific effects have best been explained by Katzenellenborgen et al. (1996) who investigated the pharmacological basis for the action of steroid hormones in both a cell and promoter specific manner (Katzenellenbogen et al., 1996). Katzenellenborgen et al. (1996) describe a three-part system consisting of ligand, receptor and effector. Results of their study show that molecular elements within the nucleus interact with the ligand-receptor complex and influence the transcriptional response to the ER activation by the ligand. It is proposed that the balance between agonism and antagonism is determined by the receptor effector coupling and not the ligand receptor coupling (O'Brian et al., 1985). An explanation is supported by other studies (Brzozowski & Pike, 1997; Paech et al., 1997). Binding of the ligand to the receptor initiates a cascade of molecular events ending in the activation or repression of a target gene. Each ligand can induce a specific conformation of the receptor activation domain leading to an interaction surface to which coactivators or corepressors are likely to bind. These can only bind to the receptor once activated by the respective ligand and provides evidence for a structural

based mechanism of gene activation or repression (Paech et al., 1997).

1.2.1.2 Tamoxifen prevention trials

For women known to be at high-risk of breast cancer, usually those with a family history, preventive endocrine therapy is an option to reduce breast cancer risk. Four breast cancer prevention trials have been performed comparing tamoxifen 20 mg/day with placebo, assessing the time to breast cancer occurrence in healthy women at high-risk of breast cancer (Table 1.1).

The Royal Marsden Study recruited 2,471 women who were randomised to tamoxifen (N = 1,238) or placebo (N = 1,233) for 5-8 years. Initially, trial results found that during the treatment phase the reduction in breast cancers was not statistically significant (HR = 0.78 95% CI (0.58-1.04), P = 0.10) (Powles et al., 1998). However, post-treatment follow-up indicated that a statistically significant reduction in ER-positive cancers was observed in the tamoxifen group compared to the placebo group (HR = 0.48, 95% CI (0.29-0.79), P = 0.004), indicating the need for long-term follow up to fully establish a preventive drug's ability to reduce risk. However, no statistically significant reductions were observed from ER-negative breast cancers (Powles et al., 2007).

The NSABP P-1 study recruited 13,388 healthy women who were deemed to be at high risk of breast cancer who were randomised to tamoxifen (N = 6,681) or to placebo (N = 6,707) for five years (Fisher et al., 1998). The NSABP P-1 study found a statistically significant 49% reduction in invasive breast carcinomas after a median follow up of 69 months (Fisher et al., 1998). This reduction increased to 69% for ER-positive disease, but no difference is observed for ER-negative disease. The magnitude of the reduction is much greater than that of any of the other tamoxifen prevention trials. Each of the risk categories within the Gail model, previous benign histology, and previous family history of breast cancer all gained benefit from tamoxifen treatment (Fisher et al., 1998, 2005).

The Italian tamoxifen prevention trial randomised 5,408 women to placebo (N = 2,708) or tamoxifen (N = 2,700), 20 mg/day orally, for five years. Initial results were similar to the Royal Marsden Study and found that during the treatment phase the reduction in breast cancers was not statistically significant (Veronesi et al., 1998). A possible

reason for this finding is that the women enrolled on this trial were all thought to be at normal risk of breast cancer due to having hysterectomy without oophorectomy or low risk due to having hysterectomy with bilateral oophorectomy before menopause. In a retrospective analysis, women were reclassified as high risk or low risk of breast cancer based on height, age at menarche, parity, age at full-term prgnancy and oophorectomy. Treatment with tamoxifen significantly lowered the incidence of ER-positive breast cancer in the high risk group (RR = 0.24 95% CI (0.10 - 0.59)); however, no statistically significant effects were observed in the low-risk group (Veronesi et al., 2003).

The results of the Italian trial are in contrast with those of IBIS-I. The difference between the Italian trial and IBIS-I is likely due to the women enrolled on the Italian study being at lower risk than the women on both the NSABP P-1 and IBIS-I trials reducing the power for observing statistically significant reductions in risk. IBIS-I enrolled a total of 7,154 women, who were at high risk of breast cancer who were then randomised to either placebo (N = 3,575) or to tamoxifen (N = 3,579) for five years. After a median follow up of 16 years, the longest median follow-up of all the breast cancer prevention trials, a statistically significant decrease of 29% (HR = 0.71, 95% CI (0.60 - 0.83), P $<$ 0.0001) in breast cancers was observed for women in the tamoxifen group compared to those in the placebo group. Reduction in breast cancer risk was similar in years 0-10 (HR = 0.72, 95% CI (0.59 - 0.88)) and after 10 years (HR = 0.69, 95% CI (0.53 - 0.91)). As expected, the largest reduction was seen in ER-positive cancers. However, no effect was observed for ER-negative breast cancer (Cuzick et al., 2015).

The increased reduction in breast cancer incidence between IBIS-I and the NSABP P-1 trials may be explained in part by the larger number of participants and therefore, greater statistical power to find a result. Additionally, the use of HRT in the other prevention trials (use of HRT was prohibited in the NSABP P-1 trial) may contribute to the difference in results. The effect of HRT on efficacy of tamoxifen is supported by results from the IBIS-I trial which found that women who used HRT during the 5 years of active tamoxifen had a less significant benefit (HRT user: HR = 0.91 (0.67 – 1.24); P = 0.55 vs non-HRT user: HR = 0.60 (0.46 – 0.79); P = 0.0002) than those who had never used HRT (P = 0.04) (Cuzick et al., 2015). The effect of HRT was highest in women who developed invasive ER-positive cancers (HRT user: HR = 0.87 (0.64 – 1.19) vs non-HRT user: 0.55 (0.42 – 0.72); P = 0.03) (Cuzick et al., 2015).

1.2.1.3 Additional SERM prevention trials

In addition to the tamoxifen prevention trials five other studies investigated other SERMs and their efficacy as chemoprevention agents and one trial studied lower doses of tamoxifen. Two trials, the multiple outcome for raloxifene evaluation (MORE/CORE) and the raloxifene use for the heart (RUTH) studies investigated raloxifene compared to placebo in postmenopausal women with osteoporosis, coronary heart disease or risk factors for coronary heart disease (Barrett-Connor et al., 2006; Dickler & Norton, 2001). An additional trial, the postmenopausal evaluation and risk-reduction with lasofoxifene (PEARL), investigated two concentrations of lasofoxifene, 0.25 mg and 0.5 mg, with placebo also in postmenopausal women with osteoporosis (Cummings et al., 2010). An increase in risk of cardiovascular disease was a known possible side effect of tamoxifen hence the use of alternative therapies in women known to be at increased risk of these events. Results from tamoxifen trials show that cardiovascular events are varied, both IBIS-I and the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 display an increase in the number of deaths linked to thromboembolism (Cuzick et al., 2007; Fisher et al., 1998). This suggests that thromboembolism is likely the most serious problem resulting from tamoxifen use. A fourth trial, the study of tamoxifen and raloxifene (STAR), investigated raloxifene versus tamoxifen in women at high-risk of developing breast cancer (Vogel et al., 2010). The GENERATIONS study compared arzoxifene with placebo in postmenopausal women with osteoporosis (Cummings et al., 2011). Finally, a trial investigating low dose tamoxifen, 5 mg/day or 10 mg every other day for three years, compared to placebo was performed to assess the efficacy of low dose tamoxifen and to investigate the number of reported side effects (DeCensi et al., 2019).

Results from both the MORE and the RUTH studies show a 58% and a 33% reduction respectively in overall breast cancer events compared to placebo (Barrett-Connor et al., 2006; Dickler & Norton, 2001). As observed in the prevention trials the reduction in ER-positive breast cancers is much larger at 76% and 55%, but no statistically significant reduction in ER-negative breast cancers is observed. Results of the PEARL study show no reduction in breast cancer incidence for women randomised to the 0.25 mg group (Cummings et al., 2010). There is a small non-statistically significant increase in ER-negative breast cancer for women in the 0.25 mg group. However, those in the 0.5 mg group had a significant reduction in overall breast cancer incidence with ER-

positive breast cancers showing the largest reduction (Cummings et al., 2010). Again, no reductions were seen for ER-negative breast cancers. The GENERATIONS study also had similar results, showing reductions in overall and ER-positive breast cancers, 58% and 70% respectively, a non-statistically significant increase in ER-negative breast cancer was noted (Cummings et al., 2011).

The STAR trial showed that tamoxifen has a greater effect on breast cancer incidence than raloxifene. Overall, the rate of breast cancer incidence was 24% lower in the tamoxifen group than the raloxifene group (Vogel et al., 2010). However, the reduction for both ER-positive and ER-negative breast cancer between the two groups was not statistically significant (Vogel et al., 2010).

The trial of low dose tamoxifen found that lower doses of tamoxifen halved the risk of new lesions and of breast cancer recurrence compared to placebo (DeCensi et al., 2019). These results are similar to those observed using the standard dose of 10 mg/day for five years. Tamoxifen reduced the risk of new lesions by 52% compared to placebo (HR = 0.48; (0.26 - 0.92); P = 0.02). No differences in quality-of-life outcomes, such as vaginal dryness or musculoskeletal events, were observed between the tamoxifen and placebo arms. However, a slight increase in daily hot flush frequency was observed (P = 0.02). Serious adverse events, such as secondary cancers, deep vein thrombosis and pulmonary embolism, were observed in very low numbers in both the tamoxifen and placebo arms (DeCensi et al., 2019).

1.2.2 Aromatase inhibitors (AIs)

The third generation non-steroidal AIs anastrozole and letrozole and the steroidal AI exemestane have shown to be superior to tamoxifen in phase III clinical trials in the adjuvant setting (Ingle & Suman, 2003; Paridaens et al., 2008). All three AIs lower oestrogen concentrations in postmenopausal women by inhibiting and deactivating aromatase, the enzyme responsible for conversion of testosterone or androstenedione to oestrogens.

Aromatase is the only source of oestrogens in postmenopausal women whose ovaries have stopped producing oestrogen. Aromatase (CYP19) is mainly expressed in granulosa cells of ovarian follicles and in lower levels in numerous tissues such as the liver,

brain and subcutaneous fats. After menopause oestrogen is produced solely from these non-glandular sources with a particular emphasis on the subcutaneous fats; therefore, oestrogen production post-menopause is correlated with BMI.

Both steroidal and nonsteroidal AIs mimic androgens, the normal substrate of aromatase, allowing them to compete for access to the binding site. Once bound the difference between the two types becomes clearer. Steroidal AIs, once bound into the enzymatic site, produce an unbreakable bond between the enzyme and the steroidal inhibitor blocking the enzymatic activity permanently. Non-steroidal inhibitors bind reversibly with the enzymatic site stopping enzymatic activity. As non-steroidal inhibitors can be removed from the enzyme binding site, this allows for renewed competition with the normal substrate. As a result of reversible binding, the success of non-steroidal inhibitors is largely dependent on the concentrations of both the normal androgen substrates and of the inhibitor itself and on the relative affinities of inhibitor and substrate to the enzyme binding site. In order for non-steroidal inhibitors to continue to work, a constant presence of inhibitors is required (Mantas et al., 2016). To compete, both non-steroidal and steroidal inhibitors must share particular moieties with androgens, which allows them to bind at the enzyme's catalytic binding site. This makes both types of inhibitor selective to the aromatase enzyme. Despite the differences in structure and function, the clinical relevance of the different mechanisms of action is yet to be understood (Buzdar, 2003).

All three third generation AIs interact with other members of the Cytochrome P450 family of drug metabolising enzymes. However, anastrozole does not show any inhibitory effect of CYP2A6 or CYP2D6, but does inhibit CYP1A2, CYP2C8&9 and CYP3A4 in decreasing magnitudes (Grimm & Dyroff, 1997). Letrozole has a strong inhibitory action on CYP2A6 and moderately inhibits CYP2C19 and CYP3A4 (Buzdar et al., 2002). Inhibition of CYP3A4 could be important if a patient is also prescribed exemestane as CYP3A4 is an important member of the metabolism of exemestane. It therefore remains possible that drug-drug interactions would exist if a patient was prescribed multiple medications concurrently. Drug-drug interaction of both anastrozole and letrozole with tamoxifen are well established with concomitant administration decreasing the plasma concentration of the AI (Dowsett et al., 1999). During the ATAC trial where anastrozole and tamoxifen were taken together, the plasma anastrozole level was 27% lower than observed in individuals taking anastrozole alone (ATAC Trialists'

Group, 2001). As a result of the drug-drug interaction and a lack of clinical evidence for a benefit from the anastrozole and tamoxifen combined, this arm of the study was halted. Interaction with CYP enzymes could form the basis of the side effect and toxicity profiles of these drugs bringing into question the suitability of AIs for long-term treatment of breast cancer (Nabholtz, 2008).

Aside from effects on CYP enzymes and the possibility that disruption to this system is responsible for side effects commonly associated with AIs, two other areas that may explain side effects are impacts on bones and on adrenal steroidogenesis. The three AIs appear to have different impacts on adrenal steroidogenesis. Data suggests that both exemestane and anastrozole have no impact on adrenal steroidogenesis. Data on anastrozole shows no impact in doses up to 10 times greater than the clinically recommended dose. This suggests that the drug has very little activity on CYP enzymes involved in areas of steroid synthesis other than the conversion of testosterone to oestrogens (Buzdar, 2003).

1.2.2.1 Aromatase inhibitor prevention trials

Two main prevention trials with AIs have been performed; IBIS-II and MAP.3 trials. Both trials were double-blind placebo-controlled trials investigating the efficacy and safety of anastrozole in the case of IBIS-II and exemestane in MAP.3 for the prevention of breast cancer (Table 1.1) (Cuzick et al., 2014; Goss et al., 2011). Both studies recruited only postmenopausal women at high-risk of breast cancer. IBIS-II recruited 3,864 postmenopausal women aged between 40 and 70 and these women were randomised to 1 mg oral anastrozole daily (N = 1,920) or matching placebo (N = 1,944) for 5 years. The MAP.3 trial recruited 4560 women who were randomly assigned to either 25 mg exemestane (N = 2,285) or placebo (N = 2,275) (Goss et al., 2011).

The primary endpoint in both studies was histologically confirmed breast cancer, which included both invasive cancers and non-invasive ductal carcinoma in situ. In the IBIS-II trial after a median follow up of five years, 40 women (2%) in the anastrozole group developed breast cancer compared to 85 (4%) in the placebo group, a reduction of over 50% (HR = 0.47, 95%CI (0.32–0.68), $P < 0.0001$). There were 18 deaths reported in the anastrozole group compared to 17 in the placebo group with no cause of death being more prevalent than another between the treatment groups. Similar results were

observed in the MAP.3 trial where, after median follow up of 35 months, 11 breast cancers were reported in the exemestane group compared to 32 in the placebo group (HR = 0.35, 95%CI (0.18 - 0.70); P = 0.002) (Goss et al., 2011).

The most recent follow-up of IBIS-II shows that the benefits of anastrozole last beyond the five-years of active therapy. A 49% reduction in breast cancer risk was observed in the anastrozole group (85 cases) compared to the placebo group (165 cases) (HR = 0.51 (0.39 – 0.66); P < 0.01). Although the reduction was greater in the first 5 years of the trial the later follow-up still found significant reductions after 5 years (50 vs 76 new cases, HR = 0.64 (0.45 – 0.91); P = 0.01), which were not significantly different from the first 5 years (P = 0.087). A 54% reduction in invasive ER-positive breast cancer was observed (HR = 0.46 (0.33 – 0.65); P < 0.01), and a 59% reduction in DCIS (HR = 0.41, (0.22 – 0.79); P < 0.01), particularly in ER-positive DCIS (HR = 0.22 (0.07 – 0.65); P < 0.01). There was still no significant difference in deaths between the two arms of the trial (anastrozole = 69, placebo = 70, HR = 0.96 (0.69 – 1.34); P = 0.82). There was no excess risk of fractures (HR = 1.04 (0.88 - 1.22); P = 0.64) or cardiovascular disease (HR = 1.41 (0.60 - 3.31)) observed. A likely explanation for the lack of statistical significance and large confidence intervals, particularly for ER-positive DCIS, may be the result of a small number of events recorded at this follow up.

Overall, both studies conclude that AIs effectively reduce the incidence of breast cancer in high-risk women (Cuzick et al., 2014, 2020; Goss et al., 2011). In the IBIS-II trial, menopausal-like side effects were reported in both the anastrozole and placebo arms; however, they were significantly higher in women randomised to anastrozole. No statistically significant increase in major adverse events such as other cancers or cardiovascular events were observed between the placebo and anastrozole groups (Cuzick et al., 2014). Therefore, there is strong support for the use of anastrozole in post-menopausal women who are at high-risk of developing breast cancer (Cuzick et al., 2014, 2020). Similar conclusions were reached for exemestane which was not associated with an increase in other cancers or cardiovascular events; however, a number of health-related quality-of-life events were noted (Goss et al., 2011).

1.2.2.2 Adjuvant AI trials

Seven major adjuvant trials have been performed comparing different AIs with tamoxifen. One trial directly compared anastrozole with tamoxifen the Arimidex, Tamoxifen Alone or in Combination Trial (ATAC) (Howell et al., 2005). The ATAC trial randomised women to 1 mg/day of anastrozole, 20 mg/day of tamoxifen or a combination for five years. Three other trials compared the switching to anastrozole compared to continued tamoxifen therapy. These trials were the Arimidex-Nolvadex (ARNO) trial 95, Austrian Breast and Colorectal Cancer Study Group 8 (ABCSG-8) and the Italian Tamoxifen Anastrozole Trial (ITAT) (Boccardo et al., 2005; Dubsky et al., 2012; Kaufmann et al., 2007). In each of the switching studies women who had been taking tamoxifen for two to three years were then randomised to either 1 mg/day anastrozole or continue tamoxifen for three further years.

The ATAC trial found that there was a significant improvement in disease-free survival in the anastrozole arm compared to the tamoxifen arm (575 vs 651, HR = 0.87, 95% CI (0.78-0.97); P = 0.01) with the greatest decrease observed in hormone receptor-positive disease (HR = 0.83 (0.73-0.94); P = 0.005) (Howell et al., 2005) The combination arm was closed early due to low efficacy. The benefit of anastrozole was observed at each follow up point after one year of therapy. After 10 years follow up anastrozole benefits versus tamoxifen are still present for both overall breast cancer and hormone receptor positive breast cancers (Cuzick et al., 2010).

Both the ARNO and the ITAT trials found an improvement in disease-free survival and recurrence-free survival for women who switched to anastrozole compared to those who remained on tamoxifen. In the ARNO trial, a 34% reduction in the risk of disease recurrence (HR = 0.66 (0.44 - 1.00); P = 0.049), and a 47% improvement in overall survival (HR = 0.53 (0.28 - 0.99); P = 0.045) were observed for switching to anastrozole compared to remaining on tamoxifen (Kaufmann et al., 2007). This was compared to a decrease of 65% in disease-free survival (HR = 0.35 (0.18 - 0.68); P = 0.001) and a 85% decrease in recurrence-free survival (HR = 0.15 (0.03 - 0.65); P = 0.003) observed in the ITAT trial (Boccardo et al., 2005). Results of the ABCSG-8 trial found no statistically significant improvement in recurrence-free survival (HR = 0.80 (0.63 - 1.01); P = 0.06), but an exploratory analysis observed a 22% improvement in distant relapse-free survival (HR = 0.78 (0.60 - 1.00); P = 0.046) (Dubsky et al., 2012). All

three trials supported the findings of the ATAC trial, namely that anastrozole improves breast cancer outcomes when compared to tamoxifen (Boccardo et al., 2005; Dubsky et al., 2012; Kaufmann et al., 2007). However, it is worth of note that the ABCSG-8 trial reached statistically opposing conclusions for recurrence-free survival and distant relapse-free survival from very similar hazard ratios and confidence intervals (Dubsky et al., 2012).

Two studies investigated the use of exemestane compared to tamoxifen. The Intergroup Exemestane Study (IES) enrolled postmenopausal women with ER-positive or ER-unknown breast cancer who were disease-free after 2-3 years of tamoxifen who were randomised to either switch to exemestane or to continue with tamoxifen for the remainder of the five-year endocrine therapy period (Coombes et al., 2007). Similarly, the Tamoxifen Exemestane Adjuvant Trial (TEAM) enrolled postmenopausal women with ER-positive breast cancer to exemestane or to tamoxifen followed by exemestane over a five year period (Van De Velde et al., 2011). The primary end point of both trials was disease-free survival. The IES study results indicate that disease-free survival favoured switching to exemestane (HR = 0.76 (0.66 - 0.88); P = 0.0001). A similar result was achieved for ER-positive disease alone (HR = 0.75 (0.65 - 0.87); P = 0.0001) (Coombes et al., 2007). The results of the IES trial were not supported by the results of the TEAM trial which observed no significant difference between the two study arms for disease-free survival (HR = 0.97 (0.88 – 1.08); P = 0.60) (Van De Velde et al., 2011).

A further study investigated the use of exemestane compared to anastrozole. The MA.27 trial randomised postmenopausal ER-positive breast cancer patients to five years of exemestane or anastrozole. The primary endpoint was event-free survival and secondary endpoints included overall survival and distant disease–free survival (Goss et al., 2013).

No difference in event free survival was observed between the exemestane and anastrozole (HR = 1.02 (0.87 – 1.18); P = 0.85). There were also no significant differences observed for either overall survival (HR = 0.93 (0.77 – 1.13); P = 0.46), or distant disease–free survival (HR = 0.95 (0.76 - 1.18); P = 0.64) (Goss et al., 2013).

Only one trial examined the use of letrozole compared to tamoxifen. The Breast International Group (BIG) 1-98 study recruited 8,010 women to a two or four-arm trial.

The four arm trial compared 5 years of monotherapy with tamoxifen (N = 1,548) or with letrozole (N = 1,546) or with sequences of 2 years of one followed by 3 years of the other (Tamoxifen – Letrozole N = 1,548, Letrozole – Tamoxifen N = 1,540)) for postmenopausal women with endocrine-responsive early invasive breast cancer (Thürlimann et al., 2005).

The first reports included all randomised patients but only included events inside two years, the time of the switch, in patients in the crossover arms. Primary results show that letrozole improves disease-free survival (HR = 0.81 (0.70 - 0.93); P = 0.0003) compared to tamoxifen. These findings led to informing the patients in the tamoxifen arm of the benefits of letrozole and offered the chance to switch therapies. Of 2,459 patients in the tamoxifen-alone treatment arm, 619 (25.2%) selectively crossed over to letrozole. The sequential treatment analysis after median follow up of 71 months showed neither of the cross over arms significantly improved disease free survival compared with letrozole monotherapy; however, women randomised to tamoxifen followed by letrozole had a greater incidence of early recurrences compared to those on letrozole monotherapy (Regan et al., 2011).

1.3 Prevalence of side effects of endocrine therapy in breast cancer prevention trials

The benefits of tamoxifen and AIs in the prevention of breast cancer have been well established. However, the effectiveness of endocrine therapy for breast cancer prevention relies on adequate uptake and adherence (Smith et al., 2016). Side effects are an important issue to women thinking about or taking endocrine therapy, as research shows that side effects are associated with poorer uptake and lower adherence (Smith et al., 2016, 2017). While increases in endometrial cancer and thrombotic events are the most serious adverse events, the number of these events is relatively small. Non-life-threatening side effects like hot flushes (HFs), gynaecological events, musculoskeletal events, headaches, nausea and vomiting also increasingly reduce the uptake of endocrine therapies. Little is known about risk factors for side effects or markers that can predict whether a woman is at increased or high risk of experiencing a particular side effect. A summary of side effects reported during each of the prevention trials using tamoxifen or aromatase inhibitors can be found in Table 1.2.

With tamoxifen prevention trials, the most frequently reported side effects were HFs, other vasomotor symptoms, and gynaecological symptoms, including vaginal discharge and irregular bleeding. Vaginal dryness is also reported by women taking tamoxifen; however, reports of vaginal dryness are not significantly different in the tamoxifen arm compared to the placebo arm (Cuzick et al., 2015; Fisher et al., 2005; Powles et al., 2007; Veronesi et al., 2007). Commonly reported side effects in the AI prevention trials included fractures, other musculoskeletal events (including arthralgia and joint stiffness), vasomotor symptoms, gynaecological symptoms and eye problems including cataracts (Cuzick et al., 2014, 2020; Goss et al., 2011). It is important to note that whilst numbers of endocrine therapy related side effects are significantly higher in women randomised to tamoxifen or anastrozole, they are also reported in large number in those randomised to placebo.

The incidence of HFs and gynaecological symptoms are significantly increased in the tamoxifen arm of all prevention trials (Table 1.2). However, tamoxifen has little impact on the rate of fractures overall and at sites linked with osteoporosis (Cuzick et al., 2007). Incidence of HFs and musculoskeletal events are significantly increased in the anastrozole arm compared to the placebo arm in both the IBIS-II and MAP.3 prevention trials. In contrast to the observations from the tamoxifen trials, incidence of vaginal dryness was significantly increased in the anastrozole arm of IBIS-II, but not MAP.3. Incidence of other gynaecological symptoms were not significantly different in women randomised to anastrozole compared to women randomised to placebo (Table 1.3). A comprehensive comparison of side effect profiles in postmenopausal women randomised to tamoxifen or anastrozole during the ATAC trial found that anastrozole was better tolerated than tamoxifen (Buzdar et al., 2006). After a median follow-up of 68 months, significantly fewer treatment-related adverse events occurred with anastrozole compared to tamoxifen (A:1884 (61%) vs T:2117 (68%); RR = 0.89 (0.86 – 0.92); P $<$ 0.0001). In addition, the number of adverse events leading to withdrawal was also lower in those randomised to anastrozole (344 (11%) vs 442 (14%); RR = 0.78 (0.68 – 0.89); P = 0.0002) as were the number of treatment-related adverse events leading to withdrawal (200 (6%) vs 274 (9%); RR = 0.73 (0.61 – 0.87); P = 0.0005) (Buzdar et al., 2006). However, these side effects only persist during the active phase of the trial and subside after the treatment phase and are not increased in the follow up period (Cuzick et al., 2007; Powles et al., 2007).

Table 1.2: Summary of the data on side effects from all the prevention trials performed with tamoxifen compared to placebo

Study name	**IBIS-I (Cuzick et al. 2002, 2007*)**				**Royal Marsden Trial (Powles et al. 2007)**			
Length of follow up	**8 Years**				**20 Years**			
Side effects	**Placebo**	**Tamox**	**RR (95% CI)**	**P-value**	**Placebo**	**Tamox**	**RR (95% CI)**	**P-value**
Numbers per group	**3575**	**3579**			**1233**	**1238**		
Vasomotor/Hot flushes	1837	2452	1.33 (1.28 - 1.39)	<0.0001*	394	598	1.51 (1.37 - 1.67)	<0.001
Vaginal discharge	503	1026	2.04 (1.85 - 2.24)	<0.0001*	167	321	1.91 (1.62 - 2.27)	<0.001
Vaginal dryness	723	782	1.08 (0.99 - 1.18)	0.10*			Not Reported	
Irregular bleeding	678	842	1.24 (1.13 - 1.36)	<0.0001*	439	496	1.13 (1.02 - 1.25)	0.02
Headaches / migraines	1030	878	0.85 (0.79-0.92)	<0.0001	244	227	0.93 (0.79 - 1.09)	0.4
All fractures	142	121	0.85 (0.67-1.08)	0.18	22	19	0.86 (0.47 - 1.58)	0.6
Breast complaints	833	612	0.73 (0.67-0.81)	<0.0001	60	65	1.08 (0.77 - 1.52)	0.7
Eye (excluding cataracts)	896	901	1.00 (0.93-1.09)	0.91	86	94	1.09 (0.82 - 1.44)	0.6
Cataracts	34	29	0.85 (0.52-1.40)	0.52	1	9	8.96 (1.13 - 70.65)	0.02
Venous thromboembolism	42	85	2.03 (1.38-3.01)	0.0002	3	8	2.66 (0.71 - 9.99)	0.2
Cerebrovascular	17	12	0.71 (0.31-1.47)	0.35	7	9	1.28 (0.48 - 3.43)	0.6
Cardiac events	71	64	0.90 (0.63-1.28)	0.54	12	10	0.83 (0.36 - 1.91)	0.7
Endometrial cancer	11	17	1.55 (0.68-3.65)	0.26	5	13	2.59 (0.93 - 7.24)	0.06

*Breakdown unavailable for 8 year follow up. Data were taken from the 2002 report and represent the reported numbers during treatment

Continued on next page

Table 1.2 – continued from previous page

Study name	NSABP P1 (Fisher et al. 1998, 2005*)				Italian Trial (Veronesi et al. 2007)			
Length of follow up	**7 Years**				**11 Years**			
Side effects	**Placebo**	**Tamox**	**RR (95% CI)**	**P-value**	**Placebo**	**Tamox**	**RR (95% CI)**	**P-value**
Numbers per group	**6498**	**6466**			**2708**	**2700**		
Vasomotor/Hot flushes	4457[a]	5276[a]	1.19 (1.17 - 1.21)	<0.0001	446	635	1.43 (1.28 - 1.59)	<0.0001
Vaginal discharge	2261[a]	3569[a]	1.59 (1.52 - 1.65)	<0.0001	173	505	2.93 (2.48 - 3.45)	<0.0001
Vaginal dryness	Not reported				269	295	1.14 (0.97-1.34)	
Irregular bleeding	Not reported				Not reported			
Headaches / migraines	Not reported				95	63	0.68 (0.50-0.94)	0.01
All fractures	116	80	0.68 (0.51-0.92)	0.01	Not reported			
Breast complaints	Not reported				Not reported			
Eye (excluding cataracts)	Not reported				Not reported			
Cataracts	929	1133	1.21 (1.10-1.34)	<0.0001	Not reported			
Venous thromboembolism	34	49	1.44 (0.91-2.30)	0.1	28	44	1.58 (0.98 - 2.52)	0.06
Cerebrovascular	50	71	1.42 (0.97-2.08)	0.05	7	12	1.72 (0.68 - 4.36)	0.25
Cardiac events	109	113	1.03 (0.79-1.36)	0.76	21	35	1.67 (0.98 - 2.86)	0.06
Endometrial cancer	17	53	3.28 (1.87-6.03)	<0.0001				

[a]Reported from first report rather than follow up as no result on quality-of-life events were reported in the 2005 report

Tamox = Tamoxifen, RR = Risk Ratio, CI = Confidence Interval

Table 1.3: Summary of side effect incidence from the prevention trials performed with anastrozole compared to placebo

Study name	**IBIS-II(Cuzick et al. 2014)**				**MAP.3 (Goss et al. 2011)**			
Date of results	**5 Years**				**3 years**			
Side effects	**Placebo**	**Anast**	**RR (95% CI)**	**P-value**	**Placebo**	**Exem**	**RR (95%CI)**	**P-value**
Numbers per group	**1944**	**1920**			**2275**	**2285**		
Vasomotor/Hot flushes	961	1090	1.15 (1.08-1.22)	<0.001	718	900	1.25 (1.15 - 1.35)	<0.001
Vaginal Dryness	304	357	1.19 (1.03-1.37)	0.01	343	352	1.02 (0.89 - 1.17)	0.68
Irregular bleeding	81	65	0.82 (0.60-1.13)	0.2				
Musculoskeletal	1124	1226	1.10 (1.05-1.16)	<0.001	606	665	1.09 (1.00 - 1.20)	0.04
All fractures	149	164	1.11 (0.90-1.38)	0.32	143	149	1.04 (0.83 - 1.30)	0.72
Eye (including cataracts)[b]	335	348	1.05 (0.92-1.21)	0.47				
Cataracts	95	90	0.96 (0.72-1.27)	0.77				
Venous thromboembolism	17	19	1.13 (0.59-2.17)	0.71	7	11	1.56 (0.61 - 4.03)	0.35
Cerebrovascular	6	3	0.51 (0.13-2.02)	0.34	11	13	1.18 (0.53 - 2.62)	0.69
Cardiac events	9	8	0.90 (0.35-2.32)	0.83	354	341	0.96 (0.84 - 1.10)	0.65
Endometrial cancer	5	3	0.61 (0.15-2.54)	0.49	8	5	0.62 (0.20 - 1.90)	0.4

[b] Reported number of eye complaints includes cataracts a deviation from the tamoxifen trials
Anast = Anastrozole, Exeme = Exemestane, RR = Risk Ratio, CI = Confidence Interval

Incidence of common side effects reported in tamoxifen trials are similar for both pre and postmenopausal women. However, this is not true for more severe adverse events such as endometrial cancer or pulmonary embolism the majority of which were reported by postmenopausal women. Gynaecological and vasomotor symptoms are significantly increased in the tamoxifen arm compared to the placebo arm for both pre and postmenopausal women (Cuzick et al., 2002). The number of reports of vaginal discharge are similar for both pre and postmenopausal women. The number of reports of vaginal bleeding in postmenopausal women are significantly lower than those observed for premenopausal women. Vaginal dryness is not statistically significantly increased in the tamoxifen arm for either premenopausal or postmenopausal women (Cuzick et al., 2002).

Women taking tamoxifen or AIs have reported a higher rate of vaginal bleeding, dryness and discharge compared to women on placebo (Cuzick et al., 2002, 2014; Day et al., 1999). Interestingly, results from three major trials comparing AIs and tamoxifen, ATAC, TEAM, and IES, reported that vaginal bleeding and discharge in the AI group were lower than observed in the tamoxifen group, which suggests that AIs may inhibit endometrial proliferation (Goss et al., 2005; Morandi et al., 2004; The ATAC (Arimidex Tamoxifen Alone or in Combination) Trialists' Group, 2002). However, comparison of gynaecological symptoms between women taking AIs and women taking tamoxifen showed that AIs cause more vaginal dryness than tamoxifen. This result is likely due to the oestrogenic effect of tamoxifen observed in the vagina and endometrium and the blocking of peripheral oestrogen production by AIs (Cella & Fallowfield, 2008; Hickey et al., 2008). 16.3% of women in the anastrozole arm of the ATAC trial reported vaginal dryness compared to 8.4% in the tamoxifen arm (Fallowfield et al., 2004). The number of HFs reported by women taking anastrozole are also lower than the number reported in the tamoxifen arm (Howell et al., 2005).

Studies have generally reported a higher incidence of both arthralgia and myalgia with AIs versus tamoxifen (Cuzick et al., 2010; Goss et al., 2011; Thürlimann et al., 2005) although it should be noted that the definition of these side effects changes between trials. Review of prevention studies including the IBIS-II study shows total fractures and the number of fractures at specific sites were not significantly different between the placebo and the anastrozole arm (Cuzick et al., 2014, 2020). During active treatment a small increase in fractures was observed (anastrozole = 198 vs placebo =

186, OR = 1.09 (0.87 – 1.35)) but following treatment completion a small reduction was observed in the anastrozole group (182 vs 187, OR = 0.98, (0.79 – 1.23)) (Cuzick et al., 2020). However, musculoskeletal events were significantly higher in the anastrozole group than the placebo group (Cuzick et al., 2014). These results are supported by the findings of the MAP.3 study where no significant differences in skeletal fractures were observed between the exemestane and placebo arms (Goss et al., 2011). While there were no significant differences between groups for mild or severe arthralgias, moderate arthralgia was more common in the anastrozole group compared to the placebo group.

The rate of arthralgia associated with AIs is important as several trials have demonstrated the superiority of AIs over tamoxifen in improving disease free survival and prevention of breast cancers (Perou et al., 2000; Trémollieres et al., 2017). However, AI induced arthralgia (AIA) can also have a large impact on compliance. One study, designed to investigate AIA incidence, found that 13% of women discontinued treatment due to arthralgia (Beckwée et al., 2017). A longer follow up study reviewing pharmacy records found that only 50-68% of women were adherent to AI therapy after three years; where adherence is set at >80% of the prescribed dose taken (Bradley et al., 2015). Further information on the role of side effects on the adherence to endocrine therapy will be presented in section 1.5.

It is clear that tamoxifen is effective in breast cancer treatment and prevention, but the increased toxicity has limited its wider use. A study of 500 women has shown that a reduced dose of 5 mg/day also decreases breast cancer proliferation and is not inferior to standard 20 mg/day dose. The low dose tamoxifen trial has also shown that the number of patients reporting side effects is also reduced. The incidence of side effects was the same in both the low dose tamoxifen arm and the placebo arm with the exception of a small increase in frequency of hot flushes with tamoxifen (P = 0.02). Reports of serious adverse events were also reduced in the low-dose trial. 12 serious adverse events with tamoxifen and 16 with placebo were reported, including one DVT or pulmonary embolism and one stage I endometrial cancer with tamoxifen and one DVT or pulmonary embolism with placebo (DeCensi et al., 2019).

The current recommended dosage of anastrozole is 1 mg/day. To the author's knowledge, no studies or trials have been performed to assess the efficacy of a lower dose of anastrozole for breast cancer prevention nor for its side effect profile.

1.4 Risk factors for endocrine therapy side effects

1.4.1 Gyanecological symptoms

During menopause, hormone concentrations, especially oestrogen, can change dramatically leading the tissues of the vagina becoming thinner, drier and less flexible, which results in vulvovaginal atrophy (VVA). Symptoms of VVA include reduced vaginal secretions, which can result in decreased lubrication and dry, fragile vulvovaginal tissues which are susceptible to injury, tearing, and bleeding. This can lead to many of the symptoms associated with endocrine therapies (Mac Bride et al., 2010). In the VVA state the vagina may become prone to inflammation, a condition known as atrophic vaginitis. This condition can also result in redness of the vagina and vaginal discharge (Lester et al., 2015).

VVA can occur at any time during a woman's life, although it is more common after menopause where it is associated with the loss of oestrogen (Mac Bride et al., 2010). In the general population VVA is reported by approximately 4% of premenopausal women but this increases dramatically to 47% in older postmenopausal women (Mac Bride et al., 2010). Additionally, abnormal uterine bleeding, which includes menorrhagia (abnormal heavy or extended menstrual bleeding), metrorrhagia (light bleeding at irregular intervals) and intermenstrual bleeding affects nearly a third of all reproductive age women (Bakkum-Gamez et al., 2011). Some subgroups report VVA more frequently; in a cohort of breast cancer survivors VVA was reported by 23.4% of premenopausal women and by 61.5% of postmenopausal women (Crandall et al., 2008).

VVA is an event that may negatively impact quality of life and is reported by women taking tamoxifen and AIs but is also common in those taking placebo. Tamoxifen acts as an oestrogen agonist or antagonist depending on the tissue and menopausal status. In premenopausal women, tamoxifen may cause VVA by acting as an oestrogen antagonist blocking the ER from the naturally high levels of circulating oestrogens. However, in postmenopausal women, tamoxifen acts as an ER agonist.

In postmenopausal women VVA symptoms were less common in the tamoxifen arm than in women receiving an AI. In the ATAC study, Cella et al. (2006) reported that cases of vaginal dryness was more common in women taking anastrozole than

in women taking tamoxifen (18.5% vs 9.1%). Women in the anastrozole group also reported more cases of dyspareunia compared to those in the tamoxifen group (17.3% vs 8.1%) (Cella et al., 2006). The incidence of gynaecological events and impact of breast cancer therapies was discussed further in section 1.3.

VVA is most often associated with hypoestrogenism, after menopause the concentration of circulating oestrogens declines with the only source through the conversion of androgens to oestrogens in adipose tissues. Systemic loss of oestrogens represents the major risk factor for increasing the incidence of VVA. Loss of oestrogen results in lower stimulation of ERα and ERβ in uterine tissues leading to the deterioration of tissues, decreased blood flow, loss of elasticity and thinning of the tissues and epithelium all key components of VVA.

Given the importance of oestrogens, it is not surprising that lower BMI has emerged as a risk factor for VVA in postmenopausal women (Huang et al., 2010). Adipose tissue plays an important role in production of postmenopausal oestrogens, and therefore, women with lower BMI should have lower circulating oestrogens and hence a higher incidence of VVA. However, a previous study in middle-aged women didn't find a significant relationship between BMI and symptoms of VVA (Gold et al., 2000). Therefore, further work is required to properly assess the relationship between BMI and other anthropometric factors on oestrogen concentrations and the incidence of VVA in postmenopausal women.

1.4.1.1 Risk factors for gynaecological symptoms

Sex Hormones

Gynaecological side effects can occur as a result of disruption to oestrogen and progesterone concentrations required to maintain healthy endometrial integrity. Mac Bride et al. (2010) showed that a low dose of oestrogen coupled to the opposing effect of progestogen, leads to the promotion of gland and stromal atrophy and is insufficient to maintain normal endometrium integrity. As a result of disruption, the endometrium is thin and prone to bleeding (Mac Bride et al., 2010).

Both tamoxifen and AIs have been found to have a significant impact on incidence of gynaecological symptoms largely due to their impact on circulating oestrogens. AIs

have been found to cause more vaginal dryness than tamoxifen, but tamoxifen increases the incidence of both vaginal discharge and irregular bleeding which suggests two different mechanisms are likely. The oestrogenic effect of tamoxifen observed in the vagina and endometrium and the blocking of peripheral oestrogen production by AIs (Cella & Fallowfield, 2008; Hickey et al., 2008).

A study performed by Love et al. (2000) in postmenopausal breast cancer patients initially given oestrogen followed by subsequent tamoxifen, identified a decrease in maturation of vaginal epithelial cells. This suggests an anti-oestrogenic effect by tamoxifen in an oestrogen rich environment (Love et al., 2000). These results are supported by the findings of Tajima et al (1979), who observed similar effects of tamoxifen in premenopausal women treated with tamoxifen for fertility problems (Polin & Ascher, 2008).

Progestins play an important role in the stabilisation of endometrial stromal and vascular extracellular matrix. Normally withdrawal of these factors results in the controlled haemorrhage of menstruation. However, in the case of irregular bleeding, long-term exposure to progestins can induce unrestrained angiogenesis, which can result in large fragile endometrial vessels, further promoting bleeding (Lockwood, 2011). While precise mechanisms have yet to be identified, angiogenesis inhibitors are believed to play an important role alongside changes in concentrations of and response to hormones (Losordo & Isner, 2001).

Androgens are known to play an important role in female sexual function (Khanjani & Panay, 2019). Baldasarre et al (2013) showed that androgen receptors are expressed in the vagina and that the expression of androgen receptors decreases with age suggesting that androgens could play a protective role in vulvovaginal atrophy (Baldassarre et al., 2013).

Witherby et al. (2011) conducted a pilot study in women with breast cancer, taking AIs and suffering from vaginal atrophy. Results showed that a 4-week course of vaginal testosterone improved signs and symptoms of vaginal atrophy without an increase in systemic oestradiol levels. This suggests a role of androgens in the basis of vulvovaginal atrophy without increasing the risk of breast cancer (Witherby et al., 2011). Dehydroepiandrosterone (DHEA) is a precursor steroid in the biosynthesis of sex steroids, levels of which decrease with age. Recent trials have suggested that oral and vagi-

nal preparations of DHEA as a daily dose can improve symptoms of VVA without increasing the concentration of sex hormones beyond those normally observed during menopause (Labrie et al., 2016).

The role of sex hormones on the incidence of gynaecological side effects could be key to minimising the effect of these symptoms and increasing adherence to endocrine therapy. Identifying concentrations of androgens which increase the risk of gynaecological symptoms will form a part of my thesis to assess the likelihood of side effects during endocrine therapy.

Baseline factors

Alongside sex hormone risk factors, other health risk factors exist which increase the risk of gynaecological symptoms. Huang et al (2010) observed a correlation between lower BMI and multiple vaginal symptoms (Huang et al., 2010). This may be explained by the fact that women with lower BMI have lower concentrations of circulating oestrogens as a result of reduced adipocyte-based aromatization of oestrone to oestradiol, reduced conversion of androstenedione to oestrone and reduced conversion of testosterone to oestradiol (Cauley et al., 1989). However, another study by Gold et al. (2000) found no significant relationship between lower BMI and vaginal dryness or dyspareunia; therefore, further research is required to confirm the relationship (Gold et al., 2000).

Smoking

Smoking has also been inversely associated with vaginal symptoms (Huang et al., 2010). While the harmful effects of smoking are well established, there are a multitude of other effects in tissues not experiencing direct smoke contact that remain relatively poorly explained. Women who smoke often behave as though they are oestrogen-deficient, which may lead to an increase in oestrogen-deficiency problems such as osteoporosis, but may prove to be beneficial in issues caused by oestrogen excess (Baron et al., 1990). There is an increasing body of evidence that at least part of the metabolic consequences of smoking is down on the altered sex-hormone metabolism in particular that of oestrogen (Tankó & Christiansen, 2004).

Single nucleotide polymorphisms

Very little work has been performed identifying SNPs associated with gynaecological symptoms. A GWAS by Burri et al. (2012) found 15 SNPs linked with female sexual

dysfunction, although none met a genome wide significance level of $P = 1 \times 10^{-8}$. Six SNPs were found to be significant at a level of $P < 3 \times 10^{-6}$ and all were associated to the locus of *EPC1* which was associated with lubrication levels (Burri et al., 2012). *EPC1* encodes the enhancer of polycomb homolog 1 and is a component of NuA4 histone acetyltransferase complex. Previous research has linked this complex to activation of oncogenes and proto-oncogene mediated growth induction, replicative senescence, apoptosis and skeletal muscle differentiation (Doyon et al., 2004). However, it is unclear why and what role it plays in decreased vaginal lubrication (Burri et al., 2012).

Tucker et al. (2005) investigated the effect of two polymorphisms of CYP3A5 (CYP3A5*3 and CYP3A5*6), an enzyme known to metabolise tamoxifen into its more potent forms, but whose impact on tamoxifen related side effects remains unknown. Using t-tests and logistic regression models, associations between the two polymorphisms and an array of side effects, including nausea, migraines, depression, vaginal discharge, vaginal dryness, insomnia and hot flushes, were investigated. No statistically significant differences in tamoxifen related side effects were observed for single or double risk alleles of both polymorphisms (Tucker et al., 2005).

1.4.2 Hot flushes

Hot flushes (HFs) are defined as periods of intense sensations of heat followed by flushing of the skin and periods of sweating. These can be followed by a sense of anxiety and palpitations (Ziv-Gal & Flaws, 2010). The frequency and duration of HFs vary during a woman's lifetime. For most women, peak occurrence of HFs happens during peri and early post menopause. About 80% of women complain about HFs during perimenopause, but for the majority of women, symptoms last for a year or less (Kronenberg, 2010). However, a small proportion of women may suffer from HFs for over ten years (Kronenberg, 1990). Approximately two-thirds of postmenopausal women have HFs, of which 10–20% with suffer severe HFs, which will severely affect daily life (Stearns et al., 2002). They may also occur in situations where a woman's oestrogen levels have dropped sharply, such as during a chemically induced menopause or after removal of the ovaries (Kronenberg, 2010). Surgically induced menopause is associated with an approximate 90% occurrence of HFs and are often more abrupt and severe than HFs in non-surgical menopause (Bachmann, 1999).

Several risk factors are associated with HFs. HFs are more common in postmenopausal women with low oestradiol concentrations. Other anthropometric factors which increase HF incidence are low BMI, little physical activity and smoking (Cochran et al., 2008). While every woman's oestrogen levels decrease after menopause, it remains an area of interest why some women do not experience HFs, others adapt, and others experience these symptoms for prolonged periods. Whether this is due to some women having a lower threshold of perception or whether there are genetic, environmental or lifestyle factors which increase or decrease the risk of HF, remains unknown (Kronenberg, 2010).

HFs are also common in breast cancer patients prescribed tamoxifen or AIs (Kronenberg, 2010). Approximately half of postmenopausal women who have had breast cancer report HFs, with about two-thirds reported as mild (Chang et al., 2016). As the majority of breast cancers occur in women older than 50, there is significant overlap between women experiencing breast cancer treatment related HFs and those experiencing menopausal induced HFs. Reports of large numbers of HFs in breast cancer patients is because breast cancer treatments, particularly endocrine therapy, disrupt oestrogen mechanisms which can contribute to or worsen the symptoms of menopause (Kligman & Younus, 2010; Puhalla et al., 2012). Although HFs are commonly reported by women randomised to either anastrozole or tamoxifen, a comparison of postmenopausal women on the ATAC trial found that women randomised to tamoxifen reported significantly more HF than those randomised to anastrozole (anastrozole:1,104 (35.75%) vs tamoxifen: 1,264 (40.9%); OR = 0.80 (0.73 – 0.89); $P < 0.0001$) (Howell et al., 2005). The following sections will introduce and discuss risk factors for HFs.

1.4.2.1 Risk factors for hot flushes

Sex hormones

Sex hormone concentrations have been hypothesised to cause the side effects commonly observed as a result of taking endocrine therapy (Emond et al., 2011; Huang et al., 2010; Mac Bride et al., 2010). In postmenopausal women, oestradiol, testosterone, SHBG and dehydroepiandrosterone sulphate (DHEA-S) may contribute to the pathogenesis of breast cancer and the occurrence of side effects (Endogenous Hormones and Breast Cancer Collaborative Group, 2011). Oestrogens in particular are often associated with the onset of HFs as shown by multiple breast cancer prevention trials using SERMS

or AIs to block or reduce the level of circulating oestrogens to prevent breast cancer (Cuzick et al., 2010, 2013).

However, oestrogen reduction alone does not explain the occurrence of HFs as there is no relationship between vaginal, urinary or circulatory concentrations of oestrogen in women with and without HFs (Freedman, 2014). Arizonović et al (2018) investigated the association between sex hormones and the presence or absence of HFs (Arizanović et al., 2018). Result of the study found that there was no significant difference in the concentrations of oestradiol, testosterone or SHBG between women who report hot flushes and those that do not. However, statistically significant differences in the concentrations of DHEA-S were observed between the two groups (Arizanović et al., 2018). Dehydroepiadrosterone (DHEA) has been used to reduce HFs by up to 50% in a small pilot study in postmenopausal women with a history of breast cancer suggesting that androgens may also influence HF incidence (Barton et al., 2006).

Additionally, despite SERMs and AIs altering the function of the normal hormonal milieu, not all women develop HFs suggesting that while withdrawal of oestrogens may be part of the aetiology it is not the whole picture. It must also be considered that rather than the absolute concentration of circulating oestrogens, the sudden change in concentration caused by oestrogen withdrawal is considered to be the cause of HFs (Mom et al., 2006). If changes in oestrogen concentrations do explain the presence or absence of HFs then if a study was performed where no change in oestrogen concentration was observed the association between oestrogen and HFs might be missed (Whiteman et al., 2003a).

Future studies should not only examine changes in oestrogen concentrations for an association with HFs but should also consider the role of other hormones and the ratio of hormones not just the absolute concentration of individual hormones. There is also a requirement for a study of genetic and lifestyle factors that could raise the risk of HFs.

Single nucleotide polymorphisms

Genetic polymorphisms in genes encoding ERα and ERβ, *ESR1* and *ESR2* respectively, are associated with tamoxifen-induced HFs. In premenopausal women, increased number of *ESR1* PvuII and XbaI polymorphisms were associated with higher HF score, incidence multiplied by severity, compared with those who had other haplotypes (Jin et

al., 2008). Postmenopausal women with *ESR1* PvuII CC polymorphisms and ESR2-02 GG genotypes had 4.6 times increases in HF scores than other postmenopausal women (Jin et al., 2008). In contrast, postmenopausal women with *ESR2* AA genotype were significantly less likely to experience tamoxifen-induced HFs than women who carried at least one *ESR2* G allele (Jin et al., 2008). Results of the study by Jin et al (2008) suggest that knowledge of menopausal status and ER genotype may help predict which women are most likely to suffer HFs during tamoxifen treatment (Jin et al., 2008).

Although the mechanism by which genetic polymorphisms may be associated with HFs is unclear, it is likely that some genetic polymorphisms are associated with altered hormone levels which in turn may be associated with the risk of HFs. A few studies have shown that polymorphisms in certain genes that encode oestrogen synthesis and metabolizing enzymes are associated with altered hormone levels. Polymorphisms in *CYP17* have been associated with higher concentrations of both oestradiol and progesterone and also higher levels of SHBG (Feigelson et al., 1998; Tworoger et al., 2004). Meanwhile a polymorphism in *CYP19* (aromatase) has been associated with lower oestradiol (Dunning et al., 2004; Somner et al., 2004).

Few studies have addressed the association of genetic polymorphism, changes to sex hormone concentrations and HFs directly. The only major study of note was that performed by Schilling et al. (2007). The investigators found that polymorphisms in *CYP19* were not significantly associated with any HFs. However, polymorphisms in *3βHSD* were associated with significantly higher risk of severe HFs and HFs which last for longer than one year. In addition to *3βHSD* polymorphisms a polymorphism in *CYP1B1* was also significantly associated with experiencing HFs compared to those without the polymorphism (Schilling et al., 2007b).

CYP2D6

Tamoxifen is extensively metabolised by many CYP isoforms into at least 22 metabolites. Two of these metabolites, 4-hydroxytamoxifen and the secondary metabolite endoxifen are largely responsible for the anti-oestrogenic action of tamoxifen due to their high affinity for the ER (Johnson et al., 2004; Mürdter et al., 2011). CYP2D6 is the major enzyme responsible for the 4-hydroxylation of tamoxifen and has a large impact on the plasma concentrations of active metabolites. Therefore, tamoxifen treatment outcomes for those with poor CYP2D6 function producing less active metabolites results in a lower rate of breast cancer reduction (Brauch et al., 2009).

This hypothesis has been well tested and a study by Jin et al. (2005) showed that genotype differences in *CYP2D6* and the use of CYP2D6 inhibitors had a significant outcome on women treated with tamoxifen (Jin et al., 2005). Jin et al. (2005) determined that plasma concentrations of endoxifen after four months of tamoxifen were statistically lower in women who had at least one copy of the variant gene. The largest effect was seen in women who are homozygous for the variant gene. This provides conclusive evidence that CYP2D6 polymorphisms or in some cases co-administration of antidepressants, such as paroxetine or fluoxetine, are significantly associated with altered tamoxifen activity.

What remains to be established is if *CYP2D6* polymorphisms have an impact on the incidence of side effects such as HFs. Several studies have been performed to assess this association, key amongst which are Zembutsu et al. (2017) and Sestak et al. (2012). Both groups sought to determine whether variants in *CYP2D6* are associated with the incidence of HFs. The Zembetsu et al. (2017) study enrolled women with hormone-receptor positive and human epidermal growth factor 2 receptor negative breast cancer receiving preoperative tamoxifen and prospectively investigated the effects of *CYP2D6* allelic variants on Ki-67 response, pathological response, and HFs. No association between HFs and *CYP2D6* variants were observed (Zembutsu et al., 2017). This is supported by the study by Sestak et al. (2012) who retrospectively investigated the association between *CYP2D6* polymorphisms and HFs in women on the IBIS-I trial. No significant difference was found between cases and controls in women with the extensive metaboliser phenotype versus the intermediate metaboliser phenotype, nor was there a significant difference between women with the extensive metaboliser phenotype versus the poor metaboliser phenotype (Sestak et al., 2012). Both studies agree that there is insufficient evidence to link *CYP2D6* variants with an increased incidence of hot flushes. However, as drug metabolism is a more complex process involving more than one enzyme variant, other tamoxifen metabolising enzymes should be investigated.

One possible reason there has been little consistency in the CYP2D6 literature is the variation in the range of tamoxifen doses, between 20-40 mg/day, duration of therapy, 1-10 years, ER status of the primary tumour and the use of CYP2D6 inhibitors (Goetz et al., 2015). Concomitant medications may have a negative impact on the recurrence and incidence of breast cancers as a result of altered tamoxifen metabolism. However,

they also have a positive impact on the incidence of side effects for the same reason. Concomitant medications therefore represent an important confounding factor when investigating the incidence of side effects as a result of altered tamoxifen metabolism (Ingle, 2013).

Despite the inconclusive results, patients with reduced CYP2D6 activity remain at risk of reduced tamoxifen efficacy or increased side effects. Guidelines on adjusting therapies based on *CYP2D6* genotypes offer the potential for improved results and whilst these have been completed for a number of medications a review for tamoxifen is still ongoing (Del Tredici et al., 2018).

CYP3A4 and CYP3A5

Another of the drug metabolism enzymes that has been investigated for its role in side effects are the CYP3A enzymes; CYP3A4 and CYP3A5. Both the *CYP3A5*3* and *6 polymorphisms show no metabolic activity, whilst *CYP3A4*22* polymorphism confers decreased metabolic activity. Therefore, the concentration of endoxifen is expected to be lower in women expressing these polymorphisms in one or more gene (de Vries Schultink et al., 2015).

Baxter et al. (2014) conducted a prospective study investigating the link between CYP3A4 polymorphisms and HF incidence. An inverse correlation between endoxifen concentration and reports of HFs was observed after adjustment for age, BMI and menopausal status. In particular *CYP3A4*22* carriers were less likely to report HFs with an odds ratio of 8.87 of reporting zero HFs compared to those with the *CYP3A4*22* variant even when compared to those with similar endoxifen concentrations (Baxter et al., 2014).

The implications of genetic polymorphisms altering the activity of tamoxifen at the primary target site in breast tissue, while also causing secondary outcomes, such as HFs, are of great importance. Particularly in the light of the results from the Study of Tamoxifen and Raloxifene (STAR) trial, which showed that tamoxifen and raloxifene are equivalent in reduction of breast cancer incidence. Given that raloxifene is not metabolised by CYP2D6, and CYP2D6 was shown to be important in the prevention setting, then it may be that a woman who is a poor metaboliser of tamoxifen due to two variant *CYP2D6* genes would be better using raloxifene rather than the current standard tamoxifen.

Baseline factors

Whilst HFs are most widely associated with reduced oestrogen concentration, several modifiable health factors are associated with the risk of HFs including alcohol consumption, smoking, and BMI (Gallicchio et al., 2006; Shobeiri et al., 2016; Tankó & Christiansen, 2004). Alcohol consumption has been linked to higher risk of HFs in both pre and postmenopausal women. However, alcohol consumption has been associated with lower risk of HFs in perimenopausal women (Schilling et al., 2007a).

The exact nature of the association between alcohol and HFs is unknown, but some studies show that alcohol consumption increases sex hormone concentrations. Proposed mechanisms for the increase in sex hormone concentrations are either through an increase in aromatase activity or via an effect on the adrenal gland (Purohit, 2000; Singletary & Gapstur, 2001).

A cross-sectional study by Onland-Moret et al. (2005) found that women who consumed more than 25g of alcohol per day had higher concentrations of oestrone (P_{trend} = 0.001), oestradiol (P_{trend} = 0.03) and DHEA-S (Ptrend = 0.18) than non-drinkers. No difference was observed in the concentrations of androstenedione, testosterone, and SHBG between women who consumed alcohol and those who did not drink (Onland-Moret et al., 2005). Further studies in postmenopausal women showed that concentrations of oestrone sulphate and oestradiol were higher in women who drank alcohol compared to those that didn't (Hankinson et al., 1995; Hines et al., 2000; Kobayashi et al., 1996; Madigan et al., 1998). However, these studies found that the results for oestrone were inconsistent (Cauley et al., 1989; Dorgan et al., 2001; Hankinson et al., 1995; Hines et al., 2000; Madigan et al., 1998; Newcomb et al., 1995). Wu et al. (2001) investigated the impact of alcohol on SHBG concentrations and found that SHBG is reduced in women who drink a moderate amount of alcohol (Wu et al., 2001). Reductions in SHBG concentrations will further increase the activity of circulating oestrogens and androgens as typically hormones bound to SHBG are inactive. Increased oestrogen concentrations and reduced SHBG concentrations would; therefore, increase the levels of bioavailable oestrogen. Studies investigating the effect of alcohol consumption on androgen concentrations are less numerous and results from these studies are inconsistent (Cauley et al., 1989; Dorgan et al., 2001; Hines et al., 2000; Madigan et al., 1998; Newcomb et al., 1995).

The inconsistency between studies may be due to small sample sizes. Inconsistencies

may also be explained by differences in length and quantity of alcohol intake, which vary between studies (Singletary & Gapstur, 2001). Furthermore, the effects of alcohol may be confounded by factors affecting oestrogen metabolism, such as smoking, a possible as four of the previously addressed studies did not adjust for smoking status (Hines et al., 2000; Madigan et al., 1998; Newcomb et al., 1995; Wu et al., 2001).

A study by Schilling et al. (2007) linked alcohol consumption to higher levels of SHBG and lower free testosterone, testosterone not bound to SHBG, compared to those who do not drink (Schilling et al., 2007a). However, neither SHBG concentration nor free testosterone levels explain the association between alcohol consumption and HFs. When the number of drinks per week was investigated, levels of DHEA-S are significantly higher in those who consume two or more drinks a week compared to those who never use alcohol (Schilling et al., 2007a).

BMI is often hypothesised as a key factor for reducing the risk of HFs as research has shown that postmenopausal women with high BMI also have higher concentrations of oestrogens due to greater conversion of androgens to oestrogens in adipose tissues. While the association of BMI with breast cancer outcomes have been well addressed, the impact of BMI on side effects particularly in the prevention setting remains under investigated (Liu et al., 2018a; Shieh et al., 2019).

The association of BMI and HFs has been addressed in healthy women with conflicting results. It has been suggested that the inconsistencies are as a result of different background levels of endogenous oestrogens and other hormones. The long-standing hypothesis is that women with increased body size have higher levels of endogenous oestrogens, due to androgen conversion in adipose tissue. Thus women with higher BMI have a lower risk of HFs compared to women with lower BMI (Øverlie et al., 2002). In contrast, some studies refute this hypothesis showing that increased BMI increases the risk of HFs (Gallicchio et al., 2005; Miller et al., 2006; Schilling et al., 2007b). Gallicchio et al. (2005) found that BMI increases the risk of any HFs including a significant increase in severe HFs in women who were very obese (BMI $\geqslant$ 30 kg/m^2) a group which had not been previously examined.

There is a very obvious contrast over the true nature of the effects of BMI on HFs. While the hypothesis that higher BMI is protective of HFs has a biological basis, it is likely that the true scenario is not as straight forward. A lack of adjustment for other

covariates, such as smoking, and not accounting for the insulating effect of adipose tissue are two issues that surround the previous work studying the effect of BMI on HFs (Thurston et al., 2008). Therefore, the importance of investigating the role of BMI on HFs within a specific population with adjustment for other covariates is essential.

Some studies found that lower BMI effects on HFs status are influenced by smoking status (Gallicchio et al., 2006; Whiteman et al., 2003b). The association between smoking and HFs has been well discussed with contrasting results. Some studies suggest that smoking is associated with HFs, but in contrast others find no association whilst others only find an association between smoking and HFs in women of low BMI (Cochran et al., 2008; Whiteman et al., 2003b). However, the weight of evidence is in favour of smoking increasing the risk of HFs or increasing the risk of more frequent and more bothersome HFs. The mechanisms through which smoking could impact the incidence of HFs is unclear, but studies have suggested several distinct pathways through which smoking may impact HFs some of which could be exacerbated by the use of endocrine therapy. These include:

i. Cigarette smoking directly depletes oestrogen concentrations by interacting with CYP enzymes responsible for metabolism of chemicals in smoke as well as oestrogen

ii. Chemicals in cigarette smoke induce mutations in or destroy ovarian follicles

iii. Chemicals in cigarette smoke may inhibit aromatase activity

iv. Reduced body weight, as a result of smoking, leading to increased hot flush risk; assuming an inverse association between BMI and HFs

It remains possible that smoking is associated with HFs through a mechanism not involving oestrogen metabolism, as several studies show that there are similar oestrogen concentrations circulating in both smokers and non-smokers (Whiteman et al., 2003b). One possible alternative is that smokers have higher levels of androgens and that higher androgen levels are responsible for the increased risk of HFs (Cochran et al., 2008).

1.4.3 Musculoskeletal events

Musculoskeletal conditions are prevalent, and their impact pervasive. They are the most common cause of severe long-term pain and physical disability, and they affect hundreds of millions of people around the world. Musculoskeletal conditions are a diverse group with regard to pathophysiology but are linked anatomically and by their association with pain and impaired physical function. They encompass a spectrum of conditions, including arthralgias, rheumatoid arthritis and osteoporosis (Woolf & Pfleger, 2003).

The incidence of many of these events increases markedly with age, and many are affected by lifestyle factors, such as obesity and lack of physical activity. Age is the strongest predictor of the development and progression of musculoskeletal events. High BMI, trauma and certain physical activities have also been associated within an increased risk (Woolf & Pfleger, 2003). Musculoskeletal conditions are common in the general population and affected approximately 18.8 million people in the UK in 2017. About 10.5 million of the total were women with those aged over 65 at the highest risk (Institute or Health Metrics and Evaluation (IHME), 2018).

Musculoskeletal events are also prevalent in women who take AIs after breast cancer. For postmenopausal women with ER-positive breast cancers, a group representing about 70% of all postmenopausal breast cancers, AIs are an important part of treatment. However, as both breast cancer and side effect risk increase with age it is important to understand the prevention and treatment of osteoporosis and other musculoskeletal events, such as arthralgia and myalgia, in high risk women (Suskin & Shapiro, 2018). In the following sections risk factors of musculoskeletal events will be discussed.

1.4.3.1 Risk factors of musculoskeletal events

Oestrogens and hormones play a crucial role in the regulation and maintenance of bone mass (Folkestad et al., 2009). Regulation occurs on both a larger scale and at a cellular level. Forces involved at a higher level include the balance of systemic hormones, such as androgens and oestrogens, and mechanical forces, such as gravity. At a cellular level, osteoblasts are required for new bone formation and osteoclasts are responsible

for bone resorption (Suskin & Shapiro, 2018). The dynamic between osteoblast and osteoclast function is key in regulating new bone formation and resorption (Boyle et al., 2003). The major regulators are osteoblasts which secrete osteoclastogenesis inhibitory factor and a receptor activator of nuclear factor kB (NF-kB) ligand RANKL. RANKL binds to RANKL receptors in osteoclast precursor cells and leads to differentiation to mature osteoclasts which stimulates the mechanisms through which bone resorption occurs (Suskin & Shapiro, 2018).

Maximum bone mass occurs at approximately 30 years of age, after which bone loss starts to occur in both women and men. Osteoporosis is determined by starting bone mass and the loss of bone mass from aging and menopause. While modifiable risk factors, such as smoking and alcohol intake exist, osteoporosis is largely genetic with other non-modifiable risk factors being age and low body mass (Kanis et al., 2019).

Single nucleotide polymorphisms

SNPs are also associated with AIA and myalgia. Associations with SNPs in *OPG*, *CYP17A1*, vitamin D receptor and *CYP2* all predict an increase in the incidence of AIA musculoskeletal events (Garcia-Giralt et al., 2013; Lintermans et al., 2016).

Ingle et al. (2010) used a GWAS study to identify SNPs which may be associated with musculoskeletal events. A nested case-control study with matching on age, treatment with exemestane or anastrozole, presence of prior adjuvant chemotherapy, receipt of celecoxib or placebo and time on study was used to assess the association. Population stratification was minimised by only using white patients. Additional covariates analysed included BMI, bisphosphonate use, previous fracture, prior HRT use, prior radiotherapy and prior taxane therapy. The GWAS study identified four SNPs on chromosome 14 with P-values of $< 1e^{-6}$. Identification of these SNPs show that they are all located on chromosome 14 and all are near the T-cell leukaemia 1A (TCL1A) gene. Assessment of the function of these SNPs with the target genes suggests that they create an oestrogen response element (ERE) (Ingle et al., 2010).

Studies have shown that SNPs linked to musculoskeletal events through expression of *TCL1A* are highly correlated with a series of genes encoding cytokines and cytokine receptors including Interleukin (IL) 17 receptor A. Knockdown of *TCL1A* results in the decreased expression of *IL17A*, but the increased expression of *IL17* with the converse also true (Liu et al., 2012). Significant correlation between *TCL1A* expression and the

cytokine receptor genes has been observed. By increasing the concentration of oestradiol, the expression of the Interleukin receptors was altered depending on the SNPs present with experiments showing that *TCL1A* is upstream of multiple Interleukin receptors.

Baseline factors

Many risk factors have been linked to a higher risk of developing musculoskeletal events. Obesity has been consistently linked to larger numbers of musculoskeletal events. A retrospective analysis of the ATAC study showed that obese women with a BMI >30 kg/m^2 reported more musculoskeletal events than women who are normal weight, BMI <25 kg/m^2, or those who are overweight with a BMI of between 25 kg/m^2 and 30 kg/m^2 (Sestak et al., 2008). Analysis of a second trial, the IES trial, supports the findings of ATAC where weight of >80 kg is a significant risk factor for arthralgia development (Miegg et al., 2012). Another study that was designed specifically to address the association between BMI and incidence of AIA. Results show that women with BMI greater than 30 the proportion of women reporting arthralgia decreases compared to those with BMI <25 kg/m^2 (Crew et al., 2007). Both these groups have significantly higher incidence than women with a BMI of 25-30 kg/m^2. It remains to be determined why overweight women are relatively protected. One hypothesis is that increased adipose tissue provides higher concentrations of oestrogen due to aromatase acting in these tissues. However, this may be counteracted by the extra stress placed on joints by increased body weight (Niravath, 2013).

Prior HRT has also been associated with increased risk of developing AIA (Sestak et al., 2008). Prior HRT use subjects were more likely to develop joint symptoms than those who never used HRT (OR = 1.72 (1.53–1.93); P < 0.0001). The analysis also determined that women who stopped using HRT less than six months prior to the start of the trial were more likely to have joint symptoms than those who stopped longer than six months before (Sestak et al., 2008).

Both weight and physical activity can impact inflammatory cytokines in particular IL17 with obesity and lack of physical exercise increasing the concentrations of IL17 increasing the risk of developing AIA (Ahmed & Gaffen, 2010; Crew et al., 2007). The increase in inflammatory cytokines may also be linked to other musculoskeletal events with higher concentrations of IL1, IL6 upregulating expression of osteoblasts. This upregulation speeds up osteoblast conversion to osteoclasts resulting in the breakdown

of bones leading to joint pain (Santen, 2011).

Sex hormones

It is commonly believed that AIA is caused by oestrogen depletion although the mechanism is currently unsubstantiated. Perimenopausal women have been shown to have a far higher incidence of arthralgia than premenopausal women; and shows how important menopausal status is due to the increased number of arthralgia, which also develop at about the same age as menopause occurs (Magliano, 2010).

Sex hormones play a crucial role in the maintenance and growth of bone in both men and women and androgen receptors are expressed in osteoblasts, and osteocytes which are important cells in the formation and maintenance of bones (Clarke & Khosla, 2009). However, androgens can also cause osteoclastic apoptosis, ultimately decreasing bone resorption (Roux & Orcel, 2000). The most important effects of testosterone on bones is through its aromatization to oestradiol, which activates ERα and ERβ in bone, decreasing bone resorption and increasing bone mineral density (Kalb et al., 2013). The studies by Kalb et al. (2013) would suggest that increased levels of testosterone, and lack of conversion to oestrogens, is key in the impact of AIs on bone density and musculoskeletal events (Kalb et al., 2013). However, little research has been performed on the role of androgens in the incidence of musculoskeletal events in women taking AIs. I aim to address this issue during my thesis by looking at a range of sex hormones and their association with side effects of AI and tamoxifen therapy.

There are two central theories why low concentrations of oestrogen may cause joint pain: (i) oestrogen has natural anti-nociceptive, a reduced perception of sensitivity to pain; (ii) cytokine mediated inflammation (Din et al., 2010).

It is well documented that a reduction in oestrogen concentration is related to inflammation in women taking AIs and experiencing musculoskeletal events via an increase in cytokine production (Islander et al., 2011). In addition, research has shown that synovial cells express aromatase which catalyses the conversion of androstenedione to oestrone and testosterone to oestradiol which decreases production of IL6 (Le Bail et al., 2001). Results of this show that AIs may increase the production of IL6 via inhibition of aromatase. IL6 is known to act as both a pro and anti-inflammatory cytokine, while it is also known to be one of the key mediators in bone loss post-menopause (D'Elia et al., 2003).

1.5 Adherence to endocrine therapy

Side effects have an important impact on the effectiveness of preventive therapies for breast cancer as success is dependent on adequate uptake and compliance to therapy. Fear of side effects is a major reason women give for not taking preventive therapy and for poor adherence to therapy. Studies show that less than 15% of eligible women choose to take preventive therapy (Ropka et al., 2010; Smith et al., 2016). The impact of side effects has been considered in recent guidelines recommending that tamoxifen, raloxifene and AIs should be limited to women at increased risk of breast cancer. For those not at increased risk the potential harm outweighs the potential benefit (US Preventive Services Task Force et al., 2019). However, side effects are not the only reason for poor uptake of preventive therapy. A meta-analysis of uptake in 26 studies including 21,423 women showed that having an abnormal biopsy, physician recommendation, higher risk of breast cancer and older age were all associated with increased uptake of preventive therapy (Smith et al., 2016). Additionally, uptake was higher in trials (25.2% (95% CI 18.3–32.2)) than in non-trial settings (8.7% (95% CI 6.8 – 10.9)) ($P < 0.001$) (Smith et al., 2016). Previous experience of side effects, particularly HFs resulted in lower uptake in one study (Yeomans-Kinney et al., 1995). However, this was not true for all studies as Yeomans-Kinney et al. (1998) found no association between previously experienced HFs and lower uptake to preventive therapy (Yeomans-Kinney et al., 1998).

Adherence to optimal therapy during the first year of active treatment was adequate; however, adherence to therapy over a five-year treatment period is sub-optimal (Smith et al., 2016). Reporting after 5-years of active treatment shows that adherence to preventive therapy ranged from 61.1% in the tamoxifen arm of the STAR trial to 80.8% in the Royal Marsden study (Powles et al., 1994; Vogel et al., 2010). However, a lower estimate of adherence to therapy was later reported for the Royal Marsden study (Powles et al., 1998). In the IBIS-I study, 65.2% of women were adherent to tamoxifen for at least 4.5 years and 74.0% were adherent to placebo during the same time (Smith et al., 2017). The rate of dropouts was highest in the first 12-18 months and decreased after this point. Reports of nausea or vomiting were associated with a decrease in adherence in the tamoxifen and the placebo arms (OR = 0.57 95% CI (0.37 - 0.86); P = 0.007) and (OR = 0.58 (0.37 - 0.93); P = 0.023) respectively. Gynaecological side

effects were also associated with a decrease in compliance in the tamoxifen arm (OR = 0.77 (0.62 - 0.97); P = 0.024). In contrast, headaches were only associated with a decrease in adherence in the placebo arm (OR = 0.62 (0.42 - 0.91); P = 0.016) (Smith et al., 2017).

It is well established that some women are sub optimally adherent to anastrozole therapy and that a large proportion of patients treated with AIs complain of joint related symptoms leading to reduced compliance (Partridge et al., 2008; Smith et al., 2016). Musculoskeletal events have been given as a major reason behind non-compliance to anastrozole, but these musculoskeletal events do not affect all women enrolled in trials and therefore a genetic basis for this variability has been hypothesised.

Full 5-year adherence in the IBIS-II study was estimated to be 68% in the anastrozole group versus 72% in the placebo group (P = 0.0047). Adverse events were given as the main reason for non-adherence in both the anastrozole group, 375 (20%); and in the placebo group 298 (15%). Additionally, patient refusal of treatment was also high (94 (5%) in the anastrozole group; 98 (5%) in the placebo group) (Cuzick et al., 2014). These drop-out rates on anastrozole and placebo are similar to those found in the MAP.3 trial after 5-years of active therapy. After 5-years, 735 (32.8%) women randomised to exemestane and 646 (28.7%) women on placebo were no longer adherent to therapy. The major reasons given in this trial for early discontinuation were toxic effects (15.4% in the exemestane groups vs. 10.8% in the placebo group, P < 0.001) and patient refusal (6.9% vs. 6.0%, P = 0.22) (Goss et al., 2011).

When determining women who are eligible for trials and for the use of prevention therapies it is important to assess their risk of breast cancer to determine the risk-benefit ratio. Most breast cancer risk assessment models use an array of risk factors such as genetic mutations, age, menopausal status, HRT use, and MD. Models that include multiple sources of information do improve risk estimates for breast cancer, but currently assessment of side effect risk is non-cohesive and identification of risk factors under investigated (US Preventive Services Task Force et al., 2019). This is the main aim of my thesis; can we identify specific risk factors for the main side effects of endocrine therapy. By identifying these risk factors, it would then be possible to assess the risk of side effects a woman might experience during endocrine therapy.

1.6 Models for predicting side effect risk

Prophylactic endocrine therapy for five years has shown decreased breast cancer risk for women at high risk (Cuzick et al., 2007, 2020; Goss et al., 2011; Veronesi et al., 2003). To have the ability to predict survival outcomes based on baseline or treatment-emergent endocrine symptoms would be clinically important. It would also be important to predict which women were likely to experience treatment-emergent endocrine symptoms and therefore be able to factor the likelihood of side effects into the initial discussion for endocrine therapy and to devise a schedule to minimise these side effects. The overall aim is to increase uptake of, and adherence to, endocrine therapy.

The main focus of this thesis is to investigate markers which predict the risk of developing side effects and to investigate the association between side effects and breast cancer incidence. I aim to investigate risk factors for side effects in more detail and aim to build a prediction model to identify women who may be at increased risk of developing these side effects during treatment. The goal is to incorporate SNPs, baseline factors and sex hormones into a tool to assist the decision-making process about endocrine therapy.

Women who have a high breast cancer risk, and would benefit from preventive intervention, but a low risk of side effects could then be included in the target population for therapy. Therefore, producing a risk model providing women with a realistic estimate of the likelihood of side effects is essential. This would provide women who are concerned about the use of endocrine therapy with more information for their decision making. The prediction of side effects as a result of endocrine therapy is a crucial task for epidemiology and public health. For efficient and effective prevention of disease medication should not only impact the target disease but minimise the disruption to quality of life (Liu et al., 2018). Models for predicting side effect risk would be useful for clinical management and for developing prevention strategies. However, currently, no prediction model for endocrine therapy side effects exists.

Multiple breast cancer prediction models exist to estimate a woman's risk of breast cancer and also to set clinical trial entry criteria and an individualised programme for breast screening (Cuzick et al., 2002, 2014; Fisher et al., 1998). Another model is the Gail model, which assesses the absolute risk of developing breast cancer (Gail et al.,

1989). Other models, such as the Tyrer-Cuzick, estimate the risk of developing breast cancer given the presence of certain risk factors when all other causes of mortality are removed (Tyrer et al., 2004). While the two types of model are similar in the short-term, over a longer period they can differ markedly (Gail & Pfeiffer, 2018).

The ideal prediction models are based on a variety of factors, have a strong genetic element and are particularly useful and widely used for women who are at high-risk. Inclusion of modifiable risk factors, such as BMI and HRT use, may provide a perspective on potential risk reduction if these exposures are avoided or reduced. Adding MD and SNPs to existing models containing epidemiological factors may also help to increase the accuracy of these models.

Polygenic risk scores (PRS) and MD have been added to prediction models to improve prediction of breast cancer risk (Michailidou et al., 2013; Tamimi et al., 2007). Recent studies have shown that including individual genetic risk variants or a PRS of multiple variants and/or MD measures significantly improves the Tyrer-Cuzick (IBIS) model (Brentnall et al., 2015, 2019; Cuzick et al., 2017; Evans et al., 2017; van Veen et al., 2019). Studies have also found that the same is true for other risk models such as the Gail model (Cheddad et al., 2014; Chen et al., 2006; Gail, 2009; Garcia-Closas et al., 2014; Maas et al., 2016; Wacholder et al., 2010). In addition, evidence supports an association between circulating oestrogens and androgens, with pre and postmenopausal breast cancer risk (Endogenous Hormones and Breast Cancer Collaborative Group, 2011, 2013). Recently, studies have shown that incorporating sex hormone concentrations can improve breast cancer risk prediction (Hüsing et al., 2017; Tworoger et al., 2014).

If baseline factors, PRS, and hormone concentrations can be combined for the identification of women who are at low risk of side effects, prediction models could further increase the target population for prevention (Khera et al 2018). An opportunity remains for prediction models with respect to side effects as there has been little work performed in this area. Determining whether SNPs and other biomarkers can help to predict the risk of developing side effects could help an individual make an informed decision regarding life-style changes or medication (Garcia-Closas et al., 2014).

Chapter 2: Aims, hypotheses and thesis scope

2.1 Hypothesis

Endocrine therapy can significantly reduce the occurrence of breast cancer in women who are at high risk. Several risk models for breast cancer have been developed, which can accurately predict those at high risk of developing breast cancer. These women are most likely to benefit from preventive endocrine therapy. This thesis investigates side effects risk factors in women taking endocrine therapy for breast cancer prevention.

It is well reported that endocrine therapy increases the risk of menopausal-like side effects, which significantly reduce a woman's quality of life. Fear of side effects is cited as a major reason behind the low uptake of preventive therapy which may lead women to refuse endocrine therapy despite its proven beneficial effects. Research in the adjuvant setting has established a number of potential risk factors for side effects and several mechanisms of action have been proposed. Adjuvant studies have also shown that women reporting early side effects have a reduced risk of breast cancer, suggesting that side effects could be used as markers of endocrine therapy efficacy.

In this thesis it is hypothesised that side effect risk can be predicted and that a risk model might help in the decision-making process about preventive endocrine therapy. Therefore, the main objectives of this thesis are to explore potential markers, including baseline measurements, genetic variants and hormonal factors to identify women who may be at increased risk of developing these side effects and to produce a model to predict side effect incidence during endocrine therapy. It is also hypothesised that, as in the adjuvant setting, women who report side effects are at lower long-term risk of breast cancer.

2.2 Objectives and scope

Many studies performed in both the preventive and adjuvant setting have reported endocrine related side effects occurring throughout the trials. Risk factors, such as sex hormones, genetic mutations, body mass index (BMI) and reproductive factors, have been used to study and evaluate breast cancer risk. However, application of these markers to side effects has been minimal despite the proposed involvement of these risk factors in side effect aetiology. Furthermore, no risk models combining these factors have been developed for side effect prediction.

To understand how these factors can increase the incidence of endocrine related side effects and to investigate whether prediction markers exist, data were utilised from two placebo-controlled randomised controlled trials (RCTs), International Breast Intervention Study (IBIS)-I and IBIS-II, which investigated the efficacy of tamoxifen or anastrozole, respectively, for prevention of breast cancer. Further details of the trials and the use of RCTs is discussed in **Chapter 3**. Also, in **Chapter 3**, the statistical techniques underpinning the analysis of the data and the suitability of these techniques is discussed.

In **Chapter 4** the association between baseline risk factors and side effects are investigated. The individual and multi-factorial relationship of BMI, menopausal status, age, smoking history, hormone replacement therapy (HRT) use and reproductive factors in association with side effects reported during the first 6-months of endocrine therapy will be assessed. Many of these anthropometric and modifiable factors can impact a woman's lifetime exposure to oestrogen and may be key in understanding the risk of side effects. I aim to investigate the association between baseline factors and early side effects and determine which factors have an impact on a woman's risk of side effects.

Chapter 5 investigates the association between single nucleotide polymorphisms (SNPs), which have links to breast cancer, with side effects from IBIS-I. The analysis has three parts, a candidate gene study, a genome-wide association study, and a multi-loci analysis. The candidate gene (CG) analysis assesses the role of 571 SNPs in genes known to be involved in tamoxifen metabolism and elimination, sex hormone metabolism, aromatase activity and oestrogen signalling via receptors. Each of these biological pathways have been previously proposed as mechanisms for side effect aetiology and as

such alterations in these pathways may impact the risk of side effects. The CG analysis is followed by an exploration of the data from a genome wide association perspective. 517,820 SNPs from an OncoArray is analysed for associations with hot flushes and gynaecological side effects. Side effect incidence at baseline to six months is assessed to determine any associations. **Chapter 5** concludes with the formation of multi-loci models for predicting side effect risk from SNPs and the use of polygenic risk scores to determine the impact of multiple risk alleles.

Chapter 6 investigates the role of sex hormone concentrations at baseline, on side effects reported during the first 12 months of active therapy, in postmenopausal women enrolled in the IBIS-I and IBIS-II trials. It has been reported that a single measurement of endogenous hormones in postmenopausal women can predict the risk of hormone responsive breast cancers for up to 20 years. Low oestrogen concentrations are also often proposed as the major mechanism behind menopausal related side effects; however, the role of androgens and sex hormone binding globulin (SHBG) are not well established. Sex hormone concentrations are analysed by enzyme linked immunosorbent assay in relation to side effect incidence. Baseline samples analyses determines concentrations of dehydroepiandrosterone – sulphate (DHEA-S), SHBG, and testosterone. Bioavailable testosterone is also calculated to assess the associations of a hormone milieu with endocrine therapy related side effects. Lastly, the association between sex hormone concentrations and baseline risk factors, such as BMI, are investigated.

Chapter 7 aims to incorporate multiple risk factors explored in Chapters 4 – 6 and develop a tool for predicting side effects utilising a wide range of risk factors to improve the performance of the model. Unlike other cancers, such as lung or cervical cancer, in which a single factor leads to the majority of cases, a large number of factors are important for determining the risk of breast cancer. Therefore, the most effective models for predicting breast cancer risk are those that contain multiple risk factors as these allow for the best stratification of women into higher or lower risk categories; here a similar model for side effects is proposed. Validation of these signatures is performed by partitioning the data sets into training and validation sets prior to model formation and using 10-fold cross-validation in the training set to define a model. The most appropriate models for this problem are unknown, as are the optimal configurations of these models. However, any selected model should use the data efficiently and the process of model development should be clear and detailed to enable reproducibility.

Therefore, two models, logistic regression and least absolute shrinkage and selection operator (LASSO) are used to predict side effect outcomes and the best method selected. Logistic regression models are widely used statistical models which allow for multivariate modelling of binary outcomes this method is selected due to its popularity in recently published literature. LASSO models offer efficient optimisation of parameters, particularly when there is a large number of covariates. The performance of the two models is compared to determine side effect outcomes from baseline risk factors and also to assess the impact of adding genomic risk factors and sex hormone risk factors to the models. The prediction performance of each of these models is compared and the models are used to predict side effects in the validation set. The best model is subsequently used to construct a side effect risk score which can be used to determine whether a women will be at high risk of side effects during the early stages of endocrine therapy.

Chapter 8 focusses on the relationship between side effects and breast cancer. Studies in the adjuvant setting have shown that side effects may predict a reduction in recurrence. Hence, the relationship between early reported side effects and the incidence of overall and oestrogen receptor-positive breast cancer is assessed in women from the IBIS-I and IBIS-II trials. The expectation is that side effects, as a result of low oestrogen concentrations, predict a reduction in breast cancer risk particularly for ER-positive disease.

Finally, **Chapter 9** sets out any further work and conclusions from this thesis. Further work includes analyses that were not feasible to complete or additional work that has been identified throughout the course of the PhD.

This work has three main overarching aims:

1. To investigate whether baseline measurements, genetic variants, and sex hormones can impact side effect development during the first 6 months of endocrine therapy using data from the IBIS-I and IBIS-II trials.

2. To combine these factors into a tool that can be used to accurately determine a woman's risk of side effects as a result of taking endocrine therapy for breast cancer prevention.

3. To assess whether early reported side effects are associated with a woman's long-term breast cancer risk.

To address these questions, side effects that were the most prominent treatment-related, predefined, known to be linked to the respective endocrine therapies, and which were reported in large numbers of women during the initial analyses of IBIS-I and IBIS-II were selected. The assessment of side effects within six months follow up enables them to be reviewed in the largest cohort of women as the dropout rates were highest during this time. Additionally, the highest incidences of side effects were reported during this six month period.

Chapter 3: General methods

This chapter introduces the two main trials which provided the data for each of the analyses in the thesis. Following this, the main statistical methods used to analyse the data are summarised, highlighting procedural decisions, and strengths and weaknesses of each chosen method. This chapter should be treated as a reference for each of the following chapters and provide supporting evidence for the statistical tools used throughout this thesis.

3.1 International Breast Cancer Intervention Study I (IBIS-I)

IBIS-I began recruitment of women at high risk of developing breast cancer in 1992. Women were randomised to receive oral tamoxifen (20 mg/day) or matching placebo. The primary endpoint was occurrence of breast cancer, including both invasive breast cancer and ductal carcinoma in situ (DCIS).

IBIS-I was designed as an international, double-blind, randomised placebo-controlled trial, which recruited pre and postmenopausal women aged 35 - 70 years between April 1992 and March 2001. Eligible women were at an increased risk of breast cancer, the magnitude of which was determined by their age at randomisation. For those aged 45 – 70y women were eligible if they had at least one of:

- First-degree relative who developed breast cancer at or before age 50y
- First-degree relative with bilateral breast cancer
- Two or more first-degree or second-degree relatives with breast cancer
- Lobular carcinoma in situ
- Atypical hyperplasia
- Nulliparous and a first-degree relative who developed breast cancer
- Benign biopsy and first-degree relative who developed breast cancer

Women were eligible from age 40y if the first degree relative with bilateral breast cancer had cancer before age 50y. For eligibility at 35y, a first-degree relative's cancer must have been diagnosed before age 40y. Women with two or more first or second-degree relatives with breast cancer were eligible from age 40y if both relatives developed breast cancer before age 50y and from age 35y if both relatives were first degree and developed breast cancer before age 50y. Additionally, risk-equivalent women, those with a strong family history, not fitting specific categories, but judged by the study chairman to be at higher risk than the minimum eligibility category were also able to join the trial (Cuzick et al., 2002).

Exclusion criteria were any previous invasive cancer with the exception of non-melanoma skin cancer, any history of deep-vein thrombosis, pulmonary embolism, or use of anti-coagulants. Women with a life expectancy of less than ten years or women who were pregnant or who wished to become pregnant were also ineligible (Cuzick et al., 2002). Use of hormone replacement therapy (HRT) was permitted during trial for treatment of menopausal symptoms, but was at the lowest concentration for control of symptoms (Cuzick et al., 2007).

7,154 women were randomised 1:1 to either placebo (N = 3,575) or to tamoxifen (N = 3,579) 20 mg/day orally for a period of five years. During the five-year treatment phase of the trial, women had a follow-up years in the clinic or by telephone every six-months. During these follow-up visits, predefined side effects and other diseases were collected, using the CRF to record the information, by asking women to grade their experience of each side effect with options being none, mild, moderate or severe. Appendix 2 contains a copy of the six-month follow up CRF form and final follow up form. The format of the six-month form was utilised at 12, 18, 24, 30, 36, 42, 48 and 54 months. The primary endpoint was the occurrence of breast cancer including invasive breast cancer and DCIS. Secondary endpoints included the occurrence of invasive oestrogen receptor-positive breast cancer, all-cause mortality, and adverse events (Cuzick et al., 2015). Baseline demographics of women included in the IBIS-I trial are shown in Table 3.1.

Table 3.1: Demographic of women enrolled on IBIS-I according to randomised treatment

Risk Factor	Tamoxifen* (N = 3,579)		Placebo* (N = 3,575)	
	(Median (IQR)		(Median (IQR)	
Age at randomisation	49.0 (45.0 - 54.0)		49.0 (46.0 - 55.0)	
Age at menarche	13.0 (12.0 - 14.0)		13.0 (12.0 - 14.0)	
Age at menopause	48.0 (44.0 - 51.0)		48.0 (42.0 - 50.0)	
Menarche to menopause	35.0 (30.0 - 38.0)		35.0 (29.0 - 38.0)	
Age at first birth	23.0 (21.0 - 26.0)		23.0 (21.0 - 26.0)	
	(N (%))		(N (%))	
Premenopausal	1613	(45.1)	1631	(45.7)
Postmenopausal	1936	(54.1)	1922	(53.8)
BMI (kg/m^2)				
< 25	1447	(40.5)	1431	(40.1)
25-35	1301	(36.4)	1334	(37.4)
>30	829	(23.2)	805	(22.6)
HRT				
Never	2093	(58.5)	2120	(59.4)
Ex-User	567	(15.9)	505	(14.2)
Current	910	(25.4)	941	(26.4)
Smoking status				
Never	1753	(49.0)	1846	(51.7)
Ex-smoker	1140	(31.9)	1077	(30.2)
Current	675	(18.9)	645	(18.1)
Parous	3111	(87.0)	3118	(87.3)
Type of menopause				
Natural menopause	1051	(29.4)	985	(27.6)
Hysterectomy or oophorectomy	327	(9.1)	318	(8.9)
Hysterectomy and single oophorectomy	161	(4.5)	159	(4.5)
Hysterectomy and bilateral oophorectomy	281	(7.9)	326	(9.1)

* 7 women Tamoxifen (N = 2), Placebo (N = 5) are randomised but no entry or follow up information is available. Percentages of women are therefore from a total of Tamoxifen N = 3,577 and Placebo N = 3,570.

The median age of women randomised to tamoxifen was 49.0 years (IQR 45.0 - 54.0). 54.1% (N = 1,936) were postmenopausal and 58.5% had never used HRT. Median BMI was 25.97 (IQR 23.31 - 29.74) and 49.0% had never smoked. Median age at menarche was 13.0 (IQR 12.0 - 14.0) and median age at menopause was 48.0 (IQR 44.0 - 51.0). 87.0% of women had at least one child and the median age at first birth was 23.0 (IQR 21.0 - 26.0) (Table 3.1).

For women randomised to placebo, the median age was 49.0 years (IQR 46.0 - 55.0). 53.8% (N = 1,922) were postmenopausal and 59.4% had never used HRT. Median BMI was 26.06 (23.23 - 29.59) and 51.7% had never smoked. Median age at menarche was

13.0 (IQR 12.0 - 14.0) and median age at menopause was 48.0 (IQR 42.0 - 50.0). 87.3% of women had at least one child and the median age at first birth was 23.0 (IQR 21.0 - 26.0) (Table 3.1). There were no significant differences between treatment groups for these variables.

In the first trial report, a median 96 months after randomisation, a total of 337 breast cancers were reported. Incidence in the tamoxifen arm was 27% lower than in the placebo arm, 142 breast cancers reported in 3,579 women in the tamoxifen group and 195 in the 3,575 women in the placebo group (RR = 0.73, 95% CI (0.58 - 0.91); P = 0.004). The risk reduction for ER-positive invasive breast cancer was 34% lower in the tamoxifen arm, 87 reported cases compared to 132 reported ER-positive invasive breast cancers in the placebo arm (RR = 0.66 (0.50 - 0.87)). No difference in risk of ER-negative invasive tumours (35 in each arm, RR = 1.00 (0.61 - 1.65)) across the entire follow-up period was observed (Cuzick et al., 2007).

In a further follow up in 2015 after a median of 16.0 years follow-up, a total of 601 breast cancers had been reported. 251 (7.0%) were reported in the tamoxifen group compared to 350 (9.8%) in the placebo group. This corresponds to a 29% reduction in breast cancer incidence in the tamoxifen arm compared to the placebo group (HR = 0.71 95% CI (0.60 - 0.83); P $<$ 0.0001). The reduction in risk increased to 34% for invasive ER-positive breast cancer (HR = 0.66 (0.54 - 0.81); P $<$ 0.0001) and 35% for DCIS (HR = 0.65 (0.43 - 1.00); P = 0.05). However, again no effect was observed for invasive ER-negative breast cancer (HR = 1.05 (0.71 - 1.57); P = 0.80) (Cuzick et al., 2015).

The protective effect of tamoxifen for any breast cancer was similar between 0-10 years after randomisation and $\geqslant$10 years after randomisation (ratio = 0.98 (0.74 - 1.30); P = 0.91) confirming a benefit of five-years of tamoxifen compared to placebo for up to 20 years. A similar pattern was observed for invasive ER-positive breast cancer (ratio = 0.94 (0.66 - 1.31); P = 0.69). However, the risk for ER-negative breast cancer was higher after 10 years compared to the first 10 years of follow-up; although the difference was not statistically significant (Cuzick et al., 2015).

3.2 International Breast Cancer Intervention Study II Prevention Trial (IBIS-II)

IBIS-II was initiated in 2003 and recruited postmenopausal women without breast cancer but at high risk of developing breast cancer and randomised these women to anastrozole (1 mg/day) or matching placebo for five-years. As with the IBIS-I trial the primary outcome of interest was breast cancer including both invasive and DCIS.

IBIS-II was an international, double-blind, randomised placebo-controlled trial, which recruited postmenopausal women aged 40–70 years between February 2003 and January 2012. Women were deemed to be postmenopausal when they were aged 60 years or older; had had a bilateral oophorectomy; were younger than 60 years, were uterine intact but had at least 12 months of amenorrhoea; or were aged less than 60 years, had no uterus, and had a concentration of follicle stimulating hormone of greater than 30 IU/L. Inclusion criteria for women aged 45 - 70y:

- First degree relative who developed breast cancer at age 50y or less
- First degree relative who developed bilateral cancer
- Two or more first or second-degree relatives who developed breast or ovarian cancer
- Nulliparous or age 30y or above at first birth, and first degree relative who developed breast cancer
- Benign biopsy with proliferative disease and first degree relative who developed breast cancer.
- Mammographic opacity covering at least 50% of the breast
- First degree relative with breast cancer at any age
- Age at menopause 55y or more
- Nulliparous or age 30y or above at first birth

For women aged 40-44

- Two or more first or second-degree relatives who developed breast cancer or ovarian cancer at age 50y or less
- First degree relative with bilateral breast cancer who developed first breast cancer at age 50y or less
- Nulliparous or age 30y or above at first birth, and first degree relative who developed breast cancer at age 40y or less
- Benign biopsy with proliferative disease and first degree relative who developed breast cancer at age 40y or less

For women in all age groups

- Lobular carcinoma in situ (LCIS)
- Atypical ductal or lobular hyperplasia in a benign lesion
- DCIS (ER-positive) diagnosed within last six months with completed adequate local treatment
- Women with a clearly apparent family history indicating appropriate increased risk

Women were eligible if they met at least one of the above criteria. In addition, women were also eligible if the Tyrer-Cuzick model indicated a 10-year absolute breast cancer risk of greater than 5%.

Exclusion criteria were: premenopausal women; any previous diagnosis of breast cancer; any invasive cancer in the previous 5 years (except for non-melanoma skin cancer or in-situ cervical cancer); present or previous use of SERMs for more than 6 months (unless as part of IBIS-I and treatment was completed at least 5 years before study entry); intention to continue HRT; prophylactic mastectomy; evidence of severe osteoporosis (T score $<$ -4 or more than two vertebral fractures); life expectancy of fewer than 10 years; psychologically or physiologically unfit for the study; or a history of gluten or lactose intolerance, or both (Cuzick et al., 2014).

Eligible women were randomised to receive 1 mg oral anastrozole or matching placebo every day for 5 years. Women enrolled on IBIS-II were followed up at 6 and 12 months and then annually during the five-year active therapy phase. During these follow-up visits, which were performed in person or via telephone, as per the IBIS-I trial, predefined side effects and other diseases were collected by asking women to grade their experience of each side effect during the last six month period with options being none, mild, moderate or severe. Appendix 2 contains a copy of the entry CRF form and the six-month follow up CRF form used for data collection at each follow up visit. The primary endpoint was histologically confirmed breast cancer (invasive cancers or DCIS). Secondary endpoints were oestrogen-receptor-positive breast cancer, breast cancer mortality, other cancers, cardiovascular disease, fractures, adverse events, and deaths not due to breast cancer. Demographics of women included on the IBIS-II trial are displayed in Table 3.2.

The median age for women randomised to anastrozole was 59.5 years (IQR 55.0 - 63.5). 52.8% had never used HRT and 55.4% had never smoked. Median BMI was 27.36 (IQR 24.2 – 31.1), median age at menarche was 13.0 (IQR 12.0 - 14.0) and median age at menopause was 50.0 (IQR 45.0 – 52.0). 85.9% of women had at least one child and the median age at first birth was 24.0 (IQR 21.0 – 27.0) (Table 3.2).

The median age for women randomised to placebo was 59.4 years (IQR 55.1 - 63.4). 52.9% had never used HRT and 58.3% had never smoked. Median BMI was 27.3 (IQR 24.4 - 31.3), median age at menarche was 13.0 (IQR 12.0 - 14.0) and median age at menopause was 50.0 (IQR 45.0 - 52.0). 84.8% of women had at least one child and the median age at first birth was 24.0 (IQR 21.0 - 27.0) (Table 3.2). There were no significant differences between treatment groups for these variables.

The first results of IBIS-II, after a median follow-up of 5 years (IQR 3.0 - 7.1) found that women in the anastrozole group had a 50% reduction in invasive breast cancer compared to the placebo group (40 cases vs 85 cases, HR = 0.47 (0.32 - 0.68); $P < 0.0001$). The risk reduction increased to 58% for invasive ER-positive breast cancer (HR = 0.42 (0.25 - 0.71); P = 0.001) and 70% for DCIS (HR = 0.30 (0.12 - 0.74); P = 0.009). No significant difference was observed in the number of ER-negative breast cancers reported between the anastrozole and placebo arm (11 vs 14, HR = 0.78 (0.35 - 1.72); P = 0.538) (Cuzick et al., 2014).

Table 3.2: Demographic of women enrolled on IBIS-II according to randomised treatment

Risk factor	Anastrozole (N = 1920)		Placebo (N = 1944)	
	(Median (IQR))		(Median (IQR))	
Age at randomisation	59.5 (55.0 - 63.5)		59.4 (55.1 - 63.4)	
Age at menarche	13.0 (12.0 - 14.0)		13.0 (12.0 - 14.0)	
Age at menopause	50.0 (45.0 - 52.0)		50.0 (45.0 - 52.0)	
Menarche to menopause	36.0 (32.0 - 39.0)		36.0 (32.0 - 39.0)	
Age at first birth	24.0 (21.0 - 27.0)		24.0 (21.0 - 27.0)	
	(N (%))		(N (%))	
BMI (kg/m^2)				
< 25	579	(30.2)	571	(29.4)
25-35	693	(36.1)	721	(37.1)
> 30	594	(30.9)	600	(30.9)
HRT				
Never	1014	(52.8)	1029	(52.9)
Ex-User	901	(46.9)	912	(46.9)
Smoking status				
Never	1064	(55.4)	1134	(58.3)
Ex-smoker	604	(31.5)	589	(30.3)
Current	223	(11.6)	198	(10.2)
Parous	1649	(85.9)	1649	(84.8)
Type of menopause				
Natural menopause	1338	(69.7)	1334	(68.6)
Hysterectomy	308	(16.0)	316	(16.3)
Oophorectomy	17	(0.9)	26	(1.3)
Hysterectomy and oophorectomy	227	(11.8)	243	(12.5)

After a median follow-up of about 11 years (131 months) women in the anastrozole group had a 49% reduction in breast cancer compared to the placebo group (85 vs 165 cases, HR = 0.51 (0.39 - 0.66); P $<$ 0.0001). The risk reduction increased to 54% for invasive ER-positive breast cancer (HR = 0.46 (0.33 - 0.65); P $<$ 0.0001) and 59% for DCIS (HR = 0.41 (0.22 - 0.79); P = 0.0081), especially in participants known to be ER-positive (HR = 0.22 (0.78 - 0.65); P = 0.0062) (Cuzick et al., 2020).

The reduction in overall breast cancer was largest in the first five-years of follow-up (HR = 0.39 (0.27 - 0.58); P $<$ 0.0001), but the reduction after the initial five-years was still significant as 50 new cases of breast cancer were reported in the anastrozole group compared to 76 in the placebo group (HR = 0.64 (0.45 - 0.91); P = 0.014). There was no statistically significant difference in event rate between the first five-years and subsequent follow-up period (P = 0.09). The same was true for invasive ER-positive

breast cancer which was reduced by 61% in the first five-years (HR = 0.39 (0.23 - 0.66); P < 0.0001), but by a slightly reduced 48% after five-years (HR = 0.52 (0.33 - 0.83); P = 0.0062). The difference between these periods was not statistically significant (P = 0.43). An initial 71% reduction in DCIS was observed (HR = 0.29 (0.11 - 0.80); P = 0.016); however, this became a 44% reduction after five-years (HR = 0.56 (0.23 - 1.32); P = 0.18). The difference between the two time periods was not statistically significant (P = 0.43). These result make IBIS-II the first and only trial to show the long-lasting effects of anastrozole for prevention of breast cancer (Cuzick et al., 2020).

3.3 Randomised controlled trials

Randomised control trials (RCT) are considered the gold standard in trial design as this type of study provides numerous advantages (Baum and Houghton 1999). RCTs are also the most stringent way of determining whether a cause-effect relation exists between the intervention and the outcome. However, results may not always mimic real-life treatment situation as women enrolled on these trials must meet a set of inclusion and exclusion criteria and the trial is conducted in a highly controlled manner and thus results are not always applicable to a wider target population.

Randomisation minimises allocation bias, selection bias, and confounding; however, these sources of bias can also be minimised through blinding. Blinding minimises performance bias and assessment bias, while allocation concealment minimises both performance and assessment bias and the prospective design of RCTs minimises recall error and selection bias (Schultz and Grimes 2002a, 2002b; Rothwell 2006). Secondly, RCTs are comparative enabling the direct comparison of the treatment to a placebo group, or other interventions such as an existing treatment in the case of new drugs, to establish efficacy and to assess the risk profile of the treatment against the alternative (Akobeng 2008; Zhang et al., 2014). In addition to assessing the effect of a treatment, different treatment strategies or dosages can also be compared. However, while these criteria maximize internal validity, at the same time these criteria limit generalisability and reduce external validity outside of the study population (Saturni et al., 2014).

When a RCT is adequately powered, both Type 1 error, where the null hypothesis is incorrectly rejected, and Type 2 error, where the null hypothesis is incorrectly accepted, are avoided, but this is also true for other study methods such as case-control

studies. However, RCTs require an analytical approach that ensures reliability and avoids methodical systematic bias (Saturni et al., 2014). This is usually obtained via an intention to treat (ITT) analysis (Hollis and Campbell 1999; Estellat et al., 2009). ITT is an analysis strategy that considers all patients in their initial randomisation groups regardless of whether they switch groups or drop out. It is important not to exclude those who do not receive the assigned therapy as these patients often do not have the same prognostic features as those receiving that therapy (Estellat et al., 2009).

3.4 Statistical methods

3.4.1 Logistic regression

Logistic regression is a widely used technique due to its simplicity, efficiency, and use of limited computational resources. Logistic regression calculates the odds of an event occurring which is the probability of an event occurring divided by the probability of an event not occurring, the impact of independent variables is usually explained in terms of odds. Logistic regression compares odds ratios for an event between treatment groups and permits easy model updating allowing for additional factors to be included in a model easily and efficiently. If using linear regression, the mean of the response variable (y) is modelled as a linear function with respect to x via Equation 3.1

$$y = \beta_0 + \beta_1 x \tag{3.1}$$

Equation 3.1: Linear function of a univariate regression model

However, this model fails when y is not a continuous variable (Park 2013). Logistic regression enables prediction of categorical outcomes transforming the odds using the natural logarithm. Equation 7.1, where p is the probability for the outcome of interest, x is the explanatory variable and β_0 and β_1 are the parameters show the structure of the logistic regression model (Peng et al., 2002).

$$logit(y) = ln(odds) = ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 x \tag{3.2}$$

Equation 3.2: Equation of the natural logarithm of a linear odds function

Taking the anti-log of both sides of Equation 7.1 and solving for p the probability of

the outcome can then be obtained (Equation 3.3).

$$p(y) = \frac{e^{(\beta_0+\beta_1 x)}}{1 + e^{(\beta_0+\beta_1 x)}} \tag{3.3}$$

Equation 3.3: Logistic equation for univariate regression

When adding multiple explanatory variables to the model the univariate model Equation 3.3 changes and takes the form of Equation 3.4 where n is the total number of variables to be included in the model.

$$p(y) = \frac{e^{(\beta_0+\beta_1 X_1+\beta_2 X_2+...+\beta_n X_n)}}{1 + e^{(\beta_0+\beta_1 X_1+\beta_2 X_2+...+\beta_n X_n)}} \tag{3.4}$$

Equation 3.4: Logistic equation for multivariate regression

When a logistic regression is calculated (Equation 3.4), the regression coefficient (β) is the estimated increase in the logged odds of the outcome per unit increase in the value of each independent variable when values of other independent variables are held constant. In order to establish a relationship between the odds and the independent variables in the logistic regression model, the odds need to be transformed to logit (log-odds) by taking the natural logarithm (ln) of odds. This is because whilst the odds ratio is constrained at the lower end, as it can't be negative, it is not at the upper end skewing the distribution (Bland and Altman 1996). The logarithmic transformation creates an independent variable which in most cases has an approximate normal distribution. It is also useful in that reversal of the categories in one variable simply reverses the sign of the log odds ratio. When presenting results of a logistic regression, the exponential of the regression coefficient *(β_x)* is taken so that odds can be more easily interpreted as the risk change per one unit increase in the independent variable given all other independent variables are held constant.

The odds of an event are the ratio of the probability (p) that an event will occur to the probability ($1 - p$) that it will not occur Equation 3.5.

$$Odds = \frac{p}{[1-p]} \tag{3.5}$$

Equation 3.5: Equation for determining the odds

To assess the relationship of each side effect (outcome variable) and risk factors (pre-

dictor variable), logistic regression is used in this thesis in a univariate and multivariate fashion for each side effect. The odds ratio (OR) is a comparative measure giving the ratio of two odds for an event usually between two different populations. For two events A and B, the corresponding odds of A occurring relative to B occurring is:

$$Odds\,Ratio\,\{A\,vs\,B\} = \frac{odds\{A\}}{odds\{B\}} = \frac{p_A((1-p_A)}{p_B((1-p_B)} \tag{3.6}$$

Equation 3.6: Equation for calculation of the odds ratio

An OR is a measure of association between an exposure and an outcome and represents the odds that an outcome (e.g. disease or disorder) will occur given a particular exposure (e.g. health behaviour, medical history, intervention), compared to the odds of the outcome occurring in the absence of that exposure. ORs express whether a particular exposure is a risk factor for an outcome of interest and can be used to compare the magnitude of various risk factors for that outcome. An OR = 1 indicates that the exposure does not affect odds of outcome. An OR $>$ 1 indicates an exposure is associated with increased odds of outcome while an OR $<$ 1 indicates an exposure associated with decreased odds of outcome (Park 2013).

Logistic regression is free from many of the major assumptions required of linear regression that are based on ordinary least squares (OLS) methods. Among these missing assumptions is linearity of relationship between independent and dependent variables. Normality of the error distribution is also not required under logistic regression; however, multivariate normality does result in a more stable reliable model. Measurement level of the independent variables can also vary under logistic regression with both continuous and categorical data handled well by logistic regression. Finally, the variation of the dependent variable does not have to been consistent across the values of the independent variable; heteroscedasticity is allowable in logistic regression (Park 2013).

However, some assumptions apply to logistic regression. Firstly, for binary logistic regression, as applied in this thesis, the dependent variable must take only the values zero and one. Since logistic regression estimates the probability of a positive outcome as ($p = 1$) the dependent variable should be coded accordingly to avoid confusion in the interpretation of the results. Secondly, each independent variable should not be correlated with other independent variables thereby reducing the multicollinearity in the model. Thirdly, whilst a linear relationship between the dependent and independent

variables is not required in logistic regression, it does require that independent variables are linearly related to the log odds of an event. Lastly, the sample size required for logistic regression should be sufficiently large to ensure adequate representation of samples in both categories of the dependent variable. Smaller sample sizes may result in a low power to detect small deviations from the logistic model (Bewick et al., 2005). During the process of logistic regression, models should not be under fitted due to insufficient variables or over fitted through inclusion of meaningless variables as both lead to unreliable conclusions.

In this thesis, logistic regression is used to calculate ORs for baseline factors, SNPs and sex hormones, thereby forming a major part of the analysis for chapters 4-6. In each of these chapters logistic regression was first used to calculate unadjusted ORs for individual variables. Multivariate logistic regression was subsequently used to adjust the univariate estimates for other variables. In chapter 4-6 the variables added for adjustment were other baseline factors such as age, BMI, HRT use, smoking history, menopausal status and randomised treatment. In chapters 4 and 6, reproductive factors such as age at menarche, age at menopause, time between menarche and menopause, and parity were also included as variables in multivariate logistic regression.

3.4.2 Testing for heterogeneity

Care must be taken when reviewing outcomes in multiple subgroups as this raises the possibility of "p-hacking", the act of creating ever smaller groups in order to find any statistically significant p-value. Therefore, testing for heterogeneity between the subgroups is important to identify different effects and ensure that significant p-values are not being reported in error.

Testing for heterogeneity investigates the null hypothesis that the effect size is the same within each subgroup (Higgins et al., 2003). The test statistic (usually Cochran's Q) is calculated from the sum of the squared deviations of each subgroup estimate from the overall estimate. P values are obtained by comparing the statistic with a chi-squared distribution with k-1 degrees of freedom; where k represents the number of subgroups (Higgins et al., 2003).

Tests for heterogeneity are known to be poor at identifying true heterogeneity between

subgroups in cases where there are small numbers of subgroups and therefore, the power of the test is low. As a result, a non-significant p-value cannot be regarded as evidence of homogeneity. In most cases the significance threshold is raised to P = 0.10 to ameliorate this issue; however, raising the significance threshold does increase the risk of making a false positive conclusion (Higgins et al., 2003). In cases where there are a large number of subgroups, especially when each subgroup is large, tests for heterogeneity can have excessive power, and a p-value of 0.05 can be achieved based on small differences between the different subgroups.

3.4.3 Genomic analysis

The genomic analysis forms the basis of chapter 5, investigating the impact of SNPs on side effects reported in the first six months of IBIS-I. The analysis uses two types of study design: a candidate gene (CG) study and a genome-wide association study (GWAS). CG studies investigate the genetic variation associated with disease within a small number of pre-specified genes, typically chosen as they form part of biological pathways of interest. In CG studies, cases of disease and controls are analysed for genetic differences found more commonly in one group or the other. In contrast, GWAS studies investigate genetic variants spanning the entire genome, rather than limiting the analysis to a relatively few number of genes. In both study types, results are typically reported as ORs.

The benefit of a CG study over a GWAS is that by predefining the SNPs and genes of interest, a large number of hypotheses are removed thereby reducing the number of statistical tests performed and increasing the statistical power to detect differences. This allows for the identification of SNPs and genes which may be associated with the outcome at a level of significance less stringent than that available if the number of statistical tests increases. However, CG studies are unavoidably biased toward the particular genes selected and biological pathways of interest, as these are hand-picked by investigators prior to analysis. As a result, the CG variants discovered may be given undue attention. There are several additional weaknesses of CG studies. One difficulty with CG studies is that differences in populations may result in the detection of alleles that differ between case and control but are unrelated to the disease. Another is that CG studies are often limited to the analysis of protein-coding regions of the genes, neglecting neighbouring genes and introns, and how these may affect gene expression

or epigenetics of CG.

The inclusion of genes which have not previously formed part of prediction panels or which might help to explain some of the variance in side effect incidence cannot be ignored and is one of the major strengths of GWAS. Investigation of additional genes and markers can help to identify novel genes, which might be involved in the trait of interest and to identify new mechanisms through which the outcome may be mediated. GWAS are also useful for identifying low frequency and rare variants, which may not previously have been identified in a CG study. However, the inclusion of high numbers of genes and markers needs to be controlled in order to avoid a high number of false positive associations. Multiple comparisons are an issue for every genomic analysis. Given the size of the genome, and the large number of variants, it is unlikely, a priori, that any variant is causally associated with a phenotype. Therefore, strong evidence is required to avoid false positive associations between variants and the phenotype (Balding 2006). As a result, GWAS are penalised for the large number of tests included, meaning genetic variants must have a more stringent p-value than the normal 0.05. In addition, GWAS may not identify the causal variant since many GWAS rely on information from previous panels for genotyping and so may only find associations for markers that are linked to the causal variant. Further discussion about significance levels in genomic analysis can be found in section 3.4.4.

As neither study type is without potential issues, the analysis presented in chapter 5 was performed using both study types. Initially a CG analysis was used to investigate the association of genes known to be involved in tamoxifen or sex hormone metabolism, or hormone receptor coding, with side effects. A GWAS study was then performed to allow for the identification of novel SNPs linked to side effects within this population. However, due to stringent multiple testing adjustments, researchers must be aware of an increased risk of false negatives. In both cases single SNPs were investigated; however, analysing single SNPs will neglect any synergistic information provided by the other SNPs in the genome. It is for this reason that multi-loci analysis and panels of SNPs were considered to provide the maximum information for an individual when assessing their risk of side effects.

3.4.3.1 Quality control

Genotyping arrays allow direct measurement of thousands of markers in an individual's genome. Analysis of these rely on high quality measurement of markers and due to the large number of loci investigated in GWAS, a small error rate can have detrimental effects. Therefore, removal of false positive associations and markers that have a high error rate is important before conducting any genomic analysis. Removal of a small number of individuals or markers from a large dataset has little impact on the power to detect an association. However, every marker removed raises the potential of overlooking a disease association and as a result could have a greater impact on the study than removal of a single individual (Anderson et al., 2010). In the analysis in Chapter 5, per-individual quality control (QC) was performed prior to per-marker QC. This prevents markers being removed due to poorly genotyped individuals but increases the risk of individuals being removed due to poorly genotyped markers. The alternative would be to run both QC steps in parallel prior to removing any markers or individuals. However, this would not avoid the possibility of unnecessary removal of data. The following sections detail the processes included in each of the QC steps.

3.4.3.2 Removal of duplicated samples

Prior to QC and association analysis of genotype data, duplicate individuals were removed from the dataset. Removal of duplicate individuals was based on the quantity of missing data for each sample. The sample with the largest amount of data was retained in the dataset. Due to the size of the genomic dataset and computational limitations, it was not possible to analyse the genomic data as one dataset. Therefore, the genotype data was split by chromosome to perform the per-individual and per-marker QC.

3.4.3.3 Per-individual quality control

Per-individual QC included: (i) sex check: for the identification of individuals with discordant sex information; (ii) heterozygosity and missing check: for the identification of individuals with outlying missing genotype and/or heterozygosity rates.

Sex check identifies individuals with discordant sex information and helps detect sample errors and samples which have a poor genotype rate. Homozygosity rates across all X-chromosomal genetic markers are calculated and compared against expected rates; 0.2 for females and 0.8 for males. Individuals whose assigned sex differs from the inferred sex from homozygosity rates are removed from the study. Further exclusions from the study were individuals with outlying missing genotype rates which aided detection of poor DNA quality or concentration. In line with previous literature, a threshold of more than 3% was set for removal of individuals with missing genotypes (The Wellcome Trust Case Control Consortium 2007; Anderson et al., 2010).

3.4.3.4 Per-marker quality control

Per-marker quality control consists of: (i) check for missing SNPs: for identifying markers with excessive missing genotype rates as a result of poor genotyping; (ii) check of Hardy-Weinberg equilibrium (HWE): for the identification of markers which show significant signs of deviation from HWE and (iii) check of minor allele frequency (MAF): for removal of marker with low MAF.

Markers with an excessive number of missing calls, are considered unreliable, and were removed. To ensure consistency with individual genotype missingness rates, the threshold for marker missingness was set at 3% after evaluation of marker missingness rate.

Markers with strong deviation from HWE are indicative of genotyping errors. Serious errors often yield extremely low p-values, markers failing HWE are identified by calculating the observed and expected heterozygote frequencies per SNP, in individuals that passed the per-individual QC process and computes the deviation of frequencies for HWE using the HWE exact test. To avoid the removal of too many SNPs, a HWE threshold of $< 1 \times 10^{-5}$ was selected for removal of SNPs replicating the data quality control protocol of several earlier studies (Anderson et al., 2010; Marees et al., 2018).

The final per-marker QC process was the identification of markers with low MAF. Those were removed as genotype calling is difficult due to the small sizes of the heterozygote and rare-homozygote clusters. Markers with low MAF were identified by calculating the frequencies for all variants that pass the per-individual QC procedure. Typically, a

MAF threshold of 1-2% is applied but due to small sample size a higher MAF threshold was required (Anderson et al., 2010). In chapter 5 analysis, the MAF frequency was set to remove markers with a MAF of <5%.

3.4.3.5 Individual and multi loci analysis with logistic regression

When testing an individual SNP for its association with a case-control outcome, contingency tables offer a simple method of displaying data. Contingency tables are a 2 × 3 matrix containing the counts of cases and controls at each genotype: wild type, heterozygote and homozygote variants, for the SNP of interest Table 3.3. The simplest analysis of SNP genotypes and case–control status at a single SNP is to test the null hypothesis of no association between rows and columns of the 2 × 3 matrix that contains the counts of the three genotypes among cases and controls (Balding 2006). The most commonly used tests for this analysis are the Pearson test and the Fisher's exact test. In most circumstances these are the same test; however, in contrast to the Pearson test which uses an approximation assuming a large sample size, the Fisher exact test uses an exact procedure for the p-value particularly when the sample size is small.

Analysis of association in Table 3.3 would produce two ORs and test statistics one for each of the 2 × 2 comparisons: wild type versus heterozygous and wild type versus homozygous.

Table 3.3: Genotypic distribution between cases and controls. Wild type – zero copies of the variant allele, heterozygous – one copy each of the variant and wild type allele, and homozygous – two copies of the variant allele (Adapted from Sasieni 1997).

	Wild type	**Heterozygous**	**Homozygous**	**Total**
Cases	R_A	R_{AB}	R_{BB}	R
Controls	S_A	S_{AB}	S_{BB}	S
Total	T_A	T_{AB}	T_{BB}	N

In the analysis of complex traits, it is often thought that the risk of disease conferred by each SNP is additive; meaning that the risk of having one variant SNP will fall between the homozygote-wild type risk and the homozygote-variant risk. However,

SNP genotypes can also be grouped into classes or models such as dominant, recessive, and multiplicative (Lewis 2002). Each model makes different assumptions about the genetic effect in the data. Assuming two alleles for each SNP, where A is a dominant allele and a is a recessive allele, a dominant model assumes that having one or more copies of the dominant allele increases risk compared to the recessive allele so Aa or AA genotypes have higher risk than an aa genotype. The same is true for a recessive model in which two copies of the A allele are required to alter risk. The multiplicative model assumes that if having a single dominant allele increases risk by 3-fold, the risk increase for two copies of the dominant allele would be 9-fold: in this case if the risk for Aa is k, the risk for AA is k^2. The additive model assumes a linear increase in risk for each copy of the A allele. Therefore, if the risk is 3-fold for Aa, the risk for AA would be 6-fold: in this case the risk for Aa is k and the risk for AA is 2k (Bush & Moore 2012).

Both the Pearson and Fisher tests have reasonable power for any of the genetic effect models. However, if the genotype risks are additive both tests lack power compared to the Cochran-Armitage trend test which is better suited to this scenario (Clarke et al., 2011). To improve the power to detect additive risks via the Pearson test it is possible to count alleles, rather than classifying according to genotype, thereby doubling each individual's contribution in a 2 × 2 table (Table 3.4).

Table 3.4: Allelic distribution between cases and controls (Adapted from Sasieni 1997).

	Variant	**Wild type**	**Total**
Cases	$R_{AB} + 2R_{BB}$	$R_{AB} + 2R_{AA}$	2R
Controls	$S_{AB} + 2S_{BB}$	$S_{AB} + 2S_{AA}$	2S
Total	$T_{AB} + 2T_{BB}$	$T_{AB} + 2T_{AA}$	2N

However, using the allelic distribution procedure is not recommended because it requires an assumption that both alleles are not statistically associated in cases and controls combined and also does not lead to interpretable risk estimates (Sasieni 1997; Balding 2006). For a more advanced analysis approach of dichotomous case/control traits in genomic studies, logistic regression is commonly used and is often the preferred approach because it allows for adjustment for clinical covariates (and other factors) and

can provide adjusted odds ratios as a measure of effect size.

For the analysis in chapter 5 the additive model was used. Additive models have reasonable power to detect both additive and dominant effects; however, it is important to note that an additive model may be underpowered to detect some recessive effects (Lettre et al., 2007).

In addition to selecting the correct model for analysis, statistical tests should be adjusted for factors known to influence the incidence of the side effect of interest. An additive model was used assuming a uniform, linear increase in risk for each copy of the variant allele (Lettre et al., 2007). Logistic regression models were adjusted for other side effect risk factors including age, menopausal status, BMI, HRT use, and smoking status to reduce spurious associations. SNP were defined as being associated with side effects if they met a significance level of 1.4×10^{-7} to control for multiple testing. Additionally, SNPs were defined as suggestively linked but require validation in other datasets if they met a pre-defined sub-threshold of 5×10^{-5} (Dudbridge and Gusnanto 2008; Burri et al., 2012). Further discussion of how statistical significance is decided in genome analysis is presented in section 3.4.4. ORs and 95% CI were calculated for all SNPs and those which met the statistical thresholds for any of the side effects were reported.

SNPs located close to each other and which are statistically significant were tested for linkage disequilibrium (LD). SNPs are said to be in LD when the frequency at which one SNP is observed with another SNP is higher or lower than what would be expected if the two loci were independent of each other. Many measures of LD have been proposed, the two commonly used measures of LD are D' and r^2 (Devlin and Risch 1995; The International HapMap Consortium 2005). D' is a measure of recombination events between markers at a population level and is scaled between 0 and 1. A D' of 1 indicates complete LD, indicating no other possible combinations between the two markers within the population and the opposite for a D' of 0 (Devlin and Risch 1995). LD is also generally reported in terms of r^2, a measure of correlation (Bush & Moore 2012). High r^2 values indicate that one allele of the first SNP is often observed with one allele of the second SNP, so convey similar information. Therefore, only one of the two SNPs needs to be genotyped to capture the allelic variation (Bush & Moore 2012). There are dependencies between these two statistics; r^2 is sensitive to the allele frequencies of the two markers and can only be high in regions of high D' (Bush &

Moore 2012).

Tag SNPs are selected to capture variation at nearby sites as alleles for these SNPs tag the surrounding stretch of LD. However, patterns of LD are population specific and therefore tag SNPs cannot be transferred from one population to another (Bush & Moore 2012). LD is exploited to optimise genetic studies, preventing genotyping SNPs that provide redundant information. Analysis of data from the HapMap project, identified that 80% of commonly occurring SNPs in European descent populations can be captured using a subset of 500,000 to one million SNPs scattered across the genome (Dong et al., 2013).

3.4.4 Significance levels in genomic analysis

Statistical tests are generally called significant and the null hypothesis is rejected if the p-value falls below a predefined alpha value, normally set to 0.05 for a single comparison. In GWAS, hundreds of thousands of tests are conducted, each one with its own false positive probability increasing the cumulative likelihood of finding one or more false positives over the entire GWAS analysis. Therefore, it is imperative to adjust for multiple testing to avoid the discovery of multiple false positives.

The simplest approach is to set the level of statistical significance at $\alpha = 0.05$, which is then adjusted using Bonferroni adjustment for multiple testing. The Bonferroni correction adjusts the alpha value from $\alpha = 0.05$ to $\alpha = (0.05/k)$ where k is the number of statistical tests conducted. This produces a genome-wide significance level of $1.4\text{x}10^{-7}$ based on testing 350,000 SNPs in the GWAS analysis in this thesis.

However, given many SNPs are correlated this genome-wide significance level can be viewed as overly protective and leads to an increase in false negative findings. Other adjustments are available to assess statistical significance. The simplest is to use a "sub-threshold" level of $1\text{x}10^{-5}$ or $5\text{x}10^{-5}$ as a predefined cut-off to identify SNPs with a suggestive association with the individual side effect (Dudbridge and Gusnanto 2008; Burri et al., 2012). This adjustment utilises known patterns in the inheritance of SNPs to reduce the threshold for statistical significance. It is important to stress that the use of a sub-threshold can only be used to identify SNPs with a suggestive association with an outcome. Any SNPs which meet this threshold must subsequently be confirmed in

independent data sets.

Alternatively, rather than controlling the family-wise error rate by adjusting the false positive rate (FPR) by the number of statistical tests to account for multiple testing, the False Discovery Rate (FDR) can be used. FDR controls the expected proportion of false positives among all signals with an FDR value below a fixed threshold, assuming that SNPs are independent (Benjamini and Hochberg 1995). This method is less conservative than Bonferroni correction.

The FDR is the rate at which features considered to be significant are actually not. In the same way that an alpha level can be set to control FPR, a threshold for FDR can also be set. For example, an FDR threshold of 0.05 is the expected proportion of false positives among all features as, or more, extreme than the observed one. The use of FDR allows for a decision to be made about how many false positives are acceptable among all features that are called significant. This is useful in exploratory analyses, such as GWAS, when a large number of features are expected to be truly significant and is not desirable to restrict discovery capacity. This generates a large number of significant features which can be confirmed in a later study.

Controlling for FDR does not imply any notion of statistical significance but is merely a method to minimize the expected proportion of false positives (Marees et al., 2018). Moreover, this method has its own limitation as SNPs and thus p-values are not independent and independence is an assumption of the FDR method (Benjamini and Hochberg 1995).

3.4.5 Variable selection: Least Absolute Shrinkage and Selection Operator (LASSO)

In a simple model with the aim of predicting observations of a response variable with a combination of predictor variables, the true parameters are unknown and need to be estimated from the sample. In the Ordinary Least Squares (OLS) approach, these parameters are estimated in such a way as to reduce the sum of the residuals to the smallest possible value to obtain the OLS parameter estimates. Two critical characteristics affect estimators: bias and variance. Bias is the difference between the true population and the expected estimator, and variance is a measure of the spread or

uncertainty of these estimates. Large values for bias and variance result in poor predictions from the model and therefore both are required to be low. While the OLS estimator is unbiased, it can have large variance particularly when the predictor variables are highly correlated with each other or there are many predictors. The solution to this problem is to reduce the variance whilst introducing some bias to the model, an approach termed regularisation. As model complexity increases, i.e. the number of predictors increases, the model's variance increases, but the bias decreases.

In order to select the minimum number of markers, which predict side effect outcome both stepwise regression and shrinkage models, such as the Least Absolute Shrinkage and Selection Operator (LASSO), can be employed to reduce the number of variables. Rather than search through all possible subsets as in best subset selection, which becomes infeasible for large sample numbers, LASSO can seek a good path through them. Forward-stepwise selection starts with the intercept, before adding parameters into the model that best improves the fit (Hastie et al., 2014). Forward-stepwise regression is computationally less intensive producing a fewer number of models than best subset selection which contains many more (Hastie et al., 2014). There are several reasons why forward-stepwise might be preferred (Hastie et al., 2014):

- Computational; for large number of variables the best subset may not be computable, but the forward stepwise sequence can always be found (even when p >> N)
- Statistical; selecting the best subset of every possible subset as with the stepwise method can lead to increased variance; forward stepwise is more constrained assessing variables individually, and as such will have lower variance, but perhaps introduce more bias

Backward-stepwise selection starts with the full model sequentially deleting variables with the least impact on the fit. Backward selection can only be used when N > p, while forward stepwise can always be used. Where N is the total number of observations (samples) and p is the number of variables. In this thesis the number of variables (p) is much greater than the number of individuals (N); therefore, backward-stepwise regression is not a suitable technique.

Subset selection retains a subset of the predictors and thereby produces an interpretable

model which possibly has a lower prediction error than the full model. However, as variables are either retained or discarded subset selection often leads to high variance, and so doesn't reduce the prediction error of the full model. Shrinkage methods are more continuous, and don't suffer as much from high variability (Hastie et al., 2014).

The LASSO represents a method of automatic variable selection, which can be used to select predictors of a target variable from a larger set of potential or candidate predictors. The LASSO formulates curve fitting as a quadratic programming problem, where the objective function penalizes the absolute size of the regression coefficients, based on the value of a tuning parameter (λ). In doing so, the LASSO can drive the coefficients of irrelevant variables to zero, thus performing automatic variable selection (Hastie et al., 2014). Once the model has been defined each of the model variables are assessed for their overall significance in a regression, as well as changes in deviance and provide p-values to decide if the parameter is supported in the model.

The choice of the tuning parameter (λ) can be achieved in one of two ways. The first is to choose the value of λ which corresponds to the smallest information criterions, e.g. Akaike Information Criterion (AIC) or Bayesian Information Criterion (BIC). The second is to adopt a more machine learning approach performing cross-validation and selecting the λ which minimises the cross-validated sum of squared-residuals. Whilst the first approach places more emphasis on the model's fit to the data, the second has a greater focus on the model's predictive performance. Therefore this thesis used the second method to determine the λ for variable selection and model development as predictive performance was more important.

Choosing λ through cross-validation requires the partition of data into K parts before training the model using K-1 parts and testing the model in the final group. This is repeated K-times, once for each of the partitions, by leaving one part out each time and training and testing the model until each partition group has been tested. This leads to the optimum value for λ that gives minimum mean cross-validated error as this value of λ is the average of the errors in each of the cross-validated folds the estimate of the error is uncertain and despite being the "best" model may be overfitted. However, it is suggested that the best value to use for λ is one that is within one standard-error of the minimum value; this acknowledges the fact that the risk curves are estimated with error, so errs on the side of fewer variables (Hastie et al., 2014). Using the value of λ at one standard error from the minimum as the selected value for λ results in a

simpler model which cannot be distinguished from the best model in terms of error given the uncertainty in the k-fold CV estimate of the error of the best model (Hastie et al., 2014).

Multi-loci analysis of the genomic data in chapter 5 was performed using LASSO. SNPs from the CG analysis and those previously identified as being associated with breast cancer were included in multi-loci modelling.

LASSO was used to select variables via 10-fold cross-validation. Area under the curve (AUC) was used to determine the best model and select the best subset of variables. Coefficients and locus for each SNP in these models were reported.

These models were then used to determine a polygenic risk score (PRS). Side effect status was modelled against the PRS score as a continuous measure and as quintiles with the third quintile serving as the reference group using a generalised linear model. Odds ratios and 95% CI for each quintile compared to the third quintile were reported.

3.4.6 Model performance classification

The diagnostic ability of models to predict outcomes has usually been determined by the confusion matrix and the receiver operating characteristic (ROC) curve (Fawcett 2006). After defining a model and selecting variables which best describe the outcome in the training set, a binary prediction can be made, of which there can be four types of outcomes: true negative, false negative, false positive, true positive. These four outcomes can be displayed in a confusion matrix sometimes known as an error or contingency matrix; the basic outline of which is shown in Figure 3.1.

		Predicted Class	
		Positive	Negative
Actual Class	Positive	True Positives (TP)	False Negatives (FN)
	Negative	False Positives (FP)	True Negatives (TN)

Figure 3.1: The basic framework of a confusion matrix

True positives (TP) are the positive cases that the model correctly identified as positive. Similarly, true negatives (TN) are the negative cases that the model correctly identified as negative. False positives (FP) are negative cases which are incorrectly identified as

positive by the model and false negatives (FN) are positive cases incorrectly identified as negative by the model.

The following measures, which are based on the confusion matrix, are commonly used to analyse the performance of classifiers, including those that are based on supervised machine learning algorithms:

- True positives (TPR), or sensitivity. This metric corresponds to the proportion of positive data points that are correctly considered as positive, with respect to all positive data points. The higher TPR, the fewer positive data points are missed.

$$Sensitivity = Recall = True\,Positive\,Rate\,(TPR) = \frac{TP}{TP + FN} \tag{3.7}$$

- False positive rate (FPR). This metric corresponds to the proportion of negative data points that are mistakenly considered as positive, with respect to all negative data points. The higher the FPR, the more negative data points are misclassified.

$$False\,Positive\,Rate\,(FPR) = \frac{FP}{FP + TN} = 1 - Sensitivity \tag{3.8}$$

Other measures that are also often associated with ROC are:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \tag{3.9}$$

$$Precision = \frac{TP}{TP + FP} \tag{3.10}$$

$$Specificity = \frac{TN}{TN + FP} \tag{3.11}$$

The FPR and the TPR can then be plotted at many different thresholds, on a single graph, with the FPR values on the x-axis and the TPR values on the y-axis to yield an ROC curve (Fawcett 2006). The area under the resulting curve is called the area under the receiver operating characteristic (AUC) which is commonly used to determine the predictive power of a model with, a higher AUC value representing the superiority of a classifier and vice versa.

3.4.7 Survival analysis

In many survival studies, the primary outcome of interest is the time between entering the study and the occurrence of an event of interest e.g. cancer or death. Survival analysis allows for the analysis of rate of disease by focussing on: the hazard *h(t)* the rate at time t; and the survivor function S(t) which can be illustrated by the survival curve and is the probability that an individual will survive, or not experience the event of interest, up to and including time t.

Chapter 8 investigates the impact of side effects occurring during the first 6-months of endocrine therapy on breast cancer incidence in the IBIS-I and IBIS-II studies was investigated. For this analysis, survival analysis is used to investigate breast cancer incidence from 6-months post randomisation up to 20-years post randomisation for IBIS-I and 12-years post randomisation for IBIS-II.

One of the difficulties in survival analysis is that the event of interest has only occurred in a subset of the study population leaving many where the time to event is unknown. This is called censoring and it may occur for one of the following reasons (Clark et al., 2003):

- a patient has not experienced the event of interest, by the end of the follow up period
- a patient is lost to follow-up during the study period
- a patient experiences a different event that makes further follow-up impossible.

Such censored survival times underestimate the true time to event, as these are unknown, and is often called right censoring. Censoring can also occur if the event of interest occurs prior to first follow-up and hence exactly when it occurred is unknown. In the analysis presented in chapter 8, the association between 6-month side effects and breast cancer is investigated. Women who have breast cancer in the first 6-months were excluded, from the analysis. Due to exclusion of women with breast cancer events prior to the 6-month follow-up point the data analysed here only contains right censored observations.

Kaplan-Meier (KM) method and KM plots are used to estimate and display the survival

function and survival curve respectively. Survival and hazard functions are described together with the KM estimation, and the Cox proportional hazards regression analysis of survival data.

3.4.7.1 Survival and hazard

Survival data is often modelled in terms of two inter-related probabilities, survival and hazard. The survival probability S(t) is the probability that an individual is without the event of interest at a future time point t from the start of the study. This is a fundamental aspect of survival analysis as the survival probabilities at different time points provide information about the time to event and describe the survival experience in the study population (Clark et al., 2003).

The hazard probability, h(t) or $\lambda(t)$ is the probability that at a certain time point, after study initiation, an event of interest is observed thereby representing the event rate for a person who has survived to the time point t (Clark et al., 2003). The hazard probability provides insight into the failure rates and provides a vehicle for specifying a survival model. In summary, the hazard relates to the incident (current) event rate, while survival reflects the cumulative non-occurrence (Clark et al., 2003).

3.4.7.2 Kaplan-Meier survival estimation

The Kaplan-Meier (KM) method allows the survival probability to be estimated from survival times of both censored and uncensored data (Kaplan and Meier 1958). As study participants have events during the follow-up period at distinct times, and as events are assumed to be independent of each other, the probability of surviving from one time point to the next can be multiplied together producing the cumulative survival probability (Clark et al., 2003). Therefore, the probability of "surviving" or being without the event of interest at time tj, can be calculated from S($t_j - 1$) the probability of being alive at $t_j - 1$ the time point prior to the current time point of interest, n_j the number of patients alive before t_j, and d_j the number of events occurring at t_j, using Equation 3.12:

$$S(t_j) = S(t_(j-1))(1 - d_j/n_j) \tag{3.12}$$

Equation 3.12: Equation defining the survival function at time tj

As the value of S(t) remains constant between time of events, the estimated probability is a step function that only changes at the time of each uncensored event. Therefore, each patient contributes information all the while they are known to be event-free (Clark et al., 2003). If every individual were to experience the event the estimator would simply reduce to the ratio of the number of individual events free at time t divided by the number of people who entered the study (Clark et al., 2003).

3.4.7.3 The hazard ratio and Cox regression

Survival of patients in two different treatment groups can be compared using nonparametric tests with the logrank test the most widely used method (Peto et al., 1977). The logrank test tests null hypothesis that the ratio of the hazard rates in the two groups is equal to 1; therefore, no difference in survival between the two groups. However, it cannot be used to investigate the effect of multiple variables on survival.

The Cox (proportional hazards or PH) model is the most commonly used approach for analysing survival time data in medical research (Cox 1972; Bradburn et al., 2003). It is a survival analysis regression model, which describes the relation between the event incidence, as expressed by the hazard function and a set of covariates. The Cox regression method estimates the hazard calculating the probability that an individual experiences the event at a particular point in time.

Mathematically, the Cox model is expressed by Equation 3.13 where the hazard function h(t) is dependent on covariates ($x_1, x_2, \ldots, x_p$), the impact of which is determined by the coefficient for each ($b_1, b_2, \ldots, b_p$). The baseline hazard (h_0) and is overall hazard if all covariates equal zero because $e_0 = 1$ (Bradburn et al., 2003). Equation 3.13 calculates the hazard at time t; therefore, the cumulative hazard (H(t)), for the risk of the event of interest from the start (time 0) to time t, can be calculated by summing all the hazards up to time t. The covariates act multiplicatively on the hazard at any point in time, resulting in the key assumption of the PH model: namely that the hazard of the event in any group is a constant multiple of the hazard in another group implying that the hazard curves for the groups are proportional and cannot cross (Cox 1972; Bradburn et al., 2003).

$$h(t) = h_0(t) \times expb_1x_1 + b_2x_2 + \ldots + b_px_p \tag{3.13}$$

Equation 3.13: Cox proportional hazards model

The hazard ratio (HR) compares the hazards at time t for each group of interest. Simplifying equation 3.13 to include only one variable, side effects experienced in the first 6-months, the hazard ratio of the side effect positive group compared to the side effect negative group can be calculated using equation 3.14

$$HR(t) = \frac{(h_0(t) \times exp(b_1))}{(h_0(t))} = exp(b_1) \tag{3.14}$$

Equation 3.14: Formula defining the hazard ratio between the side effect group and the non-side effect group

As the Cox PH model assumes that the hazard ratio remains constant through time and equals $\exp(b_1)$, the b_1 is the estimated log hazard ratio for the side effect group compared to the non-side effect group and thus once log transformed is equal to the hazard ratio.

An HR above 1 indicates that more events have occurred in the side effect group thereby decreasing the length of survival. When the hazard ratio is below 1, the covariate is negatively associated with the event and therefore increases the survival time. An HR of 1 indicates no difference in survival.

For the Cox PH method to be valid the covariate effect needs to be approximately constant throughout the duration of the study, and the proportionality assumption must hold (Bradburn et al., 2003). This means that the HR is assumed constant over time. However, if the PH assumption fails, the HR is not constant over time and becomes dependent on follow-up time (Prentice et al., 2005; Hernan 2010).

The PH assumption can be tested using graphical and analytical methods via Kaplan-Meier survival curve (KM) estimates or via correlation tests of Schoenfeld residuals and the event time respectively (Xue et al., 2013; Emmert-Streib and Dehmer 2019). These tests are among the most frequently used approaches for assessing the PH assumption and are popular as they can be calculated using standard statistical software and are easy to interpret (Xue et al., 2013).

KM estimates can show graphically whether the PH assumption holds and compares observed and expected survival curves to assess the PH assumption. The observed survival curves are calculated using stratified estimates of KM curves. Strata are obtained from the categories of the covariates and the expected survival curves for each stratum is obtained by performing a Cox PH model with each of the adjusted survival curves, see Equation 3.14 (Emmert-Streib and Dehmer 2019). A comparison is then performed between each survival curve for each covariate one at a time. Performing the analysis one covariate at a time is more stringent hence the analysis is performed this way rather than grouping all covariates together. Observed and expected survival curves for each strata are plotted together. If the two lines of the KM curve cross then the PH assumption is violated and needs to be controlled; however, if for each covariate there is no convergence, and the observed and expected survival curves are close, then the PH assumption can be said to hold (Emmert-Streib and Dehmer 2019).

Whilst the graphical method is a useful technique in the case of categorical variables and few covariates, if an exploratory variable is continuous or if there are many categorical variables the plot can be difficult to implement (Machin et al., 2006; Lee and Wang 2013). In these cases, Schoenfeld residuals are useful for the assessment of the PH assumption (Schoenfeld 1982). A Schoenfeld residual can be calculated for each exploratory variable and for each non-censored individual.

A reference vector containing the ranks of events is created with the first event receiving a value of 1, the next subject receives a value of 2, and so on until each non-censored individual is listed. A correlation test between the variables obtained in the first and second steps is then performed. The null hypothesis is that the Schoenfeld residues sum to zero and should be distributed around zero if the model is correct and the PH assumption holds. A systematic trend or a significant regression coefficient in the Schoenfeld residues in relation to the rank order of time indicates non-PH (Machin et al., 2006; Lee and Wang 2013; Emmert-Streib and Dehmer 2019).

When the PH assumption is not met, the HR estimates would not be representative over the whole intervention period. This might lead to the convergence and possible crossing of survival curves outcomes which can be common in medical research (Zewdu 2009; Li et al., 2015). Crossing survival curves are often reported when the treatment offers a short-term benefit, but no long-term advantages or when the treatments being compared have different biological mechanisms of action (Li et al., 2015). Additionally,

if different sub-populations are included and different populations respond differently to the treatment this may be another reason for the survival curves to converge or cross (Royston and Parmar 2013; Li et al., 2015).

3.4.8 Statistical software

All analyses in chapters 4, 6, 7, 8 were performed using R (R Foundation for Statistical Computing, Vienna, Austria.). In chapter 5, QC was performed using PLINK (Version 1.9) and R Version 3.6.0 through the plugin plinkQC R package (Chang et al., 2015; Meyer 2018). Statistical analysis, OR and 95% CI, for both the GWAS study and the CG study was performed using PLINK Version 1.9 (Package: PLINK[1.9], Authors: Shaun Purcell and Christopher Chang, URL: www.cog-genomics.org/plink/1.9/) (Chang et al., 2015). Output of statistical analysis was imported into R for further analysis of interactions between SNPs and treatment and for production of graphics to summarise the outputs.

In addition to the statistical software used in chapter 5, LDlink was used to plot the LDmatrix for regions of interest. Genotypes from the Central Europeans living in Utah (CEU) group of the 1000 genomes project will be used to assess the LD between different SNPs within our cohort (Machiela and Chanock 2015; The 1000 Genomes Project Consortium 2015).

3.4.9 Authors contribution

The IBIS-I study was designed by Jack Cuzick (J.C.), Anthony Howell (A.H), and John Forbes (J.F.F.) and data was collected by A.H., Simon Cawthorn, Hamish Hamed, Kaija Holli, and J.F.F. The author Michael Hale (M.H.) processed the experimental data and performed all analysis within the thesis. All results were discussed with Ivana Sestak (I.S.) and J.C. M.H. designed the studies in chapter 5 and chapter 6. Samples for the genomic analysis were selected by J.C. and Assays were carried out blindly at Genome Quebec (Montreal, Canada). M.H. performed QC of samples and all subsequent analysis. Blood samples for sex hormone measurement were selected by I.S. All sample measurement and QC was performed by M.H. prior to analysis. A more detailed breakdown of contributions to each chapter can been found in a summary table in Appendix 1.

Chapter 4: Association of baseline factors with endocrine therapy side effects

4.1 Introduction

Endocrine therapies, both selective oestrogen receptor modifiers (SERMs) and aromatase inhibitors (AIs) are highly effective at reducing the risk of oestrogen receptor positive breast cancer in women at high risk. However, they also increase the risk of menopausal related side effects which is an area of concern for women and have a significant impact on the success of preventive therapies (Thürlimann et al., 2005; Cuzick et al., 2007, 2014; Veronesi et al., 2007; Land et al., 2016). Some of these side effects are severe, for example increased risk of endometrial cancer or thromboembolism with tamoxifen, but they occur rarely. The more common side effects of endocrine therapy are menopausal-like, such as hot flushes (HFs), and night sweats; genitourinary symptoms including vaginal dryness, vaginal discharge, irregular bleeding, and joint and muscle pain (Gold et al., 2000; Woods and Mitchell 2005).

These less severe but bothersome side effects are still the subject of much interest as they are given as a major reason by eligible women for poor uptake and adherence to therapy (Ropka, Keim, & Philbrick, 2010; Smith et al., 2016). Women who experienced gynaecological, vasomotor or sexual symptoms were all less likely to be adherent after one year than those who experience no side effects (Land et al., 2016; Smith et al., 2017). Participants often believe that the risk of side effects related to SERMs or AIs are far greater than the benefits and their risk of breast cancer (Lacroix et al., 2010). Therefore, a better understanding of side effects and related risk factors is required to communicate the risk and benefits of SERMs or AIs to women at high risk of breast cancer (Vogel, 2015).

However, it is important to distinguish between symptoms in women that are due to ovarian failure, or the ageing process, and those initiated as a result of endocrine therapy. Therefore, it is important to take these factors, which increase the risk of

menopausal-like symptoms, into account during analysis.

The effect of age and menopausal status was shown in recent studies (Gold et al., 2000; Makara-Studzińska et al., 2014). A review which reported vasomotor symptom prevalence of 57% in healthy women in the general population aged 40 to 64 years, sexual dysfunction was reported by approximately 60% of women, and arthralgia by 62% (Makara-Studzińska et al., 2014). The Study of Women's Health Across the Nation (SWAN) showed that in women aged 52-55 years (N = 2,066) HFs were prevalent in 46% of women (Gold et al., 2000). In contrast, in women aged 40-43 (N = 3,493) the percentage of women reporting HFs was considerably lower at 25%. In a smaller cohort of 1,776 women from the SWAN study aged 47 to 59 years who had an intact uterus and at least one ovary and for whom assessment of body composition at the sixth annual study visit (2002–2004) was available, about 59% had vasomotor symptoms. Risk factors that were associated with these symptoms in the smaller cohort (N = 1,776) from the SWAN study were race/ethnicity, education level, menopause stage, parity, smoking status, abdominal circumference, percentage body fat, anxiety, levels of follicle-stimulating hormone and oestradiol (E2), and free E2 index (Thurston et al., 2008).

In the general population, vasomotor and gynaecological symptoms are associated with a reduction in circulating oestrogen concentrations (Guthrie et al., 1996; Kronenberg 2010; Jager et al., 2013). Postmenopausal status is associated with the development of HFs and joint symptoms in particular (Jones et al., 2015; Walitt et al., 2015; Andrews et al., 2018), but symptoms often improve with the use of HRT (Sassarini and Lumsden 2015).

Further evidence linking side effects to lower oestrogen concentrations comes from both prevention and adjuvant trials investigating both SERMs and AIs. Incidence of gynaecological side effects, HFs, venous thromboembolic events and endometrial cancers were all associated with tamoxifen for prevention or treatment of breast cancer (Cuzick et al., 2013; Lin, Zhang, & Manson, 2011). The increased risk of side effects in women taking tamoxifen has been clearly shown in prevention trials (Powles et al., 1998; Veronesi et al., 1998; Cuzick et al., 2002). Similarly, evidence of the effect of AIs on the increased incidence of arthralgia, gynaecological events and HFs have been observed (Goss et al., 2011; Cuzick et al., 2014).

In addition to menopausal status and the use of endocrine therapy, other risk factors have been associated with the menopausal-related side effects, such as BMI, smoking, and HRT use. It has been shown that women with high BMI have higher concentrations of circulating oestrogen than women with low BMI due to greater conversion of androgens to oestrogens in adipose tissues (Endogenous Hormones and Breast Cancer Collaborative Group 2011). As oestrogen levels have been shown to be negatively associated with HFs it is hypothesised that postmenopausal women with high BMI have a reduced risk of HFs compared to those with lower BMI (Wilbur et al., 1998; Øverlie et al., 2002). However, Thurston et al., (2008) showed that HFs occurred more frequently when there was a higher percentage of fat tissue, after adjustment for age, site, race, educational level, parity, HRT use in the last month, menopausal status, smoking history, and anxiety symptoms (Thurston et al., 2008). This is further supported by other studies (Gallicchio et al., 2005; Miller et al., 2006; Schilling et al., 2007), but ultimately it is unclear whether it is the actual BMI overall or fat distribution that is related to risk of developing HFs.

Lower BMI has emerged as a risk factor for gynaecological symptoms in postmenopausal women (Huang et al., 2010). However, a previous study in middle-aged women found no significant relationship between BMI and gynaecological symptoms (Gold et al., 2000). A retrospective analysis of the ATAC study showed that obese women reported more musculoskeletal events than women who were of normal weight, BMI <25 kg/m2, or those who are overweight; BMI of 25-30 kg/m^2 (Sestak et al., 2008). The IES trial supports the findings of ATAC where a weight of more than 80 kg was associated the development of arthralgia (Miegg et al., 2012).

Smoking has also been associated with an increase in menopausal-like symptoms. The association between smoking and HFs have been discussed in section 1.4.2.1 and studies report contrasting results (Maura K Whiteman et al., 2003; Cochran et al., 2008). Smoking has also been inversely associated with vaginal symptoms (Huang et al., 2010). While the harmful effects of smoking are well established, there are a multitude of other effects in tissues not experiencing direct smoke contact that remain relatively poorly explained. Women who smoke are often oestrogen-deficient, which may lead to an increase in oestrogen-deficiency problems such as osteoporosis (Baron et al., 1990).

HRT is often prescribed to relieve menopausal symptoms, such as HFs. However, prior HRT has also been associated with an increased risk of developing aromatase inhibitor

induced arthralgia (AIA) a well known side effect of AIs (Sestak et al., 2008). A retrospective analysis of the ATAC study, which randomsied women to tamoxifen, anastrozole or combination for 5-years, found that those who were randomsied to tamoxifen or anastrozole and had used HRT were more likely to develop joint symptoms than those who never used HRT. Additionally, women who stopped using HRT less than six months before the start of the trial reported a similar number of joint symptoms as those who stopped longer than six months before (Sestak et al., 2008).

Associations between risk factors and side effects are complex and their interpretation is not aided by a lack of adjustment for other covariates in previous work studying the effect of risk factors on side effects (Thurston et al., 2008). Therefore, the importance of investigating the role of each risk factor with adjustment for other covariates is essential.

Due to the negative impact that menopausal symptoms have on a woman's quality of life, new analyses are required to gain a better understanding of how potential risk factors influence side effect incidence particularly in women taking endocrine therapy for breast cancer prevention. This chapter describes the association between risk factors and the incidence of side effects in the IBIS-I (tamoxifen) and IBIS-II (anastrozole) studies. The major areas of focus will be on baseline factors such as age, menopausal status and BMI, modifiable factors: HRT use and smoking history and reproductive factors such as parity, age at menopause, age at menarche and time since menopause.

This chapter investigates the association between risk factors and the incidence of side effects in the IBIS-I (tamoxifen) and IBIS-II (anastrozole) studies. The major areas of focus will be on baseline factors such as age, and BMI, modifiable factors: HRT use and smoking history and reproductive factors such as parity, age at menopause, age at menarche and time since menopause. Side effects are often hypothesised to occur as a result of disruption to oestrogen concentrations. Many of these anthropometric and modifiable factors can impact a woman's lifetime exposure to oestrogen and may be key in understanding the risk of side effects. However, in order to determine the effect of these risk factors several factors must be taken into account. Menopausal status and treatment allocation have been identified as an important effect modifiers of side effect risk. The use of tamoxifen and anastrozole are known to increase the risk of side effects compared to women who are randomised to placebo. Additionally, menopause must also be accounted for as after menopause oestrogen concentration are greatly reduced

compared to premenopausal concentrations. It may therefore be that given these different conditions that risk factor may have differing effect pre and post-menopause and may depend on whether a woman has been randomised to tamoxifen, anastrozole or placebo. Therefore, this research separated women into distinct subgroups for analysis to identify more precisely the populations for whom the baseline factors particularly increased or decreased risks. The possibility of interactions between treatment and risk factors in pre and postmenopausal women cannot be discounted. Therefore, an interaction analysis was also performed to assess the effect of randomised treatment on risk factors.

What we already know

- Women who take endocrine therapy for breast cancer treatment or prevention are at increased risk of menopausal like side effects
- Side effects are given as one of the major reasons behind not choosing to take tamoxifen or poor adherence to endocrine therapy
- Side effects are hypothesised to be driven by oestrogen deficiency
- Side effects may be as a result of ER agonism or ER antagonism
- A large variation in reports of side effects between women

What this analysis adds

- Quantifies the incidence of key endocrine therapy-related side effects
- Assesses side effect incidence addressing the key points where incidence is high
- Establishes anthropometric, modifiable and reproductive factors which increase the risk of side effects
- Assesses the influence of endocrine therapy on how these risk factors impact side effect incidence

4.2 Methodology

4.2.1 Study population

7,154 women enrolled on the IBIS-I trial and 3,864 women enrolled on the IBIS-II trial were eligible for analysis. After exclusion of women with no side effect information at the 6 month follow-up for either HFs or gynaecological side effects in the IBIS-I trial (N = 400), and after removal of women with no side effect information for arthralgias, HFs or vaginal changes in the IBIS-II trial (N = 268), 6,754 women from IBIS-I and 3,597 women from IBIS-II remained. In addition to those without side effect information, women who died or those who developed breast cancer within the first 6 months of follow up were also excluded leaving 6,746 women in IBIS-I and 3,591 women in IBIS-II for analysis (Figure 4.1).

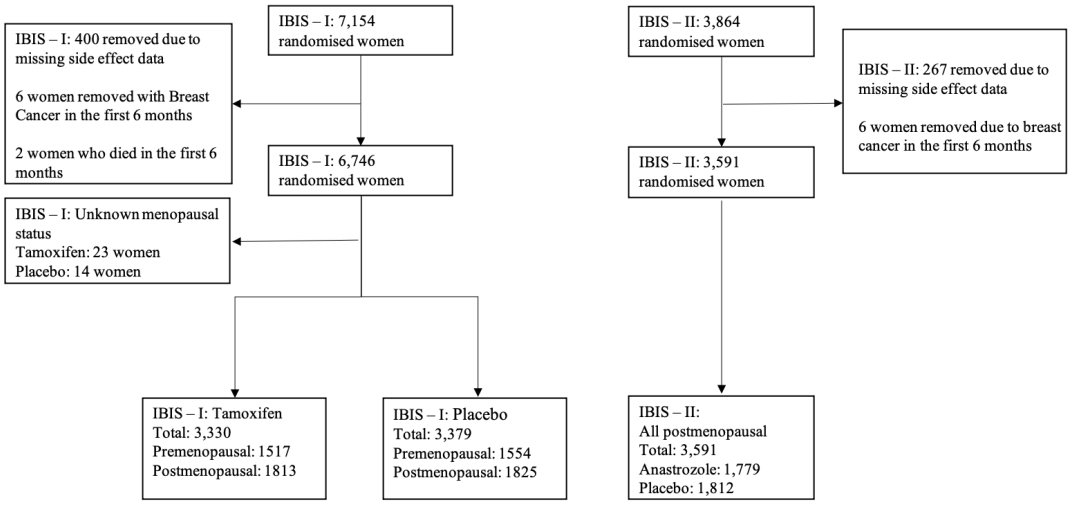


Figure 4.1: Analysis profile with the number of women remaining in each treatment arm after removal of women with missing side effect data

4.2.2 Study measures

The analysis focuses on arthralgia, HFs or gynaecological symptoms reported during the first 6 months of follow-up since they are the major side effects associated with tamoxifen or anastrozole. These side effects were mainly reported during the first 6-months of endocrine therapy and therefore are of interest with relation to baseline risk factors. Gynaecological symptoms included vaginal discharge, vaginal dryness and irregular bleeding. Women who reported side effects during the first 6 months and

those who dropped out during the first 6 months were included.

4.2.3 Statistical methods

It has been previously established that the frequencies of side effects vary by menopausal status and endocrine treatment (Stearns et al., 2002; Ziv-Gal and Flaws 2010; Cuzick et al., 2015, 2020). Therefore, the analysis in this chapter begins by establishing the effect of randomised treatment, and menopausal status for women enrolled on the IBIS-I trial. These factors alone are sufficient to alter risks and must be adjusted for. Therefore, when calculating the effect of potential risk factors on the risk of side effects within the first 6 months, each study group was split by treatment arm, and in the case of IBIS-I, menopausal status. The IBIS-II study group was analysed by treatment arm alone as all women were confirmed as postmenopausal before study entry. While risk factors are investigated within each treatment arm, the presence of interaction between treatment and risk factors cannot be discounted. To account for this scenario, interaction analyses were performed between risk factors and randomised treatment.

Where possible continuous variables were used for analysis, but were also categorised to enable comparison between subgroups. For women in IBIS-I, age at randomisation was categorised into, $\leqslant 50$ and greater than 50. However, women enrolled in IBIS-II were older and therefore the age split was higher, $\leqslant 60$ and greater than 60. Both these age categories are consistent with those used in the initial analyses of IBIS-I and IBIS-II (Cuzick et al., 2002, 2014). BMI was measured as weight in kilograms divided by height in metres squared (kg/m^2) and categorised as < 25, 25-30 and > 30. In IBIS-I, HRT use was defined as never users, current users and former users. In IBIS-II, HRT use was defined as never or ever users. Finally smoking, parity, age at first birth, age of menarche, age of menopause, type of menopause, and time since menopause were also investigated as risk factors for side effects.

Many of the reproductive risk factors, such as age at menopause, type of menopause and time since menopause, are only applicable for postmenopausal women. Furthermore, as menopausal status affects side effect risk, premenopausal and postmenopausal women were analysed separately.

In the IBIS-I trial, age of menopause was defined as the age of natural menopause or

the age of bilateral oophorectomy with or without hysterectomy. Age at menopause was subsequently split into two categories those who were postmenopausal at age 50 or younger and those who were postmenopausal at age 50 or older. Time since menopause was the age of menopause minus the age of randomisation. Time since menopause was split into three categories those who were 5 years or less since menopause at randomisation, those who were 5-10 years postmenopausal, and those who were postmenopausal for greater than 10 years. Menarche to menopause was used to determine the length of exposure to oestrogen. Menarche to menopause was calculated as the age of menopause minus the age of menarche.

Each of these risk factors are thought to affect side effect outcomes via the influence of circulating hormones, specifically their effect on circulatory concentrations of oestrogen. Through modifications of oestrogens, each of the risk factors can increase or decrease the chances of side effects.

Two main methodological issues arise in epidemiological studies of anthropometric factors. Firstly how the measurements were taken, and secondly when in life these measurements were taken (Friedenreich 2001). The use of anthropometric data taken at baseline to predict the risk of the disease occurring several years after enrolment may induce bias. In many cases, these factors are not re-measured during follow up which induces bias as changes occur throughout a woman's lifetime, for example in body weight and adiposity. In women, the rate of increase of weight or central adiposity changes at defined life periods including menarche, pregnancy and menopause (Friedenreich 2001). However, studying these factors at baseline is ideal as they represent the status before endocrine therapy was started. This then allows us to determine side effects on endocrine therapy once started.

To assess the impact of each of the risk factors logistic regression was used for univariate and multivariate analysis for each side effect. As side effect outcome was defined as a binary variable, 1 -side effect has occurred or 0 – no side effect reported, logistic regression can be used. A secondary advantage of logistic regression is that it enables the use of additional variables to assess the strength of variables after adjustment.

Logistic regression aims to determine the probability of an outcome based on a set of inputs. Univariate logistic regression was performed to assess the association of risk factors, at baseline, with the development of side effects within the first 6 months of

trial. Each risk factor was regressed against the side effect outcome and odds ratio, 95% confidence intervals and P-values reported. Multivariate analysis was subsequently performed on all variables that were univariately significant. Odds ratios 95% confidence intervals and P-values were reported. Interaction analysis was also performed using logistic regression with terms for treatment, risk factors, and interaction between treatment and individual risk factors included in the model.

4.3 Results

Baseline demographics of women in the IBIS-I study included in this analysis are shown in Table 4.1 and they were evenly distributed between the placebo and the tamoxifen arm.

After 6 months follow up, the incidence of HFs and gynaecological symptoms reported in the two groups was significantly different, with HFs approximately 3-fold increased with tamoxifen than with placebo (Tamoxifen: 51.9% vs Placebo: 26.7%, OR = 2.97 95%CI (2.68 – 3.29): P $<$ 0.0001). Incidence of gynaecological symptoms was approximately 2-fold higher in the tamoxifen group (28.5% vs 15.6%, OR = 2.15 (1.91 – 2.43); P $<$ 0.0001) compared to women in the placebo group. In both treatment arms, the majority of HFs reported were mild and only a small percentage were reported as severe.

The incidence of vaginal discharge after 6 months was 3.6-fold higher in the tamoxifen arm compared to the placebo arm (15.4% vs 4.7%, OR = 3.67 (3.06 – 4.42); P $<$ 0.0001). This was the largest increase in any of the gynaecological symptoms reported during the first six months. Vaginal dryness was the most commonly reported gynaecological symptom in women in the placebo group (N = 255, (7.6%)). The risk of vaginal dryness was significantly higher in the tamoxifen group (9.2% vs 7.6%, OR = 1.24 (1.04 – 1.47); P = 0.02). The incidence of irregular bleeding was also significantly higher in the tamoxifen group compared to the placebo group (9.6% vs 5.9%, OR = 1.68 (1.40 – 2.03); P $<$ 0.0001).

Table 4.1: Demographics of women included in this analysis from IBIS-I according to randomised treatment

	Placebo (N = 3,379)		**Tamoxifen (N = 3,330)**	
	Median (IQR)		**Median (IQR)**	
Age at randomisation (years)	49.0 (46.0 - 55.0)		50.0 (46.0 - 55.0)	
Age menarche (years)	13.0 (12.0 - 14.0)		13.0 (12.0 - 14.0)	
Age menopause (years)	49.0 (46.0 - 51.0)		49.0 (46.0 - 51.0)	
Menarche to menopause (years)	35.0 (31.0 - 38.0)		35.0 (31.0 - 38.0)	
Age at first birth (years)	23.0 (21.0 - 27.0)		23.0 (21.0 - 26.0)	
BMI (kg/m^2)	26.0 (23.2 - 29.5)		26.0 (23.3 - 29.8)	
	N (%)		**N (%)**	
Menopausal status				
Premenopausal	1554	(46.0)	1517	(45.6)
Postmenopausal	1825	(54.0)	1813	(54.4)
BMI (kg/m^2)				
< 25	1365	(40.4)	1349	(40.5)
25-30	1156	(34.2)	1096	(32.9)
> 30	761	(22.5)	780	(23.4)
HRT				
Never	2023	(59.9)	1959	(58.8)
Former	472	(14.0)	536	(16.1)
Current	883	(26.1)	833	(25.0)
Smoke				
Never	1759	(52.1)	1650	(49.6)
Ex-Smoker	1025	(30.3)	1066	(32.0)
Current	595	(17.6)	611	(18.4)
Parity				
Nulliparous	433	(12.8)	424	(12.7)
Parous	2944	(87.1)	2906	(87.3)
Type of menopause				
Natural menopause	999	(29.6)	1046	(31.4)
Hysterectomy	1210	(35.8)	1143	(34.3)
Hysterectomy and oophorectomy	202	(16.7)	209	(18.3)
Hysterectomy and bilateral oophorectomy	303	(25.0)	260	(22.8)

Continued on next page

Table 4.1 – continued from previous page

	Placebo (N = 3,379) N (%)		Tamoxifen (N = 3,330) N (%)	
6 Month side effect reports				
Hot flushes*	902	(26.7)	1727	(51.9)
Mild	749	(83.0)	1275	(73.8)
Moderate	112	(12.4)	264	(15.3)
Severe	41	(4.6)	188	(10.9)
Vaginal discharge*	160	(4.7)	512	(15.4)
Mild	143	(89.4)	447	(87.3)
Moderate	14	(8.8)	47	(9.2)
Severe	3	(1.9)	18	(3.5)
Vaginal dryness*	255	(7.6)	305	(9.2)
Mild	241	(94.5)	274	(89.8)
Moderate	11	(4.3)	25	(8.20
Severe	3	(1.2)	6	(2.0)
Irregular bleeding*	200	(5.9)	318	(9.6)
Mild	184	(92.0)	294	(92.5)
Moderate	13	(6.5)	17	(5.4)
Severe	3	(1.5)	7	(2.2)

* Total side effect percentage is of the total number in the placebo (N = 3,379) or tamoxifen (N = 3,330) arm. Severity information is given as a percentage of the total reports of each side effect.

The baseline characteristics of women in the IBIS-II analysis are shown in Table 4.2. The distribution of baseline factors between the placebo and anastrozole arms of the trial were well matched. The incidence of arthralgia after 6 months follow up was significantly higher in the anastrozole group, with a 34% increase in odds reported compared to placebo (Anstrozole: 32.7% vs Placebo: 26.6%, OR = 1.34, (1.16-1.54); P < 0.0001). However, increases in odds were confined to mild (20.2% vs 16.5%, OR = 1.34 (1.12 – 1.59); P = 0.001) and moderate (9.9% vs 8.1%, OR = 1.34 (1.07 – 1.70); P = 0.01) arthralgia. There was a 47% increase in HFs in the anastrozole group compared to the placebo group (44.7% vs 35.5%, OR = 1.47 (1.29-1.68); P < 0.0001). Mild HFs were significantly increased in the anastrozole arm compared to the placebo arm (26.9 vs 21.2%, OR = 1.48 (1.26 – 1.73); P < 0.0001), so too were moderate HFs (14.2% vs 11.0%, OR = 1.50 (1.23 – 1.85); P < 0.0001). However, severe HFs were not significantly higher in the anastrozole arm compared to the placebo arm (3.6% vs 3.2%, OR = 1.31 (0.91 – 1.89): P = 0.14).

Table 4.2: Demographics of women included in this analysis from IBIS-II according to randomised treatment allocation

	Placebo (N = 1,812)		**Anastrozole (N = 1,779)**	
	Median (IQR)		**Median (IQR)**	
Age at randomisation (years)	59.0 (54.8 - 63.0)		59.0 (55.0 - 63.0)	
Age menarche (years)	13.0 (12.0 - 14.0)		13.0 (12.0 - 14.0)	
Age menopause (years)	50.0 (45.0 - 52.0)		50.0 (45.0 - 52.0)	
Menarche to menopause (years)	36.0 (32.0 - 39.0)		36.0 (32.0 - 39.0)	
Time since menopause (years)	9.0 (5.0 - 15.0)		10.0 (4.0 - 15.0)	
Age at first birth (years)	24.0 (21.0 - 27.0)		24.0 (21.0 - 27.0)	
BMI (kg/m^2)	27.3 (24.4 - 31.3)		27.3 (24.2 - 31.0)	
	N (%)		**N (%)**	
BMI (kg/m^2)				
< 25	534	(29.5)	548	(30.8)
25-30	676	(37.3)	653	(36.7)
> 30	563	(31.1)	547	(30.7)
HRT				
Never	954	(52.6)	933	(52.4)
Ever	858	(47.4)	844	(47.4)
Smoking status				
Never	1065	(58.8)	992	(55.8)
Ex-Smoker	553	(30.5)	565	(31.8)
Current	182	(10.0)	212	(11.9)
Parity				
Nulliparous	262	(14.5)	253	(14.2)
Parous	1540	(85.0)	1520	(85.4)
Type of menopause				
Hysterectomy	614	(33.9)	593	(33.3)
Hysterectomy and oophorectomy	228	(12.6)	204	(11.5)
6 Month side effect reports				
Arthralgia[*]	482	(26.6)	581	(32.7)
Mild	299	(62.0)	360	(62.0)
Moderate	146	(30.3)	177	(30.5)
Severe	37	(7.7)	44	(7.6)
Hot flushes[*]	643	(35.5)	796	(44.7)
Mild	385	(59.9)	479	(60.2)
Moderate	200	(31.1)	253	(31.8)
Severe	58	(9.0)	64	(8.0)
Gynaecological symptoms[*]	152	(8.4)	197	(11.1)
Mild	102	(67.1)	135	(68.5)
Moderate	42	(27.6)	44	(22.3)
Severe	8	(5.3)	18	(9.1)

[*] Severity percentage is as a proportion of the number of reported symptoms rather than the total population.

A 36% increase in the number of vaginal changes in the anastrozole group was observed compared to placebo (11.1% vs 8.4%, OR = 1.36 (1.09-1.70); P = 0.01). The number of reported mild vaginal changes were significantly higher in the anastrozole arm compared to the placebo arm (7.6% vs 5.6%, OR = 1.39 (1.06 – 1.81); P = 0.02) so too were the number of severe vaginal changes in the anastrozole group were significantly higher (1.0% vs 0.4%, OR = 2.36 (1.06 – 5.76); P = 0.04) than in the placebo group. No statistically significant risk change for anastrozole was observed in reports of moderate vaginal changes.

4.3.1 Analysis of IBIS-I risk factors

4.3.1.1 Analysis of the effect of menopausal status on the incidence of side effects after 6-months of follow up

The incidence of side effects is known to increase around menopause; therefore, prior to further investigation the impact of menopausal status was investigated in the IBIS-I trial (Table 4.3).

Women who were premenopausal had significantly fewer reports of HFs within 6 months of trial entry than women who were postmenopausal at entry (34.9% vs 42.8%, OR = 0.72 (0.65 – 0.79); P < 0.0001). In contrast, premenopausal women were at increased risk of gynaecological symptoms (24.8% vs 19.8%, OR = 1.33 (1.19 – 1.50); P < 0.0001). When individual gynaecological symptoms were investigated, premenopausal women had an increased risk of irregular bleeding (13.9% vs 2.5%, OR = 6.29 (5.02 – 7.98); P < 0.0001), but a decreased risk of vaginal dryness (6.8% vs 9.8%, OR = 0.67 (0.56 – 0.83); P < 0.0001). No effect of menopausal status was observed for vaginal discharge.

When menopausal status was investigated within each arm of the IBIS-I trial, premenopausal women randomised to tamoxifen had a statistically significant lower HF risk (47.1% vs 55.8%, OR = 0.71 (0.62 - 0.81); P < 0.0001) compared to postmenopausal women randomised to tamoxifen. Conversely, premenopausal women taking tamoxifen had an increased risk of gynaecological symptoms compared to those who were postmenopausal and randomised to tamoxifen at study entry (32.3% vs 25.4%, OR = 1.41 (1.21 - 1.63); P < 0.0001). However, when each of the gynaecological symptoms was

considered independently, premenopausal women randomised to tamoxifen were at increased risk of irregular bleeding (17.5% vs 2.9%, OR = 7.20 (5.35 – 9.87); P < 0.0001) and decreased risk of vaginal dryness (7.8% vs 10.3%, OR = 0.74 (0.58 – 0.95); P = 0.02). Premenopausal women in the tamoxifen group did not have a statistically significant difference in reports of vaginal discharge (14.2% vs 16.4%, OR = 0.84 (0.70 – 1.02); P = 0.08) compared to postmenopausal women randomised to tamoxifen (Table 4.3).

Premenopausal women in the placebo group also had a statistically significant lower risk of HF (22.9% vs 29.9%, OR = 0.70 (0.59 - 0.81); P < 0.0001) compared to postmenopausal women randomised to placebo. However, as in the tamoxifen group, premenopausal women had a greater risk of gynaecological symptom incidence (17.4% vs 14.2%, OR = 1.26 (1.05 – 1.53); P = 0.01) than postmenopausal women. Premenopausal women were at significantly higher risk of irregular bleeding than postmenopausal women (10.4% vs 2.1%, OR = 5.29 (3.75 – 7.66); P < 0.0001). However, risk of vaginal dryness was significantly lower in premenopausal women in the placebo group (5.7% vs 9.2%, OR = 0.60 (0.45 – 0.78); P = 0.0001) when compared to postmenopausal women. There was no significant difference in reports of vaginal discharge (4.5% vs 4.9%, OR = 0.91 (0.66 – 1.25); P = 0.56) (Table 4.3).

Due to the effect that both treatment and menopausal status have on side effect incidence, further analysis of risk factors was conducted according to treatment allocation and menopausal status.

Interaction with allocated treatment are reported in Table 4.3. There was no evidence of an interaction between randomised treatment and menopausal status for any side effect.

Table 4.3: Odds ratios and 95% confidence intervals for the impact of menopausal status on major side effect incidence reported at the 6-month follow up during the IBIS-I trial

		Tamoxifen		Placebo		
		Events N (%)	**OR (95%CI) P-Value**	**Events N (%)**	**OR (95%CI) P-Value**	**P-value****
Hot flushes	Postmenopausal	1012 (55.8)	Reference	546 (29.9)	Reference	< 0.0001
	Premenopausal	715 (47.1)	0.71 (0.62 - 0.81) < 0.0001	356 (22.9)	0.70 (0.59 - 0.81) < 0.0001	< 0.0001
$\boldsymbol{P}_{interaction*}$					0.86	
Gynaecological symptoms	Postmenopausal	460 (25.4)	Reference	260 (14.2)	Reference	< 0.0001
	Premenopausal	490 (32.3)	1.41 (1.21 - 1.63) < 0.0001	270 (17.4)	1.27 (1.05 - 1.53) 0.01	< 0.0001
$\boldsymbol{P}_{interaction*}$					0.4	
Vaginal discharge	Postmenopausal	297 (16.4)	Reference	90 (4.9)	Reference	< 0.0001
	Premenopausal	215 (14.2)	0.84 (0.70 - 1.02) 0.08	70 (4.5)	0.91 (0.66 - 1.25) 0.56	< 0.0001
$\boldsymbol{P}_{interaction*}$					0.69	
Vaginal dryness	Postmenopausal	186 (10.3)	Reference	167 (9.2)	Reference	0.25
	Premenopausal	119 (7.8)	0.74 (0.58 - 0.95) 0.02	88 (5.7)	0.60 (0.45 - 0.78) 0.0001	0.02
$\boldsymbol{P}_{interaction*}$					0.23	
Irregular bleeding	Postmenopausal	52 (2.9)	Reference	39 (2.1)	Reference	0.15
	Premenopausal	266 (17.5)	7.20 (5.35 - 9.87) < 0.0001	161 (10.4)	5.29 (3.75 - 7.66) < 0.0001	< 0.0001
$\boldsymbol{P}_{interaction*}$					0.2	

* P-value for interaction between menopausal status and randomised treatment

** P-value for effect of randomised treatment in each menopausal group for each side effect

4.3.2 Analysis of IBIS-I risk factors for endocrine side effects in premenopausal women

4.3.2.1 Premenopausal women randomised to tamoxifen

For premenopausal women randomised to tamoxifen, risk factors which increased the risk of developing HFs were age 50 or above at randomisation, previously (but not currently) using HRT, obesity, and parity (Table 4.4). Odds of HFs increased in premenopausal women for every one year increase in age (OR/y = 1.06 (1.04 – 1.09); P $<$ 0.001), and per one unit increase in BMI (OR/(kg/m^2) = 1.02 (1.00 – 1.04); P = 0.02). Current HRT use, smoking status, being overweight (BMI between 25-30), and age at menarche were all statistically non-significant (Table 4.4). When adjusted for other variables the ORs were consistent with the univariate findings except for parity which was no longer statistically significant (Table 4.4).

Age of menarche greater than 14 was the only factor which increased the risk of developing gynaecological side effects in premenopausal women (38.0% vs 29.8%, OR = 1.44 (1.18-1.88); P = 0.002) (Table 4.4). As age of menarche was the only univariate significant variable no adjustment for other risk factors was performed.

4.3.2.2 Premenopausal women randomised to placebo

For premenopausal women randomised to placebo, odds of HFs increased for every one year increase in age (OR/y = 1.08 (1.05 – 1.11); P $<$ 0.0001). Women who were aged 50 or older at randomisation had an increased risk of developing HFs compared to those who were younger (37.1% vs 20.2%, OR = 2.33 (1.74 – 3.11); P $<$ 0.0001). Women who were former users of HRT, but not those who were current users, also had an increased risk of HF compared to those who had never used HRT (39.3% vs 21.5%, OR = 2.35 (1.48 – 3.71); P $<$ 0.0001). BMI as a continuous variable was weakly associated with a small increase in HF risk (OR/(kg/m^2) = 1.02 (1.00 – 1.04); P = 0.07). However, women who were overweight or obese were at increased risk of reporting HFs compared to those with a BMI of $<$25 (BMI 25-30: 25.7% vs 19.5%, OR = 1.42 (1.08 – 1.88); P = 0.01) and (BMI $>$30: 26.8% vs 19.5%, OR = 1.51 (1.11 – 2.04); P = 0.01) (Table 4.5). As age at first birth increased a decrease in the risk of HFs was observed (OR/y = 0.97

(0.95 – 0.99) ; P = 0.02). In a multivariate analysis in women randomised to placebo, age, former HRT use, a BMI of 25-30 or >30, all significantly increased the incidence of HFs (Table 4.5). After adjustment for other statistically significant factors, age at first birth reduced the risk of HF in premenopausal women randomised to placebo (OR/y = 0.97 (0.95 – 1.00); P = 0.04) (Table 4.5).

Higher age at randomisation increased the risk of gynaecological symptoms in premenopausal women randomised to placebo per year increase (OR/y = 1.11 (1.07 – 1.14); P < 0.0001). Additionally, when age was considered as a categorical variable, women who were aged 50 or over were at higher risk of gynaecological symptoms (28.6% vs 15.3%, OR = 1.55 (1.18 – 2.04); P = 0.002) compared to those aged under 50 (Table 4.5). After adjustment for other significant variables, both age (OR/y = 1.04 (1.02 - 1.07); P = 0.002) and age at menarche (OR/y = 1.06 (1.00 – 1.13); P = 0.05) remained statistically significant risk factors for gynaecological symptoms (Table 4.5).

Table 4.4: Odds ratios and 95% confidence intervals for hot flushes and gynaecological symptoms for premenopausal women in IBIS-I randomised to tamoxifen. Multivariate models include adjustment for other univariately significant risk factors

		Hot flushes				
		Events N (%)	**Premenopausal univariate**		**Premenopausal multivariate**	
		Total = 715	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Age (years)	Continuous (Per year)	715 (47.3)	1.06 (1.04 - 1.09)	< 0.0001	1.05 (1.03 - 1.08)	< 0.001
	<50	562 (45.0)	Reference		Reference	
	⩾50	153 (58.0)	1.68 (1.29 - 2.21)	0.0001	-	
HRT	Never	585 (45.2)	Reference		Reference	
	Current	66 (52.8)	1.36 (0.94 - 1.96)	0.10	1.07 (0.72 - 1.58)	0.75
	Ex-User	63 (68.5)	2.63 (1.69 - 4.20)	< 0.0001	2.22 (1.40 - 3.59)	<0.0001
BMI (kg/m^2)	Continuous (Per kg/m^2)	694 (47.6)	1.02 (1.00 - 1.04)	0.02	1.02 (1.00 - 1.04)	0.03
	<25	295 (44.8)	Reference		Reference	
	25-30	222 (48.1)	1.14 (0.90 - 1.45)	0.28	-	
	>30	177 (52.4)	1.36 (1.04 - 1.77)	0.02	-	
Smoking status	Never	346 (45.5)	Reference		Reference	
	Ex-Smoker	208 (47.0)	1.06 (0.84 - 1.34)	0.62	-	
	Current	160 (51.9)	1.29 (0.99 - 1.69)	0.06	-	
Parity	Nulliparous	75 (38.9)	Reference		Reference	
	Parous	640 (48.5)	1.47 (1.09 - 2.01)	0.01	1.36 (0.99 - 1.88)	0.06
Age at first birth (years)	Continuous (Per year)	640 (48.5)	0.98 (0.96 - 1.00)	0.10	-	
Age at menarche (years)	Continuous (Per year)	713 (47.2)	0.98 (0.94 - 1.06)	0.92	-	
	<14	479 (46.4)	Reference		Reference	
	⩾14	234 (48.9)	1.10 (0.89 - 1.37)	0.38	-	

Continued on next page

Table 4.4 – continued from previous page

		Gynaecological symptoms*				
		Events N (%)	**Premenopausal univariate**		**Premenopausal multivariate**	
		Total = 490	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Age (years)	Continuous (Per year)	490 (32.4)	1.00 (0.98 - 1.02)	0.98	-	
	<50	409 (32.8)	Reference		Reference	
	⩾50	81 (30.7)	0.91 (0.68 - 1.20)	0.50	-	
HRT	Never	423 (32.7)	Reference		Reference	
	Current	34 (27.2)	0.77 (0.50 - 1.15)	0.21	-	
	Ex-User	33 (35.9)	1.15 (0.73 - 1.78)	0.54	-	
BMI (kg/m^2)	Continuous (Per kg/m^2)	481 (33)	0.98 (0.96 - 1.00)	0.08	-	
	<25	221 (33.5)	Reference		Reference	
	25-30	160 (34.7)	1.05 (0.82 - 1.34)	0.74	-	
	>30	100 (29.7)	0.84 (0.63 - 1.11)	0.22	-	
Smoking status	Never	244 (32.1)	Reference		Reference	
	Ex-Smoker	146 (33.0)	1.04 (0.81 - 1.35)	0.68	-	
	Current	100 (32.5)	1.02 (0.76 - 1.35)	0.91	-	
Parity	Nulliparous	59 (30.6)	Reference		Reference	
	Parous	431 (32.7)	1.10 (0.80 - 1.54)	0.55	-	
Age at first birth (years)	Continuous (Per year)	431 (32.7)	1.00 (0.98 - 1.03)	0.68	-	
Age at menarche (years)	Continuous (Per year)	489 (32.4)	1.05 (0.99 - 1.12)	0.14	-	
	<14	307 (29.8)	Reference		Reference	
	⩾14	182 (38.0)	1.44 (1.15 - 1.81)	0.002	1.44 (1.15 - 1.81)	0.002

* Gynaecological symptoms were vaginal discharge, vaginal dryness or irregular bleeding.

Table 4.5: Odds ratios and 95% confidence intervals for hot flushes and gynaecological symptoms for premenopausal women in IBIS-I randomised to placebo. Multivariate models include adjustment for other univariately significant risk factors

		Hot flushes				
		Events N (%)	**Premenopausal univariate**		**Premenopausal multivariate**	
		Total = 356	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Age (years)	Continuous (Per year)	356 (22.9)	1.08 (1.05 - 1.11)	< 0.0001	1.08 (1.04 - 1.11)	< 0.0001
	<50	264 (20.2)	Reference		Reference	
	⩾50	92 (37.1)	2.33 (1.74 - 3.11)	< 0.0001	-	
HRT	Never	286 (21.5)	Reference		Reference	
	Current	37 (26.2)	1.30 (0.86 - 1.91)	0.2	0.98 (0.64 - 1.47)	0.91
	Ex-User	33 (39.3)	2.35 (1.48 - 3.71)	< 0.0001	2.01 (1.25 - 3.21)	0.003
BMI (kg/m^2)	Continuous (Per kg/m^2)	350 (23.2)	1.02 (1.00 - 1.04)	0.07	-	
	<25	133 (19.5)	Reference		Reference	
	25-30	124 (25.7)	1.42 (1.08 - 1.88)	0.01	1.37 (1.04 - 1.82)	0.03
	>30	93 (26.8)	1.51 (1.11 - 2.04)	0.01	1.50 (1.10 - 2.04)	0.01
Smoking status	Never	183 (22.2)	Reference		Reference	
	Ex-Smoker	99 (22.5)	1.02 (0.77 -1.34)	0.91	-	
	Current	74 (25.5)	1.20 (0.88 - 1.63)	0.25	-	
Parity	Nulliparous	42 (19.8)	Reference		Reference	
	Parous	314 (23.4)	1.24 (0.87 - 1.79)	0.25	-	
Age at first birth (years)	Continuous (Per year)	314 (23.4)	0.97 (0.95 - 0.99)	0.02	0.97 (0.95 - 1.00)	0.04
Age at menarche (years)	Continuous (Per year)	356 (23)	1.06 (0.99 - 1.13)	0.13	-	
	<14	233 (21.6)	Reference		Reference	
	⩾14	123 (26)	1.27 (0.99 - 1.64)	0.06	-	

Continued on next page

Table 4.5 – continued from previous page

		Gynaecological symptoms*				
		Events N (%) Total = 270	**Premenopausal univariate**		**Premenopausal multivariate**	
			OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
Age (years)	Continuous (Per year)	270 (17.4)	1.11 (1.07 - 1.14)	< 0.0001	1.11 (1.07 - 1.15)	< 0.0001
	<50	199 (15.3)	Reference		Reference	
	⩾50	71 (28.6)	2.23 (1.62 - 3.04)	< 0.0001	-	
HRT	Never	217 (16.4)	Reference		Reference	
	Current	31 (22.0)	1.44 (0.92 - 2.18)	0.09	1.02 (0.64 - 1.57)	0.94
	Ex-User	22 (26.2)	1.81 (1.07 - 2.97)	0.02	1.44 (0.84 - 2.38)	0.17
BMI (kg/m^2)	Continuous (Per kg/m^2)	265 (17.6)	0.99 (0.97 - 1.02)	0.57	-	
	<25	109 (16.0)	Reference		Reference	
	25-30	98 (20.3)	1.34 (0.99 - 1.810	0.06	-	
	>30	58 (16.7)	1.05 (0.74 - 1.48)	0.78	-	
Smoking status	Never	135 (16.4)	Reference		Reference	
	Ex-Smoker	86 (19.5)	1.24 (0.92 - 1.67)	0.16	-	
	Current	49 (17.0)	1.05 (0.73 - 1.49)	0.80	-	
Parity	Nulliparous	23 (10.8)	Reference		Reference	
	Parous	247 (18.4)	1.86 (1.20 - 3.00)	0.008	1.90 (1.23 - 3.09)	0.006
Age at first birth (years)	Continuous (Per year)	247 (18.4)	1.00 (0.98 - 1.030	0.81	-	
Age at menarche (years)	Continuous (Per year)	269 (17.4)	1.05 (0.98 - 1.14)	0.20	-	
	<14	179 (16.6)	Reference		Reference	
	⩾14	90 (19.0)	1.11 (0.89 - 1.55)	0.25	-	

* Gynaecological symptoms were vaginal discharge, vaginal dryness or irregular bleeding.

4.3.3 Analysis of IBIS-I risk factors for endocrine side effects in postmenopausal women

4.3.3.1 Postmenopausal women randomised to tamoxifen

In postmenopausal women randomised to tamoxifen, current use of HRT (60.9% vs 46.8%, OR = 1.77 (1.43 – 2.19); P < 0.0001) and former use of HRT (61.3% vs 46.8%, OR = 1.80 (1.41 - 2.30); P < 0.0001) increased the incidence of HFs. Women who had a hysterectomy also had a higher risk of HFs (60.2% vs 51.4%, OR = 1.43 (1.20 – 1.73); P = 0.0001) as were those who had a hysterectomy and at least one ovary removed (61.8% vs 53.8%, OR = 1.38 (1.12 – 1.73); P = 0.003) (Table 4.6). Both age per one year increase (OR/y = 0.97 (0.96 – 0.99); P = 0.0001) and time since menopause per one year increase (OR/y = 0.95 (0.92 – 0.99); P = 0.004) were associated with a lower HF risk (Table 4.6). Age at first birth also decreased the HF risk in postmenopausal women randomised to tamoxifen (OR/y = 0.97 (0.95 – 0.99); P = 0.01) (Table 4.6).

After adjustment for HRT use, age at first birth, time since menopause and hysterectomy with and without oophorectomy, increased age was no longer associated with a statistically significantly reduction the risk of HFs in postmenopausal women randomised to tamoxifen (OR = 0.98 (0.96 – 1.03); P = 0.64) (Table 4.6). In multivariate analysis, previous use of HRT remained statistically significant and increased HF risk (OR = 1.54 (1.07 – 2.23); P = 0.02); however, current HRT use was no longer statistically significant nor was age at first birth (Table 4.6). Women who were longer term postmenopausal developed significantly fewer HFs than those who were more recently postmenopausal (OR/y = 0.95 (0.92 - 0.99); P = 0.006) (Table 4.6). Women who had a hysterectomy without oophorectomy were remained at higher risk of HFs than those who had not had hysterectomy (OR = 1.50 (1.00 - 2.27); P = 0.05), but women who had had hysterectomy and oophorectomy were no longer at significantly higher risk (Table 4.6). No statistically significant interactions between risk factors were observed in postmenopausal women randomised to tamoxifen.

BMI was the only risk factor associated with gynaecological symptoms (OR = 0.97 (0.94 – 0.99); P = 0.01). When BMI was investigated as a categorical variable the reduction in risk was only for women who had a BMI over 30, (OR = 0.68 (0.51 - 0.91); P = 0.01) compared to those who had a BMI of < 25 (Table 4.6). The

reduced risk associated with BMI remained after adjustment as no other risk factors were included in the multivariate model (Table 4.6). These results were in contrast to those observed in premenopausal women for whom BMI was not a risk factor for gynaecological symptoms.

4.3.3.2 Postmenopausal women randomised to placebo

In comparision, postmenopausal women who were randomised to placebo, age was associated with reduced risk of HFs (OR/y = 0.97 (0.95 – 0.98); P < 0.0001). Current users of HRT also had reduced risk of HFs (23.4% vs 32.7%, OR = 0.63 (0.50 - 0.79); P < 0.0001) but no reduction was observed for former users of HRT (37.6% vs 32.7%, OR = 1.24 (0.96 – 1.61); P = 0.10) (Table 4.7). A decreased risk of HFs was also observed for women whose age of menarche was 14 or older (26.7% vs 31.7%, OR = 0.79 (0.63 – 0.97); P = 0.03), for longer time between menarche and menopause (OR/y = 1.01 (1.00 – 1.02); P = 0.02), and for increasing time since menopause (OR/y = 0.94 (0.92 – 0.97); P < 0.0001). An increased risk of HFs was observed for increasing BMI (OR/(kg/m^2) = 1.02 (1.00 – 1.04); P = 0.05) (Table 4.7).

After adjustment for age, age at menarche, time between menarche and menopause and time since menopause, women who were HRT users remained at lower risk of HFs (OR = 0.67 (0.47 – 0.96); P = 0.03) (Table 4.7). However, after adjustment, former users of HRT were at higher risk of HFs compared to those who had never used HRT (OR = 1.47 (1.02- 2.12); P = 0.04) (Table 4.7). Age at randomisation, BMI, age at menarche, time between menarche and menopause, and time since menopause were not associated with HF risk after adjustment for other risk factors (Table 4.7).

Only time since menopause was significantly associated with gynaecological symptoms (OR/y = 0.96 (0.93 – 1.00); P = 0.04) (Table 4.7). When time since menopause was considered as a categorical variable, women who were 5-10 years postmenopausal reported no significant change in risk of gynaecological symptoms compared to those less than five years postmenopausal (5-10 year: 16.6% vs 20.1%, OR = 0.79 (0.53 – 1.17); P = 0.24). However, those who were postmenopausal for greater than 10 years had reduced risk of gynaecological symptoms compared to women who were postmenopausal for less than five years (>10 years: 11.1% vs 20.1%, OR = 0.49 (0.29 – 0.81); P = 0.006) (Table 4.7). These findings were again in contrast to the findings

in the tamoxifen group where no effect of time since menopause on gynaecological symptom risk was observed.

4.3.3.3 Interaction analysis of risk factors and allocated treatment

Interaction with allocated treatment are reported in Table 4.3. There was no evidence of an interaction between risk factors for HFs or gynaecological symptoms in premenopausal women randomised to tamoxifen or to placebo. However, an interaction between randomised treatment and age was observed. Premenopausal women who were taking tamoxifen had a statistically significant lower risk of gynaecological symptoms as age increased compared to those who were taking placebo (OR = 0.90 (0.87 – 0.94); P < 0.0001).

Whilst no interaction was observed between risk factors for either HFs or gynaecological symptoms in postmenopausal women randomised to tamoxifen or placebo, interactions between risk factors and randomised treatment were observed. Women randomised to tamoxifen who were current or former users of HRT were at significantly higher relative risk of HFs than women randomised to placebo. Women who were current users of HRT were at 2.8-fold higher odds of HFs compared to HRT users randomised to placebo (OR = 2.81 (2.05 – 3.86); P < 0.0001). Women who were formerly taking HRT had a lower but still statistically significant higher odds of HFs compared to former HRT users randomised to placebo (OR = 1.44 (1.01 – 2.06); P = 0.04). For women who had had hysterectomy those who were randomised to tamoxifen were also had a higher relative odds of HFs compared to women randomised to placebo (OR = 1.40 (1.15 – 1.72); P = 0.001).

Randomised treatment and BMI had a statistically significant interaction with a lower risk of gynaecological symptoms in women randomised to tamoxifen compared to women randomised to placebo (OR = 0.96 (0.93 – 0.99); P = 0.02).

Table 4.6: Odds ratios and 95% confidence intervals for hot flushes for postmenopausal women in IBIS-I randomised to tamoxifen with reported side effects. Multivariate models include adjustment for other univariately significant risk factors

		HFs		
		Events N (%) Total = 1012	**Univariate OR (95% CI)P-value**	**Multivariate OR (95% CI) P-value**
Age (years)	Continuous (Per year)	1012 (55.8)	0.97 (0.96 - 0.99) 0.0001	0.98 (0.96 - 1.03) 0.64
HRT	Never	310 (46.8)	Reference	Reference
	Current	430 (60.9)	1.77 (1.43 - 2.19) $<$ 0.0001	1.36 (0.96 - 1.93) 0.08
	Ex-User	272 (61.3)	1.80 (1.41 - 2.30) $<$ 0.0001	1.54(1.07 - 2.23) 0.02
BMI (kg/m^2)	Continuous (Per kg/m^2)	985 (55.9)	1.02 (1.00 - 1.03) 0.11	-
Smoking status	Never	478 (54.0)	Reference	Reference
	Ex-Smoker	357 (57.5)	1.15 (0.94 - 1.42) 0.17	-
	Current	175 (57.8)	1.17 (0.90 - 1.52) 0.25	-
Parity	Nulliparous	128 (55.7)	Reference	Reference
	Parous	884 (55.9)	1.01 (0.76 - 1.33) 0.95	-
Age at first birth (years)	Continuous (Per year)	884 (55.9)	0.97 (0.95 - 0.99) 0.01	0.98 (0.95 - 1.01) 0.19
Age at menarche (years)	Continuous (Per year)	1010 (55.9)	0.98 (0.93 - 1.04) 0.55	-
Age at menopause (years)	Continuous (Per year)	491 (53.8)	1.00 (0.97 - 1.03) 0.87	-
Menarche to menopause (years)	Continuous (Per year)	545 (53.6)	1.00 (1.00 - 1.01) 0.48	-
Time since menopause (years)	Continuous (Per year)	491 (53.8)	0.94 (0.92 - 0.97) $<$ 0.0001	0.95 (0.92 - 0.99) 0.006
	$<$ 5	226 (62.3)	Reference	Reference
	5-10	169 (53.7)	0.70 (0.52 - 0.95) 0.02	-
	$>$ 10	96 (41.0)	0.42 (0.30 - 0.59) $<$ 0.0001	-
Hysterectomy	No	452 (51.4)	Reference	Reference
	Yes	560 (60.2)	1.43 (1.20 - 1.73) 0.0001	1.50 (1.00 - 2.27) 0.05
Hysterectomy	No	723 (53.8)	Reference	Reference
with oophorectomy	Yes	283 (61.8)	1.38 (1.12 - 1.73) 0.003	1.19 (0.61 - 2.38) 0.62

Continued on next page

Table 4.6 – continued from previous page

		Gynaecological symptoms*		
		Events N (%) Total = 460	**Univariate OR (95% CI)P-value**	**Multivariate OR (95% CI)P-value**
Age (years)	Continuous (Per year)	460 (25.5)	1.00 (0.98 - 1.02) 0.91	-
HRT	Never	180 (27.2)	Reference	Reference
	Current	163 (23.1)	0.80 (0.63 - 1.03) 0.08	-
	Ex-User	117 (26.5)	0.96 (0.73 - 1.27) 0.8	-
BMI (kg/m^2)	Continuous (Per kg/m^2)	450 (25.6)	0.97 (0.95 - 0.99) 0.008	0.97 (0.94 - 0.99) 0.01
Smoking status	Never	227 (25.7)	Reference	Reference
	Ex-Smoker	166 (26.8)	1.06 (0.84 - 1.34) 0.62	-
	Current	67 (22.2)	0.83 (0.60 - 1.12) 0.23	-
Parity	Nulliparous	59 (25.9)	Reference	Reference
	Parous	401 (25.4)	0.98 (0.71 - 1.35) 0.88	-
Age at first birth (years)	Continuous (Per year)	401 (25.4)	0.98 (0.96 - 1.01) 0.14	-
Age at menarche (years)	Continuous (Per year)	458 (25.4)	0.98 (0.93 - 1.04) 0.57	-
Age at menopause (years)	Continuous (Per year)	254 (27.9)	0.99 (0.98 - 1.02) 0.68	-
Menarche to menopause (years)	Continuous (Per year)	254 (27.9)	1.01 (1.00 - 1.01) 0.19	-
Time since menopause (years)	Continuous (Per year)	254 (27.9)	1.00 (0.97 - 1.02) 0.83	-
	< 5	101 (27.9)	Reference	Reference
	5-10	88 (28.0)	1.01 (0.72 - 1.41) 0.97	-
	> 10	65 (27.8)	0.99 (0.69 - 1.43) 0.97	-
Hysterectomy	No	238 (27.1)	Reference	Reference
	Yes	222 (23.9)	0.90 (0.74 - 1.08) 0.25	-
Hysterectomy with oophorectomy	No	343 (25.5)	Reference	Reference
	Yes	116 (25.6)	0.97 (0.71 - 1.31) 0.85	-

* Gynaecological symptoms were vaginal discharge, vaginal dryness or irregular bleeding.

Table 4.7: Odds ratios and 95% confidence intervals for hot flushes for postmenopausal women in IBIS-I randomised to placebo with reported side effects. Multivariate models include adjustment for other univariately significant risk factors

		Hot flushes		
		Events N (%) Total = 546	**Univariate OR (95% CI) P-value**	**Multivariate OR (95% CI) P-value**
Age (years)	Continuous (Per year)	546 (30.0)	0.97 (0.95 - 0.98) < 0.0001	1.00 (0.92 - 1.10) 0.95
HRT	Never	227 (32.7)	Reference	Reference
	Current	173 (23.4)	0.63 (0.50 - 0.79) < 0.0001	0.67 (0.47 - 0.96) 0.03
	Ex-User	146 (37.6)	1.24 (0.96 - 1.61) 0.10	1.47 (1.02 - 2.12) 0.04
BMI (kg/m^2)	Continuous (Per kg/m^2)	531 (30.0)	1.02 (1.00 - 1.04) 0.05	1.00 (0.97 - 1.03) 0.89
Smoking status	Never	267 (28.6)	Reference	Reference
	Ex-Smoker	175 (29.9)	1.06 (0.85 - 1.34) 0.59	-
	Current	104 (34.1)	1.29 (0.98 - 1.70) 0.07	-
Parity	Nulliparous	59 (26.7)	Reference	Reference
	Parous	486 (30.4)	1.04 (0.84 - 1.34) 0.73	-
Age at first birth (years)	Continuous (Per year)	884 (55.9)	0.97 (0.95 - 0.99) 0.01	0.98 (0.95 - 1.01) 0.19
Age at menarche (years)	Continuous (Per year)	545 (29.9)	0.97 (0.93 - 1.01) 0.12	-
Age at menopause (years)	Continuous (Per year)	266 (30.4)	1.02 (0.99 - 1.05) 0.17	-
Menarche to menopause (years)	Continuous (Per year)	266 (30.4)	1.01 (1.00 - 1.02) 0.02	0.98 (0.90 - 1.06) 0.70
Time since menopause (years)	Continuous (Per year)	266 (30.4)	0.94 (0.92 - 0.97) < 0.0001	0.93 (0.85 - 1.02) 0.13
	<5	129 (37.1)	Reference	Reference
	5-10	89 (27.9)	0.66 (0.47 - 0.91) 0.01	-
	> 10	48 (23.1)	0.51 (0.34 - 0.75) 0.007	-
Hysterectomy	No	235 (27.3)	Reference	Reference
	Yes	311 (32.4)	1.02 (0.93 - 1.13) 0.58	-
Hysterectomy with oophorectomy	No	395 (29.7)	Reference	Reference
	Yes	150 (31.1)	1.07 (0.85 - 1.34) 0.56	-

Continued on next page

Table 4.7 – continued from previous page

		Gynaecological symptoms*		
		Events N (%) Total = 260	**Univariate OR (95% CI)P-value**	**Multivariate OR (95% CI) P-value**
Age (years)	Continuous (Per year)	260 (14.3)	0.98 (0.96 -1.00) 0.09	-
HRT	Never	95 (13.7)	Reference	Reference
	Current	107 (14.4)	1.07 (0.79 - 1.43) 0.67	-
	Ex-User	58 (14.9)	1.11 (0.78 - 1.58) 0.56	-
BMI (kg/m^2)	Continuous (Per kg/m^2)	254 (14.4)	1.01 (0.98 - 1.04) 0.43	-
Smoking status	Never	129 (13.8)	Reference	Reference
	Ex-Smoker	92 (15.7)	1.16 (0.87 - 1.55) 0.30	-
	Current	39 (12.8)	0.91 (0.62 - 1.33) 0.65	-
Parity	Nulliparous	34 (15.4)	Reference	Reference
	Parous	225 (14.1)	0.86 (0.68 - 1.10) 0.18	-
Age at first birth (years)	Continuous (Per year)	225 (14.1)	1.00 (0.98 - 1.03) 0.94	-
Age at menarche (years)	Continuous (Per year)	260 (14.3)	0.99 (0.94 - 1.04) 0.61	-
Age at menopause (years)	Continuous (Per year)	146 (16.7)	0.98 (0.94 - 1.01) 0.31	-
Menarche to menopause (years)	Continuous (Per year)	146 (16.7)	1.01 (1.00 - 1.02) 0.18	-
Time since menopause (years)	Continuous (Per year)	146 (16.7)	0.96 (0.93 - 1.00) 0.04	0.96 (0.93 - 1.00) 0.04
	<5	70 (20.1)	Reference	Reference
	5-10	53 (16.6)	0.79 (0.53 - 1.17) 0.24	-
	> 10	23 (11.1)	0.49 (0.29 - 0.81) 0.006	-
Hysterectomy	No	126 (14.6)	Reference	Reference
	Yes	133 (13.9)	0.85 (0.68 - 1.03) 0.16	-
Hysterectomy with oophorectomy	No	184 (13.8)	Reference	Reference
	Yes	75 (15.5)	1.14 (0.85 - 1.52) 0.37	-

* Gynaecological symptoms were vaginal discharge, vaginal dryness or irregular bleeding.

4.3.4 Analysis of endocrine side effect risk factors in the IBIS-II study

4.3.4.1 Arthralgia events in women randomised to anastrozole

Arthralgias were reported in large numbers during the first 6 months of the IBIS-II trial in both the anastrozole and placebo arms. In a univariate analysis of women randomised to anastrozole, high BMI (37.8% vs 27.6%, OR = 1.60 (1.24 – 2.07); P = 0.0003), and age of menarche greater than 14 increased risk of arthralgia (29.3% vs 34.7%, OR = 0.78 (0.63 – 0.96); P = 0.02) (Table 4.8). However, per one year increase in age at menarche was not a statistically significant risk factor (OR/y = 0.98 (0.93 – 1.01); P = 0.30). The risk of arthralgia is positively associated with increased BMI, when BMI was considered as a continuous variable (OR/(kg/m^2) = 1.03 (1.02 – 1.05); P = 0.0003); however, no other continuous variables (age, age at menopause, age at menarche, time since menopause and time between menarche and menopause) had a significant effect on arthralgia risk. Women who had used HRT before study entry had a 21% non-significant increase in arthralgia risk compared to those who'd never used HRT (34.8% vs 30.7%, OR = 1.21 (0.99 – 1.48); P = 0.06) (Table 4.8).

After multivariate analysis adjusted for age at menarche, BMI remained associated with increased risk of arthralgia (OR = 1.03 (1.01 – 1.05); P = 0.0005) (Table 4.8). However, after adjustment for BMI women with a higher age at menarche did not have a statistically significant reduction in arthralgia risks (OR = 0.83 (0.67 – 1.03); P = 0.10) (Table 4.8).

4.3.4.2 Arthralgia events in women randomised to placebo

For women randomised to placebo, arthralgia was reported by 26.6% of women. In a univariate analysis, women who had used HRT had an increased risk of reporting arthralgia at the 6-month follow-up compared to those who had never used HRT (30.7% vs 23.0%, OR = 1.48 (1.20 – 1.83); P = 0.0002) (Table 4.9). BMI as a continuous variable was not associated with risk of arthralgia; however, women whose BMI was >30 did had an increased risk of arthralgia (30.0% vs 24.3%, OR = 1.33 (1.02 – 1.74); P = 0.04) compared to those with a BMI of <25. Former smokers were also at increased risk of developing arthralgias compared to women who had never smoked (31.8% vs

25.3%, OR = 1.38 (1.10 – 1.73); P = 0.005). Women who were current smoker had a statistically non-significant decrease in risk of arthralgia (18.7% vs 25.3%, OR = 0.68 (0.45 – 1.00); P = 0.06). Compared to women who were uterine intact, women who had been hysterectomised were also at increased risk of arthralgia (29.5% vs 25.1%, OR = 1.24 (1.00 – 1.54); P = 0.05) (Table 4.9).

After adjustment for smoking history, BMI, and hysterectomy, women randomised to placebo, HRT use (OR = 1.41 (1.13-1.75); P = 0.002) and former smokers (OR = 1.39 (1.10 – 1.74); P = 0.005) increased the risk of arthralgia in the multivariate model. In the multivariate analysis, women who were current smokers had a statistically significant decrease in arthralgia risk (OR = 0.66 (0.44 – 0.98); P = 0.05) (Table 4.9). In the placebo group, obesity did not have a statistically significant effect on the incidence of arthralgia in women randomised to placebo (OR = 1.25 (0.95 – 1.64); P = 0.11). These results highlight the importance and influence of BMI as a risk factor within the anastrozole group (Table 4.9).

Table 4.8: Odds ratios and 95% confidence intervals for arthralgia in postmenopausal women in IBIS-II randomised to anastrozole with reported side effects in the first 6 months. Multivariate analysis includes all univariately significant risk factors

		Events N (%)	Univariate Analysis		Multivariate Analysis	
		Total = 581	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (years)	Continuous (Per year)	581 (32.7)	1.00 (0.98 - 1.02)	0.88	-	
HRT	Never	286 (30.7)	Reference		Reference	
	Ever	294 (34.8)	1.21 (0.99 - 1.48)	0.06	-	
BMI (kg/m^2)	Continuous (Per kg/m^2)	576 (33)	1.03 (1.02 - 1.05)	0.0003	1.03 (1.01 - 1.05)	0.0005
Smoking status	Never	324 (32.7)	Reference		Reference	
	Ex-smoker	187 (33.1)	1.02 (0.82 - 1.27)	0.86	-	
	Current	69 (32.5)	0.99 (0.72 - 1.36)	0.97	-	
Parity	Nulliparous	501 (33)	Reference		Reference	
	Parous	79 (31.2)	0.92 (0.69 -1.23)	0.59	-	
Age at first birth (years)	Continuous (Per year)	499 (33.1)	0.98 (0.96 - 1.00)	0.14	-	
Age at menarche (years)	Continuous (Per year)	580 (32.8)	0.98 (0.93 - 1.01)	0.30	-	
	$\leqslant 14$	403 (34.7)	Reference		Reference	
	> 14	177 (29.3)	0.78 (0.63 - 0.96)	0.02	0.83 (0.67 - 1.03)	0.10
Age at menopause (years)	Continuous (Per year)	566 (32.3)	1.00 (0.99 - 1.02)	0.89	-	
Menarche to menopause (years)	Continuous (Per year)	565 (32.5)	1.00 (0.99 - 1.02)	0.57	-	
Hysterectomy	No	371 (31.3)	Reference		Reference	
	Yes	209 (35.2)	1.19 (0.97 - 1.47)	0.10	-	
Hysterectomy and oophorectomy	No	514 (32.7)	Reference		Reference	
	Yes	66 (32.4)	0.99 (0.72 - 1.34)	0.93	-	
Time since menopause (years)	Continuous (Per year)	566 (32.3)	1.00 (0.99 - 1.01)	0.81	-	

Table 4.9: Odds ratios and 95% confidence intervals for arthralgia in postmenopausal women in IBIS-II randomised to placebo with reported side effects in the first 6 months. Multivariate analysis includes all univariately significant risk factors

		Events N (%)	Univariate Analysis		Multivariate Analysis	
		Total = 482	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (years)	Continuous (Per year)	482 (26.6)	1.01 (0.99 - 1.03)	0.37	-	
HRT	Never	219 (23.0)	Reference		Reference	
	Ever	263 (30.7)	1.48 (1.20 - 1.83)	0.0002	1.41 (1.13 - 1.75)	0.002
BMI (kg/m^2)	Continuous (Per kg/m^2)	471 (26.6)	1.00 (1.00 - 1.00)	0.87	-	
	< 25	130 (24.3)	Reference		Reference	
	25-30	172 (25.4)	1.06 (0.82 - 1.38)	0.66	0.99 (0.76 - 1.30)	0.97
	> 30	169 (30.0)	1.33 (1.02 - 1.74)	0.04	1.25 (0.95 - 1.64)	0.11
Smoking status	Never	269 (25.3)	Reference		Reference	
	Ex-smoker	176 (31.8)	1.38 (1.10 - 1.73)	0.005	1.39 (1.10 - 1.74)	0.005
	Current	34 (18.7)	0.68 (0.45 - 1.00)	0.06	0.66 (0.44 - 0.98)	0.05
Parity	Nulliparous	413 (26.8)	Reference		Reference	
	Parous	68 (26.0)	0.96 (0.71 - 1.28)	0.77	-	
Age at first birth (years)	Continuous (Per year)	409 (26.6)	1.00 (0.98 - 1.02)	0.86	-	
Age at menarche (years)	Continuous (Per year)	479 (26.7)	1.02 (0.98 - 1.06)	0.37	-	
Age at menopause (years)	Continuous (Per year)	478 (26.7)	1.00 (0.99 - 1.02)	0.79	-	
Menarche to menopause (years)	Continuous (Per year)	475 (26.8)	1.00 (0.99 - 1.01)	0.95	-	
Hysterectomy	No	301 (25.1)	Reference		Reference	
	Yes	181 (29.5)	1.24 (1.00 - 1.54)	0.05	1.17 (0.93 - 1.47)	0.17
Hysterectomy and oophorectomy	No	411 (26.0)	Reference		Reference	
	Yes	71 (31.1)	1.29 (0.95 - 1.74)	0.10	-	
Time since menopause (years)	Continuous (Per year)	478 (26.7)	1.00 (0.99 - 1.02)	0.64	-	
	< 5	107 (24.7)	Reference		Reference	
	5 - 10	145 (25.9)	1.06 (0.80 - 1.42)	0.67	-	
	> 10	226 (28.4)	1.21 (0.92 - 1.58)	0.17	-	

4.3.4.3 Hot flushes in women randomised to anastrozole

In addition to arthralgias, HFs were reported by large numbers of women during the first 6 months of trial in both the anastrozole and placebo arms. In women randomised to anastrozole other factors that increased the risk of HFs were: ever using HRT (48.7% vs 41.2%, OR = 1.36 (1.13 - 1.64); P = 0.001), obesity (48.3% vs 41.6%, OR = 1.31 (1.03-1.66); P = 0.03) and women who were hysterectomised (49.4% vs 42.4%, OR = 1.33 (1.09 – 1.62); P = 0.005) (Table 4.10). Higher age at randomisation (OR/y = 0.97 (0.95 – 0.98); P < 0.0001), had a significantly reduced HF risk as did longer time since menopause (OR/y = 0.99 (0.97 – 1.00); P = 0.02) (Table 4.10).

After adjustment for other risk factors, HRT (OR = 1.46 (1.19 - 1.78); P = 0.0002) and obesity (OR = 1.32 (1.03 - 1.68); P = 0.03) remained significant risk factors for increased risk of HFs (Table 4.10). Women who had hysterectomy were also at higher risk of HFs (OR = 1.43 (1.13 – 1.81); P = 0.003). Age at randomisation was similarly associated post adjustment with a statistically significant reduction in HFs (OR = 0.97 (0.95 – 0.99); P = 0.005) (Table 4.10), as was time since menopause as a continuous variable which had a statistically significant 2% reduction in HF risk (OR = 0.98 (0.96 – 1.00); P = 0.02) (Table 4.10).

4.3.4.4 Hot flushes in women randomised to placebo

Higher age at randomisation had a decreased risk of reporting HFs at 6-months follow-up (OR/y = 0.95 (0.94 – 0.97); P < 0.0001) (Table 4.11). Women who had been postmenopausal for longer had a 2% decreased risk of HFs for each year post menopause (OR/y = 0.98 (0.97 – 0.99); P = 0.004). However, women who were more than 10 years postmenopausal were at significantly lower risk compared to those who had been postmenopausal for less than 5 years (31.0% vs 40.0%, OR = 0.67 (0.53 – 0.86); P = 0.002) (Table 4.11). In contrast, women who had formerly used HRT were at higher risk of HFs than those who had never used HRT (41.8% vs 29.8%, OR = 1.70 (1.40 – 2.06); P < 0.0001) (Table 4.11). Women who had had a hysterectomy with (42.1% vs 34.5%, OR = 1.38 (1.04 – 1.83); P = 0.03) or without oophorectomy (40.9% vs 32.7%, OR = 1.42 (1.16 – 1.74); P = 0.0006) also had higher risk of hot flushes compared to women who were not hysterectomised (Table 4.11).

For women in the placebo group of IBIS-II, after adjustment for other risk factors, use of HRT (OR = 1.85 (1.50-2.28); P < 0.0001) increased the incidence of HFs. Women who had a hysterectomy without oophorectomy were also at higher risk of HFs (OR = 1.60 (1.21 – 2.12); P = 0.001), but women who had had a hysterectomy and oophorectomy were not at increased risk (OR = 0.77 (0.54 – 1.10); P = 0.15) (Table 4.11). Time since menopause was associated with a reduction in the reports of HFs in the placebo group (OR = 0.98 (0.96 – 1.00); P = 0.01) so to was higher age at randomisation (OR = 0.96 (0.94 – 0.98); P = 0.0004) (Table 4.11). Compared to those on anastrozole, risk factors were the same and had a similar increase or decrease in HF risk with the exception of BMI which was only a risk factor for women randomised to anastrozole.

Table 4.10: Odds ratios and 95% confidence intervals for hot flushes in postmenopausal women in IBIS-II randomised to anastrozole with reported side effects in the 6 months. Multivariate analysis includes all risk factors which were significant in the univariate analysis

		Events N (%)	Univariate Analysis		Multivariate Analysis	
		Total = 796	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (years)	Continuous (Per year)	796 (44.7)	0.97 (0.95 - 0.98)	< 0.0001	0.97 (0.95 - 0.99)	0.005
HRT	Never	384 (41.2)	Reference		Reference	
	Ever	411 (48.7)	1.36 (1.13 - 1.64)	0.001	1.46 (1.19 - 1.78)	0.0002
BMI (kg/m^2)	Continuous (Per kg/m^2)	785 (44.9)	1.01 (1.00 - 1.03)	0.12	-	
	< 25	228 (41.6)	Reference		Reference	
	25-30	293 (44.9)	1.14 (0.91 - 1.44)	0.26	1.21 (0.95 - 1.54)	0.11
	> 30	264 (48.3)	1.31 (1.03 - 1.66)	0.03	1.32 (1.03 - 1.68)	0.03
Smoking status	Never	447 (45.1)	Reference		Reference	
	Ex-smoker	255 (45.1)	1.00 (0.81 - 1.23)	0.98	-	
	Current	89 (42.0)	0.88 (0.65 - 1.19)	0.41	-	
Parity	Nulliparous	683 (44.9)	Reference		Reference	
	Parous	110 (43.5)	0.94 (0.72 - 1.23)	0.67	-	
Age at first birth (years)	Continuous (Per year)	679 (45.1)	0.98 (0.96 - 1.00)	0.06	-	
Age at menarche (years)	Continuous (Per year)	789 (44.7)	0.98 (0.94 - 1.01)	0.24	-	
Age at menopause (years)	Continuous (Per year)	781 (44.6)	0.99 (0.98 - 1.01)	0.50	-	
Menarche to menopause (years)	Continuous (Per year)	774 (44.5)	1.00 (0.99 - 1.01)	0.87	-	
Hysterectomy	No	502 (42.4)	Reference		Reference	
	Yes	293 (49.4)	1.33 (1.09 - 1.62)	0.005	1.43 (1.13 - 1.81)	0.003
Hysterectomy and oophorectomy	No	692 (44.0)	Reference		Reference	
	Yes	103 (50.5)	1.30 (0.97 - 1.74)	0.08	-	
Time since menopause (years)	Continuous (Per year)	781 (44.6)	0.99 (0.97 - 1.00)	0.02	0.98 (0.96 - 1.00)	0.02
	< 5	211 (47.8)	Reference		Reference	
	5 - 10	242 (45.5)	0.91 (0.71 - 1.17)	0.46	-	
	> 10	328 (42.1)	0.79 (0.63 - 1.00)	0.05	-	

Table 4.11: Odds ratios and 95% confidence intervals for hot flushes in postmenopausal women in IBIS-II randomised to placebo with reported side effects in the 6 months. Multivariate analysis includes all risk factors which were significant in the univariate analysis

		Events N (%)	Univariate Analysis		Multivariate Analysis	
		Total = 643	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (years)	Continuous (Per year)	643 (35.5)	0.95 (0.94 - 0.97)	< 0.0001	0.96 (0.94 - 0.98)	0.0004
HRT	Never	284 (29.8)	Reference		Reference	
	Ever	359 (41.8)	1.70 (1.40 - 2.06)	< 0.0001	1.85 (1.50 - 2.28)	< 0.0001
BMI (kg/m^2)	Continuous (Per kg/m^2)	629 (35.5)	1.00 (0.98 - 1.00)	0.57	-	
Smoking status	Never	360 (33.8)	Reference		Reference	
	Ex-smoker	208 (37.6)	1.18 (0.95 - 1.46)	0.13	-	
	Current	71 (39.0)	1.25 (0.90 - 1.730	0.17	-	
Parity	Nulliparous	547 (35.5)	Reference		Reference	
	Parous	92 (35.1)	0.98 (0.74 - 1.29)	0.90	-	
Age at first birth (years)	Continuous (Per year)	544 (35.4)	0.99 (0.97 - 1.01)	0.42		
Age at menarche (years)	Continuous (Per year)	634 (35.3)	0.97 (0.92 - 1.01)	0.29	-	
Age at menopause (years)	Continuous (Per year)	634 (35.4)	0.99 (0.98 - 1.01)	0.32	-	
Menarche to menopause (years)	Continuous (Per year)	625 (35.3)	1.00 (0.98 - 1.01)	0.55	-	
Hysterectomy	No	392 (32.7)	Reference		Reference	
	Yes	251 (40.9)	1.42 (1.16 - 1.74)	0.0006	1.60 (1.21 - 2.12)	0.001
Hysterectomy and oophorectomy	No	547 (34.5)	Reference		Reference	
	Yes	96 (42.1)	1.38 (1.04 - 1.83)	0.03	0.77 (0.54 - 1.10)	0.15
Time since menopause (years)	Continuous (Per year)	634 (35.4)	0.98 (0.97 - 0.99)	0.004	0.98 (0.96 - 1.00)	0.01
	< 5	173 (40.0)	Reference		Reference	
	5 - 10	214 (38.1)	0.93 (0.72 - 1.20)	0.56	-	
	> 10	247 (31)	0.67 (0.53 - 0.86)	0.002	-	

4.3.4.5 Gynaecological symptoms in women randomised to anastrozole

The final side effect to be reported in large numbers during the first 6 months of the IBIS-II trial was vaginal changes. Vaginal changes were also reported in the IBIS-I cohort. Vaginal changes were reported in both placebo and anastrozole arms but were significantly more common in the anastrozole arm (OR = 1.36 (1.09-1.70); P = 0.01).

In the anastrozole arm, age over 60 was a statistically significant factor decreasing the risk of vaginal changes (9.0% vs 12.9%, OR = 0.67 (0.49 - 0.90); P = 0.009) (Table 4.12). Also, age as a continuous variable showed a non-statistically significant reduction of 2% (OR/y = 0.98 (0.95 – 1.00); P = 0.06). Time since menopause as a continuous variable was also associated with a decrease in vaginal symptoms (OR/y = 0.98 (0.96 – 1.00); P = 0.02) (Table 4.12). In a univariate analysis no risk factors were associated with a statistically significant increase in vaginal changes (Table 4.12).

After adjustment neither age at randomisation (OR = 0.76 (0.53 – 1.09); P = 0.13) nor time since menopause (OR/y = 0.98 (0.96 – 1.00); P = 0.12) remained significant risk factors for decreasing the risk of vaginal changes (Table 4.12). Each of these risk factors, differed from the risk factors identified in the IBIS-I postmenopausal women analysis, where no effect of age or time since menopause was observed in univariate or multivariate analysis. Additionally, no effect of obesity was observed in this analysis, a risk factor previously associated with vaginal changes in the IBIS-I postmenopausal group.

4.3.4.6 Gynaecological symptoms in women randomised to placebo

In women randomised to placebo, in a univariate analysis only women who had previously used HRT had a statistically significant increase in risk of vaginal symptoms at the 6-month follow-up visit (10.4% vs 6.6%, OR = 1.63 (1.17 – 2.30); P = 0.004) (Table 4.13). No multivariate analysis was performed for women randomised to placebo as only one variable was significant in the univariate analysis.

Table 4.12: Odds ratios and 95% confidence intervals for gynaecological symptoms in postmenopausal women in IBIS-II randomised to anastrozole with reported side effects in the first 6 months. Multivariate analysis includes all risk factors which were significant in the univariate analysis

		Events N (%)	Univariate Analysis		Multivariate Analysis	
		Total = 197	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (years)	Continuous (Per year)	197 (11.1)	0.98 (0.95 - 1.00)	0.06	-	
	$\leqslant 60$	121 (12.9)	Reference		Reference	
	> 60	76 (9.0)	0.67 (0.49 - 0.90)	0.009	0.76 (0.53 - 1.09)	0.13
HRT	Never	98 (10.5)	Reference		Reference	
	Ever	99 (11.7)	1.13 (0.84 - 1.52)	0.41	-	
BMI (kg/m2)	Continuous (Per year)	195 (11.2)	0.98 (0.96 - 1.01)	0.26	-	
Smoking status	Never	103 (10.4)	Reference		Reference	
	Ex-smoker	71 (12.6)	1.24 (0.90 - 1.71)	0.19	-	
	Current	22 (10.4)	1.00 (0.60 - 1.60)	0.99	-	
Parity	Nulliparous	169 (11.1)	Reference		Reference	
	Parous	27 (10.7)	0.96 (0.61 - 1.44)	0.83	-	
Age at first birth (years)	Continuous (Per year)	168 (11.1)	0.99 (0.95 - 1.02)	0.40	-	
Age at menarche (years)	Continuous (Per year)	196 (11.1)	0.98 (0.91 - 1.03)	0.52	-	
Age at menopause (years)	Continuous (Per year)	195 (11.1)	1.01 (0.99 - 1.04)	0.23	-	
Menarche to menopause (years)	Continuous (Per year)	194 (11.1)	1.02 (0.99 - 1.04)	0.19	-	
Hysterectomy	No	138 (11.7)	Reference		Reference	
	Yes	59 (9.9)	0.84 (0.60 - 1.15)	0.28	-	
Hysterectomy and oophorectomy	No	174 (11.1)	Reference		Reference	
	Yes	23 (11.3)	1.02 (0.63 - 1.59)	0.93	-	
Time since menopause (years)	Continuous (Per year)	195 (11.1)	0.98 (0.96 - 1.00)	0.02	0.98 (0.96 - 1.00)	0.12
	< 5	54 (12.2)	Reference		Reference	
	5 - 10	66 (12.4)	1.02 (0.69 - 1.49)	0.94	-	
	> 10	75 (9.6)	0.76 (0.53 - 1.11)	0.15	-	

Table 4.13: Odds ratios and 95% confidence intervals for gynaecological symptoms in postmenopausal women in IBIS-II randomised to placebo with reported side effects in the first 6 months. Multivariate analysis includes all risk factors which were significant in the univariate analysis

		Events N (%)	Univariate Analysis		Multivariate Analysis	
		Total = 152	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (years)	Continuous (Per year)	152 (8.4)	0.99 (0.96 - 1.02)	0.48	-	
HRT	Never	63 (6.6)	Reference		Reference	
	Ever	89 (10.4)	1.63 (1.17 - 2.30)	0.004	1.63 (1.17 - 2.30)	0.004
BMI (kg/m^2)	Continuous (Per kg/m^2)	148 (8.3)	0.93 (0.96 - 1.00)	0.43	-	
Smoking status	Never	81 (7.6)	Reference		Reference	
	Ex-smoker	53 (9.6)	1.29 (0.89 - 1.85)	0.17	-	
	Current	17 (9.3)	1.25 (0.70 - 2.11)	0.42	-	
Parity	Nulliparous	131 (8.5)	Reference		Reference	
	Parous	20 (7.6)	0.89 (0.53 - 1.42)	0.64	-	
Age at first birth (years)	Continuous (Per year)	130 (8.5)	1.01 (0.98 - 1.04)	0.57	-	
Age at menarche (years)	Continuous (Per year)	150 (8.4)	0.93 (0.84 - 1.02)	0.15	-	
Age at menopause (years)	Continuous (Per year)	151 (8.4)	1.00 (0.97 - 1.02)	0.73	-	
Menarche to menopause (years)	Continuous (Per year)	149 (8.4)	1.00 (0.98 - 1.03)	0.86	-	
Hysterectomy	No	92 (7.7)	Reference		Reference	
	Yes	60 (9.8)	1.30 (0.92 - 1.82)	0.13	-	
Hysterectomy and oophorectomy	No	131 (8.3)	Reference		Reference	
	Yes	21 (9.2)	1.12 (0.68 - 1.79)	0.63	-	
Time since menopause (years)	Continuous (Per year)	151 (8.4)	1.00 (0.98 - 1.02)	0.81	-	
	< 5	37 (8.5)	Reference		Reference	
	5 - 10	56 (10.0)	1.18 (0.77 - 1.85)	0.44	-	
	> 10	58 (7.3)	0.84 (0.55 - 1.30)	0.43	-	

4.3.4.7 Interaction analysis between risk factor and allocated treatment

No interaction between risk factors and randomised treatment were observed in postmenopausal women who reported arthralgia and who were randomised to anastrozole or to placebo. However, a weak interaction between randomised treatment and smoking was observed. Postmenopausal women who were taking placebo had a non-statistically significant higher relative odds of arthralgia if they were former smokers compared to those who were taking anastrozole (OR = 1.15 (0.98 – 1.35); P = 0.09). A weak interaction between treatment group and HRT was also observed for arthralgia. Women who were former HRT users were at non-statistically significant higher relative risk of arthralgia compared to those who were randomised to anastrozole (OR = 1.23 (0.92 – 1.64); P = 0.17).

For postmenopausal women who reported HFs, an interaction between women with hysterectomy and time since menopause was observed. Women who had had a hysterectomy were at statistically significant higher relative risk of HFs as time since menopause increased compared to those without hysterectomy (OR = 1.03 (1.00 – 1.06); P = 0.03).

No interaction between risk factors were observed in postmenopausal women randomised to placebo; however, a statistically significant interaction between randomised treatment and BMI was observed. Postmenopausal women who were taking placebo had a statistically significant lower relative odds of HFs if they were obese, BMI >30, compared to obese women who were taking anastrozole (OR = 0.68 (0.48 – 0.95); P = 0.03). Women who were former HRT users and randomised to placebo were at non-statistically significant higher relative risk of HFs compared to former HRT users who were randomised to anastrozole (OR = 1.25 (0.95 – 1.64); P = 0.10).

No interaction was observed between risk factors for women randomised to either anastrozole or placebo and relative risk of gynaecological symptoms. Additionally, no interaction between risk factors and randomised treatment was observed.

4.4 Discussion

This analysis investigated the impact of risk factors on the incidence of several side effects associated with endocrine therapy. Risk factors for HFs in postmenopausal women were similar in IBIS-I and IBIS-II. However, there was a difference in risk factors for HF between pre and postmenopausal women in the IBIS-I trial.

Menopausal status and treatment allocation have been identified as an important effect modifiers of side effect risk. Therefore, this research separated women into distinct subgroups for analysis to identify more precisely the populations for whom the baseline factors particularly increased or decreased risks. Menopausal status presents some interesting results concerning significance as a risk factor for individual side effects. The analysis showed that being premenopausal significantly increased the risk of developing gynaecological side effects, in particular irregular bleeding. This is in contrast to HFs where being premenopausal showed a statistically significant decrease. It is widely considered that most side effects of endocrine therapy, such as HFs and vaginal dryness, are ascribed to the anti-oestrogenic properties of endocrine therapy (Mourits et al., 2001). However, given that our analysis points to menopausal status affecting HFs and gynaecological side effects in contrasting manners, the agonistic or antagonistic effects of tamoxifen or anastrozole on different body tissues may be dependent on the ambient oestrogen concentration i.e. the patient's menopausal status. Menopausal status also changes the risk of HFs, vaginal dryness and irregular bleeding in the placebo groups as it does in the endocrine therapy group, further supporting the hypothesis that sex hormone concentrations should be considered.

After stratifying the analyses by randomisation arm and menopausal status, the most common factors which alter the risk of side effects when taking endocrine therapy were age at randomisation, HRT use and BMI. Other factors which change the risk of some side effects include smoking status and time since menopause. All these factors are possibly linked with decreases in oestrogen concentrations around the time that endocrine therapy is started.

Particular focus should be shown to former HRT use which increases the risk of HFs, in pre and postmenopausal women taking tamoxifen, and increases the risk of arthralgia, HFs and vaginal changes in postmenopausal women taking anastrozole. In contrast,

in the placebo group, postmenopausal women taking HRT were at lower risk of HFs compared to those who had never taken HRT or who were previous users. These effects could be because a large proportion of ex-users of HRT have stopped taking HRT recently and therefore are a group for whom the decrease in oestrogen concentrations is particularly large.

HRT is often prescribed to prevent side effects of oestrogen deficiency; in particular the alleviation of vasomotor and bone problems both of which are common in postmenopausal women. The benefit of HRT for the reduction of vasomotor and bone fractures has been well supported by studies such as the Women's Health Initiative Trial (WHI). However, the effects of oestrogen only HRT on cardiovascular disease and breast cancer in the oestrogen and progesterone trial led to the early termination of the trial.

Nevertheless, studies noted that HRT is very effective for controlling vasomotor symptoms, vaginal dryness and sleep disturbance in postmenopausal women (Welton et al., 2008; Jehan et al., 2015). The WHI investigators also found that after one year, women randomised to oestrogen alone had fewer reports of joint pain compared to placebo, (76.3% vs 79.2%, respectively $P = 0.001$) and had significantly lower joint pain scores (1.16 ± 0.87 vs 1.22 ± 0.88, mean $\pm$ SD, $P < 0.001$, respectively). In contrast, the frequency of joint swelling was higher in the oestrogen alone group (42.1% vs 39.7%, $P = 0.02$) compared to the placebo group (Chlebowski et al., 2018). However, after three years there was no statistically significant difference between the two groups for joint swelling (Chlebowski et al., 2018). An Australian study of 2130 postmenopausal women observed a positive effect of HRT on musculoskeletal symptoms. At study entry 63% complained of joint ache and myalgia; however, after one year of treatment, the proportion of women experiencing arthralgia decreased to 57% in women allocated to oestrogen plus progestin but remained the same in the placebo group (Welton et al., 2008). In contrast, a Swedish population survey of women aged 53 and 54 showed no difference in the incidence of arthralgia in HRT users and non-users (Jansson et al., 2003). The majority of studies demonstrate that HRT alleviates arthralgia in postmenopausal women, but the size of this effect appears to be small. However, results of our study indicate that current use of HRT in postmenopausal women and formerly using HRT in postmenopausal women significantly increases the risk of developing HFs in pre and postmenopausal women and also increases the risk of arthralgia in

postmenopausal women.

Previous use of HRT, in particular oestrogen therapy, results in the altered production of several hepatic proteins including SHBG increasing in concentration compared to non-users (Ropponen et al., 2005; Maggio et al., 2008). The physiological changes as a result of increased SHBG concentrations and; therefore, lower free testosterone concentrations are unclear. Testosterone is responsible for the deposition and the maintenance of skeletal muscle and administration of testosterone to elderly men has shown to increase the rate of muscle protein synthesis and muscle strength (Urban et al., 1995). In women with polycystic ovary syndrome, the concentration of testosterone, and weaker androgens like DHEA-S, are negatively associated with body mass (Douchi et al., 1999). However, in premenopausal women testosterone is positively associated with fat mass (Notelovitz 2002; Keller et al., 2011). In postmenopausal women, the decline of testosterone may be one of the factors that contribute to the loss of muscle mass and strength with increasing age and increases in rate at the time of menopause (Turcato et al., 1997; Bjørnerem et al., 2004; Maltais et al., 2009). The loss of muscle mass could lead to a decrease in physical activity and increase the risk of falls and other disabilities. Additionally, the lower body energy requirements of reduced muscle mass could lead to an increased risk of obesity and other associated diseases (Gower and Nyman 2000).

Obesity was also a significant risk factor for arthralgias and HFs in women taking AIs and HFs in women taking tamoxifen. Obese postmenopausal women tend to have higher concentrations of circulating oestrogen than those who are not obese; this is largely due to the result of the aromatisation of androgens to oestrogen in adipose tissue (Zumoff 1982). Therefore, obese women will be particularly affected by a large drop in oestrogen concentrations after starting endocrine therapy. It has also been shown that obesity is a risk factor for arthralgia, independent of endocrine therapy (Reyes et al., 2016).

This analysis supports the increased risk of arthralgia and HFs in postmenopausal women taking anastrozole and HFs in premenopausal women taking tamoxifen, with significantly more women reporting these symptoms as BMI increases.

However, prior evidence regarding the impact of BMI on HFs is conflicting. Some studies suggest that BMI and HFs have a positive association (Freeman et al., 2001; Miller

et al., 2006). However, other studies find no such association (Maura K. Whiteman et al., 2003; Gallicchio et al., 2005). In our study, BMI showed significant positive associations with HFs and arthralgia in postmenopausal women taking AI, but the impact of BMI was particularly strong in premenopausal women.

BMI as a risk factor for breast cancer is interesting because age affects its association with the disease. Greater BMI is associated with an increased risk of breast cancer for postmenopausal women (Bandera et al., 2013; Robinson et al., 2014; Shawon et al., 2017). However, for premenopausal women , greater BMI is associated with decreased breast cancer risk (Bandera et al., 2013; Robinson et al., 2014; Shawon et al., 2017).

BMI is an important risk factor because it is potentially modifiable. However, as greater BMI appears to be protective in premenopausal women, yet has the opposite effect in postmenopausal women, its consequences need to be quantified. Potischman et al. (1996) described a scenario in which obesity in young women reduces exposure to endogenous oestrogens, reducing the risk of breast cancer (Potischman et al., 1996). Potischman et al. (1996) found that while total oestradiol levels increased in postmenopausal women as BMI increased, the opposite was true of premenopausal women (Potischman et al., 1996). Reduced exposure to endogenous oestrogens would explain the increased risk of obese premenopausal women to HFs and why this effect is not seen in postmenopausal women. But further studies need to be performed to fully understand the role of BMI and age on HFs and other side effects.

Interestingly, two studies, one prospective study and one case-control study, found that the increased risk associated with higher BMI increases with time after menopause, but that the association is not evident until 10 years post menopause (MacInnis et al., 2004; John et al., 2013). In this analysis time since menopause and BMI are not significant risk factors for the same side effects in the postmenopausal group; however further stratification of postmenopausal women may show that as time since menopause increases the risk of side effects also increases at higher BMI.

Questions arise in the literature whether it is general body weight, in our case assessed by BMI, or fat distribution that is related to HFs. Recent studies have evaluated the percentages of visceral fat and total abdominal fat. The volume of total abdominal fat was significantly associated with HFs. HFs increased by 30% with every 151 cm^2 of subcutaneous fat. However, visceral fat was not significantly associated with hot

flushes (Thurston et al., 2008). In a separate study, postmenopausal women aged 50 to 79 years, participating in the Women's Health Initiative (WHI) trial, reported that a group undergoing dietary intervention to lose weight showed considerable improvement in vasomotor symptoms compared with a control group (OR = 1.14, 95%CI (1.01-1.28)). A loss of about 10% of their initial weight had a significant decrease in vasomotor symptoms while women who lost 10 kg or more showed complete improvement in moderate to severe vasomotor symptoms (Kroenke et al., 2012). In contrast to these findings, the French E3N cohort study found that women who have natural menopause and who are very thin (BMI $<$ 18.5) or very obese (BMI $>$ 30) had a higher risk of developing menopausal symptoms than those with a more normal BMI; relative risk = 0.87, RR = 0.84 respectively (Sabia et al., 2008). Therefore, the association between BMI and vasomotor symptoms has been highly controversial. Two conflicting hypotheses exist to explain the mechanism behind the association of BMI and HFs. The thermoregulatory model proposes that BMI increases the incidence of HFs and other vasomotor symptoms as increased body fat acts as an insulator making heat dissipation more difficult. The counter-argument to this is the hypothesis, which suggests that as BMI increases postmenopausal women have fewer HFs due to an increase in the concentration of circulating oestradiol as a result of increased conversion of androgens to oestrogens which takes place in body fat. The latter hypothesis is supported by observations that fat tissues produce oestrogens and that the most effective treatment for HFs and vasomotor symptoms is HRT (Freedman 2014).

In likelihood, the initiation of HFs and other side effects is as a result of a milieu of factors. Several hypothesised biological mechanisms have been postulated and investigated as to how these baseline factors might influence side effect risk. The most commonly proposed mechanism is a reduction in endogenous sex hormones (Friedenreich 2001). The role of oestrogens, and possibly androgens, are important in the aetiology of side effects. Research showed that reduced oestrogen concentrations induce and promote HFs and that the risk of developing HFs is increased among women with early menarche or late menopause (Gallicchio et al., 2015; Arizanović et al., 2018). The impact of menopause on concentrations of circulating hormones has been well established but other factors can also impact the concentrations of these hormones and have a significant impact on the incidence of side effects.

Low oestrogen concentrations and postmenopausal status are known to be associated

with reports of arthralgia; however, tamoxifen appears to have little impact on the incidence of arthralgia. This is not the case with AIs which, alongside obesity, are the major risk factors for increased risk of arthralgia in postmenopausal women. A decrease in oestrogen is the most likely underlying mechanism for joint symptoms. This hypothesis is supported by the observations that arthralgia is commonly reported among peri and postmenopausal women in the general population (Mishra and Kuh 2006). Reduction of oestradiol concentrations as a result of reduced production by aromatase inhibition has also been shown to increase the incidence of musculoskeletal events compared to placebo and tamoxifen (Cella et al., 2006; Coombes et al., 2007; Regan et al., 2011; Cuzick et al., 2015). The development of arthralgia after chemotherapy, which also causes hormone suppression, has also been well documented (Crew et al., 2007; Presant et al., 2007).

Gynaecological side effects related to endocrine therapy indicate the complex mechanism of action with both oestrogen agonist and antagonist effects produced in a variety of different tissues. As established, without oestrogen normal vaginal conditions may change. Oestrogen is vital for maintaining the vaginal epithelium and underlying tissues, without it the epithelium can thin resulting in dryness, discomfort and possible bleeding (Krause et al., 2010).

The agonist or antagonist effects of tamoxifen could depend on background oestradiol concentrations and hence menopausal status. Our results show that risk factors that increase oestrogen levels decrease the number of gynaecological side effects; therefore, it is likely that gynaecological side effects are a symptom of a reduced oestrogen environment.

Serum total oestradiol levels have been shown to decrease with increasing BMI in premenopausal women but to increase in postmenopausal women (Potischman et al., 1996; Schoemaker et al., 2014; Tin Tin et al., 2021). BMI has also been associated with an increase in breast cancer particularly in postmenopausal women (Carroll 1998; Davoodi et al., 2013). The reversal in this endogenous oestrogen level could explain the differing relation between obesity and side effects, particularly HFs before and after menopause. Studies in both pre and postmenopausal women have shown that increased concentration of testosterone and lower concentrations of SHBG are associated with obesity (Maggio et al., 2008; Cooper et al., 2015). SHBG is the major carrier of oestradiol and the concentration of free oestradiol is directly related to the concentration of SHBG. As

testosterone and oestradiol concentrations increase and SHBG concentrations decrease the level of free oestradiol and testosterone also increases (Gower and Nyman 2000; Cooper et al., 2015). Menopausal status is an additional influence on the effect of obesity on free oestradiol concentrations. Before menopause, the majority of oestrogen is produced in the ovaries as opposed to peripheral adipose tissues. Postmenopause, the production of oestrogen is mainly through the conversion of androgens in adipose tissue . There is also decreased oestrogen binding to SHBG due to the lower concentrations of SHBG in older women (Gower and Nyman 2000). These factors may contribute to a lower risk of HFs in postmenopausal women.

In postmenopausal women, this effect may be mediated through the inhibition of aromatase stopping the major source of oestrogens. As established in Chapter 1 section 1.3, without oestrogen normal vaginal conditions may change. Oestrogen is vital for maintaining the vaginal epithelium and underlying tissues, without it the epithelium can thin resulting in dryness, discomfort and possible bleeding (Krause et al., 2010).

Inhibiting the enzyme aromatase further reduces the concentration of oestrogen within the body. It seems unlikely that a system with significantly reduced oestrogen would be protective of gynaecological side effects given the importance of oestrogen in maintaining the vaginal epithelium. Tamoxifen has a tissue-dependent effect being an oestrogen antagonist in some tissues whilst being an oestrogen agonist in others. This situation may change depending on whether the woman is pre or postmenopausal as the concentration of endogenous oestrogen, androgens and progesterone may play an important role in deciding whether tamoxifen is an oestrogen agonist or antagonist (Mac Bride et al., 2010).

As with any study this analysis has strengths and weaknesses. Self-reported measures were used and only a limited number of measures were made. Prospective cohort studies are needed that have repeat assessments of anthropometric measures and associated factors over several periods as well as follow-up assessments of menopausal status. Such studies would permit an analysis of the temporal relations between breast cancer and side effect risk with the baseline measures and would reduce misclassification bias since menopausal status over time would be available.

On the issue of weight gain and fat distribution changes with menopause, there is a need to evaluate the effect of increased physical activity and fitness as a tool for the preven-

tion of changes in body composition associated with menopause and ageing in normal women. This shows a need to distinguish abdominal fat from the risk attributable to total fat gain associated with menopause.

4.5 Conclusions

The investigation of anthropometric measurements and modifiable risk factors of side effect and their associations with breast cancer risk has been actively researched and a clearer understanding of the aetiologic roles of weight, height, adiposity, fat distribution patterns, and weight change throughout a lifetime is emerging. However, gaps in the epidemiological evidence exist and further research is needed to clarify the biologic mechanisms that are operative.

The baseline factors investigated here are implicated in side effect aetiology. Several aspects of these associations and the underlying biologic mechanisms, however, require further clarification. Priorities for research should include better assessment of anthropometric characteristics. Aetiological studies of these factors and their influence on risk throughout life, with emphasis on population subgroups, such as women who are current or former users of HRT or those who have been postmenopausal for greater than 10 years, would be beneficial.

The use of HRT during the trial period of IBIS-I was in contrast to other large breast cancer prevention studies such as NSABP-P1 study and is particularly relevant as HRT is well known to increase the risk of breast cancer (Collaborative Group on Hormonal Factors in Breast Cancer, 2019). It is also interesting to consider whether the use of HRT in these studies confounds the identification of side effects, via increased oestrogen concentrations, or whether the use of HRT contributes to the aetiology of side effects as a result of large levels of disruption on oestrogen concentrations.

To investigate further the impact of HRT and other risk factors identified in this analysis, a trial primarily focussed on side effect outcomes should be performed. Such a trial should be a large prospective trial of women at high risk of breast cancer and women should be randomised to tamoxifen, anastrozole or placebo. The use of HRT on trial should be prohibited. Baseline characteristics should be measured including, age, BMI, previous use of HRT, type of HRT, length of previous HRT use, time since

HRT last taken and reproductive factors such as menopausal status, parity, age at first birth, age at menarche, age at menopause and time between menopause and randomisation. Women should be followed up every six months and questioned about the type, frequency (average number per week), and severity of predetermined side effects. Blood samples for measurement of circulating concentrations of oestradiol, testosterone and SHBG should be taken at each of the follow up points to determine the impact that endocrine therapy may be having on hormone concentrations and how hormone concentrations over time impact the number and frequency of reported side effects.

Finally, studies in the prevention setting that test the impact of genetic variants, endogenous sex hormones and weight control programs on side effect risk will provide a more definitive understanding of the underlying aetiology and biology for these associations. Chapters 5 discusses the impact of genetic factors on side effects reported during the first year of tamoxifen to determine whether single nucleotide polymorphisms in genes known to be involved in metabolic processes or receptor proteins can increase or decrease the risk of side effects. Additionally, chapter 6 investigates the role of two hormones, testosterone and dehydroepiandrosterone-sulphate, and sex hormone binding globulin and the role that these hormones play in side effect outcomes. Each of these chapters, aims to establish how each affect side effect incidence and explore these mechanisms to determine if we can predict a women's risk of side effect before starting endocrine therapy.

Chapter 5: The relationship between genetic factors and side effects in women randomised to tamoxifen

5.1 Introduction

The central aim of most human genomic studies is the identification of markers which aid the understanding of common diseases (Hunter and Drazen 2019). To this end the European Bioinformatics Institute have identified more than 70,000 associations between SNPs and disease traits (Buniello et al., 2019). Furthermore, without a strong family history of a disease, which suggests a single gene-based disorder, most diseases are complex and involve many variants of small effects contributing to a larger result.

While some genetic variants underlying rare conditions, such as Huntington's disease, show clear associations with the disease, those that might underlie common diseases have been more difficult to identify. The concept that a common disease has a more multi-factorial aetiology and complex relationship with genetic factors than a rare disease, coupled with the discovery of several susceptibility variants for common disease, has led to the development of the common disease / common variant hypothesis (Corder et al., 1993; Altshuler et al., 2000; Reich and Lander 2001).

The common disease / common variant hypothesis states that common diseases are likely influenced by common genetic variations, which raises several key implications for studying these diseases. (Hunter 2005; Bush and Moore 2012). Common diseases are typically caused by a multitude of factors such as genetic, environmental or lifestyle factors (Hunter 2005). Firstly, if single common genetic variants influence disease, the effect size for any one variant must be small relative to effect sizes found with dominant genes for rare disorders. This proposal must be true as if a SNP with high frequency in the population led directly to a disease phenotype, the majority of the population would have that phenotype. However, if the SNP caused a small alteration in disease risk, the disease and the allele would only be slightly correlated. As such, common

variants cannot have high penetrance (Bush and Moore 2012). Secondly, if common alleles have small genetic effects, but common disorders show heritability, then multiple common alleles will be needed to influence disease susceptibility. If a single SNP variant confers only a small increase in disease risk, that SNP only explains a small proportion of the total variance due to genetic factors. Therefore, the total genetic risk due to common genetic variation must be spread across multiple genetic factors (Bush and Moore 2012). These two points suggest the importance of moving towards population-based studies to be more successful in identifying panels of SNPs involved in complex diseases. Their frequency in the population and the importance of models containing multiple SNPs for better prediction of disease risk makes this a more challenging task.

Breast cancer risk assessment is often based on factors such as personal, medical and familial history, while some models also include exposure to endogenous hormones and benign breast disease. Advances in genetics and genomics has aided the of advancement and refinement of breast cancer risk assessment models. There are many different technologies, study designs and analytical tools for identifying genetic risk factors. The focus in this chapter will be on hypothesis driven Candidate Gene (CG) and hypothesis generating Genome-Wide Association (GWAS) analyses to determine the risk of developing side effects during the first six months of taking tamoxifen for breast cancer prevention.

CG studies focus on genes or genomic areas with a clearly defined hypothesis as to how this area may affect the incidence of a trait. By focussing on selected genes, it is possible to determine whether these genes are associated with the outcome of interest in the study population. GWAS are conducted without prior hypothesis and measure DNA sequence variations from across the human genome to identify genetic risk factors for diseases that are common in the population. The goal of GWAS is to use genetic risk factors to predict who is at risk of disease and to identify biological mechanisms of disease susceptibility which can be targets for new prevention and treatment strategies.

Breast cancer has many established risk factors, which may operate in unison or independently to increase an individual's chance of developing the disease. Genomic variants are integral for defining breast cancer risk with over 300 SNPs associated with breast cancer (Mavaddat et al., 2019). Individually, most SNPs have a small impact on the risk of developing breast cancer; however, a recent study suggested that combining SNPs may explain up to 18% of the risk (Michailidou et al., 2017). Three bands

of mutations have been established: high, moderate and low penetrance mutations (Mavaddat et al., 2010). Genomic studies have identified multiple susceptibility genes the most prominent being those that belong in the high penetrance category but with low frequency, such as *BRCA1* and *BRCA2*, contributing to up to 20% of hereditary breast cancer (Easton 1999). Moderate penetrance mutations, such as *ATM*, *CHEK2* and *PALB2*, tend to be protein shortening variants of DNA repair genes and have been shown to increase susceptibility to breast cancer (Easton et al., 2015). Nevertheless, the majority of the 300 SNPs associated with breast cancer are low penetrance mutations which individually only carry a small increase in risk (Mavaddat et al., 2019). However, given the prevalence of these mutations, they often occur in combination leading to a larger increase in risk.

Whilst multiple studies have investigated the associations between SNPs and breast cancer risk, little research has been undertaken to explore the relationship between SNPs and endocrine related side effects. Genes controlling biological processes, such as sex hormone or drug metabolism, and genes coding for hormone receptors, and which therefore may influence diseases outcomes and treatment success, have been proposed as possible key genes for side effect aetiology.

As described in section 1.2.1.1, tamoxifen is converted into more potent metabolites, such as N-desmethyl-tamoxifen and endoxifen, and other secondary metabolites, via two enzyme controlled pathways (Desta et al., 2004; Lyon et al., 2012; Sanchez Spitman et al., 2017). It has been hypothesised that SNPs in genes encoding these enzymes are linked to side effects. The focus of most studies has been on the role of SNPs in the major enzyme involved in tamoxifen conversion, Cytochrome P_{450} 2D6 (CYP2D6); these have produced inconsistent outcomes (Goetz et al., 2005, 2007; Sestak et al., 2012). Little work has focussed on SNPs in other key enzymes involved in any stage of tamoxifen metabolism such as CYP3A4, CYP3A5, or those involved in excretion, such as sulfotransferases or UDP-Glucuronosyltransferases.

In addition to altered tamoxifen metabolism, sex hormone concentrations are known to affect the incidence of hot flushes (HFs), gynaecological and musculoskeletal symptoms (Huang et al., 2010; Mac Bride et al., 2010; Emond et al., 2011). The majority of work on side effects has investigated lifestyle and reproductive factors and HRT and the incidence of side effects. However, sex hormones are synthesised in the body via conversion from other hormones and cholesterol. Whilst genomic analysis of *CYP19A1*

(aromatase) has been investigated, there has been little work investigating other enzymes in this complex pathway (Ingle et al., 2010; Hertz et al., 2017). It is possible that SNPs could alter the function of key enzymes in the hormone synthesis pathway thereby disrupting concentrations of important sex hormones such as testosterone and oestrogen. Therefore, the role of SNPs in enzymes, metabolising sex hormones and tamoxifen remain important areas of research which could lead to a better understanding of the aetiology of menopausal-like side effects.

It is important to examine whether SNPs within genes involved in tamoxifen and sex hormone metabolism alter gene function, and as a result increase or decrease the risk of side effects. The widespread availability of low-cost technology for measuring an individual's genetic architecture has made this possible on a much wider scale and has increased its usefulness. Clinical observations and epidemiological studies show that patients have markedly different responses to treatment, for both disease outcome and side effects associated with treatment. There is also variability in adverse events reported as a result of treatment. An example of this variability is the incidence of musculoskeletal events often experienced during AI therapy. While some women report no musculoskeletal symptoms others can become disabled from them. Musculoskeletal and vasomotor side effects are not by themselves life threatening; however, they may seriously disrupt a patient's treatment adherence. The variability in patient outcomes and adverse events could be associated with the variability identified with the metabolism and pharmacodynamic effect of AIs or SERMs (Ingle et al., 2010; Ingle 2013). Therefore, it is plausible that specific combinations of multiple SNPs might be associated with side effects, which, when inherited together, increase the risk of side effects in women receiving tamoxifen.

Combinations of low penetrance genes have been used to construct polygenic risk scores (PRS), which have been shown to be important predictors of breast cancer risk (Michailidou et al., 2017). Several studies have proposed SNP panels as an additional tool to predict breast cancer risk. Cuzick et al., (2017) investigated 88 SNPs in a nested case-control study of women enrolled in IBIS-I or the Royal Marsden Trial (Cuzick et al., 2017). In a separate study, Evans et al., (2017) used 18 SNPs coupled with known *BRCA1/2* mutations to assess whether the 18 SNPs provided further important information regarding risk of breast cancer (Evans et al., 2017). Opportunities remain for using PRS to predict side effects, as there has been little work performed on

the role of multiple gene variants and how they are associated with endocrine related symptoms. Determining whether SNPs can help to predict the risk of developing side effects would increase understanding and could help an individual make an informed decision regarding life-style changes or medication (Garcia-Closas et al., 2014).

The aim of this analysis is to investigate the relationship between SNPs and endocrine therapy related side effects using three separate analyses:

1. A CG study of known breast cancer risk SNPs from a panel of 88 SNPs previously defined by Cuzick et al., (2017) combined with genes known to be involved in sex hormone metabolism, drug metabolism pathways or genes coding for hormone receptors, which have been proposed as possible mechanisms for side effect aetiology (Cuzick et al., 2017).

2. A GWAS investigating the existence of other loci present in the OncoArray not previously associated with breast cancer prediction or hypothesised pathways to side effects. GWAS data will be explored for links to novel variants not previously part of the candidate gene study but which might help to explain the presence of side effects.

3. An investigation of multiple genetic variants through-out the genome which, in combination, may provide useful information when describing complex disease. As it is computationally intractable to analyse pairwise all SNPs genotyped, genes from the CG study will be used to select a group of SNPs which best predict side effect outcomes. These SNPs will subsequently be combined to form a PRS model for prediction side effect outcome.

What we already know

• SNPs have been identified with association to breast cancer risk

• Variability is reported in side effects observed between patients taking endocrine therapy for prevention of breast cancer

• Genetic components of side effects previously observed have not been identified

• Association between the variability observed in primary outcomes and side effects, and the variability in metabolism and pharmacodynamic effects of endocrine therapies is not known

---

What this analysis adds

• CG study to investigate the association of SNPs known to be involved in biological pathways previously suggested to be mechanisms for side effect incidence:

- Genes previously associated with predicting breast cancer risk
- Tamoxifen metabolism and excretion
- Sex Hormone metabolism
- Genes coding for receptors

• GWAS study to identify potential candidate SNPs associated with side effects for further research

• Multi-loci analysis to determine groups of SNPs associated with side effects

• Formation of a PRS from the multi-loci analysis to assess side effect risk

5.2 Methodology

5.2.1 Participants

Details of recruitment for women who were part of the IBIS-I trial have been detailed earlier in section 3.1 and in trial reports (Cuzick et al., 2002, 2007, 2015). Genotyping for IBIS-I samples was performed for all breast cancer cases and matched controls with available material (Cuzick et al., 2017). Genotypes and demographic information at study entry were available for 820 women from the IBIS-I trial. Only women from IBIS-I who were randomised to tamoxifen were included in the analysis (N = 310).

5.2.2 Genotyping assay methods

The analysis in this chapter used genotyped data previously collected for determining the impact of 88 SNPs on breast cancer risk where genotyping procedures have been previously described (Cuzick et al., 2017). Briefly, 10 mL of DNA extracted from IBIS-I blood samples were analysed in 96-well plates. Formalin-fixed paraffin-embedded block samples were analysed on a separate plate. For quality control, two internal controls with known genotypes were run on each plate. Assays were carried out blindly at Genome Quebec (Montreal, Canada), a clinical service provider–certified by Illumina; the Illumina OncoArray was used, and the Illumina HTS (high-throughput sequencing) protocol was rigorously followed (Cuzick et al., 2017). A total of 517,820 SNPs were genotyped and included at the start of the analysis.

5.2.3 Study design

The primary end point was a report of side effects during the first six months of IBIS-I by women taking tamoxifen. Side effects of interest were those considered to be linked to endocrine therapy: HFs and gynaecological symptoms (vaginal discharge, vaginal dryness and irregular bleeding). Women were selected as cases if they reported a HF or gynaecological symptom within the first six months of trial whilst controls were women who did not report the side effect during the same follow-up period. The study was designed to use all cases from IBIS-I with genotype information and follow-up information available.

Candidate genes were selected based on identification in comprehensive reviews as being involved in distinct biological processes, namely genes coding sex hormone or tamoxifen metabolism enzymes or genes coding for hormone receptors (Desta et al., 2004; Sanchez-Spitman et al., 2017; Schiffer et al., 2019). Table 5.1 summarises the candidate genes selected for analysis. Genes were grouped by function and may appear more than once if they belong to more than one functional pathway. These were added to the 88 SNPs identified by Cuzick et al. (2017) for CG analysis (Cuzick et al., 2017).

Table 5.1: Candidate genes selected for the candidate gene analysis. Genes are grouped by function and may appear more than once if they belong to more than one functional pathway

Tamoxifen metabolism genes					
CYP2D6	*CYP2C9*	*CYP3A4*	*CYP3A5*	*SULT1A1*	*SULT1A2*
SULT1E1	*UGT2B4*	*UGT2B7*	*UGT2B15*	*UGT2B17*	*CYP1A1*
CYP1A2	*CYP2A6*	*CYP2B6*	*CYP2C19*	*CYP2C8*	*CYP2E1*
ABCC2					
Sex hormone metabolism					
CYP21A2	*CYP17A1*	*CYP11A1*	*SULT2A1*	*SULT2B1*	*HSD3B1*
HSD3B2	*SRD5A1*	*SRD5A2*	*SRD5A3*	*CYP11B1*	*CYP11B2*
HSD11B1	*HSD11B2*	*UGT1A1*	*UGT2B4*	*AKR1C1*	*CYP19A1*
COMT	*HSD17B1*	*HSD17B2*	*HSD17B3*	*HSD17B4*	*HSD17B7*
HSD17B8	*HSD17B14*	*CYP1A1*	*CYP1B1*	*CYP3A4*	*UGT1A1*
UGT2B4	*SULT1A1*	*SULT131*	*SOD2*	*NAT1*	*NAT2*
GSTP1	*NQO1*				
Receptor genes					
ESR1		*ESR2*		*PGR*	

Following QC of the genotyped samples, gene locations were identified and extracted using the University of California Santa Cruz genome browser using genome build GRCh37/hg19 from February 2009 (Kent et al., 2002).

5.2.4 Analysis methods

5.2.4.1 Quality control

Prior to quality control (QC) and association analysis of genotype data, duplicated individuals were removed from the dataset (N = 14). Removal of duplicates was based on the missingness genotyping rate of the samples with the highest call rate sample retained in the dataset. Genotype data ordered by chromosome underwent per-individual and per-marker QC. The QC process is summarised in Figure 5.1.

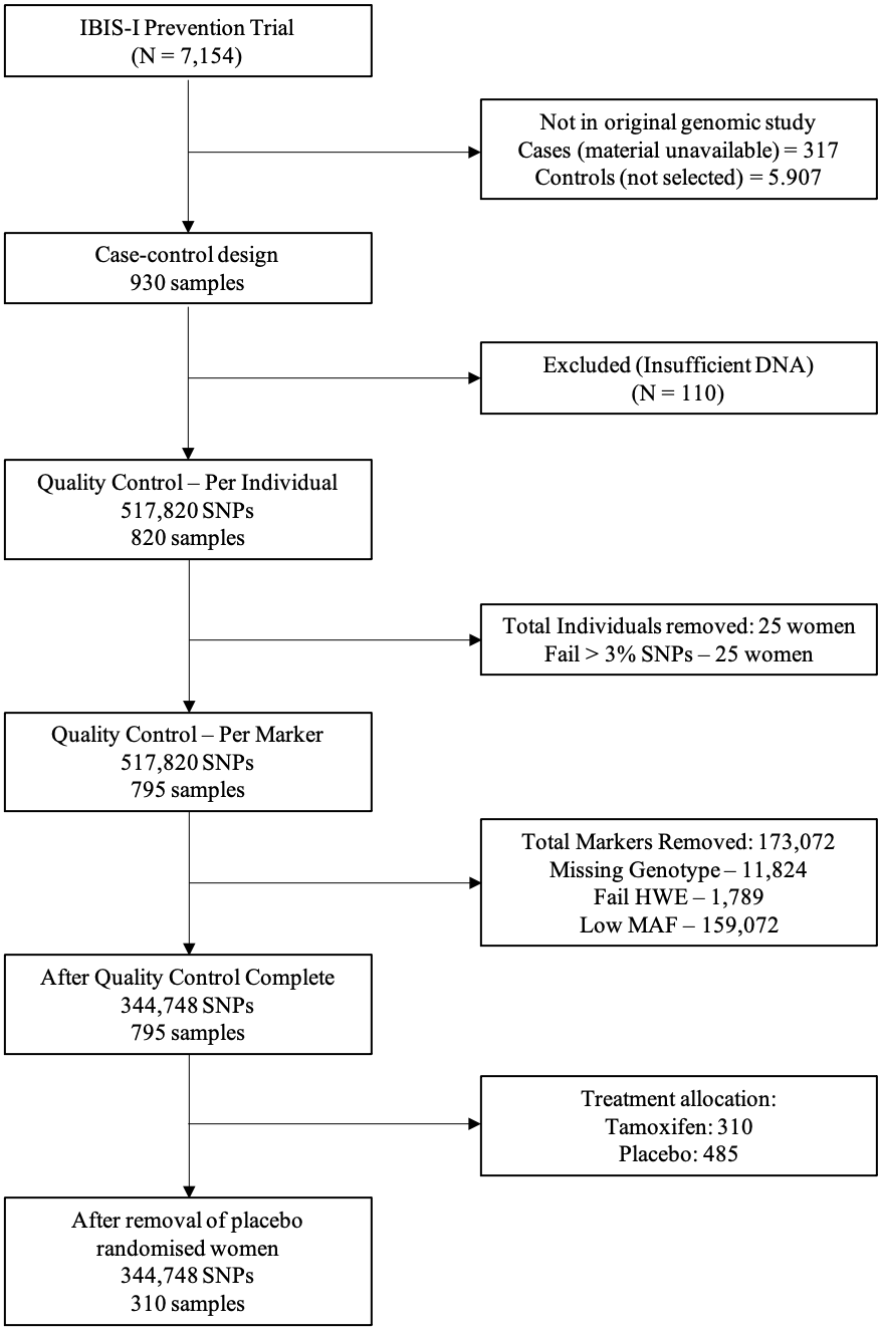


Figure 5.1: Quality control procedure prior to sample analysis

Per-individual QC included: (i) sex check: for the identification of individuals with discordant sex information; (ii) heterozygosity and missing check: for the identification of individuals with outlying missing genotype and/or heterozygosity rates. Sex check identified zero individuals with discordant sex information. 25 individuals were identified with missing genotype rates of more than 3% who were subsequently excluded from the study.

Per-marker QC was performed on 517,820 SNPs and consisted of: (i) check for missing

SNPs: for identifying markers with excessive missing genotype rates as a result of poor genotyping; (ii) check of Hardy-Weinberg equilibrium (HWE): for the identification of markers which show significant signs of deviation from HWE and (iii) check of minor allele frequency (MAF): for removal of marker with low MAF. Breakdown of marker removal by chromosome can be found in Table 5.2.

Table 5.2: Table of markers failing per marker quality control on each chromosome. HWE – Hardy-Weinberg Equilibrium, MAF – Minor Allele Frequency

Chromosome	**SNP total (Start)**	**Failed Markers**			**Remaining**
		Missing Genotype	**Fail HWE**	**Fail MAF**	**markers**
1	37,064	807	60	11,576	24,621
2	43,774	819	115	13,638	29,202
3	35,330	587	83	11,210	23,450
4	28,790	524	83	9,026	19,157
5	32,633	614	84	10,362	21,573
6	43,473	2,047	449	13,457	27,520
7	26,451	567	63	7,725	18,096
8	27,635	447	70	8,246	18,872
9	22,309	430	53	6,375	15,451
10	26,242	531	60	7,961	17,690
11	24,667	473	53	7,719	16,422
12	26,553	558	65	8,109	17,821
13	17,282	301	33	5,432	11,516
14	16,565	366	47	4,987	11,165
15	15,195	287	41	5,120	9,747
16	16,053	389	44	4,926	10,694
17	18,042	497	166	5,408	11,971
18	14,912	251	36	4,510	10,115
19	14,122	563	94	4,389	9,076
20	14,279	362	32	4,138	9,747
21	6,469	128	10	1,852	4,479
22	9,980	276	48	3,293	6,363
Total	**517,820**	**11,824**	**1,789**	**159,459**	**344,748**

A marker missingness rate of >3% were identified in 11,824 (2.3%) SNPs. This is important because serious errors in genotyping often yield extremely low p-values for heterozygosity testing. After investigation of the distribution of p-values of genotyping p-values a threshold of $P < 1\times10^{-5}$ was selected to avoid the removal of too many SNPs. 1,789 (0.3%) markers had strong deviation from HWE ($P < 1\times10^{-5}$), indicative of genotyping or genotype calling errors and were removed from the analysis. 159,459 (31.8%) markers failed to meet the 5% threshold for MAF testing and were removed.

Once per-individual and per-marker QC were performed, women who were randomised to placebo were also removed from the analysis. This left 310 individuals and 344,748 markers remaining for analysis. After QC and removal of failing markers overall genotyping rate was >0.999 for each of the chromosomes.

5.2.4.2 Statistical analysis of individual loci

A series of single-locus tests analysing each SNP for association with the side effect phenotype were performed using logistic regression, with and without the inclusion of covariates. Categorical covariates included in the multivariate logistic regression were age (<50 and ≥50), BMI (<25, 25-30, >30), HRT history (never, current, former user), baseline menopausal status (pre or postmenopausal), and smoking history (never, current, former). Odds ratio and 95% confidence intervals were produced for each SNP.

The statistical significance level for the CG study was defined as 8.3×10^{-4} (alpha of 0.05 divided by 60 genes). This significance level was selected because of LD between SNPs; therefore, for each of the candidate genes, SNPs within each gene were linked together to give a statistical significance level of 8.3×10^{-4}. Subsequently the false discovery rate (FDR) was calculated to determine the likelihood that each SNP is a false positive at the current level of significance. For the GWAS study statistical significance was defined at a genome-wide level of 1×10^{-7}. A sub-threshold of 5×10^{-5} for suggestive associations which would require validation in independent data sets was also defined to identify novel SNPs. A discussion of statistical significance levels can be found in section 3.4.4. Following identification of statistically significant SNPs, those located close to each other were tested for linkage disequilibrium. $D^{'}$ and R^2 values between each pair of SNPs were reported.

5.2.4.3 Multi-loci analysis

After initial investigation of individual SNPs, multiple loci were analysed to assess whether more than one SNP working in tandem with others might prove a better predictor for side effects than any single SNP.

Given the computational complications of analysing 350,000 SNPs in a pairwise fashion, markers were filtered to remove those hypothesised to have little effect on outcomes.

Only SNPs from an established biological context, such as a biochemical pathway or a protein family as per the CG study (see Table 5.1) or SNPs previously associated with breast cancer risk were used to assess multi-loci associations.

The dataset was partitioned into ten sets each containing a similar number of cases and controls. Least Absolute Shrinkage and Selection Operator (LASSO), was used to reduce the number of variables and to form a model for each side effect. Ten-fold cross-validation was performed with each of the ten sets used once as a test set. Model parameters were reported at the minimum lambda (λ) value (lambda.min), the option which refers to the value of λ at the lowest CV error, and at the largest value of λ which is within one standard error of the best model (lambda.1se) (Tibshirani 1996; Freidman et al., 2010). If all parameters were excluded in the lambda.1se model, then the range of λ was explored to find the model with the best prediction. The OR point estimate, calculated by the exponential of the LASSO coefficient, and locus for each SNP in each model was reported.

5.2.4.4 Polygenic risk score

Models were then used to determine a polygenic risk score (PRS). The coefficients from the LASSO model (β) were multiplied by the number of risk alleles (x) for each SNP identified in the LASSO generated models. The PRS represents the combined effect of each of the SNPs (Equation 5.1).

$$PRS = \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \beta_n x_n \tag{5.1}$$

Equation 5.1: Equation for the generation of polygenic risk score for each side effect.

The PRS was then exponentiated and subsequently normalised by dividing each exponentiated PRS score by the mean. To assess the utility of the PRS, the side effect status (0 or 1) was then modelled against the PRS and thresholds for side effect prediction identified.

5.2.4.5 Statistical software

QC was performed using PLINK (Version 1.9) and R Version 3.6.0 through the plugin plinkQC R package (Chang et al., 2015; Meyer 2018). Statistical analysis, OR and 95% CI, for both the GWAS study and the CG study was performed using PLINK Version 1.9 (Package: PLINK[1.9], Authors: Shaun Purcell and Christopher Chang, URL: www.cog-genomics.org/plink/1.9/) (Chang et al., 2015). Output of statistical analysis will be imported into R for further analysis of interactions between SNPs and treatment and for production of graphics to summarise the outputs.

In addition to the statistical software used, LDlink was used to plot the LDmatrix for regions of interest. Genotypes from the Central Europeans living in Utah (CEU) group of the 1000 genomes project will be used to assess the LD between different SNPs within our cohort (Machiela and Chanock 2015; The 1000 Genomes Project Consortium 2015).

5.2.4.6 Author contributions

Samples for the genomic analysis were selected by J.C. and assays were carried out blindly at Genome Quebec (Montreal, Canada). M.H. performed QC of samples and all subsequent analysis. A more detailed breakdown of contributions to each chapter can been found in a summary table in Appendix 1.

5.3 Results

5.3.1 Study demographics

Per individual QC was performed on 820 women randomised to tamoxifen or placebo during the IBIS-I trial. Twenty-five women were removed due to poor genotyping in greater than 5% of samples or a heterozygosity greater than three standard deviations from the mean, 485 women randomised to placebo were also removed. The demographic of women included for further analysis after removal of individuals who fail per individual QC is shown in Table 5.3.

Table 5.3: Demographic of women remaining in analysis (N = 310) after removal of failed individuals with respect to randomised treatment

Parameter	Tamoxifen (N = 310)
Median age (IQR)	50.00 (46.0-53.8)
BMI median (IQR)	25.64 (23.4-29.9)
Menopausal status	
Premenopausal	136 (43.9)
Postmenopausal	173 (55.8)
HRT use	
Never	180 (58.1)
Former	54 (17.4)
Current	76 (24.5)
Smoking history	
Never	157 (50.7)
Former	101 (32.6)
Current	52 (16.8)
Reported side effect number (N (%))	
Hot flushes	143 (46.1)
Vaginal discharge	44 (14.2)
Vaginal dryness	21 (6.8)
Irregular bleeding	29 (9.4)
Any gynaecological symptom	84 (27.1)

Subsequent per marker QC in remaining samples identified low MAF as the main reason for excluding SNPs, followed by missing genotype and failed HWE. Details for markers removed during per marker QC and the reason for the removal from the analysis are shown in Table 5.2.

5.3.2 Candidate gene study results

The CG analysis was performed on 571 SNPs from 60 genes shown in Table 5.1. The analysis was performed in 310 women randomised to tamoxifen who passed the per individual QC.

One SNP was associated with HFs at $P < 8.3\times10^{-4}$ after adjustment for menopausal status, smoking history, BMI, age, and HRT use. The SNP was rs9332220 found in a region encoding *CYP2C9* and had a 52% (OR = 0.49 95%CI (0.32 – 0.74)) reduction in HF risk. However, an FDR of 0.45 suggested that this finding may be false in a large proportion of cases. Two other SNPs, rs1934956 and rs9332242, located on chromosome 10 between 96748893 - 96828160, in the site of the *CYP2C8* and *CYP2C9* respectively, were of borderline significance but did not meet the $P < 8.3\times10^{-4}$ threshold. One of these SNPs, rs9332242, was within 5kb of rs9332220 and was also associated with a reduction in HFs (OR = 0.56 (0.34 – 0.90)) (Table 5.4).

The ORs for these SNPs were similar and both were found in the same genomic region, which raised the potential for LD between these SNPs. The investigation of LD was required to avoid overstating the number of SNPs associations or the strength of SNP associations with side effects. For this analysis the CEU data set, people of northern and western European descent living in Utah, of the 1000 genomes project was used to calculate LD between SNPs. This was to determine the rate at which each pair of SNPs are found together and thus whether the risk was a shared risk or whether each SNP is individually linked to the side effect of interest. In the case of rs9332220 and rs9332242, both SNPs are likely inherited together if AG/AG are the in-phase alleles, $D' = 1.00$ and $R^2 = 1.00$ and if CG/GA are the in-phase alleles, $D' = 1.00$ and $R^2 = 0.64$.

rs1934956 was not found in high LD with either of the other SNPs on chromosome 10, $D' = 0.70$ and $R^2 = 0.011$ with rs9332242 and $D' = 0.581$ and $R^2 = 0.012$ with rs9332220. rs1934956 was associated with a 89% increase in the risk of HFs (OR = 1.89 (1.13 – 3.16)) compared to those without the variant (Table 5.4).

No SNPs had a $P < 8.3\times10^{-4}$ for vaginal dryness or discharge, irregular bleeding or any gynaecological side effect. In each case the most associated SNP after adjustment were rs8100458 ($P = 1.08\times10^{-2}$) in the *CYP2B6* coding region for vaginal dryness,

rs1799930 (P = 1.12×10^{-3}) in the *NAT2* coding region for vaginal discharge, rs7003927 in the *NAT1* gene region (P = 4.89×10^{-3}) for irregular bleeding and chr2:202,221,239 in *ALSCR12* (P = 2.42×10^{-3}) for any gynaecological symptoms. Given that the SNP in *ALSCR12* was also significantly associated with vaginal discharge (OR = 2.51 (1.29 – 4.88); P = 6.57×10^{-3}), there is a strong likelihood that this SNP predicts the outcome of vaginal discharge within the group labelled any gynaecological symptoms. However, it was interesting to observed that the strength of association between chr2:202,221,239 in *ALSCR12* and any gynaecological symptom was stronger than the individual side effect suggesting that chr2:202,221,239 in *ALSCR12* may also be weakly associated with other gynaecological symptoms (Table 5.4).

Interestingly, with the exception of vaginal discharge, there were four different SNPs in *ESR1* reported among the top associated SNPs with gynaecological outcomes. Two SNPs in *ESR1* were reported for irregular bleeding rs1285057 (OR = 2.18 (1.24 – 3.80); P = 6.39×10^{-3}) and chr6:152,130,918 (OR = 2.24 (1.26 – 4.01); P = 6.33×10^{-3}) and one for vaginal dryness rs11393678 (OR = 2.65 (1.16 – 6.08): P = 2.10×10^{-2}). One further SNP, rs827423 was reported for any gynaecological symptom (Table 5.4). They were located on chromosome 6 between 152,103792 – 152,156,197 base pairs a region within the *ESR1* gene which codes the ERα receptor. The two SNPs associated with irregular bleeding, rs1285057 and chr6:152,130,918, found within 27Kb of each other increased the risk of irregular bleeding by approximately 2-fold which remained unchanged after adjustment for age, menopausal status, BMI, HRT and smoking status. Investigation of LD between these SNPs revealed no LD between the SNPs.

Table 5.4: Table of per allele OR and 95% confidence intervals for all SNPs with P $< 8.3\times x10^{-4}$ for an association with a side effect without and after adjustment for covariates. After adjustment for covariates the p-value for the test and the FDR are provided. For those where no SNPs were P $< 8.3\times x10^{-4}$ the top five results are displayed.

CHR	SNP	Base Pair	Allele frequency in affected / unaffected	Unadjusted	Adjusted		
				OR (95%CI) P-value	OR (95%CI) P-value	FDR	Locus
Hot flushes							
10	rs9332220	96743943	0.15 / 0.26	0.49 (0.32 - 0.74) $9.40x10^{-4}$	0.48 (0.31 - 0.74) $8.31x10^{-4}$	0.45	CYP2C9
10	rs1934956	96828160	0.15 / 0.09	1.89 (1.13 - 3.16) $1.48x10^{-2}$	2.01 (1.20 - 3.38) $8.04x10^{-3}$	0.84	CYP2C8
10	rs9332242	96748893	0.11 / 0.18	0.56 (0.34 - 0.90) $1.69x10^{-2}$	0.53 (0.32 - 0.87) $1.12x10^{-2}$	0.84	CYP2C9
15	chr15_51532647	51532647	0.06 / 0.14	0.47 (0.25 - 0.87) $1.72x10^{-2}$	0.48 (0.26 - 0.90) $2.11x10^{-2}$	0.84	CYP19A1
10	chr10_64278682	64278682	0.08 / 0.14	0.54 (0.32 - 0.92) $2.46x10^{-2}$	0.54 (0.33 - 0.90) $1.69x10^{-2}$	0.84	ZNF365
Vaginal dryness							
10	rs2050308	5258571	0.24 / 0.10	2.81 (1.29 - 6.12) $9.19x10^{-3}$	2.60 (1.16 - 5.83) $2.05x10^{-2}$	0.79	AKR1C1
10	chr10_5244295	5244295	0.29 / 0.14	2.56 (1.22 - 5.38) $1.28x10^{-2}$	2.57 (1.18 - 5.60) $1.76x10^{-2}$	0.79	AKR1C1
14	rs11627032	93104072	0.10 / 0.28	0.26 (0.09 - 0.75) $1.29x10^{-2}$	0.24 (0.08 - 0.72) $1.08x10^{-2}$	0.79	RIN3
19	rs8100458	41500213	0.17 / 0.37	0.36 (0.16 - 0.82) $1.46x10^{-2}$	0.33 (0.14 - 0.77) $1.05x10^{-2}$	0.79	CYP2B6
6	rs11393678	152151930	0.26 / 0.13	2.58 (1.18 - 5.64) $1.71x10^{-2}$	2.65 (1.16 - 6.08) $2.10x10^{-2}$	0.79	ESR1
1	rs4245739	204518842	0.43 / 0.27	2.35 (1.16 - 4.78) $1.84x10^{-2}$	2.84 (1.30 - 6.20) $8.67x10^{-3}$	0.79	MDM4
22	rs4646316	19952132	0.38 / 0.23	2.10 (1.08 - 4.09) $2.09x10^{-2}$	2.42 (1.20 - 4.89) $1.35x10^{-2}$	0.79	COMT
10	rs1934953	96797470	0.19 / 0.35	0.40 90.17 - 0.91) $2.87x10^{-2}$	0.35 (0.15 - 0.82) $1.62x10^{-2}$	0.79	CYP2C8
19	rs3745274	41512841	0.43 / 0.26	2.09 (1.11 - 3.90) $2.32x10^{-1}$	2.17 (1.12 - 4.17) $2.10x10^{-2}$	0.79	CYP2B6

Continued on next page

Table 5.4 – continued from previous page

CHR	SNP	Base Pair	Allele frequency in affected / unaffected	Unadjusted	Adjusted		
				OR (95%CI) P-value	OR (95%CI) P-value	FDR	Locus
Vaginal discharge							
8	rs1799930	18258103	0.41 / 0.23	2.30 (1.40 - 3.76) $9.80x10^{-4}$	2.31 (1.40 - 3.81) $1.12x10^{-3}$	0.61	NAT2
8	rs1041983	18257795	0.41 / 0.26	2.04 (1.25 - 3.34) $4.49x10^{-3}$	2.04 (1.23 - 3.36) $5.39x10^{-3}$	0.71	NAT2
2	chr2_202221239	202221239	0.20 / 0.10	2.37 (1.24 - 4.52) $9.03x10^{-3}$	2.51 (1.29 - 4.88) $6.58x10^{-3}$	0.71	ALSCR12
15	rs10046	51502986	0.34 / 0.48	0.56 90.34 - 0.90) $1.81x10^{-2}$	0.54 (0.33 - 0.88) $1.31x10^{-2}$	0.71	CYP19A1
15	chr15_51507508	51507508	0.34 / 0.48	0.56 (0.34 - 0.90) $1.81x10^{-2}$	0.54 (0.33 - 0.88) $1.31x10^{-2}$	0.71	CYP19A1
Irregular bleeding							
6	chr6_152130918	152130918	0.53 / 0.34	2.18 (1.26 - 3.78) $5.65x10^{-3}$	2.24 (1.26 - 4.01) $6.33x10^{-3}$	0.70	ESR1
8	rs7003927	18032110	0.33 / 0.17	2.34 (1.28 - 4.29) $5.78x10^{-3}$	2.42 (1.31 - 4.48) $4.89x10^{-3}$	0.70	NAT1
10	rs1934968	96741817	0.21 / 0.09	2.69 (1.31 - 5.55) $7.33x10^{-3}$	2.74 (1.29 - 5.83) $8.69x10^{-3}$	0.73	CYP2C9
1	chr1_162781787	162781787	0.66 / 0.48	2.26 (1.24 - 4.12) $8.01x10^{-3}$	2.34 (1.28 - 4.22) $5.63x10^{-3}$	0.70	HSD17B7
6	rs1285057	152103792	0.53 / 0.35	2.04 (1.19 - 3.50) $9.57x10^{-3}$	2.18 (1.24 -3.80) $6.39x10^{-3}$	0.70	ESR1
Any gynaecological symptoms							
2	chr2_202221239	202221239	0.17 / 0.09	2.19 (1.27 - 3.78) $4.86x10^{-3}$	2.42 (1.38 - 4.26) $2.16x10^{-3}$	0.60	ALSCR12
9	rs11788785	99031628	0.10 / 0.19	0.46 (0.26 - 0.81) $6.98x10^{-3}$	0.42 (0.24 - 0.77) $4.40x10^{-3}$	0.60	HSD17B3
9	rs11788083	99056209	0.10 / 0.18	0.49 (0.28 - 0.87) $1.51x10^{-2}$	0.47 (0.26 - 0.85) $1.21x10^{-2}$	0.60	HSD17B3
16	chr16_53855291	53855291	0.28 / 0.19	1.66 (1.10 - 2.50) $1.56x10^{-2}$	1.88 (1.22 - 2.90) $4.48x10^{-3}$	0.60	FTO
6	rs827423	152156197	0.56 / 0.45	1.53 (1.07 - 2.19) $2.00x10^{-2}$	1.53 (1.06 - 2.21) $2.40x10^{-2}$	0.60	ESR1

5.3.3 GWAS results

Previous GWAS analyses found that there were numerous SNPs associated with breast cancer or secondary treatment outcomes, which do not form part of any known pathways or genes. Therefore, a GWAS analysis was performed on the dataset to identify any additional SNPs, which may be associated with side effects. The GWAS analysis was performed on approximately 345,000 SNPs in 310 women randomised to tamoxifen. While no SNPs were significant at a genome-wide significance level (5×10^{-8}), 29 SNPs were identified which had a p-value of $< 5\times10^{-5}$ for association with any side effect (Figure 5.2, Table 5.5). Of these 29 SNPs two had p-values of $< 1\times10^{-5}$ and were located on chromosome 19 within ~13kb of each other and both associated with vaginal discharge (Table 5.5). These two SNPs had the strongest associations of any SNPs, rs147676656 and rs62113694 with p-values of 9.24×10^{-7} and 1.55×10^{-6} respectively (Table 5.5).

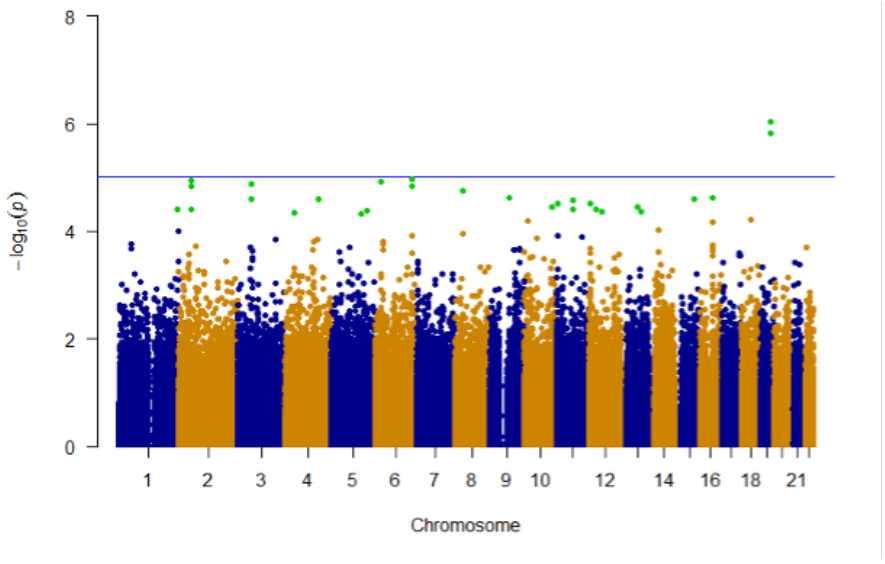


Figure 5.2: Manhattan plot of all SNPs passing QC. Blue line represents significance level of 1×10^{-5}. SNPs highlighted in green are SNPs which met a statistical significance level of 5×10^{-5} for hot flushes, vaginal discharge, vaginal dryness, irregular bleeding or any gynaecological symptom.

Both SNPs were not directly associated with a gene; however, they lie within ~30 Kb of *PSG9* and *CD177* respectively. Both *PSG9* and *CD177* have previously been identified as being involved in cell adhesion pathways. Given the close proximity of the two SNPs too each other a test for LD was performed showing that both SNPs are in high LD ($R^2 = 0.94$, $D' = 0.97$). These SNPs were not in LD with either of the

genomic regions which code for the genes *PSG9* and *CD177* meaning that these SNPs are more likely to exert their mode of action through the enhancement or inhibition of gene expression rather than altering the protein structure (Figure 5.3).

In addition, three other SNPs potentially associated with vaginal discharge were identified. These were rs1947300 on chromosome 8 (OR = 6.96 (2.87-16.87); P = 1.79×10^{-5}) which is close 17.5 Kb from the region coding for the *UNC5D* gene, rs60835808 on chromosome 11 (OR = 6.46 (2.69-15.51); P = 3.04×10^{-5}) close to the region coding for *ASCL2* and rs73040051 on chromosome 12 (OR = 6.32 (2.65-15.03); P = 3.11×10^{-5}) near to the *CCND2* gene coding region (Table 5.5).

Other regions of interest with links to side effects other than vaginal discharge were identified during the GWAS analysis (Table 5.5). Six SNPs are associated with increased risk of HFs, one SNP on chromosome 1 in the *PLD5* coding region (rs3913529: OR = 2.08 (1.47 – 2.94); P = 4.00×10^{-5}), one on chromosome 5 in the *PPP2R2B* coding region (rs178549: OR = 2.22 (1.52 – 3.25); P = 4.71×10^{-5}) and one on chromosome 13 in the *RNF219-AS1* gene region (rs9593346: OR = 2.36 (1.57 – 3.57); P = 4.30×10^{-5}). All three SNPs are associated with approximately a 2-fold increase in the risk of HFs. A further three SNPs are located within a ∼21Kb region on chromosome 2 associated with the LOC730100 region which has been found to produce non-coding RNA.

The three SNPs on chromosome 2 present a contradictory picture. Two SNPs, rs1919430 (OR = 0.44 (0.30 – 0.65); P = 3.93×10^{-5}) and rs13387812 (OR = 0.42 (0.28 – 0.62); P = 1.13×10^{-5}) were both associated with a decreased risk of HFs. The third SNP was associated with a 2.3-fold increase in risk of HFs (rs10206670: OR = 2.34 (1.59 – 3.44); P = 1.50×10^{-5}). LD analysis of the three SNPs showed that while all three have a high D' only rs1919430 and rs13387812 are in high LD $R^2 = 0.82$. Although rs10206670 was a commonly observed SNP, it was poorly linked to the other two SNPs with $R^2 = 0.39$ with rs1919430 and $R^2 = 0.36$ with rs1919430. These LD values help explain the risk effects brought by each SNP. The two SNPs in high LD, rs1919430 and rs13387812, are often inherited together and reduce the risk of HFs by approximately 60%. Alternatively, having the rs10206670 mutation leads to an increased risk of HF of 2.3-fold. The effect of having all three mutations is unknown.

Four SNPs were associated with vaginal dryness below 5×10^{-5}. However, none of

these SNPs were located in a gene coding region. None of them were located in the same region of the genome as any other SNP. While none of the SNPs from the CG were significant, and no link to *ESR1* was observed, this analysis shows one SNPs also on chromosome 6 with the closest gene *GMNN*. *GMNN* encodes a protein involved in cell cycle regulation inhibiting the incorporation of mini chromosome maintenance proteins into the pre-replication complex. Increased expression is believed to play a role in several malignancies including breast cancer.

Table 5.5: Table of all statistically significant SNPs at 5x10^{-5} level. CI – Confidence Interval, OR – Odds Ratio, SNP – Single Nucleotide Polymorphism. *Gene loci in bold contain the SNP, gene loci non-bold are the nearest gene to the SNP of interest.

Side effect	CHR	SNP Name	Base pair	Allele	OR (95% CI)	P - value	Locus*
Hot flushes	1	rs3913529	242427162	A	2.08 (1.47 - 2.94)	4.00x10^{-5}	**PLD5**
	2	rs1919430	51633618	C	0.44 (0.30 - 0.65)	3.93x10^{-5}	**LOC730100**
	2	rs10206670	51638488	G	2.34 (1.59 - 3.44)	1.50x10^{-5}	**LOC730100**
	2	rs13387812	51655345	A	0.42 (0.28 - 0.62)	1.13x10^{-5}	**LOC730100**
	5	rs178549	146295990	A	2.22 (1.52 - 3.25)	4.17x10^{-5}	**PPP2R2B**
	13	rs9593346	79183804	A	2.36 (1.57 - 3.57)	4.30x10^{-5}	**RNF219-AS1**
Vaginal discharge	8	rs1947300	35075632	A	6.96 (2.87 - 16.87)	1.79x10^{-5}	UNC5D
	11	rs60835808	2279249	G	6.46 (2.69 - 15.51)	3.04x10^{-5}	ASCL2
	12	rs73040051	4419190	G	6.32 (2.65 - 15.03)	3.11x10^{-5}	CCND2
	19	rs147676656	43809832	-	12.49 (4.56 - 34.26)	9.24x10^{-7}	PSG9
	19	rs62113694	43823065	A	12.72 (4.51 - 35.88)	1.55x10^{-6}	CD177
Vaginal dryness	4	rs10519404	137875449	A	7.36 (2.91 - 18.63)	2.53x10^{-5}	PCDH18
	5	rs3909326	123171342	T	11.05 (3.47 - 35.18)	4.80x10^{-5}	LINC01170/ CSNK1G3
	6	rs12203240	24749104	C	7.45 (3.03 - 18.30)	1.19x10^{-5}	GMNN
	12	rs11049731	28744896	A	8.98 (3.15 - 25.59)	3.98x10^{-5}	CCDC91
Irregular bleeding	9	rs73452088	81368490	G	9.23 (3.29 - 25.86)	2.39x10^{-5}	PSAT1
	10	rs12244656	118129299	C	7.34 (2.85 - 18.88)	3.58x10^{-5}	**CCDC172**
	11	rs4930643	68818936	G	6.26 (2.66 - 14.72)	2.68x10^{-5}	**TPCN2**
	11	rs11604251	68820429	T	4.71 (2.25 - 9.85)	3.90x10^{-5}	**TPCN2**
	12	rs11378140	50291551	+	4.07 (2.08 - 7.97)	4.25x10^{-5}	**FAIM2**
	16	rs66525491	52440096	T	7.28 (2.90 - 18.27)	2.41x10^{-5}	TOX3
Any gynaecological symptom	3	rs7638552	58091098	A	2.97 (1.82 - 4.84)	1.34x10^{-5}	**FLNB**
	3	rs11130613	58105995	T	2.85 (1.75 - 4.65)	2.51x10^{-5}	**FLNB**
	3	rs2362904	58112488	A	2.85 (1.75 - 4.65)	2.51x10^{-5}	**FLNB**
	4	rs17615370	38464493	G	3.90 (2.03 - 7.49)	4.59x10^{-5}	**LINC01258**
	6	rs1405696	152859775	-	2.82 (1.78 - 4.48)	1.11x10^{-5}	**SYNE1**
	6	rs2141148	152870992	T	2.78 (1.75 - 4.42)	1.50x10^{-5}	**SYNE1**
	13	rs11843180	68340875	T	3.34 (1.89 - 5.91)	3.55x10^{-5}	PCDH9
	15	rs4887053	78712699	A	3.26 (1.88 - 5.64)	2.55x10^{-5}	IREB2

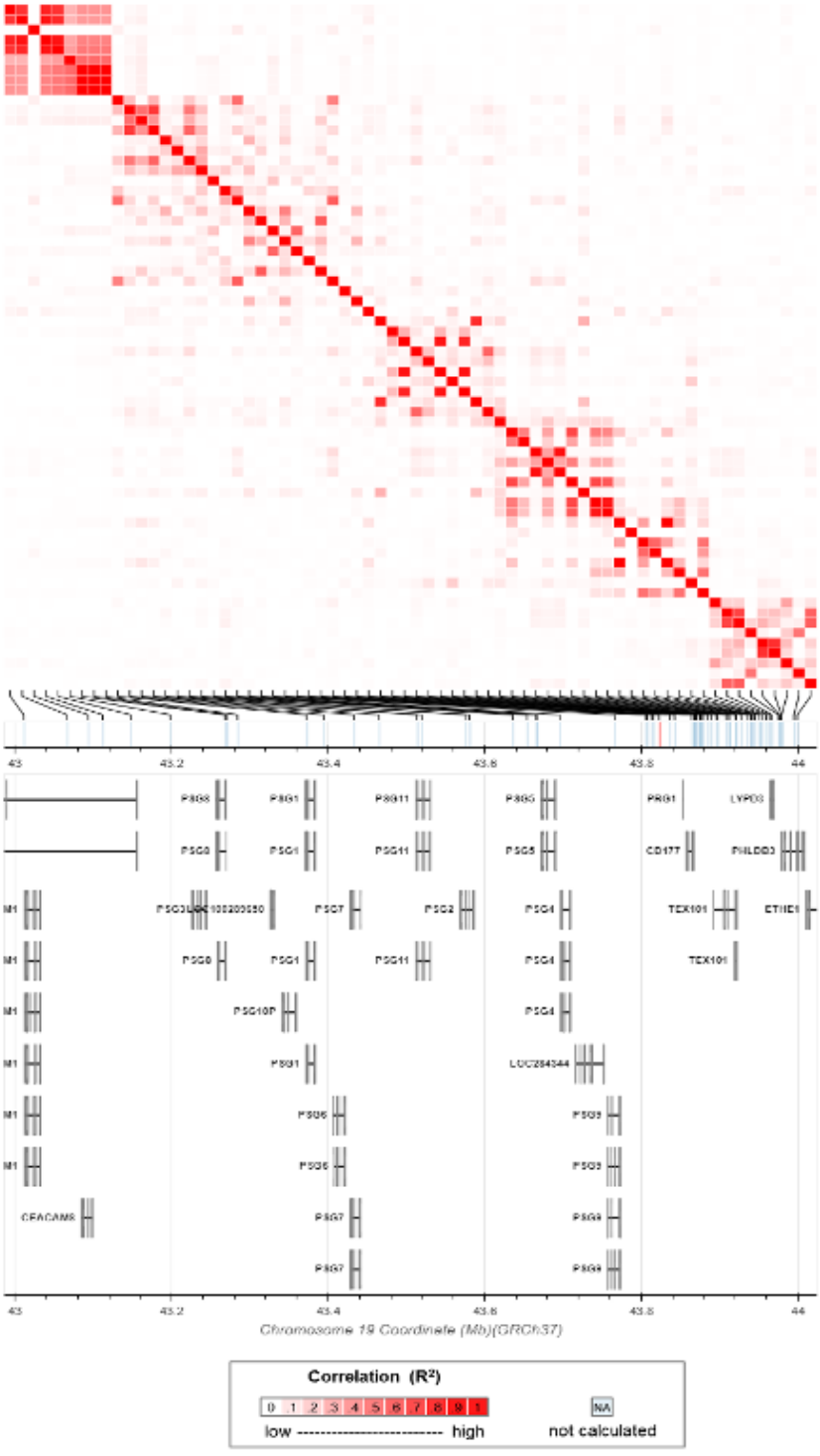


Figure 5.3: Linkage disequilibrium plot of all genotyped positions on Chromosome 19. LD patterns from Phase III of the 1000 genomes project – CEU population was used to determine linkage. Panel below the LD plot identifies genes and gene positions. Genotyped SNPs are plotted as squares, with the colour indicating the degree of pairwise LD between SNPs. Red indicates strong pairwise LD and reducing LD is displayed by the intensity, white indicates no LD.

There were six SNPs associated with irregular bleeding at $P < 5\times10^{-5}$ two of which, rs73452088 and rs66525491, were not within any gene coding regions but are close to *PSAT1* and *TOX3* respectively. Two other SNPs were located in genes *CCDC172*, rs12244656, on chromosome 10 and *FAIM2*, rs11378140, coding an apoptotic inhibitor molecule on chromosome 12. Two further SNPs, rs4930643 and rs11604251, were found 1.5Kb apart on chromosome 11 both found within the *TPCN2* gene coding region. These SNPs were associated with an increase in irregular bleeding of 6-fold (OR = 6.26 (2.66 – 14.72); $P = 2.68\times10^{-5}$) and 4.7-fold (OR = 4.71 (2.08 – 7.97); $P = 3.90\times10^{-5}$) respectively. LD analysis of these SNPs indicates that both were in high LD with $D' = 1.00$ and $R^2 = 0.91$. The *TPCN2* gene encodes a cation-selective ion channel. The protein coded localises to lysosomal membranes and allow nicotinic acid adenine dinucleotide phosphate induced calcium ion release from lysosome stores. *TPCN2* is often upregulated in cancer and also contains the cyclin gene which is a known promoter of oncogenesis. Given this known link to oncogenesis it is possible that *TPCN2* is involved in angiogenesis. The promotion of new blood vessels may well be an important mechanism through which irregular bleeding is initiated.

Finally, eight SNPs were associated with any gynaecological symptom. None of the SNPs associated with this general class of gynaecological symptoms were associated with any of the individual gynaecological symptoms. Three SNPs were grouped within 21 Kb on chromosome 3 in the gene region encoding *FLNB*. Each of the three SNPs is linked with an approximately 3-fold increased risk of any gynaecological symptom (rs7638552: OR = 2.97 (1.82 - 4.84); $P = 2.39\times10^{-5}$), (rs11130613: OR = 2.85 (1.75 - 4.65); $P = 2.51\times10^{-5}$), and (rs2362904: OR = 2.85 (1.75 - 4.65); $P = 2.51\times10^{-5}$). LD analysis of these SNPs showed that all are highly correlated with $D' > 0.97$ and $R^2 > 0.92$ in all cases. This means it is likely that the 3-fold increase in gynaecological risk is a combined risk rather than an individual risk. *FLNB* encodes a protein in the filamin family. Filamin proteins interact with glycoproteins during the vascular repair process while other mutations in these genes have been linked to several conditions affecting body growth and development. A fourth SNP is located in chromosome 4 in the region of *LINC01258* and is associated with a 4-fold increase in gynaecological symptoms (rs17615370: OR = 3.90 (2.03 - 7.49); $P = 4.59\times10^{-5}$). The pathways and impact of *LINC01258* is unknown with *LINC01258* coding a long intergenic non-protein coding RNA. Two further SNPs were found 11 Kb apart on chromosome 6 in the region coding for *SYNE1*. *SYNE1* encodes a spectrin repeat containing protein which

is expressed in skeletal muscle and peripheral blood lymphocytes. The mechanism through which *SYNE1* is linked with gynaecological symptoms is unknown. The SNPs found in this region of chromosome 6 were associated with a 2.7-fold (rs1405696: OR = 2.82 (1.78 - 4.48); P = 1.11×10^{-5}) and 2.8-fold (rs2141148: OR = 2.78 (1.75 - 4.42); P = 1.50×10^{-5}) increase in risk of gynaecological side effects. LD analysis of the two SNPs again indicated that these SNPs were in high LD ($D^{'}$ = 1.00 and R^2 = 0.98) suggesting that these risks should not be combined and that, given both are inherited together, both SNPs combined to give a 2.8-fold increase in gynaecological symptoms. Two final SNPs, rs11843180 and rs4887053, located on chromosome 13 and 15 respectively increase the risk of gynaecological symptoms by 3.3-fold (rs11843180: OR = 3.34 (1.89 - 5.91); P = 3.55×10^{-5}) and 3.2-fold (rs4887053: OR= 3.26 (1.88 - 5.64); P = 2.55×10^{-5}) respectively. However, neither of these SNPs were found in a gene coding region, 863Kb from *PCDH9* and 18Kb from *IREB2* respectively. It is therefore not known how these SNPs exert their effect and how they increase the risk of gynaecological symptoms.

5.3.4 Multi-loci results

All 571 SNPs from the CG analysis genotyped in 310 women randomised to tamoxifen were used in the multi-loci analysis. No SNPs from the GWAS analysis were included in the multi-loci analysis. Women who had no side effect information were excluded from this analysis (N = 299). SNPs were then selected using LASSO and 10-fold cross-validation to tune the model and select a subset of variables which described each of the side effects of interest. The number of variables selected by LASSO for each of the side effects are shown in Table 5.6. The LASSO coefficients of each SNP were subsequently exponentiated to give OR point estimates to assess the strength of the influence on the side effect.

The LASSO model for HFs at the lowest lambda ($\lambda = 0.080$) selected a total of one SNP. Whilst the model using the largest lambda ($\lambda = 0.092$) within one standard error of the minimum selected also contained one SNP; the AUC at $\lambda = 0.092$ was 0.55 (SE = 0.04). The single SNP within the LASSO model was rs9332220 (*CYP2C9*) which had the largest associations with HFs in the CG study. Point OR estimate at λ = 0.092 for rs9332220 was 0.97. The magnitude of OR suggested that the selected SNP was not strongly associated with HFs supporting the results of the CG analysis (Table

Table 5.6: Summary of selected variables for models defining side effects. Min – is the value of lambda.min and 1SE the lambda value at one standard error from the minimum lambda

	Threshold	Lambda	AUC	SE	Number of selected variables
Hot flushes	min	0.080	0.557	0.041	1
	1se	0.092	0.551	0.021	1
Vaginal discharge	min	0.066	0.519	0.018	0
	1se	0.066	0.519	0.018	0
	Self Select	0.024	0.630	0.047	66
Vaginal dryness	min	0.001	0.575	0.060	61
	1se	0.016	0.529	0.072	44
Irregular bleeding	min	0.006	0.597	0.061	85
	1se	0.026	0.540	0.060	28
Any gynaecological symptom	min	0.007	0.630	0.029	161
	1se	0.014	0.604	0.028	123

5.4).

The vaginal discharge model contained no SNPs selected at the optimum lambda (λ = 0.066) investigation showed that the largest lambda within one standard error was also (λ = 0.066). Investigation of the lambda range identified λ = 0.024 as the point at which the LASSO generated model had the best AUC (0.63, SE = 0.05) for the prediction of vaginal discharge. At λ = 0.024 the LASSO model selected 66 SNPs in the model. The range of OR point estimates ranged from 0.75 – 1.79 with a median and interquartile range of 1.00 (IQR: 0.96 – 1.08).

The vaginal dryness model contained 61 SNPs at the optimal lambda (λ = 0.001) and 44 SNPs at λ = 0.016. At λ = 0.016, the AUC of the LASSO model was 0.53 (SE = 0.07) which suggested the predictive potential of the model was poor. This was in concordance with the CG study which found no statistically significant SNPs associated with vaginal dryness. The OR estimates for SNPs which formed the vaginal dryness model ranged between 0.58, in a region coding for *SULT1E1*, and 1.83 for SNPs in *ESR1* and *HSD17B4* genes and had a median (IQR) of 1.06 (0.93 – 1.29). Results from the multi-loci modelling of vaginal dryness supported the result of the CG study. The strength of association between SNPs and vaginal dryness was weak, but evidence suggested that altered sex hormone metabolism and disrupted signalling at ERα may be involved in the aetiology of vaginal dryness in women randomised to tamoxifen.

The irregular bleeding model at lambda.1se (λ = 0.026) contained 28 SNPs compared

to 85 reported SNPs at the optimum lambda. ORs for the SNPs ranged from 0.72 in a region coding for *ESR1* to 1.53 for SNPs in *CYP2C9* and *NAT1* genes. The two SNPs with the largest ORs from the LASSO model, rs7003927 (*NAT1*) and rs1934968 (*CYP2C9*) were both observed in the CG study among the SNPs with the strongest association with irregular bleeding. However, it should be noted that no SNPs were significantly associated with irregular bleeding in the CG study.

When any gynaecological symptom was considered, the LASSO model contained 123 SNPs at lambda.1se ($\lambda = 0.014$) compared to 161 at the optimum lambda ($\lambda = 0.007$) (Table 5.6). At $\lambda = 0.014$, the AUC for any gynaecological symptom was 0.60 (SE = 0.03) which was the best prediction model for any of the gynaecological symptoms. OR estimates of SNPs in the model ranged from 0.51 to 2.24 and had a median (IQR) of 1.01 (0.91 – 1.17). There were five SNPs with OR estimates above 1.75, the largest of these rs1934698 (OR = 2.23) was located in the coding region of *CYP2C9* and had previously been associated with an increase in irregular bleeding. The next largest effect SNPs were chr2:202,221,239 (*ALSCR12*) and chr16:53,855,291 (*FTO*) both of which were among the SNPs with the strongest association with any gynaecological symptoms in the CG study. chr2:202,221,239 (*ALSCR12*) was also associated with an increase in vaginal discharge in the CG study. The final two SNPs which had an OR > 1.75 were chr10:5,244,295 (*AKR1C1*) which had previously been associated with vaginal dryness during the CG study and rs8017441(*ESR2*) which had not previously been associated with any gynaecological symptoms. Four SNPs had an OR of <0.60, amongst these were rs12662670 and rs1934953 (both *CYP2C8*), rs11788785 (*HSD17B3*) and rs6586714 (*NAT1*). rs1934953 had previously been associated with a decrease in vaginal dryness and rs11788785 was associated with a decrease in any gynaecological side effect in the CG study. No previous association with gynaecological symptoms had been observed for either of the other two SNPs.

After the models were defined, the coefficients for each SNP were multiplied by the number of risk alleles and the products summed, exponentiated and normalised, which generated a PRS score for each woman in the dataset. Women who were missing genomic information for any of the selected SNPs in each model were removed from the analysis. It was not possible to form a PRS score for HFs or for vaginal discharge due to the number of SNPs selected by each of the multi-loci LASSO models, N = 1 and N = 0 respectively.

There were 225 women who had complete information for all SNPs in the vaginal dryness LASSO model (reported vaginal dryness = 15, no vaginal dryness = 210). The PRS range for loci selected for predicting vaginal dryness was 0.02 – 21.73 with a median of 0.28 (IQR = 0.16 – 0.62). For women who reported vaginal dryness the range was 3.82 – 21.73 and median of 8.40 (IQR = 6.75 – 10.72) compared to a range of 0.02 – 3.25, median 0.25 (IQR = 0.14 – 0.51) for those who did not report vaginal dryness. At a threshold of 0.5 for the normalised PRS score, women who had a PRS of greater than 0.5 all reported vaginal dryness and those who had a score of less than 0.5 did not report any vaginal dryness.

297 women had complete SNP information for all SNPs in the irregular bleeding model (reported irregular bleeding = 25, no irregular bleeding = 272). The PRS range for all women was 0.15 – 6.68 and had a median of 0.75 (IQR = 0.48 – 1.22). Women who reported irregular bleeding had a PRS range of 1.22 – 6.68 and a median of 2.70 (IQR = 1.82 – 3.43) compared to a range of 0.15 – 4.34 and a median of 0.73 (IQR = 0.47 – 1.06) for women without irregular bleeding. PRS scores for each woman were rounded to the nearest 0.5 and the range of the PRS was partitioned into 10 groups in 0.5 increments from 0-5. Only one woman had a score in excess of 5 and was thus included in the highest group. Only five women (1.8%) who did not report irregular bleeding had a PRS score of greater than middle score group (2.5). The majority of women (228/272, 83.8%) who do not report irregular bleeding had a PRS score of one or lower. This was compared to 64.0% (16/25) women who reported irregular bleeding had a PRS score of 2.5 or greater.

269 women had complete SNP information for SNPs when all gynaecological symptoms were combined (reported gynaecological symptoms = 71, no gynaecological symptoms = 198). The PRS for any gynaecological symptoms had a range of 0 – 49.1 and a median of 1.00 (IQR = 0.08 – 0.68). Women who reported symptoms had a PRS range of 0.29 – 49.08 and a mean of 1.79 (IQR = 1.15 – 2.89) compared to a range of 0.00 – 0.83 and a median of 0.17 (IQR = 0.06 – 0.21) for those without any gynaecological symptom. The PRS scores were rounded to the nearest 0.5 and the range of the PRS was partitioned into ten groups in 0.5 increments from 0-5. Seven women had a score in excess of 5 and were included in the highest group (5.0). 62 women (87.3%) who reported gynaecological symptoms had a PRS score of greater than one compared to nine (12.6%) who had a score of less than one. The majority (194/198, 98.0%) of

women who do not report irregular bleeding had a PRS score of 0.5 or lower. 14/18 (77.8%) of women who had a PRS of one reported gynaecological symptoms.

These results suggested that the use of multiple loci could be used to determine risk of gynaecological symptoms. In each case women who reported gynaecological side effects had higher PRS scores than women who did not report any symptoms. These results highlighted the potential benefit of using multi-loci models to construct a PRS for prediction of side effects and that the use of genomic factors should be considered for prediction of women who could be at high-risk of side effects.

5.4 Discussion

This analysis investigated SNPs for association with the risk of side effects in women randomised to tamoxifen in the IBIS-I study. This study began with a candidate gene study of SNPs in genes which:

- Have been previously linked to breast cancer in the study population
- Are involved in tamoxifen metabolism
- Are involved in sex hormone metabolism, or
- Code for receptor proteins

In addition to these known SNPs, a discovery GWAS was performed to identify genetic factors that might contribute to risk for the development of side effects in the first six months of breast cancer prevention therapy with tamoxifen.

The hypothesis that side effects cannot be explained by a single SNP and that groups of SNPs give more information, led to a final multi-loci analysis using the SNPs from the CG study. Of the 571 SNPs analysed as part of the CG study, only 1 SNPs had an association with any side effect at $P < 8.3 \times 10^{-4}$. The SNP was associated with HFs and located in a region of chromosome 10 known as the site of *CYP2C9* the gene responsible for coding a protein involved in the primary metabolism step tamoxifen to 4-hydroxytamoxifen or N-desmethyltamoxifen.

Little work has been performed studying the genetics of menopausal-like side effects (Crandall et al., 2020). A recent review by Crandall et al. (2020) found that only one

study had previously examined *CYP2C9* variants and HFs (Crandall et al., 2020). The study by Kapoor et al. (2019) found that as CYP2C9 activity decreased, the severity of HFs also decreased (P = 0.04). However, this association was no longer significant after adjustment for hormone therapy use (Crandall et al., 2020). The findings of this analysis support those of Kapoor et al. (2019) particularly if the variant observed in this analysis reduces *CYP2C9* activity. This analysis highlights the need for further genetic investigation of secondary enzymes involved in the conversion of tamoxifen to endoxifen which may decrease the risk of HFs in women taking tamoxifen.

The major enzyme responsible for the conversion of tamoxifen to endoxifen is CYP2D6. There was no evidence to support suggestions that variants to *CYP2D6* result in a lower incidence of HFs. This finding is supported by evidence from others who also find no association of SNPs, in particular *CYP2D6* SNPs, with altered HF outcomes (Sestak et al., 2012). Sestak et al. (2012) reviewed IBIS-I data for the link between HFs *CYP2D6* phenotype and breast cancer outcomes and found no support for an association between the three in tamoxifen treated women (Sestak et al., 2012). In contrast, Goetz et al. (2007) proposed a pharmacogenomic mechanism focussing on the biotransformation of tamoxifen to its more active metabolites catalysed by *CYP2D6*. Their study shows that women who are extensive metabolisers of tamoxifen have improved relapse free survival compared with those who are poor or intermediate metabolisers (Goetz et al., 2007). Of the 377 women in the normal metaboliser group 36 experienced HFs, whereas zero women in other groups reported HFs (Goetz et al., 2005). The findings of Goetz et al. (2007) suggested it is possible that HFs were a marker of *CYP2D6* activity and the successful conversion of tamoxifen (Goetz et al., 2007).

Whilst studies of *CYP2D6* mutations provide no definitive evidence of major difference in breast cancer outcomes for women with or without a mutation, the use of genotyping to guide selection of therapy remains an interesting investigative area. Genotyping is important as the presence of particular mutations affecting gene function could aid therapy selection. Currently there are two SERMs available for use as prevention agents, tamoxifen and raloxifene. As discussed in section 1.2.1.1, tamoxifen, is extensively metabolised to more potent anti-oestrogens, 4-hydroxy tamoxifen and endoxifen. While multiple CYP enzymes contribute to the metabolic process, the rate limiting step is CYP2D6-mediated. Common genetic variations in *CYP2D6* are associated with significant reductions in endoxifen concentrations (Goetz et al., 2007); therefore, those

carrying *CYP2D6* mutations may not benefit from taking tamoxifen. As raloxifene is not metabolised via *CYP2D6* it could be that those with a *CYP2D6* mutation may benefit from taking raloxifene rather than tamoxifen as they may still get a benefit.

In contrast to no observed affect for *CYP2D6* there was statistically non-significant ($P > 8.3\times10^{-4}$) evidence to support the findings from other trials linking SNPs in aromatase (*CYP19A1*) to HF outcomes. Results of the CG study indicate that SNPs in *CYP19A1* are statistically non-significantly associated with 50% reductions in HFs and vaginal discharge. It is known that increased aromatase activity increases circulating oestrogens and reduces HFs in postmenopausal women (Warren et al., 2006; Crandall et al., 2009). While the majority of associations between *CYP19A1* mutations and side effects have been observed in women taking AIs (Fontein et al., 2014; Johansson et al., 2016). Johansson et al., (2016) also included women randomised to tamoxifen as part of the TEXT trial. The TEXT study provided evidence that in premenopausal women *CYP19A1* rs10046 variant carriers may face milder vasomotor symptoms under combined endocrine treatment (Johansson et al., 2016). This finding could be supported by results of this analysis which suggest that variants in *CYP19A1* are associated with lower risk of HFs. However, the effect was restricted to patients under ovarian function suppression combined with exemestane (treatment-by-genotype interaction, $P = 0.03$) and not tamoxifen, after adjusting for patient characteristics and concomitant medications, including the selective serotonin-reuptake inhibitors known to reduce HFs (Johansson et al., 2016). Other studies report similar responses in postmenopausal women taking exemestane as part of the TEAM trial; however, no association for tamoxifen is available (Fontein et al., 2014).

No other SNPs met the 8.3×10^{-4} threshold for association with any other side effect. However, it was noted that several SNPs found in the *ESR1* coding region were weakly associated with gynaecological symptoms. The *ESR1* gene is of particular interest to breast carcinogenesis due to its known interaction with sex hormones as it codes for ERα which regulates signal transduction of oestrogen, which is often cited as the major mechanism behind tamoxifen related side effects. Since the biological effects of oestrogen are mediated primarily through binding to ERs, genetic variants within ER genes, including *ESR1* and *ESR2* have been the focus of multiple previous epidemiologic studies (Cai, Gao, et al., 2003; Cai, Shu, et al., 2003; Zheng et al., 2003, 2009).

The identified SNPs are associated with an increase in the risk of gynaecological symptoms and are located less than 50 Kb from the coding region of *ESR1* (Koš et al., 2001). None of the SNPs identified in this analysis have been previously associated with vaginal discharge or dryness or with irregular bleeding, nor have they shown to be in LD with the two most commonly studies polymorphisms of the *ESR1* gene *Xbal* and *Pvull*. However, *ESR1* has been previously associated with vaginal integrity, identifying that in response to physical stress, the lack of *ESR1* caused deterioration of the vaginal epithelium (Li et al., 2018).

Oestrogen signalling in the vagina initiates the formation of a barrier by maintaining the thickness of the vaginal epithelium and increasing the secretion of antimicrobial peptides, cytokines and chemokines (Dimitriadis et al., 2005; Wira et al., 2005; Ochiel et al., 2008). The epithelial lining of the vagina is also covered by a layer of glycoprotein-containing mucus important for protection against infectious agents (Carson et al., 1998; Hickey et al., 2011). Oestrogen signalling stimulates vaginal epithelial cells to produce glycogen, which is then metabolized by the native microflora (Moncla et al., 2016). In postmenopausal women, reduced oestradiol levels result in fewer epithelial cells and reduced glycogen content. When vaginal epithelial cells atrophy, coupled with a lack of lubrication, the vaginal tissue becomes vulnerable to physical damage and is subsequently susceptible to infection. Additionally, the composition of the vaginal microbiota often changes in postmenopausal women due to the reduction in the nutritional substrate for the microbiota (Hummelen et al., 2011). These changes in the vaginal environment can be treated with a topical synthetic oestradiol, supporting the evidence that oestradiol signalling plays an important role in the maintenance of the immune response and vaginal homeostasis (Lethaby et al., 2016). In the absence of *ESR1*, physical stresses could exacerbate the observed effects on the vaginal epithelium. A compromised epithelial layer is likely to alter vaginal homeostasis, eliciting an immune response that differs from an individual with normal *ESR1* function.

This analysis suggests that *ESR1* activity in the vaginal epithelial cells is required to maintain proper structural integrity of the vagina and immune response, both of which are necessary for protecting the vagina against physical damage and resetting the vaginal environment. Without performing functional analysis or PCR to determine whether these SNPs reduce the efficiency of *ESR1* it is difficult to say whether these SNPs have the same effect on vaginal tissue as previously associated SNPs. However,

a link between *ESR1* and vaginal health has been established and; therefore, *ESR1* should remain a target of interest when considering the adverse effects of endocrine therapy.

An interesting GWAS study by Styrkarsdottir et al. (2008) found associations between the *ESR1* locus and bone mineral density, which is well known to be under the control of oestrogens (Rizzoli and Bonjour 1997; Abdi et al., 2017). The impact of SNPs on the outcomes of musculoskeletal events could not be assessed in this current work. However, *ESR1* is one of the most intensively studied candidate genes for osteoporosis and a meta-analysis of more than 18,000 participants indicated that the most intensively studied polymorphisms in *ESR1* had no effect on bone mineral density but were associated with a risk of fracture (Ioannidis et al., 2004; Albagha et al., 2005). The SNPs at the *ESR1* locus described here have not been previously reported to be associated with bone mineral density, nor are they in substantive LD with two of the most widely studied polymorphisms in *ESR1*: *PvuII* and *XbaI*. This reinforces the belief that *ESR1* is a key factor in the increased risk of many of the side effects experienced during endocrine therapy given the importance of *ESR1* in the mediation of oestrogen signalling.

It has also been shown that *ESR1* status in women prescribed tamoxifen can help to predict how likely a women is to experience HFs (Jin et al., 2008). In premenopausal women, increased number of *ESR1 PvuII* and *XbaI* mutations was associated with higher baseline HF incidence compared with those who had other haplotypes (P = 0.0026). After four months, postmenopausal women with *ESR1 PvuII* and *ESR2* GG genotypes had 4.6 times increases in HF scores than other postmenopausal women (56 v 12; P = 0.0007). Women who had the *ESR2* AA genotype were significantly less likely to experience tamoxifen-induced HF than women who carried at least one *ESR2* G allele (hazard ratio, 0.26; (0.10 - 0.63); P = 0.001) (Jin et al., 2008). However, there is no evidence of *ESR1* or *ESR2* mutations affecting the incidence of HFs in this study.

This was the first study investigating SNPs with side effect outcomes in breast cancer prevention. The results from a large GWAS of 344,748 SNPs and their association with HFs; individual gynaecological symptoms: vaginal discharge, vaginal dryness, irregular bleeding and combined gynaecological symptoms were reported in this chapter. There were no associations at conventional genome-wide level of significance ($P < 5\times10^{-8}$); however, suggestive associations were discovered ($P < 5\times10^{-5}$). Several studies of

similar size have considered any association with P-values $< 1\times10^{-5}$ as being suggestive (Dudbridge and Gusnanto 2008; Amundadottir et al., 2010). Here, several suggestive associations with much stronger levels of significance were identified. The strongest associations, one insertion or deletion (rs147676656; P = 9.24×10^{-7}) and one SNP (rs62113694; P = 1.55×10^{-6}), were on incidence of vaginal discharge. This potential susceptibility locus resides within a segment of the genome devoid of annotated genes, located about 35 kilo base pairs (Kbp) downstream of the *PSG9* and upstream of the *CD177* genes respectively. After determining that the two markers are in high LD and are likely inherited together, the relationship between rs62113694 and genes was assessed and the LD pattern of the region plotted. 1000 genomes phase 3 data from the caucasian population CEU shows that rs62113694, which shows association with vaginal discharge in this study, is located in different LD blocks than both *CD177* and *PSG9*. Although both *CD177* and *PSG9* are interesting candidate genes, the large distance between both loci, together with the evidence that the significant SNPs lie in other LD blocks suggest that these SNPs are tagging independent associations and that the causal polymorphism is more likely to regulate gene expression rather than the protein structure of either *PSG9* or *CD177*. Enhancers are elements of the genome that regulate gene expression of nearby or distant genes and which can be located within gene deserts (Nair et al., 2009). Recent research suggests that polymorphisms in gene deserts could impact on disease by altering an enhancer element (Ghoussaini et al., 2008; Nair et al., 2009; Wasserman et al., 2010). Thus, it is possible that the identified causal variant, likely to be rs147676656 which is marked by rs62113694 is altering an enhancer element located in the gene desert influencing either *PSG9*, *CD177* or both.

PSG9 encodes a protein which is a member of the pregnancy-specific glycoprotein (PSG) family which are closely related to the carcinoembryonic antigen cell adhesion molecule (CEACAM) gene family which are both members of the immunoglobulin superfamily and are organised as a large gene cluster. The encoded protein is thought to inhibit platelet-fibrinogen interactions. *CD177* encodes a glycosyl-phosphatidylinositol (GPI)-linked cell surface glycoprotein that plays a role in neutrophil activation. The protein can bind platelet endothelial cell adhesion molecule-1 and function in neutrophil transmigration. Among the related pathways for both genes is cell surface interaction at the vascular wall thereby making both genes biologically relevant to the vaginal discharge phenotype.

Research has documented the role of PSG proteins in the development of some cancers, such as colorectal, but no evidence exists linking them to vaginal events (Salahshor et al., 2005; Jones et al., 2016; Yang et al., 2016). Several studies have indicated that PSGs have a proangiogenic function because their expression induces endothelial tube formation (Lisboa et al., 2011). Recently, members of the PSG family were demonstrated to activate the anti-inflammatory cytokines, transforming growth factor (TGF)-β1 and TGF-β2, which are responsible for the regulation of the SMAD signalling pathway (Colland et al., 2004; Moore and Dveksler 2014). These functions are important for the main function of these proteins which is to ensure a successful pregnancy. Pregnancy success requires that the maternal immune system does not attack the foetal trophoblast cells, which are in direct contact with maternal blood and express genes derived from both the mother and the father. In addition, during pregnancy major vascular adaptations are required to guarantee foetal growth and survival (Lisboa et al., 2011).

CD177 has not been previously linked to an increased breast cancer risk, but has been associated with other disease such as Kawasaki Disease and neutropenia (Eulenberg-Gustavus et al., 2017; Huang et al., 2019). *CD177* is also reported as part of a two-gene classifier system predicting clinical outcome of colon cancer patients (Dalerba et al., 2011). *CD177* is a neutrophil-specific receptor with clinical significance in autoimmune diseases. Beyond its role in immune conditions, *CD177* facilitates neutrophil endothelial transmigration involving heterophilic PECAM-1 interactions and catalytic membrane PR3 activity (Sachs et al., 2007; Bayat et al., 2010; Kuckleburg et al., 2012).

Finding the association of *PSG9* and *CD177* with vaginal discharge is entirely new. Interestingly, given that both genes are associated with immune response and are linked to both angiogenesis and cell adhesion both genes belong to a plausible pathway for vaginal discharge. Furthermore, tamoxifen agonism of ERβ may be responsible for the regulation of both these genes. Where ERα is primarily thought to mediate the proliferative effect of oestrogens in breast tissue, the function of ERβ in endometrial tissue is more complex. There is evidence that ERβ exerts antagonistic effects on ERα action, resulting for example in reduction of cellular proliferation. The growth-inhibitory action of ERβ and the observed decline of ERβ expression during carcinogenesis have raised the hypothesis that this receptor might act as a tumour suppressor in hormone-dependent tissues like the breast (Treeck et al., 2010; Williams and Lin 2013). In a

separate study, Lattrich et al. (2014) performed a transcriptome analysis confirming increased downregulation of *CEACAM* genes, a similar gene family to the *PSG* gene family after exposure to tamoxifen and ERβ agonists. In the presence of ERβ agonists *CD177* was also shown to be strongly induced (Lattrich et al., 2014). If the expression of these genes is altered in response to tamoxifen exposure, then they could represent a plausible pathway for vaginal discharge. It then follows that vaginal discharge could be a marker for increased ERβ agonism and thus a marker for increased proliferation-inhibition and reduced risk of breast cancer.

One of the most common weaknesses in GWAS and CG studies is that multi-loci models are not considered. The current approaches to performing GWAS are most successful if the common disease/common variant assumption holds and; therefore, complex outcomes should be considered a result of many small effects over a larger number of genes. Therefore, using models to determine a group of genes through which an effect can be wrought could be important in predicting the risk of side effects during endocrine therapy. However, care must be taken to avoid overfitting models and should avoid inclusion of SNPs with negligible effects on the outcome of interest.

In this analysis the development of models and subsequent PRS scores for side effects reported during the first six months of endocrine therapy were reported. The models and PRS are based on SNPs from the CG study and included 571 SNPs. It is believed that these are the first PRS models for prediction of side effects for breast cancer prevention. As each side effect potentially has a different aetiology, the aim was to produce a model for each that allowed for specific prediction of each side effect rather than a combined side effect model.

The LASSO model derived PRS for vaginal dryness, irregular bleeding and any gynaecological symptoms identified a threshold for each side effect at which the presence or absence of the side effect could be determined. The SNPs in the gynaecological symptom models were selected from an array of genes; however, all gynaecological models contained at least one SNP from either *ESR1* or *ESR2* among the SNPs with the largest ORs in each of the models. As previously discussed, disruption to sex hormones has often been proposed as a mechanism behind gynaecological side effects with different action of oestrogen at ERα and ERβ potentially resulting in different outcomes. Tamoxifen antagonism of ERα leads to a state of hypoestrogenism and thus increased risk of VVA of which vaginal dryness is a core component (Mac Bride et al.,

2010). However, tamoxifen agonism of ERα and ERβ may lead to a thickening of the vaginal lining and increase irregular bleeding. It is therefore unsurprising that *ESR1* and *ESR2* SNPs are potential risk SNPs for gynaecological side effects.

Despite the promising identification of PRS models for gynaecological side effects, these PRS must be interpreted with caution due to a low number of each of the gynaecological symptoms. From these models alone it is not possible to determine whether PRS models aid prediction of gynaecological symptoms in women taking tamoxifen for breast cancer prevention. The SNPs selected in the models provide interesting targets for future studies containing more cases, but further validation is required.

In contrast to the gynaecological models, the LASSO model for predicting HFs selected a single SNP. A lack of SNPs in the models supports the results of the CG study and indicates that no SNPs are strongly associated with HFs.

Whilst no statistically significant SNPs were found, it may be that the sample size was not sufficient to detect small changes in risk. A larger study focussed on oestrogen receptor genes may be able to determine more precise effects of SNPs in these genes and gynaecological and HF outcomes. Though the discriminatory ability of our PRS model has been inadequate for use as a standalone tool, PRS models have considerable potential in improving risk modelling. It has been demonstrated that PRS models aid in refining the risk stratification of individuals who are already at an increased risk of developing breast cancer (Mavaddat et al., 2015; Vachon et al., 2015; Cuzick et al., 2017; Evans et al., 2017). Whilst PRS have been combined with other breast cancer risk factors, such as breast density and family history, to improve prediction of breast cancer the benefit of PRS in side effect prediction appears limited.

The present study had some methodological limitations, and the findings should be interpreted with caution. The available sample size may be one reason why the GWAS analysis did not reach conventional levels of GWA significance. When analysing large numbers of markers, it is good to have a large number of study cases. However, in this instance there was a limited number of women genotyped and; therefore, the number of study participants was lower than the ideal number. It may be possible to combine tamoxifen prevention studies to increase the number of women genotyped and increase the power for finding SNP associations, but it would be important to ensure that standard phenotype criteria were used between the studies.

Additionally, the study type may also result in lowering the chances of finding associations between SNPs and phenotypes. There are two primary classes of phenotypes: categorical (often binary case/control) or quantitative. From the statistical perspective, quantitative traits are preferred because they improve power to detect a genetic effect, and often have a more interpretable outcome; in this case we have binary outcomes for side effect status. While some common diseases have good quantitative measures for outcome assessment other disease traits do not. There is a vast difference in measurement error associated with classifying individuals as either "case" or "control" versus precisely measuring a quantitative trait. However, despite the classification of case and control, GWAS of breast cancer have been enormously successful, implicating more than 300 new genes for the disorder (Mavaddat et al., 2019). So, while quantitative outcomes are preferred, they are not required for a successful study.

The issue of LD must also be accounted for when interpreting results. Whilst helping to address whether SNPs are linked and if the SNPs are also linked to gene, the presence of LD creates two possible positive outcomes from a genetic association study:

- The SNP influencing a biological system that ultimately leads to the phenotype is directly genotyped this is referred to as a direct association
- The second possibility is that the influential SNP is not directly typed, but instead a tag SNP in high LD with the influential SNP this is referred to as an indirect association. Because of these two possibilities, a significant SNP association from a GWAS should not be assumed as the causal variant and may require additional studies to map the precise location of the influential SNP.

The major weakness of this study is that the results and models have not been validated in an independent sample. However, for validation to be useful a variety of criteria must be met in the validation set. Replication studies should have a sufficient sample size to detect the effects of the variant allele. In most cases the SNPs and effects identified in the initial GWAS are likely stronger in the GWAS sample than in the general population (Zöllner and Pritchard 2007). This means that replication samples should ideally be larger to account for the over-estimation of effect size from the initial analysis. With replication, it is important for the study to be well-powered to identify spuriously associated SNPs where the null hypothesis is most likely true. Replication studies should be conducted in an independent dataset drawn from the same population

as the GWAS, in an attempt to confirm the effect in the GWAS target population. To confirm an SNP a similar effect should be seen in the replication set from the same SNP, or an SNP in high LD with the GWAS-identified SNP.

Another possible area for future work is in gene-environment interactions. A gene environment interaction is the influence on the expression of a trait as a result of the environment. In this case it would be interesting to assess the impact of SNPs in women who were former HRT users and current HRT users to determine whether the use of HRT alters the expression of genes and the potential outcomes of this expression.

5.5 Conclusions

In summary, this analysis reports the first GWAS of tamoxifen related side effects in women at high risk of breast cancer. This has pointed to several risk alleles and the implication of cell adhesion and oestrogen signalling pathways in the aetiology of vaginal discharge. These results should encourage further replication in large and independent population-based cohorts and then biological investigation to elucidate possible mechanisms. However, identification of any key SNPs with statistically significant associations with HFs or other gynaecological symptoms was unsuccessful. Results show that there is very little association between SNPs and side effects. In cases where SNPs in the CG or GWAS are identified as being below the statistical significance level the likelihood is that these are false positives. Multi-loci models show that most of the coefficients are small and therefore any effect of SNPs on side effects is relatively small. What has been identified in this study is there are no large effects of any SNP on any of the side effects. However, that is not to say that there aren't any effects, and it remains possible that this analysis has insufficient power to find them. Therefore, investigation of the role of SNPs identified in this exploratory dataset should be investigated in a much larger study to validate these results.

Ultimately, understanding key mechanisms via this research may lead to new endocrine therapy regimes and inform clinical practice to the issue of side effects during endocrine therapy. It is possible that, in the future, SNP genotyping may facilitate not only choice of treatment, but also tailoring of type, dose, and schedule of endocrine therapy according to a risk algorithm that incorporates clinical and pathologic features; the basis of which will be discussed in chapter 7.

Chapter 6: Investigation of baseline sex hormone concentrations and early side effects incidence in women from the IBIS-I and IBIS-II studies

6.1 Introduction

Sex steroids have a critical role in the aetiology of breast cancer. Evidence for this association includes the known relationships between reproductive factors, such as early age at menarche, parity and late age at menopause, and an increased risk of breast cancer (Hankinson, Colditz and Willett, 2004). Adipose tissue is an important risk factor in postmenopausal women where it is the major source of oestrogen (Szymczak et al., 1998; Simpson, 2003). Obese postmenopausal women have a higher risk of breast cancer. Studies show this association can be explained by the relationship between body mass index (BMI) and higher levels of endogenous hormones in particular oestrogens (Endogenous Hormones and Breast Cancer Collaborative Group, 2003; Anderson and Neuhouser, 2012; Collaborative Group on Hormonal Factors in Breast Cancer, 2012). It has been reported that a single measurement of endogenous hormones in postmenopausal women can predict hormone receptor positive breast cancer risk for up to 20 years (Zhang et al., 2013).

Oestrogens promote the proliferation of cells with existing mutations or alternatively increase the opportunity for mutations by increasing cell proliferation thereby contributing to tumour growth (Henderson and Feigelson, 2000). Meanwhile, androgens have been hypothesized to increase breast cancer risk either directly, by increasing cellular growth and proliferation, or indirectly, by their conversion to oestrogen in adipose tissues by the enzyme aromatase (Liao and Dickson, 2002; Secreto, Girombelli and Krogh, 2019).

Reduced concentrations of sex hormones are often hypothesised as the cause of com-

monly observed endocrine therapy side effects, such as hot flushes (HFs), gynaecological symptoms and arthralgia (Huang et al., 2010; Mac Bride, Rhodes and Shuster, 2010; Emond et al., 2011). Most women experience these side effects within the first 12 month of taking endocrine therapy (Powles et al., 1998; Veronesi et al., 1998; Cuzick et al., 2002, 2014). Therefore, measurement of sex hormone concentrations before starting endocrine therapy might provide a marker for assessment of side effect risk.

The focus of most studies evaluating sex hormones as risk factors of endocrine therapy side effects, particularly HFs, is oestrogen concentrations. Women experience HFs as a result of dramatically decreased oestrogen concentrations due to physiological conditions such as menopause or bilateral oophorectomy (Sturdee, 2008). Additionally, the known involvement of oestrogen in thermoregulatory homeostasis in the hypothalamus, and the successful use of HRT to reduce the numbers of HFs are also key indicators for the role of oestrogens in HF incidence (MacLennan et al., 2004; Utian, 2005). The association between HFs and lower oestrogen concentrations is fairly consistent (MacLennan et al., 2004; Miller et al., 2006; Dennerstein et al., 2007; Schilling et al., 2007c; Sturdee, 2008). Nonetheless, the mechanism through which altered oestrogen concentrations influence the incidence of HFs is yet to be determined fully.

The association of other sex hormones, SHBG, and HFs has previously been investigated in obese women aged 45-54 (Schilling et al., 2007c). SHBG, oestrone, free oestradiol concentrations and a higher ratio of total androgens to total oestrogens were significantly associated with HFs. Individually, androstenedione, testosterone, and DHEAS were not associated with HFs (Schilling et al., 2007c). Testosterone concentrations have been indirectly related to the incidence of HFs and gynaecological symptoms in postmenopausal women. Conversion of testosterone to oestrogen in the adipose tissue is the major source of oestrogens in postmenopausal women and therefore, low concentrations of testosterone could lead to a low concentration of oestrogen (Hankinson and Eliassen, 2007).

The majority of circulating testosterone is bound to either SHBG or to albumin, and a relatively small quantity is unbound (Hammond, 2016; Goldman et al., 2017). The unbound, free fraction is thought to be physiologically active, or bioavailable and therefore able to interaction with the androgen receptor (Laurent et al., 2016). Although SHBG binds to other steroid hormones, its affinity for other hormones is lower, and it accounts for a lower percentage of bound hormone (Gower and Nyman, 2000). SHBG

may have an indirect effect on menopausal symptoms due to binding to testosterone and oestradiol, lowering the active circulating fraction. Studies have shown that SHBG is involved in the pathway to development of HFs through the attenuation of circulating oestrogens and androgens. However, no direct influence of SHBG on HFs has been observed (Schilling et al., 2007b).

Dehydroepiandrosterone (DHEA) is a so-called precursor hormone converted by the body into oestrogens and androgens (Figure 6.1) (Davis, Panjari and Stanczyk, 2011). Supplementation with DHEA may increase oestrogen and testosterone levels in peri and postmenopausal women, decrease menopausal symptoms and improve general wellbeing and sexual function (Scheffers et al., 2015). DHEA-S is the sulphate conjugate of DHEA. The concentration of DHEA-S in the body is more stable and provides a good estimate of the amount of DHEA in circulation. Menopausal side effects may be alleviated by administration of DHEA. Studies have shown that the use of DHEA may increase oestrogen and testosterone levels in peri and postmenopausal women and ease their symptoms leading to improved quality of life (Davis, Panjari and Stanczyk, 2011; Pluchino et al., 2013). However, a recent review found no evidence that DHEA improves quality of life (Scheffers et al., 2015). There is some evidence which suggests an association with androgenic side effects: mainly acne and unwanted hair growth (Scheffers et al., 2015).Therefore, investigating DHEA-S concentrations as an alternative for DHEA concentrations may provide some insight into whether women with higher DHEA have fewer side effects and a better quality of life.

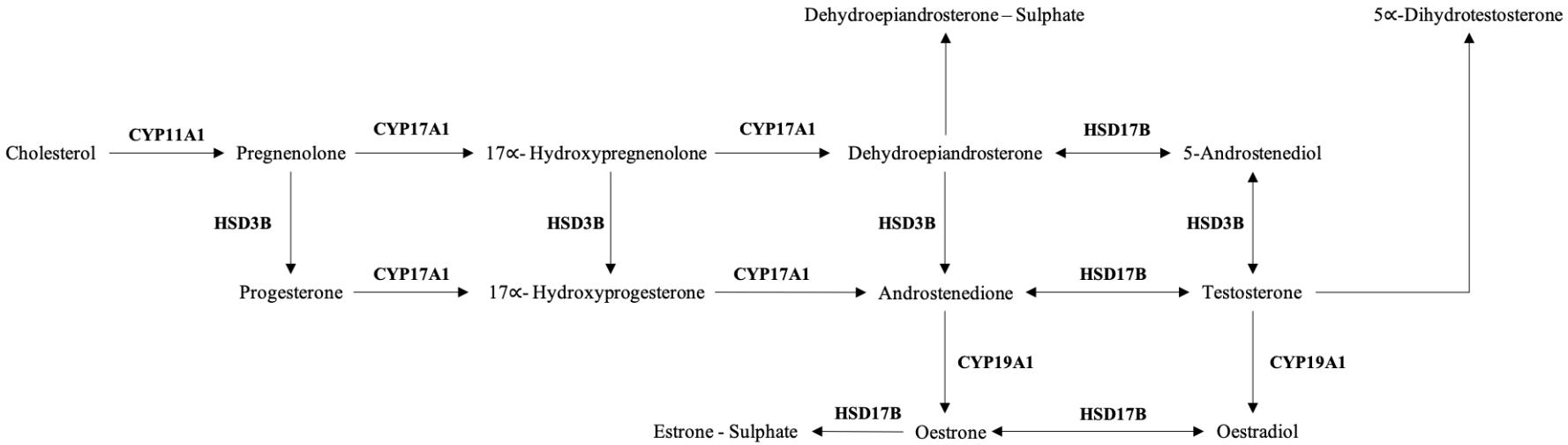


Figure 6.1: Sex steroid hormone biosynthesis and metabolism pathway

Other risk factors must also be considered as they can have a significant impact on sex hormone concentrations. HRT use increases the levels of circulating oestrogens, and androgens if a combined therapy is used. One of the consequences of HRT use is the exposure of the liver to extremely high concentrations of oestrogen and subsequently the alteration in production of several hepatic proteins. Of particular interest is the increased concentrations of SHBG in women who use oral oestrogen therapy, when compared with nonusers (Ropponen et al., 2005; Waaseth et al., 2008). Oral HRT is accompanied by elevations in SHBG concentrations; however, no evidence of increased SHBG concentrations has been observed for transdermal HRT (Serin et al., 2001; Samsioe, 2002). The physiological ramifications of altered SHBG production with HRT is only partially understood. But it remains plausible that an increase in SHBG with HRT use could lower circulating concentrations of bioavailable testosterone.

The association between BMI and circulating sex hormones, particularly oestradiol, have been established in the realm of breast cancer risk in postmenopausal women. An analysis of eight prospective studies have shown that breast cancer risk increased with increasing BMI ($P_{trend} = 0.002$), but that the increase in RR was substantially reduced after adjustment for serum oestrogen concentrations (Endogenous Hormones and Breast Cancer Collaborative Group, 2003). The risk of developing breast cancer was also moderately reduced after adjusting for SHBG. In contrast, adjustment for the androgens (androstenedione, DHEA, DHEAS, and testosterone) had little effect on the risk. These results support the hypothesis that the increase in breast cancer risk with increasing BMI among postmenopausal women is largely the result of the associated increase in oestrogens, particularly free oestradiol. The results also suggest that the association between BMI and side effects of endocrine therapy could be mediated through sex hormone disruption and should be further investigated (Endogenous Hormones and Breast Cancer Collaborative Group, 2003).

In a separate analysis of 13 prospective studies, concentrations of all sex hormones were significantly lower in older than younger postmenopausal women (Endogenous Hormones and Breast Cancer Collaborative Group, 2011). The largest reduction was observed in DHEAS concentrations; in contrast, SHBG concentrations were higher in older women (Endogenous Hormones and Breast Cancer Collaborative Group, 2011). In obese women all hormone concentrations, apart from SHBG, were higher compared to lean women, with the largest difference for free oestradiol.

Smokers of at least 15+ cigarettes per day had higher levels of all hormones than non-smokers, with the largest difference for androstenedione, testosterone and calculated free testosterone (Endogenous Hormones and Breast Cancer Collaborative Group, 2011). These associations may be due to cigarette smokers being, on average, thinner than non-smokers, and as such the authors note that differences in oestrogens were accentuated by the adjustment for BMI. Clear associations of smoking with circulating oestrogens have not been established in every study, but most have observed that some androgens were higher in smokers than in non-smokers (Baron et al., 1995; Manjer, Johansson and Lenner, 2005).

The effects of cigarette smoking has a clear anti-oestrogenic effect in women (Baron, Vecchia and Levi, 1990; Tankó and Christiansen, 2004). The number of menopausal symptoms, such as HFs, are reported more commonly by smokers (Staropoli et al., 1998). Changes in hepatic oestrogen metabolism induced by smoking are the likely cause for these observations (Kapoor and Jones, 2005). Smoking enhances the 2-hydroxylation pathway of oestradiol metabolism leading to an increase in 2-hydroxyestrogen concentrations (Michnovicz et al., 1986; Napoli et al., 2005). These compounds have minimal oestrogenic activity and are quickly removed from circulation. In smokers, concentrations of SHBG are higher. In circulation approximately 38% of oestrogens bind SHBG whilst a further 60% is bound to albumin, the remainder is free unbound fraction. Therefore, given the increase in oestrogen metabolism and an increase in SHBG concentrations lower concentrations of biologically active oestrogens are observed (Kapoor and Jones, 2005).

All hormones were higher in women who drank at least 20 g of alcohol per day compared to those who don't drink. The strongest positive association between sex hormone concentration and alcohol consumption was for DHEAS. In contrast, SHBG was lower in women who drank compared to non-drinkers. The association between alcohol consumption and oestrogens, androgens, and SHBG were emphasised after adjustment for BMI. Hormone concentrations were not strongly related to any of the reproductive factors tested including age at menarche, parity, age at first full-term pregnancy, nor was there any association with family history of breast cancer (Endogenous Hormones and Breast Cancer Collaborative Group, 2011).

While evidence for the association between sex hormones, general quality of life and breast cancer risk in postmenopausal women is well established this is not the case

for premenopausal women taking endocrine therapy. For premenopausal women, the evidence of association between breast cancer risk factors and sex hormone concentrations can be contradictory. This association is further complicated by the presence of fewer data and by large fluctuations in hormone concentrations during the menstrual cycle (Endogenous Hormones and Breast Cancer Collaborative Group, 2013). It is therefore necessary to review the relationship between breast cancer risk factors and sex hormones in premenopausal women separately from postmenopausal women.

In a recent review in premenopausal women, concentrations of seven hormones were investigated for their association with numerous breast cancer risk factors. Age was associated with decreasing concentrations of all sex hormones with concentrations lower in older women than younger women; however, SHBG was higher in older women compared to younger women (Endogenous Hormones and Breast Cancer Collaborative Group, 2013). Total oestadiol was inversely associated with BMI; however, free oestradiol was positively associated with BMI which was likely due to the strong inverse association of SHBG with BMI (Endogenous Hormones and Breast Cancer Collaborative Group, 2013).

For premenopausal women, many of the reproductive factors were not strongly associated with sex hormone concentrations. Although parity showed an inverse association with calculated free testosterone, is was not associated with any other sex hormones or SHBG and none of the hormones or SHBG was associated with age at menarche or family history of breast cancer (Endogenous Hormones and Breast Cancer Collaborative Group, 2013). Women who had previously used hormonal contraceptives had 7% lower concentrations of oestradiol and oestrone whilst androstenedione was reduced by 5%, and SHBG by 4% compared to women who had never used hormonal contraceptives (Endogenous Hormones and Breast Cancer Collaborative Group, 2013).

Determining the association between circulating sex steroid hormones, SHBG and side effects can provide further insight into aetiology and may ultimately help identify women who would be at high-risk of side effects during preventive endocrine therapy. The impact of sex hormones and SHBG on side effects has not previously been assessed in women at high-risk of breast cancer so this study represents a novel analysis. This analysis focusses on the association between testosterone, DHEAS, and SHBG and side effects reported by postmenopausal women during the first year of endocrine therapy. Given the action of anastrozole inhibiting the conversion of testosterone to

oestradiol, higher concentrations of testosterone may be a marker for poor conversion and; therefore, these women could be at higher risk of side effects due to lower oestrogen concentrations. Additionally, binding to SHBG inactivates testosterone and oestrogens, as such a high concentration of SHBG could also lead to an increased risk of side effects. The primary aim of this analysis is to determine whether concentration of these key sex hormones at baseline are linked to side effect outcomes during the first year of endocrine therapy. This analysis also investigates sex hormone concentrations in the placebo arm of the IBIS-I and IBIS-II trials which enables us to establish this effect in women without therapy and therefore whether the outcomes are linked to the action of therapy on sex hormone concentrations. The association between lifestyle factors and sex hormones are also considered.

What we already know

- Sex hormones and SHBG are associated with breast cancer in both pre and postmenopausal women.
- Concentrations of sex hormones and SHBG are affected by lifestyle factors such as BMI, HRT and smoking.
- Circulating concentrations of sex hormones and SHBG have been associated with menopausal symptoms such as HF, gynaecological symptoms and musculoskeletal events in postmenopausal women.

What this analysis adds

- Individual sex hormones at baseline as predictors of side effects in women randomised to tamoxifen, anastrozole or placebo as part of IBIS-I or IBIS-II.
- Association with other risk factors for side effects. The investigation of sex hormone concentrations with anthropometric or reproductive risk factor groups.

6.2 Methodology

6.2.1 Study population

This was a case-control study and used side effects reported within the first year of the trial which was in contrast to other analyses in this thesis. Case-control status was set for the first 12 months; however, during delays in testing due to COVID-19 it was decided that the 6-month follow up was a better time frame for other analyses. Unfortunately, samples had already been picked and a large number tested meaning a change in case definition was not possible. Therefore, in order to retain as many samples as possible in the analysis the 12-month side effect case control status has been retained.

Cases were women who reported hot flushes, gynaecological symptoms or musculoskeletal symptoms within the first 12-months of starting endocrine therapy. Controls were women who reported no side effects during the initial 12-months of endocrine therapy. Postmenopausal women were matched 1:1, matching for randomised treatment and age, plus or minus 1 year, as both of these factors are known to influence side effects and sex hormone concentrations.

Women enrolled on the IBIS-II were the primary population with a smaller secondary population of women enrolled on IBIS-I. Postmenopausal women were selected from either trial who had blood collected at study entry and for whom side effect status was known up to and including the 12-month follow-up visit. In addition, women from the IBIS-I study were excluded if they used HRT during the first 12 months of follow-up. Women selected for this study had similar baseline demographics as the overall study populations.

Premenopausal women from IBIS-I were excluded from the analysis to ensure that samples from both trials were of the same menopausal status and due to the wide variation in sex hormone concentrations which occur through the menstrual cycle.

6.2.2 Sample size calculation

Sample size calculations were based on reported data from Arizanović et al. (2018) who studied the changes in sex hormones in association with the experience of HFs in menopausal women (Arizanović et al., 2018). In addition, calculated effect sizes and total sample sizes are displayed alongside the mean and standard deviations of hormone concentrations in the case and control groups. Assumptions made are of an alpha (two-sided) error of 0.05 and a power of 0.8 (Table 6.1).

Calculations show a sample size of 750 patients would be needed to detect an association between sex hormone concentrations and HF outcomes at a power of at least 80% for testosterone, DHEA-S and SHBG. A sample size of this magnitude would be able to predict a statistically significant effect for oestradiol with a power of 41%.

In total 934 samples from IBIS-II and 400 samples from IBIS-I were selected. HFs, gynaecological symptoms (vaginal discharge, vaginal dryness and irregular bleeding), and joint or muscle pain were considered in this analysis. In IBIS-I, cases were women reporting HF or gynaecological symptoms within the first 12 months of follow-up. In IBIS-II women who reported HF, gynaecological or musculoskeletal side effects within the first 12 months were considered as cases. Conversely, controls were women who did not report any side effect during the first 12 months of therapy.

Table 6.1: Baseline hormonal concentrations of women with and without hot flushes (Arizanović et al., 2018)

Sex Hormone	**Hot flushes** **N=24**	**No Hot flushes** **N=12**	**Effect Size**	**Total Required Sample Size** **a = 0.05, 1-b = 0.8**
Oestradiol (pmol/L)	31.68 ± 14.52	33.24 ± 9.76	0.126	1905
Testosterone (nmol/L)	1.28 ± 0.71	1.15 ± 0.55	0.205	742
DHEA-S (nmol/L)	3.60 ± 2.49	1.88 ± 1.27	0.87	41
SHBG (nmol/L)	56.13 ± 37.78	73.96 ± 38.64	0.467	148
Oestradiol (pmol/L) (day)	28.17 ± 10.06	30.63 ± 13.56	0.206	727

To reduce bias in selection and measurement, samples were selected by an independent

statistician. Identities of cases and controls and study participant numbers remained blinded until after measurement of hormone concentrations was completed.

6.2.3 Exclusion of oestradiol from analysis

Oestradiol plays a key role in the development of side effects of endocrine therapy. However, in this analysis oestradiol was not considered as a marker due to the number of samples that would be required to identify a statistically significant difference based on available data. Secondly, best technique for measurement of sex hormones remains liquid chromatography – mass spectrometry (LC-MS), which is capable to accurately measure low concentration of sex hormones in serum samples. However, the cost of LC-MS for sample analysis was far in excess of available resources which precluded its use for oestradiol measurement in these samples (Faupel-Badger et al., 2010). Resources dictated that the best method available for determining the concentration of sex hormones in serum samples was enzyme linked immunosorbant assays (ELISA) (Professor M. Dowsett and Dr C. Reuter, personal communication, May 2019). In postmenopausal women the concentration of oestradiol is very low and therefore ELISA is unlikely to accurately or consistently measure the concentration of oestradiol in the samples (Vesper et al., 2014).

6.2.3.1 Suitability of ELISA for analysis of sex hormones

For the routine determination of individual or panels of steroids LC–MS/MS (Liquid Chromatography - Tandem Mass Spectrometry) is now the method of choice; while in research laboratories the combination of GC–MS (Gas Chromatography - Mass Spectrometry) and LC–MS/MS provides a cutting-edge research tool for endocrinology (Taylor, Keevil and Huhtaniemi, 2015). However, the ELISA method does offer considerable advantages over conventional chemical-analytical procedures. ELISA has excellent sensitivity to the specific hormone assay, it is fast, allows for high sample turnover, requires small sample volumes, has a wide range of applications, and requires no specialist skills. Additionally, the relatively cost of machinery required for testing, coupled with relatively fast measurement, and low quantification limit make ELISA an excellent method for sex hormone measurement (Manickum and John, 2015). However, there are disadvantages inherent in using the ELISA methods. Key among these

is that ELISA is not 100% specific and is vulnerable to cross-reactivity to other hormones within each assay. Additionally, ELISA is not suitable for small loads, and only one substance/analyte can be analysed at one time (Manickum and John, 2015).

However, a good and well-validated ELISA is not inferior to LC-MS for the detection of sex hormone concentrations. When selecting ELISA as an assay the choice was driven by the measurement performance dictated by the clinical need, the suitability of ELISA as an assay, and not by assay technology. Also, whilst GC- and LC-MS methods are major technical advancements, they do not provide a solution to all steroid measurements and analysis problems. Whilst this is also true for ELISA, the emphasis must be placed on the importance of assay validation and quality control, rather than focussing on methodological aspects of sex hormone measurement (Taylor, Keevil and Huhtaniemi, 2015).

6.2.4 Enzyme linked immunosorbent assay (ELISA) protocols

6.2.4.1 General principles of ELISA

The aim of ELISA analysis is to accurately measure the concentration of a target molecule in blood plasma or serum. Each ELISA plate was pre-coated with a capture antibody directed towards a unique antigenic site on the molecule of interest. Samples were added to each well and were immobilised by the capture antibodies on the well surface and were complexed with an enzyme conjugate. Immobilisation and ability to wash makes ELISA a powerful tool for measuring a specific analyte. A colorimetric substrate was added to each well and incubated to produce a complex, which determines the amount of antigen attached to the surface. The highly specific interaction between the antibody and the antigen is the key element of detection. A stop solution was then added, and the colour produced in each well measured to determine the concentration of the target molecule in each sample. The exact method for each target molecule is different. Section 6.2.5 details the protocols for each of the assays used.

6.2.4.2 Sample preparation and measurement

Determination of sex hormone concentrations were obtained via ELISA with no previous hormone extraction step. Inclusion of a purification step would allow the assay to be more specific than a direct non-extraction assay. However, with the selection of the correct buffer to release the sex hormones from albumin and SHBG, direct assays are a reliable assay for sex hormone measurement (Stanczyk et al., 2003; Rosner et al., 2013).

Each sample was run once with the exception of 20 samples, which were run in duplicate to assess intra- and inter-assay variation. Each plate of 80 samples was run alongside two blank control samples and two duplicates of six or seven calibration standards per plate which were used to create standard curves against which sample concentrations were read.

Although only a single baseline blood sample was analysed per study subject, a single blood sample was been found to reflect long-term hormone levels. In postmenopausal women, the correlation over a two to three year period for the steroid hormones ranged from 0.5 to 0.9 (Toniolo et al., 1994; Hankinson et al., 1995; Muti et al., 1996).

For analysis of testosterone and DHEA-S a direct ELISA assay was used. For measurement of SHBG concentrations a sandwich ELISA was used. The sections below summarise how each of the different assays perform.

6.2.4.3 Direct ELISA assay

The direct detection method requires a labelled primary antibody that reacts directly with the antigen. Direct detection is performed with an antigen that is directly immobilised on the assay plate in competition with the untagged target protein. The enzyme substrate reaction causes an inverse association as the strength of the signal is inversely correlated to the concentration of target protein (Figure 6.2). High detection readings are as a result of high conversion of substrate corresponding to low levels of bound target protein.

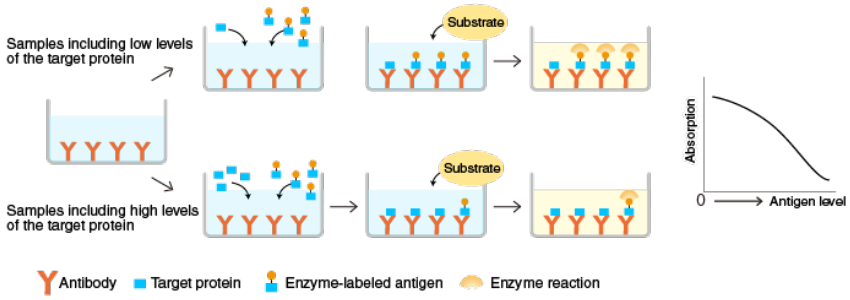


Figure 6.2: Illustration of direct ELISA method used for measurement of testosterone and DHEA-S concentrations (MBL Life Science, 2017).

6.2.4.4 Sandwich ELISA assay

The most powerful ELISA assay format is the sandwich assay where the analyte is measured bound between two primary antibodies - the capture antibody and the detection antibody. The sandwich format is used because it is sensitive and robust (Figure 6.3).

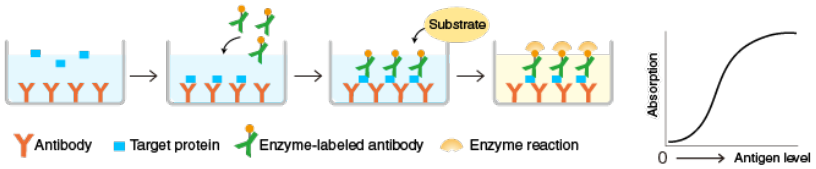


Figure 6.3: Illustration of the procedure for sandwich ELISA. This procedure was used for the determination of SHBG concentrations (MBL Life Science, 2017).

The secondary antibody has specificity for the target protein. In a sandwich ELISA, it is critical that the secondary antibody be specific for the detection primary antibody only (and not the capture antibody) or the assay will not be specific for the antigen. Generally, this is achieved by using capture and primary antibodies from different host species. In contrast to the direct ELISA detection, the measured absorption in sandwich ELISA increases as the concentration of target protein increases (Figure 6.3).

6.2.5 Sex hormone assay procedures

Storage

All ELISA kits, including required chemicals unless specified, were supplied by Abcam (Cambridge, UK) and contained wells for 96 tests per kit. Testosterone and DHEA-S

ELISA kits were stored at 4°C immediately after delivery and SHBG kits were stored at -20°C.

After initial collection, blood samples were frozen at -80°C. After sample identification samples were removed from storage but maintained at -80°C until testing. Multiple freeze-thaw cycles of samples were avoided whenever possible.

6.2.5.1 Testosterone ELISA protocol

Before staring the assay, all samples and reagents were equilibrated to room temperature (18 - 25°C) for one hour. Thawed samples were inverted several times prior to testing. Initial assay results indicated that no dilution of samples for testosterone assay was necessary with all readings within the standard calibration range.

Each run included a calibration curve consisting of a blank and six calibration fluids run in duplicate. Details of the calibration concentrations are shown in Table 6.2.

Table 6.2: Table of calibrators for the calibration curve to determine testosterone concentrations during each run

Calibrator	Concentration (ng/mL)	Quantity per well (μL)
Blank	0	25
1	0.2	25
2	0.5	25
3	1	25
4	2	25
5	6	25
6	16	25

25μL of each calibrator, control and sample were dispensed into appropriate individual wells. To avoid high background, samples or standards were added to the well before the addition of the antibody cocktail. 200μL Horse Radish Peroxidase (HRP) enzyme conjugate was then added into each well and mixed thoroughly for 10 seconds ensuring complete mixing. All samples were mixed thoroughly and gently to avoid foaming or bubbles when mixing or reconstituting components. This was to improve accuracy of each reading and reduce variation between duplicates. The plate was then incubated without covering for 60 minutes at room temperature (18.5°C) on a plate shaker set at 300 rpm. Following incubation, the plate was briskly shaken to remove the well content and the plate blotted with absorbent paper. The wash solution, 1170mL of deionized water was added to 30mL of 40X concentrated wash solution (Abcam, Cam-

bridge UK), to make a final volume of 1200mL. Wells were rinsed three times with diluted wash solution (400μL per well). To finish the wash procedure wells were struck sharply on absorbent paper to remove residual droplets. Complete removal of all solutions and buffers during wash steps was necessary to minimize background. 200μL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution was added to each well avoiding foaming or bubbles when mixing to ensure more accurate measuring. The plate was sealed and incubated for 15 minutes at room temperature on a plate shaker at 300rpm. The enzymatic reaction was stopped by adding 100μL of stop solution to each well in the same order as the TMB substrate solution was added avoiding foaming or bubbles when mixing components. The optical density (OD) of each well at 450 $\pm$ 10nm was then determined with a microtiter plate reader immediately after adding the stop solution.

6.2.5.2 Dehydroepiandrosterone sulphate ELISA protocol

Before starting the assay, all samples and reagents were equilibrated to room temperature (18 - 25°C) for one hour. Thawed samples were inverted several times prior to testing.

Samples for DHEA-S assay all require a 1:50 dilution prior to analysis. 20μL of each sample was added to 980μL of serum diluent and mixed thoroughly prior to running the assay. After dilution all readings were within the standard calibration range.

Each run included a calibration curve consisting of a blank well for measuring background, two control samples and six calibration fluids run in duplicate. Details of the calibration concentrations are shown in Table 6.3.

Table 6.3: Table of calibrators for the calibration curve to determine testosterone concentrations during each run

Calibrator	Concentration (μg/mL)	Quantity per well (μL)
Control A		30
Control B		30
0	0	30
1	0.1	30
2	0.4	30
3	1	30
4	2	30
5	10	30
Blank well	0	0

30μL of each calibrator, control and sample were dispensed into appropriate individual wells. To avoid high background, samples or standards were added to the well before the addition of the antibody cocktail. A single blank well containing no sample, control or calibrator was kept for analysis of background. 100μL HRP enzyme conjugate was then added into each well except the blank well. The plate was then incubated by covering with a foil for 60 minutes at 37°C. Following incubation, the plate was briskly shaken to remove the well content and the plate blotted with absorbent paper. The wash solution, 450mL of deionized water was added to 50mL of 10X concentrated wash solution, to make a final volume of 500 mL as specified by the kit supplier. Wells were rinsed five times with diluted wash solution (350μL per well). Soak time between wash cycles was 20 seconds. To finish the wash procedure wells were tapped sharply on absorbent paper to remove residual droplets. Complete removal of all solutions and buffers during wash steps is necessary to minimize background and to improve precision of the assay. 100μL of TMB substrate solution was added to each well avoiding foaming or bubbles when mixing to ensure more accurate measuring. The plate was sealed and then incubated for 15 minutes at room temperature in the dark. The enzymatic reaction was stopped by adding 100μL of stop solution to each well in the same order and rate as the TMB substrate solution was added. The plate was then shaken gently at 300rpm for five minutes. The OD of each well was then determined at 450nm with a microtiter plate reader immediately after the five minutes of shaking.

6.2.5.3 Sex Hormone Binding Globulin ELISA protocol

Reagent Preparation

Prior to starting the assay all reagents were equilibrated to room temperature (18-25°C). Due to instability of prepared solution only as much reagent as needed for the run was prepared.

Lyophilized recombinant Human SHBG standard (0.002 nmol)

The SHBG standard solution was prepared no more than two hours prior to the experiment. 1mL of sample diluent buffer was added to create a 2nmol/L of Human SHBG stock solution. The solution was kept at room temperature for 10 minutes and mixed thoroughly.

Biotinylated anti-Human SHBG antibody

The solution was prepared no more than two hours prior to the experiment. Biotinylated anti-Human SHBG antibody was diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

Avidin-Biotin-Peroxidase Complex (ABC)

The solution was prepared no more than one hour prior to the experiment. Avidin-Biotin Peroxidase Complex (ABC) was diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

The ABC working solution and TMB colour developing agent were kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly to avoid marginal effect of plate incubation due to temperature difference; the reaction may be stronger in the marginal wells.

Phosphate Buffered Saline (0.01M) (PBS)

0.01M Phosphate buffered saline (PBS) was used for washing the ELISA plate to remove unbound proteins or antibodies which would therefore raise the background and reduce accuracy. PBS was supplied by Severn Biotech (Kidderminster, UK).

The sample diluent buffer, antibody diluent buffer, ABC diluent buffer, TMB chromogenic solution and the TMB stop solution were all ready-to-use as supplied.

Standard preparation

Starting with the 2nmol/L stock solution 300μL was pipetted into 300μL of sample diluent buffer to form a 1nmol/L standard solution. This process was continued to make five further standards of 0.5nmol/L, 0.25nmol/L, 0.125nmol/L, 0.0625nmol/L and 0.03125nmol/L respectively. Standard solutions were used within two hours. Table 6.4 details standard solutions and preparation.

Table 6.4: SHBG calibration standards and volumes of each solution required to make each standard

Tube	Volume of Tube	Volume of Diluent buffer (μL)	Concentration (nmol/L)
1	300μL of 2 nmol/L stock solution	0	2
2	300μL of tube #1	300	1
3	300μL of tube #2	300	0.5
4	300μL of tube #3	300	0.25
5	300μL of tube #4	300	0.125
6	300μL of tube #5	300	0.0625
7	300μL of tube #6	300	0.03125

Sample preparation

Previous analysis showed that SHBG concentrations were likely to be in in the region of 50-100nmol/L (Burger et al., 2000; Maggio et al., 2008; Arizanović et al., 2018).

For accurate measurement, high target protein concentration (20nmol/L - 200nmol/L) samples were diluted 1:100 with sample diluent buffer. All samples should be mixed thoroughly and gently, and multiple freeze/thaw of samples should be avoided.

Assay procedure

100μL per well of the 2nmol/L, 1nmol/L, 0.5nmol/L, 0.25nmol/L, 0.125nmol/L, 0.0625nmol/L and 0.03125nmol/L Human SHBG standard solutions were aliquoted into the pre-coated 96-well plate. Additionally, 100μL of the sample diluent buffer was added to the control well to act as a negative control. 100μL of each properly diluted sample of human serum was added to each remaining empty well. The plate was sealed with an adhesive cover and incubated at 37°C for 90 minutes. Plates were properly sealed or covered during incubation steps to prevent evaporation of reagents from the wells. After 90 minutes the cover was removed, plate content discarded, and the plate blotted onto paper towels. At no time were the wells allowed to completely dry as this would result in inactivation of the active components on plate.

100μL of biotinylated anti-human SHBG antibody working solution was added into each well, the plate sealed with a new adhesive cover and incubated at 37°C for 60 minutes. The plate was then washed three times with 350μL 0.01M PBS each time letting the washing buffer stay in the wells for one minute. Washing was performed using an automated washer (TECAN, Männedorf, Switzerland). Prior to the first wash the well contents were aspirated without touching the side walls. After the final cycle the washing buffer was discarded, and the plate blotted onto paper towels or other absorbent material. Complete removal of all solutions and buffers during wash steps is necessary to minimize background.

After washing 100μL of newly prepared ABC working solution was added to each well. The plate was sealed with a new adhesive cover and returned to the incubator at 37°C for 30 minutes. Following incubation, the plate was washed five times with 0.01M PBS and each time let washing buffer stay in the wells for 1-2 minutes as per the previous wash cycle. After the final cycle the washing buffer was discarded, and the plate blotted with paper towel.

90μL of prepared TMB color developing agent was added to each well, the plate sealed and incubated at 37°C in dark. After 20 minutes 100μL of prepared TMB stop solution was added to each well and the colour changes into yellow immediately. The O.D.

absorbance at 450 nm was measured using a in a microplate reader (Dynex Opsys MR, Worthing, UK) within two minutes of adding the TMB Stop Solution.

6.3 Statistical principles and considerations

Baseline characteristics for all patients are shown by study and treatment arm. Differences between the trial arms for each characteristic were also be assessed. Baseline characteristics reported were age, BMI, smoking history, parity, HRT use, age of menarche, age of menopause, type of menopause, and time since menopause.

The analysis was based on women who had reported side effects during the first 12 months (cases) and compared them against women to those who had not experienced any side effects over the same period (controls).

6.3.1 Calibration curves and determination of sample hormone concentrations

6.3.1.1 Testosterone

The average absorbance values for each of the controls, calibrators and patient samples were calculated. A calibration curve was then constructed plotting the mean absorbance value of each calibrator against its known concentration. The concentration of each sample was then determined from the calibration curve using the average absorbance value from the tested sample.

The best curve fit for the calibration data was a four-parameter logistic model. It was important to gain the best fit model and to apply the model to each of the calibration curves. Using a four-parameter logistic curve, the concentration of each of the samples were read directly from the calibration curve. Samples with concentrations higher than that of the highest calibrator were further diluted or reported as >16ng/mL. For the calculation of the concentrations, this dilution factor was taken into account.

6.3.1.2 Bioavailable testosterone (bioT)

A large proportion of testosterone is tightly bound to SHBG and is therefore physiologically inactive. A further 20–30% of testosterone is bound loosely to albumin and only a small percentage of testosterone is free in the serum. The albumin-bound and free fraction are physiologically available to the body tissues and is known as bioavailable testosterone (bioT).

Concentrations of SHBG vary widely in healthy women and are related to variables such as diet, BMI, and age (Maggio et al., 2008). Therefore, measurement of bioT is valuable for correct assessment of the bioactive fraction of testosterone.

BioT (pmol/L) was calculated from serum concentrations of total testosterone and SHBG using the method of Morris et al. (2004) (Equation 6.1) (Morris et al., 2004). This method is based on the concentration of SHBG, and total testosterone as determined in a sample of normal men (Morris et al., 2004; De Ronde et al., 2006). Although the algorithm was developed based on measurements in men, De Ronde et al. (2006) repeated the analyses in 415 premenopausal women and obtained results similar to those for men (De Ronde et al., 2006). In most publications no arguments are given for the choice of a particular method for calculating bioT although, choosing a particular set of constants will obviously influence results of calculated bioT concentrations and thus might influence results of analyses. Moreover, it is doubtful whether algorithms composed and validated in one laboratory can be applied to samples from an unrelated laboratory that uses different assay techniques; however, as determining bioT concentrations from ELISA testing is not possible, algorithms offer the best alternative to physical testing (De Ronde et al., 2006).

$$bioT(pmol/L) = (e^{(-0.266+0.955xln[TT]-0.228xln[SHBG])}) \times 1000 \qquad (6.1)$$

Equation 6.1: Equation for the calculation of bioavailable testosterone (bioT)

6.3.1.3 SHBG

The standard curve can be plotted as the relative $O.D_{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X) (Equation 6.2).

$$(Relative\, O.D_{450}) = (O.D_{450}\, of\, each\, well) - (O.D_{450}\, of\, Zero\, well). \quad (6.2)$$

Equation 6.2: Equation for the calculation of SHBG concentrations

The SHBG concentration of the samples can be interpolated from the standard curve. The samples measured were diluted, therefore, the $O.D_{450}$ of each well was multiplied by 100, the dilution factor, to obtain the sample serum concentration before dilution.

6.3.1.4 DHEA-S

The mean background subtracted absorbance for each point of the standard curve and each sample was calculated. The mean value of absorbance of the standards was plotted against the concentration of each sample. The best-fit curve, a four-parameter logistic curve, was then plotted through the points. The values of the samples were interpolated from the standard curve to obtain the corresponding values of the concentrations expressed in μg/mL.

6.3.2 Coefficient of variability (CV or %CV)

The high-quality quantification data achievable with ELISA is advantageous compared to more qualitative methods like immunohistochemistry and Western blotting. However, demonstrating that assays are well run, and measurements are precise and accurate is crucial. Reliability of assays was assessed through a standardised method, coefficient of variability (CV).

The CV is a numerical ratio which describes the level of variability within a population independent of the absolute values of the observations. As the absolute values measured during ELISA vary, a standardised approach such as %CV is required to assess the precision of a laboratory technique. CV was calculated by dividing the standard deviation of a set of measurements by the mean of the set which is then expressed as a percentage of variation to the mean. In ELISA data interpretation, %CV can highlight inconsistencies among sample replicates; directly reflecting the performance of the assay.

Two types of %CVs were used to express the precision of immunoassay results: intra-

assay CV and inter-assay CV. Intra-assay CV is a measure of the variance between data points within an assay; sample replicates within the same plate. Inter-assay CV is a measure of the variance between runs of sample replicates on different plates that can be used to assess plate-to-plate consistency. Desired thresholds for overall reliability of immunoassay results were set at, inter-assay %CV <15% and intra-assay %CV <10%. The causes of high %CV in ELISAs is varied. Human technical error such as inaccurate pipetting technique, splashing of reagents between wells, drying out of the wells, or inconsistent sample handling (variability due to freeze-thaw cycles among samples) are potential causes. Additionally, high %CV can be the result of machine error such as usage of uncalibrated automated machine pipettes, uncalibrated plate readers, and inappropriate plate reader software settings to analyse samples. Lastly, plate, sample, and reagent contamination can lead to a high %CV. Cross-contamination between reagents can occur from handling errors leading to bacterial or fungal contamination of samples and reagents derived from compromised sterility.

During the assay procedure, every opportunity to reduce %CV was taken. During incubation, plates were incubated away from drafts to minimise temperature variation across the plate and ensure even heat distribution allowing the analyte to bind in an equivalent manner across the plate. During incubation, particularly at temperatures above room temperature plates were always covered to prevent wells from drying out. The wash protocol was run according to kit manufacturer guidelines and maintained from plate-to-plate to minimise inter-assay CVs.

Results in Table 6.5 present the mean CV for all samples. Of the 21 serum samples measured for testosterone CV, 18 had a CV under 10% for intra-assay variance and 19 and 17 had variance under 15% for inter-assay run one and two respectively. Overall, the CV for both intra- and inter-assay variance for testosterone were below the desired thresholds and indicate that assays were accurate and repeatable. Twenty samples were run in duplicate to assess both inter- and intra-assay variation for DHEA-S due to an additional calibration standard required on each plate. Eighteen of the tested serum samples were below the 10% CV for intra-assay variance and 16 and 15 samples were below the 15% threshold for inter-assay run one and two respectively. The overall CV for both intra- and inter-assay variance was below the desired threshold for DHEA-S measurements. Twenty serum samples for assessment of SHBG CV were run but five wells failed; therefore, CV was calculated over the remaining 15 samples. Eight

of the samples had an individual CV below 10% with a further two samples within 0.5%. Overall, intra-assay CV was above the desired 10% threshold. Whilst this is not ideal, samples with a high concentration of SHBG have the largest CV and are the main cause behind the increased CV. The high concentrations of SHBG in these samples approached the method's upper limit of detection. The high CVs at these concentrations demonstrate the expected loss of precision when quantifying samples at the extreme ends of the assay's range. This likely points to an error in dilution and highlights the importance of ensuring good dilution. Ten and 11 serum samples have a CV below the desired 15% inter-assay variation cut off for run one and two respectively. Each of the CV for inter-assay variance are below the 15% desired threshold.

Table 6.5: Coefficient of variance for intra- and inter-assay variation of testosterone, SHBG and DHEA-S ELISA kits

Sex Hormone	Number of samples	Intra-assay	Inter-assay 1	Inter-assay 2
Testosterone	21	6.71	8.91	9.63
SHBG	15	12.95	13.36	11.52
DHEA-S	20	6.97	11.12	11.82

6.3.3 Analysis of sex hormone association with side effects and baseline factors

Hormone and SHBG concentrations were ln-transformed to normalise distributions. If required hormone values recorded as zero were given a small non-zero value to allow for log transformation. Odds ratios and 95% Confidence intervals were reported for each of the aims. Statistical significance was at the 0.05 level and all P-values were two-sided.

Concentrations of sex hormones and SHBG on continuous scale were compared to side effect outcomes using conditional logistic regression and ORs and 95% confidence intervals were reported. Subsequently, covariates which may impact sex hormone concentrations were added to form an adjusted logistic regression model. Covariates included were BMI, HRT use history, smoking history, and prior hysterectomy.

Subsequently, sex hormone and SHBG concentrations were analysed by quintile for their association with side effects. Quintiles for each sex hormone and SHBG were calculated and the lowest quintile used as the reference for risk comparison. Heterogeneity

was assessed by Wald test for trend across the quintiles.

As a secondary outcome, correlations between sex hormone concentrations and risk factors including age ($\leqslant$55, >55), BMI (<25, 25-30, >30), HRT use (never, ever), smoking history (never, current, ever), parous (yes, no), type of menopause (natural, oophorectomy, hysterectomy and hysterectomy with oophorectomy), age at menarche ($\leqslant$14, >14), age of menopause ($\leqslant$50, >50) and time since menopause ($\leqslant$5, 5-10, >10) were investigated. Prior to baseline factor assessment, the impact of randomised treatment was assessed. Additional analysis of age, BMI, age of menarche, age of menopause and time since menopause as continuous variables was performed to assess the incremental effects of these variables. Variance between the categories listed above was assessed and trends between sex hormone concentrations and risk factors were reported. Distributions of each sex hormone for each sub-group were assessed for normality using Shapiro-Wilk test for normality. Kruskal-Wallis rank sum tests were used to assess the differences in sex hormone concentrations and each of the baseline factors as at least one sub-group was found to violate the assumption of normality. Where Kruskal-Wallis was significant and baseline factors had more than two levels, Dunn's *post hoc* test with Hochberg adjustment for multiple comparisons was used to determine between which categories the significant difference occurred.

6.4 Results

6.4.1 Baseline characteristics

A total of 400 women from the IBIS-I trial had baseline blood samples tested for sex hormone concentrations. 63.5% (N = 254) of women in the IBIS-I group were randomised to tamoxifen and 36.5% (N = 146) were randomised to placebo (Table 6.6).

In the tamoxifen group, the median age was 53 (IQR = 50.0 – 58.0) and the median BMI was 26.3 (IQR = 23.5 – 29.7). 65.7% (N = 167) had never used HRT and 46.5% (N = 118) had never smoked (Table 6.6). Median age at menopause was 47.0 (IQR = 38.0 – 50.0) and 64.2% had a natural menopause. 35.8% (N = 91) were hysterectomised with 62.6% (N = 57) retaining both ovaries. 24.2% (N = 22) and 12.1% (N = 11) of women in the tamoxifen group had hysterectomy with single and bilateral oophorectomy respectively (Table 6.6).

Women randomised to placebo had a median age of 53 (IQR = 50.0 – 58.0) and a median BMI 25.7 (IQR = 23.4 – 28.7). 63% (N = 92) had never used HRT and 47.9% (N = 70) had never smoked. (Table 6.6). Median age at menopause was 48.0 (IQR = 44.0 – 50.0) and 41.1% (N = 60) had undergone a hysterectomy with 63.3% (N = 38) retaining both ovaries. 23.3% (N = 14) and 11.7% (N = 7) of women in the placebo group had hysterectomy with single and bilateral oophorectomy respectively (Table 6.6).

Table 6.6: Baseline characteristics of all participants from the IBIS-I and IBIS-II trials included in the sex hormone analysis

	IBIS-I Participants (N = 400)				**IBIS-II Participants (N = 968)**			
	Tamoxifen (N = 254 (63.5%))		**Placebo (N = 146 (36.5%))**		**Anastrozole (N = 580 (59.9%))**		**Placebo (N = 388 (40.1%))**	
Cases:Controls	127 : 127		73 : 73		290 : 290		194 : 194	
Age (years) Median (IQR)	53.0 (50.0-58.0)		53.0 (50.0-58.0)		59.0 (55.0 - 63.0)		57.0 (53.0 - 62.0)	
HRT use								
Never	167	(65.7)	92	(63.0)	293	(50.5)	191	(49.2)
Ever	87	(21.8)	54	(37.0)	286	(49.3)	197	(50.8)
BMI (kg/m^2) Median (IQR)	26.2 (23.5-29.7)		25.7 (23.4-28.7)		27.3 (24.4 - 30.9)		27.0 (24.5 - 31.6)	
< 25	105	(41.3)	59	(40.4)	177	(30.5)	113	(29.1)
25-30	79	(31.1)	56	(38.4)	214	(36.9)	144	(37.1)
> 30	61	(24.0)	23	(15.8)	172	(29.7)	121	(31.2)
Smoking history								
Never	118	(46.5)	70	(47.9)	329	(56.7)	236	(60.8)
Former	95	(37.4)	43	(29.5)	194	(33.4)	105	(27.1)
Current	41	(16.1)	33	(22.6)	55	(9.5)	43	(11.1)
Parous	219	(86.2)	128	(87.7)	504	(86.9)	313	(80.7)
Age of menarche (Years) Median (IQR)	13.0 (12.0-14.0)		13.0 (12.0-14.0)		13.0 (12.0 - 14.0)		13.0 (12.0 - 14.0)	
Age of menopause (Years) Median (IQR)	47.0 (38.0-50.0)		48.0 (44.0-50.0)		50.0 (45.0 - 52.0)		49.0 (45.0 - 52.0)	
Time since menopause (Years) Median (IQR)	8.0 (3.0-19.0)		5.0 (2.0-11.0)		9.0 (4.0 - 15.0)		9.0 (4.0 - 14.0)	
< 5	57	(22.4)	43	(29.5)	154	(26.6)	102	(26.3)
5 - 10	42	(16.5)	25	(17.1)	173	(29.8)	125	(32.2)
> 10	64	(25.2)	26	(17.8)	242	(41.7)	158	(40.7)

Of the 968 women from the IBIS-II trial 59.9% (N = 580) were randomised to anastrozole and 40.1% (N = 388) were randomised to placebo (Table 6.6).

For women randomised to anastrozole the median age was 59 (IQR = 55.0 – 63.0) and the median BMI 27.3 (IQR = 24.4 – 30.9). 50.5% (N = 293) had never used HRT and 30.5% (N = 177) had never smoked (Table 6.6). Median age at menopause was 50.0 (IQR = 45.0 – 52.0) and 29.8% (N = 173) had a previous hysterectomy with 65.3% (N = 113) retaining at least one ovary. 34.7% (N = 60) of hysterectomised women in the anastrozole group had bilateral oophorectomy (Table 6.6).

Women randomised to placebo had a median age of 57 (IQR = 53.0 – 62.0) and a median BMI 27.0 (IQR = 24.5 – 31.6). 49.2% (N = 191) had never used HRT and 60.8% (N = 236) had never smoked. (Table 6.6). Median age at menopause was 49.0 (IQR = 45.0 – 52.0) and 68% had a natural menopause. 35.6% (N = 138) were hysterectomised with 58.7% (N = 81) retaining at least one ovary. 41.3% (N = 57) of hysterectomised women in the placebo group had bilateral oophorectomy (Table 6.6).

Concentrations of each sex hormone was derived from the OD recorded for each sample well compared to the calibration standard for each plate via the methods described in section 6.3.1. Where more than one measurement was available for a sample the mean of the measurements was taken. Concentrations of hormones were then converted to SI units (nmol/L) where required.

Concentrations of testosterone and DHEA-S were calculated for each sample with no known failures. However, SHBG concentrations were unavailable for 108 samples due to failures. For 28 samples across four plates this was due to well readings falling below that of the blank well. In addition, the calibration standards for one plate failed and therefore all of the samples on this plate failed testing (N = 80). This plate was re-analysed and concentrations of these samples were an order of magnitude larger than samples from all other plates. Therefore, concentrations for these samples were not recorded. BioT concentrations were only available for women with SHBG hence analyses with both SHBG and bioT were performed with a reduced number of samples. Only one IBIS-I sample had a failed SHBG and bioT measurement. This sample, and its matching case were removed prior to analysis. For women from the IBIS-II trial, four women were missing data for all four sex hormone concentrations. These women and the matched cases or controls were removed from the analysis. A further 103 women

were missing SHBG and bioT measurements. For these sex hormones women with missing values were excluded from the analysis as were the matched case or control. Therefore, for SHBG and bioT 778 women were included in the analysis.

6.4.2 IBIS-I results

6.4.2.1 Correlations between hormones

In women randomised to tamoxifen, concentrations of all the androgens were positively correlated with each other (Figure 6.4, Panel A). Partial correlation coefficients ranged between 0.56 for the correlation between ln-DHEA-S and ln-bioT and 0.91 between ln-testosterone and ln-bioT. Ln-SHBG was inversely correlated with ln-bioT (r = -0.54) and weakly inversely correlated with ln-T (r = -0.13) and ln-DHEA-S (r = -0.12) (Figure 6.4, Panel A). All values are statistically significant below P <0.01 except the correlation between ln-SHBG and ln-T (P = 0.04) and the partial correlation between ln-SHBG and ln-DHEA-S (P = 0.06).

Similar correlations between androgens were seen in women randomised to placebo. Ln-bioT was strongly correlated with ln-T (r = 0.90) and ln-DHEA-S (r = 0.52) (Figure 6.4, Panel B). The correlation between ln-testosterone and ln-DHEA-S was also similar to the tamoxifen group (r = 0.58). ln-SHBG was strongly inversely correlated with ln-bioT (r = -0.57) and was partially inversely correlated with ln-T (r = -0.15) and ln-DHEA-S (r = -0.07) (Figure 6.4, Panel B). All values are statistically significant below P <0.01 except the partial correlation between ln-SHBG and ln-T (P = 0.07) and no significant correlation between ln-SHBG and ln-DHEA-S (P = 0.38).

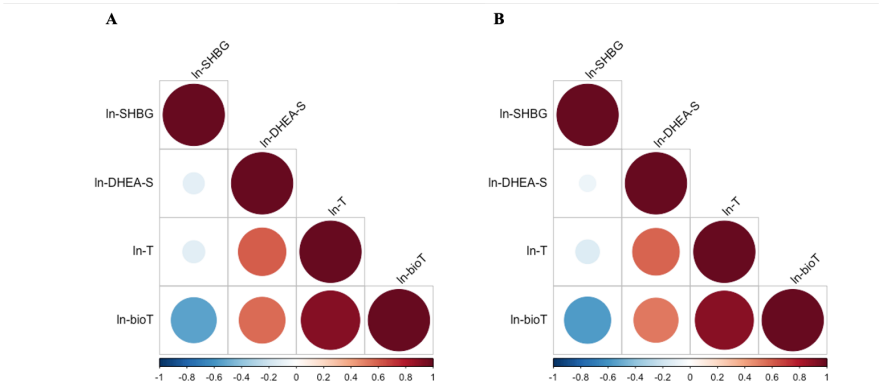


Figure 6.4: Correlations between hormones and SHBG. Panel A: Women randomised to Tamoxifen. Panel B: Women randomised to placebo. Abbreviations: DHEA-S = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin; T = Testosterone; bioT = bioavailable testosterone; ln = ln-transformed.

6.4.2.2 Sex hormone concentrations in the tamoxifen and placebo arms of IBIS-I

SHBG and bioT concentrations were both significantly different between case and controls in the tamoxifen arm and the placebo arm ($P < 0.01$). Circulating SHBG concentrations were significantly lower in the cases than the controls for both tamoxifen and placebo groups. In contrast, concentrations of bioT were significantly higher in cases compared to controls (Table 6.7). No statistically significant difference in concentrations of testosterone or DHEA-S were observed between cases and controls in either the tamoxifen or placebo arm (Table 6.7).

Table 6.7: Means and standard deviations (SD) of ln-transformed circulating concentrations of sex hormones and SHBG in postmenopausal women by randomisation and case control status. P-values are Kruskal-Wallis test with Dunn's post-hoc adjustment for multiple comparisons P-values

	Tamoxifen			Placebo		
	Cases Mean (SD) (N = 127)	Controls Mean (SD) (N = 127)	P-value	Cases Mean (SD) (N = 73)	Controls Mean (SD) (N = 73)	P-value
Testosterone (nmol/L)	0.76 (0.40)	0.69 (0.42)	0.30	0.66 (0.37)	0.53 (0.35)	0.14
DHEA-S (nmol/L)	7.99 (0.77)	8.01 (0.71)	0.92	7.99 (0.67)	7.82 (0.87)	0.92
SHBG (nmol/L)	4.61 (0.65)	5.07 (0.65)	<0.01	4.43 (0.63)	5.02 (0.38)	<0.01
Calculated Bioavailable Testosterone (pmol/L)	6.04 (0.45)	5.84 (0.45)	<0.01	6.02 (0.36)	5.71 (0.35)	<0.01

6.4.2.3 IBIS-I: sex hormone and SHBG associations with side effect outcomes

There was a significant increase in risk of side effects per one unit increase in ln-transformed values of testosterone (OR = 1.81 (1.09 – 3.00); P = 0.02) and bioT (OR = 4.27 (2.42 – 7.54); P < 0.01) in all women (Table 6.8). Whilst a one unit increase in ln-transformed SHBG values at baseline was associated with a statistically significant decrease in developing side effects (OR = 0.22 (0.13 – 0.35); P < 0.01), no effect for ln-DHEA-S in relation to side effects was observed (Table 6.8).

Women randomised to tamoxifen, had a statistically significant increase in side effect risk with increasing bioT (OR = 2.81 (1.51 – 5.22); P < 0.01). However, a one-unit change in ln-transformed testosterone concentrations was not associated with any statistically significant increase in side effect risk (OR = 1.51 (0.84 – 2.72); P = 0.17). Ln-SHBG remained a risk reduction factor for side effects in the tamoxifen group (OR = 0.32 (0.19 – 0.53); P < 0.01) (Table 6.8).

An increase in risk of side effects was observed for testosterone (OR = 2.90 (1.05 – 8.03); P = 0.04), DHEA-S (OR = 1.48 (0.88 – 2.49); P = 0.14) and bioT (OR = 16.02 (3.96 – 64.82); P < 0.01) in women who received placebo (Table 6.8). No statistically significant association was observed per one ln-transformed unit increase in DHEA-S. In the placebo group, the increased risk associated per one unit increase in ln-bioT is greater than the increased risk observed in the overall population and the tamoxifen group. Additionally, the decreased risk of side effects associated with ln-SHBG is limited to the tamoxifen group only (OR = 1.83 (0.79 – 4.24); P = 0.16) (Table 6.8).

When the analysis was adjusted for baseline risk factors identified as significant in multivariate models in chapter 4, BMI, HRT use, and prior hysterectomy, there was a significant effect of baseline SHBG and bioT on the risk of side effects (OR = 0.21 (0.12 – 0.35); P < 0.01 and (OR = 3.85 (2.15 – 6.90); P < 0.01) respectively. When analysed by randomised treatment arm, no change in the risk of side effects for any of the sex hormones or SHBG was observed after adjustment for other risk factors (Table 6.8).

Table 6.8: Unadjusted and adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG as a continuous variable and side effects in all women and those randomised to tamoxifen or placebo in the IBIS-I trial

	Overall (N = 399)		Tamoxifen Only (N = 254)		Placebo Only (N = 145)	
Unadjusted	**OR (95%CI)**	**P-value**	**OR (95%CI)**	**P-value**	**OR (95%CI)**	**P-value**
Ln T	1.81 (1.09 - 3.00)	0.02	1.51 (0.84 - 2.72)	0.17	2.90 (1.05 - 8.03)	0.04
Ln DHEA-S	1.12 (0.83 - 1.50)	0.46	0.97 (0.67 - 1.39)	0.85	1.48 (0.88 - 2.49)	0.14
Ln SHBG	0.22 (0.13 - 0.35)	< 0.01	0.32 (0.19 - 0.53)	< 0.01	0.07 (0.02 - 0.23)	< 0.01
Ln Bio T	4.27 (2.42 - 7.54)	< 0.01	2.81 (1.51 - 5.22)	< 0.01	16.02 (3.96 - 64.82)	< 0.01
	Overall (N = 380)		**Tamoxifen Only (N = 244)**		**Placebo Only (N = 136)**	
Adjusted	**OR (95%CI)**	**P-value**	**OR (95%CI)**	**P-value**	**OR (95%CI)**	**P-value**
Ln T	1.64 (0.97 - 2.77)	0.07	1.38 (0.74 - 2.58)	0.31	2.20 (0.73 - 86.65)	0.16
Ln DHEA-S	1.02 (0.74 - 1.40)	0.91	0.89 (0.60 - 1.32)	0.56	1.30 (0.71 - 2.37)	0.39
Ln SHBG	0.21 (0.12 - 0.35)	< 0.01	0.32 (0.19 - 0.55)	< 0.01	0.02 (0.00 - 0.16)	< 0.01
Ln Bio T	3.85 (2.15 - 6.90)	< 0.01	2.60 (1.36 - 4.97)	< 0.01	14.94 (3.20 - 69.82)	<0.01

Ln T = Natural log of testosterone concentrations, Ln DHEA-S = Natural log of Dehydroepiandrosterone - sulphate concentrations, Ln SHBG = Natural log of sex hormone binding globulin concentrations, Ln BioT = Natural log of bioavailable testosterone concentrations,

6.4.2.4 Analysis of sex hormones by quintile

After analysis of sex hormone concentrations as continuous variables, each of the sex hormones were divided into quintiles and the risk of reporting side effects was calculated for each quintile using the first quintile as the reference group.

In women randomised to tamoxifen, compared to the lowest quintile, a significant decrease in side effects was observed with increasing quintiles of ln-SHBG (Figure 6.5). Women in quintiles 3-5 of ln-SHBG concentrations were at significantly lower risk of side effects than those in the lowest quintile (3^{rd} quintile: OR = 0.24 (0.08 – 0.74); P = 0.01), (4^{th} quintile: OR = 0.07 (0.02 – 0.25); P < 0.01) and (5^{th} quintile: OR = 0.05 (0.01 – 0.21); P < 0.01) respectively (Figure 6.5). Women in the 2^{nd} quintile had a non-statistically significant increase in side effects compared to those in the lowest quintile.

The opposite trend was observed for ln-bioT which displayed an increased risk of side effects as the concentration of ln-bioT increased. Women in the 2^{nd} and 3^{rd} quintiles of ln-bioT concentrations had no statistically significant change in side effect risk (Figure 6.5). But women in the 4^{th} and 5^{th} quintiles for ln-bioT had an increase in side effect risk compared to those in the lowest quintile (4^{th} quintile: OR = 2.11 (0.92 – 4.84); P = 0.08) and (OR = 6.40 (2.14 – 19.12); P < 0.01) respectively.

Trends for ln-testosterone and ln-DHEA-S were less clear. Quintiles of ln-testosterone followed a U-shape whereby the 3^{rd} quintile (OR = 0.88 (0.38 – 2.01); P = 0.76) conferred a lower risk of side effects than the 2^{nd}, 4^{th} and 5^{th} quintiles (Figure 6.5). However, women in none of the higher quintiles had a statistically significant increase in side effect risk compared to women in the lowest quintile. For ln-DHEA-S, women in each quintile had a small reduction in risk compared to those in the lowest quintile. Women in the 3^{rd} quintile (OR = 0.42 (0.17 – 1.02); P = 0.06) had the lowest risk of side effects; however, this was not statistically significant compared to the lowest quintile (Figure 6.5).

In women randomised to placebo, a significant decrease in side effects was observed in all quintiles of ln-SHBG (Figure 6.6). Women in quintiles 3-5 of ln-SHBG concentrations had similar low risk levels compared to those in the lowest quintile (3^{rd} quintile: OR = 0.01 (<0.01 – 0.16); P < 0.01), (4^{th} quintile: OR = <0.01 (<0.01 – 0.12); P < 0.01)

and (5^{th} quintile: OR = <0.01 (<0.01 – 0.06); P < 0.01) respectively (Figure 6.6). Women in the 2^{nd} quintile also had a statistically significant decrease in side effects compared to those in the lowest quintile (OR = 0.05 (<0.01 – 0.68); P = 0.02) (Figure 6.6).

For ln-bioT women in the highest quintile had an increased risk of side effects (OR = 83.40 (5.71 – 1218.88); P < 0.01). Women in the 2^{nd} – 4^{th} quintiles of ln-bioT concentrations had a non-statistically significant increase in side effect risk (Figure 6.6).

Trends for ln-testosterone and ln-DHEA-S were less clear. All quintiles of ln-testosterone had increased risk of side effects compared to the lowest quintile. The 3^{rd} quintile conferred a smallest increase in risk (OR = 1.13 (0.36 – 3.62); P = 0.83). The increase in risk in the 2^{nd} and 4^{th} quintiles are similar to that of the 3^{rd} quintile (Figure 6.6). Women in the 5^{th} quintile had a higher risk than other quintiles (OR = 3.05 (0.83 – 11.24); P = 0.09), but this was not-statistically significant compared to women in the lowest quintile. For ln-DHEA-S, women in each quintile had a similar increase in risk compared to those in the lowest quintile. However, none of the quintiles had a statistically significant increase in risk (Figure 6.6).

Testosterone	N	Events	Odds Ratio (95% CI)
1st Quintile	51	20	Reference
2nd Quintile	51	29	1.88 (0.77 - 4.57)
3rd Quintile	50	23	0.88 (0.38 - 2.01)
4th Quintile	51	27	1.61 (0.74 - 3.48)
5th Quintile	51	28	1.67 (0.73 - 3.81)
		P-value	**0.60**
DHEA-S			
1st Quintile	51	27	Reference
2nd Quintile	51	24	0.59 (0.24 - 1.43)
3rd Quintile	50	21	0.42 (0.17 - 1.02)
4th Quintile	52	27	0.78 (0.34 - 1.82)
5th Quintile	50	28	0.89 (0.35 - 2.26)
		P-value	**0.50**
SHBG			
1st Quintile	51	40	Reference
2nd Quintile	51	37	1.09 (0.34 - 3.52)
3rd Quintile	50	26	0.24 (0.08 - 0.74)
4th Quintile	51	13	0.07 (0.02 - 0.25)
5th Quintile	51	11	0.05 (0.01 - 0.21)
		P-value	**< 0.01**
Bioavailable Testosterone			
1st Quintile	52	19	Reference
2nd Quintile	50	19	0.85 (0.35 - 2.06)
3rd Quintile	50	23	0.86 (0.36 - 2.07)
4th Quintile	51	30	2.11 (0.92 - 4.84)
5th Quintile	51	36	6.4 (2.14 - 19.12)
		P-value	**0.04**

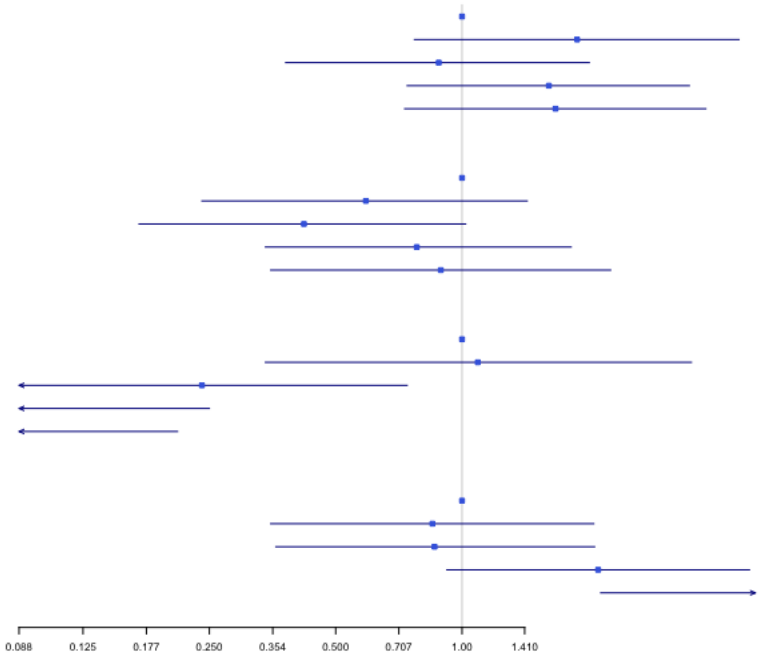


Figure 6.5: Adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG by quintile and side effects in women randomised to tamoxifen. Sex hormones adjusted for BMI, HRT use, and prior hysterectomy. CI = Confidence Intervals, DHEA-S = Dehydroepiandrosterone-sulphate, N = Number, SHBG = Sex Hormone Binding Globulin.

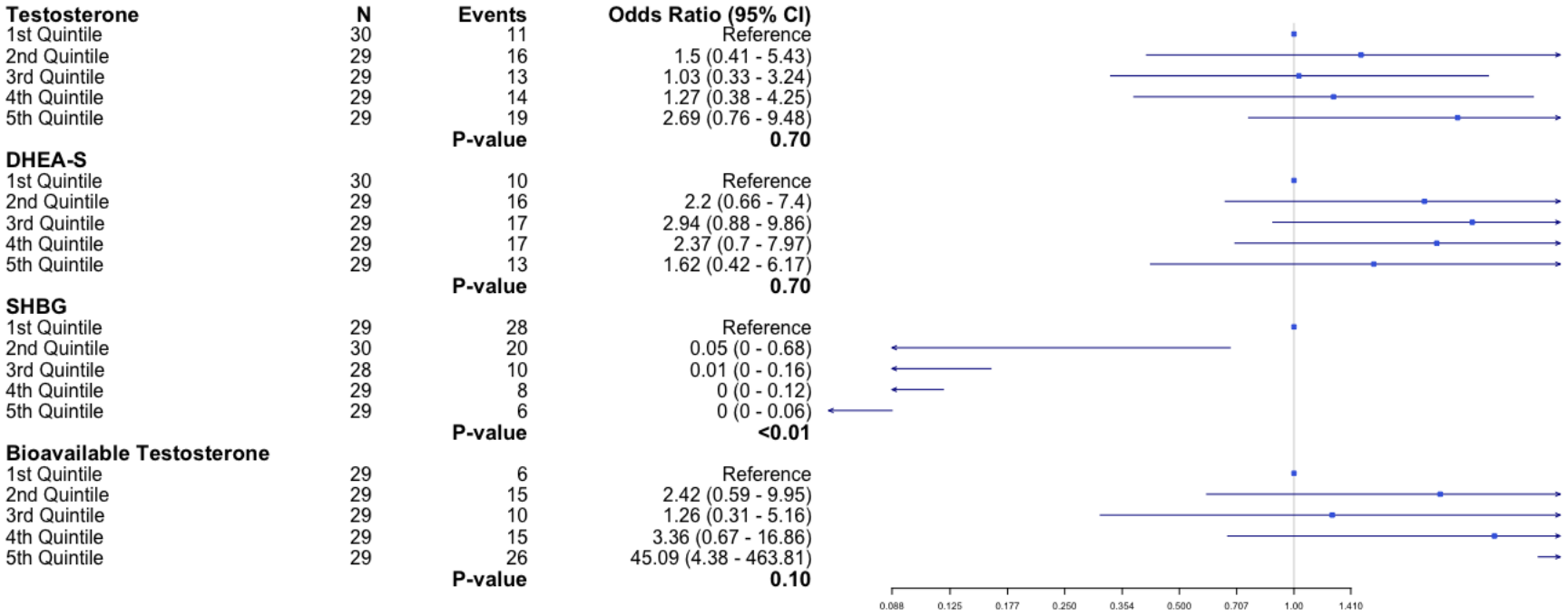


Figure 6.6: Adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG by quintile and side effects in women randomised to placebo. Sex hormones adjusted for BMI, HRT use, and prior hysterectomy. CI = Confidence Intervals, DHEA-S = Dehydroepiandrosterone-sulphate, N = Number, SHBG = Sex Hormone Binding Globulin.

6.4.2.5 IBIS-I: sex hormone association with side effect risk factors

In section 4.3.3, age, BMI, and several reproductive factors, such as age at menarche were identified as risk factors for side effects. Some of these risk factors are thought to mediate their action through sex hormone. In the following section, associations of sex hormones with these risk factors were investigated. Women who reported side effects were analysed to establish associations between side effect risk factors and sex hormones (N = 200). Given the effect of randomisation on side effect incidence, the analysis was performed by treatment group (tamoxifen (N = 127) and placebo (N = 73)).

Age

All of the androgens (all $P < 0.01$) were associated with a small statistically significant decrease in concentration as age increased in women randomised to tamoxifen. As women aged, there was a non-statistically significant increase in ln-SHBG concentrations ($P = 0.39$) per year. When age was categorised as sub-groups, concentrations of ln-transformed testosterone, DHEA-S and bioT were significantly higher in younger women (below 55 years) compared to those aged over 55 (Table 6.9). Mean SHBG concentrations were consistent in both age groups with those aged over 55 showing a small non-statistically significant increase in concentrations.

In women randomised to placebo, older women had significantly lower concentrations of ln-DHEA-S ($P < 0.01$) per year, but no statistically significant association was observed for ln-testosterone ($P = 0.10$), ln-SHBG ($P = 0.91$) or ln-bioT ($P = 0.06$). Sub-group analysis of age showed that only ln-DHEA-S had statistically significant lower concentrations in those aged over 55 compared to those younger than 55. No statistically significant differences for ln-testosterone, ln-SHBG or ln-bioT were observed between women aged less than 55 and women who were older.

Lifestyle and modifiable factors

In women randomised to tamoxifen, women with higher BMI had significantly lower concentrations of ln-SHBG in women randomised to tamoxifen ($P = 0.01$); however, BMI was not associated with any statistically significant changes in any of the ln-transformed sex hormone concentrations. Analysis of BMI by category showed no significant difference in mean concentration for any of the sex hormones (Table 6.9). Sub-group analysis of ln-SHBG indicated that concentrations decreased by 4.4% in

overweight women and by 5.6% in obese women, compared to women with a BMI < 25 (Table 6.9).

In women randomised to placebo, increasing BMI was not associated with any change in ln-transformed sex hormone or SHBG concentrations. Sub-group analysis of ln-SHBG and BMI showed that, compared to women who had BMI < 25, women who were overweight (BMI 25-30) and those who were obese (BMI > 30) had a nearly statistically significant 4.1% and 4.8% reduction in ln-SHBG concentrations respectively. Whilst no statistically significant changes in testosterone concentrations were observed, a non-statistically significant increase in ln-bioT was observed in overweight and obese women compared to those who had a BMI < 25.

Previous use of HRT had no effect on any ln-transformed sex hormone or SHBG in women randomised to tamoxifen (Table 6.9). In contrast, previous HRT use was associated with a statistically significant decrease in ln-transformed testosterone, DHEA-S and calculated bioT concentrations compared to women randomised to placebo who had never taken HRT. Ln-testosterone was 26.8% lower in women who had previously used HRT whilst ln-bioT was 3.4% lower in the same group of women. Smoking was not associated with any statistically significant changes in any ln-transformed sex hormone or ln-SHBG in either the tamoxifen or the placebo group (Table 6.9).

Table 6.9: Means, 95% confidence intervals and Kruskal-Wallis chi-squared p-values for variance of sex hormone and sex hormone binding globulin concentrations and age, BMI, HRT smoking and reproductive factors

	N	Mean Testosterone (95%CI)	Mean DHEA-S (95%CI)	Mean SHBG (95%CI)	Mean Bioavailable Testosterone (95%CI)
Age (years)					
⩽55	77	0.84 (0.76 - 0.93)	8.23 (8.09 - 8.37)	4.60 (4.44 - 4.76)	6.12 (6.02 - 6.22)
> 55	50	0.63 (0.52 - 0.74)	7.62 (7.38 - 7.86)	4.62 (4.46 - 4.79)	5.91 (5.79 - 6.03)
Kruskal-Wallis P-Value		<0.01	<0.01	0.66	0.01
BMI (kg/m2)					
< 25	56	0.74 (0.63 - 0.84)	7.98 (7.8 - 8.16)	4.73 (4.58 - 4.87)	5.98 (5.86 - 6.1)
25-30	39	0.82 (0.67 - 0.96)	8.01 (7.73 - 8.3)	4.53 (4.33 - 4.73)	6.12 (5.96 - 6.28)
> 30	28	0.74 (0.6 - 0.88)	8.07 (7.74 - 8.39)	4.48 (4.15 - 4.82)	6.06 (5.9 - 6.22)
Kruskal-Wallis P-Value		0.36	0.59	0.02	0.21
Previous HRT use					
Never	81	0.81 (0.73 - 0.89)	8.00 (7.84 - 8.17)	4.61 (4.49 - 4.74)	6.08 (5.99 - 6.18)
Ever	46	0.67 (0.54 - 0.81)	7.96 (7.73 - 8.20)	4.61 (4.38 - 4.84)	5.96 (5.81 - 6.11)
Kruskal-Wallis P-Value		0.09	0.69	0.85	0.13
Smoking status					
Never	63	0.78 (0.69 - 0.87)	8.07 (7.88 - 8.26)	4.61 (4.43 - 4.78)	6.06 (5.96 - 6.17)
Ever	64	0.74 (0.63 - 0.85)	7.91 (7.71 - 8.10)	4.61 (4.46 - 4.77)	6.02 (5.89 - 6.14)
Kruskal-Wallis P-Value		0.51	0.21	0.75	0.60
Parity					
Nulliparous	17	0.83 (0.59 - 1.07)	8.11 (7.77 - 8.44)	4.56 (4.32 - 4.8)	6.12 (5.87 - 6.37)
Parous	110	0.75 (0.68 - 0.82)	7.97 (7.82 - 8.12)	4.62 (4.49 - 4.75)	6.03 (5.94 - 6.11)
Kruskal-Wallis P-Value		0.76	0.68	0.68	0.72

Continued on next page

Table 6.9 – continued from previous page

	N	Mean Testosterone (95%CI)	Mean DHEA-S (95%CI)	Mean SHBG (95%CI)	Mean Bioavailable Testosterone (95%CI)
Age at menarche (years)					
⩽ 14	85	0.81 (0.72 - 0.90)	8.05 (7.90 - 8.20)	4.58 (4.43 - 4.73)	6.09 (5.99 - 6.20)
>14	41	0.66 (0.56 - 0.75)	7.85 (7.57 - 8.13)	4.67 (4.49 - 4.84)	5.93 (5.81 - 6.05)
Kruskal-Wallis P-Value		0.02	0.35	0.29	0.02
Age at menopause (years)					
⩽50	58	0.80 (0.71 - 0.90)	8.06 (7.85 - 8.27)	4.55 (4.32 - 4.77)	6.10 (5.99 - 6.21)
>50	35	0.62 (0.48 - 0.76)	7.75 (7.46 - 8.05)	4.72 (4.59 - 4.85)	5.87 (5.72 - 6.03)
Kruskal-Wallis P-Value		0.02	0.04	0.30	0.05
Time since menopause (years)					
<5	36	0.77 (0.63 - 0.91)	8.10 (7.80 - 8.41)	4.60 (4.37 - 4.83)	6.05 (5.89 - 6.21)
5 - 10	23	0.65 (0.49 - 0.81)	7.92 (7.65 - 8.20)	4.73 (4.42 - 5.05)	5.9 (5.71 - 6.09)
>10	34	0.75 (0.62 - 0.88)	7.79 (7.49 - 8.09)	4.54 (4.29 - 4.80)	6.05 (5.9 - 6.2)
Kruskal-Wallis P-Value		0.39	0.12	0.99	0.45
Type of menopause					
Natural	92	0.73 (0.65 - 0.81)	7.94 (7.77 - 8.11)	4.61 (4.46 - 4.76)	6.01 (5.92 - 6.11)
Hysterectomy	15	0.84 (0.55 - 1.14)	8.14 (7.84 - 8.44)	4.77 (4.52 - 5.01)	6.07 (5.76 - 6.39)
Bilateral oophorectory	5	0.53 (0.11 - 0.95)	7.69 (6.78 - 8.60)	4.62 (4.29 - 4.95)	5.81 (5.41 - 6.22)
Kruskal-Wallis P-Value		0.40	0.46	0.81	0.49

Reproductive and other factors

Parity was not associated with any of the hormones nor with ln-SHBG in either women randomised to tamoxifen or those randomised to placebo (Table 6.9). A younger age at menarche was also not associated with any change in ln-transformed hormones nor with ln-SHBG in either women randomised to tamoxifen or those randomised to placebo. In the tamoxifen group, women with an age of menarche of greater than 14 had a statistically significant 22.7% reduction in ln-testosterone and a statistically significant 2.7% reduction in ln-bioT compared to women whose age of menarche was 14 or below (Table 6.9). Women in the placebo group showed different outcomes as no difference in any ln-transformed sex hormone or ln-SHBG concentrations were observed.

Later age at menopause was not associated with any statistically significant changes for any of the ln-sex hormones or ln-SHBG in either the tamoxifen or the placebo group. When analysed as a categorical variable, women in the tamoxifen group whose age at menopause was greater than 50 had a statistically significant reduction in ln-transformed testosterone, DHEA-S and bioT concentrations (Table 6.9). There was no association between age at menopause and ln-SHBG concentrations in the tamoxifen group. In women randomised to placebo, age at menopause had no association to any of the sex hormones or SHBG.

Increased time since menopause was not associated with any change in ln-transformed sex hormone or ln-SHBG concentrations in women randomised to either tamoxifen or placebo. Investigation of time since menopause in sub-groups indicated that, in women randomised to tamoxifen, time since menopause was not associated with any change in ln-transformed sex hormone or SHBG concentrations. (Table 6.9). In women randomised to placebo, ln-DHEA-S, concentrations were 8.7% higher, in women who had had their menopause within the last five years compared to women who had had their menopause 5-10 or more years previously. The other hormones and ln-SHBG were not associated with time since menopause in either the tamoxifen or placebo group.

Ln-transformed sex hormone concentrations and SHBG did not differ significantly by type of menopause for women randomised to tamoxifen or placebo (Table 6.9). However, in women randomised to placebo, women who had a bilateral oophorectomy had 14.5% higher ln-SHBG concentrations compared to those who had a hysterectomy, but this was not statistically significant.

6.4.3 IBIS-II results

6.4.3.1 Correlations between sex hormones

In women randomised to anastrozole, concentrations of all the androgens were positively correlated with each other (Figure 6.7, Panel A). Correlation coefficients ranged between 0.38 between ln-DHEA-S and ln-bioT and 0.78 between ln-testosterone and ln-bioT. Ln-SHBG was inversely correlated with ln-bioT (r = -0.68). Ln-SHBG was not correlated with either ln-T (r = -0.08) or ln-DHEA-S (r < -0.01) (Figure 6.7, Panel A). Values are statistically significant below P <0.01 except the correlation between ln-SHBG and ln-T (P = 0.09) and ln-DHEA-S (P = 0.97).

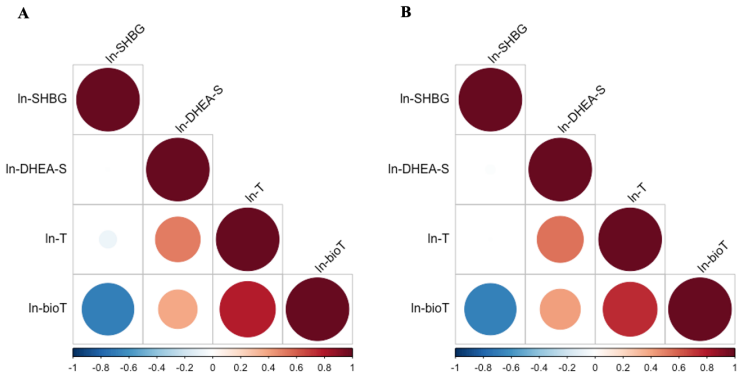


Figure 6.7: Correlations between hormones and SHBG. Panel A: Women randomised to Anastrozole. Panel B: Women randomised to placebo. Abbreviations: DHEA-S = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin; T = Testosterone; bioT = bioavailable testosterone; ln = ln-transformed.

Correlations between androgens in the placebo group were consistent with those in the anastrozole group. Ln-bioT was strongly correlated with ln-testosterone (r = 0.74) and ln-DHEA-S (r = 0.41) (Figure 6.7, Panel B). The correlation between ln-testosterone and ln-DHEA-S was also similar to the anastrzole group (r = 0.54). ln-SHBG was strongly inversely correlated with ln-bioT (r = -0.67) and was not correlated with ln-testosterone (r < -0.01) and ln-DHEA-S (r = -0.02) (Figure 6.7, Panel B). All values are statistically significant below P <0.01 except the correlation between ln-SHBG and ln-testosterone (P = 0.98) and ln-DHEA-S (P = 0.71).

6.4.3.2 Sex hormone concentrations in the anastrozole and placebo arms of IBIS-II

For women in the IBIS-II study no statistically significant differences in the concentration of ln-testosterone, ln-DHEA-S or ln-bioT between case and controls were observed in either the placebo or the anastrozole group. Ln-SHBG concentrations were significantly higher in cases compared to controls in the anastrozole arm; however, there were no statistically significant differences in mean ln-SHBG concentrations between cases and controls in the placebo arm (Table 6.10).

Table 6.10: Means and standard deviations (SD) of ln-transformed circulating concentrations of sex hormones and SHBG in postmenopausal women by randomisation and case control status. P-values are Kruskal-Wallis test with Dunn's post-hoc adjustment for multiple comparisons P-values

	Anastrozole (N = 580)			Placebo (N = 388)		
	Cases Mean (SD) (N = 290)	Controls Mean (SD) (N = 290)	P-value	Cases Mean (SD) (N = 194)	Controls Mean (SD) (N = 194)	P-value
Testosterone (nmol/L)	0.67 (0.36)	0.67 (0.43)	0.96	0.62 (0.40)	0.65 (0.40)	0.96
DHEA-S (nmol/L)	7.88 (0.74)	7.87 (0.74)	0.92	7.81 (0.77)	7.86 (0.77)	0.92
SHBG (nmol/L)	4.60 (1.23)	4.86 (1.05)	0.02	4.32 (1.36)	4.44 (0.96)	0.8
Calculated Bioavailable Testosterone (pmol/L)	5.97 (0.51)	5.89 (0.54)	0.68	5.99 (0.55)	5.99 (0.45)	0.68

6.4.3.3 IBIS-II: Sex hormone and SHBG associations with side effect outcomes

There was a non-significant decrease in risk of side effects per one unit increase in ln-transformed values of SHBG (OR = 0.87 (0.76 – 1.01); P = 0.07) in all women (Table 6.11). No statistically significant effect was observed for ln-transformed concentrations of testosterone, DHEA-S or bioT (Table 6.11).

In women randomised to anastrozole, ln-SHBG had a statistically significant reduction in side effect risk (OR = 0.81 (0.66 – 1.00); P = 0.05). A one-unit change in any ln-transformed sex hormone concentration still had no statistically significant change in side effect risk. None of the ln-transformed sex hormones nor ln-SHBG concentrations were risk factors for side effects in the placebo group (Table 6.11).

Table 6.11: Unadjusted and adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG as a continuous variable and side effects in all women and those randomised to anastrozole or placebo in the IBIS-II trial.

	Unadjusted					
	Overall (N = 856)		**Anastrozole Only (N = 508)**		**Placebo Only (N = 348)**	
	OR (95%CI)	**P-value**	**OR (95%CI)**	**P-value**	**OR (95%CI)**	**P-value**
Ln T	0.93 (0.68 - 1.28)	0.66	1.01 (0.67 - 1.51)	0.98	0.82 (0.49 - 1.38)	0.46
Ln DHEA-S	0.98 (0.83 - 1.15)	0.79	1.01 (0.82 - 1.26)	0.91	0.93 (0.71 - 1.21)	0.57
Ln SHBG	0.87 (0.76 - 1.01)	0.07	0.81 (0.66 - 1.00)	0.05	0.94 (0.77 - 1.15)	0.55
Ln BioT	1.05 (0.80 - 1.38)	0.72	1.19 (0.82 - 1.73)	0.35	0.90 (0.60 - 1.35)	0.61
	Adjusted					
	Overall (N = 828)		**Anastrozole Only (N = 491)**		**Placebo Only (N = 337)**	
	OR (95%CI)	**P-value**	**OR (95%CI)**	**P-value**	**OR (95%CI)**	**P-value**
Ln T	1.11 (0.79 - 1.58)	0.54	1.15 (0.73 - 1.79)	0.55	1.12 (0.63 - 1.98)	0.70
Ln DHEA-S	1.01 (0.85 - 1.21)	0.88	1.09 (0.86 - 1.39)	0.48	1.00 (1.00 - 1.00)	0.92
Ln SHBG	0.84 (0.72 - 0.98)	0.03	0.79 (0.63 - 1.00)	0.05	0.90 (0.74 - 1.16)	0.49
Ln BioT	1.21 (0.90 - 1.63)	0.22	1.33 (0.87 - 2.04)	0.18	1.05 (0.66 - 1.67)	0.82

Ln T = Natural log of testosterone concentrations, Ln DHEA-S = Natural log of Dehydroepiandrosterone - sulphate concentrations, Ln SHBG = Natural log of sex hormone binding globulin concentrations, Ln BioT = Natural log of bioavailable testosterone concentrations,

For women on the IBIS-II trial, adjustment for BMI, HRT use, smoking history, and prior hysterectomy, factors previously multivariate significant for side effect risk, did not alter the results except for ln-SHBG which, after adjustment, had a statistically significant decrease in the risk of side effects in the whole study population (OR = 0.84 (0.72 – 0.98); P = 0.03). No association with side effects was observed for ln-testosterone, DHEA-S or bioT after adjustment (Table 6.11).

When sex hormones were analysed within each treatment arm, only ln-SHBG was significantly associated with side effect outcomes in the anastrozole arm (OR = 0.79 (0.63 – 1.00); P = 0.05) but not in the placebo arm (OR = 0.90 (0.74 – 1.16); P = 0.49) (Table 6.11). No association with side effects was observed for ln-testosterone, DHEA-S or bioT after adjustment for other risk factors in either the anastrozole or the placebo groups (Table 6.11).

6.4.3.4 Sex hormone analysis by quintile

After analysis of sex hormone concentrations as continuous variables, each of the sex hormones were divided into quintiles and the risk of reporting side effects was calculated for each quintile using the first quintile as the reference group.

In women randomised to anastrozole, an inverse association between risk and ln-SHBG concentrations was observed with risk of side effects decreasing as ln-SHBG concentrations increase. Women in the 2^{nd} and 3^{rd} quintiles of ln-SHBG concentrations had a 40% and 43% non-statistically significant lower risk of side effects than those in the lowest quintile (2^{nd} quintile: OR = 0.59 (0.33 – 1.08); P = 0.09), (3^{rd} quintile: OR = 0.57 (0.31 – 1.06); P = 0.08) (Figure 6.8). Women in the 4^{th} quintile had a non-statistically significant decrease in side effects compared to those in the lowest quintile. Women in the 5^{th} quintile of ln-SHBG concentrations had a statistically significant 67% decrease in side effect risk compared to women in the lowest quintile (OR = 0.33 (0.15 – 0.76); P = 0.01) (Figure 6.8).

The opposite was observed for ln-testosterone and ln-bioT which displayed an increased risk in higher concentrations compared to the lowest quintile. The quintiles of ln-testosterone followed a U-shaped curve, women in the 3^{rd} and 4^{th} quintiles (OR = 1.44 (0.83 – 2.51); P = 0.20) and (OR = 1.30 (0.77 – 2.28); P = 0.36) respectively were at

higher risk of side effects than those in the 2^{nd} (OR = 1.13 (0.66 – 1.93); P = 0.65) and 5^{th} quintiles (OR = 1.10 (0.64 – 1.90); P = 0.73) (Figure 6.8).

Women in all quintiles of ln-bioT had an increased risk of side effects. Women whose ln-bioT concentrations were in the 2^{nd}, 3^{rd} and 5^{th} quintiles had a statistically significant increase in side effect risk (2^{nd} quintile: OR = 2.14 (1.09 – 4.20); P = 0.03), (3^{rd} quintile: OR = 3.00 (1.45 – 6.19); P = 0.002) (5^{th} quintile: OR = 2.06 (1.01 – 4.20); P = 0.05). Although women whose ln-bioT concentrations were in the 4^{th} quintile for ln-bioT had non-statistically significant increased risk compared to those in the lowest quintile (Figure 6.8). For ln-DHEA-S, all quintiles were associated with a small non-statistically significant increase in side effect risk compared to those in the lowest quintile (Figure 6.8).

In women randomised to placebo, a small increase in side effects was observed in all quintiles of ln-SHBG; however, risk increases were not statistically significant (Figure 6.9). Higher quintiles of ln-bioT or ln-DHEA-S were not associated with a statistically significant increase in the risk of reporting side effects compared to those in the lower quintile of each sex hormone (Figure 6.9). Women in the 3^{rd} quintile of ln-testosterone concentrations had a statistically significant 2-fold increase in side effect risk (OR = 2.36 (1.12 – 5.00); P = 0.02) (Figure 6.9). No other quintiles of ln-testosterone had a statistically significant risk increase.

Testosterone	N	Events	Odds Ratio (95% CI)
1st Quintile	116	54	Reference
2nd Quintile	118	61	1.13 (0.66 - 1.93)
3rd Quintile	114	62	1.44 (0.83 - 2.51)
4th Quintile	116	59	1.30 (0.74 - 2.28)
5th Quintile	116	54	1.10 (0.64 - 1.90)
		P-value	**0.08**
DHEA-S			
1st Quintile	116	55	Reference
2nd Quintile	116	63	1.48 (0.85 - 2.60)
3rd Quintile	116	59	1.03 (0.59 - 1.79)
4th Quintile	116	57	1.38 (0.78 - 2.46)
5th Quintile	116	56	1.26 (0.72 - 2.23)
		P-value	**0.05**
SHBG			
1st Quintile	102	61	Reference
2nd Quintile	101	50	0.60 (0.33 - 1.08)
3rd Quintile	103	43	0.57 (0.31 - 1.06)
4th Quintile	100	55	0.75 (0.35 - 1.60)
5th Quintile	102	37	0.33 (0.15 - 0.76)
		P-value	**0.01**
Bioavailable Testosterone			
1st Quintile	103	38	Reference
2nd Quintile	100	52	2.15 (1.10 - 4.20)
3rd Quintile	102	58	3.00 (1.45 - 6.19)
4th Quintile	101	47	1.56 (0.77 - 3.16)
5th Quintile	102	51	2.06 (1.01 - 4.20)
		P-value	**0.01**

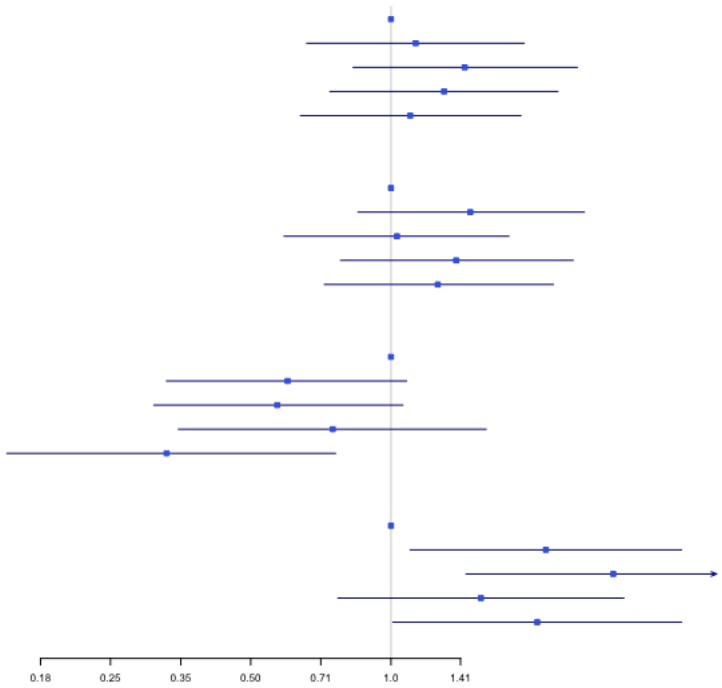


Figure 6.8: Adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG by quintile and side effects in women randomised to anastrozole. Sex hormones adjusted for BMI, HRT use, smoking history, and hysterectomy. CI = Confidence Intervals, DHEA-S = Dehydroepiandrosterone-sulphate, N = Number, SHBG = Sex Hormone Binding Globulin.

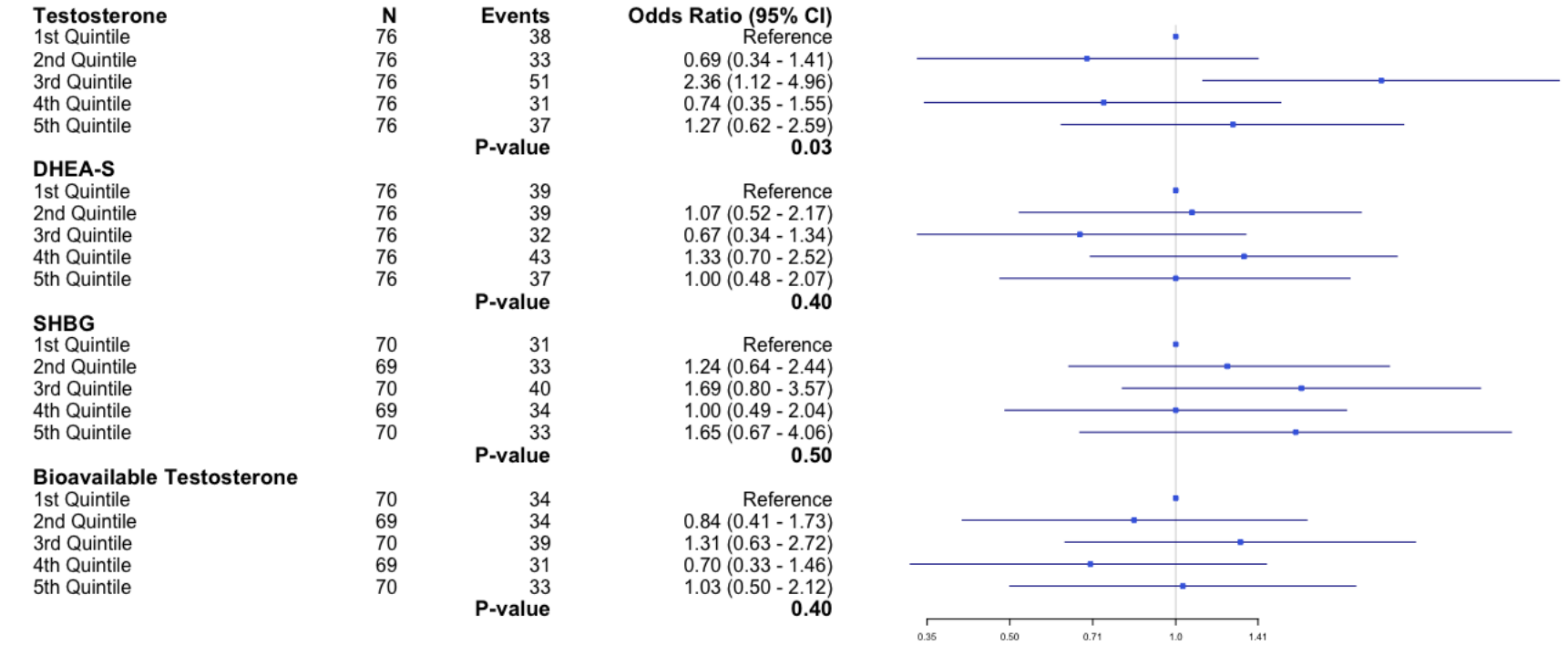


Figure 6.9: Adjusted odds ratios and 95% confidence intervals for the association of the logarithm of each sex hormone or SHBG by quintile and side effects in women randomised to placebo. Sex hormones adjusted for BMI, HRT use, smoking history, and hysterectomy. CI = Confidence Intervals, DHEA-S = Dehydroepiandrosterone-sulphate, N = Number, SHBG = Sex Hormone Binding Globulin.

6.4.3.5 IBIS-II: sex hormone association with side effect risk factors

In chapter 4 section 4.3.4, risk factors for side effects were identified. In the following section, associations of sex hormones with these risk factors were investigated. Risk factors were available for all women. No sex hormone information was available for eight women who were removed from the analysis. Additionally, only women reporting side effects were analysed and these women were analysed by randomised treatment which left N = 290 women in the anastrozole analysis and N = 190 women in the placebo analysis.

Age

In women randomised to anastrozole, a statistically significant decrease in ln-DHEA-S ($P < 0.01$) concentrations was observed as age increased. Concentrations of ln-testosterone, bioT and SHBG had no statistically significant associations with increased age. Sub-categorised age showed, concentrations of ln-testosterone and ln-bioT were similar in the two age sub-categories (Table 6.12). Ln-DHEA-S concentrations increased by 3.8% ($P < 0.01$) in women aged over 60 years compared to women aged below 60 years. Mean ln-SHBG concentrations were consistent in women in both age categories (Table 6.12). In women randomised to placebo, age was not significantly associated with any ln-transformed sex hormones or ln-SHBG (all $P > 0.10$).

Lifestyle and modifiable factors

For women randomised to anastrozole, increasing BMI was associated with statistically significant decrease in ln-SHBG ($P = 0.05$) and increased ln-bioT ($P = 0.03$) in women randomised to anastrozole. BMI was not associated with any statistically significant changes in ln-DHEA-S or ln-testosterone. Analysis of BMI by category showed a significant difference in mean ln-transformed concentration for SHBG and bioT (Table 6.12). Sub-group analysis of ln-SHBG and BMI showed that, compared to women who had BMI < 25, women who were obese (BMI > 30) had a statistically significant 7.9% reduction in ln-SHBG concentrations ($P < 0.01$). Obese women also had a statistically significant 5.4% ($P < 0.01$) reduction in SHBG concentrations compared to overweight women. No statistically significant decrease was observed between overweight women and those with BMI < 25. Ln-bioT had a statistically significant 3.6% increase between women with BMI < 25 and obese women ($P < 0.01$) (Table 6.12).

In women randomised to placebo, per one unit increase in BMI was not associated

with any change in ln-transformed sex hormone or SHBG concentrations. Sub-group analysis of ln-SHBG and BMI showed that, compared to women who had BMI < 25, women who were obese (BMI > 30) had a statistically significant 12.7% reduction in ln-SHBG concentrations ($P < 0.01$). Compared to women who were overweight (BMI 25-30), those with BMI > 30 had a statistically significant 4.1% reduction in ln-SHBG concentrations ($P = 0.02$).

BMI also had a non-statistically significant increase in ln-bioT concentrations in women who were obese compared to those who were overweight (BMI 25-30) ($P = 0.06$). However, no statistically significant increase was observed in overweight or obese women compared to women whose BMI was <25.

Previous use of HRT was not associated with any change in ln-transformed sex hormones or SHBG concentrations in women randomised to anastrozole or to placebo (Table 6.12).

Smoking history was not associated with any statistically significant changes in any ln-transformed sex hormone or SHBG in the anastrozole group (Table 6.12). However, in the placebo group, smoking was associated with a 12.0% ($P = 0.02$) decrease in ln-SHBG concentrations between former smokers and women who were currently smoking and a 5.1% decrease between never smokers and former smokers ($P < 0.01$).

Reproductive and other factors

Parity was not associated with any of the hormones nor with SHBG in either women randomised to anastrozole or those randomised to placebo (Table 6.12).

Age at menarche as a continuous variable was not associated with decreased or increased ln-transformed sex hormone or SHBG concentrations in women randomised to anastrozole or placebo. In the anastrozole group, women with an age of menarche of greater than 14 had no statistically significant association with any change in sex hormone or SHBG concentration compared to those whose age of menarche was 14 or below (Table 6.12). In the placebo group, women whose age of menarche was greater than 14 had a statistically significant 5.0% reduction in ln-testosterone concentrations and a statistically significant 0.7% reduction in ln-bioT compared to women whose age at menarche was 14 or below. Additionally, women whose age of menarche was greater than 14 had a statistically significant 2.5% increase in ln-DHEA-S concentrations compared to women whose age at menarche was 14 or below.

Age at menopause was only associated with a statistically significant reduction in ln-DHEA-S concentrations compared to those whose age of menopause was less than 50 in women randomised to placebo. Age at menopause had no association to any of the other ln-transformed sex hormones or SHBG in the women randomised to placebo nor was it associated with any ln-transformed sex hormones or SHBG in the anastrozole arm. Similar results were observed for time since menopause which was only associated with decreased concentrations of ln-DHEA-S in women who have been postmenopausal for more than 10 years compared to those who have been postmenopausal for less than five years in women randomised to placebo.

Type of menopause had weak association with concentrations of ln-SHBG in women randomised to anastrozole but not to any other hormones or to any sex hormones in the placebo group. Compared with women with a natural menopause those with hysterectomy had concentrations of ln-SHBG which were 7.4% lower. Women who had a hysterectomy had 12.1% lower ln-SHBG concentrations than those who had bilateral oophorectomy (Table 6.12).

Table 6.12: Means, 95% confidence intervals and Kruskal-Wallis chi-squared p-values for variance of sex hormone and sex hormone binding globulin concentrations and age, BMI, HRT smoking and reproductive factors

	N	Mean testosterone (95%CI)	Mean DHEA-S (95%CI)	Mean SHBG (95%CI)	Mean bioavailable testosterone (95%CI)
Age (Years)					
⩽60	112	0.67 (0.59 - 0.74)	7.70 (7.56 - 7.84)	4.57 (4.32 - 4.82)	5.97 (5.86 - 6.08)
> 60	178	0.67 (0.62 - 0.72)	7.99 (7.88 - 8.09)	4.62 (4.45 - 4.79)	5.96 (5.90 - 6.03)
Kruskal-Wallis P-Value		0.77	<0.01	0.91	0.94
BMI (kg/m2)					
< 25	81	0.63 (0.56 - 0.70)	7.82 (7.64 - 7.99)	4.80 (4.56 - 5.05)	5.86 (5.77 - 5.95)
25-30	104	0.62 (0.56 - 0.69)	7.90 (7.75 - 8.05)	4.69 (4.46 - 4.93)	5.91 (5.81 - 6.00)
> 30	100	0.74 (0.66 - 0.81)	7.89 (7.75 - 8.02)	4.45 (4.25 - 4.66)	6.07 (5.97 - 6.16)
Kruskal-Wallis P-Value		0.12	0.90	<0.01	<0.01
HRT use					
Never	130	0.71 (0.65 - 0.77)	7.91 (7.79 - 8.03)	4.55 (4.30 - 4.80)	6.02 (5.93 - 6.11)
Ever	159	0.64 (0.58 - 0.70)	7.84 (7.72 - 7.97)	4.64 (4.48 - 4.80)	5.93 (5.85 - 6.00)
Kruskal-Wallis P-Value		0.08	0.37	0.63	0.27
Smoking					
Never	170	0.70 (0.64 - 0.76)	7.89 (7.77 - 8.00)	4.71 (4.56 - 4.86)	5.96 (5.88 - 6.03)
Current	29	0.72 (0.63 - 0.81)	8.02 (7.82 - 8.22)	3.94 (3.07 - 4.81)	6.18 (5.93 - 6.43)
Ever	89	0.60 (0.52 - 0.67)	7.80 (7.64 - 7.96)	4.60 (4.36 - 4.84)	5.91 (5.81 - 6.01)
Kruskal-Wallis P-Value		0.08	0.50	0.17	0.19
Parity					
Nulliparous	39	0.64 (0.52 - 0.76)	7.82 (7.60 - 8.05)	4.67 (4.15 - 5.19)	5.93 (5.72 - 6.14)
Parous	249	0.67 (0.63 - 0.72)	7.88 (7.79 - 7.98)	4.59 (4.45 - 4.74)	5.97 (5.91 - 6.03)
Kruskal-Wallis P-Value		0.89	0.39	0.10	0.34

Continued on next page

Table 6.12 – continued from previous page

	N	Mean testosterone (95%CI)	Mean DHEA-S (95%CI)	Mean SHBG (95%CI)	Mean bioavailable testosterone (95%CI)
Age at menarche (Years)					
$\leqslant 14$	198	0.66 (0.61 - 0.71)	7.83 (7.72 - 7.93)	4.57 (4.37 - 4.77)	5.98 (5.90 - 6.05)
> 14	90	0.68 (0.61 - 0.76)	7.98 (7.83 - 8.13)	4.68 (4.54 - 4.81)	5.93 (5.85 - 6.02)
Kruskal-Wallis P-Value		0.42	0.06	0.82	0.92
Age at menopause (Years)					
$\leqslant 50$	137	0.67 (0.61 - 0.73)	7.87 (7.73 - 8.01)	4.46 (4.20 - 4.73)	6.01 (5.91 - 6.11)
> 50	145	0.68 (0.62 - 0.74)	7.89 (7.78 - 8.00)	4.73 (4.61 - 4.85)	5.94 (5.87 - 6.01)
Kruskal-Wallis P-Value		0.74	0.83	0.29	0.55
Time since menopause (Years)					
< 5	75	0.72 (0.64 - 0.80)	8.01 (7.87 - 8.15)	4.53 (4.17 - 4.88)	6.03 (5.91 - 6.15)
5 - 10	91	0.67 (0.59 - 0.75)	7.91 (7.74 - 8.07)	4.71 (4.55 - 4.88)	5.94 (5.84 - 6.03)
> 10	116	0.65 (0.59 - 0.72)	7.78 (7.64 - 7.92)	4.55 (4.32 - 4.79)	5.97 (5.87 - 6.07)
Kruskal-Wallis P-Value		0.31	0.12	0.75	0.84
Type of menopause					
Natural	210	0.69 (0.64 - 0.74)	7.93 (7.83 - 8.03)	4.62 (4.46 - 4.79)	5.97 (5.91 - 6.04)
Hysterectomy	42	0.66 (0.55 - 0.77)	7.67 (7.42 - 7.92)	4.30 (3.82 - 4.78)	6.06 (5.87 - 6.26)
Bilateral oophorectory	34	0.60 (0.48 - 0.72)	7.80 (7.56 - 8.05)	4.82 (4.57 - 5.07)	5.82 (5.67 - 5.97)
Kruskal-Wallis P-Value		0.32	0.20	0.05	0.21

6.5 Discussion

Sex hormones, in particular oestrogen, are recognised as stimulating breast tissue and increasing the risk of breast cancer (Endogenous Hormones and Breast Cancer Collaborative Group, 2003, 2011). There are concerns surrounding androgen concentrations and breast cancer risk. However, the evidence from epidemiological studies is conflicting. Some studies have shown an increased incidence of breast cancer associated with endogenous testosterone concentrations (Kaaks et al., 2005; Endogenous Hormones and Breast Cancer Collaborative Group, 2011; James et al., 2011; Schernhammer et al., 2013; Zhang et al., 2013). Other studies have found no association between androgen concentrations and breast cancer risk (Zeleniuch-Jacquotte et al., 2005; Adly et al., 2006; Danforth et al., 2010). A further study has found testosterone levels to be protective against breast cancer (Farhat et al., 2011).

Alongside the risk of sex hormones for breast cancer numerous studies have evaluated sex steroid hormones as risk factors for menopausal symptoms, which are commonly reported as side effects of endocrine therapy (MacLennan et al., 2004; Utian, 2005; Sturdee, 2008). There has been a keen focus on oestrogens in the aetiology of menopausal symptoms for a number of reasons. Among these are the high prevalence of women who experience HFs at times when oestrogen levels dramatically decrease due to various physiological conditions such as menopause. Oestrogen is known to be involved in thermoregulatory homeostasis acting on the hypothalamus, and the success of oestrogen-based treatments, such as HRT, in easing HFs (MacLennan et al., 2004; Utian, 2005; Sturdee, 2008). Additionally, the association between menopausal-like side effects and ER modifiers such as tamoxifen, and AIs such as anastrozole is consistent with the hypothesis that oestrogen disruption is an important part of HF and menopausal side effect aetiology (Veronesi et al., 2003; Fallowfield et al., 2004; Goss et al., 2011; Cuzick et al., 2015, 2020).

The mechanisms through which altered oestrogens, androgens and SHBG on the incidence of menopausal-like side effects of endocrine therapy remain poorly understood. Most menopausal symptoms are believed to be not purely the product of disrupted oestrogen equilibrium as no correlation is observed between serum oestrogen concentration and the frequency or severity of HFs. Furthermore, menopausal symptoms, including HFs, relent over time after menopause a period when oestrogen levels are

still declining. Therefore, it may be that rate of oestrogen depletion rather than actual decrease is more important in causation of HFs (Sturdee et al., 2017). In addition to oestrogen withdrawal, the action of oestrogens in the brain and other pituitary hormones, gonadotropins, and anti-mullerian hormones has been suggested but never proven (Sturdee et al., 2017). Interestingly, testosterone has been shown to relieve side effects of aromatase inhibitor therapy in cancer survivors (Jankovic-Karasoulos et al., 2009; Witherby et al., 2011). The use of testosterone therapy for these symptoms is being prescribed at an increasing rate; however, its long-term effect in the breast need to be further elucidated (Glaser and Dimitrakakis, 2013).

This chapter documented the results of an analyses investigating the role of sex hormones, specifically testosterone, DHEA-S, SHBG and calculated bioavailable testosterone (bioT) on side effect incidence experienced during the first year of endocrine therapy. The outcomes from analysis show that in the IBIS-I study the risk of side effects increased at higher concentrations of ln-testosterone and ln-bioT and decreased at higher concentrations of ln-SHBG. However, once adjusted for covariates, testosterone was no longer associated with an increase in side effect risk. Analysis of sex hormone concentrations by quintile identified that, for women randomised to tamoxifen, women in the highest ln-bioT quintile had an increase in side effect risk compared to those in the lowest quintile. Women whose ln-SHBG concentrations were in the 3^{rd}-5^{th} quintiles had a statistically significant decrease in side effect risk. For women randomised to placebo, all quintiles of SHBG had reduced risk of side effects compared to those in the lowest quintile. Additionally, those in the 5^{th} quintile of ln-bioT had increased risk of side effects. In the IBIS-II analysis only SHBG was associated with side effect with decreased side effect risk as SHBG concentrations increased after adjustment for covariates. Quintile analysis identified that for women randomised to placebo, women in the middle (third) testosterone quintile had a 2-fold increase in side effect risk compared to those in the lowest quintile. For women randomised to anastrozole, women whose ln-SHBG was in the highest quintile had a significant reduction in side effect risk, but those in the 2^{nd} or 3^{rd} quintile for ln-bioT had an increased risk.

These results are consistent with other reports, which observed no difference between the levels of ln-testosterone or ln-DHEA-S in women with side effects, including HF, and women without side effects (Randolph et al., 2005; Thurston and Joffe, 2011). The study population of both the Randolph et al. (2005) and the Thurston and Joffe.

(2011) studies were premenopausal women who were followed through the menopausal transition. As a result, it is likely that these women have higher concentrations of ln-testosterone and ln-DHEA-S than women who are postmenopausal due to the decreasing concentrations of these hormones with age. It is therefore somewhat surprising that the results of this analysis in postmenopausal women are consistent with these younger women.

In contrast, other studies have observed that higher levels of testosterone and DHEA-S seem to protect against menopausal symptoms in postmenopausal women (Øverlie et al., 2002). Whilst the study population of Øverlie et al.(2002) were premenopausal women who were followed through the menopausal transition the protective effect of testosterone and DHEA-S were observed in women who were two years postmenopausal.

It is possible that the lack of association observed between DHEA-S and testosterone concentrations and side effect can be at least partially explained by evidence that DHEA-S and testosterone levels remain relatively consistent in women aged 50 and older, an observation supported by previous studies (Burger, 2006). Testosterone levels remain stable during the later menopausal stages because it is synthesised and secreted by ovarian stromal cells and adrenal glands during this time (Lasley et al., 2002; Fogle et al., 2007). Alternatively, these conflicting observations may be due to differences in study sample, data analysis, or definition of side effects.

Analysis of ln-bioT by quintile shows that, in IBIS-I, the risk of side effects is highest in the 5^{th} quintile of ln-bioT concentrations indicating that higher concentrations of ln-bioT increase the risk of side effects. The results for ln-bioT in the IBIS-II population are similar, with a non-statistically significant increase in risk observed in the quintiles of ln-bioT. Analysis of ln-testosterone shows that in women randomised to anastrozole or tamoxifen increased concentrations of ln-testosterone have no association with side effect outcomes. However, the result in the IBIS-II analysis for ln-testosterone in the placebo group shows that the women at highest risk are those in the 3^{rd} quintile compared to those in the lowest quintile.

Previous studies have linked a polymorphism in CYP19 (aromatase) gene with higher testosterone and DHEA-S levels (Schilling et al., 2007b). For women with higher testosterone concentrations, determination of aromatase performance could provide interesting insight into side effect risk. It is possible that higher testosterone and

subsequently bioT is as a result of poorer conversion to oestrogen resulting in lower oestrogen concentrations which could then be the major cause of side effects.

A secondary concern regarding higher concentrations of testosterone is that testosterone is the major substrate for oestradiol and could therefore have a stimulatory effect at the oestrogen receptor (ER). However, this had been shown to not be the case and that anastrozole combined with testosterone has been shown to prevent aromatisation and provides adequate levels of testosterone without elevating oestradiol in breast cancer survivors (Glaser, 2010; Glaser and Dimitrakakis, 2012).

While testosterone is known to be an active androgen in middle aged women, there is some debate as to whether bioT is the better predictor of health outcomes compared to total testosterone (Rosner et al., 2007; Basaria, 2015). Since bioT has SHBG as a denominator the observation that bioT could be a better predictor than testosterone may be in large part due to the effect of SHBG on side effects. Given the strength of the effect of SHBG in the IBIS-I analysis, it is unsurprising that greater concentrations of bioT in the IBIS-I analysis are associated with an increase in side effect risk. It is also important to remember that androgens suppress SHBG expression. Therefore, it would be expected that testosterone and the other androgens would have the opposite effect on side effect outcome. Further evidence is available that supports the role of androgens, particularly testosterone, in the prevalence of HFs, via their influence on neurotransmitters and dysfunction of thermoregulatory centres in the brain (Emond et al., 2011).

Norepinephrine is believed to be the primary neurotransmitter regulating the thermoregulatory set point disruption of which can trigger heat loss mechanisms (Hasegawa et al., 2005; Kozyreva, Meyta and Khramova, 2015). Circulating plasma concentrations of norepinephrine and its metabolites increase before and during HFs, whilst injection of norepinephrine can increase core body temperature and induce a heat loss response (Hasegawa et al., 2005).

Evidence exists showing that both oestrogen and testosterone can stimulate endorphin production and thus may exert a modulatory effect on norepinephrine release. A model that explains the role of testosterone in side effect incidence, particularly HFs, is that testosterone leads to an increase in endorphin and catecholestrogen levels and culminates in increased hypothalamic norepinephrine and serotonin release. Norepinephrine

and serotonin then lower the set point in the thermoregulatory nucleus, which allows heat loss mechanisms to be triggered by subtle changes in core body temperature.

The ratio of bioT and other androgens to free and total oestrogens is another important factor to consider. In a previous study, the ratio of total androgens, the combination of androstenedione and testosterone, to total oestrogens, oestradiol plus oestrone, was significantly higher in women reporting HFs compared to those without HFs (Schilling et al., 2007c). However, this observed association may not be directly related to the concentration of total or free androgens, but the result of known associations between women with HFs having lower oestrogen concentrations than women without HFs (Øverlie et al., 2002; Gallicchio et al., 2005, 2006; Randolph et al., 2005).

The role of SHBG and its action on suppression of sex steroid activity is well known. However, several additional effects of SHBG have been proposed. Some have argued that higher levels of SHBG are compensated *in vivo* by hypothalamic-pituitary feedback, resulting in higher total sex steroid concentrations (De Ronde et al., 2005). There is continuing debate over whether - and through which mechanisms - SHBG regulates total, free and/or bioavailable sex steroid concentrations and their physiological responses (De Ronde et al., 2005; Khosla, 2006). These unanswered questions bear clinical relevance because SHBG concentrations fluctuate and several epidemiological studies have even suggested effects of SHBG independent from total or free sex hormone concentrations on menopausal symptoms (Randolph et al., 2005; Schilling et al., 2007c; Thurston and Joffe, 2011).

Evidence from the IBIS-I analysis group suggests that women with higher SHBG concentrations are at lower risk of side effects. This is supported by the IBIS-II results, although these results were not statistically significant. The association of SHBG with menopausal symptoms is the focus of few studies with contrasting outcomes. Our findings are in contrast to a previous study which reported no change in SHBG concentrations in women who reported HFs (Thurston and Joffe, 2011). The result differences may be due to study populations as the Thurston and Joffe analysis reported results from the study of women's health across the nation (SWAN) for which the study population focussed on premenopausal women as opposed to postmenopausal women in this analysis. This reason is further strengthened as our findings are in agreement with two studies investigating sex hormone concentrations and menopausal symptoms in postmenopausal women. Schilling et al. (2007c) found that SHBG concentrations

were significantly lower in postmenopausal women who experienced HFs within the last 30 days and for whom HFs occur weekly (Schilling et al., 2007c). A study by Randolph et al. (2005) also found that vasomotor symptoms decreased at higher SHBG concentrations in postmenopausal women (Randolph et al., 2005).

An inverse association between SHBG and menopausal symptoms would seem to be counter intuitive under the commonly proposed mechanism that HFs and other side effects are mainly because of oestrogen deficiency. It is known that oestrogens and androgens bound to SHBG are physiologically inactive. An increase in SHBG concentrations should result in an increase in bound oestrogens and androgens reducing their ability to bind with ERs or act in the brain increasing the risk of HFs and other menopausal symptoms. Indeed, this scenario certainly seems to be the best explanation for the observations of the Randolph et al. (2005) study that higher SHBG concentrations result in higher risk of side effects (Randolph et al., 2005). However, this does not satisfactorily explain our results; a potential explanation for which involves the interaction between BMI, SHBG and leptin.

Leptin is a protein whose main function is regulating energy balance via action in the brain on appetite and energy expenditure and is expressed by adipocytes in relation to body fat (Alexander et al., 2010). Leptin is secreted in a pulsatile manner and has a diurnal rhythm, with a peak at night and a nadir in the morning (Alexander et al., 2010). Leptin concentrations are also affected by menopause. An investigation by Sowers et al. (2008) found that during menopause leptin levels are higher in non-obese postmenopausal women than in non-obese premenopausal women (Sowers et al., 2008). In addition to the changes observed through menopause, high leptin levels are known to suppress ovarian steroid production (Zachow and Magoffin, 1997; Montgomery Rice et al., 1999). Leptin levels have been found to be positively associated with reports of HFs ($P = 0.04$) and are positively associated with BMI, whilst it has an inverse correlation with concentrations of SHBG ($P < 0.0001$) (Alexander et al., 2010). It is because of these reasons that leptin has been suggested to play a role in HFs and why it could be linked to a decrease in risk of side effects in this analysis.

The mechanism of action leading to HFs may involve leptin's action in the brain on specific receptors in the hypothalamus, a region associated with energy expenditure (Cakmak et al., 2005; Klok, Jakobsdottir and Drent, 2007). Luheshi et al. (1999) reported that the injection of leptin peripherally increases core body temperature (Luheshi et al.,

1999). According to previous studies, an increase in core body temperature precedes HFs in postmenopausal women (Freedman, 2014). If higher SHBG concentrations are a marker for lower leptin concentrations, then this may be one explanation for the decreased risk of side effects seen in these analyses. However, for this hypothesis to be confirmed, detailed investigation of leptin concentrations within these samples would need to be performed.

Associations of sex hormones and SHBG with other baseline risk factors of side effects in both the endocrine therapy and placebo group of both IBIS-I and IBIS-II were investigated. The results suggest that circulating concentrations of sex hormones are associated with several of the well-established or suspected risk factors for side effects. Within the IBIS-I analysis, the strongest associations were with age where concentrations of all the sex hormones but not SHBG were lower in older women compared to younger women. Age at menarche was strongly associated with decreases in testosterone and bioT in women who had an age of menarche older than 14 compared to those 14 or under.

In the IBIS-II study, the strongest associations were with age which indicated lower sex hormone concentration and higher SHBG concentrations. In contrast to the IBIS-I study, BMI was also associated with a decrease in SHBG concentrations and an increase in bioT. Time since menopause was also strongly associated with decreases in all sex hormones whilst those with hysterectomy had higher concentrations of bioT and lower concentrations of SHBG than those who had a natural menopause without hysterectomy.

The decrease in sex hormone concentrations, and the increase in SHBG with age are consistent with previously reported associations between age and these sex hormones in postmenopausal women (Endogenous Hormones and Breast Cancer Collaborative Group, 2011). The relatively consistent concentrations of testosterone, bioT and SHBG between the two age categories is likely due to the time since menopause and sex hormones reaching their postmenopausal levels. This is further enforced by the association of time since menopause and each of the sex hormones which shows that the largest changes are between women who are newly menopausal (<5 years) and those who have been menopausal for longer. This is consistent with the review of sex hormone studies performed by the Endogenous Hormones and Breast Cancer Collaborative Group (EHBCCG) who also observed no change in hormone concentrations in women who

had been postmenopausal for longer (Endogenous Hormones and Breast Cancer Collaborative Group, 2011).

Other risk factors that are associated with sex hormone concentrations are hysterectomy and oophorectomy. Previous studies from the EHBCCG have shown that postmenopausal women who have had a bilateral oophorectomy have lower circulating androgen concentrations than postmenopausal women with intact ovaries; our analyses support these relationships. Clinical studies have shown that androgen concentrations in ovarian veins are higher than in peripheral circulation thereby demonstrating that ovaries secrete androgens (Lebbe and Woodruff, 2013; Franks and Hardy, 2018). As a result of this women without ovaries will have lower androgen concentrations than those who have retained their ovaries. Women in the IBIS-II group randomised to anastrozole who had undergone a bilateral oophorectomy had testosterone concentrations about 25% lower and bioT concentrations 3.7% lower than those of naturally postmenopausal women. Women who had undergone a hysterectomy had SHBG concentrations lower than those of women who had a natural menopause or women who had undergone a bilateral oophorectomy. This is in contrast to previous studies which found no change in SHBG concentrations between women who have had a hysterectomy and those that have not (Kotsopoulos et al., 2015).

Other breast cancer risk factors identified by the EHBCCG as being associated with sex hormone concentrations were BMI and previous HRT use (Endogenous Hormones and Breast Cancer Collaborative Group, 2011). Results from the IBIS-II analysis support these findings as BMI was associated with a reduction in SHBG and an increase in bioT in obese women compared to those with BMI < 25. Additionally, previous HRT use was associated with decreased concentrations of all sex hormones, but not SHBG. The reason why androgens increase with BMI is not clear. One potential mechanism is that as BMI increases there are associated increases in insulin, which may stimulate androgen production in the ovarian stroma, whereas adrenal androgen synthesis is not known to be stimulated by insulin (Barber et al., 2006). To further support this potential mechanism, results suggested that androgens decreased in women who had undergone a bilateral oophorectomy and therefore for whom most androgen synthesis must take place in the adrenals. SHBG also decreased with increasing BMI, as has been consistently observed in previous studies (Baglietto et al., 2009; Cooper et al., 2015). There is evidence to suggest that the decrease in SHBG may also be due to increased

insulin concentrations which inhibit SHBG synthesis in the liver (Daka et al., 2013). The finding that previous HRT use was associated with lower concentration of testosterone, DHEA-S and bioT supports the findings of Chan et al. (2008) who observed a reduction in postmenopausal women (Chan et al., 2008). Whilst the reason why this association exists is not fully known, it is possible that the associations with HRT may exist due to the fact that women with relatively low endogenous hormone levels may have more menopausal symptoms, and therefore be more likely to be prescribed hormonal therapy (Chan et al., 2008).

In contrast to the result of IBIS-II, results of the IBIS-I analysis show no association between BMI or previous HRT use and sex hormone or SHBG concentrations. This may be due to a smaller number of women in this analysis and therefore the analysis was not sufficiently powered to find such associations.

When interpreting the results of this analysis, its strengths and limitations must be considered. A major strength of this work is that the study was specifically designed to examine side effects reported within the first year of endocrine therapy. This study is a novel examination of side effects and their association with sex hormone and SHBG concentrations which had not previously been performed in women at high risk of breast cancer. This study also benefits from a consistent method of sample analysis and does not rely on data from a wide array of groups. This is important as inconsistent procedures such as the type of sex hormone assay used (with or without a purification step) may influence results.

The major limitation of this study is that it was not possible for resource reasons to measure oestrogen concentrations in the baseline blood samples. It has been well documented that oestrogen plays a key role in the incidence of side effects and not being able to measure them was disappointing. Other limitations of this study include the use of only one blood sample measured for sex steroid hormone levels. Another limitation of this analysis is that the data are taken at baseline, and therefore cannot be used to attribute causality, and that some other potentially important factors such as physical activity and diet were not included in the analyses.

This study adds to the existing evidence surrounding the impact of sex hormone concentrations on early reported side effects. This study furthers the evidence that androgen concentrations, in particular ln-bioT, may play an important role in the aetiology of

side effects. However, the direction of the effect of ln-SHBG observed in this study is contrary to that which is expected and, as such, highlights the need for further studies.

A prospective study, as described in chapter 4, with baseline hormone measurements followed by regular hormone measurement through active therapy, in particular the first year of active therapy, would enable more detailed analysis of the role of sex hormones in side effects aetiology. Multiple hormone measurements would provide potential answers to whether side effects are as a result of hormone concentrations dropping below a threshold, or if the size of the decreases in hormone concentrations has a role in side effect aetiology. Additionally, the rate of sex hormone reductions could be addressed and a more complete picture of the role of sex hormones on side effect outcomes could be achieved.

6.6 Conclusions

Results of this analysis show that bioT and SHBG are associated with side effects occurring in the first year of taking endocrine therapy. Therefore, they should be considered as markers for risk of side effects and could be used to better inform women who are considering taking endocrine therapy for prevention of breast cancer. Sex hormone concentrations are also associated with several established or suspected risk factors for side effects and may mediate the effects of these factors on side effect risk. Not being able to measure oestrogen concentrations is disappointing, but the results presented in this chapter highlights the need to perform further testing and to accurately measure oestrogen concentrations in these samples to properly assess the impact that oestrogens have on side effects. Further work investigating concentrations of oestrogen and leptin should be performed to gain further insight into mechanisms of menopausal-like side effects. Additionally, genetic testing of the samples should be undertaken to assess the possibility that those with high testosterone have lower oestradiol because of lack of conversion via aromatase due to polymorphisms within the gene encoding aromatase.

Chapter 7: Prediction models for early reported side effects of endocrine therapy

7.1 Introduction

Prophylactic endocrine therapy for five years has shown to decrease breast cancer incidence in women at high risk (Cuzick et al., 2015, 2020; Goss et al., 2011; Veronesi et al., 2003). However, a large number of women who take endocrine therapy for breast cancer prevention also report side effects, particularly in the early stages of active therapy (Cuzick et al., 2015, 2020; Goss et al., 2011; Powles et al., 2007; Regan et al., 2011; Veronesi et al., 2007). Side effects concerns are commonly reported as a major reason for poor uptake and adherence to endocrine therapy (Ropka et al., 2010; Smith et al., 2016). As such, identification of patients who are at high risk of early side effects and who may benefit from close surveillance or management of side effects is clinically important. Identification of women at high risk of side effects would enable the potential of side effects to be factored into the initial discussion for endocrine therapy and to devise a schedule to minimise these side effects. The overall aim is to increase the number of women, at high risk of breast cancer, who choose to take endocrine therapy for prevention and subsequently to increase adherence to therapy.

To achieve this aim, producing a risk model which gives women a realistic estimate of their likelihood of side effects could be beneficial (Juraskova et al., 2014). This would provide women who are concerned about the use of endocrine therapy with more information for their decision making. For efficient and effective prevention of disease medication should not only impact the target disease but minimise the disruption to quality of life (Liu et al., 2018). Models for predicting side effect risk would be useful for clinical management and for developing prevention strategies. However, currently, no prediction models combining risk factors for endocrine therapy side effects exists.

Multiple risk factors for side effects have been established. Throughout chapters 4-6 of this thesis risk factors for side effects have been identified and their effects quantified.

These risk factors were divided into baseline risk factors; including age, age of menarche, age of menopause, time since menopause, the use of hormone replacement therapy, body mass index, and smoking; genetic factors, and sex hormone factors. The use of clinical risk factors, such as age and family history to determine the risk of breast cancer has been established (Antoniou et al., 2008; Gail et al., 1989; Tyrer et al., 2004). Studies have also shown that the addition of polygenic risk scores (PRS) and mammographic density to prediction models can improve prediction of breast cancer risk (Brentnall et al., 2015, 2019; Cuzick et al., 2017; Evans et al., 2017; Michailidou et al., 2013; Tamimi et al., 2007; van Veen et al., 2019). Recently, studies have shown that incorporating sex hormone concentrations can also improve breast cancer risk prediction (Hüsing et al., 2017; Tin Tin et al., 2021; Tworoger et al., 2014). Therefore, it is important to investigate multiple risk factors for side effects in more detail and build prediction models to identify women who may be at increased risk of developing side effects during treatment. The incorporation of genomic factors and sex hormones into a tool for use in the decision-making process about endocrine therapy should also be explored.

To this end, machine learning and statistical modelling algorithms have been used in a wide range of applications, including disease modelling (Ming et al., 2019; Uddin et al., 2019). Machine learning algorithms use an array of statistical and computational algorithms to learn from and find patterns in large and complex datasets, and the most common form of these algorithms are for supervised learning. Supervised models are developed using a dataset with a known outcome variable and subsequently the model can be used to predict the outcome of unlabelled examples based on other known information about that sample. Whilst there is growing applicability and effectiveness of supervised algorithms for predictive disease modelling, no models exist for prediction of side effects during endocrine therapy for breast cancer prevention or treatment.

In a recent review Uddin et al. (2019) investigated the use of different approaches for the prediction of diseases including breast cancer and compared which algorithms performed best (Uddin et al., 2019). The most commonly used machine learning techniques across five breast cancer studies comparing multiple machine learning methods for breast cancer prediction, were artificial neural networks (ANN), logistic regression (LR) and decision trees (Ahmad et al., 2013; Ayer et al., 2010; Delen et al., 2005; Lundin et al., 1999; Yao et al., 2013). In the case of the Ayer et al. (2010) and Lundin et al. (1999) studies, ANN and LR models had similar predictive performance for

breast cancer risk (Ayer et al., 2010; Lundin et al., 1999). Ayer et al. (2010) study compared the use of ANN and LR models for breast cancer risk prediction in 18,269 patients (17,924 women and 345 men) (Ayer et al., 2010). Mammographic descriptors, demographic risk factors and breast cancer history were mapped to 36 discrete variables which were used to train the models. The LR model, using a forward selection method to select breast cancer predictors at a cut-off of $P < 0.001$, achieved an AUC of 0.963 (SD $\pm$ 0.009) using six of the 36 variables. In comparison, the ANN model, using 36 variables, achieved an AUC of 0.965 (SD $\pm$ 0.001). Both models outperformed radiologists working unaided ($P < 0.001$); however, no external validation was performed (Ayer et al., 2010). Lundin et al. (1999) used ANN models to investigate 5, 10 and 15 year risk of breast cancer in 951 breast cancer patients who were divided into a training set of 651 and a validation set of 300 patients (Lundin et al., 1999). Eight variables, tumour size, axillary nodal status, histological type, mitotic count, nuclear pleomorphism, tubule formation, tumour necrosis and age were used to train the ANN model. The ANN models for 5-, 10- and 15-year breast-cancer-specific survival had a higher AUC than the LR models in the independent validation dataset; however, results were similar at each time point, ANN = 0.909, 0.886 and 0.883, compared to LR = 0.897, 0.862 and 0.858 at 5-, 10- and 15-years respectively. External validation was not performed for either the ANN models nor the logistic regression models (Lundin et al., 1999).

During this thesis LR has been used to test and select variables associated with side effects, and given its use in numerous breast cancer studies, LR was used for the formation of prediction models. LR can be used to examine the relationship of one or multiple variables and a binary outcome and has been used for predicting breast cancer outcomes in multiple studies and compared well against other algorithms (Ayer et al., 2010; Delen et al., 2005; Lundin et al., 1999). One major issue with LR, and other statistical models, is that when a large number of variables are considered, regression models can overfit to the data leading to a model that is well fit to the training data but does not predict outcomes well in a validation set or real-world data (Kim et al., 2018). It is therefore necessary to reduce the number of variables and obtain accurate predictions particularly in this case where a combination of clinical data, genetic factors and sex hormones were investigated.

To avoid overfitting with large number of variables some methods only use variables

known to be statistically significant for the outcome of interest. However, the use of stepwise logistic regression also helps selection of the most important variables by adding variables sequentially to an empty model and checking all variables in the model to ensure that all variables are still significant this method removes redundant variables and reduces the risk of model overfitting. The use of stepwise logistic regression was previously discussed in section 3.4.5 as being suboptimal for regression models with numerous covariates, hence the use of LASSO, in chapter 5 to select SNPs associated with side effects. LASSO is a commonly used tool for variable selection to reduce the number of variables (Hastie et al., 2014). In the following analysis LASSO will be used to develop prediction models with a variable selection step prior to model training to determine whether all statistically significant variables should be included in the models or whether simpler models can be used.

The main aim of this study was to develop a model for prediction of early side effects of endocrine therapy and to identify women who are at high risk of developing side effects. LR and LASSO were used for model formation before being compared to see which performs best in an internal validation data set from IBIS- I or IBIS-II. Secondary analyses were the addition of genomic and sex hormone data to baseline factor models to assess the impact of a combination multiple risk factors on side effect risk prediction. In addition, a simple side effect risk score based on logistic regression coefficients was developed for use in a clinical setting using easily available measurements to produce a risk score for side effects.

What we already know

- Models can be used to predict those at high risk of breast cancer
- Multiple techniques have been used to select variables for these models
- Providing information about side effect risk at the study of endocrine therapy may increase uptake and adherence

---

What this analysis adds

- Investigation of statistical models for the prediction of early side effects
- Assesses the impact of adding sex hormone and genetic information to a clinical model
- Defines the best method for variable selection and derives a model
- Validation of the best models in a subset of data not used for model training
- Development of simple side effect risk score to identify women at high side effect risk

7.2 Methodology

7.2.1 Study populations

Data from the double-blinded, randomised, placebo-controlled IBIS-I and IBIS-II trials were used (Cuzick et al., 2002, 2014, 2015, 2020). The IBIS-I cohort consisted of a total of 7,154 women were randomised to placebo (N = 3,575) or tamoxifen (N = 3,579) for five years and were followed up every six months during the active treatment period. Details of the trial design, methodology and primary outcomes have been published elsewhere and study inclusion and exclusion criteria described in section 3.1 and in previous publications (Cuzick et al., 2002, 2007, 2015). The IBIS-II study randomised 3,864 postmenopausal women aged 40–70 years to either 1 mg oral anastrozole or matching placebo to be taken every day for 5-years. Inclusion and exclusion criteria are provided in full in section 3.2 and in previous publications (Cuzick et al., 2014, 2020). Follow up visits occurred at 6 months, and 12 months, and then annually for 5-years. The type of follow-up visit over the 5 years varied and consisted of a mixture of clinic visits, annual questionnaires, and also record linkage systems in the UK (Cuzick et al., 2014, 2020).

Menopausal status for women on the IBIS-I trial was measured at study entry only. Menopausal status was assessed by "self-reports" and no hormone testing was performed to definitively assess menopausal status. As a result, it was not possible to assess changes in menopausal status between baseline and 6 months. Therefore, the split menopausal analysis in the IBIS-I trial is based on baseline menopausal status and no correction was made for women who became postmenopausal during the first 6 months. Hormone testing was performed on women in the IBIS-II trial and women with follicle stimulating hormone of greater than 30 IU/L were ineligible for participation on the trial ensuring a trial population of only postmenopausal women (Cuzick et al., 2014).

In the IBIS-I analysis, the major side effects of interest were hot flushes and gynaecological symptoms as these were the major side effects associated with tamoxifen (Cuzick et al., 2002; Cuzick et al., 2015). The side effects were reported on a 6-month basis and were collected on a presence or absence basis. Side effect severity (mild, moderate, or severe) was also collected at each follow up point. In the IBIS-II analysis, the major

side effects were hot flushes, gynaecological symptoms and arthralgia as these were the most commonly reported side effects of anastrozole (Cuzick et al., 2014, 2020). During IBIS-II side effects were collected at 6- and 12-months after which side effects were collected at the annual follow-up visits. For both studies the cut-off point for side effect reporting was the six-months follow-up in line with other analyses within this thesis. Side effect events occurring after this date were not included.

Both datasets consisted of data related to specific side effects including HFs, gynaecological symptoms (vaginal discharge and dryness, and irregular bleeding) and additionally arthralgia in the IBIS-II dataset. Baseline factors such as age at randomisation, BMI, HRT history, and smoking history; reproductive factors including age at menarche and menopause, time since menopause, parity, and age at first birth were recorded at study entry.

7.2.2 Statistical methods

7.2.2.1 Data preparation

For IBIS-I all women whose side effect status was known and who did not develop breast cancer or die before the 6-month follow up point were included. After exclusion of 6 women who developed breast cancer, 2 women who died, and 400 women who had missing side effect status 6 months after randomisation, 6,746 women remained for analysis. Women randomised to tamoxifen but with unknown menopausal status were also excluded from this analysis (N = 23) as were women randomised to placebo (N = 3,393). Remaining women (N = 3,330) were then analysed by menopausal status. Figure 7.1 summarises the women remaining in the analysis.

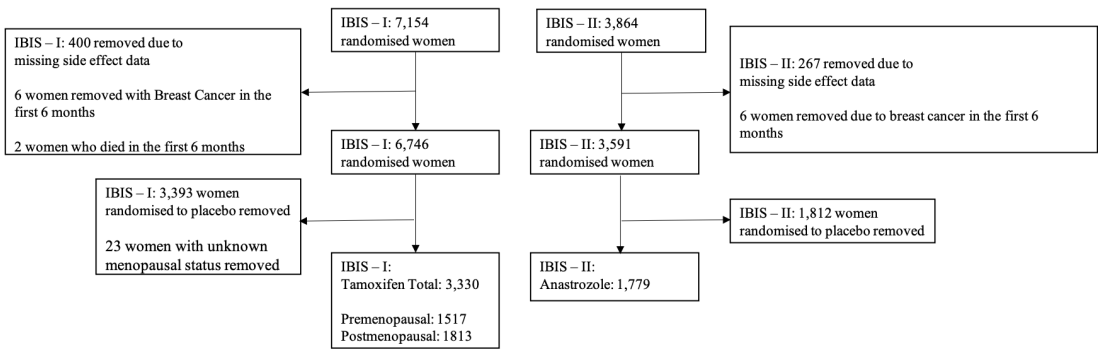


Figure 7.1: Consort diagram displaying study numbers for the IBIS-I and IBIS-II analysis and number of women removed from each analysis

For the primary IBIS-II analysis all women whose side effect status was known and who did not develop breast cancer before the 6-month follow up point were included. 6 women developed breast cancer, 0 women died, and 267 women had missing side effect status 6 months after randomisation and were excluded leaving 3,591 women for this analysis. After removal of women randomised to placebo 1,779 women randomised to anastrozole remained in the analysis group (Figure 7.1).

7.2.2.2 Variable selection

Analysis of baseline risk factors, chapter 4, genomic risk factors, chapter 5 and sex hormones chapters 6 identified numerous risk factors for pre and postmenopausal women randomised to tamoxifen on the IBIS-I trial and for postmenopausal women randomised to anastrozole on the IBIS-II trial. Only risk factors identified as multivariate significant in chapter 4, and chapter 6, were included. When available, continuous variables were used rather than categorical variables.

No SNPs from the candidate gene study were significantly linked to any side effect. Therefore, 195 SNPs identified in the multi-loci analysis, section 5.3.4, were added to the baseline factor dataset and their impact on side effect prediction models evaluated.

7.2.2.3 Missing data

Each dataset was explored for missing data. An analysis of premenopausal women randomised to tamoxifen on the IBIS-I trial showed that <1% of variable data was missing. On inspection most missing data was confined to BMI (N = 54). In addition, data was missing for HRT (N = 2) and age at menarche (N = 2). Analysis of postmenopausal women in the IBIS-I dataset identified 8% of missing data with most missing data was confined to time since menopause (N = 901). A small quantity of missing data was also observed for BMI (N = 51). The IBIS-II dataset of postmenopausal women randomised to anastrozole showed that approximately 1% of all variable data was missing. Missing data was observed in time since menopause (N = 26), and BMI (N = 31), but a small number of women had missing data for HRT (N = 2) and prior hysterectomy (N = 2).

Given the previous association of the above variables with side effect incidence in each group, see section 4.3, these variables were retained in the dataset and missing data

was imputed using multivariate imputation by chained equations implemented through the MICE package in R (van Buuren et al., 2011). The imputation of missing data process was as follows. Each variable was initially imputed using the mean of observed values for each variable in the dataset. Mean imputations acted as placeholders prior to further imputation. For the variable with the fewest missing values, each of the placeholder imputations was then returned to missing. The variable with missing values became the dependent variable in a regression model and other variables without missing data became independent variables of the regression model. Missing values were then imputed using predictions from the regression model. Following imputation, the next variable with the fewest missing values was modelled using the same technique as described above. Both observed and predicted values from previously imputed variables were used in the regression model. The process was run five times for each variable and the imputation for each variable updated each time. The repeating the process was to ensure convergence in the distribution of the imputation parameters thereby producing consistent imputations for each variable accounting for any statistical uncertainty in the imputations (van Buuren et al., 2011).

7.2.2.4 Addition of genomic information to prediction models

Genotyping of IBIS-I samples was performed for all breast cancer cases and matched controls with available material; genotyping procedures have been previously described (Cuzick et al., 2017). Briefly, 10 mL of DNA extracted from IBIS-I blood samples were analysed in plates of 96-well plates. Formalin-fixed paraffin-embedded block samples were analysed on a separate plate. For quality control, two internal controls with known genotypes were run on each plate. Assays were carried out blindly at Genome Quebec (Montreal, Canada), a clinical service provider–certified by Illumina; the Illumina OncoArray was used, and the Illumina HTS (high-throughput sequencing) protocol was rigorously followed (Jack Cuzick, Brentnall, & Dowsett, 2017).

Genotypes and demographic information at study entry were available for 820 pre and postmenopausal women from the IBIS-I trial. Only women from IBIS-I who were randomised to tamoxifen and who had a genotype rate of >97% were included in the analysis (N = 310).

131 premenopausal women (Train = 103, Test = 25) were included in this analysis

and 169 women in the postmenopausal analysis (Train = 136, Test = 33). A subsection of 195 SNPs, which had been identified by LASSO as being associated with side effects from the candidate gene group (N = 571), were subsequently added to the baseline factors to assess the impact of genomic factors on prediction models. Genomic information was not available for women in the IBIS-II cohort.

7.2.2.5 Addition of baseline sex hormone concentrations to prediction models

Baseline sex hormone concentration were assessed in chapter 6. The details of the sex hormone analysis were provided in more detail in chapter 6. Briefly, baseline concentrations of dehydroepiandrosterone-sulphate (DHEA-S), testosterone, and sex hormone binding globulin (SHBG) were analysed using enzyme linked immunosorbent assay. Postmenopausal women were matched on a 1:1 basis matching for randomised treatment and age ± 1 year. Postmenopausal women were selected from either trial who had blood collected before study entry and for whom side effect status was known up to and including the 12-month follow-up visit. Women were selected as cases if they reported any of the major side effects; hot flushes, vaginal discharge, vaginal dryness, or irregular bleeding, for IBIS-I and hot flushes, gynaecological symptoms, or arthralgia for IBIS-II. Controls were women who reported no side effects. Concentrations of sex hormones were ln-transformed to normalise the distributions and were investigated for association with side effects outcomes in the first 12 months of IBIS-I or IBIS-II.

Analysis in chapter 6 showed that ln-SHBG and ln-bioT were both associated with side effect outcomes in postmenopausal women randomised to tamoxifen, section 6.4.2. Therefore, data for both these sex hormones was added to the baseline data and used to train models using each of the procedures used for determining models using baseline data to determine the effect of adding sex hormone factors to the prediction models. Analysis of postmenopausal women randomised to anastrozole during IBIS-II showed only ln-SHBG was associated with side effect outcomes, see section 6.4.3. Therefore, ln-SHBG was the only variable from the sex hormone study added to the IBIS-II baseline factor data set. No sex hormone measurements were taken for premenopausal women in the IBIS-I study so the addition of sex hormones to the original baseline factor model was not possible.

Sex hormone concentrations were available for 224 postmenopausal women (train set = 172, validation set = 52) randomised to tamoxifen as part of the IBIS-I study and for 524 postmenopausal women (train set = 445, validation set = 109) randomised to anastrozole during the IBIS-II study. Due to large variation in sex hormone concentrations in premenopausal women sex hormone measurements were not measured in premenopausal women. Therefore, data was not available for this subgroup.

7.2.2.6 Dataset partition and 10-fold cross-validation

To minimise bias associated with random sampling of validation and training datasets stratified 10-fold cross-validation was used, a common procedure in research (Zhang et al., 2015). In 10-fold cross-validation, the complete dataset is randomly split into 10 subsets of approximately equal size. The classification model is trained and tested 10 times. Each time the model is trained on all but one-fold and tested on the remaining single fold Figure 7.2.

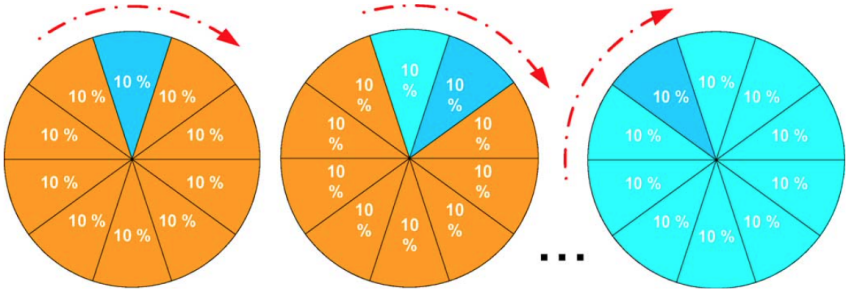


Figure 7.2: Depiction of the ten-fold cross-validation procedure used for model development (Delen et al., 2005)

The overall accuracy of the cross-validation is the average of each of the 10 individual accuracy measures. Since the cross-validation accuracy depends on the random assignment of the individual cases into 10 distinct folds, it is required to stratify each of the folds. In stratified 10-fold cross-validation, each fold is created to contain approximately the same proportion of cases or non-cases as the original dataset. Studies show that the use of stratified cross-validation compared to regular 10-fold cross-validation generate results with lower bias and lower variance (Kohavi, 1995). Empirical studies have shown that 10 can be an optimal number of folds optimising the test time and minimising the bias and variance (Bengio et al., 2004; Kohavi, 1995; Zhang et al., 2015).

7.2.2.7 Prediction models

There is no consensus method for selection of statistical models to predict outcomes. However, any selected model should use the data efficiently and the process of model development should be clear and detailed to enable reproducibility (Philips et al., 2004). Two classification models: logistic regression and LASSO, were used for model development. Logistic regression (LR) models are widely used statistical models which allow for multivariate modelling of binary variables. LR models were selected for inclusions in this study due to their popularity in the recently published literature. LR and LASSO, have been discussed in depth in section 3.4.1 and 3.4.5 respectively.

In both of the prediction models, prior to training the data all variables were pre-processed to centre and scale the data. Centring the data ensured that each of the predictor variables had a mean of 0 which enabled easier interpretation of the model intercept. Scaling the data put each of the predictor variables on the same scale which avoided very small coefficients for variables for predictors which had larger scales and made interpretation of regression coefficients easier. In addition to modelling, multivariate LR models estimated coefficients for each of the predictors in the model and weights each predictor against the other predictors in the model quantifying the contribution of each predictor to the modelled outcome. Coefficients from the LR model were used to form a risk score against which a woman's risk of side effects could be determined, see section 7.1.2.9.

7.2.2.8 Classifier performance

The area under the receiver operating characteristic curve (AUC) was used as a measure of accuracy of the predictor models (Metz, 1978). The AUC indicates how well a prediction model discriminates between women with side effect and women without side effects. The ROC curve for all models using the probabilities generated using the 10-fold cross-validation technique was attained, and the AUC of each ROC calculated. The mean AUC, sensitivity, and specificity of the 10 models was subsequently calculated and reported. After training, the models were validated in the pre-partitioned validation set. When validating the model in the validation set a confusion matrix was obtained. The AUC, sensitivity and specificity were calculated using the formulas in section 3.4.6.

Validation was first performed at a decision threshold of 0.5. A ROC curve was then made to determine the optimum prediction threshold for classification of outcomes in the validation set. The optimum threshold was determined using Youden's J statistic calculated by:

$$Youden's\, J = Sensitivity + Specificity - 1 \tag{7.1}$$

Equation 7.1: Equation for calculation of Youden's J statistic for summarising the performance of a diagnostic test.

Youden's J statistic is often used alongside ROC analysis. The maximum value of the index was used to select the optimum cut-off point. The statistic value ranges from zero to one where zero indicates a model predicting equal numbers of true positives and false positives and one indicates a perfect model which predicts no false positives or false negatives (Youden, 1950). After determining the optimum prediction threshold, the AUC, sensitivity and specificity of the "best" model were reported.

The Delong method was used to compare the AUCs of the baseline risk factor models and the baseline risk factor models including genomic or sex hormone data, as this method accounts for potential correlation between ROC curves which have been obtained from the same data (Delong et al., 1988). The Delong test calculates the AUC of each model in addition to the variance and covariance of each model. A z-statistic and p-value for the test statistic are subsequently calculated comparing two AUCs in a two-sided test (Delong et al., 1988).

7.2.2.9 Development of a side effect risk (SER) score

After models were trained and validated, the LR model had the superior performance. The availability of regression coefficients from LR also enabled the formation of a risk score against which side effect outcomes were modelled.

Coefficients from the logistic regression models using baseline factors only were subsequently used to create a side effect risk (SER) score. The number of women reporting or not reporting side effects at each score was reviewed and the score divided into quartiles classifying women as high risk, high intermediate risk, low intermediate risk,

or low risk. The number of women reporting side effects in each risk quartile was investigated. The SER score was developed in the training data set and then tested in the validation set.

7.2.2.10 Outcomes

The primary analysis was to develop models for predicting the incidence of side effects within the first 6 months of starting endocrine therapy. Side effects were defined as a binary dependent variable representing the presence or absence of side effects during the first 6-months. For women in the IBIS-I trial the side effects combined were hot flushes, vaginal discharge, vaginal dryness, and irregular bleeding. For women in the IBIS-II trial the combined side effects were hot flushes, arthralgias, and gynaecological symptoms including vaginal dryness, bleeding, uterine prolapse and vulvovaginal pruritus.

The decision to combine side effects was based on a review of the literature. Studies show that all side effects are associated with a decrease in adherence particularly in women randomised to endocrine therapy (Land et al., 2016; Smith et al., 2017). Additionally, no threshold exists at which side effects are considered tolerable. Evidence suggests that women discontinue treatment based on any severity; although there is evidence to suggest that the trend increases as symptom severity increases (Land et al., 2016; Smith et al., 2016, 2017). Therefore, for ease of understanding side effects were represented as a yes / no binary outcome. Prediction models were developed and compared to determine which was the best model for side effect prediction. Models were then used to predict side effects in a validation dataset, 20% of total data, previously partitioned from the original dataset.

Secondary analyses included the addition of genetic and sex hormone factors to the baseline factor models. Secondary endpoints were assessment of the impact of these factors on the predictive ability of these models. A simple risk score for side effects in group was developed based on regression coefficients to assess which women may be identified as being at high risk of side effects. The following results section is presented as model training and validation using baseline factors, investigation of the impact of genetic and sex hormone data on the models, and finally development and validation of a side effect risk score for each subgroup of women.

7.3 Results

7.3.1 Study demographics

A total of 3,330 women from the IBIS-I trial were included in the analysis. 45.6% (N = 1,517) of women in the IBIS-I group were premenopausal and 54.4% (N = 1,813) were postmenopausal (Table 7.1). In the premenopausal group, the median age was 46y (IQR = 43.0 – 48.0) and the median BMI was 25.5 kg/m^2 (IQR = 22.8 – 29.4), 85.6% (N = 1,299) had never used HRT and 50.3% (N = 763) had never smoked. Median age at menarche was 13.0y (IQR = 12.0 – 14.0) (Table 7.1).

Postmenopausal women from IBIS-I had a median age of 54y (IQR = 50.0 – 59.0) and a median BMI 26.3 kg/m2 (IQR = 23.7 – 30.0). 36.5% (N = 662) had never used HRT and 48.9% (N = 887) had never smoked (Table 7.1). Median age at menarche was 13.0y (IQR = 12.0 – 14.0) and median age at menopause was 50.0y (IQR = 45.0 – 52.0). 51.4% (N = 932) had a hysterectomy of which 49.2% (N = 459) had also removed at least one ovary (Table 7.1).

A total of 1,779 women from the IBIS-II trial were included in the analysis. The median age was 59.0y (IQR = 55.0 – 63.0) and the median BMI was 27.3 kg/m2 (IQR = 24.2 – 31.0). 52.6% (N = 935) had never used HRT and 55.8% (N = 992) had never smoked. Median age at menarche was 13.0y (IQR = 12.0 – 14.0) and 85.4% (N = 1,520) had at least one child. Median age at menopause was 50.0y (IQR = 45.0 – 52.0) and 32.8% (N = 594) had had a hysterectomy with 65.6% (N = 389) removing at least one ovary (Table 7.1).

Table 7.1: Baseline characteristics of all participants in each of the analysis groups defined by trial on which a woman was enrolled and menopausal status within the IBIS-I trial

	IBIS-I		IBIS-II
	Premenopausal (N = 1,517)	**Postmenopausal (N = 1,813)**	**Postmenopausal (N = 1,779)**
	Median (IQR)	**Median (IQR)**	**Median (IQR)**
Age at randomisation (years)	46.0 (43.0 - 48.0)	54.0 (50.0 - 59.0)	59.0 (55.0 - 63.0)
Age of menarche (years)	13.0 (12.0 - 14.0)	13.0 (12.0 - 14.0)	13.0 (12.0 - 14.0)
Age of menopause (years)	NA	49.0 (46.0 - 52.0)	50.0 (45.0 - 52.0)
Time since menopause (years)	NA	7.0 (3.0 - 13.0)	10.0 (4.0 - 15.0)
	N (%)	**N (%)**	**N (%)**
HRT use			
Never	1,299 (85.6)	662 (36.5)	935 (52.6)
Former	92 (6.1)	444 (24.5)	844 (47.4)
Current	126 (8.3)	707 (39.0)	NA
BMI (kg/m^2) Median (IQR)	25.5 (22.8 - 29.4)	26.4 (23.7 - 30.0)	27.3 (24.3 - 31.0)
Smoking history			
Never	763 (50.3)	887 (48.9)	992 (55.8)
Former	445 (29.3)	621 (34.3)	565 (31.8)
Current	308 (20.3)	303 (16.7)	212 (11.9)
Type of menopause			
Hysterectomy	NA	932 (51.4)	594 (32.8)
Hysterectomy and oophorectomy	NA	459 (25.3)	389 (21.9)
Sex Hormones			
LnSHBG[a] (nmol/L) Median (IQR)	NA	6.7 (6.1 - 7.3)	4.6 (4.2 - 5.0)
LnBioT[a] (pmol/L) Median (IQR)	NA	5.9 (5.7 - 6.2)	6.0 (5.7 - 6.2)

[a] Number of women with available lnSHBG and lnBioT data was 224 for IBIS-I postmenopausal women and 554 for IBIS-II postmenopausal women. LnSHBG was not measured for premenopausal women.

7.3.2 Statistically significant risk factors of side effects

Analysis of baseline risk factors, chapter 4, genomic risk factors, chapter 5 and sex hormones chapters 6 identified numerous risk factors for pre and postmenopausal women randomised to tamoxifen on the IBIS-I trial, and for postmenopausal women randomised to anastrozole on the IBIS-II trial (Table 7.2).

For premenopausal women in IBIS-I, age, former use of HRT, and BMI were all associated with hot flush risk. Age at menarche was associated with gynaecological symptoms. Sex hormones were not measured for premenopausal women. Genomic information was available for 131 women. The effect of 197 SNPs identified by LASSO in chapter 5 were assessed as a secondary outcome (Table 7.2).

HRT use, both current and former, hysterectomy and time since menopause were associated with hot flush risk in postmenopausal women randomised to tamoxifen. Additionally, BMI was associated with gynaecological symptoms. Both ln-SHBG concentrations (OR = 0.32 (0.19 – 0.55); P $<$ 0.0001) and ln-bioT concentrations (OR = 2.38 (1.23 – 4.58); P = 0.004) were associated with side effects in postmenopausal women randomised to tamoxifen. Hormone measurements were available for 224 women randomised to tamoxifen during IBIS-I and were investigated as a secondary outcome for the effect of sex hormones in prediction models for side effects. Genomic information was available for 196 postmenopausal women randomised to tamoxifen and were also assessed as a secondary analysis. Data for both sex hormone and genomic information was available for a total 44 women. Since not enough samples were available to assess the impact of genomic and sex hormone concentration, they were not included in this analysis (Table 7.2).

Table 7.2: A summary of statistically significant baseline risk factors for pre and postmenopausal women randomised to tamoxifen in IBIS-I or anastrozole in IBIS-II trials. All OR are from multivariate models, only risk factors which were statistically significant in full multivariate models are shown and included in these final models. For full model results see chapter 4 tables 4-4, 4-6, 4-8, 4-10, 4-12.

	IBIS-I				**IBIS-II**	
	Premenopausal		**Postmenopausal**		**Postmenopausal**	
	OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value
Hot Flushes						
Age (years)	1.05 (1.03 - 1.08)	< 0.001	-		0.97 (0.95 - 0.99)	0.005
HRT (Current)	-		1.42 (1.03 - 1.96)	0.03	1.46 (1.19 - 1.78)	0.0002
HRT (Ex-user)	2.22 (1.40 - 3.59)	< 0.001	1.43 (1.01 - 1.98)	0.04		
BMI (kg/m^2)	1.02 (1.00 - 1.04)	0.03	-		1.32 (1.03 - 1.68)	0.03
Time since menopause (years)	-		0.95 (0.92 - 0.99)	0.004	0.98 (0.96 - 1.00)	< 0.01
Hysterectomy	-		1.57 (1.06 - 2.34)	0.03	1.43 (1.13 - 1.81)	< 0.01
Gynaecological symptoms						
Age at menarche (years)	1.44 (1.15 - 1.81)	0.002	-		-	
BMI (kg/m^2)	-		0.97 (0.94 - 0.99)	0.01	-	
Arthralgia						
BMI(kg/m^2)	NA		NA		1.03 (1.01 - 1.05)	< 0.01

[-] Risk factor not included in the multivariate model
[NA] Risk factor not applicable for the subpopulation

Age, HRT use, BMI, time since menopause and hysterectomy were all associated with hot flush risk in postmenopausal women randomised to anastrozole. While age and time since menopause were also associated with gynaecological symptoms univariately, neither were associated with gynaecological symptoms in a multivariate model. Women who were older than 60 at randomisation were at a non-statistically significant decrease in gynaecological symptoms (OR = 0.76 (0.53 – 1.09); P = 0.13) compared to those younger than 60 at randomisation. Age as a continuous variable was not associated with gynaecological risk in a univariate analysis (OR/y = 0.98 (0.95 – 1.00); P = 0.06). Time since menopause as a continuous variable was not associated with gynaecological side effects after adjustment for age at randomisation (OR/y = 0.98 (0.96 – 1.00); P = 0.12) in postmenopausal women randomised to anastrozole. Time since menopause as a categorical risk factor was also not significantly associated with gynaecological events. BMI, and age at menarche were associated with arthralgia univariately, but after multivariate analysis only BMI remained significantly associated with arthralgia. Ln-SHBG (OR = 0.79 (0.63 – 0.99); P = 0.04) was the only sex hormone associated with side effects in women randomised to anastrozole and measurements were available for 554 women. Genomic factors were not available for postmenopausal women on the IBIS-II trial (Table 7.2).

7.3.3 Prediction of side effects in IBIS-I premenopausal women

7.3.3.1 Model development for premenopausal women using cross-validation

The training set consisted of 80% of available samples (N = 1,214) and was selected from premenopausal women randomised to tamoxifen. 59.4% (N = 721) of women in the training set reported side effects within the first 6-months of starting endocrine therapy. In this analysis, each model was evaluated based on the measures discussed is section 7.1.2.8 (AUC, sensitivity, and specificity). Results were achieved using 10-fold cross-validation for each model and the overall model performance reported is the mean of results obtained from the test dataset (the 10th segment) for each fold.

For premenopausal women randomised to tamoxifen in IBIS-I, the LR model without variable selection used all four variables identified as statistically significant in previous

chapters. The final LR model for premenopausal women was log (odds of side effect) = – 0.13[HRT (never, ever, current)] – 0.15[Age (years)] + 0.01[Age at menarche (years)] + 0.003[BMI (kg/m^2)].

At a cut-off threshold of 0.594, the LR model had an AUC of 0.56 (95%CI: 0.53 – 0.59), a sensitivity of 0.46 and a specificity of 0.63 (Table 7.3). In comparison, the LASSO model selected only two of the four variables: HRT and age. There was consistency between the variable selection model and full LR model as HRT and age had the largest regression coefficients in the full LR model. The LASSO model had similar performance metrics to the full LR model. The LASSO model had an AUC of 0.56 (0.54 – 0.58), a sensitivity of 0.43 and a specificity of 0.66 (Table 7.3). The regression coefficients for HRT (0.17) and age (0.03) in the LASSO model contrasted with the full LR model in that both were positive. This is likely due to the two positive coefficient variables in the LR model being excluded in the LASSO model.

Table 7.3: Predictive performance expressed as the area under the ROC curve, sensitivity, and specificity of the logistic regression and LASSO models for the prediction of side effects, in the 10-fold cross-validation training and validation sets for premenopausal women randomised to tamoxifen in IBIS-I

Model			**LR**		**LASSO**	
Model Training (N events = 721) (N total = 1,214)						
Number of variables			4		2	
AUC (95%CI)			0.56 (0.53 - 0.59)		0.56 (0.0.54 - 0.58)	
Sensitivity			0.46		0.43	
Specificity			0.63		0.66	
Model validation (N events = 180) (N total = 303)						
Predict-	**TP**	**FP**	140	113	140	113
ions	**FN**	**TN**	40	10	40	10
Threshold			0.611		0.491	
AUC (95%CI)			0.59 (0.52 - 0.65)		0.59 (0.52 - 0.65)	
Sensitivity			0.78		0.78	
Specificity			0.08		0.08	

LR = Logistic Regression, LASSO = Least absolute Shrinkage and Selection Operator, AUC = Area under the receiver operating characteristic, 95%CI = 95% Confidence Interval, TP = True Positive, FP = False Positive, TN = True Negative, FN = False Negative.

7.3.3.2 Validation of trained premenopausal models

The validation set contained 303 premenopausal women randomised to tamoxifen of whom 180 (59.4%) experienced side effects during the first 6 months of the IBIS-I trial.

At a cut-off threshold of 0.611, the LR model had an AUC of 0.59 95%CI (0.52 – 0.65) (Table 7.3). The threshold for the cut-off was selected using Youden's index. The LR model correctly predicted 140 (77.7%) women who experienced side effects, but incorrectly predicted side effects in 113 (91.9%) women who did not report side effects.

At a cut-off threshold of 0.491, the LASSO model made the same predictions as the LR model. The LASSO model correctly predicted side effects in 140 (77.7%) women and no side effects correctly in 10 (8.1%) women. This gave a model accuracy of 0.57 (0.51 – 0.63) and a sensitivity and specificity of 0.54 and 0.59 respectively (Table 7.3).

Neither of the prediction models were able to predict the side effect outcomes with a high degree of accuracy. In both cases, the models predicted more cases of side effects than observed in the dataset.

7.3.3.3 Addition of genomic data to baseline prediction models

A total of 131 premenopausal women (train = 103, validation = 28) randomised to tamoxifen during the IBIS-I study had available genomic data for 195 SNPs. The validation set had a lower number of women than optimal; however, as the analysis was exploratory, results of the validation were reported with the acknowledgement that validation required repeating in a larger dataset.

When genomic information was combined with the other baseline information, the best 10-fold cross-validation trained LR model had a mean AUC of 0.53, sensitivity 0.45 and specificity 0.58. In comparison to the LR model, the LASSO model used only seven variables. In addition to BMI, the other six variables were SNPs and included chr17:77,781,387 (no gene), rs1285057 (*ESR1*) and rs3736599 (*SULT1E1*), rs3775777 (*SULT1E1*), chr16:53,855,291 (*FTO*), rs1052131 (*SULT2B1*). The model resulted in an AUC of 0.64 (0.58 - 0.70) which outperformed the LR model.

The models did not perform well in the validation set. The overall accuracy of the LR model was not improved compared to the models without genomic factors. AUC for the full LR model was 0.53 (0.33 – 0.73). The LR model correctly predicted 10 (55.6%) of women who reported side effects and 5 (50.0%) women who did not report side effects. Although the LASSO model had the best AUC in the training models, the prediction performance in the validation set was poor. The LASSO AUC was 0.51

95%CI (0.25 – 0.76) and had a sensitivity of 0.78 and a specificity of 0.50. The LASSO model correctly predicted 14 (77.7%) cases of side effects, but only correctly predicted 5 (50.0%) of women who do not experience side effects.

When genomic information was combined with the other baseline information, the AUC of the LR prediction had no improvement compared to the baseline factor only model (D = 1.47, P = 0.14). This suggested that the addition of genomic information to the premenopausal prediction model did not increased the prediction ability of the LR model compared to the model containing only baseline factors.

7.3.3.4 Side effect risk score in premenopausal women randomised to tamoxifen

The final LR model developed using baseline factors was log (odds of side effect) = – 0.13[HRT (never, ever, current)] – 0.15[Age (years)] + 0.01[Age at menarche (years)] + 0.003[BMI (kg/m^2)]. The model was subsequently used to produce a side effect risk (SER) score for each of the women and side effect risk was modelled over the range of the risk score. The model was first developed in the training data set before it was applied to the validation set to determine how well the score identified women at high risk of side effects.

A score of LR coefficients multiplied by each variable, produced a risk score with a range -10.29 – -5.11 and a median (IQR) of -6.91 (-6.47 – -7.35). The percentage of women reporting or not reporting side effects at each point was then calculated (Figure 7.3).

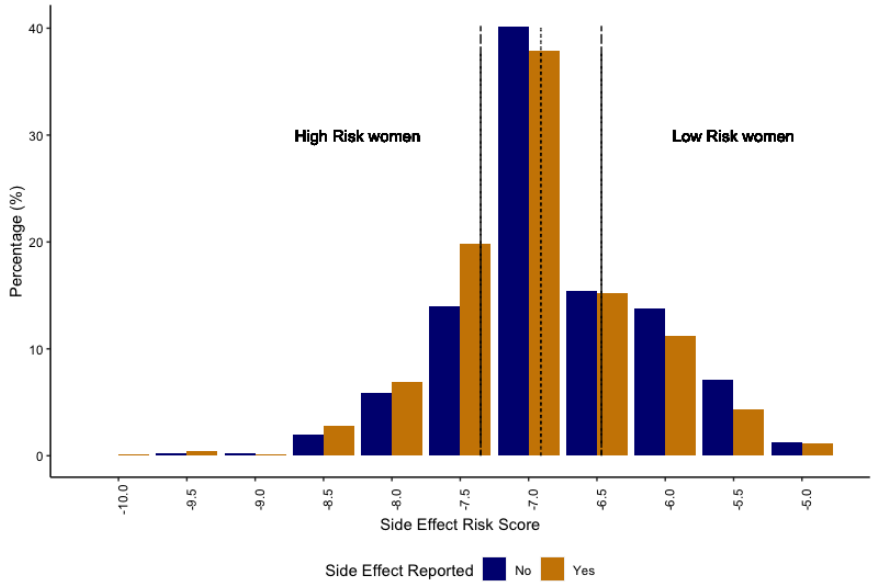


Figure 7.3: Side effect risk score for premenopausal women with and without side effects. Bars are percentage of the affected or unaffected population at each score. Solid vertical lines are the 1^{st} and 3^{rd} quartiles, and the dotted line is the median

Quartiles with equal numbers of women were calculated. Women with the lowest risk scores in the first quartile (SER score < -7.35) were at highest risk of side effects. Of the 304 women who were in the first quartile, 66.4% reported side effects. Those in the fourth quartile (SER score > -6.47) had the lowest risk of side effects, 52.8% (160/303). Women who had a risk score in the second quartile were at high intermediate risk of side effects 185/304 (60.9%) and those in the third quartile were at low intermediate risk of side effects 160/303 (57.4%). The ability of the score to predict those who have a lower risk of side effects was poorer than the prediction for higher risk women. Side effect risk stratification based on the SER score for premenopausal women had poor discrimination and significant improvement in the derivation of the score and identification of risk factors would be required for accurate prediction.

The SER score performed better in the validation cohort. 57/80 (71.3%) of women in the high-risk group (SER score below -7.35) reported side effects. Of the 88 women whose score was greater than -6.47, and therefore in the low-risk group, the number of women reporting side effects and not reporting side effects was almost equal: 47 (53.4%) vs 41 (46.6%) respectively. The intermediate risk quartiles had similar number of women reporting side effect, 55.4% in the high intermediate risk group compared to 57.4% in the lower intermediate risk group. These findings confirmed those found

in the training set, namely that the discrimination of the SER score was poor. The prediction of women at high risk of side effects was possible; however, the identification of those without side effects was more difficult.

7.3.4 Prediction of side effects in IBIS-I postmenopausal women

7.3.4.1 Model development for postmenopausal women using cross-validation

The training set consisted of 1,451 samples selected from postmenopausal women randomised to tamoxifen. 922 (63.5%) women in the training set reported side effects within the first 6-months of starting endocrine therapy. Four baseline factors were used to form prediction models for side effects in postmenopausal women: HRT use, time since menopause, BMI and whether a woman had a hysterectomy. All four variables were used for LR model development. The final LR model for postmenopausal women was log (odds of side effect) = – 0.17[HRT (never, ever, current)] - 0.08[Hysterectomy (yes, no)] + 0.13[Time since menopause (years)] + 0.04[BMI (kg/m^2)]. The threshold for cut-off, calculated using Youden's statistic was 0.624. At the optimum cut-off, the LR model had an AUC of 0.56 (0.53 – 0.60), and a sensitivity of 0.47 and specificity of 0.64 (Table 7.4).

The LASSO model selected only HRT and time since menopause of the four variables. The regression coefficients in the LASSO model were in contrast to the coefficients in the LR model. The LASSO coefficient for HRT was 0.06 compared to -0.17 in the LR model and the coefficient for time since menopause was -0.0002 compared to + 0.13 in the LR model. However, the AUC for the LASSO model was similar to the LR model 0.56 (0.53 – 0.59) (Table 7.4). The sensitivity of the LASSO model was 0.81 and the specificity 0.30 (Table 7.4).

Table 7.4 shows the AUC (95%CI), sensitivity and specificity of each model. Performance metrics were calculated using the respective formulas presented in section 3.3.6.

Table 7.4: Predictive performance expressed as the area under the ROC curve, sensitivity, and specificity of the logistic regression and LASSO models for the prediction of side effects, in the 10-fold cross-validation training and validation sets for postmenopausal women randomised to tamoxifen in IBIS-I

Model			LR		LASSO	
Model Training (N events = 922) (N total = 1,451)						
Number of variables			4		2	
AUC (95%CI)			0.55 (0.53 - 0.58)		0.56 (0.53 - 0.59)	
Sensitivity			0.64		0.81	
Specificity			0.47		0.30	
Model validation (N events = 230) (N total = 362)						
Predict-	**TP**	**FP**	126	49	117	44
ions	**FN**	**TN**	104	83	113	88
Threshold			0.641		0.563	
AUC (95%CI)			0.59 (0.53 - 0.65)		0.60 (0.54 - 0.66)	
Sensitivity			0.55		0.51	
Specificity			0.63		0.67	

LR = Logistic Regression, LASSO = Least absolute Shrinkage and Selection Operator, AUC = Area under the receiver operating characteristic, 95%CI = 95% Confidence Interval, TP = True Positive, FP = False Positive, TN = True Negative, FN = False Negative.

7.3.4.2 Validation of trained postmenopausal models

The postmenopausal women validation set contained 362 postmenopausal women of whom 230 (63.5%) experienced a side effect during the first 6 months of the IBIS-I trial. The LR model had a similar AUC to that previously observed for the premenopausal women 0.59 (0.53 – 0.65) (Table 7.4). The LR model correctly predicted 126/230 (54.8%) women who experienced side effects. However, the LR model correctly predicted 83/132 (62.9%) women who did not report side effects. Compared to the premenopausal model, the postmenopausal LR model had superior prediction of women who did not report side effects, but had inferior performance predicting side effect cases.

The LASSO model was able to correctly predict a larger number of women not reporting side effect 88/132 (66.6%) but was less good at identifying correctly women with side effects, 117/230 (50.9%), compared to the LR model. The LASSO model had an AUC of 0.60 (0.54 – 0.66) and a sensitivity and specificity of 0.51 and 0.67 respectively (Table 7.4).

7.3.4.3 Addition of genomic information to the IBIS-I postmenopausal women prediction model

Genotyping of IBIS-I samples was performed for all breast cancer cases and matched controls with available material; genotyping procedures have been previously described (Cuzick et al., 2017). A total of 169 postmenopausal women (train = 136, validation = 33) randomised to tamoxifen during the IBIS-I study had available genomic data for 195 SNPs identified as associated with side effects in the genomic analysis performed in chapter 5. As noted previously during the premenopausal women analysis, a larger cohort would provide scope for a more conclusive analysis and as such care should be taken when eliciting the meaning of these results.

When genomic information was combined with the other baseline information, the 10-fold cross-validation trained LR model had an AUC of 0.53, a sensitivity of 0.54 and a specificity of 0.51. Compared to the baseline factor only model, the addition of genomic information only increased the specificity of the models, but the addition of genomic factors led to a decrease in both AUC and sensitivity compared to the baseline factor only model.

In comparison with the LR model, the LASSO model selected 35 variables of the 199 variables. 34 of the variables selected were SNPs and only HRT remained from the baseline factor model. The final LASSO model had an AUC of 0.52 (0.46 – 0.59) which was similar to the AUC of the full LR model. It is interesting to note that the SNP with the largest OR in the LASSO model is rs1273196 a SNP in the ESR2 coding region which codes for ERβ.

None of the models performed well in the validation set. The validation set available consisted of 33 women of whom 20 (60.6%) experienced side effects. The large number of variables in the models rendered the validation set too small for conclusive validation; therefore, results from validation should be interpreted with caution. The LASSO model had a better AUC than the LR model (0.66 95%CI (0.47 – 0.86)) and predicted 15 (75.0%) of women with side effects and 8 (61.5%) women without side effects correctly.

The prediction performance of the LR was not improved compared to the model without genomic factors. The AUC of the LR model with baseline factors and genomic information was 0.53 (0.33 – 0.73). 12/20 (60.0%) of women with side effects were mis-

classified as not having side effects by the LR model with genomic factors. However, 9/13 (69.2%) women who do not report side effects were correctly classified.

When genomic information was combined with the other baseline information, the AUC of the LR prediction was not significantly different compared to the baseline factor only model (D = 0.65, P = 0.51).

7.3.4.4 Addition of baseline sex hormone concentrations to the IBIS-I postmenopausal women prediction model

A total of 224 postmenopausal women (train = 172, validation = 52) randomised to tamoxifen during the IBIS-I study had sex hormone data available from the analysis in chapter 6. Women were selected as cases if they reported any of the major side effects; hot flushes, vaginal discharge, vaginal dryness or irregular bleeding, and controls were women who reported no side effects. Concentrations of dehydroepiandrosterone-sulphate (DHEA-S), testosterone, sex hormone binding globulin (SHBG), and calculated bioavailable testosterone (bioT) were measured, and ln-transformed concentrations investigated for association with side effects, section 6.4.2. Only ln-SHBG and ln-bioT had a statistically significant association with side effects and were therefore the only sex hormones added to the baseline factor dataset giving a total of 6 variables.

When sex hormone concentrations and baseline information were modelled using LR, the 10-fold cross-validation trained full LR model, at the optimum prediction threshold of 0.607 calculated from Youden's J statistic, had an AUC of 0.79 (0.72 – 0.86) a sensitivity of 0.70 and a specificity of 0.78. The validation set contained 52 women of whom 23 (44.2%) reported side effects. In the validation set, the LR model had an AUC of 0.70 (0.56 - 0.84). The LR model with four baseline factors; HRT, BMI, time since menopause, hysterectomy; with the addition of ln-SHBG and ln-bioT concentrations, correctly predicted 18 (78.3%) women with side effects, and 19 (65.5%) women without side effects. Compared to the LR model without sex hormone concentrations, the addition of sex hormones to baseline factors showed a statistically significant increase in the AUC (D = -4.66, P $<$ 0.001).

In contrast, the LASSO model contained only one variable, ln-SHBG concentration. The model AUC was 0.77 (0.70 - 0.84). The LASSO model had a sensitivity of 0.84,

and a specificity of 0.62. Testing the LASSO model in the validation set showed an improvement over baseline factors alone. Using ln-SHBG concentrations, the LASSO model had an AUC of 0.70 (0.56 – 0.85). The LASSO model, at a prediction threshold of 0.363, correctly predicted 18/29 (62.1%) women with side effects and 19/23 (82.6%) women without side effects. The performance of the LASSO model in the validation set was equal to the performance of the LR model despite ln-SHBG being the only risk factor included.

Analysis of all models with sex hormones showed that where possible sex hormone concentrations, particularly SHBG concentrations should be included in prediction models for postmenopausal women randomised to tamoxifen as this information increased the prediction potential of the models.

7.3.4.5 Side effect risk score in postmenopausal women randomised to tamoxifen

The full LR model using baseline factors only was log (odds of side effect) = – 0.17[HRT (never, ever, current)] - 0.08[Hysterectomy (yes, no)] + 0.13[Time since menopause (years)] + 0.04[BMI (kg/m^2)] and had the best prediction in the validation set. Despite the benefit of sex hormone concentrations to overall prediction, the formation of a score which used only easily obtained data was created in anticipation that SHBG may not be known for a large proportion of women and thus would not be useful to include in a risk score. The regression coefficients for the full LR model were used to produce a risk score for each of the women and side effect risk was modelled over the range of the risk score.

A score of LR coefficients multiplied by each variable, produced a risk score with a range 0.12 – 5.37 with a median and (IQR) of 1.46 (1.03 – 2.05). After calculation of the SER score for each woman the percentage of women from two side effect groups were plotted against the SER score (Figure 7.4).

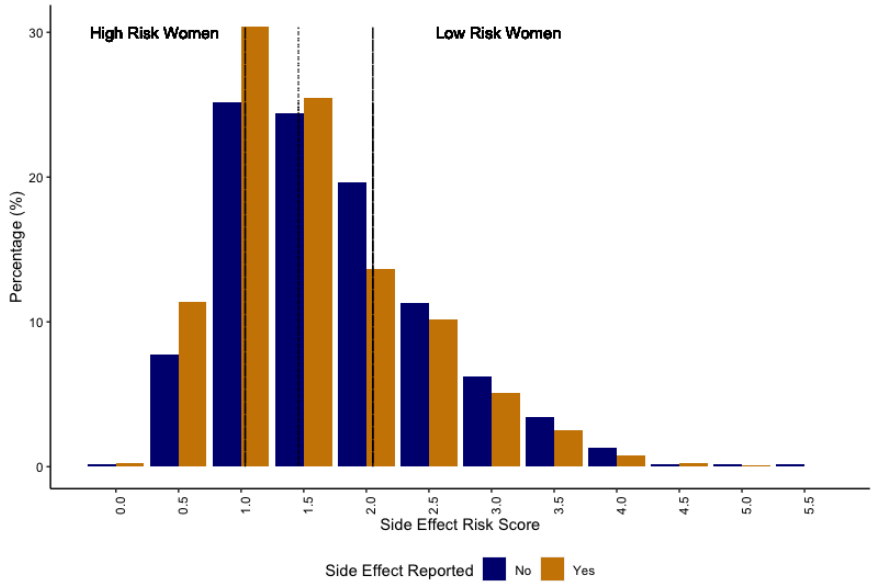


Figure 7.4: Side effect risk score for postmenopausal women with and without side effects. Bars are percentage of the affected or unaffected population at each score. Solid vertical lines are the 1^{st} and 3^{rd} quartiles, and the dotted line is the median

Women in the high-risk group had a SER score of 1.03 or lower and contained a larger proportion of women reporting side effects than those without, 249/363 (68.6%). However, as observed when the SER score was used to determine risk in premenopausal women, the ability of the score to prediction those who have a lower risk of side effects was not good. Of the 362 women with a SER score above 2.05 and therefore considered to be in the low-risk group, 40.9% had no side effects compared to 59.1% who reported side effects. The intermediate risk category was split into high intermediate and low intermediate risk, and in contrast to the premenopausal score categories women in each group reported different number of side effects. Of those in the high-intermediate risk group, 246/363 (67.8%) of women reported side effects compared to 213/363 (58.7%) of women in the low-intermediate risk group. These findings indicate that the use of a binary threshold at the median SER score could be used in postmenopausal women to identify those at high-risk or low risk. Exploration of a threshold at the median found, 68.2% of women in the high-risk group (495/726) reported a side effect compared to 58.9% in the low-risk group. These results were similar to those found when using quartiles.

The score was subsequently tested in the validation cohort (N = 362). 72/104 (69.2%) of women who had a SER score in the high-risk group reported side effects. Of the

101 women with a SER score in the low-risk group, 61 (60.4%) reported side effects. Interestingly, 69.4% of women in the high-intermediate risk group also reported side effects compared to only 55.3% in the low-intermediate risk group. The use of the median as a threshold in the validation group found that 58.1% of women in the low-risk group reported side effects compared to 69.3% in the high-risk group.

7.3.5 IBIS-II

7.3.5.1 Model development for postmenopausal women randomised to anastrozole using cross-validation

The analysis of IBIS-II data followed the same protocol as IBIS-I data. Models of women randomised to anastrozole were achieved using 10-fold cross-validation and the overall model performance was the average of results obtained from the test dataset (the 10th fold) for each fold. Each model was evaluated based on the measures discussed is section 7.1.7.5 (AUC, sensitivity and specificity).

The training set consisted of 1,424 samples selected from postmenopausal women randomised to anastrozole. 835 (58.6%) women in the training set reported side effects within the first 6-months of starting endocrine therapy. Five variables had a statistically significant association with side effects in women randomised to anastrozole in the IBIS-II study: age, BMI, HRT use, time since menopause and prior hysterectomy. The LR model, at a threshold of 0.581 calculated from Youden's J statistic, had an AUC of 0.58 95%CI(0.55 – 0.61) and had sensitivity of 0.57 and specificity of 0.55 (Table 7.5). The final LR model was for postmenopausal women was log (odds of side effect) = – 0.14[HRT (never, ever)] + 0.13[Time since menopause (years)] – 0.12[BMI (kg/m^2)] – 0.13[Hysterectomy (yes, no)] + 0.08[Age (years)].

The LASSO regression also included all five variables after training. Regression coefficients in the LASSO model were similar to those of the LR model as HRT had the largest coefficient and age had the smallest. At the optimum threshold of 0.349, the LASSO model had an AUC of 0.57 (0.54 – 0.60) a sensitivity of 0.54 and specificity of 0.60 which were broadly similar to the LR model (Table 7.5).

Table 7.5: Predictive performance expressed as the area under the ROC curve, sensitivity, and specificity of the logistic regression and LASSO models for the prediction of side effects, in the 10-fold cross-validation training and validation sets for postmenopausal women randomised to anastrozole in IBIS-II

Model			LR		LASSO	
Model Training (N events = 835) (N total = 1,424)						
Number of variables			5		5	
AUC (95%CI)			0.58 (0.55 - 0.61)		0.57 (0.54 - 0.60)	
Sensitivity			0.57		0.54	
Specificity			0.55		0.60	
Model validation (N events = 208) (N total = 355)						
Predict-	**TP**	**FP**	129	54	126	58
ions	**FN**	**TN**	79	93	82	89
Threshold			0.578		0.339	
AUC (95%CI)			0.64 (0.58 - 0.70)		0.63 (0.57 - 0.69)	
Sensitivity			0.63		0.61	
Specificity			0.62		0.61	

LR = Logistic Regression, LASSO = Least absolute Shrinkage and Selection Operator, AUC = Area under the receiver operating characteristic, 95%CI = 95% Confidence Interval, TP = True Positive, FP = False Positive, TN = True Negative, FN = False Negative.

7.3.5.2 Validation of trained postmenopausal models

Each of the tested models had similar predictive potential amongst postmenopausal women randomised to anastrozole in the IBIS-II trial. Models were subsequently validated in a pre-partitioned validation set. The validation set of postmenopausal women contained 355 women of whom 208 (58.6%) experienced side effects during the first 6 months on trial.

The optimum threshold for prediction was calculated to be 0.578. At this threshold the LR model had an AUC of 0.64 (0.58 – 0.70) (Table 7.5). The LR model correctly predicted side effects in a large number of women who reported side effects 129/208 (62.0%), but also predicted side effects in women who did not reported any symptoms. The LR model correctly predicted 93 (63.3%) women without side effects. The LASSO model, at a prediction threshold of 0.339 had a similar performance to the LR model. The LASSO model had an AUC of 0.63 (0.57 – 0.69) and had a similar prediction performance for women who did not report any side effects (89/147: 60.5%) and those who did report side effects 126/208 (60.5%) compared to the LR model (Table 7.5).

7.3.5.3 Addition of sex hormone information to IBIS-II prediction models

A total of 507 postmenopausal women (train = 407, validation = 100) randomised to anastrozole during the IBIS-II study had sex hormone data available from the analysis in chapter 6. Women were selected as cases if they reported any of the major side effects; hot flushes, gynaecological symptoms or arthralgia, and controls were women who reported no side effects. Concentrations of DHEA-S, testosterone, SHBG, and bioT were measured, and ln-transformed concentrations investigated for association with side effects, section 6.4.3. Ln-SHBG was the only sex hormone which was significantly associated with side effect outcomes and was thus the only sex hormone added to the baseline factors models for a maximum number of five variables for model training. 253 (62.2%) women in the training set reported side effects in the first 6-months of taking anastrozole and 70 (70.0%) women in the validation set reported side effects in the same period.

When sex hormone information was combined with the other baseline information, the 10-fold cross-validation trained LR model had a mean AUC of 0.63 (0.58 – 0.69) a sensitivity of 0.67 and a specificity of 0.55. In agreement with IBIS-I postmenopausal prediction models, to which the addition of sex hormone concentrations showed improvement in trained model AUC, the addition of sex hormone data to postmenopausal model in women randomised to anastrozole had a small improvement in prediction performance.

The LASSO model used only two of five variables: HRT and BMI. At a prediction threshold of 0.414, the LASSO model had an AUC of 0.60 (0.55 – 0.66) a sensitivity of 0.66 and a specificity of 0.53. It is interesting to note that the LASSO model did not select ln-SHBG concentrations in the model unlike the LASSO model used for predicting side effects in postmenopausal women randomised to tamoxifen. This likely reflects the strength of the association between ln-SHBG and side effect outcomes observed in chapter 6, but prompts the need for further investigation into the role of SHBG concentrations in the incidence of side effects.

When the models were tested in the validation set, the LR model had an AUC of 0.63 (0.51 – 0.75). The LR model correctly predicted 36/70 (51.4%) women with side effects and had sensitivity of 0.51, and correctly predicted 23/30 (76.7%) women without side

effects giving a specificity of 0.77. Compared to the LR model without sex hormone concentrations, the addition of sex hormones to baseline factors had no statistically significant increase in the AUC of the predicted values (D = -1.40, P = 0.163).

In comparison to the LR model, the LASSO model had a marginally lower predictive performance than the baseline factor only model reporting an AUC of 0.62 (0.50 – 0.74). The sensitivity of the LASSO model was worse than the LASSO model using all baseline factors and correctly identified only 22/70 (31.4%) women with side effects. However, the LASSO model had a better predictive performance for women without side effects and correctly identified 28/30 (93.3%) women without side effects. The poor discriminatory ability of the LASSO model to identify women who reported side effects meant that it was inferior when compared to the LR model.

7.3.5.4 Side effect risk score for postmenopausal women randomised to anastrozole

The full LR model using baseline factors was log (odds of side effect) = – 0.14[HRT (never, ever)] + 0.13[Time since menopause (years)] – 0.12[BMI (kg/m^2)] – 0.13[Hysterectomy (yes, no)] + 0.08[Age (years)] and had the best prediction potential and was used to develop a side effect risk score for postmenopausal women randomised to anastrozole.

A score of LR coefficients multiplied by each variable, produced a risk score with a range -3.93 – 8.40 with a median and (IQR) of 2.59 (1.75 – 3.75). Women who had a SER score of less than 1.75 were in the high-risk quartile in which 227/356 (63.8%) of women reported side effects. Of women who had a score of greater than 3.75 and who were in the low-risk quartile, 54.8% reported side effects and 45.2% did not report side effects. 60.1% of women in the high-intermediate risk group reported side effects compared to 55.3% of women in the low-intermediate risk group. The total side effect risk (SER) score for each woman and the percentage of women from two side effect groups are shown in Figure 7.5

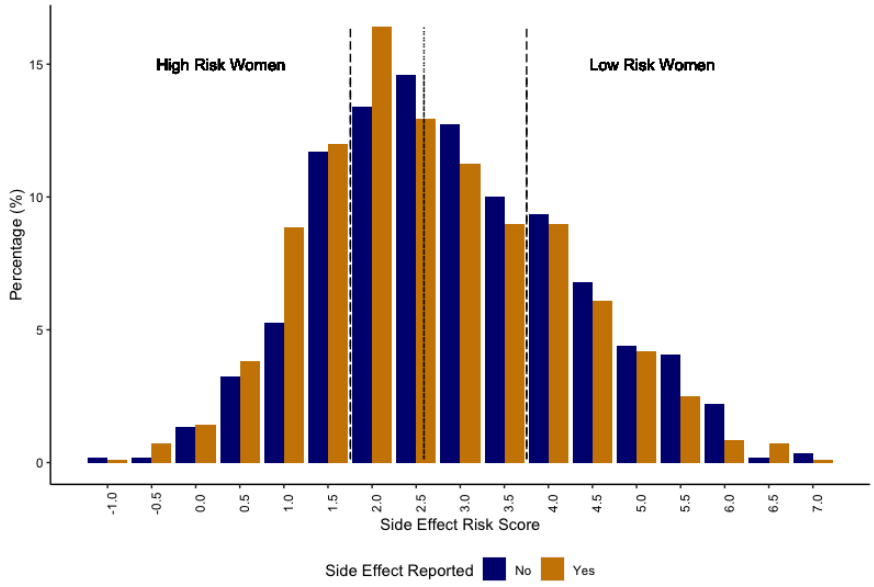


Figure 7.5: Side effect risk score for postmenopausal women with and without side effects. Bars are percentage of the affected or unaffected population at each score. Solid vertical lines are the 1^{st} and 3^{rd} quartiles, and the dotted line is the median

Using the SER score, women in the validation set (N = 355) were tested. Women who had a SER score in the high-risk group had a much larger proportion of women reporting side effects than those without, 70.1% (68/97). In contrast to the SER scores for pre and postmenopausal women randomised to tamoxifen, the women with a SER score in the low-risk group the majority did not report side effects 54/102 (52.9%). Of the 78 women in the high-intermediate risk group, 60.3% (47/78) reported a side effect compared to 57.7% (45/78) in the low intermediate risk group. The SER score in postmenopausal women randomised to anastrozole was better at identifying low risk women than the SER score for either premenopausal or postmenopausal women randomised to tamoxifen. However, the SER score, despite a large effort across a range of areas, showed that currently no useful factors have been found that predict risk of side effects accurately in women taking anastrozole for breast cancer prevention. Identification of risk factors and the development of a risk score should remain an important area of focus to overcome the negative impact that side effects have on the uptake and adherence to endocrine therapy.

7.4 Discussion

The use of endocrine therapy for women at high risk of breast cancer has been highly effective as both SERMs and AIs have been shown to decrease the risk of breast cancer incidence in women at high risk, (Cuzick et al., 2002, 2014, 2020; Goss et al., 2011; Key et al., 2001; Powles et al., 1998; Veronesi et al., 1998). While the benefits of SERMs and AIs are clearly shown in clinical trials, these trials also highlight the issue of side effects. Side effects are an important issue to women thinking about or taking endocrine therapy, as research shows that side effects are associated with poorer uptake and lower adherence (Smith et al., 2016, 2017). Non-life-threatening side effects like hot flushes (HFs), gynaecological symptoms, and musculoskeletal events reduce the uptake of endocrine therapies. Currently, little is known about whether a woman is at increased or high risk of experiencing a particular side effect.

This analysis examined combinations of risk factors to predict early side effects in women randomised to tamoxifen or anastrozole. The mean AUC, sensitivity, and specificity of two different models were calculated from each test fold in 10-fold cross-validation, compared and used to predict side effect outcomes in a validation set. Subsequently, genomic variants and baseline sex hormone concentrations were individually combined with baseline risk factors to determine the impact of genetic or sex hormone variables for predicting side effect incidence. A side effect risk (SER) score was developed using logistic regression coefficients to identify women who were at high or low risk of early side effects. The analysis shows how statistical modelling could be used to predict side effect risk and to give women considering using endocrine therapy valuable information on which to base her decision. However, despite investigations across a range of areas, currently no significant risk factors have been found to predict side effect risk. The range of side effects reported and the high-risk of menopausal-like side effect in women aged 50 and over means that any definitive prediction currently remains out of reach. Thus, clearer data on the association of the identified risk factors and further efforts to develop clinically useful models for side effect prediction are required to produce a tool which provides accurate information about side effect risk to women when choosing to take endocrine therapy.

In each of the three subgroups tested; the LR models using risk factors collected at study entry, demonstrated a similar or higher AUC, sensitivity and specificity compared

to the LASSO models. The addition of genomic information to the IBIS-I models resulted in a decrease in the AUC of all models in IBIS-I postmenopausal women but had minimal effect in IBIS-I premenopausal women. Concentrations of ln-SHBG and ln-BioT increased the AUC of each of the IBIS-I and IBIS-II postmenopausal models; however, the effect was larger in the IBIS-I models likely reflecting the outcomes of chapter 6 demonstrating that ln-SHBG had a much stronger association with side effects reported in IBIS-I than those reported in IBIS-II.

There is little evidence to suggest a significant role of genomic factors in the aetiology of side effects confirming the outcomes of chapter 5 which found only a weak suggestive association of SNPs with side effects. Therefore, it is unsurprising that the addition of genomic information does not significantly affect the predictive potential of any of the current prediction models. These results also support the finding of others which suggest little involvement of genetic factors on side effect outcomes (Sestak et al., 2012). There is however opportunity for further investigation into the role of SNPs in the incidence of side effects, and the potential importance and use of genomic factors in prediction models should not be precluded due to a current lack of knowledge.

The impact of sex hormone concentrations in the improvement of prediction of the models through cross-validated training also supports the known involvement and importance of sex hormones in side effect incidence (Emond et al., 2011; Huang et al., 2010; Mac Bride et al., 2010). During the sex hormone analysis, chapter 6, ln-SHBG had the largest effect on side effect incidence in postmenopausal women taking tamoxifen during IBIS-I and had a significant but smaller effect on reducing side effects in postmenopausal women randomised to anastrozole during IBIS-II.

The addition of sex hormones in both cases aided the classification of side effects by reducing the number of false positive predictions of side effects. However, analysis of the effect of adding sex hormone concentrations to showed that only in the case of postmenopausal women in IBIS-I did sex hormones significantly affect the prediction performance. These results are likely a reflection of the size of association between sex hormone concentrations and side effects observed in chapter 6. This would suggest that although the inclusion of sex hormones benefits the prediction performance of models, further work is needed to understand the role that sex hormones play in the incidence of side effects. Given the benefits of adding androgen or SHBG concentrations to the prediction models, measuring baseline concentrations of oestrogen could be highly

beneficial for predicting side effects and could have a significant effect on the prediction performance of side effect risk models.

Currently, the risk factors included are not suitable for accurate prediction of side effect outcomes and so we must search for more informative risk factors. At the forefront of these new risk factors is oestradiol concentrations. As the majority of side effects are proposed as being as a result of lower oestrogen concentrations, or of large variation in oestradiol concentrations, the next developmental step for these models should be the inclusion of oestradiol concentrations. Accurate measurement of oestradiol may help to identify women who are at high risk of side effects and thus offers the potential for managing these side effects or implementation of endocrine therapy regimes which minimise the risk of side effects in the high risk group.

Pre-treatment identification of women who are at high risk of side effects could allow for better management of side effects or for a personalised endocrine therapy regime to increase uptake and increase the number of women who are adherent to a full course of therapy. Therefore, the formation of a predictive SER score is important to identify women who are at high-risk of side effects and ensure any disruption to quality-of-life is minimised. Predicting side effect risk prior to starting endocrine therapy may enable women to balance the risk of side effects versus the risk of breast cancer. Currently, side effects are offered as a reason for not taking endocrine therapy; however, if models could predict a lower risk, then women may be more inclined to weigh the benefits and limitation of preventive endocrine therapy. Women at lower risk of side effects would, naturally, be more inclined to accept preventive therapy. The same might be true for women with higher risk, as better knowledge about side effect would enable a more tailored regime minimising the risk of side effects whilst also reducing the long term risk of breast cancer.

The use of baseline risk factors is important as currently there is a paucity of information surrounding side effects and concern over side effects is a major reason given for not choosing endocrine therapy. Therefore, it is essential to provide an assessment of side effect risk before beginning endocrine therapy to assuage women's concerns prior to the period during which side effects are most likely.

However, as shown by the current models, accurate prediction of early side effects using baseline risk factors is not possible. As such, it is important to consider the predictive

ability of a model and to balance the risk of false positives and false negatives. In a case such as this a model that under predicts side effect risk is more beneficial to the overall aim than a model that over predicts risk. Over prediction of side effects could have the opposite effect to that which is desired, discouraging women to choose endocrine therapy. However, whilst under prediction is not recommended, if this encourages women to try endocrine therapy, options remain for managing any subsequent side effects prior to women discontinuing therapy.

This is the first study producing a side effect risk score for endocrine therapy and aims to utilise easily assessable risk factors identified throughout the thesis to determine which women are at high risk. Whilst the SER score could identify women who might be at higher risk of side effect, lower scores were not indicative of lower risk of side effects. This may be because models used to develop the score did not have a high predictive accuracy for side effects during model training. Particularly, the specificity was low as many of the women who did not have side effects were incorrectly classified. This is likely due to the nature of the side effects under study. Large numbers of women report menopausal side effects naturally; therefore, defining women who are at low risk of these symptoms is particularly difficult. As a result, the best development opportunities for these models may be in prediction of severe side effects. Severe events are much more likely to be reported by women taking endocrine therapy than women taking placebo, and whilst evidence suggests that there is no "acceptable" level of side effects, studies have identified a trend between side effect severity and adherence (Land et al., 2016; Smith et al., 2016, 2017). Therefore, prediction of severe side effects may present the best opportunity to further develop side effect prediction models and scores.

These prediction models are the first to address the question of whether side effects of endocrine therapy can be predicted. As both the LASSO model and the LR model had similarly poor predictive ability, it is important to address how the two modelling methods compared to assess which offers the best future development options. While drawing a general conclusion regarding which is the better model is difficult, as results are strongly based on the specific data set used to train the models it is pertinent to highlight that LASSO methods provide more parsimonious models with minor loss of prediction performance. Currently, LASSO methods are underused in this field while LR methods are more common. LASSO methods should be considered as an alternative in future studies.

There are some accompanying limitations to this analysis. During the preparation of the data sets, the imputation of non-complete cases may have led to bias entering the dataset. Unfortunately, machine learning algorithms are unable to deal with missing data giving the options of imputation or removal of samples without complete information. Whilst imputation may have introduced bias into the data set removing samples with data not missing at random would also have altered the data set. Lack of independent data sets with which to validate this data was also a limitation. Whilst the models have been validated in a set of unknown data the best test of how well these models have been trained would be to run them on an independent data set. Unfortunately, these data sets are not readily available and therefore testing in an independent data set was not possible.

Significant strengths of the analysis include the novelty of the approach applying modelling techniques to individual side effect risk prediction which had not been previously investigated in this setting. Also, by comparing predictive accuracy across LR and LASSO models multiple techniques have been reviewed to develop side effect models exploring the use of genomic information and sex hormone concentrations.

In this analysis, the models were developed using the full model approach, whereby all a priori selected candidate predictors were included in the multivariable analyses. Choice of predictors was based on evidence generated in previous chapters showing that each of the variables was associated with increasing or decreasing side effects. The benefit of this approach is that it avoids predictor selection bias (incorrectly including spurious predictors in the final model) and overfitting (Moons et al., 2012).

The development of the first side effect risk score aimed to predict women at high risk of side effects is a strength and should continue to be developed. Achieving this aim will require time and energy, but side effects remain an important barrier to breast cancer prevention therefore further work understanding side effects is essential. The lack of information at present regarding side effect risk is in part responsible for the suboptimal use of endocrine therapy for breast cancer prevention. If the score accuracy can continue to be improved, it provides an opportunity to increase the use of endocrine therapy whilst promoting quality-of-life measures. Any improvement in knowledge achieved via the use of modelling for accurate classification of women with and without side effects compared to the current situation could have a stark impact on the attitude towards preventive endocrine therapy.

7.5 Conclusions

Predictive models have helped revolutionise personalised medicine contributing to early identification and prevention of disease in high-risk individuals based on known epidemiological and clinical risk factors. The development of accurate breast cancer risk models has informed clinical care for over 20 years and has enabled the development of risk management strategies, including: lifestyle changes, endocrine therapy for prevention, and screening strategies based on personal risk. However, until now, no attention has been paid to providing women with information which can help to address fears about quality-of-life which can reduce clinician prescription of endocrine therapy, prevent uptake by women when offered endocrine therapy and reduce adherence to the full course of therapy.

Results through-out this thesis show that prediction may be possible. However, currently, the identified risk factors and risk models are not able to accurately predict side effect risk. Whilst development of risk models will take time and focus, the impact of side effects remains an important barrier to breast cancer prevention and therefore further work on side effects is essential.

The models described in this analysis offer the exciting prospect of producing a guide to the problem of side effects. This is the first step in developing new risk prediction approaches and further explores diverse risk factors. Models are not limited to a specific number of risk factors but have the flexibility to change or incorporate additional variables. The improvement in predictive accuracy achieved in this study should be further explored and developed with prospective databases and additional risk factors. By continuing development of models and increased understanding of risk factors for early endocrine therapy side effects a commitment can be made to ensuring that women have the information required to make educated decisions about their health and offers a further tool in the battle against increasing breast cancer rates across the globe.

Chapter 8: Endocrine therapy related side effects and their impact on breast cancer incidence in the IBIS-I and IBIS-II studies

8.1 Introduction

Side effects may play an important role in breast cancer prevention. Long-term follow up of prevention trials show that five years of preventive therapy can decrease the incidence of any breast cancer by up to 29%, after 16 years of follow-up with tamoxifen, and 49% with anastrozole, after 12 years follow-up. However there are also associated side effects of these therapies (Cuzick et al., 2015, 2020). The preventive effects of both tamoxifen and anastrozole last well beyond the active treatment phase. Long-term follow-up studies suggest that benefits of five years of endocrine therapy last at least 20 years for tamoxifen and 12 years for anastrozole (Cuzick et al., 2015, 2020). Minor side effects of endocrine therapy only persist during the active phase of the trial, and subside after the treatment phase and do not increase in the follow up period (Cuzick et al., 2007; Powles et al., 2007).

Several studies in the adjuvant setting have investigated the association between side effects and breast cancer outcomes (Cuzick et al., 2008; Mortimer et al., 2008; Fontein et al., 2013; Huober et al., 2014; Stearns et al., 2015). These studies suggest that side effects could be a marker for predicting therapy benefit (Yoo et al., 2018). However, side effects are associated with a decrease in adherence to therapy, which has also been shown to reduce the benefit gained from taking endocrine therapy (Makubate et al., 2013; McCowan et al., 2013; Smith et al., 2016).

Sex hormone concentrations are often proposed as the cause of side effects from endocrine therapy (Huang et al., 2010; Mac Bride, Rhodes and Shuster, 2010; Emond et al., 2011). In postmenopausal women, oestradiol, testosterone, sex hormone binding globulin (SHBG) and dehydroepiandrosterone-sulphate (DHEA-S) may contribute to the pathogenesis of breast cancer and the occurrence of side effects (Endogenous Hor-

mones and Breast Cancer Collaborative Group, 2011). Decreased oestrogen concentrations are often commonly associated with hot flushes (HFs) as shown by multiple breast cancer prevention trials using SERMs to block oestrogen receptors and prevent binding with circulating oestrogens or anastrozole to inhibit aromatase to prevent breast cancer (Cuzick et al., 2010, 2013, 2014, 2020). Due to these proposed mechanisms, side effects have been suggested as markers of endocrine therapy efficacy and improved survival times of breast cancer recurrence.

Studies in the adjuvant setting have shown that endocrine therapy side effects are associated with a reduction in breast cancer recurrence when compared to women who do not report any side effects. These observations suggest that side effects might be a marker for predicting therapy benefit (Yoo et al., 2018). The Women's Healthy Eating and Living (WHEL) trial reported that women with breast cancer experiencing HFs at baseline were less likely to have a recurrence, compared to those that did not report these symptoms (12.9% vs 21%, P = 0.01) (Mortimer et al., 2008).

The Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial showed that newly reported early joint symptoms with or without vasomotor symptoms in the tamoxifen arm were associated with a greater decrease in breast cancer recurrence compared to no symptoms (HR = 0.58 95% CI (0.45 - 0.74); P < 0.0001) (Cuzick et al., 2008). The ATAC researchers also observed that women randomised to anastrozole who experience newly reported joint symptoms, and had no vasomotor symptoms, had a 35% reduction in the incidence of breast cancer compared to those who did not experience side effects (HR = 0.65 (0.47 - 0.90); P = 0.009) (Cuzick et al., 2008). When vasomotor symptoms were considered alongside joint symptoms the reduction in risk remained the same (HR = 0.65 (0.50 - 0.85); P = 0.001). However, no statistically significant reductions were observed in women who reported vasomotor symptoms either alone or in combination with joint symptoms. When either side effect was reported, reductions were smaller at only 21% (HR = 0.79 (0.67 - 0.94); P = 0.006). Reports of experiencing both side effects indicated a reduction in breast cancer incidence of 44% (HR = 0.56 (0.37 - 0.87); P = 0.01) compared to those who reported no side effects (Cuzick et al., 2008).

Fontein et al. (2013), found similar effects in the Tamoxifen, Exemestane Adjuvant Multinational (TEAM) trial (Fontein et al., 2013). A reduction in breast cancer events for women who reported vasomotor symptoms was observed (HR = 0.73 (0.62-0.87); P < 0.001). However, gynaecological symptoms had a non-statistically significant re-

duction in disease-free survival (HR = 0.77 (0.59 - 1.01); P = 0.058) (Fontein et al., 2013).

Results from the Breast International Group (BIG) 1-98 trial support the results from the TEAM trial identifying an 18% reduction in recurrence (HR = 0.82 (0.70 - 0.96)) in women who reported vasomotor symptoms (Huober et al., 2014). In contrast, Stearns et al. (2015) reported no statistically significant differences in recurrence free survival in women who experienced vasomotor symptoms or musculoskeletal events at three months, six months and 12 months after treatment initiation, compared to those that do not report side effects (Stearns et al., 2015). Result showed a reduction in breast cancer recurrence of 38% in women reporting symptoms at three months (HR = 0.62 (0.33 - 1.18); P = 0.15). However, the reduction decreased for women reporting symptoms at six and 12 months to 20% (HR = 0.80 (0.59 - 1.07); P = 0.13) and 19% (HR = 0.81 (0.63 - 1.03); P = 0.09) respectively (Stearns et al., 2015). These findings provide evidence of an association between side effects and breast cancer and it is therefore important to further study the relationship in the preventive setting.

The association of side effects and breast cancer outcomes is far from certain. Four observational studies reported conflicting results following investigation of the impact of tamoxifen related side effects and the effect on breast cancer incidence. In two case-control studies, women who reported vasomotor symptoms had a lower risk of breast cancer compared to those who did not report vasomotor symptoms (Huang et al., 2011; Fei et al., 2013). Additionally, a cohort study, including 108 incidences of breast cancer, also observed that vasomotor symptoms were associated with a decrease in breast cancer incidence (Hart et al., 2016). In contrast, a second larger cohort study, with 348 breast cancer cases reported, found no association between vasomotor symptoms and breast cancer incidence (Johanneke Van Den Berg et al., 2014).

Persistence of side effects has also drawn attention with some studies in healthy women showing that longer term side effects can predict breast cancer outcomes and are even associated with symptom severity and type of breast cancer (Chlebowski et al., 2019). Results from the Women's Health Initiative (WHI) study indicate that healthy women not taking endocrine therapy but reporting vasomotor symptoms for longer than 10 years had a statistically significant increase in breast cancer incidence compared to those who do not suffer vasomotor symptoms (Chlebowski et al., 2019). Interestingly, women with long-term vasomotor symptoms have an increased risk of ER-negative

breast cancers (Chlebowski et al., 2019). This suggests women who are long-term oestrogen deficient have an increased risk of cancers, which rely on stimulation other than oestrogen to grow and develop. This poses an interesting question as to whether side effects which occur early during preventive endocrine therapy and are thought to be the result of low oestrogen concentrations can be used to predict breast cancer outcomes in the future.

This chapter investigates whether the occurrence of HFs and gynaecological symptoms, including vaginal discharge ad vaginal dryness, in the IBIS-I trial and HFs, gynaecological changes and arthralgia in the IBIS-II trial, within the first six months of trial initiation, predicts breast cancer risk in the prevention setting. These symptoms are linked to the mechanisms of drug metabolism or the effect of an oestrogen deficient environment. Therefore, these side effects could be used as markers to predict a subsequent reduction in breast cancer incidence. The impact of the side effects in subgroups of women categorised by treatment allocation and menopausal status at baseline are addressed as well as the role of side effect severity on breast cancer outcomes.

What we already know

- Side effects, particularly HFs, have shown in the adjuvant setting that they can be markers for tamoxifen efficacy
- HFs and joint symptoms in women taking tamoxifen have shown to reduce the recurrence of breast cancer
- In women taking anastrozole vasomotor and joint symptoms have shown reduced breast cancer recurrence
- Persistent vasomotor symptoms ($>$ 10 years) in the adjuvant setting are associated with an increase in breast cancer incidence

What this analysis adds

- The impact of side effects in the prevention setting to assess their role in breast cancer risk
- Reviews side effects risk on all breast cancers and ER-positive breast cancers
- Review side effects by menopausal status and time since menopause

8.2 Methodology

8.2.1 Study population

This analysis used data from the double-blinded, randomised, placebo-controlled IBIS-I and IBIS-II trials. Details of the trial design, methodology and primary outcomes have been published elsewhere and study inclusion and exclusion criteria described in section 3.1 - 3.2 and in previous publications (Cuzick et al., 2002, 2007, 2014, 2015, 2020). For IBIS-I, the cut-off date was the 1st May 2014 and for IBIS-II the cut-off date was the 30th September 2019 in line with previously reported long-term follow up of each trial (Cuzick et al., 2015, 2020). Breast cancer events occurring after this date were not included in the analysis.

Data for breast cancers during active follow up were reported during follow-up visits. Data for breast cancers outside the active follow-up period, as well as deaths, were obtained from annual follow-up questionnaires. In the UK, cancers and deaths were also reported by the Office for National Statistics. In non-UK centres, annual questionnaires, annual clinic visits, or hospital notes were used to collect these data, supplemented by a national registry in Finland (Cuzick et al. 2015). Follow-up time was calculated from time of randomisation to breast cancer event, death, or final follow-up date.

In IBIS-I, side effects were reported on a six month basis and were reported by participants at each follow-up visit for five years (Cuzick et al., 2002, 2007, 2015). Self-reported side effect severity (mild, moderate or severe) was also collected at each follow up point. For IBIS-II side effects were collected at six and 12-months after which side effects were collected at the annual follow-up visits (Cuzick et al., 2014, 2020). No information regarding the number of side effects experienced within each six month period was collected for either study.

Menopausal status in the IBIS-I study was assessed by self-reports and no hormone testing was performed to definitively assess menopausal status. As a result, it was not possible to effectively assess change in menopausal status between baseline and six months. Therefore, all menopausal analysis in the IBIS-I trial was referenced to baseline menopausal status and no correction was made for women who became post-

menopausal during the first six months. In the IBIS-II trial, women with follicle stimulating hormone of greater than 30 IU/L were ineligible for participation on the trial, as this concentration was considered to indicate premenopausal status.

For IBIS-I, all women whose side effect status was known and who did not develop breast cancer before the six month follow up point were included in the analysis. Six women who developed breast cancer and two women who died during the first six months after randomisation were excluded from the analysis, as were 400 women who had missing side effect status over the same period, leaving 6,746 women for this analysis. The IBIS-II analysis included all women whose side effect status was known and who did not develop breast cancer before the six month follow up point. Six women who developed breast cancer, and 267 women who had missing side effect status six months after randomisation were excluded leaving 3,591 women for this analysis (Figure 8.1). For both analyses, follow-up time began at the six month time point to enable side effects reported during this time period to be measured.

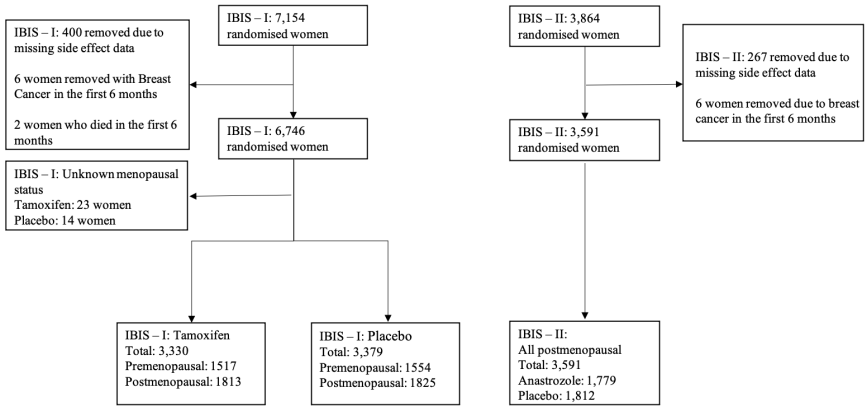


Figure 8.1: Analysis profile with the number of women remaining in each treatment arm after removal of women with missing side effect data and those who had breast cancer or died in the first six months of trial.

8.2.2 Statistical analysis

The primary objective was to establish whether side effects reported by women randomised to tamoxifen or anastrozole during the first six months were predictive of breast cancer occurrence. In this analysis, the side effects of interest were HFs and gynaecological symptoms in the IBIS-I cohort and HFs, gynaecological changes and arthralgia in the IBIS-II cohort, as these were the major side effects associated with tamoxifen and anastrozole respectively (Cuzick et al., 2015, 2020). The primary endpoint was breast cancer (invasive and ductal carcinoma in situ). Follow-up time was

calculated from time of randomisation to breast cancer event, death, or final follow-up date.

Side effects are believed to be a result of oestrogen deficiency; therefore, a secondary objective was to assess how side effects affected oestrogen receptor (ER)-positive breast cancer incidence as ER-positive breast cancer was the breast cancer sub-type which responds best to endocrine therapy. In the secondary analysis, women who had ER-negative breast cancer or women whose ER status was unknown were censored at the time of breast cancer but not classified as an ER-positive breast cancer event. Other secondary objectives included an analysis of side effect impact on all breast cancer and ER-positive breast cancer in the tamoxifen group of IBIS-I and the anastrozole group of IBIS-II according to menopausal status and time since menopause.

In the IBIS-I analysis, women with unknown menopausal status were excluded (N = 37; Tamoxifen N = 23, Placebo N = 14). Postmenopausal women were classified as newly menopausal if time between menopause and randomisation was five years or less, menopausal for 5-10 years, or greater than ten years postmenopausal. Of the 1,813 women on tamoxifen who were postmenopausal, 363 women had been postmenopausal for less than five years, 315 women have been postmenopausal for 5-10 years and 234 women postmenopausal for greater than ten years at study entry. Time since menopause was unknown for 901 women who were excluded from the analysis.

Women in the IBIS-II population were all postmenopausal at study entry. Of the 1,779 women randomised to anastrozole for whom information on side effects during the first six months was available and who did not have any breast events during the first six months 438 had been postmenopausal for less than five years at study entry, 534 had been postmenopausal for 5-10 years and 776 were postmenopausal for greater than ten years. Twenty six women had an unknown time since menopause and were excluded from this secondary analysis.

The third objective was to assess the severity of symptoms reported by women during the first six months of tamoxifen or anastrozole therapy to determine whether those who suffer from more severe symptoms have a greater impact on all breast cancer incidence and ER-positive breast cancers.

The final objective was to investigate the association between side effects and any breast cancer and ER-positive breast cancer occurrence in those randomised to placebo

in the IBIS-I and IBIS-II populations. Side effect impact in the placebo group of IBIS-I was also investigated by menopausal status. Thirty seven women had unknown menopausal status and were excluded from the analysis. All women enrolled on IBIS-II are confirmed postmenopausal so no analysis by menopausal status was required.

For all primary and secondary analyses, Cox proportional hazard models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for associations between side effects and breast cancer incidence. Kaplan-Meier curves were plotted for all breast cancer and ER-positive breast cancer incidence in the tamoxifen and anastrozole arms of IBIS-I and IBIS-II respectively dependent on side effect status at six months after therapy start. Chi-squared tests were used to assess the heterogeneity between menopausal subgroups in the IBIS-I analysis.

8.3 Results

8.3.1 Baseline characteristics

In the IBIS-I group baseline characteristics were evenly distributed between the two treatment arms and are shown in Table 8.1. Median follow up time was 16.6 years (IQR 14.8 - 18.2) and median age was 49.0 (IQR 46.0 - 55.0). 54.2% of women were postmenopausal, 59.4% never used hormone replacement therapy before trial entry, and 50.7% were never smokers (Table 8.1). 39.1% (N = 2,629) women experienced HFs within the first six months and 22.1% (N = 1480) women experienced gynaecological symptoms. Women randomised to tamoxifen reported an approximate three-fold increase in the odds of reporting HFs during the first six months (Tamoxifen: 51.9% vs Placebo: 26.7%, OR = 2.97 95%CI (2.68 – 3.29): $P < 0.0001$). An approximate 3.5-fold increase in vaginal discharge (15.4% vs 4.7%, OR = 3.67 (3.06 – 4.42); $P < 0.0001$) and a 20% increase in vaginal dryness (9.2% vs 7.6%, OR = 1.24 (1.04 – 1.47); P = 0.02) were reported at the six months visit by women randomised to tamoxifen when compared to placebo. Most HFs were mild, 83.0% and 73.8% of women randomised to placebo and tamoxifen respectively. 89.4% of vaginal discharge reported in the placebo group were mild compared to 87.3% of reported vaginal discharge symptoms in the tamoxifen group. 94.5% of vaginal dryness reports in the placebo group and 89.8% of vaginal dryness symptoms in the tamoxifen group were mild.

In the IBIS-II analysis, baseline characteristics were evenly distributed between the two treatment arms and are shown in Table 8.1. Median follow up time was 11.3 years and median age was 59. 47.4% of women in the placebo and anastrozole groups had used hormone replacement therapy before trial entry (Table 8.1). In the anastrozole arm, 32.7% (N = 581) women experienced arthralgia within the first six months the majority of which, 62.0% (N = 360), were mild. Reports of arthralgia in the anastrozole group were significantly larger than those reported in the placebo group (anastrozole = 581 vs placebo = 482, OR = 1.34, (1.16-1.54); P $<$ 0.01). 44.7% of women randomised to women anastrozole experienced HFs; a statistically significant 47% increased risk compared to the placebo group (anastrozole = 796 vs placebo = 642, OR = 1.47 (1.29-1.69); P $<$ 0.01). The majority of HFs were mild, 60.2% and 59.8% of women randomised to anastrozole and placebo respectively. Women randomised to anastrozole reported a 36% increase in the odds of reporting vaginal changes during the first six months (197 vs. 152, OR = 1.36 (1.09-1.70); P $<$ 0.01) compared to women in the placebo group. 68.5% of vaginal changes reported in the anastrozole group were mild and 67.1% of reported symptoms in the placebo group were mild.

Table 8.1: Baseline characteristics for the women eligible for analysis as part of IBIS-I or IBIS-II

	IBIS-I Population				IBIS-II Population			
Patient Demographic	**Placebo (N = 3,379)**		**Tamoxifen (N = 3,330)**		**Placebo (N = 1,812)**		**Anastrozole (N = 1,779)**	
	No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**	**No.**	**(%)**
Number of reported breast cancers	376	(11.1)	269	(8.1)	147	(8.1)	84	(4.7)
Median follow up (IQR) (years)	16.6 (14.8 - 18.2)		16.7 (14.9 - 18.3)		11.3 (9.1 - 13.4)		11.3 (9.3 - 13.4)	
Median age (IQR) (years)	49.0 (46.0 - 55.0)		50.0 (46.0 - 55.0)		59.0 (54.5 - 63.0)		59.0 (55.0 - 63.0)	
Menopausal status								
Premenopausal	1,554	(46.0)	1,517	(45.6)	NA		NA	
Postmenopausal	1,825	(54.0)	1,813	(54.4)	1,812	(100)	1,779	(100)
BMI (kg/m^2)								
Median (IQR)	26.0 (23.2 - 29.5)		26.0 (23.3 - 29.8)		27.3 (24.4 - 31.3)		27.3 (24.2 - 31.0)	
< 25	1,365	(40.4)	1,349	(40.5)	534	(29.5)	548	(30.8)
25-30	1,156	(34.2)	1,096	(32.9)	676	(37.3)	653	(36.7)
> 30	761	(22.5)	780	(23.4)	563	(31.1)	547	(30.7)
HRT use								
Never	2,023	(59.9)	1,959	(58.8)	953	(52.6)	933	(52.4)
Current	883	(26.1)	833	(25.0)	NA		NA	
Ex-User	472	(13.9)	536	(16.1)	858	(47.4)	844	(47.4)

8.3.2 IBIS-I

8.3.2.1 Side effects and breast cancer outcomes in women randomised to tamoxifen during the IBIS-I study

No significant effect of HFs for the prediction of breast cancer was observed when compared to women not reporting these symptoms at the six month visit (HR = 1.27 (0.98 - 1.64); P = 0.07) (Figure 8.2 Panel a, Table 8.2). However, those reporting severe HFs (HR = 1.62 (0.99 - 2.64); P = 0.06) were at higher risk of developing breast cancer than those who reported mild (HR = 1.23 (0.93 - 1.62); P = 0.14) or moderate (HR = 1.23 (0.77 - 1.96); P = 0.39) HFs compared to no HFs. Similarly, no significant difference in breast cancer incidence was observed in women who reported vaginal discharge (HR = 0.73 (0.50 - 1.08); P = 0.12) or vaginal dryness (HR = 0.89 (0.57 - 1.41); P = 0.63) at six months compared to those without these symptoms (Figure 8.2 Panel b and c, Table 8.2). Due to small numbers of severe gynaecological events, and a lack of breast cancer events, symptom severity did not impact the risk of breast cancer. However, women who reported moderate severity vaginal dryness symptoms had a non-statistically significant increase in breast cancer incidence compared to those without symptoms (HR = 1.77 (0.56 - 5.54); P = 0.33) which was not observed in those reporting mild vaginal dryness symptoms (HR = 0.84 (0.51 - 1.38); P = 0.49).

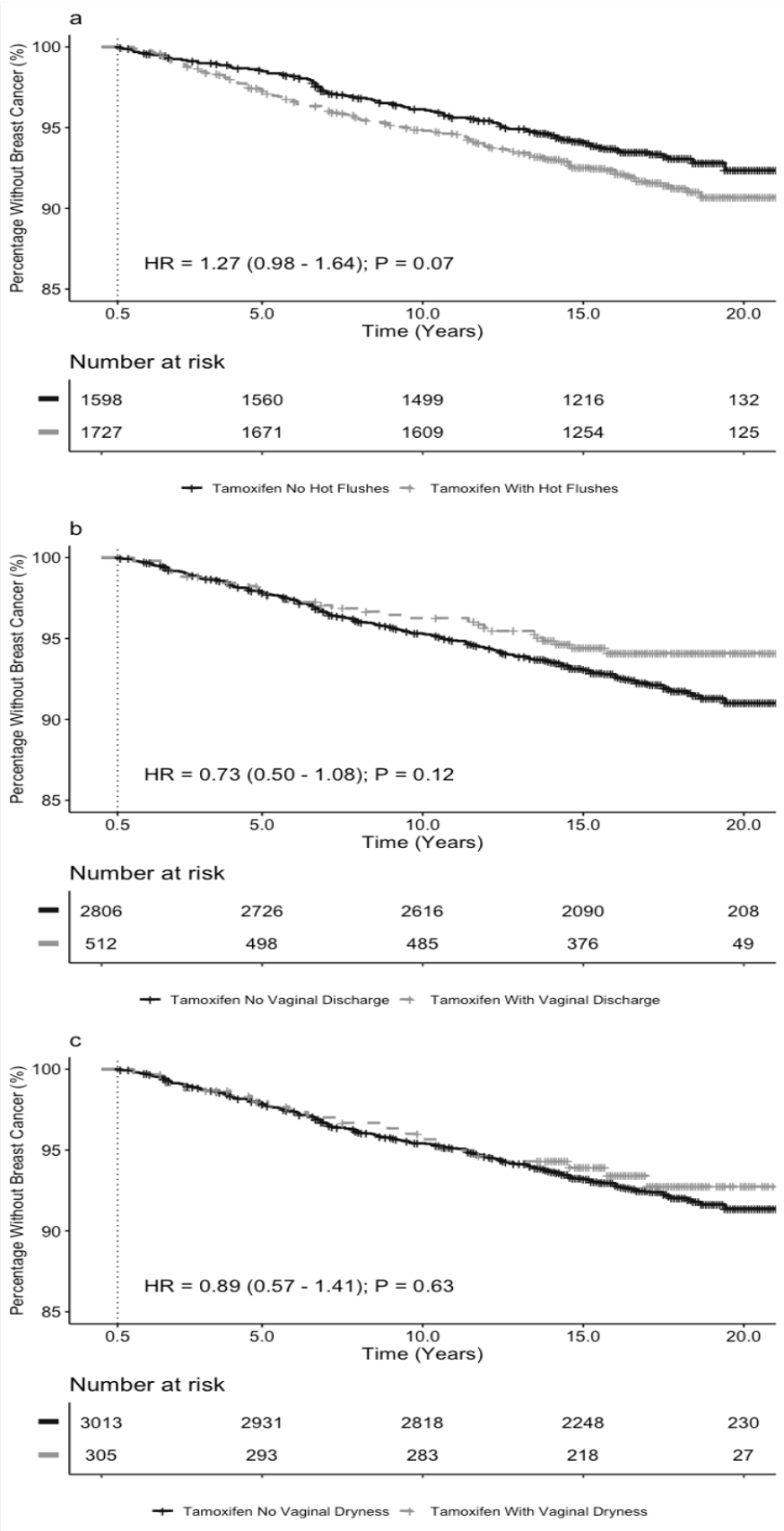


Figure 8.2: Kaplan-Meier graphs for breast cancer incidence in the tamoxifen arm with and without side effects. A – Hot flushes, B – Vaginal discharge, C – Vaginal dryness.

Table 8.2: Hazard ratios, 95% confidence intervals and P-values for breast cancer incidence within treatment arm comparison according to side effects for overall, premenopausal and postmenopausal women

Treatment arm	Side effect status	Overall		Postmenopausal		Premenopausal	
		HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value
Hot flushes							
Tamoxifen	None (N = 1,598)	Reference		Reference		Reference	
	Yes (N = 1,727)	1.27 (0.98-1.64)	0.07	1.61 (1.13-2.28)	0.01	0.91 (0.62-1.35)	0.65
Vaginal discharge							
Tamoxifen	None (N = 2,806)	Reference		Reference		Reference	
	Yes (N = 512)	0.73 (0.50-1.08)	0.12	0.64 (0.38-1.07)	0.09	0.88 (0.49-1.57)	0.66
Vaginal dryness							
Tamoxifen	None (N = 3,013)	Reference		Reference		Reference	
	Yes (N = 305)	0.89 (0.57-1.41)	0.63	0.90 (0.51-1.59)	0.71	0.85 (0.40-1.84)	0.69

8.3.2.2 Impact of side effects on breast cancer incidence in pre and postmenopausal women randomised to tamoxifen in the IBIS-I trial

Postmenopausal women who reported HFs at six months had a statistically significant increase in breast cancer compared to those without HFs (HR = 1.61 (1.13 - 2.28); P = 0.01) (Figure 8.3 Panel a, Table 8.2). Breast cancer risk increased as severity of HFs increased in postmenopausal women. Women reporting mild HFs had a statistically significant increase in breast cancer risk (HR = 1.49 (1.02 - 2.17); P = 0.04). Breast cancer risk increased for women reporting moderate (HR = 1.80 (1.02 - 3.17); P = 0.04) and severe (HR = 2.06 (1.15 - 3.69); P = 0.01) HFs compared to those not reporting any HFs. Neither gynaecological symptom predicted breast cancer incidence in postmenopausal women (vaginal discharge: HR = 0.64 (0.38 - 1.07); P = 0.09; vaginal dryness: HR = 0.90 (0.51 - 1.59); P = 0.71) (Figure 8.3 Panel b,c, Table 8.2). No association between HFs and breast cancer was observed for premenopausal women (HR = 0.91 (0.62 - 1.35); P = 0.65) (Table 8.2). Similarly, vaginal discharge and vaginal dryness were not predictive of breast cancer compared to women not reporting these symptoms (Table 8.2). No statistically significant observations in either pre or postmenopausal women were observed for either vaginal discharge or vaginal dryness due to small numbers of severe symptoms reported.

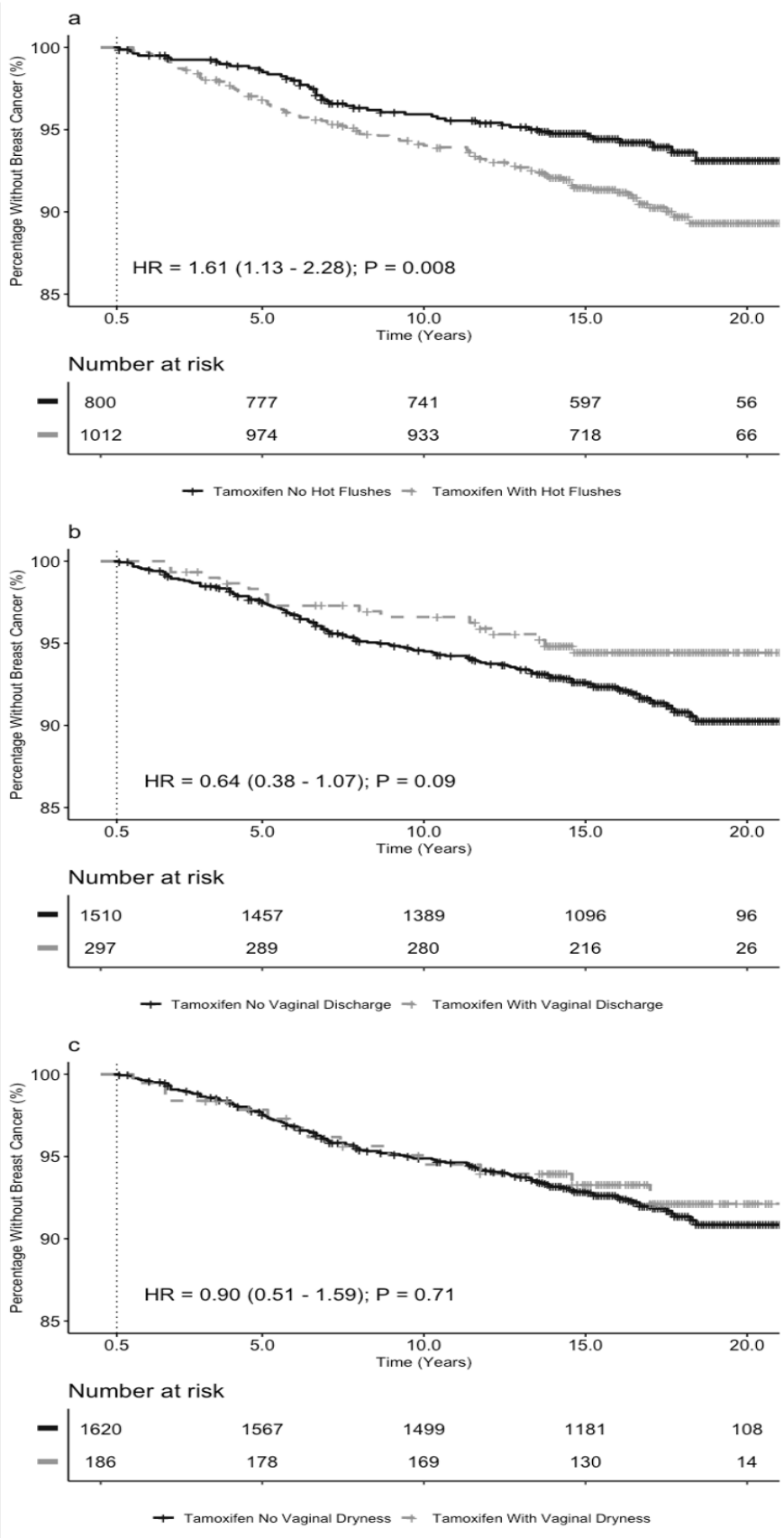


Figure 8.3: Kaplan-Meier graphs for breast cancer incidence in the tamoxifen arm with and without side effects in postmenopausal women. A – Hot flushes, B – Vaginal discharge, C – Vaginal dryness

8.3.2.3 Impact of side effects on ER-positive breast cancers in women randomised to tamoxifen

A secondary endpoint of the analysis was ER-positive breast cancer. Women who reported HFs had a statistically significant 38% increase in ER-positive breast cancer compared to those without HFs at six months (HR = 1.38 (1.01 - 1.87); P = 0.04) (Table 8.3). When HFs were analysed by severity, no statistically significant increase in breast cancer incidence was observed for the overall population of women randomised to tamoxifen.

In postmenopausal women, an association between HFs and ER-positive breast cancer was observed (HR = 1.82 (1.19 – 2.80); P = 0.01) (Table 8.3). When analysed by severity, mild HFs (HR = 1.78 (1.13 - 2.81); P = 0.01) had a statistically significant increase in the risk of breast cancer when compared to no HFs. Women reporting moderate (HR = 1.94 (0.97 – 3.87); P = 0.06) and severe HFs (HR = 1.94 (0.92 - 4.10); P = 0.08) had a non-statistically significant increase in breast cancer risk compared to women who did not report HFs. Postmenopausal women who reported either vaginal discharge or vaginal dryness had a non-significant decrease in ER-positive breast cancer compared to those not reporting this symptom (Table 8.3). No significant effects were seen in premenopausal women (Table 8.3). Similar results were observed according to severity of side effects. Due to a lack of severe or moderate gynaecological symptoms in premenopausal women, the risk of ER-positive breast cancer in this sub-group was not reportable. A test for heterogeneity between menopausal status and any side effect was not significant (all $P_{heterogeneity} > 0.05$).

Table 8.3: Hazard ratios, 95% confidence intervals and P-values for ER-positive breast cancer incidence within treatment arm comparison according to side effects for overall, premenopausal and postmenopausal women

Treatment Arm	Side effect status	Overall		Postmenopausal		Premenopausal	
		HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value
Hot flushes							
Tamoxifen	None (N = 1,698)	Reference		Reference		Reference	
	Yes (N = 1,727)	1.38 (1.01-1.87)	0.04	1.82 (1.19-2.80)	0.01	0.96 (0.61-1.52)	0.88
Vaginal discharge							
Tamoxifen	None (N = 2,806)	Reference		Reference		Reference	
	Yes (N = 512)	0.68 (0.42-1.09)	0.11	0.56 (0.29-1.09)	0.09	0.84 (0.42-1.69)	0.62
Vaginal dryness							
Tamoxifen	None (N = 3,013)	Reference		Reference		Reference	
	Yes (N = 305)	0.89 (0.51-1.53)	0.67	0.79 (0.38-1.62)	0.51	1.04 (0.45-2.39)	0.94

8.3.2.4 Influence of time since menopause on breast cancer outcomes in women on the IBIS-I study

Women randomised to tamoxifen who were postmenopausal for less than five years before randomisation had a non-significant increase in all breast cancers (HR = 2.02 (0.91 - 4.49); P = 0.08) and an almost 3-fold non-statistically significant increase in ER-positive breast cancer (HR = 2.58 (0.97 - 6.88); P = 0.06) if they reported symptoms at the six month follow-up visit. Women who were postmenopausal for more than ten years before randomisation, had a statistically significant increase in any breast cancer if they reported HFs (HR = 4.35 (1.18 – 16.06); P = 0.03). However, the increase was not maintained for ER-positive breast cancers (HR = 4.37 (0.88 – 21.64); P = 0.07). There was no association between side effects and breast cancer outcome was observed for women who had been postmenopausal for between five and ten prior to randomisation. No association between gynaecological side effect and time since menopause was observed.

8.3.2.5 Side effects and breast cancer outcomes in the IBIS-I placebo group

In women randomised to placebo, HFs did not predict overall breast cancer incidence (HR = 0.98, (0.77 - 1.24); P = 0.87) or ER-positive breast cancer (HR = 0.98 (0.75 - 1.29); P = 0.89) compared to those not reporting HFs (Table 8.4). Neither of the gynaecological symptoms predicted breast cancer incidence: vaginal discharge (HR = 0.76 (0.44 - 1.38); P = 0.33) and vaginal dryness (HR = 1.02 (0.69 - 1.50); P = 0.94) (Table 8.4) compared to those not reporting gynaecological symptoms in the placebo group.

Postmenopausal women who reported any side effect had no statistically significant increase in breast cancer incidence compared to those not reporting these symptoms for either all breast cancer or ER-positive breast cancers (Table 8.4).

Premenopausal women who experienced HFs had a non-significant decrease in breast cancer incidence compared to those without HFs for all breast cancers (HR = 0.92 (0.62 - 1.36); P = 0.67) and ER-positive breast cancers (HR = 1.00 (0.64 - 1.57); P = 0.99) (Table 8.4). Gynaecological symptoms, particularly vaginal dryness, did

not predict breast cancer nor ER-positive breast cancer incidence in premenopausal women randomised to placebo compared to those who did not experience symptoms, (Table 8.4). However, vaginal discharge in this sub-group of women did suggest a non-statistically significant decrease in all breast cancer (HR = 0.27 (0.07 - 1.10); P = 0.07) and ER-positive breast cancer (HR = 0.37 (0.09 – 1.51); P = 0.17) compared to premenopausal women who do not report any vaginal discharge in the first six months of IBIS-I (Table 8.4).

Heterogeneity testing of subgroups for all breast cancers and ER-positive breast cancer indicated that all subgroups were similar (all $P_{heterogeneity} > 0.05$).

Table 8.4: Hazard ratios, 95% confidence intervals and P-values for breast cancer incidence within treatment arm comparison according to side effects for overall, premenopausal and postmenopausal women in IBIS-I

		Overall		Postmenopausal		Premenopausal	
Treatment arm	**Side effect status**	**HR (95%CI)**	**P-value**	**HR (95%CI)**	**P-value**	**HR (95%CI)**	**P-value**
All breast cancer							
Hot flushes							
Placebo	None	Reference		Reference		Reference	
	Yes	0.98 (0.77-1.24)	0.87	0.99 (0.74-1.35)	0.97	0.92 (0.62-1.36)	0.67
Vaginal discharge							
Placebo	None	Reference		Reference		Reference	
	Yes	0.76 (0.44-1.38)	0.33	1.12 (0.61-2.05)	0.72	0.27 (0.07-1.10)	0.07
Vaginal dryness							
Placebo	None	Reference		Reference		Reference	
	Yes	1.02 (0.69-1.50)	0.94	1.10 (0.69-1.74)	0.70	0.80 (0.37-1.70)	0.56
ER- positive breast cancer							
Hot flushes							
Placebo	None	Reference		Reference		Reference	
	Yes	0.98 (0.75-1.29)	0.89	0.94 (0.66-1.32)	0.71	1.00 (0.64-1.57)	0.99
Vaginal discharge							
Placebo	None	Reference		Reference		Reference	
	Yes	0.76 (0.41-1.44)	0.41	1.03 (0.51-2.11)	0.93	0.37 (0.09-1.51)	0.17
Vaginal dryness							
Placebo	None	Reference		Reference		Reference	
	Yes	1.04 (0.67-1.63)	0.86	0.97 (0.56-1.68)	0.91	1.11 (0.51-2.38)	0.79

8.3.3 IBIS-II

8.3.3.1 IBIS-II analysis of side effects and breast cancer incidence in women randomised to anastrozole

No significant effect of arthralgia on breast cancer incidence was observed when compared to women in the anastrozole group not reporting these symptoms at the six month visit (HR = 1.39 (0.89 - 2.15); P = 0.15) (Figure 8.4). However, those who reported mild arthralgia events (HR = 1.56 (0.95 - 2.55); P = 0.08) were at higher, but not significant, risk of breast cancer than those who reported moderate (HR = 1.24 (0.61 – 2.52); P = 0.55) or severe (HR = 0.54 (0.08 – 3.94); P = 0.55) arthralgia compared to no arthralgia.

HFs reported at the six month visit were associated with a non-statistically significant reduction in breast cancer incidence when compared to those not reporting these symptoms (HR = 0.92 (0.59 - 1.41); P = 0.69) (Figure 8.4). Women who reported moderate HFs (HR = 0.48 (0.21 - 1.14); P = 0.09) were at lower risk of breast cancer than those who reported mild (HR = 1.10 (0.68 - 1.78); P = 0.69) or severe (HR = 1.23 (0.44 - 3.42); P = 0.69) HFs compared to no HFs.

Similarly, no significant difference in breast cancer incidence was observed in women who reported vaginal changes (HR = 0.73 (0.34 - 1.59); P = 0.43) at six months compared to those without these symptoms (Figure 8.4). Despite small numbers of severe vaginal changes reported, symptom severity did not appear to impact the risk of breast cancer. Women who report mild or moderate severity vaginal changes have a non-statistically significant decrease in breast cancer incidence compared to those without symptoms (HR = 0.75 (0.31 - 1.87); P = 0.54) and (HR = 0.48 (0.07 - 3.47); P = 0.47) respectively. However, women who had severe vaginal changes had a non-statistically significant increase in the risk of breast cancer events (HR = 1.14 (0.16 - 8.18); P = 0.90).

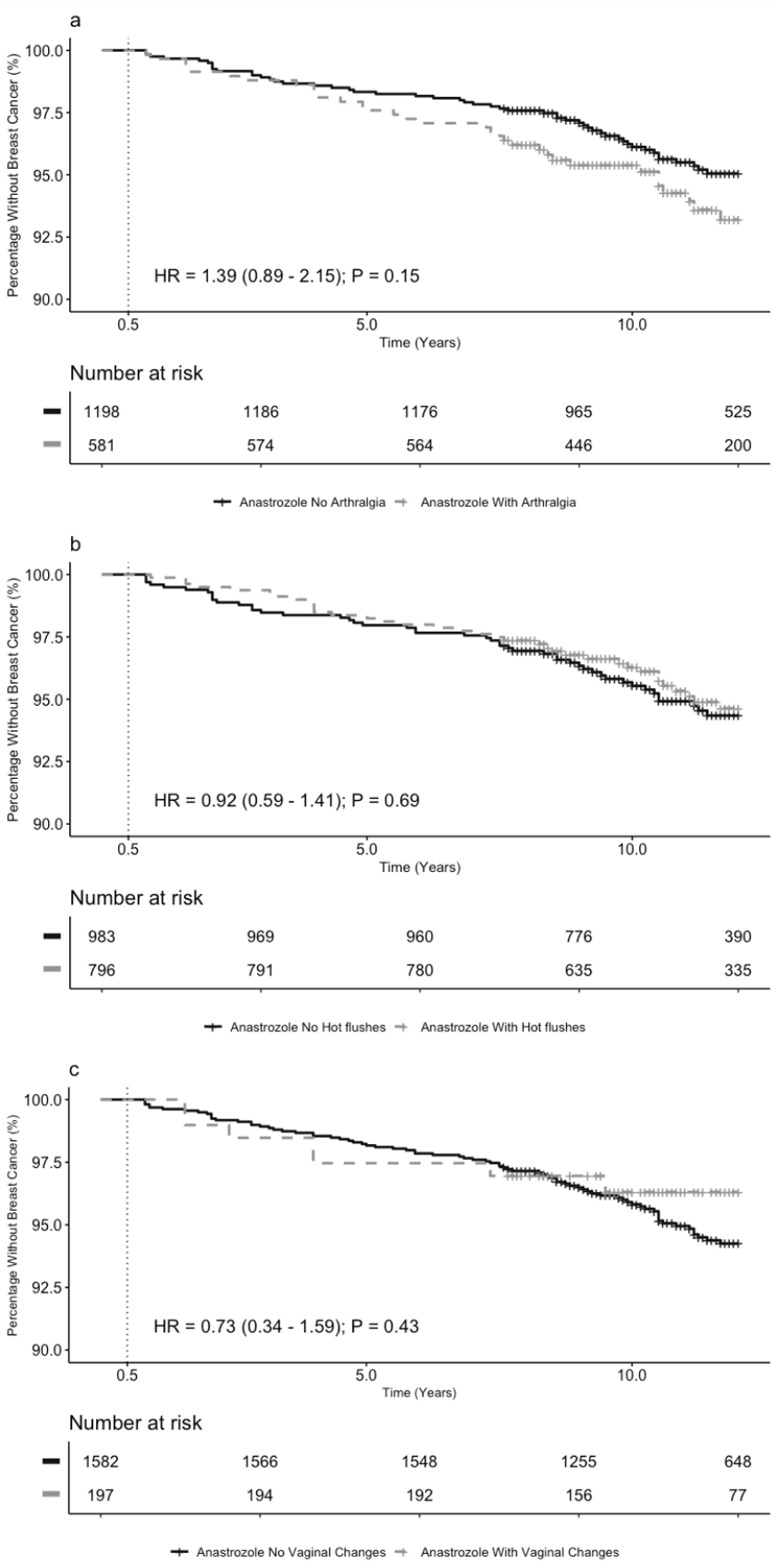


Figure 8.4: Kaplan-Meier graphs for breast cancer incidence in the IBIS-II anastrozole arm with and without side effects in postmenopausal women. A –Arthralgia, B – Hot flushes, C – Gynaecological symptoms.

8.3.3.2 Impact of side effects on ER-positive breast cancer in women randomised to anastrozole

A secondary endpoint of the IBIS-II analysis was ER-positive breast cancer. Women in the anastrozole group who experienced arthralgia in the first six months had a statistically significant 90% increase in the risk of ER-positive breast cancers compared to those who did not report any symptoms (HR = 1.90 (1.11 - 3.27); P = 0.02) (Figure 8.5). Women who reported mild arthralgia events (HR = 2.35 (1.31 - 4.21); P < 0.01) were at higher risk of breast cancer than those who reported moderate arthralgia (HR = 1.50 (0.62 - 3.62); P = 0.37). Due to a lack of severe arthralgia events and no ER-positive events in this group, the risk of breast cancer in this sub-group was not reportable.

Women who had HFs had a non-statistically significant decrease in the risk of breast cancer (HR = 0.74 (0.42 - 1.29); P = 0.29) compared to those without HFs (Figure 8.5). Analysis of HFs by severity of symptom showed that severity did not have any effect on ER-positive breast cancer risk. Women who reported mild or severe HFs had a non-statistically significant decrease in the risk of ER-positive breast cancer (HR = 0.86 (0.46 - 1.62); P = 0.65) and (HR = 0.90 (0.22 - 3.73); P = 0.88) compared to no reported HFs respectively. Women who reported moderate HFs had a non-statistically significant 54% decrease in ER- positive breast cancer risk (HR = 0.46 (0.16 - 1.31); P = 0.15).

Gynaecological symptoms experienced during the first six months of anastrozole therapy was not significantly associated with a decrease in ER-positive breast cancer risk (HR = 0.66 (0.24 - 1.82); P = 0.42) (Figure 8.5). Due to the lack of moderate or severe vaginal events, analysis of breast cancer risk by severity was not possible. Women who had mild gynaecological symptoms had a non-statistically significant reduction in ER-positive breast cancer risk (HR = 0.95 (0.34 - 2.63); P = 0.92).

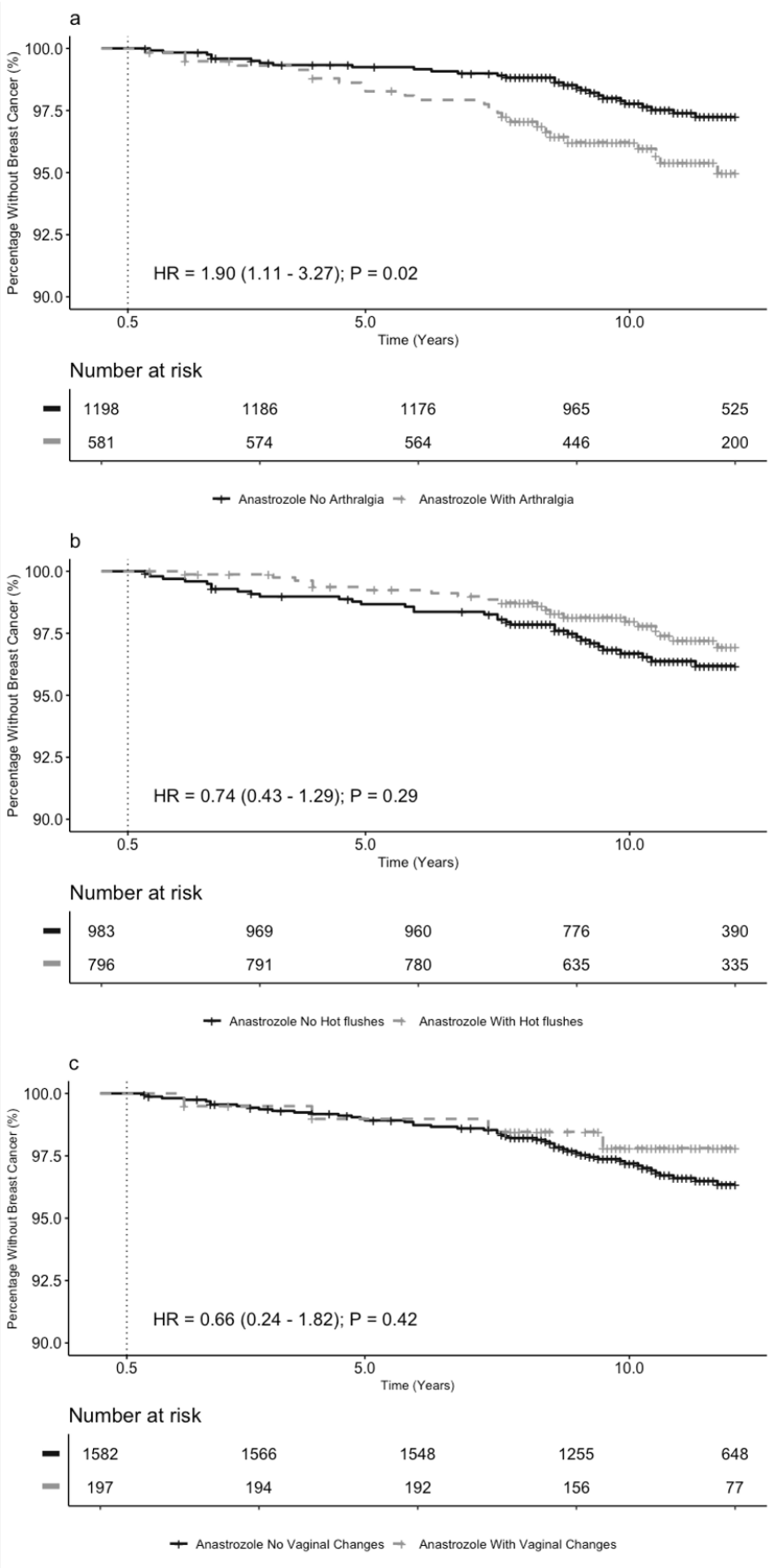


Figure 8.5: Kaplan-Meier graphs for ER-positive breast cancer incidence in the IBIS-II anastrozole arm with and without side effects in postmenopausal women. A –Arthralgia, B – Hot flushes, C – Gynaecological symptoms.

8.3.3.3 Influence of time since menopause on breast cancer outcomes in women on the IBIS-II study

Women on anastrozole who were postmenopausal for less than five years before randomisation had a non-significant increase in all breast cancers (HR = 1.94 (0.92 - 4.08); P = 0.08) and an approximate 3.5-fold increase in ER-positive breast cancer (HR = 3.36 (1.37 - 8.23); P < 0.01) if they reported arthralgia symptoms at the six month follow-up visit. Women who reported HFs and gynaecological symptoms had a non-statistically significant decrease in any breast cancer and ER-positive breast cancer compared to those without HFs (Table 8.5).

For women who were postmenopausal for between five and ten years before randomisation, no associations between side effects and breast cancer or ER-positive breast cancer outcomes were observed. This was also the case for women who were greater than ten year postmenopausal at study entry (Table 8.5).

Table 8.5: Hazard ratios and 95% confidence intervals for breast cancer incidence given the presence of side effects compared to no reported side effects at 6-month depending on type of breast cancer and time since menopause in women from IBIS-II

All breast cancers							
		Arthralgia		**Hot flushes**		**Gynaecological symptoms**	
	Events	**HR 95%CI**	**P-Value**	**HR 95%CI**	**P-Value**	**HR 95%CI**	**P-Value**
$<$ 5 years	N = 28	1.94 (0.92 - 4.08)	0.08	0.60 (0.28 - 1.31)	0.20	0.56 (0.13 - 2.36)	0.43
5-10 years	N = 19	1.98 (0.80 - 4.86)	0.14	2.10 (0.83 - 5.33)	0.12	1.32 (0.38 - 4.52)	0.66
$>$ 10 years	N = 36	0.81 (0.39 - 1.68)	0.57	0.75 (0.38 - 1.48)	0.40	0.54 (0.13 - 2.23)	0.39
ER-positive breast cancers							
		Arthralgia		**Hot flushes**		**Gynaecological symptoms**	
	Events	**HR 95%CI**	**P-Value**	**HR 95%CI**	**P-Value**	**HR 95%CI**	**P-Value**
$<$ 5 years	N = 20	3.36 (1.37 - 8.23)	$<$ 0.01	0.59 (0.23 - 1.47)	0.26	0.81 (0.19 - 3.49)	0.78
5-10 years	N = 12	2.22 (0.71 - 6.87)	0.17	1.72 (0.55 - 5.43)	0.35	0.64 (0.08 - 4.93)	0.67
$>$10 years	N = 21	1.05 (0.42 - 2.59)	0.92	0.53 (0.21 - 1.37)	0.19	0.46 (0.06 - 3.41)	0.45

8.3.3.4 Side effects and breast cancer outcomes in the IBIS-II placebo group

Within the IBIS-II trial women randomised to placebo, women who reported arthralgia in the first six months of trial had a non-statistically significant reduction in the incidence of all breast cancer and ER-positive breast cancer (HR = 0.75 (0.50 - 1.11); P = 0.15) and (HR = 0.79 (0.50 - 1.25); P = 0.32) respectively (Table 8.6).

HFs did not predict breast cancer incidence compared to those not reporting HFs (HR = 1.06 (0.76 - 1.48); P = 0.73) nor do HFs predict ER-positive breast cancer (HR = 1.24 (0.84 - 1.81), P = 0.28) (Table 8.6). Additionally, gynaecological symptoms did not predict all or ER-positive breast cancer incidence (HR = 1.07 (0.60 - 1.89); P = 0.82) and (HR = 1.24 (0.66 - 2.31); P = 0.51) compared to those not reporting gynaecological symptoms (Table 8.6).

Table 8.6: Hazard ratios, 95% confidence intervals and P-values for breast cancer incidence within the placebo group of IBIS-II

		All breast cancer		ER-positive breast cancer	
Treatment arm	Side effect status	HR (95%CI)	P-value	HR (95%CI)	P-value
Arthralgia					
Placebo	None (N = 1,329)	Reference		Reference	
	Yes (N = 482)	0.75 (0.50 - 1.11)	0.15	0.79 (0.50 - 1.25)	0.32
Hot flushes					
Placebo	None (N = 1,169)	Reference		Reference	
	Yes (N = 642)	1.06 (0.76 - 1.48)	0.73	1.24 (0.84 - 1.81)	0.28
Gynaecological symptoms					
Placebo	None (N = 1,659)	Reference		Reference	
	Yes (N = 152)	1.07 (0.60 - 1.89)	0.82	1.24 (0.66 - 2.31)	0.51

8.4 Discussion

In this analysis, results from a retrospective analysis of the IBIS-I and IBIS-II trials, investigating whether early endocrine symptoms reported during the first six months of trial by women randomised to tamoxifen, anastrozole or placebo were predictive of breast cancer occurrence are presented. Prophylactic endocrine therapy for five years has shown decreased breast cancer risk for women at high risk (Veronesi et al., 2003; Cuzick et al., 2007, 2020; Goss et al., 2011). The ability to predict survival outcomes based on treatment-emergent endocrine symptoms would be clinically important. Retrospective analyses of the ATAC, TEAM, and BIG 1-98 trials have suggested that side effects of endocrine therapy may be associated with superior survival outcomes (Cuzick et al., 2008; Fontein et al., 2013; Huober et al., 2014). However, analysis of the MA.27 trial found that treatment-emergent symptoms had no significant impact on recurrence free survival (Stearns et al., 2015).

In contrast to the other adjuvant trials, an analysis by Chlebowski et al. (2019) of postmenopausal women in the WHI found that women who reported vasomotor symptoms, particularly persistent vasomotor symptoms, were more likely to develop breast cancer than those not reporting these symptoms (Chlebowski et al., 2019). Whilst these results would seem to support those found in the IBIS-I population, women taking part in the WHI were not taking tamoxifen or AIs for prevention or treatment of breast cancer and so are more consistent with the placebo group. Results from both placebo groups indicate that there are no statistically significant associations between any side effects and breast cancer incidence in either IBIS-I or IBIS-II.

In the IBIS-I analysis, findings suggest that early reported symptoms did not predict overall breast cancer occurrence, a finding supported by the results of the IBIS-II analysis. However, within the IBIS-I study a significant positive association between HFs and ER-positive breast cancer, specifically in postmenopausal women randomised to tamoxifen was observed. Similarly, in the IBIS-II study, there was a statistically significant association between mild arthralgia and an increase in ER-positive breast cancer incidence in women randomised to anastrozole. Results from the placebo groups of both trials show no statistically significant associations between any side effect and breast cancer incidence.

The results of the IBIS-I analysis contrast with previous investigations. In a systematic review and meta-analysis of the ATAC, TEAM, BIG1-98, WHEL, IES and MA.27 trials breast cancer patients who experienced endocrine treatment-related symptoms had a lower recurrence rate than those not reporting these symptoms (Yoo et al., 2018). This relationship persisted despite numerous changes including different types of symptoms - vasomotor symptoms and/or musculoskeletal symptoms, the time at which the side effects were evaluated (three months or 12 months after initiation of endocrine treatment), inclusion of patients with baseline symptoms, or exclusive inclusion of postmenopausal women. In all analyses similar HRs, of 0.69 to 0.80, were reported for the impact of side effects on breast cancer recurrence (Yoo et al., 2018).

Observational studies have also found conflicting results where vasomotor symptoms were associated with a decrease in breast cancer incidence in two case-control studies and one cohort study (Huang et al., 2011; Fei et al., 2013; Hart et al., 2016) but no association was observed in a second cohort study (Johanneke Van Den Berg et al., 2014).

In both the IBIS-I and IBIS-II studies, contrary to the original hypothesis, HFs did not predict tamoxifen or anastrozole efficacy for breast cancer, particularly ER-positive breast cancer in women who were postmenopausal. One explanation for the positive association in the IBIS-I analysis might be found in oestrogen concentrations. At menopause oestrogen concentrations rapidly decrease but as women continue aging oestrogen concentrations decrease at a much slower rate. Therefore, women who are recently postmenopausal at study entry, less than five years, would have higher oestrogen concentrations than those who are long-term postmenopausal. These women may therefore be at higher risk of breast cancer and side effects due to higher oestrogen concentrations and greater disruption of oestrogen signalling by tamoxifen. This hypothesis is supported by findings from the IBIS-I analysis that only women who were postmenopausal for less than five years had a 2.8-fold statistically significant increase in ER-positive breast cancers and a statistically non-significant 90% increase in all breast cancers in those reporting side effects. Women who had been postmenopausal for greater than five years no statistically significant associations were observed.

In further support of the menopausal hypothesis, these results show no evidence of an association between HFs and breast cancer incidence in premenopausal women. This is in agreement with studies by Johanneke Van den Berg et al. (2014) where no association

between HFs and breast cancer was observed in premenopausal women (Johanneke Van Den Berg et al., 2014). Additionally, Chapman et al. (2011) evaluated the role of emergent symptoms in premenopausal women randomly assigned to tamoxifen or placebo in the MA.12 trial (Chapman et al., 2011). Analysis of the MA.12 trial also observed that treatment-emergent symptoms did not impact recurrence free survival (P = 0.90) (Chapman et al., 2011).

What is interesting is the disparity in the result of the IBIS-I and IBIS-II analysis. A possible reason for this may be the different allowance for the use of HRT in each of the studies. Women in IBIS-I were allowed to continue the use of HRT throughout the trial; however, women on the IBIS-II trial had to cease HRT prior to starting anastrozole (Cuzick et al., 2002, 2014). It is possible that the difference observed in the results between IBIS-I and IBIS-II analyses with regards HFs may be due to the cessation of HRT. Inclusion of HRT history into Cox models did not change the reported hazard ratios for side effect impact on all breast cancer nor ER-positive breast cancer. Analysis of a subgroup of IBIS-I women who had never previously used HRT showed that, in contrast to previous results, reporting HFs did not have a statistically significant increase in breast cancer risk. However, results remained close to previous significance levels in postmenopausal women for all breast cancer (HR = 1.51 (0.87 – 2.63); P = 0.14) and ER-positive breast cancer (HR = 1.94 (0.98 – 3.86); P = 0.06) suggesting that the differences in trial outcomes cannot be explained by the use of HRT alone. HRT withdrawal is frequently associated with the appearance of rebound symptoms and improved breast cancer outcomes, which may explain the disparate results between the IBIS-I analysis and the IBIS-II analysis.

The effects of withdrawal generally occur promptly and would be recorded at baseline therefore effects during the first six months of trial would not be classed as new symptoms. In IBIS-II, baseline vasomotor symptoms were not recorded and therefore it was not possible to test whether the HFs recorded in the first six months are new or recurrent symptoms. In ATAC, which did not require an HRT washout period before random assignment, only 9.5% and 9.3% of patients who started treatment had baseline vasomotor and joint symptoms, respectively (Stearns et al., 2015). In the ATAC analysis, only the three month symptom data were investigated, and 55% of patients receiving either anastrozole or tamoxifen developed symptoms by three months. Among these ATAC participants, the emergence of vasomotor symptoms was partic-

ularly pronounced in those who had received prior HRT (P = 0.003) (Cuzick et al., 2008). Additionally, the reduction in breast cancer recurrence was most pronounced in those reporting new symptoms (Cuzick et al., 2008). Therefore, is it possible that the prior use HRT use up until, or near, the start of the IBIS-II trial could have confounded these results.

The mechanism underlying the relationship between endocrine treatment-related symptoms and improved survival remains unclear. There is a strong focus on oestrogen and the incidence of HFs due to the high prevalence of women who experience HFs as a result of dramatically decreased oestrogen concentrations as a result of physiological conditions such as menopause or bilateral oophorectomy (Sturdee, 2008). It is possible that women with a high concentration of circulating oestrogens are more sensitive to endocrine therapy and as a result experience more HFs than those with lower concentrations. Higher concentrations of oestrogens may put these women at higher risk of breast cancer due to increased lifetime exposure and therefore may be the link between increased HFs and a higher risk of breast cancer. It has also been hypothesised that it is not the concentration of circulating oestrogens which leads to HFs but the extent to which they are reduced. Women who are newly postmenopausal or those who have recently been taking HRT will have higher circulating oestrogen concentrations than those who are long-term postmenopausal and therefore may be at higher risk of both breast cancer and of developing menopausal-like symptoms.

HFs are not the only reported side effects in this analysis which have a statistically significant increase in breast cancer incidence. The analysis of women randomised to anastrozole on the IBIS-II study shows that arthralgias reported in the first six months which are associated with an increase in breast cancer incidence particularly ER-positive breast cancer. Whilst the aetiology of HFs has been well discussed, the aetiology of musculoskeletal symptoms is more obscure. However, several hypotheses have been reported. Disruption of oestrogen concentrations, the increased sensation of pain stimuli (antinociceptive effects) due to oestrogen deficiency, accumulation of fluid in and around the joints, and autoimmunity increasing joint inflammation are some of the possible sources of musculoskeletal symptoms (Gaillard and Stearns, 2011; Honda et al., 2011). AI-associated musculoskeletal symptoms may also be as a result of pharmacogenetic or genetic factors (Henry, Giles and Stearns, 2008). Connecting these causes to the association between musculoskeletal symptoms and increased breast

cancer with endocrine therapy requires additional investigation.

The use of pharmacological interventions to manage side effects should also be considered when investigating the relationship of side effects and breast cancer incidence as symptoms may be influenced by concomitant use of interventions alleviating symptoms (Henry, Giles and Stearns, 2008). Women suffering from HFs may be prescribed treatment to alleviate these symptoms e.g. Selective Serotonin Reuptake Inhibitors (SSRIs) or HRT (Cella and Fallowfield, 2008). Both of these treatments would increase the risk of developing breast cancer due to the new influx of oestrogens associated with HRT and inhibition of CYP2D6 a key enzyme in the conversion of tamoxifen to its more potent metabolite endoxifen by SSRIs. This provides a potential link between the reports of vasomotor symptoms and an increased risk of breast cancer.

Furthermore, the effects of arthralgia pain medications may have confounded results in the IBIS-II trial. Details of aspirin use and other nonsteroidal anti-inflammatory drugs (NSAIDs), which may have been prescribed to manage symptoms, were not rigorously collected in either the ATAC or the IBIS-II trial. However, in the ATAC study, 12% of patients reported the use of cyclooxygenase-2 inhibitors (Stearns et al., 2015). The use of NSAIDS and cyclooxygenase-2 inhibitors in the ATAC trial could have been directed toward controlling symptoms, which may have improved adherence and thus led to improved breast cancer outcomes.

Despite this variety of approaches and evidence, the link between endocrine treatment-related symptoms and treatment efficacy may be simply the result of differences in adherence. In the ATAC trial, a nonsignificant increase in patient drug adherence was observed in patients reporting symptoms (88%) compared with those with no symptoms (84%) (Cuzick et al., 2008). Physical symptoms are only recorded when patients report them, and a hypothesis that patients reporting their symptoms might be more likely to take their pills than those who do not report symptoms is a possible explanation. However, it has been established that side effects are a major reason for nonadherence to endocrine therapy (Smith et al., 2016). 63.9% of women enrolled on IBIS-I were adherent to the full five years of endocrine therapy and 65.7% were adherent in the IBIS-II study (Cuzick et al., 2007; Sestak et al., 2018). Furthermore, another study reported higher rates of symptoms in nonadherent patients (Brett et al., 2018). Both lower adherence due to side effects and the increased rate of side effects in non-adherent patients challenge the hypothesis that the benefits seen in other trials are as a result of

better adherence. The issue of adherence was not the focus of this analysis and further studies investigating the impact of adherence on breast cancer outcomes in women who experience side effects should be undertaken.

The difficulty with accounting for adherence lies within the measure of adherence and the length of time that a woman is adherent. The measure of adherence can be through direct, measurement of drug or metabolite levels or indirect such as pill-counts, electronic databases, self-reported via questionnaires, or electronic monitoring systems (Anghel et al., 2019). There are advantages and disadvantages to each of these various techniques for measuring adherence, and whilst we cannot tell if patients actually took the medication, women at high-risk of breast cancer are probably motivated to be adherent, as reflected in the high levels of adherence reported in the IBIS-I and IBIS-II trials.

In addition to pill counts, the time when a woman becomes non-adherent must be considered. If side effects are reported at six months but a woman remains adherent until 24-30 months, can this be associated with the previously reported side effects? This situation is difficult to argue and make a fixed assessment of adherence complicated. A future study could investigate the impact of side effects and adherence on breast cancer outcomes, but any such study may benefit from an adherence scale in which pill counts and time on therapy are included as a score to determine how adherent the participant was to therapy.

Analysis of both the IBIS-I and IBIS-II datasets found no significant effects for the association between gynaecological symptoms and breast cancer incidence. Since oestrogen is vital for the maintenance of vaginal epithelium and underlying tissues, without it the epithelium can thin resulting in dryness, discomfort and possible bleeding (Krause et al., 2010). It is possible that tamoxifen provides a pseudo-oestrogenic effect on the vagina increasing secretions without the presence of oestrogen. The incidence of vaginal discharge may represent the successful conversion of tamoxifen to more potent metabolites and thus a better breast cancer outcome. Conversely, vaginal dryness would suggest that there is little oestrogenic action of tamoxifen on the vagina translating to a lack of efficacy of tamoxifen and no reduction in breast cancer incidence. Very few women in the IBIS-II study reported gynaecological symptoms. This may be due to the mode of action of anastrozole which, as opposed to tamoxifen, does not bind to the ER leaving the ER binding site clear. Free ERs may allow some stimulation by

androgens, precursors of oestrogens, minimising some of the vaginal issues reported in tamoxifen studies. A combination of few reports of gynaecological symptoms and an absence of oestrogens due to AIs could result in any effects being difficult to observe due to a small number of events in each case.

Endocrine therapy significantly reduces the number of breast cancer events in women at high risk when compared to a group of similar risk women on placebo (Cuzick et al., 2015, 2020). Despite the impact of side effects on quality of life, endocrine therapy should be promoted and used as an effective tool for reducing a woman's risk of breast cancer. What these analyses highlight is that side effects are a real concern in the prevention of breast cancers. Potential mechanisms behind these outcomes have been suggested but remain poorly understood. Nevertheless, strategies must be put in place for management of side effects whilst retaining the benefit of endocrine therapy. These strategies could include personalised endocrine therapy regimes targeting-specific populations, who are at lower risk of serious side effects and women with high-risk benign breast lesions who derive greater benefit from anti-oestrogens. Identifying women who are at high risk of side effects is the main focus of this thesis. The identified risk factors, and prediction tools developed through-out this thesis could be important for the identification of women at high risk of side effects. Using these tools to minimise toxicities with the use of treatment holidays, lower doses of tamoxifen or switching to alternative endocrine therapies, such as AIs, rather than "treating" symptoms and risking drug interactions negating the benefits of tamoxifen should be explored. Lower doses of tamoxifen, 5 mg/d rather than 20 mg/d, for a shorter period of time, three years vs five years, have shown to be effective at reducing breast cancer events (HR = 0.48 (0.26 – 0.92); P = 0.02) whilst having limited toxicity (DeCensi et al., 2019). Lower doses of tamoxifen are therefore a possible option for women who experience a high number of side effects, but would benefit from preventive endocrine therapy.

The major strength of this analysis is that it is among the first to investigate the relationship between early reported side effects and breast cancer incidence in the preventive setting for women randomised to tamoxifen and anastrozole. The analysis benefits from data from two large clinical placebo-controlled trials both of which have long-term follow-up and detailed assessment of breast cancer outcomes and side effects at each six monthly follow-up visit. Analyses focused on the relationship between early side effects reported at the six month follow up visit and breast cancer incidence because

the vast majority of side effects are first reported within the first six months. This analysis is also amongst the first to compare the effects of tamoxifen and anastrozole side effects and their impact on breast cancer incidence, a subject that has previously only been addressed in the adjuvant setting.

The limitations of this analysis include that all side effects were self-reported and based on previously established toxicity outcomes. While this analysis focussed on the most common endocrine therapy side effects which were previously believed to be suitable markers for endocrine therapy efficacy, it remains possible that other previously unknown side effects exist that have a different impact on breast cancer outcomes. Presence of side effects prior to study entry were not collected and therefore could not be accounted for in this study. This means that it was not possible to identify new side effects that may be as a result of starting endocrine therapy and those which were a continuation of those experienced during menopause. Concomitant medications were also not accounted for in this analysis. Whilst adherence to therapy was known at the six month follow-up, we have not assessed full five year adherence and breast cancer outcome; however, the impact of side effects on adherence has been addressed in previous publications (Smith et al., 2016; Sestak et al., 2018). Due to the amount of variation that should be considered to account for adherence, the impact of adherence to endocrine therapy in women who have experienced different severities of side effects, particularly HFs, should be considered in a separate analysis. Finally, onset of side effects later in the treatment period may contribute to breast cancer outcomes and might be a possible explanation for some of the findings in this analysis.

8.5 Conclusions

In summary, the majority of studies of patients who received tamoxifen or AI reported a relationship between emergence of vasomotor and musculoskeletal symptoms and survival outcomes. This study, examining two types of endocrine therapy, failed to show a positive association between symptoms and breast cancer outcome. Indeed, results of this analysis suggests that HFs have a weak association with overall breast cancer outcome for women on tamoxifen. In postmenopausal women randomised to tamoxifen the association of HFs with breast cancer incidence was stronger particularly in those who have been postmenopausal for under five years. In particular HFs were associated

with a significant increase in ER-positive breast cancer was observed if women were postmenopausal for less than five years before study entry. Women taking anastrozole who report arthralgias have a weak positive association with overall breast cancer outcomes and a statistically significant positive association was observed for ER-positive breast cancer. There was no statistically significant association between HFs and overall breast cancer or ER-positive breast cancer in women randomised to anastrozole. Results show that gynaecological symptoms reported by women randomised to tamoxifen or anastrozole were not good markers for prediction of subsequent breast cancer incidence. The findings of this analysis are in contrast to those observed in the adjuvant setting and highlight the need for further study to fully understand the relationship between side effect and breast cancer outcomes.

The findings of this analysis are in no way meant to support non prescription of endocrine therapy to women at high risk of breast cancer. Preventive endocrine therapy is a vital opportunity to decrease a woman's risk of future breast cancer and suitable for women at high risk. This analysis highlights the importance of side effects in achieving the goal of preventing breast cancer and shows that predictive tools are required to determine a woman's risk of endocrine therapy side effects to ensure that women are better informed of the side effect risks prior to starting endocrine therapy. Bothersome side effects should be managed as effectively as possible to support continuation and adherence to therapy, and it is premature to counsel patients on whether to continue or change their endocrine therapy based on side effects alone.

Chapter 9: Conclusions and further work

9.1 Conclusions and findings

This thesis investigated the association between genetic, hormonal and clinical risk factors and the incidence of menopausal-like side effects of endocrine therapy. The primary thesis hypothesis was that baseline measurements, genetic variants and hormonal factors could be established to identify women who may be at increased risk of developing side effects and subsequently combine risk factors to produce a model to predict side effect incidence during endocrine therapy. It is also hypothesised that, as in the adjuvant setting, women who report side effects are at lower long-term risk of breast cancer and that the presence of side effects is a marker that endocrine therapy is effective.

This thesis tested these hypotheses over five chapters: chapters 4-6 assessing risk factors for side effects, chapter 7 describing prediction models for side effects and chapter 8 exploring the impact of side effects on breast cancer incidence. An introduction and description of study aims, methods, results, discussions and conclusions were presented separately for each chapter. In this final chapter, the findings of the thesis are summarised and areas for future research are identified and discussed. Additionally, the impact of the thesis and target groups who may benefit from the work of the thesis are discussed.

Chapter 1 introduced the current scientific position regarding breast cancer outlining incidence, risk factors and the ongoing efforts to combat breast cancer including a summary of the major prevention and adjuvant trials and introducing two main endocrine therapies used for prevention of first breast cancer and prevention of breast cancer recurrence. Chapter 1 also introduced the issue of endocrine therapy side effects, their incidence in major prevention trials, potential risk factors and their impact on reduction of uptake and adherence to endocrine therapy. The literature regarding breast cancer prediction models and their application to side effect prediction was also summarised and highlighted.

Chapter 2 contained the rationale for the thesis and the study aims and objectives for each chapter.

Chapter 3 summarised the general methods used throughout the thesis, identified where techniques were used, and why these methods were appropriate for each analysis. It was included as a reference chapter to inform chapters 4-8.

Chapter 4 assessed whether clinical baseline factors, are risk factors for side effects. The aim was to investigate whether randomised treatment, menopausal status, age, BMI, HRT use, smoking status, and reproductive factors, increased or decreased the risk of side effects. Women who were randomised to tamoxifen had an approximate three-fold increase in HF incidence (OR = 2.95 95%CI (2.66 – 3.26): $P < 0.01$) and a two-fold increase in gynaecological symptoms (OR = 2.13 (1.90 – 2.41); $P < 0.01$) compared to women randomised to placebo. Women randomised to anastrozole had a 47% increase in HFs (OR = 1.47 (1.29 - 1.68); $P < 0.01$), a 36% increase in the number of gynaecological symptoms (OR = 1.36 (1.09 - 1.70); $P = 0.01$ and a 34% increase in arthralgia (OR = 1.34 (1.16 - 1.54); $P < 0.0001$) compared to women randomised to placebo. Within the IBIS-I trial, menopausal status was also found to be an important risk factor for side effects. Premenopausal women in the IBIS-I trial had significantly fewer reports of HFs (OR = 0.71 (0.65 – 0.79); $P < 0.001$) but were at increased risk of gynaecological symptoms (OR = 1.33 (1.19 – 1.50); $P < 0.001$) than women who were postmenopausal. Given the influence of these risk factors, women were subsequently partitioned into subgroups by menopausal status and randomised treatment to assess other risk factors. The most common risk factors for side effects when taking either tamoxifen or anastrozole were age at randomisation, HRT use and BMI. Other factors which affect side effect incidence in some subgroups, but not all, were age at menarche, smoking (current), time since menopause and previous hysterectomy. It is likely that all these factors mediate their effects via sex hormone concentrations in pre and postmenopausal women (Endogenous Hormones and Breast Cancer Collaborative Group, 2011, 2013). The anti-oestrogenic properties of endocrine therapy are usually considered the key reason for side effects (Mourits et al., 2001). However, this analysis found that menopausal status affects HFs and gynaecological side effects, in contrasting manners, raising the possibility that different side effects may be dependent on factors beyond the ambient oestrogen concentration. Given the high importance of menopausal status, and of time since menopause in postmenopausal women, there was

evidence to suggest that alongside oestrogen concentrations the rate at which oestrogen was decreased was also an important factor for the incidence of side effects. There was also evidence that given the different effects of tamoxifen on HFs and gynaecological symptoms that HFs, vaginal bleeding and vaginal dryness may be due to antagonist action at the ER, but that vaginal discharge may be due to agonism of the ER.

Chapter 5 investigated the association of SNPs with side effects in women taking tamoxifen. This analysis was among the first to investigate SNPs across multiple genes both individually and in combination to assess genetic impact on side effects. The analysis had three main components: (1) a CG study investigating SNPs within genes known to be involved in tamoxifen or hormone metabolism or for receptor coding, (2) a GWAS study to identify novel SNPs for association with side effects, (3) investigation of multi-loci to form a polygenic risk score for side effects. Results showed that there was little association between SNPs and side effects. In most cases the SNPs identified in the CG or GWAS did not reach the bonferroni corrected statistical significance level of $8.3\text{x}10^{-4}$ and were likely false positives. The analysis did corroborate the results of Sestak et al. (2012) by confirming that there was no association of SNPs within the CYP2D6 gene and side effects reported by women taking tamoxifen (Sestak et al., 2012). However, this analysis was the first GWAS of tamoxifen related side effects in women at high risk of breast cancer and has identified two risk alleles in *PSG9* and *CD177* which implicate cell adhesion and oestrogen signalling pathways in the aetiology of vaginal discharge. The multi-loci analysis used least absolute shrinkage and selection operator for variable selection to create a polygenic risk score (PRS) for side effects. Although, the PRS for HFs could not be formed as only one SNP was selected, PRS scores for gynaecological symptoms, particularly vaginal dryness and irregular bleeding were illuminating. Using a PRS score with 44 SNPs to identify women at risk of vaginal dryness, women with a score below a threshold of 0.5 reported no side effects and those above all reported vaginal dryness. A 28 SNP PRS for irregular bleeding also showed that most women without irregular bleeding (228/272, 83.8%) had a score of less than 1, compared to 64.0% (16/25) women who reported irregular bleeding who had a score of greater than 2.5. In both cases PRS show potential for identifying women who are at risk of gynaecological side effects and those who are at lower risk. Multi-loci models showed that the coefficients of each SNP were small, suggesting that any effect of SNPs on side effects in relatively small. Key findings of this study were that there were no large effects of any SNP on any of the side effects. However, it remains possi-

ble that this analysis had insufficient power to find statistically significant associations. These results should encourage further replication in large and independent cohorts and biological investigation to elucidate possible mechanisms.

Chapter 6 focussed on the association between ln-transformed concentrations of testosterone, DHEAS, and SHBG and side effects in postmenopausal women randomised to tamoxifen and placebo arms of the IBIS-I trial and the anastrozole and placebo arms of the IBIS-II trial. The impact of sex hormones and SHBG on side effects had not previously been assessed in women at high-risk of breast cancer so this study represented a novel analysis. The association between lifestyle factors and sex hormones was also investigated. Results showed that after adjustment for BMI, HRT use, hysterectomy, and smoking status there was a significant effect of baseline ln-SHBG and ln-bioT on the risk of side effects in all women on the IBIS-I trial (OR = 0.21 (0.12 – 0.35); P $<$ 0.01) and (OR = 3.85 (2.15 – 6.90); P $<$ 0.01) respectively. These results were maintained after accounting for randomisation status. In the IBIS-I analysis ln-testosterone, ln-DHEAS and ln-bioT concentrations were all found to be associated with age, and age at menopause; ln-testosterone and ln-bioT were also associated with age at menarche. Ln-SHBG was inversely associated with BMI. The association of SHBG with menopausal symptoms is the focus of few studies and those available reach contrasting outcomes. Whilst the inverse association between SHBG and menopausal symptoms would seem to be counter intuitive the interaction between BMI, SHBG and leptin was proposed as a potential mechanism. Leptin levels have been found to be positively associated with reports of HFs (P = 0.04) and are positively associated with BMI, whilst leptin also has an inverse correlation with concentrations of SHBG (P $<$ 0.0001) (Alexander et al., 2010). It was based on these reasons that leptin was suggested as playing a role in HFs and why it could be linked to a decrease in risk of side effects in this analysis. However, for this hypothesis to be confirmed, detailed investigation of leptin concentrations within these samples would need to be performed (see section 9.1.2). Results in the IBIS-II trial showed that after adjustment only ln-SHBG was associated with the risk of side effects in all women (OR = 0.84 (0.72 – 0.98); P = 0.03). Accounting for randomisation showed that the association only remained in women randomised to anastrozole (OR = 0.79 (0.63 – 1.00); P = 0.05) but not in women randomised to placebo. In the IBIS-II analysis group, only ln-DHEAS was positively associated with age, ln-SHBG was inversely associated with BMI and ln-bioT positively associated with BMI. These results showed ln-SHBG concentrations

are associated with side effects occurring in the first year of taking endocrine therapy with some evidence of ln-bioT concentrations also serving as a marker for side effects. As such they should be considered as side effect risk factors and could be used to better inform women who are considering taking endocrine therapy for prevention of breast cancer. Sex hormone concentrations are also associated with several established or suspected risk factors for side effects and may mediate the effects of these factors on side effect risk.

The chapter 7 analysis investigated the use of statistical modelling to form a prediction model using clinical, genetic and sex hormone markers to predict side effect outcomes. Models have been formed to predict breast cancer outcomes, but no models exist for the prediction of side effects. Two algorithms were used to train models using 10-fold cross-validation and model accuracy was tested in the 10th fold. Models were subsequently validated in a pre-partitioned test set. In each subgroup, pre and post-menopausal women randomised to tamoxifen and postmenopausal women randomised to anastrozole, LASSO and logistic regression models had weak predictive performance when using baseline clinical risk factors. Addition of sex hormone concentrations to the models improved the predictive ability of the models. However, inclusion of SNPs did not improve the model predictions. Validation of the combined models in a separate validation set found that in general models could predict women who were at risk of side effects but were poor at identifying those who do not experience side effects (sensitive not specific). A side effect risk (SER) score developed using the logistic regression coefficients showed that currently the identified risk factors cannot accurately predict the risk of early side effects in women taking tamoxifen or anastrozole and requires significant future development. Predictive models have helped revolutionise personalised medicine and the development of algorithms offer an exciting prospect of producing an accurate guide to the problem of side effects. While this was the first step in developing new risk prediction approaches, the incorporation of new risk factors should be further explored and duplicated with prospective and independent datasets. Side effects remain an important barrier to effective prevention of breast cancer and thus further work to understand risk factors is imperative.

The investigation in chapter 8 sought to explore whether the occurrence of side effects after six months of tamoxifen or anastrozole predicts long-term breast cancer risk in the prevention setting. The impact of side effects in subgroups of women categorised by

treatment allocation and, where necessary, menopausal status at baseline was assessed. The role of the severity of side effects on breast cancer outcomes was also explored. Multiple studies in the adjuvant setting have shown a positive association between side effects and breast cancer outcomes and suggest that early side effects could be a marker for predicting therapy benefit and a long-term reduction in breast cancers (Cuzick et al., 2008; Fontein et al., 2013; Huober et al., 2014; Mortimer et al., 2008; Stearns et al., 2015). However, the impact of side effects in the prevention setting had not previously been investigated. Results of analyses in this chapter, failed to corroborate the positive association between symptoms and a reduction in breast cancer incidence observed in the adjuvant setting. Indeed, results from the IBIS-I trial, in women randomised to tamoxifen suggested a weak association between hot flushes and an overall increase in breast cancer incidence (HR = 1.26 (0.98 - 1.62); P = 0.08), which was strongest in older, postmenopausal women (HR = 1.59 (1.12 - 2.26); P = 0.01) and for ER-positive disease in postmenopausal women (HR = 1.81 (1.19 - 2.74); P = 0.01). No side effects had an association with overall or ER-positive breast cancers in premenopausal women randomised to tamoxifen. For women taking anastrozole as part of the IBIS-II trial, arthralgia had a weak association with overall breast cancer incidence and a statistically significant increase in ER-positive breast cancer incidence (HR = 1.90 (1.11 - 3.27); P = 0.02). For women randomised to anastrozole, early reported hot flushes showed a non-statistically significant reduction in breast cancer incidence. Results show that gynaecological symptoms were not good markers for prediction of subsequent breast cancer incidence. These findings are important as they suggest that the experience of early side effects has a significant negative impact on breast cancer outcomes. A possible reason is adherence to therapy as it is well known that side effects reduce adherence (Ropka et al., 2010; Smith et al., 2016). These results show the importance of communicating side effect risk and management of symptoms in women at high risk of breast cancer. The findings of this chapter are in contrast to those observed in the adjuvant setting and highlight the need for further study, including the role of adherence and concomitant medications, to fully understand the relationship between side effect and breast cancer outcomes in the prevention setting.

9.2 Further research: identifying risk factors for side effects

The work performed in this thesis generated several hypotheses and revealed several avenues for further research: (1) increased focus on genetic mutations and how they impact side effects, (2) measurement of oestrogen concentrations in postmenopausal women in order to identify how baseline oestrogen concentrations affect side effect incidence, (3) exploration of the link between mammographic density and side effects of endocrine therapy including whether mammographic density is related to sex hormone concentrations, (4) further development and validation of the prediction models developed in chapter 7 with the ultimate aim of incorporating it into established breast cancer risk models. The following sections 9.2.1 - 9.2.4 describe in more detail what could be done in each case.

9.2.1 Investigation of genetic features

The analysis performed in this thesis suggested that few SNPs have an association with side effect risk and that observed associations were weak. However, the analysis performed was in a small data set and thus results need to be confirmed in a larger independent data set. Using the results of the analysis performed to target genes would reduce the number of statistical tests required. This may lead to better identification of SNPs associated with side effects.

In the current analysis, SNP data was only available for women from the IBIS-I trial; therefore, extending this analysis to the IBIS-II trial is necessary. Using data from the IBIS-I trial has enabled the formation of an 88 SNP PRS for breast cancer and has provided some weak associations with side effects. Extending the SNP analysis to the IBIS-II trial would enable validation of the SNP breast cancer score, identify any SNPs associated with side effects and test the weak SNP associations observed in the current IBIS-I analysis. It may also allow for the identification of novel SNPs for both breast cancer and side effects.

No link was discovered between tamoxifen metabolism genes and side effect risk. However, there was no information about how SNPs in tamoxifen metabolism genes affect the concentration of tamoxifen metabolites, including endoxifen, in circulation. A combined analysis assessing SNPs in tamoxifen metabolism genes, the circulating con-

centration of endoxifen and side effects should be performed to investigate whether the metabolism of tamoxifen significantly affects the risk of side effects. If a link is established, this may have a significant impact on the type of endocrine therapy recommended for women carrying these known metabolism modifying SNPs.

Finally, investigations regarding epigenetic modifications should be performed. Epigenetics is often described as the missing link between the genome and the environment. An epigenetic analysis would allow for the investigation of how the environment can affect side effect risk. Establishing epigenetic mutations of genes may help to identify key pathways to side effects and to suggest possible lifestyle alteration through which side effects could be managed or their impact reduced.

9.2.2 Continuation of sex hormone study

The most pressing area for investigation after the completion of the sex hormone study is the analysis of baseline oestrogen concentrations and the association with early reported side effects in all samples previously tested for androgen and SHBG concentrations. It was disappointing that obtaining accurate measurements of oestrogen concentrations in the current analysis was not possible, but the results presented in chapter 6, highlight the need to perform further testing and to accurately measure oestrogen concentrations in these samples to properly assess the impact of oestrogens on side effects. Liquid-chromatography mass-spectrometry (LC-MS) should be used to measure concentrations of oestrogens in postmenopausal women as it is important to ensure that results are accurate and repeatable. Additionally, sex hormone concentration should be linked to genetic testing of the samples to explore whether those with high testosterone have lower oestrogen concentrations due to polymorphisms within the gene encoding aromatase. A combined genomic and sex hormone analysis could also be used to assess the impact of SNPs in genes encoding hormone metabolism enzymes to assess the impact of reduced metabolism and elimination of sex hormones.

In chapter 6, leptin was suggested as a potential mediation factor for the action of SHBG on side effects. Investigation of leptin concentrations should be performed to test this hypothesis. Therefore, LC-MS should be used to determine leptin concentration in all samples to adjudge whether this remains a likely pathway to side effects or whether it is oestrogen binding effects of SHBG which are responsible for side effect aetiology.

9.2.3 Mammographic density and the association with menopausal-like side effects during tamoxifen therapy for breast cancer risk reduction

Mammographic density (MD), has been well-established as a risk factor for breast cancer (McCormack et al., 2006). Studies show that breast cancer risk increases 3–5-fold for women whose breasts are >75% dense tissue compared to women with <10% density (Barlow et al., 2006; Boyd et al., 2007; Chen et al., 2006; Harvey et al., 2004). Therefore, MD has to been added to breast cancer prediction models to aid the accuracy of these models in assessing a woman's risk of breast cancer (Brentnall et al., 2019).

MD has been extensively investigated for the prediction of breast cancer risk and its inclusion in risk prediction models has improved sensitivity (Boyd et al., 2011; Duffy et al., 2018; Vachon et al., 2007). Nevertheless, the association between MD and the risk of endocrine related side effects, such as hot flushes or gynaecological symptoms has not been investigated.

Establishing whether there is a link between MD and side effects and whether there is an association between MD and sex hormone concentrations should be a focus of investigation (Figure 9.1).

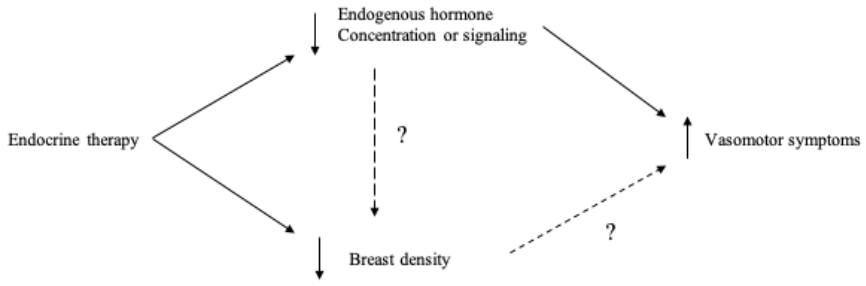


Figure 9.1: Proposed interaction of endocrine therapy, sex hormones and mammographic density in relation to vasomotor incidence in women taking tamoxifen for the prevention of breast cancer

The primary objective would be to investigate whether MD at baseline predicts side effects during the first 6 months in women randomised to tamoxifen or placebo in the IBIS-I study or anastrozole and placebo in the IBIS-II study. MD could be used as a continuous and a categorical variable with cut-offs at 0, 1-10, 11-25, 26-50, 51-75, and 76-100% to reflect the groupings used in previous studies (Cuzick et al., 2011).

MD is likely confounded by BMI, age, and possibly other risk factors (Boyd et al., 2006; Checka et al., 2012). Thus, to assess a woman's risk of developing side effects in association with her MD, it will be necessary to take into account these risk factors (Baglietto et al., 2014; Warwick et al., 2014).

A secondary objective should be the investigation of whether serum concentrations of testosterone, dehydroepiandrosterone – sulphate (DHEAS) and sex hormone binding globulin (SHBG) are associated with %MD at baseline.

9.2.4 Development and evaluation of side effect prediction models

The side effect prediction models designed in chapter 7 are the first step in developing risk models for the prediction of side effects. The current models provide a foundation for further development through the inclusion of new risk factors, such as mammographic density, genetic modifications or epigenetic modifications. If accurate and reliable models can be formed there is an opportunity to give women important information regarding their side effect risk and the potential to increase uptake and adherence to endocrine therapy. Once an accurate prediction of incidence can be achieved it may then be possible to develop risk models to determine the severity of side effects; however, this will require larger data sets and a consistent measure of side effect severity to train models. To refine and develop these models, new independent data sets are required for testing and exploration of potential new risk factors.

9.2.5 Implications of using data from RCTs and the need for real-world evidence

RCTs are considered the gold standard in trial design and are also the most stringent way of determining whether a cause-effect relation exists between the intervention and the outcome. However, results may not always mimic real-life treatment situation as women enrolled on these trials must meet highly selective inclusion and exclusion criteria consequently result from RCTs may lack generalisability.

For this reason, concurrent execution of real-world evidence (RWE), in addition to RCT, needs to be established; and should ideally be developed in unity with RCTs rather than competing relationship. For example, if an RCT focuses on the efficacy

of a drug, the RWE can focus on the epidemiology, effectiveness, safety, or costs of treatment related to the drug. A retrospective chart review across multiple UK hospitals would provide information regarding patient characteristics for those who chose endocrine therapy, and would give details regarding the type and number of side effects, particularly if these led to discontinuation of therapy.

9.3 Impact of research

The findings in this thesis have potential to impact a number of settings that may improve breast cancer prevention. The main impact of side effects is that reduced quality-of-life means that relatively few women choose preventive therapy, so prevention is an often-missed opportunity to make an impact and reduce the number of breast cancer diagnoses. The identification and increased understanding of risk factors which can be used to predict particular side effects and the production of a risk model for side effects that can be used when discussing potential prevention routes with women at high risk of breast cancer could have a wide-ranging impact:

(1) Women at high risk of breast cancer - To guide chemoprevention options for women at high risk of breast cancer with an easily understandable measure of side effect risk
The predicted risks helping clinicians and women at high-risk of breast cancer take informed decisions when faced with the options of risk reducing chemoprevention. Women classified as high-risk of developing breast cancer offered chemoprevention with tamoxifen or aromatase inhibitors could be informed of how likely they are to develop particular side effects.

Optimising risk factors and a prediction model could help to identify those who are at high-risk of side effects and therefore those who may not adhere to a full course of endocrine therapy. This could lead to a more personalised approach to endocrine therapy to manage side effects and ensure a balance between quality-of-life and breast cancer prevention strategies. Information regarding their risk of breast cancer could also be useful to help women make more informed lifestyle choices, such as diet, exercise and smoking, that could reduce the risks of developing side effects.

When meeting their clinician, women could engage with the predictive tool, either through a clinician led programme or an app that women can use to assess their risk

of side effects and monitor side effects whilst taking endocrine therapy. The success of this will be determined by the production of a well-designed, accurate and intuitive assessment for side effects. It is of utmost importance that at a daunting and stressful time women have an easy to use and understandable tool to assess their side effect risks and to aid the decision-making process about the best preventive therapy strategy.

(2) Clinicians - Change in clinical practice and policy to improve quality of patient care
Primary care physicians rarely prescribe prevention therapies with analysis suggesting that underuse of these agents is correlated to development of adverse events. Effectively defining side effect risk could aid the selection of the best preventive agent by balancing personalised side effect risk with the prevention benefit afforded by the therapy of choice, thereby optimising the treatment choice for women by considering multiple drugs in accordance with their individual side effect profile. Reducing these medication side effects could increase the acceptance of prevention medication by both patients and physicians.

Rather than viewing side effects as problems they should be seen as a challenge that can be overcome. To do this effectively clinicians need to play a strong role. Engaging clinicians is vitally important as they are the link between assessment of side effects and the patients themselves; therefore, clinicians must be involved from the start of planning on how to bring the side effect assessment tools to the attention of women. Through involvement of clinicians and the use of their data to see how addressing side effect risk could alter patients' decision outcomes, it would be hoped that clinicians can become partners rather than customers in the delivery of an application that helps to achieve a common purpose.

(3) Social and economic benefits - Societal change through understanding of side effects and at-risk populations and economic benefit via a reduction in breast cancer cases
The identification of side effect risk factors and the production of a prediction tool raises two more general benefits: firstly, the more women who take prevention therapies the more "acceptable" these therapies become. Secondly, if the number of breast cancer cases reduces this alleviates some strain on health care services.

By supporting women at the start of endocrine therapy via discussions to enable a proper assessment of side effect risk the hope would be an increased number of women choosing preventive endocrine therapy with less concerns about the risks involved. An

increased uptake of endocrine therapy improves its perception as a preventive measure and therefore more women may choose to take endocrine therapy. With the established preventive effects of endocrine therapy, increased uptake could lead to women being able to stay in the workplace, and hence remain economically active, and stay out of hospitals and other centres required for cancer treatment. This provides more time to focus on those that need further intervention or those for whom endocrine therapy has been unsuccessful. A health economics analysis would prove beneficial in this case to assess how well this intervention works.

To conclude, this thesis found multiple risk factors associated with side effects and which could be combined to form a risk prediction tool to identify women at high-risk of side effects when starting preventive endocrine therapy. These risk factors and prediction tools could be used to personalise prevention therapies ensuring a balance between the risks and benefits of endocrine therapy; however, further work is required to make accurate prediction possible. Future work must explore ways in which the findings stemming from this project can be communicated to health care professionals and to other public and patient groups is key to ensuring that women who can benefit from breast cancer prevention therapies have access to as much information as possible when making life changing decisions.

Bibliography

Abdi, F., Mobedi, H., Bayat, F., Mosaffa, N., Dolatian, M., and Tehrani, F. R. (2017). The effects of transdermal estrogen delivery on bone mineral density in postmenopausal women: A meta-analysis. *Iranian Journal of Pharmaceutical Research*, 16(1):380–389.

Abdolell, M., Tsuruda, K. M., Lightfoot, C. B., Payne, J. I., Caines, J. S., and Iles, S. E. (2016). Utility of relative and absolute measures of mammographic density vs clinical risk factors in evaluating breast cancer risk at time of screening mammography. *British Journal of Radiology*, 89(1059):20150522.

Adly, L., Hill, D., Sherman, M. E., Sturgeon, S. R., Fears, T., Mies, C., Ziegler, R. G., Hoover, R. N., and Schairer, C. (2006). Serum concentrations of estrogens, sex hormone-binding globulin, and androgens and risk of breast cancer in postmenopausal women. *International Journal of Cancer*, 119(10):2402–2407.

Ahmad, L. G., Eshlaghy, A. T., Poorebrahimi, A., Ebrahimi, M., and Ravavi, A. R. (2013). Using three machine learning techniques for predicting breast cancer recurrence. *Journal of Health and Medical Informatics*, 4(2):2–4.

Ahmed, M. and Gaffen, S. L. (2010). IL17 in obesity and adipogenesis. *Cytokine Growth Factor Review*, 21(6):449–453.

Akobeng, A. K. (2008). Assessing the validity of clinical trials. *Journal of Pediatric Gastroenterology and Nutrition*, 47(3):277–282.

Albagha, O. M., Pettersson, U., Stewart, A., McGuigan, F. E., MacDonald, H. M., Reid, D. M., and Ralston, S. H. (2005). Association of oestrogen receptor α gene polymorphisms with postmenopausal bone loss, bone mass, and quantitative ultrasound properties of bone. *Journal of Medical Genetics*, 42(3):240–246.

Alexander, C., Cochran, C. J., Gallicchio, L., Miller, S. R., Flaws, J. A., and Zacur, H. (2010). Serum leptin levels, hormone levels, and hot flashes in midlife women. *Fertility and Sterility*, 94(3):1037–1043.

Ali, S., Mondal, N., Choudhry, H., Rasool, M., Pushparaj, P. N., Khan, M. A., Mahfooz, M., Sami, G. A., Jarullah, J., Ali, A., and Jamal, M. S. (2016). Current management strategies in breast cancer by targeting key altered molecular players. *Frontiers in Oncology*, 6(45).

Alowami, S., Troup, S., Al-Haddad, S., Kirkpatrick, I., and Watson, P. H. (2003). Mammographic density is related to stroma and stromal proteoglycan expression. *Breast Cancer Research : BCR*, 5(5):129–135.

Altshuler, D., Hirschhorn, J. N., Klannemark, M., Lindgren, C. M., Vohl, M. C., Nemesh, J., Lane, C. R., Schaffner, S. F., Bolk, S., Brewer, C., Tuomi, T., Gaudet, D., Hudson, T. J., Daly, M., Groop, L., and Lander, E. S. (2000). The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nature Genetics*, 26(1):76–80.

Amos, C. I., Dennis, J., Wang, Z., Byun, J., Schumacher, F. R., Gayther, S. A., Casey, G., Hunter, D. J., Sellers, T. A., Gruber, S. B., Dunning, A. M., Michailidou, K., Fachal, L., Doheny, K., Spurdle, A. B., Li, Y., Xiao, X., Romm, J., Pugh, E., ..., and Easton, D. F. (2017). The oncoarray consortium: A network for understanding the genetic architecture of common cancers. *Cancer Epidemiology Biomarkers and Prevention*, 26(1):126–135.

Amundadottir, L., Kraft, P., Stolzenberg-Solomon, R. Z., Charles, S., Petersen, G. M., Arslan, A. A., Bueno-de Mesquita, H. B., Helzlsouer, K., Jacobs, E. J., Lacroix, A., Zheng, W., González, C. A., Hallmans, G., and Hankinson, S. E. (2010). Locus associated with susceptibility to pancreatic cancer. *Nature Genetics*, 41(9):986–990.

Anderson, C. A., Pettersson, F. H., Clarke, G. M., Cardon, L. R., Morris, A. P., and Zondervan, K. T. (2010). Data quality control in genetic case-control association studies. *Nature Protocols*, 5(9):1564–1573.

Anderson, G. L. and Neuhouser, M. L. (2012). Obesity and the risk for premenopausal and postmenopausal breast cancer. *Cancer Prevention Research*, 5(4):515–521.

Andrews, P., Steultjens, M., and Riskowski, J. (2018). Chronic widespread pain prevalence in the general population: A systematic review. *European Journal of Pain*, 22((2018)):5–18.

Anghel, L. A., Farcas, A. M., and Oprean, R. N. (2019). An overview of the common methods used to measure treatment adherence. *Medicine and Pharmacy Reports*, 92(2):117–122.

Antoniou, A. C., Cunningham, A. P., Peto, J., Evans, D. G., Lalloo, F., Narod, S. A., Risch, H. A., Eyfjord, J. E., Hopper, J. L., Southey, M. C., Olsson, H., Johannsson, O., Borg, A., Passini, B., Radice, P., Manoukian, S., Eccles, D. M., Tang, N., Olah, E., ..., and Easton, D. F. (2008). The BOADICEA model of genetic susceptibility to breast and ovarian cancers: Updates and extensions. *British Journal of Cancer*, 98(8):1457–1466.

Antoniou, A. C. and Easton, D. F. (2006). Models of genetic susceptibility to breast cancer. *Oncogene*, 25(43):5898–5905.

Arizanović, Z., Vujović, S., Ivović, M., Tančić-Gajić, M., Marina, L., Stojanović, M., and Micić, D. (2018). Hot flush values of gonadotropins and estradiol in the menopause. *Serbian Archives of Medicine*, pages 1–8.

Astley, S. M., Harkness, E. F., Sergeant, J. C., Warwick, J., Stavrinos, P., Warren, R., Wilson, M., Beetles, U., Gadde, S., Lim, Y., Jain, A., Bundred, S., Barr, N., Reece, V., Brentnall, A. R., Cuzick, J., Howell, T., and Evans, D. G. (2018). A comparison of five methods of measuring mammographic density: A case-control study. *Breast Cancer Research*, 20(1):1–13.

ATAC Trialists' Group (2001). Pharmacokinetics of anastrozole and tamoxifen alone, and in combination, during adjuvant endocrine therapy for early breast cancer in postmenopausal women: a sub-protocol of the Arimidex and Tamoxifen Alone or in Combination (ATAC) trial. *British journal of cancer*, 85(3):317–324.

Ayer, T., Chhatwal, J., Alagoz, O., Kahn, C. E., Woods, R. W., and Burnside, E. S. (2010). Informatics in radiology: Comparison of logistic regression and artificial neural network models in breast cancer risk estimation. *Radiographics*, 30(1):13–22.

Bachmann, G. A. (1999). Vasomotor flushes in menopausal women. *American Journal of Obstetrics and Gynecology*, 180(3):S312–316.

Baglietto, L., English, D. R., Hopper, J. L., MacInnis, R. J., Morris, H. A., Tilley, W. D., Krishnan, K., and Giles, G. G. (2009). Circulating steroid hormone concentrations in postmenopausal women in relation to body size and composition. *Breast Cancer Research and Treatment*, 115(1):171–179.

Baglietto, L., Krishnan, K., Stone, J., Apicella, C., Southey, M. C., English, D. R., Hopper, J. L., and Giles, G. G. (2014). Associations of mammographic dense and nondense areas and body mass index with risk of breast cancer. *American Journal of Epidemiology*, 179(4):475–483.

Bakkum-Gamez, J. N., Laughlin, S. K., Jensen, J. R., Akogyeram, C. O., and Pruthi, S. (2011). Challenges in the gynecologic care of premenopausal women with breast cancer. *Mayo Clinic Proceedings*, 86(3):229–240.

Baldassarre, M., Perrone, A. M., Giannone, F. A., Armillotta, F., Battaglia, C., Costantino, A., Venturoli, S., and Meriggiola, M. C. (2013). Androgen receptor expression in the human vagina under different physiological and treatment conditions. *International Journal of Impotence Research*, 25(1):7–11.

Balding, D. J. (2006). A tutorial on statistical methods for population association studies. *Nature Reviews Genetics*, 7(10):781–791.

Bandera, E. V., Chandran, U., Zirpoli, G., Ciupak, G., Bovbjerg, D. H., Jandorf, L., Pawlish, K., Freudenheim, J. L., and Ambrosone, C. B. (2013). Body size in early life and breast cancer risk in African American and European American women. *Cancer causes & control*, 24(12):2231–2243.

Barber, T. M., McCarthy, M. I., Wass, J. A., and Franks, S. (2006). Obesity and polycystic ovary syndrome. *Clinical Endocrinology*, 65(2):137–145.

Barcellos-Hoff, M. H. and Medina, D. (2005). New highlights on stroma-epithelial interactions in breast cancer. *Breast Cancer Research*, 7(1):33–36.

Barlow, W. E., White, E., Ballard-Barbash, R., Vacek, P. M., Titus-Ernstoff, L., Carney, P. A., Tice, J. A., Buist, D. S., Geller, B. M., Rosenberg, R., Yankaskas, B. C., and Kerlikowske, K. (2006). Prospective breast cancer risk prediction model for women undergoing screening mammography. *Journal of the National Cancer Institute*, 98(17):1204–1214.

Baron, J. A., Comis, R. L., Cryns, V., Brinck-Johnson, T., and Mercer, N. G. (1995). The effect of cigarette smoking on Adrenal Cortical Hormones. *The Journal of Pharmacology and Experimental therapeutics*, 272(1):151–155.

Baron, J. A., Vecchia, C. L., and Levi, F. (1990). The antiestrogenic effect of cigarette smoking in women. *American Journal of Obstetrics and Gynecology*, 162(2):502–514.

Barrett-Connor, E., Mosca, L., Collins, P., Geiger, M. J., Grady, D., Kornitzer, M., McNabb, M. A., and Wenger, N. K. (2006). Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. *New England Journal of Medicine*, 355(2):125–137.

Barton, D. L., Loprinzi, C., Atherton, P. J., Kottschade, L., Collins, M., Carpenter, P., Adjei, A., Rummans, T. A., Shanafelt, T., Christensen, B., and Sloan, J. (2006). Dehydroepiandrosterone for the treatment of hot flashes: A pilot study. *Supportive Cancer Therapy*, 3(2):91–97.

Basaria, S. (2015). Testosterone levels for evaluation of androgen deficiency. *JAMA - Journal of the American Medical Association*, 313(17):1749–1750.

Baum, M. and Houghton, J. (1999). Contribution of randomised controlled trials to understanding and management of early breast cancer. *British Medical Journal*, 319(7209):568–571.

Baxter, S. D., Teft, W. A., Choi, Y. H., Winquist, E., and Kim, R. B. (2014). Tamoxifen-associated hot flash severity is inversely correlated with endoxifen concentration and CYP3A422. *Breast Cancer Research and Treatment*, 145(2):419–428.

Bayat, B., Werth, S., Sachs, U. J., Newman, Debra, K., Newman, P. J., and Santoso, S. (2010). Neutrophil transmigration mediated by the neutrophil-specific antigen CD177 Is influenced by the endothelial S536 N dimorphism of platelet endothelial cell adhesion molecule-1. *Journal of Immunology*, 184(7):3889–3896.

Beckwée, D., Leysen, L., Meuwis, K., and Adriaenssens, N. (2017). Prevalence of aromatase inhibitor-induced arthralgia in breast cancer: a systematic review and meta-analysis. *Supportive Care in Cancer*, 25(5):1673–1686.

Bengio, Y. and Grandvalet, Y. (2004). No unbiased estimator of the variance of K-fold cross-validation. *Journal of Mechine Learning Research*, 5:1089–1105.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate : A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodolgical)*, 57(1):289–300.

Beral, V., Reeves, G., Bull, D., and Green, J. (2011). Breast cancer risk in relation to the interval between menopause and starting hormone therapy. *Journal of the National Cancer Institute*, 103(4):296–305.

Bertrand, K. A., Eliassen, A. H., Hankinson, S. E., Rosner, B. A., and Tamimi, R. M. (2018). Circulating hormones and mammographic density in premenopausal women. *Hormones and Cancer*, 9(2):117–127.

Bewick, V., Cheek, L., and Ball, J. (2005). Statistics review 14: Logistic regression. *Critical Care*, 9(1):112–118.

Bjørnerem, Å., Straume, B., Midtby, M., Fønnebø, V., Sundsfjord, J., Svartberg, J., Acharya, G., Øian, P., and Berntsen, G. K. (2004). Endogenous sex hormones in relation to age, sex, lifestyle factors, and chronic diseases in a general population: The Tromsø study. *Journal of Clinical Endocrinology and Metabolism*, 89(12):6039–6047.

Bland, J. M. and Altman, D. G. (1996). Transforming data. *British Medical Journal*, 312:770.

Bleyer, A. and Welch, H. G. (2012). Effect of three decades of screening mammography on breast-cancer incidence. *New England Journal of Medicine*, 367(21):1998–2005.

Boccardo, F., Rubagotti, A., Puntoni, M., Guglielmini, P., Amoroso, D., Fini, A., Paladini, G., Mesiti, M., Romeo, D., Rinaldini, M., Scali, S., Porpiglia, M., Benedetto, C., Restuccia, N., Buzzi, F., Franchi, R., Massidda, B., Distante, V., Amadori, D., and Sismondi, P. (2005). Switching to anastrozole versus continued tamoxifen treatment of early breast cancer: Preliminary results of the Italian Tamoxifen Anastrozole Trial. *Journal of Clinical Oncology*, 23(22):5138–5147.

Bodicoat, D. H., Schoemaker, M. J., Jones, M. E., McFadden, E., Griffin, J., Ashworth, A., and Swerdlow, A. J. (2014). Timing of pubertal stages and breast cancer risk: The Breakthrough Generations Study. *Breast Cancer Research*, 16(1):R18.

Boyd, N., Martin, L., Stone, J., Little, L., Minkin, S., and Yaffe, M. (2002a). A longitudinal study of the effects of menopause on mammographic features. *Cancer Epidemiology Biomarkers and Prevention*, 11(10):1048–1053.

Boyd, N. F., Byng, J. W., Jong, R. A., Fishell, E. K., Little, L. E., Miller, A. B., Lockwood, G. A., Tritchler, D. L., and Yaffe, M. J. (1995). Quantitative classification of mammographic densities and breast cancer risk: Results from the canadian national breast screening study. *Journal of the National Cancer Institute*, 87(9):670–675.

Boyd, N. F., Dite, G. S., Stone, J., Gunasekara, A., English, D. R., McCredie, M., Giles, G. G., Tritchler, D. L., Chiarelli, A. M., Yaffe, M. J., and Hopper, J. L. (2002b).

Heritiability of mammographic density, a risk factor for breast cancer. *The New England Journal of Medicine*, 347(12):886–894.

Boyd, N. F., Guo, H., Martin, L. J., Sun, L., Stone, J., Fishell, E., Jong, R. A., Hislop, G., Chiarelli, A., Minkin, S., and Yaffe, M. J. (2007). Mammographic density and the risk and detection of breast cancer. *The New England Journal of Medicine*, 356:227–236.

Boyd, N. F., Jensen, H. M., Cooke, G., and Han, H. L. (1992). Relationship between mammographic and histological risk factors for breast cancer. *Journal of the National Cancer Institute*, 84(15):1170–1179.

Boyd, N. F., Lockwood, G. A., Byng, J. W., Tritchler, D. L., and Yaffe, M. J. (1998). Mammographic densities and breast cancer risk. *Cancer Epidemiology Biomarkers and Prevention*, 7(12):1133–1144.

Boyd, N. F., Martin, L. J., Sun, L., Guo, H., Chiarelli, A., Hislop, G., Yaffe, M., and Minkin, S. (2006). Body size, mammographic density, and breast cancer risk. *Cancer Epidemiology Biomarkers and Prevention*, 15(11):2086–2092.

Boyd, N. F., Martin, L. J., Yaff, M. J., and Minkin, S. (2011). Mammographic density and breast cancer risk: current understanding and future prospects. *Breast Cancer Research*, 13(223).

Boyd, N. F., Stone, J., Vogt, K. N., Connelly, B. S., Martin, L. J., and Minkin, S. (2003). Dietary fat and breast cancer risk revisited: A meta-analysis of the published literature. *British Journal of Cancer*, 89(9):1672–1685.

Boyle, W. J., Simonet, W. S., and Lacey, D. L. (2003). Osteoclast differentiation and activation. *Nature*, 423(5):337–342.

Bradburn, M. J., Clark, T. G., Love, S. B., and Altman, D. G. (2003). Survival Analysis Part II: Multivariate data analysis – an introduction to concepts and methods. *British Journal of Cancer*, 89(3):431–436.

Brand, J. S., Humphreys, K., Li, J., Karlsson, R., Hall, P., and Czene, K. (2018). Common genetic variation and novel loci associated with volumetric mammographic density. *Breast Cancer Research*, 20:30.

Brandt, K. R., Scott, C. G., Miglioretti, D. L., Jensen, M. R., Mahmoudzadeh, A. P., Hruska, C., Ma, L., Wu, F. F., Cummings, S. R., Norman, A. D., Engmann, N. J.,

Shepherd, J. A., Winham, S. J., Kerlikowske, K., and Vachon, C. M. (2019). Automated volumetric breast density measures: Differential change between breasts in women with and without breast cancer. *Breast Cancer Research*, 21:118.

Brauch, H., Mürdter, T. E., Eichelbaum, M., and Schwab, M. (2009). Pharmacogenomics of tamoxifen therapy. *Clinical Chemistry*, 55(10):1770–1782.

Bray, F., Ferlay, J., Laversanne, M., Brewster, D. H., Gombe Mbalawa, C., Kohler, B., Piñeros, M., Steliarova-Foucher, E., Swaminathan, R., Antoni, S., Soerjomataram, I., and Forman, D. (2015). Cancer incidence in five continents: Inclusion criteria, highlights from Volume X and the global status of cancer registration. *International Journal of Cancer*, 137(9):2060–2071.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 68:394–424.

Bray, F., McCarron, P., and Parkin, D. M. (2004). The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Research*, 6(6):229–239.

Breast Cancer Now (2018). Breast Cancer Now.

Brentnall, A. R., Cohn, W. F., Knaus, W. A., Yaffe, M. J., Cuzick, J., and Harvey, J. A. (2019). A case-control study to add volumetric or clinical mammographic density into the Tyrer-Cuzick breast cancer risk model. *Journal of Breast Imaging*, 1(2):99–106.

Brentnall, A. R., Harkness, E. F., Astley, S. M., Donnelly, L. S., Stavrinos, P., Sampson, S., Fox, L., Sergeant, J. C., Harvie, M. N., Wilson, M., Beetles, U., Gadde, S., Lim, Y., Jain, A., Bundred, S., Barr, N., Reece, V., Howell, A., Cuzick, J., and Evans, D. G. R. (2015). Mammographic density adds accuracy to both the Tyrer-Cuzick and Gail breast cancer risk models in a prospective UK screening cohort. *Breast Cancer Research*, 17:147.

Brett, J., Fenlon, D., Boulton, M., Hulbert-Williams, N. J., Walter, F. M., Donnelly, P., Lavery, B., Morgan, A., Morris, C., and Watson, E. (2018). Factors associated with intentional and unintentional non-adherence to adjuvant endocrine therapy following breast cancer. *European Journal of Cancer Care*, 27(1).

Brewer, H. R., Jones, M. E., Schoemaker, M. J., Ashworth, A., and Swerdlow, A. J. (2017). Family history and risk of breast cancer: an analysis accounting for family structure. *Breast Cancer Research and Treatment*, 165(1):193–200.

Bromley, S. E., De Vries, C. S., and Farmer, R. D. (2004). Utilisation of hormone replacement therapy in the United Kingdom. A descriptive study using the general practice research database. *BJOG: An International Journal of Obstetrics and Gynaecology*, 111(4):369–376.

Brzozowski, A. M. and Pike, A. C. W. (1997). Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature*, 389(16):753–758.

Buell, P. (1973). Changing incidence of breast cancer in japanese-american women. *Journal of the National Cancer Institute*, 51(5):1479–1483.

Buniello, A., Macarthur, J. A., Cerezo, M., Harris, L. W., Hayhurst, J., Malangone, C., McMahon, A., Morales, J., Mountjoy, E., Sollis, E., Suveges, D., Vrousgou, O., Whetzel, P. L., Amode, R., Guillen, J. A., Riat, H. S., Trevanion, S. J., Hall, P., Junkins, H., ..., and Parkinson, H. (2019). The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Research*, 47(D1):D1005–D1012.

Burger, H. G. (2006). Physiology and endocrinology of the menopause. *Medicine*, 34(1):27–30.

Burger, H. G., Dudley, E. C., Cui, J., Dennerstein, L., and Hopper, J. L. (2000). A prospective longitudinal study of serum testosterone, dehydroepiandrosterone sulfate, and sex hormone-binding globulin levels through the menopause transition. *Journal of Clinical Endocrinology and Metabolism*, 85(8):2832–2838.

Burri, A., Hysi, P., Clop, A., Rahman, Q., and Spector, T. D. (2012). A Genome-Wide Association Study of female sexual dysfunction. *PLoS ONE*, 7(4):e35041.

Burton, A., Maskarinec, G., Perez-Gomez, B., Vachon, C., Miao, H., Lajous, M., López-Ridaura, R., Rice, M., Pereira, A., Garmendia, M. L., Tamimi, R. M., Bertrand, K., Kwong, A., Ursin, G., Lee, E., Qureshi, S. A., Ma, H., Vinnicombe, S., Moss, S., ..., and McCormack, V. (2017). Mammographic density and ageing: A collaborative pooled analysis of cross-sectional data from 22 countries worldwide. *PLoS Medicine*, 14(6):e1002335.

Bush, W. S. and Moore, J. H. (2012). Genome-Wide Association Studies. *PLoS Computational Biology*, 8(12):e1002822.

Butler, L. M., Gold, E. B., Greendale, G. A., Crandall, C. J., Oestreicher, N., Jr, C. P. Q., and Habel, L. A. (2008). Menstrual and reproductive factors in relation to mammographic density: the Study of Women's Health Across the Nation (SWAN). *Breast Cancer Research and Treatment*, 112(1):165–174.

Buzdar, A., Howell, A., Cuzick, J., Wale, C., Distler, W., Hoctin-Boes, G., Houghton, J., Locker, G. Y., and Nabholtz, J. M. (2006). Comprehensive side-effect profile of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: long-term safety analysis of the ATAC trial. *Lancet Oncology*, 7(8):633–643.

Buzdar, A. U., Robertson, J. F., Eiermann, W., and Nabholtz, J. M. (2002). An overview of the pharmacology and pharmacokinetics of the newer generation aromatase inhibitors anastrozole, letrozole, and exemestane. *Cancer*, 95(9):2006–2016.

Byrne, C., Schairer, C., Wolfe, J., Parekh, N., Salane, M., Brinton, L. A., Hoover, R., and Haile, R. (1995). Mammographic features and breast cancer risk: Effects with time, age, and menopause status. *Journal of the National Cancer Institute*, 87(21):1622–1629.

Cai, Q., Gao, Y. T., Wen, W., Shu, X. O., Jin, F., Smith, J. R., and Zheng, W. (2003a). Association of breast cancer risk with a GT dinucleotide repeat polymorphism upstream of the estrogen receptor-α gene. *Cancer Research*, 63(18):5727–5730.

Cai, Q., Shu, X. O., Jin, F., Dai, Q., Wen, W., Cheng, J. R., Gao, Y. T., and Zheng, W. (2003b). Genetic polymorphisms in the estrogen receptor α gene and risk of breast cancer: Results from the Shanghai breast cancer study. *Cancer Epidemiology Biomarkers and Prevention*, 12(9):853–859.

Cakmak, A., Posaci, C., Dogan, E., Caliskan, S., Guclu, S., and Altunyurt, S. (2005). Raloxifene increases serum leptin levels in postmenopausal women: A prospective study. *American Journal of Obstetrics and Gynecology*, 193(2):347–351.

Cancer Research UK (2019a). Breast Cancer Incidence. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/incidence-invasivecollapseOne.

Cancer Research UK (2019b). Breast Cancer Survival. Available from:

https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/survival.

Cancer Research UK (2020). Cancer Research UK Breast Cancer Statisitcs. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer.

Carroll, K. K. (1998). Obesity as a risk factor for certain types of cancer. *Lipids*, 33(11):1055–1059.

Carson, D. D., DeSouza, M. M., Kardon, R., Zhou, X., Lagow, E., and Julian, J. A. (1998). Mucin expression and function in the female reproductive tract. *Human Reproduction Update*, 4(5):459–464.

Cauley, J. A., Gutai, J. P., Kuller, L. H., Ledonne, D., and Powell, J. G. (1989). The epidemiology of serum sex hormones in postmenopausal women. *American Journal of Epidemiology*, 129(6):1120–1131.

Cella, D., Fallowfield, L., Barker, P., Cuzick, J., Locker, G., Howell, A., and On behalf of the ATAC Trialists' Group (2006). Quality of life of postmenopausal women in the ATAC ("Arimidex", tamoxifen, alone or in combination) trial after completion of 5 years' adjuvant treatment for early breast cancer. *Breast Cancer Research and Treatment*, 100(3):273–284.

Cella, D. and Fallowfield, L. J. (2008). Recognition and management of treatment-related side effects for breast cancer patients receiving adjuvant endocrine therapy. *Breast Cancer Research and Treatment*, 107(2):167–180.

Chan, M. F., Dowsett, M., Folkerd, E., Wareham, N., Luben, R., Welch, A., Bingham, S., and Khaw, K. T. (2008). Past oral contraceptive and hormone therapy use and endogenous hormone concentrations in postmenopausal women. *Menopause*, 15(2):332–339.

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., and Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience*, 4:7.

Chang, H.-Y., Jotwanic, A. C., Lai, Y.-H., Jensend, M. P., Syrjalae, K. L., Fannf, J. R., and Gralow, J. (2016). Hot flashes in breast cancer survivors: Frequency, severity and impact. *Breast*, 27(6):116–121.

Chang, M. (2012). Tamoxifen resistance in breast cancer. *Biomolecules and Therapeutics*, 20(3):256–267.

Chapman, J.-A., Shepherd, L., Maitre, A. L., Pritchard, K., Graham, B., Gelmon, K., and Bramwell, V. (2011). Effect of treatment emergent symptoms on relapse free survival: NCIC CTG MA.12 a randomized placebo-controlled trial of tamoxifen after adjuvant chemotherapy in pre-menopausal women in early breast cancer. In *Cancer Research*.

Checka, C. M., Chun, J. E., Schnabel, F. R., Lee, J., and Toth, H. (2012). The relationship of mammographic density and age: Implications for breast cancer screening. *American Journal of Roentgenology*, 198(3):292–295.

Cheddad, A., Czene, K., Shepherd, J. A., Li, J., Hall, P., and Humphreys, K. (2014). Enhancement of mammographic density measures in breast cancer risk prediction. *Cancer Epidemiology Biomarkers and Prevention*, 23(7):1314–1323.

Chen, J., Pee, D., Ayyagari, R., Graubard, B., Schairer, C., Byrne, C., Benichou, J., and Gail, M. H. (2006). Projecting absolute invasive breast cancer risk in white women with a model that includes mammographic density. *Journal of the National Cancer Institute*, 98(17):1215–1226.

Chen, J.-H., Chang, Y.-C., Chang, D., Wang, Y.-T., Nie, K., Chang, R.-F., Nalcioglu, O., Huang, C.-S., and Su, M.-Y. (2011). Reduction of breast density following tamoxifen treatment evaluated by 3-D MRI : preliminary study. *Magnetic Resonance Imaging*, 29(1):91–98.

Chlebowski, R. T., Cirillo, D. J., Eaton, C. B., Stefanick, M. L., Pettinger, M., Carbone, L. D., Johnson, K. C., Simon, M. S., Woods, N. F., and Wactawski-Wende, J. (2018). Estrogen alone and joint symptoms in the Women's Health Initiative randomized trial. *Menopause*, 25(11):1313–1320.

Chlebowski, R. T., Hendrix, S. L., Langer, R. D., Stefanick, M. L., Gass, M., Lane, D., Rodabough, R. J., Gilligan, M. A., Cyr, M. G., Thomson, C. A., Khandekar, J., Petrovitch, H., McTiernan, A., and for the WHI Investigators (2003). Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: The Women's Health Initiative randomized trial. *Journal of the American Medical Association*, 289(24):3243–3253.

Chlebowski, R. T., Mortimer, J. E., Crandall, C. J., Pan, K., Manson, J. A. E., Nelson, R., Johnson, K. C., Vitolin, M. Z., Lane, D., Wactawski-Wende, J., Kwan, K., and Stefanick, M. L. (2019). Persistent vasomotor symptoms and breast cancer in the Women's Health Initiative. *Menopause*, 26(6):578–587.

Chow, C. K., Venzon, D., Jones, E. C., Premkumar, A., O'Shaughnessy, J., and Zujewski, J. (2000). Effect of tamoxifen on mammographic density. *Cancer Epidemiology Biomarkers and Prevention*, 9(9):917–921.

Clark, T. G., Bradburn, M. J., Love, S. B., and Altman, D. G. (2003). Survival Analysis Part I: Basic concepts and first analyses. *British Journal of Cancer*, 89(2):232–238.

Clarke, B. L. and Khosla, S. (2009). Androgens and bone. *Steroids*, 74(3):296–305.

Clarke, G. M., Anderson, C. a., Pettersson, F. H., Cardon, L. R., and Andrew, P. (2011). Basic statistical analysis in genetic case-control studies. *Nature protocols*, 6(2):121–133.

Cochran, C. J., Gallicchio, L., Miller, S. R., Zacur, H., and Flaws, J. A. (2008). Cigarette smoking, androgen levels, and hot flushes in midlife women. *Obstet gynecol*, 112(5):1037–1044.

Colditz, G. A. and Bohlke, K. (2014). Priorities for the primary prevention of breast cancer. *CA: A Cancer Journal for Clinicians*, 64(3):186–194.

Colditz, G. A., Rosner, B. A., and Speizer, F. E. (1996). Risk factors for breast cancer according to family history of breast cancer. *Journal of the National Cancer Institute*, 88(6):365–371.

Collaborative Group on Hormonal Factors in Breast Cancer. (1996). Breast cancer and hormonal contraceptives: Collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet*, 347(9017):1713–1727.

Collaborative Group on Hormonal Factors in Breast Cancer (2001). Familial breast cancer- collaborative reanalysis of individual data from 52 epidemiological studies including 58 209 .pdf. *Lancet*, 358:1389–1399.

Collaborative Group on Hormonal Factors in Breast Cancer (2012). Menarche, menopause, and breast cancer risk: Individual participant meta-analysis, including 118

964 women with breast cancer from 117 epidemiological studies. *The Lancet Oncology*, 13(11):1141–1151.

Collaborative Group on Hormonal Factors in Breast Cancer, Group, C., and Cancer, B. (2019). Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence. *The Lancet*, 394(10204):1159–1168.

Colland, F., Jacq, X., Trouplin, V., Mougin, C., Groizeleau, C., Hamburger, A., Meil, A., Wojcik, J., Legrain, P., and Gauthier, J. M. (2004). Functional proteomics mapping of a human signaling pathway. *Genome Research*, 14(7):1324–1332.

Coombes, C., Hall, E., Gibson, L. J., Paridaens, R., Jassem, J., Delozier, T., Jones, S. E., Alvarez, I., Bertelli, G., Ortmann, O., Coates, A. S., Bajetta, E., Dodwell, D., Coleman, R. E., Fallowfield, L. J., Mickiewicz, E., Andersen, J., Lønning, P. E., Cocconi, G., ..., and Bliss, J. M. (2004). A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. *The New England Journal of Medicine*, 350(11):1081–1092.

Coombes, R., Kilburn, L., Snowdon, C., Paridaens, R., Coleman, R., Jones, S., Jassem, J., Van de Velde, C., Delozier, T., Alvarez, I., Del Mastro, L., Ortmann, O., Diedrich, K., Coates, A., Bajetta, E., Holmberg, S., Dodwell, D., Mickiewicz, E., Andersen, J., ..., and Bliss, J. (2007). Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial. *Lancet*, 369(9561):559–570.

Cooper, L. A., Page, S. T., Amory, J. K., Anawalt, B. D., and Matsumoto, M. (2015). The association of obesity with sex hormone-binding globulin is stronger than the association with ageing – implications for the interpretation of total testosterone measurements. *Clinical Endocrinology*, 83(6):828–833.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., and Pericak-Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*, 261(5123):921–923.

Cox, D. R. (1972). Regression models and life-tables. *Journal of the Royal Statistical Society. Series B (Methodolgical)*, 34(2):187–220.

Crandall, C. J., Diamant, A. L., Maglione, M., Thurston, R. C., and Sinsheimer, J. (2020). Genetic variation and hot flashes: A systematic review. *Journal of Clinical Endocrinology and Metabolism*, 105(12):e4907 – e4957.

Crandall, C. J., Guan, M., Laughlin, G. A., Ursin, G. A., Stanczyk, F. Z., Ingles, S. A., Barrett-Connor, E., and Greendale, G. A. (2008). Increases in serum estrone sulfate level are associated with increased mammographic density during menopausal hormone therapy. *Cancer Epidemiology Biomarkers and Prevention*, 17(7):1674–1681.

Crandall, C. J., Sehl, M. E., Crawford, S. L., Gold, E. B., Habel, L. A., Butler, L. M., Sowers, M. F. R., Greendale, G. A., and Sinsheimer, J. S. (2009). Sex steroid metabolism polymorphisms and mammographic density in pre- and early perimenopausal women. *Breast Cancer Research*, 11(4):R51.

Crew, K. D., Greenlee, H., Capodice, J., Raptis, G., Brafman, L., Fuentes, D., Sierra, A., and Hershman, D. L. (2007). Prevalence of joint symptoms in postmenopausal women taking aromatase inhibitors for early-stage breast cancer. *Journal of Clinical Oncology*, 25(25):3877–3883.

Cummings, S. R., Ensrud, K., Delmas, P. D., LaCroix, A. Z., Vukicevic, S., Reid, D. M., Goldstein, S., Sriram, U., Lee, A., Thompson, J., Armstrong, R. a., Thompson, D. D., Powles, T., Zanchetta, J., Kendler, D., Neven, P., and Eastell, R. (2010). Lasofoxifene in postmenopausal women with osteoporosis. *The New England Journal of Medicine*, 362(8):686–696.

Cummings, S. R., McClung, M., Reginster, J. Y., Cox, D., Mitlak, B., Stock, J., Amewou-Atisso, M., Powles, T., Miller, P., Zanchetta, J., and Christiansen, C. (2011). Arzoxifene for prevention of fractures and invasive breast cancer in postmenopausal women. *Journal of Bone and Mineral Research*, 26(2):397–404.

Cuzick, J., Brentnall, A., and Dowsett, M. (2017a). SNPs for breast cancer risk assessment. *Oncotarget*, 8(59):1–2.

Cuzick, J., Brentnall, A. R., Segal, C., Byers, H., Reuter, C., Detre, S., Lopez-Knowles, E., Sestak, I., Howell, A., Powles, T. J., Newman, W. G., and Dowsett, M. (2017b). Impact of a panel of 88 single nucleotide polymorphisms on the risk of breast cancer in high-risk women: Results from two randomized tamoxifen prevention trials. *Journal of Clinical Oncology*, 35(7):743–750.

Cuzick, J., Forbes, J., Edwards, R., Baum, M., Cawthorn, S., Coates, A., Hamed, H., Howell, A., and Powles, T. (2002). First results from the International Breast Cancer Intervention Study (IBIS-I): A randomised prevention trial. *The Lancet*, 360:817–824.

Cuzick, J., Forbes, J. F., Sestak, I., Cawthorn, S., Hamed, H., Holli, K., and Howell, A. (2007). Long-term results of tamoxifen prophylaxis for breast cancer-96-month follow-up of the randomized IBIS-I trial. *Journal of the National Cancer Institute*, 99(4):272–282.

Cuzick, J., Sestak, I., Baum, M., Buzdar, A., Howell, A., Dowsett, M., and Forbes, J. F. (2010). Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. *The Lancet Oncology*, 11(12):1135–1141.

Cuzick, J., Sestak, I., Bonanni, B., Costantino, J. P., Cummings, S., DeCensi, A., Dowsett, M., Forbes, J. F., Ford, L., LaCroix, A. Z., Mershon, J., Mitlak, B. H., Powles, T., Veronesi, U., Vogel, V., and Wickerham, D. L. (2013). Selective oestrogen receptor modulators in prevention of breast cancer: An updated meta-analysis of individual participant data. *The Lancet*, 381(9880):1827–1834.

Cuzick, J., Sestak, I., Cawthorn, S., Hamed, H., Holli, K., Howell, A., and Forbes, J. F. (2015). Tamoxifen for prevention of breast cancer: Extended long-term follow-up of the IBIS-I breast cancer prevention trial. *The Lancet Oncology*, 16(1):67–75.

Cuzick, J., Sestak, I., Cella, D., and Fallowfield, L. (2008). Treatment-emergent endocrine symptoms and the risk of breast cancer recurrence: a retrospective analysis of the ATAC trial. *The Lancet Oncology*, 9(12):1143–1148.

Cuzick, J., Sestak, I., Forbes, J. F., Dowsett, M., Cawthorn, S., Mansel, R. E., Loibl, S., Bonanni, B., Evans, D. G., and Howell, A. (2020). Use of anastrozole for breast cancer prevention (IBIS-II): long-term results of a randomised controlled trial. *The Lancet*, 395(10218):117–122.

Cuzick, J., Sestak, I., Forbes, J. F., Dowsett, M., Knox, J., Cawthorn, S., Saunders, C., Roche, N., Mansel, R. E., Von Minckwitz, G., Bonanni, B., Palva, T., and Howell, A. (2014). Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): An international, double-blind, randomised placebo-controlled trial. *The Lancet*, 383(9922):1041–1048.

Cuzick, J., Warwick, J., Pinney, E., Duffy, S. W., Cawthorn, S., Howell, A., Forbes, J. F., and Warren, R. M. (2011). Tamoxifen-induced reduction in mammographic

density and breast cancer risk reduction: A nested case-control study. *Journal of the National Cancer Institute*, 103(9):744–752.

Cuzick, J., Warwick, J., Pinney, E., Warren, R. M., and Duffy, S. W. (2004). Tamoxifen and breast density in women at increased risk of breast cancer. *Journal of the National Cancer Institute*, 96(8):621–628.

Daka, B., Rosen, T., Jansson, P. A., Råstam, L., Larsson, C. A., and Lindblad, U. (2013). Inverse association between serum insulin and sex hormone-binding globulin in a population survey in Sweden. *Endocrine Connections*, 2(1):18–22.

Dalerba, P., Kalisky, T., Sahoo, D., Rajendran, P. S., Rothenberg, M. E., Leyrat, A. A., Sim, S., Okamoto, J., Johnston, D. M., Qian, D., Zabala, M., Bueno, J., Neff, N. F., Wang, J., Shelton, A. A., Visser, B., Hisamori, S., Shimono, Y., Van De Wetering, M., ..., and Quake, S. R. (2011). Single-cell dissection of transcriptional heterogeneity in human colon tumors. *Nature Biotechnology*, 29(12):1120–1127.

Dall, G. V. and Britt, K. L. (2017). Estrogen effects on the mammary gland in early and late life and breast cancer risk. *Frontiers in Oncology*, 7:110.

Danforth, K. N., Eliassen, A. H., Tworoger, S. S., Missmer, S. A., Barbieri, R. L., Rosner, B. A., Colditz, G. A., and Hankinson, S. E. (2010). The association of plasma androgen levels with breast, ovarian and endometrial cancer risk factors among postmenopausal women. *International Journal of Cancer*, 126(1):199–207.

Dartois, L., Fagherazzi, G., Baglietto, L., Boutron-Ruault, M. C., Delaloge, S., Mesrine, S., and Clavel-Chapelon, F. (2016). Proportion of premenopausal and postmenopausal breast cancers attributable to known risk factors: Estimates from the E3N-EPIC cohort. *International Journal of Cancer*, 138(10):2415–2427.

Davis, S. R., Panjari, M., and Stanczyk, F. Z. (2011). DHEA replacement for postmenopausal women. *Journal of Clinical Endocrinology and Metabolism*, 96(6):1642–1653.

Davoodi, S. H., Malek-Shahabi, T., Malekshahi-Moghadam, A., Shahbazi, R., and Esmaeili, S. (2013). Obesity as an important risk factor for certain types of cancer. *Iranian Journal of Cancer Prevention*, 6(4):186–194.

Day, R., Ganz, P. a., Costantino, J. P., Cronin, W. M., Wickerham, D. L., and Fisher, B. (1999). Health-related quality of life and tamoxifen in breast cancer prevention: a

report from the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 17(9):2659–2669.

De Ronde, W., Van Der Schouw, Y. T., Pierik, F. H., Pols, H. A., Muller, M., Grobbee, D. E., Gooren, L. J., Weber, R. F., and De Jong, F. H. (2005). Serum levels of sex hormone-binding globulin (SHBG) are not associated with lower levels of non-SHBG-bound testosterone in male newborns and healthy adult men. *Clinical Endocrinology*, 62(4):498–503.

De Ronde, W., Van Der Schouw, Y. T., Pols, H. A., Gooren, L. J., Muller, M., Grobbee, D. E., and De Jong, F. H. (2006). Calculation of bioavailable and free testosterone in men: A comparison of 5 published algorithms. *Clinical Chemistry*, 52(9):1777–1784.

de Vries Schultink, A. H., Zwart, W., Linn, S. C., Beijnen, J. H., and Huitema, A. D. (2015). Effects of pharmacogenetics on the pharmacokinetics and pharmacodynamics of tamoxifen. *Clinical Pharmacokinetics*, 54(8):797–810.

DeCensi, A., Puntoni, M., Guerrieri-Gonzaga, A., Caviglia, S., Avino, F., Cortesi, L., Taverniti, C., Pacquola, M. G., Falcini, F., Gulisano, M., Digennaro, M., Cariello, A., Cagossi, K., Pinotti, G., Lazzeroni, M., Serrano, D., Branchi, D., Campora, S., Petrera, M., ..., and Bonanni, B. (2019). Randomized placebo controlled trial of low-dose tamoxifen to prevent local and contralateral recurrence in breast intraepithelial neoplasia. *Journal of Clinical Oncology*, 37(19):1629–1637.

DeFriend, D. J., Anderson, E., Bell, J., Wilks, D. P., West, C. M., Mansel, R. E., and Howell, A. (1994). Effects of 4-hydroxytamoxifen and a novel pure antioestrogen (ICI 182780) on the clonogenic growth of human breast cancer cells in vitro. *British Journal of Cancer*, 70(2):204–211.

Del Tredici, A. L., Malhotra, A., Dedek, M., Espin, F., Roach, D., dan Zhu, G., Voland, J., and Moreno, T. A. (2018). Frequency of CYP2D6 alleles including structural variants in the United States. *Frontiers in Pharmacology*, 9:305.

Delen, D., Walker, G., and Kadam, A. (2005). Predicting breast cancer survivability: A comparison of three data mining methods. *Artificial Intelligence in Medicine*, 34(2):113–127.

D'Elia, H. F., Mattsson, L.-A. A., Ohlsson, C., Nordborg, E., and Carlsten, H. (2003). Hormone replacement therapy in rheumatoid arthritis is associated with lower serum

levels of soluble IL-6 receptor and higher insulin-like growth factor 1. *Arthritis research & therapy*, 5(4):202–209.

Delong, E. R., DeLong, D. M., and Clarke-Pearson, D. L. (1988). Comparing the areas under two or more correlated receiver operating characteristic curves : A nonparametric approach. *Biometrics*, 44(3):837–845.

Dennerstein, L., Lehert, P., Guthrie, J. R., and Burger, H. G. (2007). Modeling women's health during the menopausal transition: A longitudinal analysis. *Menopause*, 14(1):53–62.

Desta, Z., Ward, B. A., Soukhova, N. V., and Flockhart, D. A. (2004). Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: Prominent roles for CYP3A and CYP2D6. *Journal of Pharmacology and Experimental Therapeutics*, 310(3):1062–1075.

Devlin, B. and Risch, N. (1995). Linkage disequilibrium measures for fine-scale mapping: A comparison. *Genomics*, 29:311–322.

Dickler, M. N. and Norton, L. (2001). The MORE trial: multiple outcomes for raloxifene evaluation–breast cancer as a secondary end point: implications for prevention. *Annals of the New York Academy of Sciences*, 949:134–42.

Dimitriadis, E., White, C. A., Jones, R. L., and Salamonsen, L. A. (2005). Cytokines, chemokines and growth factors in endometrium related to implantation. *Human Reproduction Update*, 11(6):613–630.

Din, O. S., Dodwell, D., Wakefield, R. J., and Coleman, R. E. (2010). Aromatase inhibitor-induced arthralgia in early breast cancer: What do we know and how can we find out more? *Breast Cancer Research and Treatment*, 120(3):525–538.

Diorio, C., Brisson, J., Bérubé, S., and Pollak, M. (2008). Genetic polymorphisms involved in insulin-like growth factor (IGF) pathway in relation to mammographic breast density and IGF levels. *Cancer Epidemiology Biomarkers and Prevention*, 17(4):880–888.

Diorio, C., Pollak, M., Byrne, C., Morin, C., Brisson, J., Yaffe, M., and Cote, G. (2005). Insulin-Like Growth Factor-I , IGF-Binding Protein-3 , and Mammographic Breast Density. *Cancer Epidemiology Biomarkers and Prevention*, 14(5):1065–1074.

Dong, X., Zhong, T., Xu, T., Xia, Y., Li, B., Li, C., Yuan, L., Ding, G., and Li, Y. (2013). Evaluating coverage of exons by HapMap SNPs. *Genomics*, 101(1):20–23.

Dorgan, J. F., Baer, D. J., Albert, P. S., Judd, J. T., Brown, E. D., Corle, D. K., Campbell, W. S., Terryl, J., Tejpar, A. A., Clevidence, A., Giffen, C. A., Chandler, D. W., Frank, Z., and Taylor, P. R. (2001). Serum Hormones and the Alcohol – Breast Cancer Association in Postmenopausal Women Background : Alcohol ingestion is associated with an increased risk of breast cancer in most epidemiologic studies . Results , however , are heterogeneous at lower levels. *Cancer*, 93:710–715.

Dorgan, J. F., Longcope, C., Stephenson, H. E., Falk, R. T., Miller, R., Franz, C., Kahle, L., Campbell, W. S., Tangrea, J. A., and Schatzkin, A. (1996). Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. *Cancer Epidemiology Biomarkers and Prevention*, 5(7):533–539.

Douchi, T., Yamamoto, S., Oki, T., Maruta, K., Kuwahata, R., and Nagata, Y. (1999). Serum androgen levels and muscle mass in women with polycystic ovary syndrome. *Obstetrics and Gynecology*, 94(3):337–340.

Dowsett, M. and Folkerd, E. (2015). Reduced progesterone levels explain the reduced risk of breast cancer in obese premenopausal women: a new hypothesis. *Breast Cancer Research and Treatment*, 149:1–4.

Dowsett, M., Pfister, C., Johnston, S. R., Miles, D. W., Houston, S. J., Verbeek, J. A., Gundacker, H., Sioufi, A., and Smith, I. E. (1999). Impact of tamoxifen on the pharmacokinetics and endocrine effects of the aromatase inhibitor letrozole in postmenopausal women with breast cancer. *Clinical Cancer Research*, 5(9):2338–2343.

Doyon, Y., Selleck, W., Lane, W. S., Tan, S., and Cote, J. (2004). Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. *Molecular and Cellular Biology*, 24(5):1884–1896.

Dubsky, P. C., Jakesz, R., Fitzal, F., Singer, C. F., Rudas, M., Bartsch, R., Steger, G. G., Gnant, M., Filipcic, L., Stierer, M., Sevelda, P., Mlineritsch, B., Greil, R., Pöstlberger, S., Samonigg, H., Stöger, H., Luschin-Ebengreuth, G., Kwasny, W., Haider, K., ..., and Tausch, C. (2012). Tamoxifen and anastrozole as a sequencing strategy: A randomized controlled trial in postmenopausal patients with endocrine-responsive early breast cancer from the Austrian Breast and Colorectal Cancer Study Group. *Journal of Clinical Oncology*, 30(7):722–728.

Dudbridge, F. and Gusnanto, A. (2008). Estimation of significance thresholds for genomewide association scans. *Genetic Epidemiology*, 32(3):227–234.

Duffy, S. W., Morrish, O. W., Allgood, P. C., Black, R., Gillan, M. G., Willsher, P., Cooke, J., Duncan, K. A., Michell, M. J., Dobson, H. M., Maroni, R., Lim, Y. Y., Purushothaman, H. N., Suaris, T., Astley, S. M., Young, K. C., Tucker, L., and Gilbert, F. J. (2018). Mammographic density and breast cancer risk in breast screening assessment cases and women with a family history of breast cancer. *European Journal of Cancer*, 88:48–56.

Dumas, I. and Diorio, C. (2010). Polymorphisms in genes involved in the estrogen pathway and mammographic density. *BMC Cancer*, 10:636.

Dunning, A. M., Dowsett, M., Healey, C. S., Tee, L., Luben, R. N., Folkerd, E., Novik, K. L., Kelemen, L., Ogata, S., Pharoah, P. D., Easton, D. F., Day, N. E., and Ponder, B. A. (2004). Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *Journal of the National Cancer Institute*, 96(12):936–945.

Easton, D. F. (1999). How many more breast cancer predisposition genes are there? [commentary].

Easton, D. F., Bishop, D. T., Ford, D., Crockford, G. P., Haites, N., Milner, B., Allan, L., King, M. C., Bowcock, A., Anderson, L., Ponder, B. A., Peto, J., Smith, S., Anderson, K., Ford, D., Stratton, M., Sobol, H., and Mazoyer, S. (1993). Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. *Am J Hum Genet*, 52(4):678–701.

Easton, D. F., Pharoah, P. D., Antoniou, A. C., Tischkowitz, M., Tavtigian, S. V., Nathanson, K. L., Devilee, P., Meindl, A., Couch, F. J., Southey, M., Goldgar, D. E., Evans, D. G. R., Chenevix-Trench, G., Rahman, N., Robson, M., Domchek, S. M., and Foulkes, W. D. (2015). Gene-panel sequencing and the prediction of breast-cancer risk. *New England Journal of Medicine*, 372(23):2243–2257.

(EBCTCG), E. B. C. T. C. G. (1998). Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet (London, England)*, 351(9114):1451–67.

(EBCTCG) Early Breast Cancer Trialists' Collaborative Group. (2011). Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: Patient-level meta-analysis of randomised trials. *The Lancet*, 378:771–784.

Egan, K. M., Stampfer, M. J., Rosner, B. A., Trichopoulos, D., Newcomb, P. A., Trentham-Dietz, A., Longnecker, M. P., Mittendorf, R., Greenberg, E. R., and Willett, W. C. (1998). Risk factors for breast cancer in women with a breast cancer family history. *Cancer Epidemiology Biomarkers and Prevention*, 7(5):359–364.

Emmert-Streib, F. and Dehmer, M. (2019). Introduction to survival analysis in practice. *Machine Learning and Knowledge Extraction*, 1(3):1013–1038.

Emond, J. A., Patterson, R. E., Natarajan, L., Laughlin, G. A., Gold, E. B., and Pierce, J. P. (2011). Sex hormone concentrations and the risk of breast cancer recurrence in postmenopausal women without hot flashes. *Cancer Epidemiology Biomarkers and Prevention*, 20(5):939–945.

Endogenous Hormones and Breast Cancer Collaborative Group (2003). Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *Journal of the National Cancer Institute*, 95(16):1218–1226.

Endogenous Hormones and Breast Cancer Collaborative Group (2011). Circulating sex hormones and breast cancer risk factors in postmenopausal women: Reanalysis of 13 studies. *British Journal of Cancer*, 105(5):709–722.

Endogenous Hormones and Breast Cancer Collaborative Group and Key, T. (2013). Sex hormones and risk of breast cancer in premenopausal women: A collaborative reanalysis of individual participant data from seven prospective studies. *The Lancet Oncology*, 14(10):1009–1019.

Endogenous Hormones and Breast Cancer Collaborative Group, Key, T. J., Appleby, P. N., Reeves, G. K., Roddam, A., Dorgan, J. F., Longcope, C., Stanczyk, F. Z., Stephenson, H. E., Falk, R. T., Miller, R., Schatzkin, A., Allen, D. S., Fentiman, I. S., Wang, D. Y., Dowsett, M., Thomas, H. V., Hankinson, S. E., Toniolo, P., Akhmedkhanov, A., Koenig, K., Shore, R. E., Zeleniuch-Jacquotte, A., Berrino, F., Muti, P., Micheli, A., Krogh, V., Sieri, S., Pala, V., Venturelli, E., Secreto, G., Barrett-Connor, E., Laughlin, G. A., Kabuto, M., Akiba, S., Stevens, R. G., Neriishi, K., Land, C. E., Cauley, J. A., Kuller, L. H., Cummings, S. R., Helzlsouer, K. J., Alberg, A. J., Bush, T. L., Comstock, G. W., Gordon, G. B., and Miller, S. R. (2003). Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *Journal of the National Cancer Institute*, 95(16):1218–1226.

Estellat, C., Torgerson, D. J., and Ravaud, P. (2009). How to perform a critical analysis of a randomised controlled trial. *Best Practice and Research: Clinical Rheumatology*, 23(2):291–303.

Eulenberg-Gustavus, C., Bähring, S., Maass, P. G., Luft, F. C., and Kettritz, R. (2017). Gene silencing and a novel monoallelic expression pattern in distinct CD177 neutrophil subsets. *Journal of Experimental Medicine*, 214(7):2089–2101.

Evans, D. G., Brentnall, A., Byers, H., Harkness, E., Stavrinos, P., Howell, A., Newman, W. G., Cuzick, J., Astley, S., Wilson, M., French, D., Harvie, M., Watterson, D., Fox, J., Sampson, S., Ingham, S., Sahin, S., and Fox, L. (2017). The impact of a panel of 18 SNPs on breast cancer risk in women attending a UK familial screening clinic: A case-control study. *Journal of Medical Genetics*, 54(2):104–110.

Fallowfield, L., Cella, D., Cuzick, J., Francis, S., Locker, G., and Howell, A. (2004). Quality of life of postmenopausal women in the Arimidex, Tamoxifen, Alone or in Combination (ATAC) Adjuvant Breast Cancer Trial. *Journal of Clinical Oncology*, 22(21):4261–4271.

Farhat, G. N., Cummings, S. R., Chlebowski, R. T., Parimi, N., Cauley, J. A., Rohan, T. E., Huang, A. J., Vitolins, M., Hubbell, F. A., Manson, J. E., Cochrane, B. B., Lane, D. S., and Lee, J. S. (2011). Sex hormone levels and risks of estrogen receptor-negative and estrogen receptor-positive breast cancers. *Journal of the National Cancer Institute*, 103(7):562–570.

Faupel-Badger, J. M., Fuhrman, B. J., Xu, X., Falk, R. T., Keefer, L. K., Veenstra, T. D., Hoover, R. N., and Ziegler, R. G. (2010). Comparison of liquid chromatography-mass spectrometry, radioimmunoassay, and enzyme-linked immunosorbent assay methods for measurement of urinary estrogens. *Cancer Epidemiology Biomarkers and Prevention*, 19(1):292–300.

Fawcett, T. (2006). An introduction to ROC analysis. *Pattern Recognition Letters*, 27(8):861–874.

Fei, C., DeRoo, L. A., Sandler, D. P., and Weinberg, C. R. (2013). Menopausal symptoms and the risk of young-onset breast cancer. *European Journal of Cancer*, 49(4):798–804.

Feigelson, H. S., Shames, L. S., Pike, M. C., Coetzee, G. A., Stanczyk, F. Z., and Henderson, B. E. (1998). Cytochrome P450c17α gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. *Cancer Research*, 58(4):585–587.

Fisher, B., Costantino, J. P., Wickerham, D. L., Cecchini, R. S., Cronin, W. M., Robidoux, A., Bevers, T. B., Kavanah, M. T., Atkins, J. N., Margolese, R. G., Runowicz, C. D., James, J. M., Ford, L. G., and Wolmark, N. (2005). Tamoxifen for the prevention of breast cancer: Current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *Journal of the National Cancer Institute*, 97(22):1652–1662.

Fisher, B., Costantino, J. P., Wickerham, D. L., Redmond, C. K., Kavanah, M., Cronin, W. M., Vogel, V., Robidoux, A., Dimitrov, N., Atkins, J., Daly, M., Wieand, S., Tan-Chiu, E., Ford, L., and Wolmark, N. (1998). Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *JNCI: Journal of the National Cancer Institute*, 90(18):1371–1388.

Flach, P. and Wu, S. (2003). Repairing concavities in ROC curves. In *UK Workshop on Computational Intelligence. University of Bristol*, pages pp. 38–44.

Fogle, R. H., Stanczyk, F. Z., Zhang, X., and Paulson, R. J. (2007). Ovarian androgen production in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism*, 92(8):3040–3043.

Folkestad, L., Bjarnason, N. H., Bjerregaard, J. K., and Brixen, K. (2009). The effect of aromatase inhibitors on bone metabolism. *Basic and Clinical Pharmacology and Toxicology*, 104(1):3–10.

Fontein, D. B., Houtsma, D., Hille, E. T., Seynaeve, C., Putter, H., Meershoek-Klein Kranenbarg, E., Guchelaar, H. J., Gelderblom, H., Dirix, L. Y., Paridaens, R., Bartlett, J. M., Nortier, J. W., and Van de Velde, C. J. (2012). Relationship between specific adverse events and efficacy of exemestane therapy in early postmenopausal breast cancer patients. *Annals of Oncology*, 23(12):3091–3097.

Fontein, D. B., Houtsma, D., Nortier, J. W., Baak-Pablo, R. F., Kranenbarg, E. M. K., Van Der Straaten, T. R., Putter, H., Seynaeve, C., Gelderblom, H., Van De Velde, C. J., and Guchelaar, H. J. (2014). Germline variants in the CYP19A1 gene are related to specific adverse events in aromatase inhibitor users: A substudy of Dutch patients in the TEAM trial. *Breast Cancer Research and Treatment*, 144(3):599–606.

Fontein, D. B., Seynaeve, C., Hadji, P., Hille, E. T., Van De Water, W., Putter, H., Kranenbarg, E. M. K., Hasenburg, A., Paridaens, R. J., Vannetzel, J. M., Markopoulos, C., Hozumi, Y., Bartlett, J. M., Jones, S. E., Rea, D. W., Nortier, J. W., and Van De Velde, C. J. (2013). Specific adverse events predict survival benefit in patients treated with tamoxifen or aromatase inhibitors: An international tamoxifen exemestane adjuvant multinational trial analysis. *Journal of Clinical Oncology*, 31(18):2257–2264.

Franks, S. and Hardy, K. (2018). Androgen action in the ovary. *Frontiers in Endocrinology*, 9:452.

Freedman, R. R. (2014). Menopausal hot flashes: mechanisms, endocrinology, treatment. *J Steroid Biochem Mol Biol*, 142(July):115–120.

Freeman, E. W., Sammel, M. D., Grisso, J. A., Battistini, M., Garcia-Espagna, B., and Hollander, L. (2001). Hot flashes in the late reproductive years: Risk factors for African American and Caucasian women. *Journal of Women's Health and Gender-Based Medicine*, 10(1):67–76.

Freidman, J., Hastie, T., and Tibshirani, R. (2010). Regularization paths for generalized linear models via coordinate descent. *Journal of Statistical Software*, 33(1):1–22.

Friedenreich, C. M. (2001). Review of anthropometric factors and breast cancer risk. *European Journal of Cancer Prevention*, 10(1):15–32.

Friedman, L., Ostermeyer, E. A., Szabo, C. I., Dowd, P., Lynch, E. D., Rowell, S. E., and King, M.-c. (1994). Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nature Genetics*, 8:399–404.

Gail, M. H. (2009). Value of adding single-nucleotide polymorphism genotypes to a breast cancer risk model. *Journal of the National Cancer Institute*, 101(13):959–963.

Gail, M. H., Brinton, L. A., Byar, D. P., Corle, D. K., Green, S. B., Schairer, C., and Mulvihill, J. J. (1989). Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *Journal of the National Cancer Institute*, 81(24):1879–1886.

Gail, M. H. and Pfeiffer, R. M. (2018). Breast cancer risk model requirements for counseling, prevention, and screening. *Journal of the National Cancer Institute*, 110(9):994–1002.

Gaillard, S. and Stearns, V. (2011). Aromatase inhibitor-associated bone and musculoskeletal effects: New evidence defining etiology and strategies for management. *Breast Cancer Research*, 13(2):205.

Gallicchio, L., Miller, S. R., Kiefer, J., Greene, T., Zacur, H. A., and Flaws, J. A. (2015). Risk factors for hot flashes among women undergoing the menopausal transition: Baseline results from the Midlife Women's Health Study. *Menopause*, 22(10):1098–1107.

Gallicchio, L., Miller, S. R., Visvanathan, K., Lewis, L. M., Babus, J., Zacur, H., and Flaws, J. A. (2006). Cigarette smoking, estrogen levels, and hot flashes in midlife women. *Maturitas*, 53(2):133–143.

Gallicchio, L., Visvanathan, K., Miller, S. R., Babus, J., Lewis, L. M., Zacur, H., and Flaws, J. A. (2005). Body mass, estrogen levels, and hot flashes in midlife women. *American Journal of Obstetrics and Gynecology*, 193(4):1353–1360.

Gapstur, S. M., López, P., Colangelo, L. A., Wolfman, J., Van Horn, L., and Hendrick, R. E. (2003). Associations of breast cancer risk factors with breast density in hispanic women. *Cancer Epidemiology Biomarkers and Prevention*, 12(10):1074–1080.

Garcia-Closas, M., Gunsoy, N. B., and Chatterjee, N. (2014). Combined associations of genetic and environmental risk factors: Implications for prevention of breast cancer. *Journal of the National Cancer Institute*, 106(11):dju305.

Garcia-Giralt, N., Rodríguez-Sanz, M., Prieto-Alhambra, D., Servitja, S., Torres-Del Pliego, E., Balcells, S., Albanell, J., Grinberg, D., Diez-Perez, A., Tusquets, I., and Nogués, X. (2013). Genetic determinants of aromatase inhibitor-related arthralgia: The B-ABLE cohort study. *Breast Cancer Research and Treatment*, 140(2):385–395.

Ghosh, K., Hartmann, L. C., Reynolds, C., Visscher, D. W., Brandt, K. R., Vierkant, R. A., Scott, C. G., Radisky, D. C., Sellers, T. A., Pankratz, V. S., and Vachon, C. M. (2010). Association between mammographic density and age-related lobular involution of the breast. *Journal of Clinical Oncology*, 28(13):2207–2212.

Ghoussaini, M. and Pharoah, P. D. (2009). Polygenic susceptibility to breast cancer : current state-of-the-art. *Future Oncology*, 5(5):689–701.

Glaser, R. L. (2010). Subcutaneous testosterone-anastrozole implant therapy in breast cancer survivors. In *ASCO Breast Cancer Symposium Hormones and Hormone Receptor Biology*, page 221.

Glaser, R. L. and Dimitrakakis, C. (2012). Subgroups of patients treated with an aromatase inhibitor (anastrozole) delivered subcutaneously in combination with testosterone. In *9th European Congress on Menopause and Andropause.*, page S31.

Glaser, R. L. and Dimitrakakis, C. (2013). Reduced breast cancer incidence in women treated with subcutaneous testosterone, or testosterone with anastrozole: A prospective, observational study. *Maturitas*, 76(4):342–349.

Goetz, M. P., Knox, S. K., Suman, V. J., Rae, J. M., Safgren, S. L., Ames, M. M., Visscher, D. W., Reynolds, C., Couch, F. J., Lingle, W. L., Weinshilboum, R. M., Fritcher, E. G. B., Nibbe, A. M., Desta, Z., Nguyen, A., Flockhart, D. A., Perez, E. A., and Ingle, J. N. (2007). The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. *Breast Cancer Research and Treatment*, 101(1):113–121.

Goetz, M. P., Rae, J. M., Suman, V. J., Safgren, S. L., Ames, M. M., Visscher, D. W., Reynolds, C., Couch, F. J., Lingle, W. L., Flockhart, D. A., Desta, Z., Perez, E. A., and Ingle, J. N. (2005). Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *Journal of Clinical Oncology*, 23(36):9312–9318.

Goetz, M. P., Sun, J. X., Suman, V. J., Silva, G. O., Perou, C. M., Nakamura, Y., Cox, N. J., Stephens, P. J., Miller, V. A., Ross, J. S., Chen, D., Safgren, S. L., Kuffel, M. J., Ames, M. M., Kalari, K. R., Gomez, H. L., Gonzalez-Angulo, A. M., Burgues, O., Brauch, H. B., ..., and Yelensky, R. (2015). Loss of heterozygosity at the CYP2D6 locus in breast cancer: Implications for germline pharmacogenetic studies. *Journal of the National Cancer Institute*, 107(2):2–9.

Gold, E. B., Sternfeld, B., Kelsey, J. L., Brown, C., Mouton, C., and Reame, N. (2000). Relation of demographic and lifestyle factors to symptoms in a multi- racial / ethnic population of women 40 – 55 years of age. *American Journal of Epidemiology*, 152(5).

Goldman, A. L., Bhasin, S., Wu, F. C., Krishna, M., Matsumoto, A. M., and Jasuja, R. (2017). A reappraisal of testosterone's binding in circulation: Physiological and clinical implications. *Endocrine Reviews*, 38(4):302–324.

Goss, P. E., Ingle, J. N., Alés-Martínez, J. E., Cheung, A. M., Chlebowski, R. T., Wactawski-Wende, J., McTiernan, A., Robbins, J., Johnson, K. C., Martin, L. W., Winquist, E., Sarto, G. E., Garber, J. E., Fabian, C. J., Pujol, P., Maunsell, E., Farmer, P., Gelmon, K. A., Tu, D., Richardson, H., and for the NCIC CTG MAP.3

Study Investigator (2011). Exemestane for breast-cancer prevention in posmenopausal women. *The New England Journal of Medicine*, 364(25):2381–2391.

Goss, P. E., Ingle, J. N., Martino, S., Robert, N. J., Muss, H. B., Piccart, M. J., Castiglione, M., Tu, D., Shepherd, L. E., Pritchard, K. I., Livingston, R. B., Davidson, N. E., Norton, L., Perez, E. A., Abrams, J. S., Cameron, D. A., Palmer, M. J., and Pater, J. L. (2005). Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: Updated findings from NCIC CTG MA.17. *Journal of the National Cancer Institute*, 97(17):1262–1271.

Goss, P. E., Ingle, J. N., Pritchard, K. I., Ellis, M. J., Sledge, G. W., Budd, G. T., Rabaglio, M., Ansari, R. H., Johnson, D. B., Tozer, R., D'Souza, D. P., Chalchal, H., Spadafora, S., Stearns, V., Perez, E. A., Liedke, P. E., Lang, I., Elliott, C., Gelmon, K. A., Chapman, J. A. W., and Shepherd, L. E. (2013). Exemestane versus anastrozole in postmenopausal women with early breast cancer: NCIC CTG MA.27 - A randomized controlled phase III trial. *Journal of Clinical Oncology*, 31(11):1398–1404.

Gower, B. A. and Nyman, L. (2000). Associations among oral estrogen use, free testosterone concentration, and lean body mass among postmenopausal women. *Journal of Clinical Endocrinology and Metabolism*, 85(12):4476–4480.

Gram, I. T., Funkhouser, E., and Tabar, L. (1995). Reproductive and menstrual factors in relation to mammographic parenchymal patterns among perimenopausal women. *British Journal of Cancer*, 71(3):647–650.

Greendale, G. A., Huang, M. H., Ursin, G., Ingles, S., Stanczyk, F., Crandall, C., Laughlin, G. A., Barrett-Connor, E., and Karlamangla, A. (2007). Serum prolactin levels are positively associated with mammographic density in postmenopausal women. *Breast Cancer Research and Treatment*, 105(3):337–346.

Grimm, S. W. and Dyroff, M. C. (1997). Inhibition of human drug metabolizing cytochromes P450 by anastrozole, a potent and selective inhibitor of aromatase. *Drug Metabolism and Disposition*, 25(5):598–602.

Guo, Y. P., Martin, L. J., Hanna, W., Banerjee, D., Miller, N., Fishell, E., Khokha, R., and Boyd, N. F. (2001). Growth factors and stromal matrix proteins associated with mammographic densities. *Cancer Epidemiology Biomarkers and Prevention*, 10(3):243–248.

Guthrie, J. R., Dennerstein, L., Hopper, J. L., and Burger, H. G. (1996). Hot flushes, menstrual status, and hormone levels in a population-based sample of midlife women. *Obstetrics and Gynecology*, 88(3):437–442.

GWAS Catalog (2018). GWAS Catalog 2018 09.01.2018.

Hammond, G. L. (2016). Plasma steroid-binding proteins: Primary gatekeepers of steroid hormone action. *Journal of Endocrinology*, 230(1):R13–R25.

Hankinson, S. E., Colditz, G. A., and Willett, W. C. (2004). Towards an integrated model for breast cancer etiology. The lifelong interplay of genes, lifestyle, and hormones. *Breast Cancer Research*, 6(5):213–218.

Hankinson, S. E. and Eliassen, A. H. (2007). Endogenous estrogen, testosterone and progesterone levels in relation to breast cancer risk. *Journal of Steroid Biochemistry and Molecular Biology*, 106(1-5):24–30.

Hankinson, S. E., Manson, J. E., Willett, W. C., and Speizer, F. E. (1995a). Reproducibility of plasma hormone levels in postmenopausal women over a 2-3-year period. *Cancer Epidemiology Biomarkers and Prevention*, 4(6):649–654.

Hankinson, S. E., Willett, W. C., Manson, J. E., Hunter, D. J., Colditz, G. A., Stampfer, M. J., Longcope, C., and Speizer, F. E. (1995b). Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. *Journal of the National Cancer Institute*, 87(17):1297–1302.

Hart, V., Reeves, K. W., Sturgeon, S. R., Reich, N. G., Sievert, L. L., Kerlikowske, K., Ma, L., Shepherd, J., Tice, J. A., Mahmoudzadeh, A. P., Malkov, S., and Sprague, B. L. (2015). The effect of change in body mass index on volumetric measures of mammographic density. *Cancer Epidemiology Biomarkers and Prevention*, 24(11):1724–1730.

Hart, V., Sturgeon, S. R., Reich, N., Sievert, L. L., Sybil, L., Gold, E. B., Avis, N. E., and Reeves, K. W. (2016). Menopausal vasomotor symptoms and the incident breat cancer risk in the Study of Women's Health Across the Nation. *Cancer Causes and Control*, 27(11):1333–1340.

Harvey, J. A. and Bovbjerg, V. E. (2004). Quantitative assessment of mammographic breast density: Relationship with breast cancer risk. *Radiology*, 230(1):29–41.

Harvey, J. A., Pinkerton, J. A. V., and Herman, C. R. (1997). Short-term cessation of hormone replacement therapy and improvement of mammographic specificity. *Journal of the National Cancer Institute*, 89(21):1623–1625.

Hasegawa, H., Meeusen, R., Sarre, S., Diltoer, M., Piacentini, M. F., and Michotte, Y. (2005). Acute dopamine/norepinephrine reuptake inhibition increases brain and core temperature in rats. *Journal of Applied Physiology*, 99(4):1397–1401.

Hastie, T., Tibshirani, R., and Friedman, J. (2014). *The elements of statistical learning*. Springer, second edition.

Hawes, D., Downey, S., Pearce, C. L., Bartow, S., Wan, P., Pike, M. C., and Wu, A. H. (2006). Dense breast stromal tissue shows greatly increased concentration of breast epithelium but no increase in its proliferative activity. *Breast Cancer Research*, 8(2):R24.

Heath, I. (2014). Breast cancer screening causes more harm than good: No. *Journal of primary health care*, 6(1):79–80.

Henderson, B. E. and Feigelson, H. S. (2000). Hormonal carcinogenesis. *Carcinogenesis*, 21(3):427–433.

Henderson, V. W. and Lobo, R. A. (2012). Hormone therapy and the risk of stroke: perspectives ten years after the Women's Health Initiative trials. *Climacteric*, 15(3):229–234.

Henry, N. L., Giles, J. T., and Stearns, V. (2008). Aromatase inhibitor–associated musculoskeletal symptoms: Etiology and strategies for management. *Oncology*, 22(12):1401–1408.

Hernan, M. A. (2010). The hazard of hazard ratios. *Epidemiology*, 21(1):13–15.

Hertz, D. L., Henry, N. L., and Rae, J. M. (2017). Pharmacogenomics germline genetic predictors of aromatase. *Pharmacogenomics*, 18(5):481–499.

Hickey, D. K., Patel, M. V., Fahey, J. V., and Wira, C. R. (2011). Innate and adaptive immunity at mucosal surfaces of the female reproductive tract: Stratification and integration of immune protection against the transmission of sexually transmitted infections. *Journal of Reproductive Immunology*, 88(2):185–194.

Hickey, M., Saunders, C., Partridge, A., Santoro, N., Joffe, H., and Stearns, V. (2008). Practical clinical guidelines for assessing and managing menopausal symptoms after breast cancer. *Annals of Oncology*, 19(10):1669–1680.

Higgins, J. P. T., Thompson, S. G., Deeks, J. J., and Altman, D. G. (2003). Measuring inconsistency in meta-analysis. *BMJ*, 327:557–560.

Hines, L. M., Hankinson, S. E., Smith-Warner, S. A., Spiegelman, D., Kelsey, K. T., Colditz, G. A., Willett, W. C., and Hunter, D. J. (2000). A prospective study of the effect of alcohol consumption and ADH3 genotype on plasma steroid hormone levels and breast cancer risk. *Cancer Epidemiology Biomarkers and Prevention*, 9(10):1099–1105.

Hollis, S. and Campbell, F. (1999). What is meant be intention to treat analysis? Survey of published randomised controlled trials. *BMJ*, 319:670–674.

Holst, F., Stahl, P. R., Ruiz, C., Hellwinkel, O., Jehan, Z., Wendland, M., Lebeau, A., Terracciano, L., Al-Kuraya, K., Jänicke, F., Sauter, G., and Simon, R. (2007). Estrogen receptor alpha (ESR1) gene amplification is frequent in breast cancer. *Nature Genetics*, 39(5):655–660.

Honda, J., Kanematsu, M., Nakagawa, M., Takahashi, M., Nagao, T., Tangoku, A., and Sasa, M. (2011). Joint symptoms, aromatase inhibitor-related adverse reactions, are indirectly associated with decreased serum estradiol. *International Journal of Surgical Oncology*, 2011:951260.

Howell, A., Cuzick, J., Baum, M., Buzdar, A., Dowsett, M., Forbes, J. F., Hoctin-Boes, G., Houghton, J., Locker, G. Y., and Tobias, J. S. (2005). Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*, 365(9453):60–62.

Hu, R., Hilakivi-Clarke, L., and Clarke, R. (2015). Molecular mechanisms of tamoxifen-associated endometrial cancer (Review). *Oncology Letters*, 9(4):1495–1501.

Huang, A. J., Moore, E. E., Boyko, E. J., Lin, F., Vittinghoff, E., and Fihn, S. D. (2010). Vaginal symptoms in postmenopausal women: self-reported severity, natural history, and risk factors. *Menopause*, 17(1):121–126.

Huang, Y., Malone, K. E., Cushing-Haugen, K. L., Daling, J. R., and Li, C. I. (2011).

Relationship between menopausal symptoms and risk of postmenopausal breast cancer. *Cancer Epidemiology Biomarkers and Prevention*, 20(2):379–388.

Huang, Y. H., Lo, M. H., Cai, X. Y., Liu, S. F., and Kuo, H. C. (2019). Increase expression of CD177 in Kawasaki disease. *Pediatric Rheumatology Online Journal*, 17(1):13.

Hummelen, R., Macklaim, J. M., Bisanz, J. E., Hammond, J. A., McMillan, A., Vongsa, R., Koenig, D., Gloor, G. B., and Reid, G. (2011). Vaginal microbiome and epithelial gene array in post-menopausal women with moderate to severe dryness. *PLoS ONE*, 6(11):e26602.

Hunter, D. J. (2005). Gene-environment interactions in human diseases. *Nature Reviews Genetics*, 6(4):287–298.

Hunter, D. J. and Drazen, J. M. (2019). Has the genome granted our wish yet? *New England Journal of Medicine*, 380(25):2391–2393.

Huober, J., Cole, B., Rabaglio, M., Giobbie-Hurder, A., Wu, J., Ejlertsen, B., Bonnefoi, H., Forbes, J., Neven, P., Láng, I., Smith, I., Wardley, A., Price, K., Goldhirsch, A., Coates, A., Colleoni, M., Gelber, R., and Thürlimann, B. (2014). Symptoms of endocrine treatment and outcome in the BIG 1-98 study. *Breast Cancer Research and Treatment*, 143(1):159–169.

Hüsing, A., Fortner, R. T., Kühn, T., Overvad, K., Tjønneland, A., Olsen, A., Boutron-Ruault, M. C., Severi, G., Fournier, A., Boeing, H., Trichopoulou, A., Benetou, V., Orfanos, P., Masala, G., Pala, V., Tumino, R., Fasanelli, F., Panico, S., De Mesquita, H. B. B., ..., and Kaaks, R. (2017). Added value of serum hormone measurements in risk prediction models for breast cancer for women not using exogenous hormones: Results from the EPIC cohort. *Clinical Cancer Research*, 23(15):4181–4189.

Ingle, J. N. (2013). Pharmacogenomics of endocrine therapy in breast cancer. *Journal of Human Genetics*, 58(6):306–312.

Ingle, J. N., Schaid, D. J., Goss, P. E., Liu, M., Mushiroda, T., Chapman, J. A. W., Kubo, M., Jenkins, G. D., Batzler, A., Shepherd, L., Pater, J., Wang, L., Ellis, M. J., Stearns, V., Rohrer, D. C., Goetz, M. P., Pritchard, K. I., Flockhart, D. A., Nakamura, Y., and Weinshilboum, R. M. (2010). Genome-wide associations and functional genomic studies of musculoskeletal adverse events in women receiving aromatase inhibitors. *Journal of Clinical Oncology*, 28(31):4674–4682.

Ingle, J. N. and Suman, V. J. (2003). Aromatase inhibitors versus tamoxifen for management of postmenopausal breast cancer in the advanced disease and neoadjuvant settings. *Journal of Steroid Biochemistry and Molecular Biology*, 86(3-5):313–319.

Ingle, J. N., Suman, V. J., Johnson, P. A., Krook, J. E., Mailliard, J. A., Wheeler, R. H., Loprinzi, C. L., Perez, E. A., Jordan, V. C., and Dowsett, M. (1999). Evaluation of tamoxifen plus letrozole with assessment of pharmacokinetic interaction in postmenopausal women with metastatic breast cancer. *Clinical Cancer Research*, 5(7):1642–1649.

Institute or Health Metrics and Evaluation (IHME) (2018). *Findings from the Global Burden of Disease Study 2017.* IHME, Seattle, WA.

International Agency for Research on Cancer (2018). Estimated age-standardised incidence rates (World) in 2018, breast, females, all ages.

Ioannidis, J. P., Ralston, S. H., Bennett, S. T., Brandi, M. L., Grinberg, D., Karassa, F. B., Langdahl, B., Van Meurs, J. B., Mosekilde, L., Scollen, S., Albagha, O. M., Bustamante, M., Carey, A. H., Dunning, A. M., Enjuanes, A., Van Leeuwen, J. P., Mavilia, C., Masi, L., McGuigan, F. E., ..., and Uitterlinden, A. G. (2004). Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. *Journal of the American Medical Association*, 292(17):2105–2114.

Islander, U., Jochems, C., Lagerquist, M. K., Forsblad-d'Elia, H., and Carlsten, H. (2011). Estrogens in rheumatoid arthritis; the immune system and bone. *Molecular and Cellular Endocrinology*, 335(1):14–29.

Iwasaki, M., Otani, T., Inoue, M., Sasazuki, S., and Tsugane, S. (2007). Body size and risk for breast cancer in relation to estrogen and progesterone receptor status in Japan. *Annals of Epidemiology*, 17(4):304–312.

Jager, N. G. L., Koornstra, R. H. T., Vincent, A. D., van Schaik, R. H. N., Huitema, A. D. R., Korse, T. M., Schellens, J. H. M., Linn, S. C., and Beijnen, J. H. (2013). Hot flashes are not predictive for serum concentrations of tamoxifen and its metabolites. *BMC Cancer*, 13:612.

James, R. E., Lukanova, A., Dossus, L., Becker, S., Rinaldi, S., Tjønneland, A., Olsen, A., Overvad, K., Mesrine, S., Engel, P., Clavel-Chapelon, F., Chang-Claude, J., Vrieling, A., Boeing, H., Schütze, M., Trichopoulou, A., Lagiou, P., Trichopoulos, D., Palli,

D., ..., and Kaaks, R. (2011). Postmenopausal serum sex steroids and risk of hormone receptor-positive and -negative breast cancer: A nested case-control study. *Cancer Prevention Research*, 4(10):1626–1635.

Jankovic-Karasoulos, T., Birrell, S., Cops, E., Jindal, S., Ochnik, A., Thomas, M., Tilley, W., and Hickey, T. (2009). Evaluation of testosterone supplementation during anastrozole therapy in a breast explant model. *Cancer Research*, 69(24 Supplement):Abstract Nr 4089.

Jansson, C., Johansson, S., Lindh-Åstrand, L., Hoffmann, M., and Hammar, M. (2003). The prevalence of symptoms possibly related to the climacteric in pre- and postmenopausal women in Linköping, Sweden. *Maturitas*, 45(2):129–135.

Jehan, S., Masters-Isarilov, A., Salifu, I., Zizi, F., Jean-Louis, G., Pandi-Perumal, S. R., Gupta, R., Brzezinski, A., and McFarlane, S. I. (2015). Sleep disorders in postmenopausal women. *Journal of sleep disorders & therapy*, 4(5):212.

Jin, Y., Desta, Z., Stearns, V., Ward, B., Ho, H., Lee, K. H., Skaar, T., Storniolo, A. M., Li, L., Araba, A., Blanchard, R., Nguyen, A., Ullmer, L., Hayden, J., Lemler, S., Weinshilboum, R. M., Rae, J. M., Hayes, D. F., and Flockhart, D. A. (2005). CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. *Journal of the National Cancer Institute*, 97(1):30–39.

Jin, Y., Hayes, D. F., Li, L., Robarge, J. D., Skaar, T. C., Philips, S., Nguyen, A., Schott, A., Hayden, J., Lemler, S., Storniolo, A. M., Flockhart, D. A., and Stearns, V. (2008). Estrogen receptor genotypes influence hot flash prevalence and composite score before and after tamoxifen therapy. *Journal of Clinical Oncology*, 26(36):5849–5854.

Johanneke Van Den Berg, M., Mishra, G. D., Van Der Schouw, Y. T., and Herber-Gast, G. C. M. (2014). Vasomotor menopausal symptoms are not associated with incidence of breast cancer in a population-based cohort of mid-aged women. *European Journal of Cancer*, 50(4):824–830.

Johansson, H., Gray, K. P., Pagani, O., Regan, M. M., Viale, G., Aristarco, V., Macis, D., Puccio, A., Roux, S., Maibach, R., Colleoni, M., Rabaglio, M., Price, K. N., Coates, A. S., Gelber, R. D., Goldhirsch, A., Kammler, R., Bonanni, B., Walley, B. A., ..., and Abraham, J. (2016). Impact of CYP19A1 and ESR1 variants on early-onset side effects during combined endocrine therapy in the TEXT trial. *Breast Cancer Research*, 18(1):110.

John, E. M., Phipps, A. I., and Sangaramoorthy, M. (2013). Body size, modifying factors, and postmenopausal breast cancer risk in a multiethnic population: The san francisco bay area breast cancer study. *SpringerPlus*, 2(1):239.

Johnson, M. D., Zuo, H., Lee, K. H., Trebley, J. P., Rae, J. M., Weatherman, R. V., Desta, Z., Flockhart, D. A., and Skaar, T. C. (2004). Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Research and Treatment*, 85(2):151–159.

Jones, G. T., Atzeni, F., Beasley, M., Flüß, E., Sarzi-Puttini, P., and Macfarlane, G. J. (2015). The prevalence of fibromyalgia in the general population: A comparison of the American College of Rheumatology 1990, 2010, and modified 2010 classification criteria. *Arthritis and Rheumatology*, 67(2):568–575.

Jones, K., Ballesteros, A., Mentink-Kane, M., Warren, J., Rattila, S., Malech, H., Kang, E., and Dveksler, G. (2016). PSG9 stimulates increase in FoxP3+ regulatory T-cells through the TGF-β1 pathway. *PLoS ONE*, 11(7):e0158050.

Jordan, V. C. (2007). New insights into the metabolism of tamoxifen and its role in the treatment and prevention of breast cancer. *Steroids*, 72(13):829–842.

Juraskova, I., Butow, P., Bonner, C., Bell, M. L., Smith, A. B., Seccombe, M., Boyle, F., Reaby, L., Cuzick, J., and Forbes, J. F. (2014). Improving decision making about clinical trial participation-a randomised controlled trial of a decision aid for women considering participation in the IBIS-II breast cancer prevention trial. *British Journal of Cancer*, 111(1):1–7.

Kaaks, R., Berrino, F., Key, T., Rinaldi, S., Dossus, L., Biessy, C., Secreto, G., Amiano, P., Bingham, S., Boeing, H., Bas Bueno de Mesquita, H., Chang-Claude, J., Clavel-Chapelon, F., Fournier, A., van Gils, C. H., Gonzalez, C. A., Gurrea, A. B., Critselis, E., Khaw, K. T., ..., and Riboli, E. (2005). Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). *Journal of the National Cancer Institute*, 97(10):755–765.

Kalb, S., Mahan, M. A., Elhadi, A. M., Dru, A., Eales, J., Lemos, M., and Theodore, N. (2013). Pharmacophysiology of bone and spinal fusion. *Spine Journal*, 13(10):1359–1369.

Kanis, J. A., Cooper, C., Rizzoli, R., and Reginster, J. (2019). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporosis International*, 30:3–44.

Kaplan, E. L. and Meier, P. (1958). Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association*, 53(282):457.

Kapoor, D. and Jones, T. H. (2005). Smoking and hormones in health and endocrine disorders. *European Journal of Endocrinology*, 152(4):491–499.

Katzenellenbogen, J. A., O'Malley, B. W., and Katzenellenbogen, B. S. (1996). Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. *Molecular Endocrinology*, 10(2):119–131.

Kaufmann, M., Jonat, W., Hilfrich, J., Eidtmann, H., Gademann, G., Zuna, I., and von Minckwitz, G. (2007). Improved overall survival in postmenopausal women with early breast cancer after anastrozole initiated after treatment with tamoxifen compared with continued tamoxifen: The ARNO 95 study. *Journal of Clinical Oncology*, 25(19):2664–2670.

Kawamura, T. and Sobue, T. (2005). Comparison of breast cancer mortality in five countries: France, Italy, Japan, the UK and the USA from the WHO Mortality Database (1960-2000). *Japanese Journal of Clinical Oncology*, 35(12):758–759.

Kelemen, L. E., Sellers, T. A., and Vachon, C. M. (2008). Can genes for mammographic density inform cancer etiology? *Nature Reviews Cancer*, 8(10):812–823.

Keller, B. M., McCarthy, A. M., Chen, J., Armstrong, K., Conant, E. F., Domchek, S. M., and Kontos, D. (2015). Associations between breast density and a panel of single nucleotide polymorphisms linked to breast cancer risk: A cohort study with digital mammography. *BMC Cancer*, 15:143.

Keller, J. L., Casson, P. R., and Toth, M. J. (2011). Relationship of androgens to body composition, energy and substrate metabolism and aerobic capacity in healthy, young women. *Steroids*, 76(12):1247–1251.

Kelsey, J. L., Gammon, M. D., and John, E. M. (1993). Reproductive factors and breast cancer. *Epidemiologic Reviews*, 15(1):36–47.

Kent, W. J., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., Zahler, A. M., and Haussler, a. D. (2002). The human genome browser at UCSC. *Genome Research*, 12(6):996–1006.

Key, T. J., Verkasalo, P. K., and Banks, E. (2001). Epidemiology of breast cancer. *Lancet Oncology*, 2(3):133–140.

Khan, Q. J., Kimler, B. F., O'Dea, A. P., Zalles, C. M., Sharma, P., and Fabian, C. J. (2007). Mammographic density does not correlate with Ki-67 expression or cytomorphology in benign breast cells obtained by random periareolar fine needle aspiration from women at high risk for breast cancer. *Breast Cancer Research*, 9(3):3–9.

Khanjani, S. and Panay, N. (2019). Vaginal estrogen deficiency. *The Obstetrician & Gynaecologist*, 21(1):37–42.

Khera, A. V., Chaffin, M., Aragam, K. G., Haas, M. E., Roselli, C., Choi, S. H., Natarajan, P., Lander, E. S., Lubitz, S. A., Ellinor, P. T., and Kathiresan, S. (2018). Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nature Genetics*, 50(9):1219–1224.

Khosla, S. (2006). Editorial: Sex hormone binding globulin: Inhibitor or facilitator (or both) of sex steroid action? *Journal of Clinical Endocrinology and Metabolism*, 91(12):4764–4766.

Kim, S. M., Kim, Y., Jeong, K., Jeong, H., and Kim, J. (2018). Logistic LASSO regression for the diagnosis of breast cancer using clinical demographic data and the BI-RADS lexicon for ultrasonography. *Ultrasonography*, 37(1):36–42.

Kleinbaum, D. G. and Klein, M. (2010). *Logistic regression*. Springer-Verlag New York Inc., New York, 3rd edition.

Kligman, L. and Younus, J. (2010). Management of hot flashes in women with breast cancer. *Current Oncology*, 17(1):81–86.

Klok, M. D., Jakobsdottir, S., and Drent, M. L. (2007). The role of leptin and ghrelin in the regulation of food intake and body weight in humans: A review. *Obesity Reviews*, 8:21–34.

Kobayashi, S., Inoue, S., Hosoi, T., Ouchi, Y., Shiraki, M., and Orimo, H. (1996). Association of bone mineral density with polymorphism of the estrogen receptor gene. *Journal of Bone and Mineral Research*, 11(3):306–311.

Kohavi, R. (1995). A study of cross-validation and bootstrap for accuracy estimation and model selection. *International Joint Conference of Artificial Intelligence*, (June).

Koš, M., Reid, G., Denger, S., and Gannon, F. (2001). Minireview: Genomic organization of the human ERα gene promoter region. *Molecular Endocrinology*, 15(12):2057–2063.

Kotsopoulos, J., Shafrir, A. L., Rice, M., Hankinson, S. E., Eliassen, H., Tworoger, S. S., and Narod, S. A. (2015). The relationship between bilateral oophorectomy and plasma hormone levels in postmenopausal women. *Hormonal Cancers*, 6(1):54–63.

Kozyreva, T. V., Meyta, E. S., and Khramova, G. M. (2015). Effect of the sympathetic nervous system co-transmitters ATP and norepinephrine on thermoregulatory response to cooling. *Temperature*, 2(1):121–128.

Krause, M., Wheeler, T. L., Richter, H. E., Snyder, T. E., and Richter, H. E. (2010). Systemic effects of vaginally administered estrogen therapy: A review. *Female Pelvic Medicine and Reconstructive Surgery*, 16(3):188–195.

Krishnan, K., Baglietto, L., Stone, J., Simpson, J. A., Severi, G., Evans, C. F., MacInnis, R. J., Giles, G. G., Apicella, C., and Hopper, J. L. (2017). Longitudinal study of mammographic density measures that predict breast cancer risk. *Cancer Epidemiology Biomarkers and Prevention*, 26(4):651–660.

Kroenke, C. H., Caan, B. J., Stefanick, M. L., Brzyski, R., Johnson, K. C., Leblanc, E., Lee, C., Croix, A. Z. L., Park, H. L., and Stacy, T. (2012). Effects of a dietary intervention and weight change on vasomotor symptoms in the Women's Health Initiative. *Menopause*, 19(9):980–988.

Kronenberg, F. (1990). Hot flashes: Epidemiology and physiology. *Annals of the New York Academy of Sciences*, 592(1):52–86.

Kronenberg, F. (2010). Menopausal hot flashes: a review of physiology and biosociocultural perspective on methods of assessment. *The Journal of Nutrition*, 140(7):1380S–1385S.

Kuchenbaecker, K. B., Hopper, J. L., Barnes, D. R., Phillips, K. A., Mooij, T. M., Roos-Blom, M. J., Jervis, S., Van Leeuwen, F. E., Milne, R. L., Andrieu, N., Goldgar, D. E., Terry, M. B., Rookus, M. A., Easton, D. F., and Antoniou, A. C. (2017). Risks

of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. *JAMA - Journal of the American Medical Association*, 317(23):2402–2416.

Kuckleburg, C. J., Tilkens, S. M., Santoso, S., and Newman, P. J. (2012). Proteinase 3 contributes to transendothelial migration of NB1- positive neutrophils. *Journal of Immunology*, 188(5):2419–2426.

Labrie, F., Archer, D. F., Koltun, W., Vachon, A., Young, D., Frenette, L., Portman, D., Montesino, M., Côté, I., Parent, J., Lavoie, L., Beauregard, A., Martel, C., Vaillancourt, M., Balser, J., and Moyneur, É. (2016). Efficacy of intravaginal dehydroepiandrosterone (DHEA) on moderate to severe dyspareunia and vaginal dryness, symptoms of vulvovaginal atrophy, and of the genitourinary syndrome of menopause. *Menopause*, 23(3):243–256.

Land, S. R., Walcott, F. L., Liu, Q., Wickerham, D. L., Costantino, J. P., and Ganz, P. A. (2016). Symptoms and QOL as predictors of chemoprevention adherence in NRG oncology/ NSABP trial P-1. *Journal of the National Cancer Institute*, 108(4):djv365.

Lasley, B. L., Santoro, N., Randolf, J. F., Gold, E. B., Crawford, S., Weiss, G., McConnell, D. S., and Sowers, M. F. (2002). The relationship of circulating dehydroepiandrosterone, testosterone, and estradiol to stages of the menopausal transition and ethnicity. *Journal of Clinical Endocrinology and Metabolism*, 87(8):3760–3767.

Lattrich, C., Schüler, S., Häring, J., Skrzypczak, M., Ortmann, O., and Treeck, O. (2014). Effects of a combined treatment with tamoxifen and estrogen receptor β agonists on human breast cancer cell lines. *Archives of Gynecology and Obstetrics*, 289(1):163–171.

Laurent, M. R., Hammond, G. L., Blokland, M., Jardí, F., Antonio, L., Dubois, V., Khalil, R., Sterk, S. S., Gielen, E., Decallonne, B., Carmeliet, G., Kaufman, J. M., Fiers, T., Huhtaniemi, I. T., Vanderschueren, D., and Claessens, F. (2016). Sex hormone-binding globulin regulation of androgen bioactivity in vivo: Validation of the free hormone hypothesis. *Scientific Reports*, 6:35539.

Le Bail, J. C., Liagre, B., Vergne, P., Bertin, P., Beneytout, J. L., and Habrioux, G. (2001). Aromatase in synovial cells from postmenopausal women. *Steroids*, 66(10):749–757.

Lebbe, M. and Woodruff, T. K. (2013). Involvement of androgens in ovarian health and disease. *Molecular Human Reproduction*, 19(12):828–837.

Lee, E. T. and Wang, J. W. (2013). *Statistical methods for survial data analysis*. John Wiley & Sons, Hoboken, New Jersey, 1st edition.

Lester, J., Pahouja, G., Andersen, B., and Lustberg, M. (2015). Atrophic vaginitis in breast cancer survivors: A difficult survivorship issue. *Journal of Personalized Medicine*, 5(2):50–66.

Lethaby, A., Ayeleke, R., and Roberts, H. (2016). Local oestrogen for vaginal atrophy in postmenopausal women (Review). *Cochrane Database of Systematic Reviews*, (8).

Lettre, G., Lange, C., and Hirschhorn, J. N. (2007). Genetic model testing and statistical power in population- based association studies of quantitative traits. *Genetic Epidemiology*, 31(8):358–362.

Lewis, C. M. (2002). Genetic association studies: design, analysis and interpretation. *Briefings in bioinformatics*, 3(2):146–153.

Li, H., Han, D., Hou, Y., Chen, H., and Chen, Z. (2015). Statistical inference methods for two crossing survival curves: A comparison of methods. *PLoS ONE*, 10(1):e0116774.

Li, S., Herrera, G. G., Tam, K. K., Lizarraga, J. S., Beedle, M. T., and Winuthayanon, W. (2018). Estrogen action in the epithelial cells of the mouse vagina regulates neutrophil infiltration and vaginal tissue integrity. *Scientific Reports*, 8(1):11247.

Liao, D. J. and Dickson, R. B. (2002). Roles of androgens in the development, growth, and carcinogenesis of the mammary gland. *Journal of Steroid Biochemistry and Molecular Biology*, 80(2):175–189.

Lim, Y. C., Desta, Z., Flockhart, D. A., and Skaar, T. C. (2005). Endoxifen (4-hydroxy-N-desmethyl-tamoxifen) has anti-estrogenic effects in breast cancer cells with potency similar to 4-hydroxy-tamoxifen. *Cancer Chemotherapy and Pharmacology*, 55(5):471–478.

Lin, J. H., Zhang, S. M., and Manson, J. E. (2011). Predicting adherence to tamoxifen for breast cancer adjuvant therapy and prevention. *Cancer Prevention Research*, 4(9):1360–1365.

Lintermans, A., Van Asten, K., Jongen, L., Van Brussel, T., Laenen, A., Verhaeghe, J., Vanderschueren, D., Lambrechts, D., and Neven, P. (2016). Genetic variant in the osteoprotegerin gene is associated with aromatase inhibitor-related musculoskeletal toxicity in breast cancer patients. *European Journal of Cancer*, 56:31–36.

Lippi, G., Mattiuzzi, C., and Montagnana, M. (2017). BRCA population screening for predicting breast cancer: For or against. *Annals of Translational Medicine*, 5(13):275.

Lisboa, F. A., Warren, J., Sulkowski, G., Aparicio, M., David, G., Zudaire, E., and Dveksler, G. S. (2011). Pregnancy-specific glycoprotein 1 induces endothelial tubulogenesis through interaction with cell surface proteoglycans. *Journal of Biological Chemistry*, 286(9):7577–7586.

Liu, K., Zhang, W., Dai, Z., Wang, M., Tian, T., Liu, X., Kang, H., Guan, H., Zhang, S., and Dai, Z. (2018a). Association between body mass index and breast cancer risk: Evidence based on a dose–response meta-analysis. *Cancer Management and Research*, 10:143–151.

Liu, L., Yu, Y., Fei, Z., Li, M., Wu, F. X., Li, H. D., Pan, Y., and Wang, J. (2018b). An interpretable boosting model to predict side effects of analgesics for osteoarthritis. *BMC Systems Biology*, 12(Suppl 6):105.

Liu, M., Wang, L., Bongartz, T., Hawse, J. R., Markovic, S. N., Schaid, D. J., Mushiroda, T., Kubo, M., Nakamura, Y., Kamatani, N., Goss, P. E., Ingle, J. N., and Weinshilboum, R. M. (2012). Aromatase inhibitors, estrogens and musculoskeletal pain: Estrogen-dependent T-cell leukemia 1A (TCL1A) gene-mediated regulation of cytokine expression. *Breast Cancer Research*, 14:R41.

Lockwood, C. J. (2011). Mechanisms of normal and abnormal endometrial bleeding. *Menopause*, 18(4):408–411.

Losordo, D. W. and Isner, J. M. (2001). Estrogen and angiogenesis. A review. *Arteriosclerosis Thrombosis and Vascular Biology*, 21:6–12.

Love, R. R., Kurtycz, D. F., Dumesic, D. A., Laube, D. W., and Yang, Y. (2000). The effects of tamoxifen on the vaginal epithelium in postmenopausal women. *Journal of Women's Health*, 9(5):559–563.

Luheshi, G. N., Gardner, J. D., Rushforth, D. A., Loudon, A. S., and Rothwell, N. J. (1999). Leptin actions on food intake and body temperature are mediated by IL-1. *Proceedings of the National Academy of Sciences of the United States of America*, 96(12):7047–7052.

Lundin, M., Lundin, J., Burke, H. B., Toikkanen, S., Pylkkanen, L., and Joensuu,

H. (1999). Artificial neural networks applied to survival prediction in breast cancer. *Oncology*, 57:281–286.

Lyon, E., Gastier Foster, J., Palomaki, G. E., Pratt, V. M., Reynolds, K., Fernanda Sábato, M., Scott, S. A., and Vitazka, P. (2012). Laboratory testing of CYP2D6 alleles in relation to tamoxifen therapy. *Genetics in Medicine*, 14(12):990–1000.

Ma, H., Bernstein, L., Pike, M. C., and Ursin, G. (2006). Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: A meta-analysis of epidemiological studies. *Breast Cancer Research*, 8(4):R43.

Ma, H., Henderson, K. D., Sullivan-Halley, J., Duan, L., Marshall, S. F., Ursin, G., Horn-Ross, P. L., Largent, J., Deapen, D. M., Lacey, J. V., and Bernstein, L. (2010). Pregnancy-related factors and the risk of breast carcinoma in situ and invasive breast cancer among postmenopausal women in the California Teachers Study cohort. *Breast Cancer Research*, 12:R35.

Maas, P., Barrdahl, M., Joshi, A. D., Auer, P. L., Mia, M., Milne, R. L., Schumacher, F. R., Anderson, W. F., Check, D., Chattopadhyay, S., Baglietto, L., Berg, Christine, D., Chanock, S. J., Cox, D. G., Figueroa, J. D., Gail, M., Graubard, B. I., Haiman, C. A., ..., and Chatterjee, N. (2016). Breast cancer risk from modifiable and non-modifiable risk factors among white women in the United States. *JAMA oncology*, 2(10):1295–1302.

Mac Bride, M. B., Rhodes, D. J., and Shuster, L. T. (2010). Vulvo-vaginal atrophy. *Mayo Clinic Proc*, 85(1):87–94.

Machiela, M. J. and Chanock, S. J. (2015). LDlink: A web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics*, 31(21):3555–3557.

Machin, D., Cheung, Y. B., and Parmar, M. K. B. (2006). *Survival analysis. A practical approach*. John Wiley & Sons, Chichester, UK, 2nd edition.

MacInnis, R. J., English, D. R., Haydon, A. M., Hopper, J. L., Gertig, D. M., and Giles, G. G. (2004). Body size and composition and risk of postmenopausal breast cancer. *Cancer Epidemiology Biomarkers and Prevention*, 13(12):2117 –2125.

MacLennan, A. H., Broadbent, J., Lester, S., and Moore, V. (2004). Oral oestrogen

and combined oestrogen/progestogen therapy versus placebo for hot flushes (Review). *Cochrane Database of Systematic Reviews*, (4).

Madigan, M. P., Troisi, R., Potischman, N., Dorgan, J. F., Brinton, L. A., and Hoover, R. N. (1998). Serum hormone levels in relation to reproductive and lifestyle factors in postmenopausal women (United States). *Cancer Causes and Control*, 9(2):199–207.

Maggi, A., Ciana, P., Belcredito, S., and Vegeto, E. (2004). Estrogens in the nervous system: Mechanisms and nonreproductive functions. *Annual Review of Physiology*, 66:291–313.

Maggio, M., Lauretani, F., Basaria, S., Ceda, G. P., Bandinelli, S., Metter, E. J., Bos, A. J., Ruggiero, C., Ceresini, G., Paolisso, G., Artoni, A., Valenti, G., Guralnik, J. M., and Ferrucci, L. (2008). Sex hormones binding globulin levels across the adult lifespan in women - The role of body mass index and fasting insulin. *Journal of Endocrinological Investigation*, 31(7):597–601.

Magliano, M. (2010). Menopausal arthralgia: Fact or fiction. *Maturitas*, 67(1):29–33.

Makara-Studzińska, M. T., Krys-Noszczyk, K. M., and Jakiel, G. (2014). Epidemiology of the symptoms of menopause - An intercontinental review. *Przeglad Menopauzalny*, 13(3):203–211.

Makubate, B., Donnan, P. T., Dewar, J. A., Thompson, A. M., and McCowan, C. (2013). Cohort study of adherence to adjuvant endocrine therapy, breast cancer recurrence and mortality. *British Journal of Cancer*, 108(7):1515–1524.

Malkin, D. (2011). Li-Fraumeni Syndrome. *Genes and Cancer*, 2(4):475–484.

Maltais, M. L., Desroches, J., and Dionne, I. J. (2009). Changes in muscle mass and strength after menopause. *Journal of Musculoskeletal Neuronal Interactions*, 9(4):186–197.

Manickum, T. and John, W. (2015). The current preference for the immuno-analytical ELISA method for quantitation of steroid hormones (endocrine disruptor compounds) in wastewater in South Africa. *Analytical and Bioanalytical Chemistry*, 407(17):4949–4970.

Manjer, J., Johansson, R., and Lenner, P. (2005). Smoking as a determinant for plasma levels of testosterone, androstenedione, and DHEAs in postmenopausal women. *European Journal of Epidemiology*, 20(4):331–337.

Mantas, D., Kostakis, J. D., and Markopoulos, C. (2016). Aromatase inhibitors: A comprehensive review in mechanisms of action, side effects and treatment in post-menopausal early breast cancer patients. *Hellenic Journal of Surgery*, 88(4):245–251.

Marees, A. T., de Kluiver, H., Stringer, S., Vorspan, F., Curis, E., Marie-Claire, C., and Derks, E. M. (2018). A tutorial on conducting genome-wide association studies: Quality control and statistical analysis. *International Journal of Methods in Psychiatric Research*, 27(2):e1608.

Mavaddat, N., Antoniou, A. C., Easton, D. F., and Garcia-Closas, M. (2010a). Genetic susceptibility to breast cancer. *Molecular Oncology*, 4(3):174–191.

Mavaddat, N., Michailidou, K., Dennis, J., Lush, M., Fachal, L., Lee, A., Tyrer, J. P., Chen, T. H., Wang, Q., Bolla, M. K., Yang, X., Adank, M. A., Ahearn, T., Aittomäki, K., Allen, J., Andrulis, I. L., Anton-Culver, H., Antonenkova, N. N., Arndt, V., ..., and Easton, D. F. (2019). Polygenic risk scores for prediction of breast cancer and breast cancer subtypes. *American Journal of Human Genetics*, 104:21–34.

Mavaddat, N., Pharoah, P. D., Blows, F., Driver, K. E., Provenzano, E., Thompson, D., MacInnis, R. J., Shah, M., Easton, D. F., and Antoniou, A. C. (2010b). Familial relative risks for breast cancer by pathological subtype: A population-based cohort study. *Breast Cancer Research*, 12:R10.

MBL Life Science (2017). The principle and method of ELISA. Available from: https://ruo.mbl.co.jp/bio/e/support/method/elisa.html.

McCormack, V. A. and Dos Santos Silva, I. (2006). Breast density and parenchymal patterns as markers of breast cancer risk: A meta-analysis. *Cancer Epidemiology Biomarkers and Prevention*, 15(6):1159–1169.

McCormack, V. A., Dowsett, M., Folkerd, E., Johnson, N., Palles, C., Coupland, B., Holly, J. M., Vinnicombe, S. J., Perry, N. M., and Dos Santos Silva, I. (2009). Sex steroids, growth factors and mammographic density: A cross-sectional study of UK postmenopausal Caucasian and Afro-Caribbean women. *Breast Cancer Research*, 11(3):R38.

McCowan, C., Wang, S., Thompson, A. M., Makubate, B., and Petrie, D. J. (2013). The value of high adherence to tamoxifen in women with breast cancer: A community-based cohort study. *British Journal of Cancer*, 109(5):1172–1180.

McTiernan, A., Martin, C. F., Peck, J. D., Aragaki, A. K., Chlebowski, R. T., Pisano, E. D., Wang, C. Y., Brunner, R. L., Johnson, K. C., Manson, J. A. E., Lewis, C. E., Kotchen, J. M., Hulka, B. S., Alving, B., Rossouw, J., Pottern, L., Prentice, R., Anderson, G., LaCroix, A., ..., and Bonds, D. (2005). Estrogen-plus-progestin use and mammographic density in postmenopausal women: Women's health initiative randomized trial. *Journal of the National Cancer Institute*, 97(18):1366–1376.

Metz, C. E. (1978). Basic principles of ROC analysis. *Seminars in Nuclear Medicine*, 8(4):283–298.

Meyer, H. (2018). *plinkQC: Genotype quality control in genetic association studies.*

Michailidou, K., Beesley, J., Lindstrom, S., Canisius, S., Dennis, J., Lush, M., Maranian, M. J., Bolla, M. K., Wang, Q., Shah, M., Perkins, B. J., Czene, K., Eriksson, M., Darabi, H., Brand, J. S., Bojesen, S. E., Nordestgaard, B. G., Flyger, H., Nielsen, S. F., ..., and Behrens, S. (2015). Genome-wide association analysis of more than 120,000 indviduals identifies 15 new susceptibility loci for breast cancer. *Nature Genetics*, 47(4):373–380.

Michailidou, K., Hall, P., Gonzalez-Neira, A., Ghoussaini, M., Dennis, J., Milne, R. L., Schmidt, M. K., Chang-Claude, J., Bojesen, S. E., Bolla, M. K., Wang, Q., Dicks, E., Lee, A., Turnbull, C., Rahman, N., Fletcher, O., Peto, J., Gibson, L., dos Santos Silva, I., ..., and Easton, D. F. (2013). Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nature Genetics*, 45(4):353–361.

Michailidou, K., Lindström, S., Dennis, J., Beesley, J., Hui, S., Kar, S., Lemaçon, A., Soucy, P., Glubb, D., Rostamianfar, A., Bolla, M. K., Wang, Q., Tyrer, J., Dicks, E., Lee, A., Wang, Z., Allen, J., Keeman, R., Eilber, U., ..., and Easton, D. F. (2017). Association analysis identifies 65 new breast cancer risk loci. *Nature*, 551(7678):92–94.

Michnovicz, J. J., Hershcopf, R. J., Naganuma, H., Bradlow, H. L., and Fishman, J. (1986). Increased 2-Hydroxylation of Estradiol as a possible Mechanism for the Anti-Estrogenic Effect of Cigarette Smoking. *New England Journal of Medicine*, 315(1305):521–522.

Miegg, J. S. D., Morden, J. P., Bliss, J. M., Coombes, R. C., Van de Velde, C. J., Mieog, J. S. D., Morden, J. P., Bliss, J. M., Coombes, R. C., and Van de Velde, C. J. (2012). Carpal tunnel syndrome and musculoskeletal symptoms in postmenopausal women with early breast cancer treated with exemestane or tamoxifen after 2-3 years

of tamoxifen: A retrospective analysis of the Intergroup Exemestane Study. *The Lancet Oncology*, 13(4):420–432.

Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M., Ding, W., Bell, R., Rosenthal, J., Hussey, C., Tran, T., McClure, M., Frye, C., Hattier, T., Phelps, R., Haugen-Strano, A., ..., and Skolnick, M. H. (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science*, 266(5182):66–71.

Miller, S. R., Gallicchio, L. M., Lewis, L. M., Babus, J. K., Langenberg, P., Zacur, H. A., and Flaws, J. A. (2006). Association between race and hot flashes in midlife women. *Maturitas*, 54(3):260–269.

Million Women Study Collaborators (2003). Breast cancer and hormone-replacement therapy in the Million Women Study. *The Lancet*, 362:419–427.

Ming, C., Viassolo, V., Probst-Hensch, N., Chappuis, P. O., Dinov, I. D., and Katapodi, M. C. (2019). Machine learning techniques for personalized breast cancer risk prediction: Comparison with the BCRAT and BOADICEA models. *Breast Cancer Research*, 21:75.

Mishra, G. and Kuh, D. (2006). Perceived change in quality of life during the menopause. *Social Science and Medicine*, 62(1):93–102.

Mitchell, G., Antoniou, A. C., Warren, R., Peock, S., Brown, J., Davies, R., Mattison, J., Cook, M., Warsi, I., Evans, D. G., Eccles, D., Douglas, F., Paterson, J., Hodgson, S., Izatt, L., Cole, T., Burgess, L., Eeles, R., and Easton, D. F. (2006). Mammographic density and breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Cancer Research*, 66(3):1866–1872.

Modugno, F., Ngo, D. L., Allen, G. O., Kuller, L. H., Ness, R. B., Vogel, V. G., Costantino, J. P., and Cauley, J. A. (2006). Breast cancer risk factors and mammographic breast density in women over age 70. *Breast Cancer Research and Treatment*, 97(2):157–166.

Mom, C. H., Buijs, C., Willemse, P. H., Mourits, M. J., and De Vries, E. G. (2006). Hot flushes in breast cancer patients. *Critical Reviews in Oncology/Hematology*, 57(1):63–77.

Moncla, B. J., Chappell, C. A., Debo, B. M., and Meyn, L. A. (2016). The effects of hormones and vaginal microflora on the glycome of the female genital Tract: Cervical-vaginal fluid. *PLoS ONE*, 11(7):1–17.

Montgomery Rice, V., Limback, S. D., Roby, K. F., and Terranova, P. F. (1999). Tumor necrosis factor alpha inhibition of follicle-stimulating hormone- induced granulosa cell estradiol secretion in the human does not involve reduction of cAMP secretion but inhibition at post-cAMP site(s). *Endocrine*, 10(1):19–23.

Moons, K. G., Kengne, A. P., Woodward, M., Royston, P., Vergouwe, Y., Altman, D. G., and Grobbee, D. E. (2012). Risk prediction models: Development, internal validation, and assessing the incremental value of a new (bio)marker. *Heart*, 98(9):683–690.

Moore, T. and Dveksler, G. S. (2014). Pregnancy-specific glycoproteins: Complex gene families regulating maternal-fetal interactions. *International Journal of Developmental Biology*, 58(2-4):273–280.

Morandi, P., Rouzier, R., Altundag, K., Buzdar, A. U., Theriault, R. L., and Hortobagyi, G. (2004). The role of aromatase inhibitors in the adjuvant treatment of breast carcinoma: The M. D. Anderson Cancer Center evidence-based approach. *Cancer*, 101(7):1482–1489.

Morris, P. D., Malkin, C. J., Channer, K. S., and Jones, T. H. (2004). A mathematical comparison of techniques to predict biologically available testosterone in a cohort of 1072 men. *European Journal of Endocrinology*, 151(2):241–249.

Mortimer, J. E., Flatt, S. W., Parker, B. A., Gold, E. B., Wasserman, L., Natarajan, L., and Pierce, J. P. (2008). Tamoxifen, hot flashes and recurrence in breast cancer. *Breast Cancer Research and Treatment*, 108(3):421–426.

Mourits, M. J., De Vries, E. G., Willemse, P. H., Ten Hoor, K. A., Hollema, H., and Van Der Zee, A. G. (2001). Tamoxifen treatment and gynecologic side effects: A review. *Obstetrics and Gynecology*, 97(5):855–866.

Mürdter, T. E., Schroth, W., Bacchus-Gerybadze, L., Winter, S., Heinkele, G., Simon, W., Fasching, P. A., Fehm, T., Eichelbaum, M., Schwab, M., and Brauch, H. (2011). Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. *Clinical Pharmacology and Therapeutics*, 89(5):708–717.

Muti, P., Trevisan, M., Micheli, A., Krogh, V., Bolelli, G., Sciajno, R., and Berrino, F. (1996). Reliability of serum hormones in premenopausal and postmenopausal women over a one-year period. *Cancer Epidemiology Biomarkers and Prevention*, 5(11):917–922.

Nabholtz, J.-M. (2008). Long-term safety of aromatase inhibitors in the treatment of breast cancer. *Therapeutics and clinical risk management*, 4(1):189–204.

Nair, R. P., Duffin, K. C., Helms, C., Ding, J., Stuart, P. E., Goldgar, D., Gudjonsson, J. E., Li, Y., Tejasvi, T., Feng, B. J., Ruether, A., Schreiber, S., Weichenthal, M., Gladman, D., Schrodi, S. J., Prahalad, S., Guthery, S. L., and Fischer, J. (2009). Genomewide scan reveals association of psoriasis with IL-23 and NF-κB pathways. *Nature Genetics*, 41(2):199–204.

Napoli, N., Donepudi, S., Sheikh, S., Rini, G. B., and Armamento-Villareal, R. (2005). Increased 2-hydroxylation of estrogen in women with a family history of osteoporosis. *Journal of Clinical Endocrinology and Metabolism*, 90(4):2035–2041.

Nelson, H. D., Pappas, M., Cantor, A., Haney, E., and Holmes, R. (2019). Risk assessment, genetic counseling, and genetic testing for BRCA-related cancer in women: Updated evidence report and systematic review for the US Preventive Services Task Force. *JAMA - Journal of the American Medical Association*, 322(7):666–685.

Newcomb, P. A., Klein, R., Klein, B. E., Haffner, S., Mares-Perlman, J., Cruickshanks, K. J., and Marcus, P. M. (1995). Association of dietary and life-style factors with sex hormones in postmenopausal women. *Epidemiology*, 6(3):326–328.

Nguyen, T. L., Schmidt, D. F., Makalic, E., Dite, G. S., Stone, J., Apicella, C., Bui, M., MacInnis, R. J., Odefrey, F., Cawson, J. N., Treloar, S. A., Southey, M. C., Giles, G. G., and Hopper, J. L. (2013). Explaining variance in the Cumulus mammographic measures that predict breast cancer risk: A twins and sisters study. *Cancer Epidemiology Biomarkers and Prevention*, 22(12):2395–2403.

Niravath, P. (2013). Aromatase inhibitor-induced arthralgia: A review. *Annals of Oncology*, 24(6):1443–1449.

Notelovitz, M. (2002). Androgen effects on bone and muscle. *Fertility and Sterility*, 77(No.4 SUPPL. 4):34–41.

O'Brian, C., Liekamp, R. M., Solomon, D. H., and Weinstein, I. B. (1985). Inhibition of Protein Kinase C by tamoxifen. *Cancer Research*, 45:2462 – 2465.

Ochiel, D., Fahey, J., Ghosh, M., Haddad, S., and Wira, C. (2008). Innate immunity in the female reproductive tract: Role of sex hormones in regulating uterine epithelial cell protection against pathogens. *Current Women's Health Reviews*, 4(2):102–117.

Onland-Moret, N. C., Peeters, P. H., Van Der Schouw, Y. T., Grobbee, D. E., and Van Gils, C. H. (2005). Alcohol and endogenous sex steroid levels in postmenopausal women: A cross-sectional study. *Journal of Clinical Endocrinology and Metabolism*, 90(3):1414–1419.

Øverlie, I., Moen, M. H., Holte, A., and Finset, A. (2002). Androgens and estrogens in relation to hot flushes during the menopausal transition. *Maturitas*, 41(1):69–77.

Oza, A. M. and Boyd, N. F. (1993). Mammographic parenchymal patterns: A marker of breast cancer risk. *Epidemiologic Reviews*, 15(1):196–208.

Paech, K., Scanlan, T. S., Webb, P., Kushner, P. J., Kuiper, G. G. J. M., Nilsson, S., and Gustafsson, J.-Å. (1997). Differential ligand activation of estrogen receptors ERα and ERrβ at AP1 sites. *Science*, 277(5331):1508–1510.

Paige, L. A., Christensen, D. J., Gron, H., Norris, J. D., Gottlin, E. B., Padilla, K. M., Chang, C. Y., Ballas, L. M., Hamilton, P. T., McDonnell, D. P., and Fowlkes, D. M. (1999). Estrogen receptor (ER) modulators each induce distinct conformational changes in ER α and ER β. *Proceedings of the National Academy of Sciences of the United States of America*, 96(7):3999–4004.

Paridaens, R. J., Dirix, L. Y., Beex, L. V., Nooij, M., Cameron, D. A., Cufer, T., Piccart, M. J., Bogaerts, J., and Therasse, P. (2008). Phase III study comparing exemestane with tamoxifen as first-line hormonal treatment of metastatic breast cancer in postmenopausal women: The European Organisation for Research and Treatment of Cancer Breast Cancer Cooperative Group. *Journal of Clinical Oncology*, 26(30):4883–4890.

Park, H. A. (2013). An introduction to logistic regression: From basic concepts to interpretation with particular attention to nursing domain. *Journal of Korean Academy of Nursing*, 43(2):154–164.

Parker, M. G., Arbuckle, N., and Dauvois, S. (1993). Structure and function of the estrogen receptor. *Annals of the New York Academy of Sciences*, 684(1):119–126.

Partridge, A. H., LaFountain, A., Mayer, E., Taylor, B. S., Winer, E., and Asnis-Alibozek, A. (2008). Adherence to initial adjuvant anastrozole therapy among women with early-stage breast cancer. *Journal of Clinical Oncology*, 26(4):556–562.

Peng, C. Y. J., Lee, K. L., and Ingersoll, G. M. (2002). An introduction to logistic regression analysis and reporting. *Journal of Educational Research*, 96(1):3–14.

Perou, C. M., Sorlie, T., Eisen, M. B., Rijn, M. V. D., Jeffrey, S. S., Rees, C. A., Pollack, J. R., Ross, D. T., Johnsen, H., Akslen, L. A., Fluge, I., Pergamenschikov, A., Williams, C., Zhu, S. X., Lùnning, P. E., Brown, P. O., and Botstein, D. (2000). Molecular portraits breast cancer. *Nature*, 406:747–752.

Peto, R., Pike, M. C., Armitage, P., Breslow, N. E., Cox, D. R., Howard, S. V., Mantel, N., McPherson, K., Peto, J., and Smith, P. G. (1977). Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *British Journal of Cancer*, 35(1):1–39.

Pettersson, A., Hankinson, S. E., Willett, W. C., Lagiou, P., Trichopoulos, D., and Tamimi, R. M. (2011). Nondense mammographic area and risk of breast cancer. *Breast Cancer Research*, 13(5).

Pharoah, P. D., Lipscombe, J. M., Redman, K. L., Day, N. E., Easton, D. F., and Ponder, B. A. (2000). Familial predisposition to breast cancer in a British population: Implications for prevention. *European Journal of Cancer*, 36(6):773–779.

Philips, Z., Ginnelly, L., Sculpher, M., Claxton, K., Golder, S., Riemsma, R., Woolacoot, N., and Glanville, J. (2004). Review of guidelines for good practice in decision-analytic modelling in health technology assessment. *Health Technology Assessment*, 8(36).

Pike, M. C., Krailo, M. D., Henderson, B. E., Casagrande, J. T., and Hoel, D. G. (1983). 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. *Nature*, 303(5920):767–770.

Pike, M. C., Spicer, D., Dahmoush, L., and Press, M. F. (1993). Estrogens progestogens normal breast cell proliferation and breast cancer risk. *Epidemiologic Reviews*, 15(1):17–35.

Pluchino, N., Carmignani, A., Cubeddu, A., Santoro, A., Cela, V., and Alcalà, T. E. (2013). Androgen therapy in women: For whom and when. *Archives of Gynecology and Obstetrics*, 288(4):731–737.

Polin, S. A. and Ascher, S. M. (2008). The effect of tamoxifen on the genital tract. *Cancer Imaging*, 8(1):135–145.

Potischman, N., Swanson, C. A., Siiteri, P., and Hoover, R. N. (1996). Reversal of relation between body mass and endogenous estrogen concentrations with menopausal status. *Journal of the National Cancer Institute*, 88(11):756–758.

Powles, T., Eeles, R., Ashley, S., Easton, D., Chang, J., Dowsett, M., Tidy, A., Viggers, J., and Davey, J. (1998). Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. *Lancet*, 352(9122):98–101.

Powles, T., Jones, A. L., Ashley, S., O'Brien, M. E., Tidy, A., Treleavan, J., Cosgrove, D., Nash, A. G., Sacks, N., and Baum, M. (1994). The Royal Marsden Hospital pilot tamoxifen chemoprevention trial. *Breast Cancer Research and Treatment*, 31(1):73–82.

Powles, T. J., Ashley, S., Tidy, A., Smith, I. E., and Dowsett, M. (2007). Twenty-year follow-up of the Royal Marsden randomized, double-blinded tamoxifen breast cancer prevention trial. *Journal of the National Cancer Institute*, 99(4):283–290.

Prentice, R. L., Pettinger, M., and Anderson, G. L. (2005). Statistical issues arising in the Women's Health Initiative. *Biometrics*, 61(4):920–921.

Presant, C. A., Bosserman, L., Young, T., Vakil, M., Horns, R., Upadhyaya, G., Ebrahimi, B., Yeon, C., and Howard, F. (2007). Aromatase inhibitor-associated arthralgia and/or bone pain: Frequency and characterization in non-clinical trial patients. *Clinical Breast Cancer*, 7(10):775–778.

Puhalla, S., Bhattacharya, S., and Davidson, N. E. (2012). Hormonal therapy in breast cancer: A model disease for the personalization of cancer care. *Molecular Oncology*, 6(2):222–236.

Purohit, V. (2000). Can alcohol promote aromatization of androgens to estrogens? A review. *Alcohol*, 22(3):123–127.

Randolph, J. F., Sowers, M. F., Bondarenko, I., Gold, E. B., Greendale, G. A., Bromberger, J. T., Brockwell, S. E., and Matthews, K. A. (2005). The relationship

of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. *Journal of Clinical Endocrinology and Metabolism*, 90(11):6106–6112.

Ravdin, P. M., Cronin, K. A., Howlader, N., Berg, C. D., Chlebowski, R. T., Feuer, E. J., Edwards, B. K., and Berry, D. A. (2007). The becrease in breast-cancer Incidence in 2003 in the United States. *New England Journal of Medicine*, 356(16):1670–1674.

Reeves, G. K., Pirie, K., Beral, V., Green, J., Spencer, E., and Bull, D. (2007). Cancer incidence and mortality in relation to body mass index in the Million Women Study: Cohort study. *British Medical Journal*, 335(7630):1134–1145.

Regan, M. M., Price, K. N., Giobbie-Hurder, A., Thürlimann, B., and Gelber, R. D. (2011). Interpreting Breast International Group (BIG) 1-98: a randomized, double-blind, phase III trial comparing letrozole and tamoxifen as adjuvant endocrine therapy for postmenopausal women with hormone receptor-positive, early breast cancer. *Breast cancer research : BCR*, 13(3):209.

Reich, D. E. and Lander, E. S. (2001). On the allelic spectrum of human disease. *Trends in Genetics*, 17(9):502–510.

Reyes, C., Leyland, K. M., Peat, G., Cooper, C., Arden, N. K., and Prieto-Alhambra, D. (2016). Association between overweight and obesity and risk of clinically diagnosed knee, hip, and hand osteoarthritis: A population-based cohort study. *Arthritis and Rheumatology*, 68(8):1869–1875.

Rizzoli, R. and Bonjour, J. P. (1997). Hormones and bones. *Lancet*, 349(SUPPL.1):20–23.

Robinson, W. R., Tse, C. K., Olshan, A. F., and Troester, M. A. (2014). Body size across the life course and risk of premenopausal and postmenopausal breast cancer in Black women, the Carolina Breast Cancer Study, 1993–2001. *Cancer Causes and Control*, 25(9):1101–1117.

Ropka, M. E., Keim, J., and Philbrick, J. T. (2010). Patient decisions about breast cancer chemoprevention: A systematic review and meta-analysis. *Journal of Clinical Oncology*, 28(18):3090–3095.

Ropponen, A., Aittomäki, K., Vihma, V., Tikkanen, M. J., and Ylikorkala, O. (2005). Effects of oral and transdermal estradiol administration on levels of sex hormone-

binding globulin in postmenopausal women with and without a history of intrahepatic cholestasis of pregnancy. *Journal of Clinical Endocrinology and Metabolism*, 90(6):3431–3434.

Rosner, W., Auchus, R. J., Azziz, R., Sluss, P. M., and Raff, H. (2007). Position statement: Utility, limitations, and pitfalls in measuring testosterone: An endocrine society position statement. *Journal of Clinical Endocrinology and Metabolism*, 92(2):405–413.

Rosner, W., Hankinson, S. E., Sluss, P. M., Vesper, H. W., and Wierman, M. E. (2013). Challenges to the measurement of estradiol: An endocrine society position statement. *Journal of Clinical Endocrinology and Metabolism*, 98(4):1376–1387.

Rothwell, P. M. (2006). Factors that can affect the external validity of randomised controlled trials. *PLoS Clinical Trials*, 1(1):e9.

Roux, S. and Orcel, P. (2000). Bone loss: Factors that regulate osteoclast differentiation: An update. *Arthritis Research*, 2(6):451–456.

Royston, P. and Parmar, M. K. B. (2013). Restricted mean survival time: an alternative to the hazard ratio for the design and analysis of randomized trials with a time-to-event outcome. *BMCMedical Research Methodology*, 13(152).

Sabia, S., Fournier, A., Mesrine, S., Boutron-Ruault, M. C., and Clavel-Chapelon, F. (2008). Risk factors for onset of menopausal symptoms. Results from a large cohort study. *Maturitas*, 60(2):108–121.

Sachs, U. J., Andrei-Selmer, C. L., Maniar, A., Weiss, T., Paddock, C., Orlova, V. V., Eun, Y. C., Newman, P. J., Preissner, K. T., Chavakis, T., and Santoso, S. (2007). The neutrophil-specific antigen CD177 is a counter-receptor for platelet endothelial cell adhesion molecule-1 (CD31). *Journal of Biological Chemistry*, 282(32):23603–23612.

Saika, K. and Sobue, T. (2009). Epidemiology of breast cancer in Japan and the US. *Japan Medical Association Journal*, 52(1):39–44.

Salahshor, S., Goncalves, J., Chetty, R., Gallinger, S., and Woodgett, J. R. (2005). Differential gene expression profile reveals deregulation of pregnancy specific β1 glycoprotein 9 early during colorectal carcinogenesis. *BMC Cancer*, 5(66).

Samsioe, G. (2002). The role of ERT/HRT. *Best Practice and Research: Clinical Obstetrics and Gynaecology*, 16(3):371–381.

Sanchez Spitman, A. B., Moes, D. J., Gelderblom, H., Dezentje, V. O., Swen, J. J., and Guchelaar, H. J. (2017). Effect of CYP3A4*22, CYP3A5*3, and CYP3A combined genotypes on tamoxifen metabolism. *European Journal of Clinical Pharmacology*, 73(12):1589–1598.

Santen, R. J. (2011). Effect of endocrine therapies on bone in breast cancer patients. *Journal of Clinical Endocrinology and Metabolism*, 96(2):308–319.

Sasieni, P. D. (1997). From genotypes to genes : Doubling the sample size. *Biometrics*, 53(4):1253–1261.

Sassarini, J. and Lumsden, M. A. (2015). Oestrogen replacement in postmenopausal women. *Age and Ageing*, 44(4):551–558.

Saturni, S., Bellini, F., Braido, F., Paggiaro, P., Sanduzzi, A., Scichilone, N., Santus, P. A., Morandi, L., and Papi, A. (2014). Randomized controlled trials and real life studies. Approaches and methodologies: A clinical point of view. *Pulmonary Pharmacology and Therapeutics*, 27(2):129–138.

Scheffers, C., Armstrong, S., Cantineau, A., Farquhar, C., and Jordan, V. (2015). Dehydroepiandrosterone for women in the peri- or postmenopausal phase (Review). *Cochrane database of Systematic Reviews*, (1).

Schernhammer, E. S., Sperati, F., Razavi, P., Agnoli, C., Sieri, S., Berrino, F., Krogh, V., Abbagnato, C. A., Grioni, S., Blandino, G., Schunemann, H. J., and Muti, P. (2013). Endogenous sex steroids in premenopausal women and risk of breast cancer: The ORDET cohort. *Breast Cancer Research*, 15(3):R46.

Schiffer, L., Barnard, L., Baranowski, E. S., Gilligan, L. C., Taylor, A. E., Arlt, W., Shackleton, C. H., and Storbeck, K. H. (2019). Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: A comprehensive review. *Journal of Steroid Biochemistry and Molecular Biology*, 194(May):105439.

Schilling, C., Gallicchio, L. M., Miller, S. R., Langenberg, P., Zacur, H., and Flaws, J. A. (2007a). Current alcohol use, hormone levels, and hot flashes in midlife women. *Fertility and Sterility*, 87(6):1483–1486.

Schilling, C., Gallicchio, L. M., Miller, S. R., Langenberg, P., Zacur, H., and Flaws,

J. A. (2007b). Genetic polymorphisms, hormone levels, and hot flashes in midlife women. *Maturitas*, 57(2):120–131.

Schilling, C., Gallicchio, L. M., Miller, S. R., Langenberg, P., Zacur, H., and Flaws, J. A. (2007c). Relation of body mass and sex steroid hormone levels to hot flushes in a sample of mid-life women. *Climacteric*, 10(1):27–37.

Schoemaker, M. J., Folkerd, E. J., Jones, M. E., Rae, M., Allen, S., Ashworth, A., Dowsett, M., and Swerdlow, A. J. (2014). Combined effects of endogenous sex hormone levels and mammographic density on postmenopausal breast cancer risk: Results from the breakthrough generations study. *British Journal of Cancer*, 110(7):1898–1907.

Schoenfeld, D. (1982). Partial residuals for the proportional hazards regression model. *Biometrika*, 69(1):239–241.

Schultz, K. F. and Grimes, D. A. (2002a). Allocation concealment in randomised trails: Defending against deciphering. *The Lancet*, 359:614–618.

Schultz, K. F. and Grimes, D. A. (2002b). Generation of allocation sequences in randomised trials: chance not choice. *The Lancet*, 359:515–519.

Secreto, G., Girombelli, A., and Krogh, V. (2019). Androgen excess in breast cancer development: Implications for prevention and treatment. *Endocrine-Related Cancer*, 26(2):R81–R94.

Serin, I. S., Özçelik, B., Başbuğ, M., Aygen, E., Kula, M., and Erez, R. (2001). Long-term effects of continuous oral and transdermal estrogen replacement therapy on sex hormone binding globulin and free testosterone levels. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, 99(2):222–225.

Sestak, I., Cuzick, J., Sapunar, F., Eastell, R., Forbes, J. F., Bianco, A. R., and Buzdar, A. U. (2008). Risk factors for joint symptoms in patients enrolled in the ATAC trial: a retrospective, exploratory analysis. *The Lancet Oncology*, 9(9):866–872.

Sestak, I., Kealy, R., Nikoloff, M., Fontecha, M., Forbes, J. F., Howell, A., and Cuzick, J. (2012). Relationships between CYP2D6 phenotype, breast cancer and hot flushes in women at high risk of breast cancer receiving prophylactic tamoxifen: Results from the IBIS-I trial. *British Journal of Cancer*, 107(2):230–233.

Sestak, I., Smith, S. G., Howell, A., Forbes, J. F., and Cuzick, J. (2018). Early participant-reported symptoms as predictors of adherence to anastrozole in the International Breast Cancer Intervention Studies II. *Annals of Oncology*, 29(2):504–509.

Shang, Y. and Brown, M. (2002). Molecular determinants for the tissue specificity of SERMs. *Science*, 295(5564):2465–2468.

Shawon, S. R., Eriksson, M., and Li, J. (2017). Body size in early life and risk of breast cancer. *Breast Cancer Research*, 19(1):84.

Shieh, Y., Scott, C. G., Jensen, M. R., Norman, A. D., Bertrand, K. A., Pankratz, V. S., Brandt, K. R., Visscher, D. W., Shepherd, J. A., Tamimi, R. M., Vachon, C. M., and Kerlikowske, K. (2019). Body mass index, mammographic density, and breast cancer risk by estrogen receptor subtype. *Breast Cancer Research*, 21(1):48.

Shiovitz, S. and Korde, L. A. (2015). Genetics of breast cancer: A topic in evolution. *Annals of Oncology*, 26(7):1291–1299.

Shobeiri, F., Jenabi, E., Poorolajal, J., and Hazavehei, S. M. M. (2016). The association between body mass index and hot flash in midlife women: A meta-analysis. *Journal of Menopausal Medicine*, 22:14–19.

Sickles, E. A., D'Orsi, C. J., Bassett, L. W., and Al, E. (2013). ACR BI-RADS® mammography. In: ACR BI-RADS® Atlas, Breast Imaging Reporting and Data System. Reston, VA. *American College of Radiology*.

Simpson, E. R. (2003). Sources of estrogen and their importance. *Journal of Steroid Biochemistry and Molecular Biology*, 86(3-5):225–230.

Sims, N. A., Dupont, S., Krust, A., Clement-Lacroix, P., Minet, D., Resche-Rigon, M., Gaillard- Kelly, M., and Baron, R. (2002). Deletion of estrogen receptors reveals a regulatory role for estrogen receptors-β in bone remodeling in females but not in males. *Bone*, 30(1):18–25.

Singletary, K. W. and Gapstur, S. M. (2001). Alcohol and breast cancer. Review of epidemiologic and experimental evidence and potential mechanisms. *JAMA - Journal of the American Medical Association*, 286(17):2143–2151.

Sisti, J. S., Bernstein, J. L., Lynch, C. F., Reiner, A. S., Mellemkjaer, L., Brooks, J. D., Knight, J. A., Bernstein, L., Malone, K. E., Woods, M., Liang, X., and John, E. M.

(2015). Reproductive factors, tumor estrogen receptor status and contralateral breast cancer risk: results from the WECARE study. *SpringerPlus*, 4(825).

Sivaraman, L. and Medina, D. (2002). Hormone-induced protection against breast cancer. *Journal of Mammary Gland Biology and Neoplasia*, 7(1):77–92.

Skol, A. D., Sasaki, M. M., and Onel, K. (2016). The genetics of breast cancer risk in the post-genome era: Thoughts on study design to move past BRCA and towards clinical relevance. *Breast Cancer Research*, 18:99.

Smith, S. G., Sestak, I., Forster, A., Partridge, A., Side, L., Wolf, M. S., Horne, R., Wardle, J., and Cuzick, J. (2016). Factors affecting uptake and adherence to breast cancer chemoprevention: a systematic review and meta-analysis. *Annals of Oncology*, 27(4):575–590.

Smith, S. G., Sestak, I., Howell, A., Forbes, J., and Cuzick, J. (2017). Participant-reported symptoms and their effect on long-term adherence in the international breast cancer intervention study I (IBIS I). *Journal of Clinical Oncology*, 35(23):2666–2673.

Soguel, L., Durocher, F., Tchernof, A., and Diorio, C. (2017). Adiposity, breast density, and breast cancer risk: Epidemiological and biological considerations. *European Journal of Cancer Prevention*, 26(6):511–520.

Somner, J., McLellan, S., Cheung, J., Mak, Y. T., Frost, M. L., Knapp, K. M., Wierzbicki, A. S., Wheeler, M., Fogelman, I., Ralston, S. H., and Hampson, G. N. (2004). Polymorphisms in the P450 c17 (17-Hydroxylase/17,20-Lyase) and P450 c19 (Aromatase) Genes: Association with Serum Sex Steroid Concentrations and Bone Mineral Density in Postmenopausal Women. *Journal of Clinical Endocrinology and Metabolism*, 89(1):344–351.

Sowers, M. F. R., Wildman, R. P., Mancuso, P., Eyvazzadeh, A. D., Karvonen-Gutierrez, C. A., Rillamas-Sun, E., and Jannausch, M. L. (2008). Change in adipocytokines and ghrelin with menopause. *Maturitas*, 59(2):149–157.

Spicer, D. V., Ursin, G. A., Parisky, Y. R., Pearce, J. G., Shoupe, D., Pike, A., and Pike, M. C. (1994). Changes in mammographic density induced by a hormonal contraceptive designed to reduce breast cancer risk. *Journal of the National Cancer Institute*, 86(6):431–436.

Stanczyk, F. Z., Cho, M. M., Endres, D. B., Morrison, J. L., Patel, S., and Paulson, R. J. (2003). Limitations of direct estradiol and testosterone immunoassay kits. *Steroids*, 68(14):1173–1178.

Staropoli, C. . A., Flaws, J. A., Bush, T., and Moulton, A. W. (1998). Predictors of menopausal hot flashes. *Journal of Women's Health*, 7(9):1149–1155.

Stearns, V., Chapman, J. A. W., Le Maitre, A., Kundapur, J., Shepherd, L. E., Pritchard, K. I., Ma, C. X., Ellis, M. J., Ingle, J. N., Budd, G. T., Rabaglio, M., Sledge, G. W., Liedke, P. E., and Goss, P. E. (2015). Treatment-associated musculoskeletal and vasomotor symptoms and relapse-free survival in the NCIC CTG MA.27 adjuvant breast cancer aromatase inhibitor trial. *Journal of Clinical Oncology*, 33(3):265–271.

Stearns, V., Johnson, M. D., Rae, J. M., Morocho, A., Novielli, A., Bhargava, P., Hayes, D. F., Desta, Z., and Flockhart, D. A. (2003). Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *Journal of the National Cancer Institute*, 95(23):1758–1764.

Stearns, V., Ullmer, L., Lo'pez, J. F., Smith, Y., Isaacs, C., and Hayes, D. F. (2002). Hot flushes. *Lancet*, 360:1851–1861.

Stone, J., Dite, G. S., Gunasekara, A., English, D. R., McCredie, M. R., Giles, G. G., Cawson, J. N., Hegele, R. A., Chiarelli, A. M., Yaffe, M. J., Boyd, N. F., and Hopper, J. L. (2006). The heritability of mammographically dense and nondense breast tissue. *Cancer Epidemiology Biomarkers and Prevention*, 15(4):612–617.

Sturdee, D. W. (2008). The menopausal hot flush-Anything new? *Maturitas*, 60(1):42–49.

Sturdee, D. W., Hunter, M. S., Maki, P. M., Gupta, P., Sassarini, J., Stevenson, J. C., and Lumsden, M. A. (2017). The menopausal hot flush: a review. *Climacteric*, 20(4):296–305.

Styrkarsdottir, U., Halldorsson, B. V., Gretarsdottir, S., Gudbjartsson, D. F., Walters, G. B., Ingvarsson, T., Jonsdottir, T., Saemundsdottir, J., Center, J. R., Nguyen, T. V., Bagger, Y., Gulcher, J. R., Eisman, J. A., Christiansen, C., Sigurdsson, G., Kong, A., Thorsteinsdottir, U., and Stefansson, K. (2008). Multiple genetic loci for bone mineral density and fractures. *New England Journal of Medicine*, 358(22):2355–2365.

Suskin, J. and Shapiro, C. L. (2018). Osteoporosis and musculoskeletal complications related to therapy of breast cancer. *Gland Surgery*, 7(4):411–423.

Suzuki, R., Iwasaki, M., Inoue, M., Sasazuki, S., Sawada, N., Yamaji, T., Shimazu, T., and Tsugane, S. (2011). Body weight at age 20 years, subsequent weight change and breast cancer risk defined by estrogen and progesterone receptor status-the Japan public health center-based prospective study. *International Journal of Cancer*, 129(5):1214–1224.

Suzuki, R., Iwasaki, M., Inoue, M., Sasazuki, S., Sawada, N., Yamaji, T., Shimazu, T., Tsugane, S., Sobue, T., Hanaoka, T., Ogata, J., Baba, S., Mannami, T., Okayama, A., Kokubo, Y., Miyakawa, K., Saito, F., Koizumi, A., Sano, Y., ..., and Kono, S. (2010). Alcohol consumption-associated breast cancer incidence and potential effect modifiers: The Japan Public Health Center-based prospective study. *International Journal of Cancer*, 127(3):685–695.

Szymczak, J., Milewicz, A., Thijssen, J. H., Blankenstein, M. A., and Daroszewski, J. (1998). Concentration of sex steroids in adipose tissue after menopause. *Steroids*, 63:319–321.

Taira, N., Arai, M., Ikeda, M., Iwasaki, M., Okamura, H., Takamatsu, K., Nomura, T., Yamamoto, S., Ito, Y., and Mukai, H. (2016). The Japanese breast cancer society clinical practice guidelines for epidemiology and prevention of breast cancer, 2015 edition. *Breast Cancer*, 23:343–356.

Tamimi, R. M., Byrne, C., Colditz, G. A., and Hankinson, S. E. (2007). Endogenous hormone levels, mammographic density, and subsequent risk of breast cancer in postmenopausal women. *Journal of the National Cancer Institute*, 99(15):1178–1187.

Tamimi, R. M., Cox, D., Kraft, P., Colditz, G. A., Hankinson, S. E., and Hunter, D. J. (2008). Breast cancer susceptibility loci and mammographic density. *Breast Cancer Research*, 10(4):R66.

Tamimi, R. M., Hankinson, S. E., Colditz, G. A., and Byrne, C. (2005). Endogenous sex hormone levels and mammographic density among postmenopausal women. *Cancer Epidemiology Biomarkers and Prevention*, 14(11):2641–2647.

Tankó, L. B. and Christiansen, C. (2004). An update on the antiestrogenic effect of smoking: A literature review with implications for researchers and practitioners. *Menopause*, 11(1):104–109.

Taylor, A. E., Keevil, B., and Huhtaniemi, I. T. (2015). Mass spectrometry and immunoassay: How to measure steroid hormones today and tomorrow. *European Journal of Endocrinology*, 173(2):D1–D12.

The 1000 Genomes Project Consortium (2015). A global reference for human genetic variation. *Nature*, 526:68–74.

The ATAC (Arimidex Tamoxifen Alone or in Combination) Trialists' Group (2002). Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early-stage breast cancer. *The Lancet*, 359(9):2131–2139.

The Breast International Group (BIG) 1-98 Collaborative Group (2005). A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *The New England Journal of Medicine*, 353(26):2747–2757.

The International HapMap Consortium (2005). A haplotype map of the human genome. *Nature*, 437(27):1299–1320.

The Premenopausal Breast Cancer Collaborative Group. (2018). Association of body mass index and age with subsequent breast cancer risk in premenopausal women. *JAMA Oncology*, 4(11):1–10.

The Wellcome Trust Case Control Consortium (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*, 447(7):661–678.

Thurston, R. C. and Joffe, H. (2011). Vasomotor symptoms and menopause: Findings from the Study of Women's Health across the Nation. *Obstetrics and Gynecology Clinics of North America*, 38(3):489–501.

Thurston, R. C., Sowers, M. F. R., Chang, Y., Sternfeld, B., Gold, E. B., Johnston, J. M., and Matthews, K. A. (2008). Adiposity and reporting of vasomotor symptoms among midlife women. *American Journal of Epidemiology*, 167(1):78–85.

Tibshirani, R. (1996). Regression shrinkage and selection via the Lasso. *Journal of the Royal Statistical Society. Series B (Methodolgical)*, 58(1):267–288.

Tin Tin, S., Reeves, G. K., and Key, T. J. (2021). Endogenous hormones and risk of invasive breast cancer in pre- and post-menopausal women: findings from the UK Biobank. *British Journal of Cancer*.

Titus-Ernstoff, L., Tosteson, A. N., Kasales, C., Weiss, J., Goodrich, M., Hatch, E. E., and Carney, P. A. (2006). Breast cancer risk factors in relation to breast density (United States). *Cancer Causes and Control*, 17(10):1281–1290.

Toniolo, P., Koenig, K. L., Pasternack, B. S., Banerjee, S., Rosenberg, C., Shore, R. E., Strax, P., and Levitz, M. (1994). Reliability of measurements of total, protein-bound, and unbound estradiol in serum. *Cancer Epidemiology Biomarkers and Prevention*, 3(1):47–50.

Toniolo, P. G., Levitz, M., Zeleniuch-jacquotte, A., Banerjee, S., Koenig, K. L., Shore, R. E., Strax, P., and Pasternack, B. S. (1995). A prospective study of endogenous estrogens and breast cancer in postmenopausal women. *Journal of the National Cancer Institute*, 87(3):190–197.

Torres-Mejía, G., De Stavola, B., Allen, D. S., Pérez-Gavilán, J. J., Ferreira, J. M., Fentiman, I. S., and Dos Santos Silva, I. (2005). Mammographic features and subsequent risk of breast cancer: A comparison of qualitative and quantitative evaluations in the Guernsey prospective studies. *Cancer Epidemiology Biomarkers and Prevention*, 14(5):1052–1059.

Travis, R. C. and Key, T. J. (2003). Oestrogen exposure and breast cancer risk. *Breast Cancer Research*, 5:239–247.

Treeck, O., Lattrich, C., Springwald, A., and Ortmann, O. (2010). Estrogen receptor beta exerts growth-inhibitory effects on human mammary epithelial cells. *Breast Cancer Research and Treatment*, 120(3):557–565.

Trémollieres, F. A., Ceausu, I., Depypere, H., Lambrinoudaki, I., Mueck, A., Pérez-López, F. R., van der Schouw, Y. T., Senturk, L. M., Simoncini, T., Stevenson, J. C., Stute, P., and Rees, M. (2017). Osteoporosis management in patients with breast cancer: EMAS position statement. *Maturitas*, 95:65–71.

Tucker, A. N., Tkaczuk, K. A., Lewis, L. M., Tomic, D., Lim, C. K., and Flaws, J. A. (2005). Polymorphisms in cytochrome P4503A5 (CYP3A5) may be associated with race and tumor characteristics, but not metabolism and side effects of tamoxifen in breast cancer patients. *Cancer Letters*, 217(1):61–72.

Turcato, E., Zamboni, M., De Pergola, G., Armellini, F., Zivelonghi, A., Bergamo-Andreis, I. A., Giorgino, R., and Bosello, O. (1997). Interrelationships between weight

loss, body fat distribution and sex hormones in pre- and postmenopausal obese women. *Journal of Internal Medicine*, 241(5):363–372.

Tworoger, S. S., Chubak, J., Aiello, E. J., Ulrich, C. M., Atkinson, C., Potter, J. D., Yasui, Y., Stapleton, P. L., Lampe, J. W., Farin, F. M., and Stanczyk, F. Z. (2004). Association of CYP17, CYP19, CYP1B1, and COMT polymorphisms with serum and urinary sex hormone concentrations in postmenopausal women. *Cancer Epidemiology, Biomarkers & Prevention*, 13(1):94–101.

Tworoger, S. S., Eliassen, A. H., Missmer, S. A., Baer, H., Rich-Edwards, J., Michels, K. B., Barbieri, R. L., Dowsett, M., and Hankinson, S. E. (2006). Birthweight and body size throughout life in relation to sex hormones and prolactin concentrations in premenopausal women. *Cancer Epidemiology Biomarkers & Prevention*, 15(12):2494–2501.

Tworoger, S. S., Zhang, X., Eliassen, A. H., Qian, J., Colditz, G. A., Willett, W. C., Rosner, B. A., Kraft, P., and Hankinson, S. E. (2014). Inclusion of endogenous hormone levels in risk prediction models of postmenopausal breast cancer. *Journal of Clinical Oncology*, 32(28):3111–3117.

Tyrer, J., Duffy, S. W., and Cuzick, J. (2004). A breast cancer prediction model incorporating familial and personal risk factors. *Statistics in Medicine*, 23(7):1111–1130.

Uddin, S., Khan, A., Hossain, M. E., and Moni, M. A. (2019). Comparing different supervised machine learning algorithms for disease prediction. *BMC Medical Informatics and Decision Making*, 19(281).

Urban, R. J., Bodenburg, Y. H., Gilkison, C., Foxworth, J., Coggan, A. R., Wolfe, R. R., and Ferrando, A. (1995). Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. *American Journal of Physiology*, 269(5 Pt 1):E820 – E826.

Ursin, G., Lillie, E. O., Lee, E., Cockburn, M., Schork, N. J., Cozen, W., Parisky, Y. R., Hamilton, A. S., Astrahan, M. A., and Mack, T. (2009). The relative importance of genetics and environment on mammographic density. *Cancer Epidemiology Biomarkers and Prevention*, 18(1):102–112.

Ursin, G., Palla, S. L., Reboussin, B. A., Slone, S., Wasilauskas, C., Pike, M. C., and Greendale, G. A. (2004). Post-treatment change in serum estrone predicts mammographic percent density changes in women who received combination estrogen and progestin in the postmenopausal estrogen/progestin interventions (PEPI) trial. *Journal of Clinical Oncology*, 22(14):2845–2848.

Ursin, G. and Qureshi, S. A. (2009). Mammographic density - A useful biomarker for breast cancer risk in epidemiologic studies. *Norsk Epidemiologi*, 19(1):59–68.

US Preventive Services Task Force (2019). Medication use to reduce risk of breast cancer: US Preventive Services Task Force Recommendation statement. *Jama*, 322(9):857–867.

Utian, W. H. (2005). Psychosocial and socioeconomic burden of vasomotor symptoms in menopause: A comprehensive review. *Health and Quality of Life Outcomes*, 3(47).

Vachon, C. M., Pankratz, V. S., Scott, C. G., Haeberle, L., Ziv, E., Jensen, M. R., Brandt, K. R., Whaley, D. H., Olson, J. E., Heusinger, K., Hack, C. C., Jud, S. M., Beckmann, M. W., Schulz-Wendtland, R., Tice, J. A., Norman, A. D., Cunningham, J. M., Purrington, K. S., Easton, D. F., ..., and Couch, F. J. (2015). The contributions of breast density and common genetic variation to breast cancer risk. *Journal of the National Cancer Institute*, 107(5).

Vachon, C. M., van Gils, C. H., Sellers, T. A., Ghosh, K., Pruthi, S., Brandt, K. R., and Pankratz, V. S. (2007). Mammographic density, breast cancer risk and risk prediction. *Breast Cancer Research*, 9(217).

van Buuren, S. and Groothuis-Oudshoorn, K. (2011). MICE: Multivariate imputation by chained equations in R. *Journal of Statistical Software*, 45(3):2–20.

Van De Velde, C. J., Rea, D., Seynaeve, C., Putter, H., Hasenburg, A., Vannetzel, J. M., Paridaens, R., Markopoulos, C., Hozumi, Y., Hille, E. T., Kieback, D. G., Asmar, L., Smeets, J., Nortier, J. W., Hadji, P., Bartlett, J. M., and Jones, S. E. (2011). Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): A randomised phase 3 trial. *The Lancet*, 377(9762):321–331.

van Veen, E. M., Brentnall, A. R., Byers, H., Harkness, E. F., Astley, S. M., Sampson, S., Howell, A., Newman, W. G., Cuzick, J., and Evans, D. G. R. (2019). Use of single-nucleotide polymorphisms and mammographic density plus classic risk factors for breast cancer risk prediction. *JAMA Oncology*, 4(4):476–482.

Veronesi, U., Maisonneuve, P., Costa, A., Sacchini, V., Maltoni, C., Robertson, C., Rotmensz, N., and Boyle, P. (1998). Prevention of breast cancer with tamoxifen: Preliminary findings from the Italian randomised trial among hysterectomised women. *Lancet*, 352(9122):93–97.

Veronesi, U., Maisonneuve, P., Rotmensz, N., Bonanni, B., Boyle, P., Viale, G., Costa, A., Sacchini, V., Travaglini, R., D'Aiuto, G., Oliviero, P., Lovison, F., Gucciardo, G., del Turco, M. R., Muraca, M. G., Pizzichetta, M. A., Conforti, S., and Decensi, A. (2007). Tamoxifen for the prevention of breast cancer: Late results of the Italian randomized tamoxifen prevention trial among women with hysterectomy. *Journal of the National Cancer Institute*, 99(9):727–737.

Veronesi, U., Rotmensz, N., Costa, A., Sacchini, V., Travaglini, R., Aiuto, D., Lovison, F., Gucciardo, G., Grazia, M., Antonietta, M., Decensi, A., and Robertson, C. (2003). Italian randomized trial among women with hysterectomy : Tamoxifen and cancer in high-risk women. *Journal of the National Cancer Institute*, 95(2):160–165.

Vesper, H. W., Botelho, J. C., Vidal, M., Rahmani, Y., Thienpont, L. M., and Caudill, S. P. (2014). High variability in serum estrodiol measurements in men and women. *Steroids*, 82:7–13.

Vinogradova, Y., Coupland, C., and Hippisley-Cox, J. (2019). Use of hormone replacement therapy and risk of venous thromboembolism: Nested case-control studies using the QResearch and CPRD databases. *BMJ (Online)*, 364(k4810).

Vogel, V. G., Costantino, J. P., Wickerham, D. L., Cronin, W. M., Cecchini, R. S., Atkins, J. N., Bevers, T. B., Fehrenbacher, L., Pajon, E. R., Wade, J. L., Robidoux, A., Margolese, R. G., James, J., Runowicz, C. D., Ganz, P. A., Reis, S. E., McCaskill-Stevens, W., Ford, L. G., Jordan, V. C., and Wolmark, N. (2010). Update of the national surgical adjuvant breast and bowel project Study of Tamoxifen and Raloxifene (STAR) P-2 trial: Preventing breast cancer. *Cancer Prevention Research*, 3(6):696–706.

Waaseth, M., Bakken, K., Dumeaux, V., Olsen, K. S., Rylander, C., Figenschau, Y., and Lund, E. (2008). Hormone replacement therapy use and plasma levels of sex hormones in the Norwegian Women and Cancer Postgenome Cohort - A cross-sectional analysis. *BMC Women's Health*, 8(1).

Wacholder, S., Hartge, P., Prentice, R., Garcia-Closas, M., Feigelson, H. S., Diver, W. R., Thun, M. J., Cox, D. G., Hankinson, S. E., Kraft, P., Rosner, B., Berg, C. D., Brinton, L. A., Lissowska, J., Sherman, M. E., Chlebowski, R., Kooperberg, C., Jackson, R. D., Buckman, D. W., ..., and Hunter, D. J. (2010). Performance of common genetic variants in breast-cancer risk models. *New England Journal of Medicine*, 362(11):986–993.

Walitt, B., Nahin, R. L., Katz, R. S., Bergman, M. J., and Wolfe, F. (2015). The prevalence and characteristics of fibromyalgia in the 2012 national health interview survey. *PLoS ONE*, 10(9):e0138024.

Walker, K., Fletcher, O., Johnson, N., Coupland, B., McCormack, V. A., Folkerd, E., Gibson, L., Hillier, S. G., Holly, J. M., Moss, S., Dowsett, M., Peto, J., and Silva, I. D. S. (2009). Premenopausal mammographic density in relation to cyclic variations in endogenous sex hormone levels, prolactin, and insulin-like growth factors. *Cancer Research*, 69(16):6490–6499.

Warren, R., Skinner, J., Sala, E., Denton, E., Dowsett, M., Folkerd, E., Healey, C. S., Dunning, A., Doody, D., Ponder, B., Luben, R. N., Day, N. E., and Easton, D. (2006). Associations among mammographic density, circulating sex hormones, and polymorphisms in sex hormone metabolism genes in postmenopausal women. *Cancer Epidemiology Biomarkers and Prevention*, 15(8):1502–1508.

Warwick, J., Birke, H., Stone, J., Warren, R. M., Pinney, E., Brentnall, A. R., Duffy, S. W., Howell, A., and Cuzick, J. (2014). Mammographic breast density refines Tyrer-Cuzick estimates of breast cancer risk in high-risk women: Findings from the placebo arm of the International Breast Cancer Intervention Study I. *Breast Cancer Research*, 16(451).

Wasserman, N. F., Aneas, I., and Nobrega, M. A. (2010). An 8q24 gene desert variant associated with prostate cancer risk confers differential in vivo activity to a MYC enhancer. *Genome Research*, 20(9):1191–1197.

Weber, B. L. and Nathanson, K. L. (2000). Low penetrance genes associated with increased risk for breast cancer. *European Journal of Cancer*, 36(10):1193–1199.

Weinshilboum, R. M. and Wang, L. (2017). Pharmacogenomics: Precision medicine and drug response. *Mayo Clinic Proceedings*, 92(11):1711–1722.

Welton, A. J., Vickers, M. R., Kim, J., Ford, D., Lawton, B. A., MacLennan, A. H., Meredith, S. K., Martin, J., and Meade, T. W. (2008). Health related quality of life after combined hormone replacement therapy: Randomised controlled trial. *British Medical Journal*, 337(7669):550–553.

Whiteman, M. K., Staropoli, C. A., Benedict, J. C., Borgeest, C., and Flaws, J. A. (2003a). Risk factors for hot flashes in midlife women. *Journal of Women's Health*, 12(5):459 – 472.

Whiteman, M. K., Staropoli, C. A., Langenberg, P. W., McCarter, R. J., Kjerulff, K. H., and Flaws, J. A. (2003b). Smoking, body mass, and hot flashes in midlife women. *Obstetrics and Gynecology*, 101(2):264–272.

Wilbur, J., Miller, A. M., Montgomery, A., and Chandler, P. (1998). Sociodemograhic characteristics, biological factors, and symptom reporting in midlife women. *Menopause*, 5(1):43–51.

Williams, C. and Lin, C. Y. (2013). Oestrogen receptors in breast cancer: Basic mechanisms and clinical implications. *Ecancermedicalscience*, 7(370).

Wira, C. R., Fahey, J. V., Sentman, C. L., Pioli, P. A., and Shen, L. (2005). Innate and adaptive immunity in female genital tract: Cellular responses and interactions. *Immunological Reviews*, 206:306–335.

Witherby, S., Johnson, J., Demers, L., Mount, S., Littenberg, B., Maclean, C. D., Wood, M., and Muss, H. (2011). Topical testosterone for breast cancer patients with vaginal atrophy related to aromatase inhibitors: A phase I/II study. *The Oncologist*, 16(4):424–431.

Woods, N. F. and Mitchell, E. S. (2005). Symptoms during the perimenopause: Prevalence, severity, trajectory, and significance in women's lives. *American Journal of Medicine*, 118(12 SUPPL. 2):14–24.

Woolf, A. D. and Pfleger, B. (2003). Burden of major muscoloskeletal conditions. *Bulletin of the World Health Organization*, 81(9):646–656.

Wooster, R., Neuhausen, S. L., Mangion, J., Quirk, Y., Ford, D., Collins, N., Nguyen, K., Seal, S., Tran, T., Averill, D., Fields, P., Marshall, G., Narod, S., Lenoir, G. M., Lynch, H., Feunteun, J., Devilee, P., Cornelisse, C. J., Menko, F. H., ..., and Strat-

ton, M. R. (1994). Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science*, 265(5181):2088–2090.

World Cancer Research Fund (2017). Diet, nutrition, physical activity and breast cancer. Continuous update project expert report 2017. Technical report.

Writing Group for the Women's Health Initiative Investigators (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women. *Jama*, 288(3):321–333.

Wu, F., Ames, R., Evans, M. C., France, J. T., and Reid, I. R. (2001). Determinants of sex hormone-binding globulin in normal postmenopausal women. *Clinical Endocrinology*, 54(1):81–87.

Wu, X., Hawse, J. R., Subramaniam, M., Goetz, M. P., Ingle, J. N., and Spelsberg, T. C. (2009). The tamoxifen metabolite, endoxifen, is a potent antiestrogen that targets estrogen receptor a for degradation in breast cancer cells. *Cancer Research*, 69(5):1722–1727.

Xue, X., Xie, X., Gunter, M., Rohan, T. E., Wassertheil-Smoller, S., Ho, G. Y., Cirillo, D., Yu, H., and Strickler, H. D. (2013). Testing the proportional hazards assumption in case-cohort analysis. *BMC Medical Research Methodology*, 13(88).

Yang, L., Hu, S., Tan, J., Zhang, X., Yuan, W., Wang, Q., Xu, L., Liu, J., Liu, Z., Jia, Y., and Huang, X. (2016). Pregnancy-specific glycoprotein 9 (PSG9), a driver for colorectal cancer, enhances angiogenesis via activation of SMAD4. *Oncotarget*, 7(38):61562–61574.

Yao, D., Yang, J., and Zhan, X. (2013). A novel method for disease prediction: Hybrid of random forest and multivariate adaptive regression splines. *Journal of Computers (Finland)*, 8(1):170–177.

Yeomans-Kinney, A., Richards, C., Vernon, S. W., Vogel, V. G., Kinney, A. Y., Richards, C., Vernon, S. W., Vogel, V. G., Yeomans-Kinney, A., Richards, C., Vernon, S. W., and Vogel, V. G. (1998). The effect of physician recommendation on enrollment in the breast cancer chemoprevention trial. *Preventive Medicine*, 27(5):713–719.

Yoo, T.-K., Jang, M.-j., Lee, E., Moon, H.-G., Noh, D.-Y., and Han, W. (2018). Endocrine treatment-related symptoms and patient outcomes in breast cancer: A meta-analysis. *Journal of Breast Cancer*, 21(1):37–44.

Youden, W. J. (1950). Index for rating diagnostic tests. *Cancer*, 3(1):32–35.

Zachow, R. J. and Magoffin, D. A. (1997). Direct intraovarian effects of leptin: Impairment of the synergistic action of insulin-like growth factor-I on follicle-stimulating hormone-dependent estradiol-17β production by rat ovarian granulosa cells. *Endocrinology*, 138(2):847–850.

Zeleniuch-Jacquotte, A., Gu, Y., Shore, R. E., Koenig, K. L., Arslan, A. A., Kato, I., Rinaldi, S., Kaaks, R., and Toniolo, P. (2005). Postmenopausal levels of sex hormones and risk of breast carcinoma in situ: Results of a prospective study. *International Journal of Cancer*, 114(2):323–327.

Zembutsu, H., Nakamura, S., Akashi-Tanaka, S., Kuwayama, T., Watanabe, C., Takamaru, T., Takei, H., Ishikawa, T., Miyahara, K., Matsumoto, H., Hasegawa, Y., Kutomi, G., Shima, H., Satomi, F., Okazaki, M., Zaha, H., Onomura, M., Matsukata, A., Sagara, Y., ..., and Nakamura, Y. (2017). Significant effect of polymorphisms in CYP2D6 on response to tamoxifen therapy for breast cancer: A prospective multicenter study. *Clinical Cancer Research*, 23(8):2019–2026.

Zewdu, A. M. (2009). Survival curve convergences and crossings: How frequent are they in medical research? A study of five medical journals.

Zhang, X., Tworoger, S. S., Eliassen, A. H., and Hankinson, S. E. (2013). Postmenopausal plasma sex hormone levels and breast cancer risk over 20 years of follow-up. *Breast Cancer Research and Treatment*, 137(3):88–892.

Zhang, Y. and Yang, Y. (2015). Cross-validation for selecting a model selection procedure. *Journal of Econometrics*, 187(1):95–112.

Zhang, Z., Peluso, M. J., Gross, C. P., Viscoli, C. M., and Kernan, W. N. (2014). Adherence reporting in randomized controlled trials. *Clinical Trials*, 11(2):195–204.

Zheng, S. L., Zheng, W., Chang, B. L., Shu, X. O., Cai, Q., Yu, H., Dai, Q., Xu, J., and Gao, Y. T. (2003). Joint effect of estrogen receptor β sequence variants and endogenous estrogen exposure on breast cancer risk in Chinese women. *Cancer Research*, 63(22):7624–7629.

Zheng, W., Long, J., Gao, Y.-T., Li, C., Zheng, Y., Xiang, Y.-B., Wen, W., Levy, S., Deming, S. L., Haines, J. L., Gu, K., Fair, A. M., Cai, Q., Lu, W., and Shu, X.-O.

(2009). Genome-wide association study identifies a novel breast cancer susceptibility locus at 6q25.1. *Nature Genetics*, 41(3):324–328.

Ziegler, R. G., Hoover, R. N., Pike, M. C., Hildesheim, A., Nomura, A. M., West, D. W., Wu-williams, A. H., Kolonel, L. N., Horn-ross, P. L., Rosenthal, J. F., and Hyer, M. B. (1993). Migration patterns and breast cancer risk in Asian-American women. *Journal of the National Cancer Institute*, 85(22):1819–1827.

Ziv, E., Tice, J., Smith-Bindman, R., Shepherd, J., Cummings, S., and Kerlikowske, K. (2004). Mammographic density and estrogen receptor status of breast cancer. *Cancer Epidemiology Biomarkers and Prevention*, 13(12):2090–2095.

Ziv-Gal, A. and Flaws, J. A. (2010). Factors that may influence the experience of hot flushes by healthy middle-aged women. *Journal of Women's Health*, 19(10):1905–1914.

Zöllner, S. and Pritchard, J. K. (2007). Overcoming the winner's curse: Estimating penetrance parameters from case-control data. *American Journal of Human Genetics*, 80(4):605–615.

Zumoff, B. (1982). Relationship of obesity to blood estrogens. *Cancer Research*, 42(8 Suppl.):3289–3292.

Zurrida, S. and Veronesi, U. (2015). Milestones in breast cancer treatment. *Breast Journal*, 21(1):3–12.

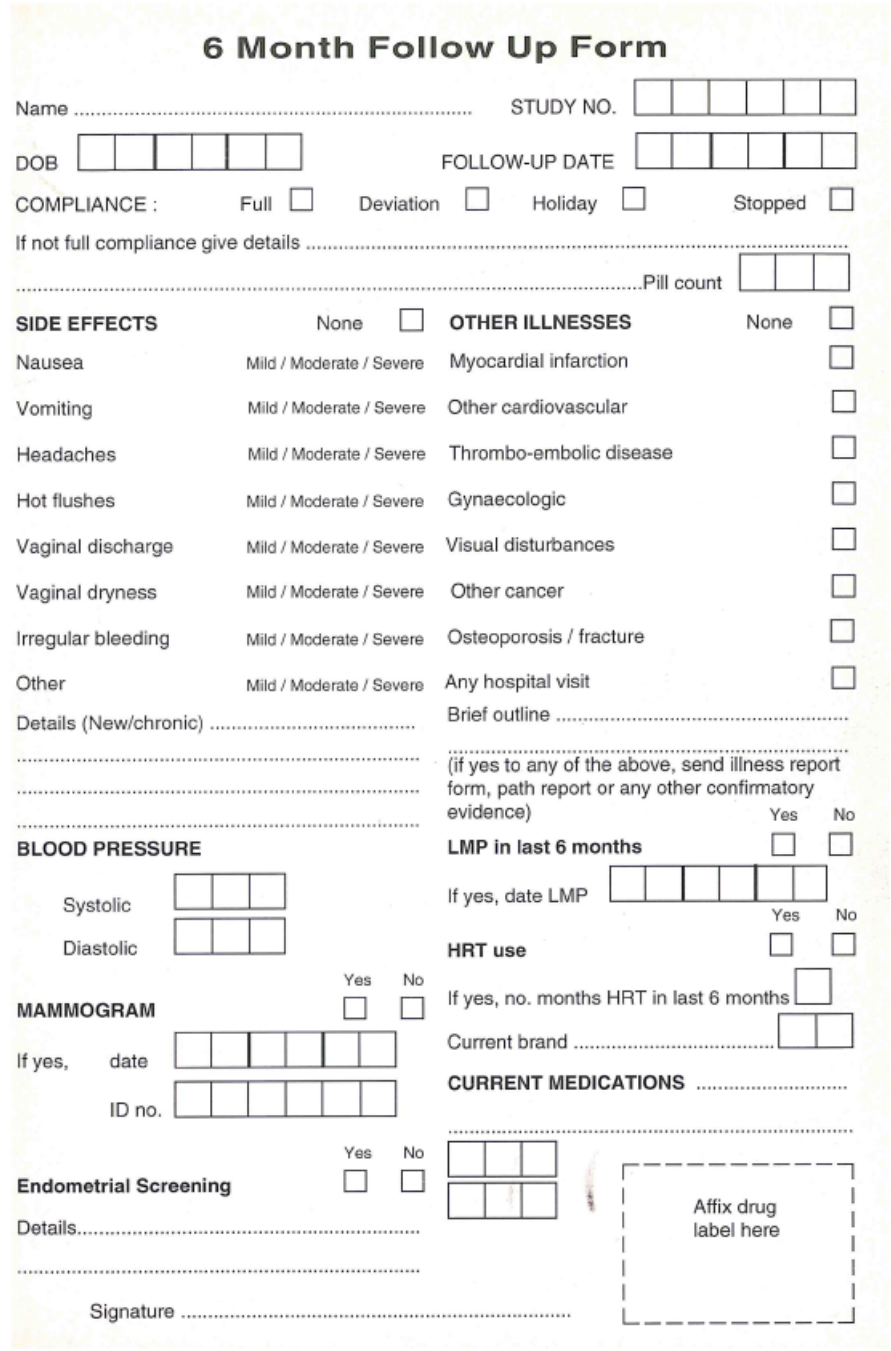
Chapter A: Appendix 1

Table A.1: Author's contribution to each chapter

	Chapter 4	Chapter 5	Chapter 6	Chapter 7	Chapter 8
IBIS-I Study design	Jack Cuzick (J.C.), Anthony Howell (A.H), and John Forbes (J.F.F.)				
IBIS-II Study design	J.C., J.F.F., Simon Cawthorn (S.C.), Mitch Dowsett (M.D.), Robert E Mansel (R.E.M.), Sibylle Loibl (S.L.), Bernardo Bonanni (B.B.)				
IBIS-I data collection	A.H., S.C., Hamish Hamed, Kaija Holli, and J.F.F.				
IBIS-II data collection	J.C., J.F.F., S.C., R.E.M., S.L., B.B., D Gareth Evans, A.H.				
Study design	Michael Hale (M.H.)	M.H.	M.H.	M.H.	M.H.
Data cleaning	M.H.	M.H.	M.H.	M.H.	M.H.
Quality control	M.H.	M.H.	M.H.	M.H.	M.H.
Sample indentification	NA	J.C.	Ivana Sestak	NA	NA
Sample picking	NA	J.C.	M.H.	NA	NA
Genotyping or sample measurement	NA	Myriad Genetics	M.H.	NA	NA
Calculation of hormone concentrations from raw data	NA	NA	M.H.	NA	NA
Data analysis	M.H.	M.H.	M.H.	M.H.	M.H.
Manuscript preparation					M.H.*

* All other authors provided comments and input for final manuscript submission. These included Ivana Sestak, J.C., A.H. and M.D.

Chapter B: Appendix 2



6 Month Follow Up Form

Name ..... STUDY NO.

DOB FOLLOW-UP DATE

COMPLIANCE : Full Deviation Holiday Stopped

If not full compliance give details .....

.....Pill count

SIDE EFFECTS None

Nausea Mild / Moderate / Severe

Vomiting Mild / Moderate / Severe

Headaches Mild / Moderate / Severe

Hot flushes Mild / Moderate / Severe

Vaginal discharge Mild / Moderate / Severe

Vaginal dryness Mild / Moderate / Severe

Irregular bleeding Mild / Moderate / Severe

Other Mild / Moderate / Severe

Details (New/chronic) .....

.....

.....

.....

BLOOD PRESSURE

Systolic

Diastolic

MAMMOGRAM Yes No

If yes, date

ID no.

Endometrial Screening Yes No

Details.....

.....

Signature .....

OTHER ILLNESSES None

Myocardial infarction

Other cardiovascular

Thrombo-embolic disease

Gynaecologic

Visual disturbances

Other cancer

Osteoporosis / fracture

Any hospital visit

Brief outline .....

.....

(if yes to any of the above, send illness report form, path report or any other confirmatory evidence)

LMP in last 6 months Yes No

If yes, date LMP

HRT use Yes No

If yes, no. months HRT in last 6 months

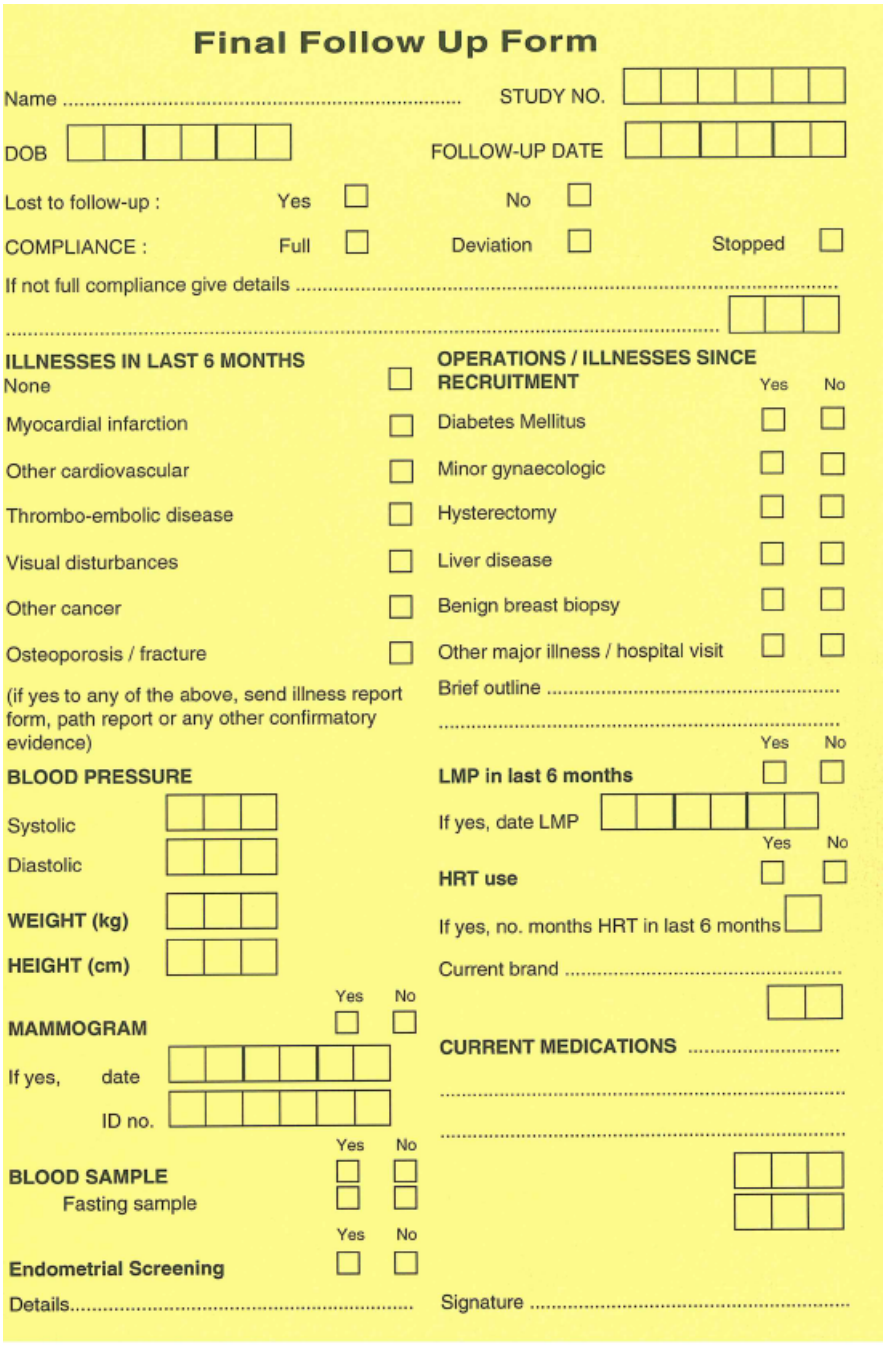
Current brand .....

CURRENT MEDICATIONS .....

.....

Affix drug label here

Figure B.1: IBIS-I six month follow up CRF form



Final Follow Up Form

Name ..... STUDY NO.

DOB FOLLOW-UP DATE

Lost to follow-up : Yes No

COMPLIANCE : Full Deviation Stopped

If not full compliance give details .....

.....

ILLNESSES IN LAST 6 MONTHS

None

Myocardial infarction

Other cardiovascular

Thrombo-embolic disease

Visual disturbances

Other cancer

Osteoporosis / fracture

(if yes to any of the above, send illness report form, path report or any other confirmatory evidence)

OPERATIONS / ILLNESSES SINCE RECRUITMENT

	Yes	No
Diabetes Mellitus		
Minor gynaecologic		
Hysterectomy		
Liver disease		
Benign breast biopsy		
Other major illness / hospital visit		

Brief outline .....

.....

BLOOD PRESSURE

Systolic

Diastolic

WEIGHT (kg)

HEIGHT (cm)

MAMMOGRAM Yes No

If yes, date

ID no.

BLOOD SAMPLE Yes No

Fasting sample

Endometrial Screening Yes No

Details.....

LMP in last 6 months Yes No

If yes, date LMP

HRT use Yes No

If yes, no. months HRT in last 6 months

Current brand .....

CURRENT MEDICATIONS .....

.....

.....

Signature .....

Figure B.2: IBIS-I final follow up CRF form

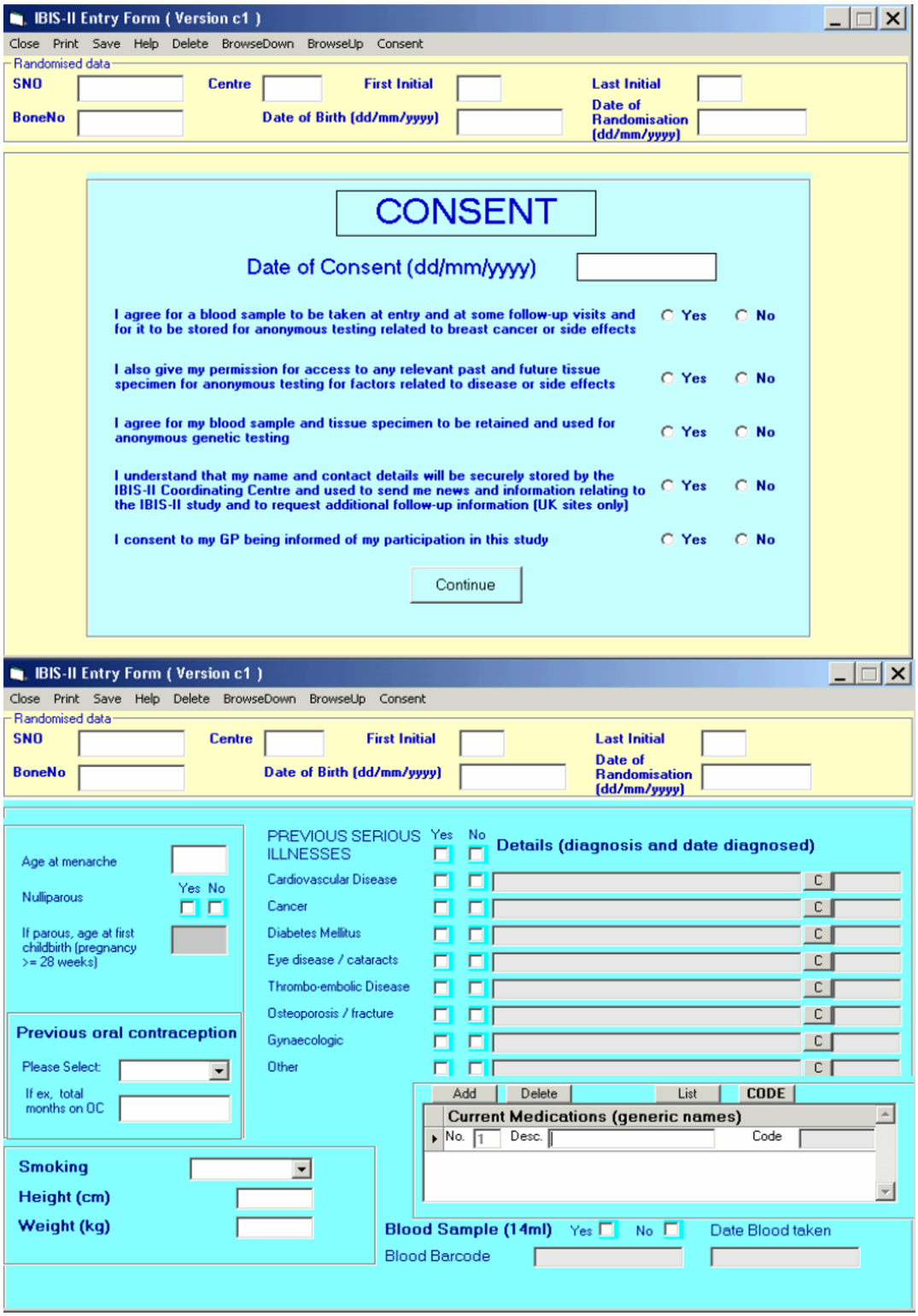


Figure B.3: IBIS-II Entry CRF form

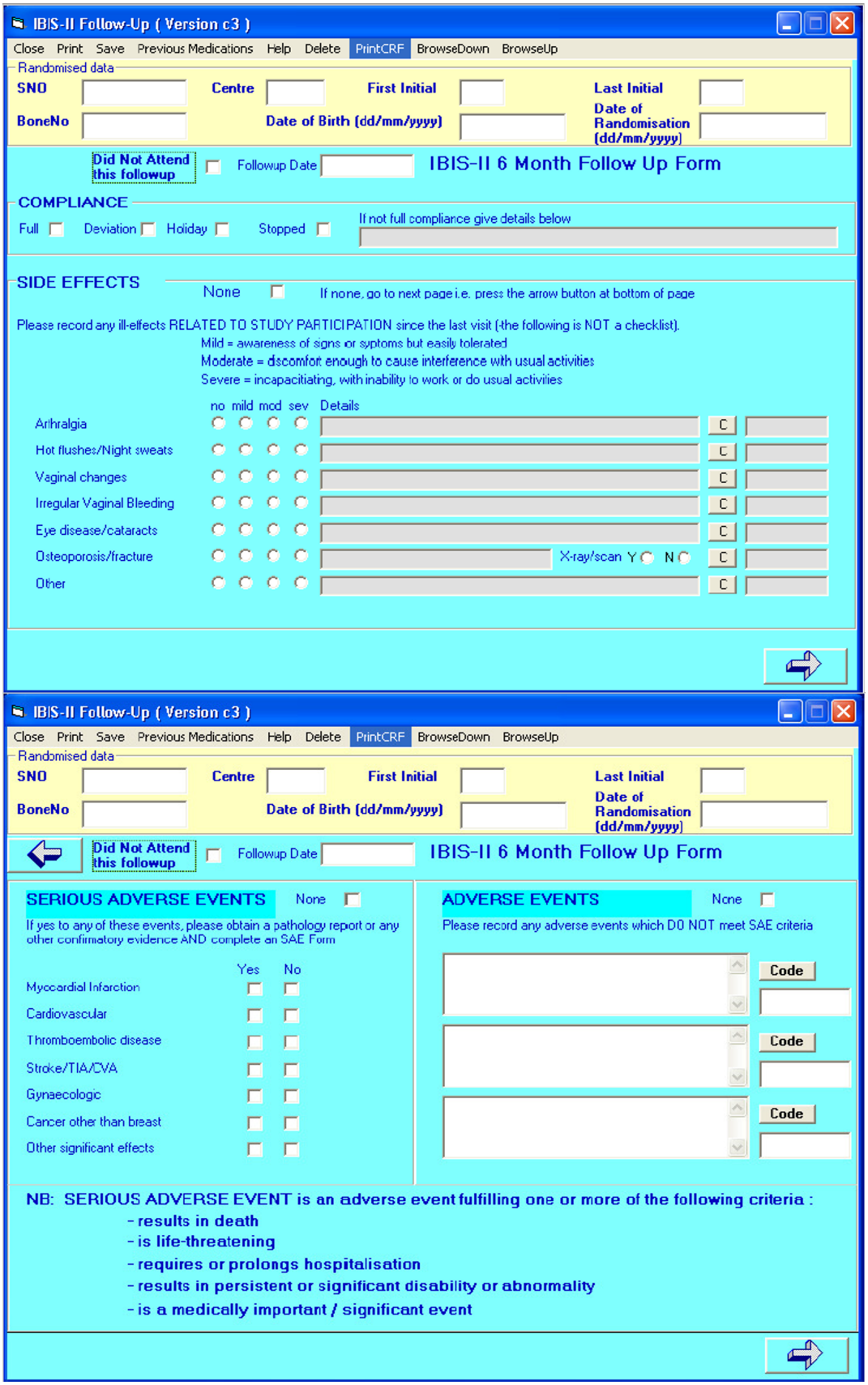


Figure B.4: IBIS-II six month follow up CRF form (continued on following page)

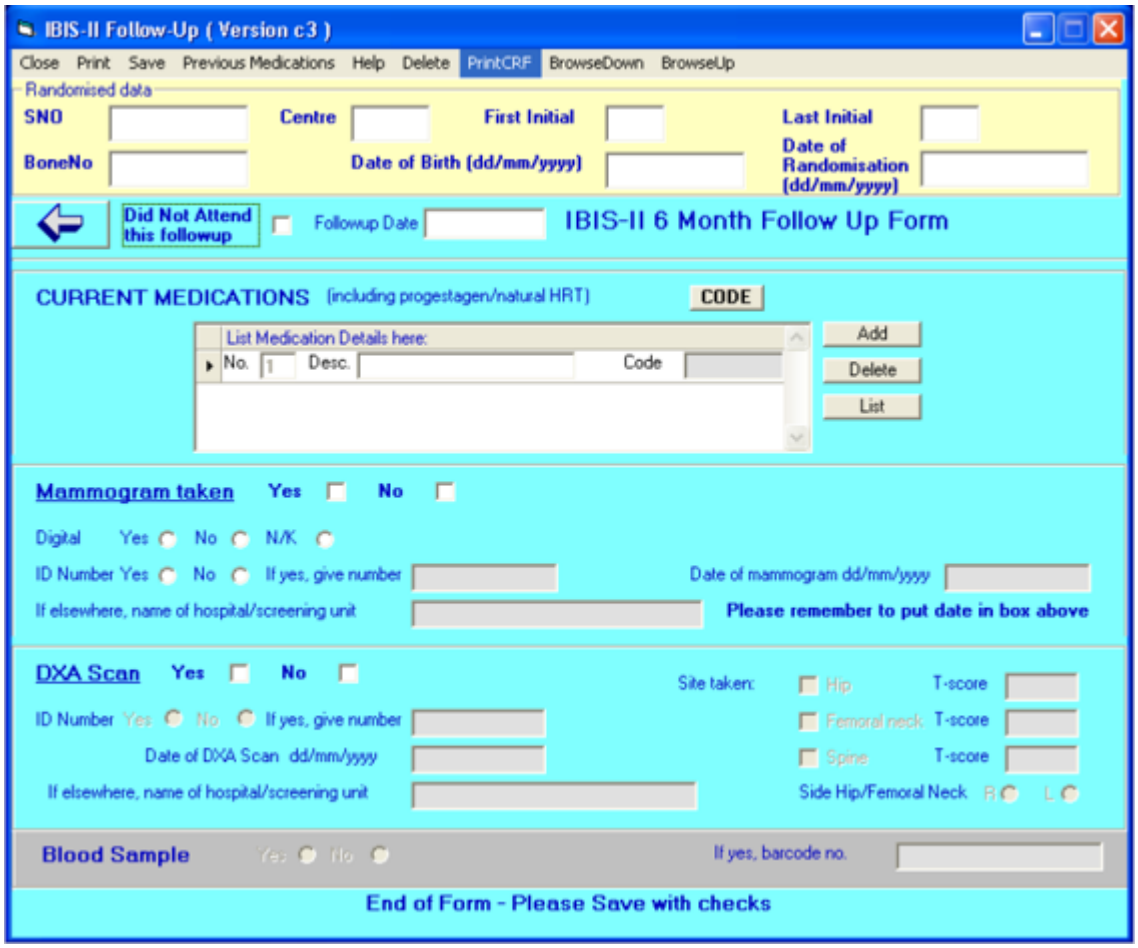


Figure B.5: IBIS-II six month follow up CRF form continued

IBIS-II Prevention Final Follow-up CRF			
SNO:	Patient initials:	Date of birth:	Date of randomisation:

Follow-up General Information

Follow-up date:

Status

Status: ☐ Alive ☐ Dead ☐ Lost contact completely

Date of last known contact:

Reason for withdrawal:

Adverse events /side effects ☐ Breast cancer ☐ Other cancer ☐ Died ☐ Ineligible ☐

Lost to follow-up ☐ Never started ☐ Non-compliant ☐ Clinician decision ☐

Patient decision ☐ Completed trial fully ☐ Unblinded – Anastrozole* ☐ Unblinded – Placebo ☐

Other ☐

Please note: A Final Form should NOT be completed if the patient is unblinded and wishes to continue on open-label Anastrozole.

Compliance (up until withdrawal)

Compliance: ☐ Full ☐ Deviation ☐ Holiday ☐ Stopped

Further details:

Date medication stopped:

Mammogram

Mammogram taken: ☐ Yes ☐ No Digital: ☐ Yes ☐ No ☐ Not known

Mammogram ID: ☐ Yes ☐ No If yes, please provide ID number:

Mammogram date:

Hospital/screening unit (if elsewhere):

DXA

DXA scan: ☐ Yes ☐ No

Figure B.6: IBIS-II final follow up CRF form (Page 1 of 4)

DXA scan ID: ☐ Yes ☐ No If yes, please provide ID number:

DXA date:

Taken at hip: ☐ Yes ☐ No If yes, please provide T-score:

Taken at femoral neck: ☐ Yes ☐ No If yes, please provide T-score:

Taken at spine: ☐ Yes ☐ No If yes, please provide T-score:

Side (hip/femoral neck): ☐ Left ☐ Right

Hospital/screening unit (if elsewhere):

Blood

A blood sample is only required if the participant was on trial medication for more than 6 months but less than 1 year OR more than 4 years but less than 5 years.

Blood sample: ☐ Yes ☐ No

Blood barcode:

Serious Adverse Events

Are there any of the following SAEs to report?

Myocardial Infarction:	☐ Yes ☐ No	Cardiovascular:	☐ Yes ☐ No
Thromboembolic disease:	☐ Yes ☐ No	Stroke / TIA / CVA:	☐ Yes ☐ No
Gynaecological:	☐ Yes ☐ No	Cancer other than breast:	☐ Yes ☐ No
Other significant effects:	☐ Yes ☐ No		

Please complete an SAE CRF for each SAE reported above.

Side Effects

Are there any side effects to report?

	No Mild Mod Sev	Details
Arthralgia	☐☐☐☐	
Hot flushes /night sweats	☐☐☐☐	

Figure B.7: IBIS-II final follow up CRF form (Page 2 of 4)

	No Mild Mod Sev	Details
Irregular vaginal bleeding	☐☐☐☐	
Eye diseases /cataracts	☐☐☐☐	
Osteoporosis /fracture	☐☐☐☐	X-ray/scan: ☐Yes ☐No
Other:	☐☐☐☐	
Vaginal changes	☐☐☐☐	

Adverse Events

Any adverse events which DO NOT meet SAE criteria? ☐Yes ☐No

If yes, please provide details:

Current Medications

Please add details of current medications:

Figure B.8: IBIS-II final follow up CRF form (Page 3 of 4)

Information Received
Participant has been informed of the newly reported common side effect of anastrozole, sensory disturbances (including paraesthesia, taste loss and taste perversion) ☐Yes ☐No Note: If 'no' selected: Please inform the participant of the newly reported common side effects of anastrozole, sensory disturbances (including paraesthesia, taste loss and taste perversion) at the next opportunity
Confirm Receipt of Results Letter
Check participant has received and signed letter informing them of the first main results of IBIS-II Prevention. ☐ If letter not received by post, it should be printed and signed by participant at follow-up appointment.

Figure B.9: IBIS-II final follow up CRF form (Page 4 of 4)

IBIS-II Prevention Death CRF			
SNO:	Patient initials:	Date of birth:	Date of randomisation:
General Information			
Date of death:			
Cause of death:			
Place of death known? ☐Yes ☐No			
If yes, please provide details:			
Was a post mortem performed? ☐Yes ☐No ☐Not know			
Evidence of Breast Cancer			
Any evidence of breast cancer before death? ☐Yes ☐No ☐Not known			

Figure B.10: IBIS-II notification of death CRF form