Use of multiple Single Nucleotide Polymorphism (SNP) testing to predict breast cancer risk in a familial screening clinic

Running title: SNP testing in familial breast cancer

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Abstract

Background: Familial-risk clinics have been set up worldwide to offer intensive screening and preventive strategies. Whilst genetic testing for *BRCA1/2* provides important risk information for a minority, use of common breast cancer risk genetic variants has not yet been implemented, but may have relevance to a much larger number of women.

Methods: A case-control study was designed to assess effects of the first 18 single nucleotide polymorphisms (SNP18) identified through genome-wide-association studies on breast cancer incidence. Pre-defined polygenic risk scores for the general population (SNP18), *BRCA*1 (SNP3) and *BRCA*2 carriers (SNP13) were obtained by multiplying normalised risk per-allele estimates.

Results: SNP18 genotyping was performed in 2,055 samples including 451 women with breast cancer (364 prospective). SNP18 was predictive in the non-*BRCA*1/2 group (inter-quartile range odds ratio 1.55 (95%CI 1.30-1.87), AUC-0.59 (0.55-0.62), O/E 96%). Findings were similar for women in prospective sub-sample, and unaffected by adjustment for classical risk factors. There was some evidence to support the use of SNP3 (*BRCA1* carriers, AUC 0.62 (95%CI 0.55–0.70)) and SNP12 (*BRCA*2 carriers, AUC 0.55 (0.48-0.62)), but general population SNP18 weights performed worse for these groups (*BRCA*1 AUC 0.52(0.44–0.59), *BRCA*2 AUC-0.53(0.45-0.60)). Using unadjusted SNP18 scores in 914 non-*BRCA1/2* unaffected women resulted in over half moving risk categories.

Conclusion: SNP18 may be used to refine risk assessment for women already at increased familial risk without *BRCA*1*/2* mutations. Unadjusted SNP18 lifetime risk adjustment results

in many non-*BRCA1/2* women moving risk category. Different weightings are required for female *BRCA1/2* mutation carriers.

Impact: SNP18 can be used in a familial breast cancer clinic for non-*BRCA1/2* carriers.

Key Words: *BRCA1*, *BRCA2*, Single Nucleotide Polymorphisms (SNPs), Polygenic risk score (PRS), breast cancer

INTRODUCTION

Breast cancer is the most common malignancy among women [1]. It is approximately twice as common in first-degree relatives of affected women compared with the general population [2,3], indicating that breast cancer risk has a substantial inherited component [2-4]. Mutations in *BRCA1* and *BRCA2* have been previously identified as a cause of hereditary breast cancer, but they account for only around 20% of the familial component [5,6]. Pathogenic mutations in these tumour suppressor genes leads to substantially increased inherited predisposition to breast cancer with lifetime risks of up to 60-90% [7-9]. Further high-risk genes include *TP53, CDH1, PTEN, STK11* and *PALB2,* but mutations in these are extremely rare and make up only a tiny proportion (1-2%) of cases of inherited breast cancer [6]. Although a number of moderate risk genes have also been identified conferring a 2-3 fold relative risk of breast cancer that accounts for about 5% of the familial component, genotyping of these has not yet been proven to have utility in risk prediction [6].

Large scale genome-wide association studies (GWAS) have focused on identifying a large number of breast cancer susceptibility alleles with much lower effect sizes [10-13]. Altogether over 94 single nucleotide polymorphisms (SNPs) have now been associated with

breast cancer risk [6], the SNPs at 18 loci identified in 2010 account for about two thirds of the familial component attributed by the identified associated variants [6,12]. Though the modest risk associated with these variants suggest that a significant percentage of familial risk in breast cancer remains unexplained with many susceptibility variants yet to be discovered [6].

There is evidence to suggest that some of these genetic variants also alter the breast cancer risk for those with *BRCA1* or *BRCA2* mutations [13-16]. Antoniou et al determined that nine of the common breast cancer susceptibility SNPs (at the *TOX3, FGFR2, MAP3K, LSP1*, 2q35, *SLC4A7*, 1p11.2, 5p12, 6q25.1 loci) were associated with altered penetrance in *BRCA2* mutation carriers [14,15]. More recent work from the CIMBA consortium has confirmed a contribution of SNPs to breast cancer risk in *BRCA1* carriers [16]. We have previously assessed SNP18 [12] in *BRCA1* and *BRCA2* mutation carriers showing that using the Turnbull weightings appears to predict risk in *BRCA2,* but not *BRCA1* carriers [17]. Here we assess the utility of SNP18 on a familial screening clinic with subdivision of women into those with and without *BRCA1* or *BRCA2* mutations.

MATERIALS AND METHODS

Study design (Family History Risk-FH-risk)

A case-control study was designed to assess the predictive value of a combined SNP panel in women at increased risk of breast cancer due to their family history. As many cases of breast cancer as possible from a family-history clinic in Manchester UK were recruited to provide DNA. Women also attending the clinic but without a breast cancer diagnosis were

invited to contribute DNA as controls. Analysis of the SNP risk score was stratified by *BRCA1/2* testing. A statistical model was used to account for differences between phenotypic risk factors in non-*BRCA1/2* cases and controls at entry to the clinic, in order to minimise possible bias due to these confounding factors. The study had more than 90% power to detect an effect of the SNP score.

Setting

Women included had been referred to the Genesis Prevention Centre in South Manchester for risk assessment and breast screening between 1987 and 2014. All breast cancers that occurred after entry to a family-history clinic between Jun-1990 and Mar-2014 were identified as potential cases, in addition to those previously diagnosed with breast cancer before they entered the clinic. Participants were contacted between Nov-2010 and Oct-2013 to obtain consent and DNA samples. Those with DNA already taken were consented for use of the sample. Deceased cases (n=75), (49/75 (65%) with previous DNA collected), did not require consent. Those that had not previously provided a blood sample provided a sample after full informed consent. Women unaffected by breast cancer were asked to consent to SNP testing and were given results using a polygenic risk score (PRS) described below. Ethics approval for the study was through the North Manchester Research (08/H1006/77) and University of Manchester ethics committees (08229).

Participants

Women were eligible for this study as cases if they had been diagnosed with breast cancer before or after entry to the clinic. Diagnosis of breast cancer was confirmed by hospital records or the North West Cancer Intelligence Service (NWCIS).

Eligibility for controls was based on attending the clinic at the time of recruitment, and by matching age at mammogram to cases. The reason for matching cases and controls on age at mammogram was to ensure that an age when disease-free and at risk of breast cancer was balanced between cases and controls, and for a possible future study of mammographic density. Dates of last follow-up were either date of breast cancer diagnosis or date the woman was last in contact with the department or other NHS service, date of risk reducing mastectomy or date of death.

Assay methods

DNA was extracted from fresh blood lymphocyte samples provided by women consenting to FH-Risk or existing DNA samples were used. *BRCA1/2* mutation testing was carried out when clinically indicated using DNA Sanger sequence and Multiple Ligation dependent Probe Amplification(MLPA) analysis. Relatives of those identified with *BRCA1/2* mutation carriers were then offered cascade screening for the family specific genetic mutation. Women recruited to FH-risk without prior testing were offered *BRCA1/2* testing if appropriate. All women were genotyped for 18 SNPs that have been shown to be associated with breast cancer risk in the general population (*FGFR2, CASP8, TOX3, MAP3K*, 2q, *CDKN2A*, 10q22, *COX11*, *NOTCH*, 11q13, 10q21, *SLC4A7*, 6q25.1, 8q24, *RAD51L1, LSP1*, 5p12, 10q) as previously described [17].

Risk factors

Women with breast cancer diagnosed in clinic were offered full mutation testing for *BRCA1* and *BRCA2,* and all women with at least a 10% likelihood of a *BRCA1* or *BRCA2* mutation (using the Manchester score [21]) have been tested.

SNP18 was based on published per SNP odds ratios and risk allele frequencies (RAF) from the iCOGS (collaborative oncological gene-environment study) analysis [13], where *BRCA1/2* carriers risks were based on analysis from CIMBA (consortium of investigators of modifiers of *BRCA1/2*), and non-carriers were from a BCAC (breast cancer association consortium) analysis. When published odds ratios were not available for a given SNP these were sought through the iCOGs database (Antoniou A personal communication). The assumptions used are given in supplementary tables. We calculated the odds ratio for each of the three SNP genotypes (no risk alleles, 1 risk allele, and 2 risk alleles), assuming independence and normalising by an assumed risk allele frequency. To obtain an overall breast cancer SNP risk score for each woman, we multiplied the odds ratios for each of her 18 genotypes together.

Phenotypic risk factors of breast cancer was assessed through a questionnaire completed in the clinic and saved onto a computer database. The Tyrer-Cuzick (TC) model version 7.02 was used to calculate risk from the information recorded [18] for non-*BRCA1/2* carriers and the complete database. The risk factors used in the model were family history pedigrees of breast and ovarian cancer up to second-degree relatives, height, weight, age at first child, and age at menarche and menopause. Hormone replacement therapy use and benign breast disease were not used because they were not reliably recorded on the database. Women were censored at date of death, date of breast cancer, date of risk-reducing

mastectomy or date of last follow up whichever was earlier and expected risk of breast cancer from cohort entry until that time was computed. A manual assessment of lifetime risk was also used, based on a life-tables approach [19,20]. This used categories that are relevant to UK NICE guidelines [22] of average 8-16%, moderate 17-29%, high 30-39% and very high 40%+ lifetime risk.

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Statistical analysis methods

Hardy-Weinberg equilibrium (HWE) for each SNP was tested by assessing the observed number of homozygotes against expected using a binomial distribution. Assay failures were ignored in the SNP score by imputing a relative risk of 1.0 when they occurred. Differences in phenotypic risk factors at entry between the *non-BRCA1/2* cases and controls and the complete cohort were tabulated. A Wilcoxon test was used for differences in age at entry for cases and controls. Analysis was stratified by *BRCA1/2* testing groups. The main test statistic was a univariate likelihood-ratio χ^2 (df=1) of the log PRS; confidence intervals for odds ratios were based on profile likelihood. The predictive ability of the log SNP scores were assessed using logistic regression. In non-*BRCA1/2* carriers and prospective cases the model also included the logarithm absolute risk from the Tyrer-Cuzick model (v7) over the follow-up period for each woman. Spearman correlation was calculated between 10-year risk and SNP18 in controls. Unadjusted area under the ROC curve (AUC) was used as a secondary measure of discrimination with DeLong confidence intervals. Risk was also presented in groups using observed and expected odds ratios. Expected odds ratios were calculated using the predicted relative risk r_i (PRS) and case-control fraction $\pi=n_0/n_1$, where

n_1,n_0 are the number of cases and controls respectively. Specifically, by using the ratio of expected number of cases $\sum_{i=1}^{n_j} r_i\pi(1+r_i\pi)^{-1}$ and controls $\sum_{i=1}^{n_j} (1+\pi r_i)^{-1}$ for the n_j subjects in group *j*, all divided by the case-control fraction (π). Confidence intervals for observed divided by expected odds ratios used Wilson's method for the binomial parameter. A plot of observed vs expected relative risk from the SNP score in deciles used a trend line from logistic regression. Analysis was carried out in GNU R version 3.1.1.

Results

Data

Between 1987-2012 9222 women were seen at the Family History clinic to assess breast cancer risk and initiate screening if appropriate. Eighty seven women had been diagnosed with breast cancer prior to being seen leaving 9135 for prospective analysis. There were 489 prospective breast cancers. DNA samples were obtained from 451/576 (77%) women with breast cancer. There were 16,832 years of follow up (median 7.9 years) from recruitment to the clinic to the last follow up or breast cancer. The median year of entry to the clinic for the prospective cases was 1996 (IQR 1993-2002), it was 2004 (IQR 1998-2009) for controls.

The composition of the sample by *BRCA1/2* testing (individual and family) is shown in Table 2. For the prospective analysis 87 cases were excluded because they were diagnosed at or before entry to the clinic. This left 1969 women who attended the family-history clinic and

were breast cancer free at baseline including 803 with a family *BRCA1/2* mutation, with 364 cases (112 with *BRCA1/2*) and 1605 controls (691 *BRCA1/2*). Table 3 shows a comparison of the distribution of phenotypic risk factors and 10-yr risk at baseline in non *BRCA1/2* carriers. The non-*BRCA1/2* controls were at a slightly higher risk than the overall cohort, being older and with a more substantial family history of the disease. The non-*BRCA1/2* controls were also younger at entry than non-*BRCA1/2* cases (P<0.001). The *BRCA1/2* cases and controls had a similar age at entry (controls: median 39, IQR 32-46, cases: 37, 33-45; P=0.3).

Quality control of the genotyping was satisfactory: the call rate for each SNP was more than 98%, and HWE was verified separately by *BRCA1/2* testing group (supplementary material).

Analysis and presentation

Table 4 presents the results when using the SNP scores as a continuous predictor. It shows that SNP18 in the non-*BRCA1/2* carrier group was a significant factor (LR-χ2 22.7, P<0.001), with an inter-quartile range odds ratio of 1.55 (95%CI 1.30-1.87) and AUC 0.59 (0.55-0.63). Findings were similar when restricted to the prospective sub-sample. SNP18 was uncorrelated with Tyrer-Cuzick 10-year risks (Spearman correlation 0.01 in controls, P=0.7), and was similarly predictive when adjusted for Tyrer-Cuzick risk over the period from entry to last follow-up (IQR-OR 1.58, 95%CI 1.29-1.89). SNP scores in the *BRCA1/2* groups suggested that they might refine risk, but analysis was limited by sample size and the strength of the predictor. SNP18 score performed worse than the *BRCA1/2*-specific weights used by SNP3 and SNP13 especially for *BRCA1*.

Figure 1a illustrates the performance of SNP18 in non-*BRCA1/2* carriers by showing the distribution in cases and controls. Some analysis of SNP18 quintiles is shown in Table 5.

There was more than a two-fold increase in risk between the bottom and top quintiles of SNP18 in non-carriers. The predicted risk was also close to expected, being 96% (95% CI 56 – 136%) of expected in the complete data. This excellent calibration is further illustrated in Figure 1b,c. The predicted risk was 100% (95%CI 57-142%) of expected after adjustment for risk from classical factors. When using lifetime risk categories (calculated by lifetables[18-20]) relevant to UK NICE guidelines [22] of average 8-16%, moderate 17-29%, high 30-39% and very high 40%+ there was a substantial proportion of the unaffected non-*BRCA1/2* population that moved risk category if an unadjusted PRS was used. Of 914 women more than half 475 (52%) moved category with 432 (25.4%) moving up a category and 443 (26.6%) moving down. Using the upper threshold of risk for MRI screening of 25% lifetime risk in North America 32/174 (18.4%) moved up into the MRI category, whereas 149/740 (20%) moved down out of this category.

DISCUSSION

The current report demonstrates that using a PRS from genotyping SNP18 was highly predictive of breast cancer risk in our familial risk clinic once families with *BRCA1* and *BRCA2* mutations had been excluded, and the observed matched expected risk. We also observed the relationship after adjustment for risk from classical factors. This has important implications for provision of breast cancer risk information in the familial situation. It implies that iCOGs weightings can be used to assess risks in women already at increased risk from their family history. It would therefore be reasonable to use SNP18 to modify risk predictions from this model. If unaffected women are undergoing genetic testing for *BRCA1* and *BRCA2* mutations either as stand-alone tests or part of a panel, use of multiple SNP testing could be considered at the same time. Even if using a 10% threshold for *BRCA1/2*

testing (many clinicians now test far below this), the great majority of women will receive a negative 'uninformative' test, which with only a 10% predicted element from *BRCA1/2* will only, in most instances slightly reduce their predicted risk of breast cancer as most of their imputed familial risk will not have been from the probability of a *BRCA1/2* mutation. For these women a SNP PRS could provide a more meaningful result once *BRCA1* and *BRCA2* mutations have been excluded as it is much more likely that they were at risk of non *BRCA1/2* familial risk factors. Indeed this is only the second independent confirmation that a SNP PRS can be used to predict breast cancer risk accurately in a familial cohort outside the iCOGs consortium [23]. Use of SNP18 PRS in an unadjusted fashion as supported by our data results in over half of women moving NICE risk category. A substantial proportion of 18-20% of women with familial risk also move either side of the North American MRI screening threshold. A recent study from the Australian Family registry showed that using a 77 SNP PRS added significantly to the prediction from risk algorithms including BOADICEA, BRCAPRO and Tyrer-Cuzick [24], in 750 cases and 405 controls. For instance improving the AUC of Tyrer-Cuzick from 0.57 to 0.63.

Indeed it is likely that use of a PRS may have more additional value than extended gene mutation panel tests of moderate and highly penetrant genes. In a study of 198 women referred for *BRCA1/2* testing 57 (29%) harboured pathogenic mutations in *BRCA1/2*[25]. A further 16 had what were classified as pathogenic mutations in the extended panel of 42 genes. However, the concept of what were 'actionable' (identifies pathogenic mutations in genes that substantially affect risk) mutations is debateable. The authors concluded that 15 women (11% of the non *BRCA1/2*) had mutations that were actionable. However, four of these were *MUTYH1* heterozygous mutations that do not even confer a 2-fold risk of

colorectal cancer and no clear increase risk of breast cancer. Many of the remainder were genes that still have an unknown breast cancer risk that almost certainly do not reach a high risk definition (>40% lifetime risk)-*NBN, BLM, SLX4,* PRSS1*,* and *ATM.* Only three in fact met high risk criteria and two of these were for other cancers –*CDKN2a* (melanoma, pancreas) and *MLH1* (colorectal, endometrial) with only *CDH1* potentially meeting a high risk definition for breast cancer. However, nearly all subjects carried a variant of 'uncertain significance' in at least one gene with an average of two per woman. Therefore in broader terms only three women (2%) without a *BRCA1/2* mutation had a really useful result from the extended panel test with the problem of how to counsel nearly all of them on an uncertain result. In contrast a SNP PRS would provide an adjusted overall risk for all women testing negative for *BRCA1/2*.

In this analysis the combined risk SNP odds ratios from iCOGS were used. These were derived from large case-control studies of the general population[13,24]. Many of the SNPs have not been validated for risk assessment in *BRCA1/2* carriers, with 15 of SNP18 not having yet been shown to have validity for *BRCA1* mutation carriers[14,15]. For *BRCA1* this is not surprising where the majority of cancers are oestrogen receptor negative (most of SNP18 are associated with oestrogen receptor positive breast cancer) in contrast to the general population and *BRCA2*. To provide an accurate risk for *BRCA1/2* a different weighting would be required for each SNP for each gene with many SNPs that would be used in a population setting dropping out of the panel as they have not yet been validated for either *BRCA1* and or *BRCA2*. The prediction in table 4 shows that SNP18 with iCOGS weightings for *BRCA1* has a non-significant prediction that improved by using just the three SNPs validated with CIMBA weightings. Similarly, although there was partial prediction in

BRCA2 carriers from SNP18, this improved by using CIMBA *BRCA*2 weightings. We have previously used *BRCA1* weightings in 3 SNPs validated for *BRCA1*[14,15] in a dataset of 462 *BRCA1* carriers with 269 cancers which showed no validity for the three SNPs[17]. The results for SNP3 in *BRCA1* carriers in the present dataset were driven by rs3757318 in *ESR1*. The individual effect for this SNP was larger than expected (supplementary material). A limitation of the analysis is that many of the *BRCA*1 cohort were part of the EMBRACE project, and were therefore used (with many other samples) to estimate the odds ratios for SNP3 from analysis through CIMBA. Analysis excluding these is very limited, and there are only 8 *BRCA1* controls. However the results (supplementary material) at least show that odds ratios are in the correct direction for those who were not part of EMBRACE.

The present study has some limitations. Not all of the non BRCA group had been tested for *BRCA1/2* and therefore will include a small number of mutation carriers. However, this number will be small as all were below the NHS testing threshold of 10% *a priori* risk of mutation detection in England and Wales [22]. Amongst the 124 untested prospective breast cancers we would estimate no more than 4-5% would harbour a *BRCA1/2* mutation; approximately 6 women. Similarly amongst the untested unaffected women we would assess an even smaller proportion are likely to harbour *BRCA1/2* mutations. As such this should have a very small effect on our results.

In summary we believe the current study provides good evidence for utility of multiple SNP testing in the familial risk clinic which may be enhanced further by assessing more recently identified risk association SNPs [24,25]. We would advise using the iCOGs weightings in women at high risk of a *BRCA1/2* mutation only if *BRCA1/2* testing has already been undertaken and has proven negative or is undertaken at the same time. If women test

positive for *BRCA1* or *BRCA2* then different algorithms need to be used if a SNP risk prediction element is to be used to refine their risk estimate.

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Disclosure

Prof D Gareth Evans has received a one off consultancy fee from Astazeneca. The authors declare no other conflicts of interest

Contribution

Conception DGE, AH, JC; data acquisition HB, PS, DGE; data analysis AB, JC, DGE EH; manuscript writing: all; approval of final version: all

References

1. Cancer Research UK website http://www.cancerresearchuk.org/cancer-info/cancerstats/incidence/commoncancers/ accessed February 2016
2. Claus EB, Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. Am J Hum Genet 1991;48(2):232-242.
3. Newman B, Austin MA, Lee M, King M. Inheritance of human breast cancer: Evidence for autosomal dominant transmission in high-risk families. *ProcNatlAcadSci* 1988;85:3044-3048.
4. Peto J, Mack TM. High constant incidence in twins and other relatives of women with breast cancer. Nat Genet 2000;26(4):411-414.
5. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. Mol Oncol. 2010;4(3):174-91.
6. Eccles SA, Aboagye EO, Ali S, Anderson AS, Armes J, Berditchevski F, Blaydes JP, Brennan K, Brown NJ, Bryant HE, Bundred NJ, Burchell JM, Campbell AM, Carroll JS, Clarke RB, Coles CE, Cook GJ, Cox A, Curtin NJ, Dekker LV, Silva IdSdos.S, Duffy SW, Easton DF, Eccles DM, Edwards DR, Edwards J, Evans D, Fenlon DF, Flanagan JM, Foster C, Gallagher WM, Garcia-Closas M, Gee JM, Gescher AJ, Goh V, Groves AM, Harvey AJ, Harvie M, Hennessy BT, Hiscox S, Holen I, SJ, Howell A, Hubbard G, Hulbert-Williams N, Hunter MS, Jasani B, Jones LJ, Key TJ, Kirwan CC, Kong A, Kunkler IH, Langdon SP, Leach MO, Mann DJ, Marshall JF, Martin L, Martin SG, Macdougall JE, Miles DW, Miller WR, Morris JR, Moss SM, Mullan P, Natrajan R, O'Connor JP, O'Connor R, Palmieri C, Pharoah PD, Rakha EA, Reed E, Robinson SP, Sahai E, Saxton JM, Schmid P, Smalley MJ, Speirs V, Stein R, Stingl J, Streuli CH, Tutt AN, Velikova G, Walker RA, Watson CJ, Williams KJ, Young LS, Thompson AM. Critical research gaps

and translational priorities for the successful prevention and treatment of breast cancer. Breast Cancer Res. 2013;15(5):R92

7. Evans DG, Shenton A, Woodward E, Lalloo F, A Howell, Maher ER. Penetrance estimates for BRCA1 and BRCA2 based on genetic testing in a Clinical Cancer Genetics service setting. BMC Cancer 2008;8(1):155
8. Mavaddat N, Peock S, Frost D, Ellis S, Platte R, Fineberg E, Evans DG, Izatt L, Eeles RA, Adlard J, Davidson R, Eccles D, Cole T, Cook J, Brewer C, Tischkowitz M, Douglas F, Hodgson S, Walker L, Porteous ME, Morrison PJ, Side LE, Kennedy MJ, Houghton C, Donaldson A, Rogers MT, Dorkins H, Miedzybrodzka Z, Gregory H, Eason J, Barwell J, McCann E, Murray A, Antoniou AC, Easton DF, EMBRACE. Cancer Risks for BRCA1 and BRCA2 Mutation Carriers: Results From Prospective Analysis of EMBRACE.J Natl Cancer Inst. 2013;105(11):812-22
9. Evans DG, Harkness E, Lalloo F, Howell A. Long-term prospective clinical follow-up after BRCA1/2 presymptomatic testing: BRCA2 risks higher than in adjusted retrospective studies.J Med Genet. 2014;51(9):573-80.
10. Easton DF, Pooley KA, Dunning AM, Pharoah PDP, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen C.-Y, Wu P.-E, Wang H.-C, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo K.-Y, Noh D.-Y, Ahn S.-H, Hunter DJ,

Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low Y.-L, Bogdanova N, Schurmann P, Dork T, Tollenaar RAEM, Jacobi CE, Devilee P, Klijn JGM, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MWR, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, van den Ouweland A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko Y.-D, Spurdle AB, Beesley J, Chen X, Mannermaa A, Kosma V.-M, Kataja V, Hartikainen J, Day NE, Cox DR, Ponder BAJ. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007; 2447:1087-1093

11. Stacey SN, Manolescu A, Sulem P, Rafnar T, Gudmundsson J, Gudjonsson SA, Masson G, Jakobsdottir M, Thorlacius S, Helgason A, Aben KK, Strobbe LJ, Albers-Akkers MT, Swinkels DW, Henderson BE, Kolonel LN, Le Marchand L, Millastre E, Andres R, Godino J, Dolores M, Polo E, Tres A, Mouy M, Saemundsdottir J, Backman VM, Gudmundsson L, Kristjansson K, Bergthorsson JT, Kostic J, Frigge ML, Geller F, Gudbjartsson D, Sigurdsson H, Jonsdottir T, Hrafnkelsson J, Johannsson J, Sveinsson T, Myrdal G, Niels H, Jonsson T, von Holst S, Werelius B, Margolin S, Lindblom A, Mayordomo JI, Haiman CA, Kiemeney LA, Th O, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. Nature Genetics 2007;39(7):865-86
12. Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranian M, Seal S, Ghoussaini M, Hines S, Healey CS, Hughes D, Warren-Perry M, Tapper W,

Eccles D, Evans DG, Hooning M, Schutte M, van den Ouweland A, Houlston R, Ross G, Langford C, Pharoah PDP, Stratton MR, Dunning AM, Rahman N, Easton DF. Genome-wide association study identifies five new breast cancer susceptibility loci. Nature Genetics 2010; 42(6): 504-507

13. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, Schmidt MK, Chang-Claude J, Bojesen SE, Bolla MK, Wang Q, Dicks E, Lee A, Turnbull C, Rahman N, Fletcher O, Peto J, Gibson L, dos Santos Silva I, Nevanlinna H, Muranen TA, Aittomaki K, Blomqvist C, Czene K, Irwanto A, Liu J, Waisfisz Q, Meijers-Heijboer H, Adank M, van der Luijt RB, Hein R, Dahmen N, Beckman L, Meindl A, Schmutzler RK, Muller-Myhsok B, Lichtner P, Hopper JL, Southey MC, Makalic E, Schmidt DF, Uitterlinden AG, Hofman A, Hunter DJ, Chanock SJ, Vincent D, Bacot F, Tessier DC, Canisius S, Wessels LFA, Haiman CA, Shah M, Luben R, Brown J, Luccarini C, Schoof N, Humphreys K, Li J, Nordestgaard BG, Nielsen SF, Flyger H, Couch FJ, Wang X, Vachon C, Stevens KN, Lambrechts D, Moisse M, Paridaens R, Christiaens M.-R, Rudolph A, Nickels S, Flesch-Janys D, Johnson N, Aitken Z, Aaltonen K, Heikkinen T, Broeks A, Veer LJ, van der Schoot CE, Guenel P, Truong T, Laurent-Puig P, Menegaux F, Marme F, Schneeweiss A, Sohn C, Burwinkel B, Zamora MP, Perez JI, Pita G, Alonso MR, Cox A, Brock IW, Cross SS, Reed MWR, Sawyer EJ, Tomlinson I, Kerin MJ, Miller N, Henderson BE, Schumacher F, Le Marchand L, Andrulis IL, Knight JA, Glendon G, Mulligan AM, Lindblom A, Margolin S, Hooning MJ, Hollestelle A, van den Ouweland AMW, Jager A, Bui QM, Stone J, Dite GS, Apicella C, Tsimiklis H, Giles GG, Severi G, Baglietto L, Fasching PA, Haeberle L, Ekici AB, Beckmann MW, Brenner H, Muller H, Arndt V, Stegmaier

C, Swerdlow A, Ashworth A, Orr N, Jones M, Figueroa J, Lissowska J, Brinton L, Goldberg MS, Labreche F, Dumont M, Winqvist R, Pylkas K, Jukkola-Vuorinen A, Grip M, Brauch H, Hamann U, Bruning T, Radice P, Peterlongo P, Manoukian S, Bonanni B, Devilee P, Tollenaar RAEM, Seynaeve C, van Asperen CJ, Jakubowska A, Lubinski J, Jaworska K, Durda K, Mannermaa A, Kataja V, Kosma V.-M, Hartikainen JM, Bogdanova NV, Antonenkova NN, Dork T, Kristensen VN, Anton-Culver H, Slager S, Toland AE, Edge S, Fostira F, Kang D, Yoo K.-Y, Noh D.-Y, Matsuo K, Ito H, Iwata H, Sueta A, Wu AH, Tseng C.-C, Van Den Berg D, Stram DO, Shu X.-O, Lu W, Gao Y.-T, Cai H, Teo SH, Yip CH, Phuah SY, Cornes BK, Hartman M, Miao H, Lim WY, Sng J.-H, Muir K, Lophatananon A, Stewart-Brown S, Siriwanarangsan P, Shen C.-Y, Hsiung C.-N, Wu P.-E, Ding S.-L, Sangrajrang S, Gaborieau V, Brennan P, McKay J, Blot WJ, Signorello LB, Cai Q, Zheng W, Deming-Halverson S, Shrubsole M, Long J, Simard J, Garcia-Closas M, Pharoah PDP, Chenevix-Trench G, Dunning AM, Benitez J, Easton DF. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet. 2013 Apr;45(4):353-61,

14. Antoniou AC, Beesley J, McGuffog L, Sinilnikova OM, Healey S, Neuhausen SL, Ding YC, Rebbeck TR, Weitzel JN, Lynch HT, Isaacs C, Ganz PA, Tomlinson G, Olopade OI, Couch FJ, Wang X, Lindor NM, Pankratz VS, Radice P, Manoukian S, Peissel B, Zaffaroni D, Barile M, Viel A, Allavena A, Dall'Olio V, Peterlongo P, Szabo CI, Zikan M, Claes K, Poppe B, Foretova L, Mai PL, Greene MH, Rennert G, Lejbkowicz F, Glendon G, Ozcelik H, Andrulis IL, Thomassen M, Gerdes A.-M, Sunde L, Cruger D, Jensen UB, Caligo M, Friedman E, Kaufman B, Laitman Y, Milgrom R, Dubrovsky M, Cohen S, Borg A, Jernström H, Lindblom A,

Rantala J, Stenmark-Askmalm M, Melin B, Nathanson K, Domchek S, Jakubowska A, Lubinski J, Huzarski T, Osorio A, Lasa A, Durán M, Tejada M.-I, Godino J, Benitez J, Hamann U, Kriege M, Hoogerbrugge N, van der Luijt RB, van Asperen CJ, Devilee P, Meijers-Heijboer EJ, Blok MJ, Aalfs CM, Hogervorst F, Rookus M, Cook M, Oliver C, Frost D, Conroy D, Evans DG, Lalloo F, Pichert G, Davidson R, Cole T, Cook J, Paterson J, Hodgson S, Morrison PJ, Porteous ME, Walker L, Kennedy MJ, Dorkins H, Peock S, Godwin AK, Stoppa-Lyonnet D, de Pauw A, Mazoyer S, Bonadona V, Lasset C, Dreyfus H, Leroux D, Hardouin A, Berthet P, Faivre L, Loustalot C, Noguchi T, Sobol H, Rouleau E, Nogues C, Frénay M, Vénat-Bouvet L, Hopper JL, Daly MB, Terry MB, John EM, Buys SS, Yassin Y, Miron A, Goldgar D, Singer CF, Dressler AC, Gschwantler-Kaulich D, Pfeiler G, Hansen TVO, Jønson L, Agnarsson BA, Kirchhoff T, Offit K, Devlin V, Dutra-Clarke A, Piedmonte M, Rodriguez GC, Wakeley K, Boggess JF, Basil J, Schwartz PE, Blank SV, Toland AE, Montagna M, Casella C, Imyanitov E, Tihomirova L, Blanco I, Lazaro C, Ramus SJ, Sucheston L, Karlan BY, Gross J, Schmutzler R, Wappenschmidt B, Engel C, Meindl A, Lochmann M, Arnold N, Heidemann S, Varon-Mateeva R, Niederacher D, Sutter C, Deissler H, Gadzicki D, Preisler-Adams S, Kast K, Schönbuchner I, Caldes T, de la Hoya M, Aittomäki K, Nevanlinna H, Simard J, Spurdle AB, Holland H, Chen X, Platte R, Chenevix-Trench G, Easton DF. Common breast cancer susceptibility alleles and the risk of breast cancer for *BRCA1* and *BRCA2* mutation carriers: implications for risk prediction. Cancer Res 2010; 70(23): 9742-9754

15. Antoniou AC, Kartsonaki C, Sinilnikova OM, Soucy P, McGuffog L, Healey S,

Lee A, Peterlongo P, Manoukian S, Peissel B, Zaffaroni D, Cattaneo E, Barile M, Pensotti V, Pasini B, Dolcetti R, Giannini G, Putignano ALL, Varesco L, Radice P, Mai PL, Greene MH, Andrulis IL, Glendon G, Ozcelik H, Thomassen M, Gerdes A.-MM, Kruse TA, Birk Jensen U, Crüger DG, Caligo MA, Laitman Y, Milgrom R, Kaufman B, Paluch-Shimon S, Friedman E, Loman N, Harbst K, Lindblom A, Arver B, Ehrencrona H, Melin B, SWE-BRCA Nathanson KL, Domchek SM, Rebbeck T, Jakubowska A, Lubinski J, Gronwald J, Huzarski T, Byrski T, Cybulski C, Gorski B, Osorio A, Ramón y Cajal T, Fostira F, Andrés R, Benitez J, Hamann U, Hogervorst FB, Rookus MA, Hooning MJ, Nelen MR, van der Luijt RB, van Os TA, van Asperen CJ, Devilee P, Meijers-Heijboer HE, Gómez Garcia EB, HEBON Peock S, Cook M, Frost D, Platte R, Leyland J, Evans DG, Lalloo F, Eeles R, Izatt L, Adlard J, Davidson R, Eccles D, Ong K.-rR, Cook J, Douglas F, Paterson J, Kennedy MJ, Miedzybrodzka Z, EMBRACE Godwin A, Stoppa-Lyonnet D, Buecher B, Belotti M, Tirapo C, Mazoyer S, Barjhoux L, Lasset C, Leroux D, Faivre L, Bronner M, Prieur F, Nogues C, Rouleau E, Pujol P, Coupier I, Frénay M, CEMO Study Collaborators Hopper JL, Daly MB, Terry MB, John EM, Buys SS, Yassin Y, Miron A, Goldgar D, Breast Cancer Family Registry Singer CF, Tea M.-KK, Pfeiler G, Dressler ACC, Hansen Tv, Jønson L, Ejlertsen B, Barkardottir RBB, Kirchhoff T, Offit K, Piedmonte M, Rodriguez G, Small L, Boggess J, Blank S, Basil J, Azodi M, Toland AEE, Montagna M, Tognazzo S, Agata S, Imyanitov E, Janavicius R, Lazaro C, Blanco I, Pharoah PD, Sucheston L, Karlan BY, Walsh CS, Olah E, Bozsik A, Teo S.-HH, Seldon JL, Beattie MS, van Rensburg EJ, Sluiter MD, Diez O, Schmutzler RK, Wappenschmidt B, Engel C, Meindl A, Ruehl I, Varon-Mateeva R, Kast K,

Deissler H, Niederacher D, Arnold N, Gadzicki D, Schönbuchner I, Caldes T, de la Hoya M, Nevanlinna H, Aittomäki K, Dumont M, Chiquette J, Tischkowitz M, Chen X, Beesley J, Spurdle AB, kConFab investigators Neuhausen SL, Chun Ding Y, Fredericksen Z, Wang X, Pankratz VS, Couch F, Simard J, Easton DF, Chenevix-Trench G, CIMBA. Common alleles at 6q25.1 and 1p11.2 are associated with breast cancer risk for BRCA1 and BRCA2 mutation carriers. Human Molecular Genetics 2011; 20(16): 3304-3321

16. Couch FJ, Wang X, McGuffog L, Lee A, Olswold C, Kuchenbaecker KB, Soucy P, Fredericksen Z, Barrowdale D, Dennis J, Gaudet MM, Dicks E, Kosel M, Healey S, Sinilnikova OM, Lee A, Bacot F, Vincent D, Hogervorst FB, Peock S, Stoppa-Lyonnet D, Jakubowska A, kConFab Investigators Radice P, Katharina R, SWE-BRCA Domchek SM, Piedmonte M, Singer CF, Friedman E, Thomassen M, Ontario Cancer Genetics Network Hansen TV, Neuhausen SL, Szabo CI, Blanco I, Greene MH, Karlan BY, Garber J, Phelan CM, Weitzel JN, Montagna M, Olah E, Andrulis IL, Godwin AK, Yannoukakos D, Goldgar DE, Caldes T, Nevanlinna H, Osorio A, Beth M, Daly MB, van Rensburg EJ, Hamann U, Ramus SJ, Ewart A, Caligo MA, Olopade OI, Tung N, Claes K, Beattie MS, Southey MC, Imyanitov EN, Tischkowitz M, Janavicius R, John EM, Kwong A, Diez O, Balmaña J, Barkardottir RB, Arun BK, Rennert G, Teo S.-HH, Ganz PA, Campbell I, van der Hout AH, van Deurzen CH, Seynaeve C, Gómez Garcia EB, van Leeuwen FE, Meijers-Heijboer HE, Gille JJ, Ausems MG, Blok MJ, Ligtenberg MJ, Rookus MA, Devilee P, Verhoef S, van Os TA, Wijnen JT, HEBON EMBRACE Frost D, Ellis S, Fineberg E, Platte R, Evans GG, Izatt L, Eeles RA, Adlard J, Eccles DM, Cook J, Brewer C, Douglas F, Hodgson S, Morrison PJ,

Side LE, Donaldson A, Houghton C, Rogers MT, Dorkins H, Eason J, Gregory H, McCann E, Murray A, Calender A, Hardouin A, Berthet P, Delnatte C, Nogues C, Lasset C, Houdayer C, Leroux D, Rouleau E, Prieur F, Damiola F, Sobol H, Coupier I, Venat-Bouvet L, Castera L, Gauthier-Villars M, Léoné M, Pujol P, Mazoyer S, Bignon Y.-JJ, GEMO Study Collaborators Złowocka-Perłowska E, Gronwald J, Lubinski J, Durda K, Jaworska K, Huzarski T, Spurdle AB, Viel A, Peissel B, Bonanni B, Melloni G, Ottini L, Papi L, Varesco L, Grazia M, Peterlongo P, Volorio S, Manoukian S, Pensotti V, Arnold N, Engel C, Deissler H, Gadzicki D, Gehrig A, Kast K, Rhiem K, Meindl A, Niederacher D, Ditsch N, Plendl H, Preisler-Adams S, Engert S, Sutter C, Varon-Mateeva R, Wappenschmidt B, Weber BH, Arver B, Stenmark-Askmalm M, Loman N, Rosenquist R, Einbeigi Z, Nathanson KL, Rebbeck TR, Blank SV, Cohn DE, Rodriguez GC, Small L, Friedlander M, Bae-Jump VL, Fink-Retter A, Rappaport C, Gschwantler-Kaulich D, Pfeiler G, Tea M.-KK, Lindor NM, Kaufman B, Paluch SS, Laitman Y, Skytte A.-BB, Gerdes A.-MM, Sokilde I, Traasdahl S, Kruse TA, Birk U, Vijai J, Sarrel K, Robson M, Kauff N, Marie A, Glendon G, Ozcelik H, Ejlertsen B, Nielsen FC, Jønson L, Andersen MK, Chun Y, Steele L, Foretova L, Teulé A, Lazaro C, Brunet J, Angel M, Mai PL, Loud JT, Walsh C, Lester J, Orsulic S, Narod SA, Herzog J, Sand SR, Tognazzo S, Agata S, Vaszko T, Weaver J, Stavropoulou AV, Buys SS, Romero A, de la Hoya M, Aittomäki K, Muranen TA, Duran M, Chung WK, Lasa A, Dorfling CM, Miron A, BCFR Benitez J, Senter L, Huo D, Chan SB, Sokolenko AP, Chiquette J, Tihomirova L, Friebel TM, Agnarsson BA, Lu KH, Lejbkowicz F, James PA, Hall P, Dunning AM, Tessier D, Cunningham J, Slager SL, Wang C, Hart S, Stevens K, Simard J, Pastinen T,

Pankratz VS, Offit K, Easton DF, Chenevix-Trench G, Antoniou AC, CIMBA. Genome-wide association study in BRCA1 mutation carriers identifies novel loci associated with breast and ovarian cancer risk. PLoS Genet. 2013;9(3):e1003212.

17. Ingham SL, Warwick J, Byers H, Lalloo F, Newman WG, Evans DG. Is multiple SNP testing in BRCA2 and BRCA1 female carriers ready for use in clinical practice? Results from a large Genetic Centre in the UK.Clin Genet. 2013;84(1):37-42
18. Amir E, Evans DG, Shenton A, Lalloo F, Moran A, Boggis C, Wilson M, Howell A.. Evaluation of Breast Cancer Risk Assessment Packages in the Family History Evaluation and Screening Programme. J Med Genet 2003; 40(11):807-14.
19. Evans DGR, Lalloo F. Risk assessment and management of high risk familial breast cancer. J Med Genet 2002; 39: 865-71.
20. Evans DGR, Ingham S, Dawe S, Roberts L, Lalloo F, Brentnall AR, Stavrinos P, Howell A. Breast cancer risk assessment in 8,824 women attending a family history evaluation and screening programme. Fam Cancer. 2014;13(2):189-96.
21. Evans DG, Lalloo F, Cramer A, Jones EA, Knox F, Amir E, Howell A. Addition of pathology and biomarker information significantly improves the performance of the Manchester scoring system for BRCA1 and BRCA2 testing. J Med Genet. 2009;46(12):811-7.
22. McIntosh A, Shaw C, Evans G Turnbull N, Bahar N, Barclay M et al. (2004 updated 2006, 2013) Clinical Guidelines and Evidence Review for The

Classification and Care of Women at Risk of Familial Breast Cancer, London: National Collaborating Centre for Primary Care/University of Sheffield. NICE guideline http://www.nice.org.uk/guidance/cg164

23. Mavaddat N, Pharoah PDP, Michailidou K, Tyrer J, Brook MN, Bolla MK, Wang Q, Dennis J, Dunning AM, Shah M, Luben R, Brown J, Bojesen SE, Nordestgaard BG, Nielsen SF, Flyger H, Czene K, Darabi H, Eriksson M, Peto J, dos Santos-Silva I, Dudbridge F, Johnson N, Schmidt MK, Broeks A, Verhoef S, Rutgers EJ, Swerdlow A, Ashworth A, Orr N, Schoemaker MJ, Figueroa J, Chanock SJ, Brinton L, Lissowska J, Couch FJ, Olson JE, Vachon C, Pankratz VS, Lambrechts D, Wildiers H, Van Ongeval C, van Limbergen E, Kristensen V, Grenaker Alnæs G, Nord S, Borresen-Dale A.-L, Nevanlinna H, Muranen TA, Aittomäki K, Blomqvist C, Chang-Claude J, Rudolph A, Seibold P, Flesch-Janys D, Fasching PA, Haeberle L, Ekici AB, Beckmann MW, Burwinkel B, Marme F, Schneeweiss A, Sohn C, Trentham-Dietz A, Newcomb P, Titus L, Egan KM, Hunter DJ, Lindstrom S, Tamimi RM, Kraft P, Rahman N, Turnbull C, Renwick A, Seal S, Li J, Liu J, Humphreys K, Benitez J, Pilar Zamora M, Arias Perez JI, Menéndez P, Jakubowska A, Lubinski J, Jaworska-Bieniek K, Durda K, Bogdanova NV, Antonenkova NN, Dörk T, Anton-Culver H, Neuhausen SL, Ziogas A, Bernstein L, Devilee P, Tollenaar RAEM, Seynaeve C, van Asperen CJ, Cox A, Cross SS, Reed MWR, Khusnutdinova E, Bermisheva M, Prokofyeva D, Takhirova Z, Meindl A, Schmutzler RK, Sutter C, Yang R, Schürmann P, Bremer M, Christiansen H, Park-Simon T.-W, Hillemanns P, Guénel P, Truong T, Menegaux F, Sanchez M, Radice P, Peterlongo P, Manoukian S, Pensotti V, Hopper JL, Tsimiklis H, Apicella C, Southey MC, Brauch H, Brüning T, Ko Y.-D,

Sigurdson AJ, Doody MM, Hamann U, Torres D, Ulmer H.-U, Försti A, Sawyer EJ, Tomlinson I, Kerin MJ, Miller N, Andrulis IL, Knight JA, Glendon G, Marie Mulligan A, Chenevix-Trench G, Balleine R, Giles GG, Milne RL, McLean C, Lindblom A, Margolin S, Haiman CA, Henderson BE, Schumacher F, Le Marchand L, Eilber U, Wang-Gohrke S, Hooning MJ, Hollestelle A, van den Ouweland AMW, Koppert LB, Carpenter J, Clarke C, Scott R, Mannermaa A, Kataja V, Kosma V.-M, Hartikainen JM, Brenner H, Arndt V, Stegmaier C, Karina Dieffenbach A, Winqvist R, Pylkäs K, Jukkola-Vuorinen A, Grip M, Offit K, Vijai J, Robson M, Rau-Murthy R, Dwek M, Swann R, Annie Perkins K, Goldberg MS, Labrèche F, Dumont M, Eccles DM, Tapper WJ, Rafiq S, John EM, Whittemore AS, Slager S, Yannoukakos D, Toland AE, Yao S, Zheng W, Halverson SL, González-Neira A, Pita G, Rosario Alonso M, Álvarez N, Herrero D, Tessier DC, Vincent D, Bacot F, Luccarini C, Baynes C, Ahmed S, Maranian M, Healey CS, Simard J, Hall P, Easton DF, Garcia-Closas M. Prediction of breast cancer risk based on profiling with common genetic variants. J Natl Cancer Inst. 2015; 8;107(5).

24. Dite GS, MacInnis RJ, Bickerstaffe A, Dowty JG, Allman R, Apicella C, Milne RL, Tsimiklis H, Phillips KA, Giles GG, Terry MB, Southey MC, Hopper JL. Breast Cancer Risk Prediction Using Clinical Models and 77 Independent Risk-Associated SNPs for Women Aged Under 50 Years: Australian Breast Cancer Family Registry. Cancer Epidemiol Biomarkers Prev. 2016;25(2):359-65
25. Kurian AW, Hare EE, Mills MA, Kingham KE, McPherson L, Whittemore AS, McGuire V, Ladabaum U, Kobayashi Y, Lincoln SE, Cargill M, Ford JM. Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk

assessment.JClinOncol. 2014;32(19):2001

26. Brentnall AR, Evans DG, Cuzick J. Distribution of breast cancer risk from SNPs and classical risk factors in women of routine screening age in the UK. Br J Cancer. 2014; 110(3):827-8.

Table 1: SNP used with odds ratios per allele and weightings derived from iCOGS dataset [13].

SNP	Chromosome	Position	Ref	EAF	EOR
rs614367	11	69328764	C	0.15	1.21
rs704010	10	80841148	T	0.62	0.92
rs713588	10	5886962	G	0.44	0.99
rs889312	5	56031884	C	0.72	0.89
rs909116	11	1941946	T	0.49	0.93
rs1011970	9	22062134	G	0.17	1.05
rs1156287	17	53076799	G	0.71	1.07
rs1562430	8	128387852	T	0.43	0.90
rs2981579	10	123337335	A	0.60	0.79
rs3757318	6	151914113	G	0.07	1.16
rs3803662	16	52586341	A	0.74	0.81
rs4973768	3	27416013	C	0.47	1.09
rs8009944	14	69039588	C	0.74	0.96
rs9790879	5	44899885	C	0.60	0.92
rs10995190	10	64278682	G	0.16	0.86
rs11249433	1	121280613	A	0.40	1.09
rs13387042	2	217905832	A	0.49	0.88
rs10931936	2	202143928	T	0.72	0.96

Footnote: EAF is the expected risk allele frequency (COGS, EOR is the expected per-allele odds ratio (COGS)

Table 2: *BRCA1/2* testing groups

Family	Testing	Controls	Cases	Controls(%)	Cases(%)
No *BRCA1/2*	Low risk, not tested	656	126	40.9	27.9
No *BRCA1/2*	Family tested negative	258	158	16.1	35.0
BRCA1		201	79	12.5	17.5
BRCA1	Proband negative	141	2	8.8	0.4
BRCA2		204	78	12.7	17.3
BRCA2	Proband negative	145	8	9.0	1.8

Table 3: Phenotypic risk characteristics at entry

Factor	Description	Complete cohort	Control (No *BRCA1/2*)	Prospective case (No *BRCA1/2*)
Database	Available	10,088*	1,176	260
questionnaire	Unavailable		24 (2%)	1 (<1%)
Age Entry (yr)	Median (IQR)	39 (33-46)	40 (35-46)	43 (37-48)
Menopause	Pre(%)	6103 (60%)	728 (62%)	169 (65%)
	Peri(%)	709 (7%)	58 (5%)	20 (8%)
	Post(%)	1051 (10%)	148 (13%)	44 (17%)
	Unknown(%)	2225 (22%)	242 (21%)	27 (10%)
BMI (kg/m^2)	Median (IQR)	24.0 (21.8-27.3)	24.4 (22.1-28.0)	23.8 (21.7-26.2)
	Unknown (%)	3715 (37%)	381 (32%)	56 (22%)
Parity	Unknown	1086 (11%)	179 (15%)	32 (12%)
	Nulliparous	4511 (45%)	661 (56%)	138 (53%)
	Parous	4491 (45%)	336 (29%)	90 (35%)
Age first child (parous)	Median (IQR)	25 (21-29)	25 (21-29)	25 (22-30)

First-degree	1	6727 (67%)	728 (62%)	168 (65%)
relatives (n (%))	2 or more	1246 (12%)	207 (18%)	61 (23%)
Tyrer-Cuzick 10y%	Median (IQR)	3.17% (1.73-5.09%)	3.86% (2.31-6.03%)	4.40% (2.98-6.14%)

***Number seen up to 2014**

Table 4: Results with use of PRS as continuous risk factor.

	IQR (controls)	IQR-OR	95% CI	LR-χ^2	AUC	95% CI
All cases						
SNP18 (NOT BRCA)	0.81 - 1.27	1.55	1.30 - 1.87	22.7	0.59	0.55 - 0.63
SNP3 (BRCA1)	0.94 - 0.99	1.38	1.16 - 1.67	12.7	0.62	0.55 - 0.70
SNP13 (BRCA2)	0.79 - 1.14	1.32	0.91 - 1.93	2.1	0.55	0.48 - 0.62
SNP18 (BRCA1)	0.81 - 1.17	0.96	0.71 - 1.30	0.1	0.52	0.44 - 0.59
SNP18 (BRCA2)	0.74 - 1.20	1.19	0.80 - 1.77	0.7	0.53	0.45 - 0.60
Prospective cases only						
SNP18 (Not BRCA)	0.81 - 1.27	1.55	1.29 - 1.87	21.3	0.59	0.55 - 0.63
-- adjusted for TC risk		1.56	1.29 - 1.89	21.7		
SNP3 (BRCA1)	0.94 - 0.99	1.44	1.17 - 1.76	12.6	0.64	0.55 - 0.72
SNP13 (BRCA2)	0.79 - 1.15	1.44	0.90 - 2.31	2.3	0.57	0.47 - 0.66

IQR, inter-quartile range; OR, odds ratio; CI, confidence interval; LR- χ^2, likelihood ratio test statistic; AUC, area under the receiver operating characteristic; TC, Tyrer-Cuzick absolute riskp

Table 5 SNP18 results by quintile in non-*BRCA1/2* carriers

Quintile	SNP18 cutpoint	Cancer	n	OR (Observed)	OR (Expected)	O/E (95% CI)
All cases						
1		49	307	0.78	0.66	1.18 (0.91-1.51)
2	0.78	38	296	0.60	0.86	0.70 (0.52-0.93)
3	0.94	58	299	0.98	1.03	0.95 (0.75-1.19)
4	1.11	67	296	1.19	1.24	0.96 (0.78-1.18)
5	1.37	82	296	1.56	1.62	0.96 (0.80-1.15)
Prospective cases only						
1		42	300	0.75	0.66	1.14 (0.86-1.49)
2	0.78	34	286	0.62	0.86	0.72 (0.53-0.98)
3	0.94	54	301	1.01	1.03	0.98 (0.76-1.24)
4	1.11	57	286	1.14	1.24	0.92 (0.73-1.16)
5	1.37	74	288	1.59	1.62	0.98 (0.80-1.18)

SNP18 cutpoint, lower limit of quantile; OR, odds ratio; O/E observed odds ratio divided by expected.

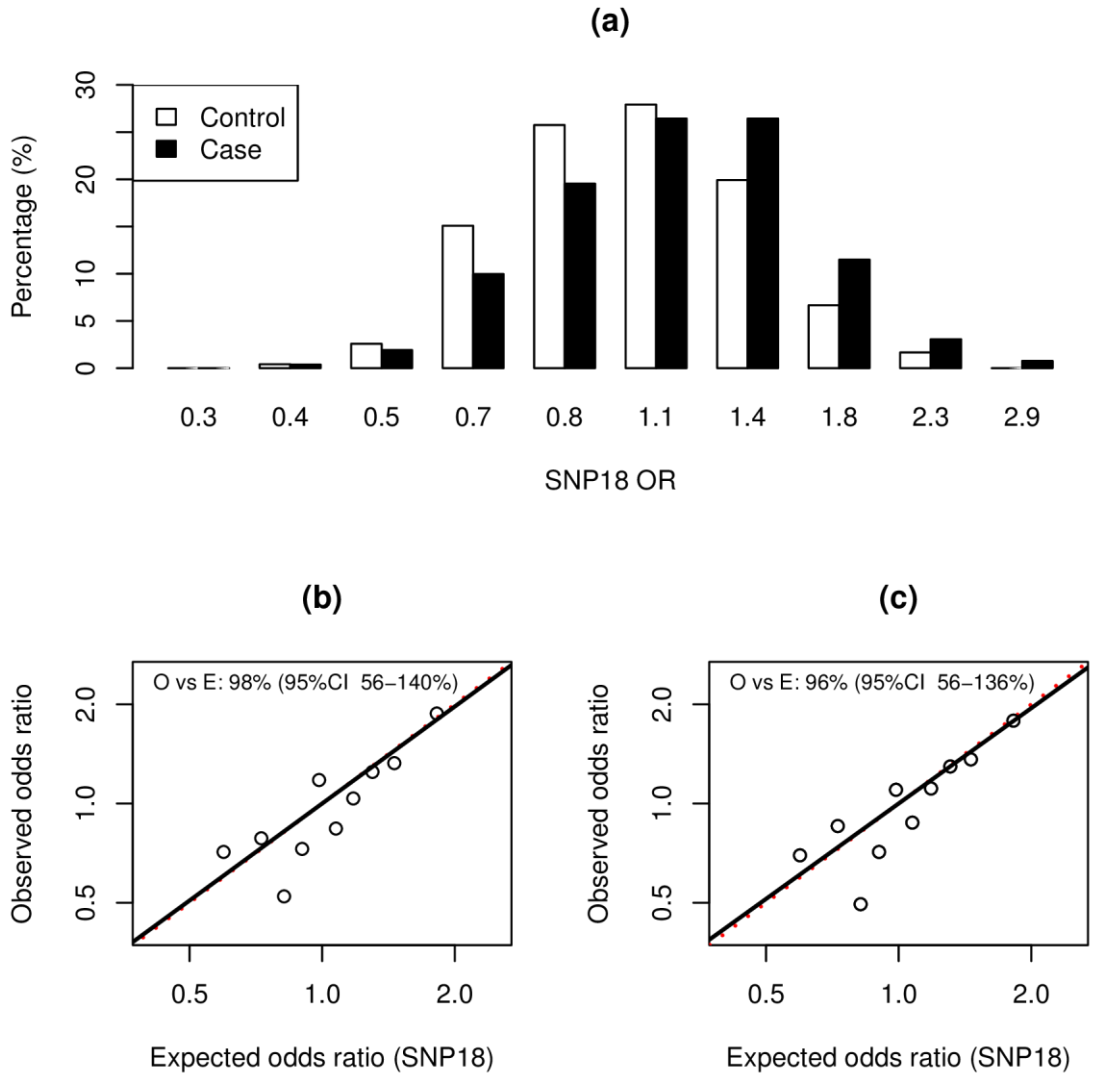

Figure 1: Results for non-*BRCA1/2* group. Panel (a) shows a histogram of SNP18 in controls and prospective cases; then the observed vs expected odds ratio is plotted in panel (b) for prospective cases and (c) all cases. In panels (b) and (c) the points (o) are observed and expected in each decile, the solid line (—) is from a logistic regression fit to the individual-level data, the dashed line is the 45-degree diagonal.

Supplementary material

Table S1: SNPs genotyped including non-prospective cancers but excluding BRCA1/2 carriers

SNP	gg(0)	gG(0)	GG(0)	gg(1)	gG(1)	GG(1)	EAF	RAF	EOR	OR	Fail	Ph ctl	Ph case	O/E ctl	O/E case
rs614367	834	331	34	204	81	9	0.15	0.17	1.21	1.01	1 (<1%)	0.895	0.819	1.00	1.01
rs704010	428	580	192	96	137	61	0.62	0.59	0.92	0.85	0 (0%)	0.875	0.326	0.99	1.05
rs713588	219	588	389	43	136	115	0.44	0.42	0.99	0.81	4 (<1%)	0.927	0.755	1.00	1.01
rs889312	562	506	119	124	133	36	0.72	0.68	0.89	0.85	14 (1%)	0.755	0.928	1.01	1.00
rs909116	259	593	347	57	149	87	0.49	0.46	0.93	0.94	2 (<1%)	0.828	0.679	1.01	0.97
rs1011970	832	328	40	192	94	7	0.17	0.17	1.05	1.10	1 (<1%)	0.476	0.488	1.01	0.97
rs1156287	86	492	596	26	112	155	0.71	0.72	1.07	1.01	27 (2%)	0.364	0.433	0.98	1.03
rs1562430	209	567	418	41	137	116	0.43	0.40	0.90	0.85	6 (<1%)	0.480	0.914	1.02	1.00
rs2981579	389	586	224	73	153	68	0.60	0.56	0.79	0.78	1 (<1%)	0.879	0.518	1.00	0.96
rs3757318	1012	176	10	243	49	2	0.07	0.08	1.16	1.11	2 (<1%)	0.724	0.951	1.00	1.00
rs3803662	597	500	96	147	128	19	0.74	0.71	0.81	1.04	7 (<1%)	0.629	0.320	0.99	0.95
rs4973768	340	587	268	63	164	66	0.47	0.48	1.09	1.15	6 (<1%)	0.609	0.047	1.01	0.88
rs8009944	632	477	88	143	125	26	0.74	0.72	0.96	0.87	3 (<1%)	0.922	0.924	1.00	0.99
rs9790879	420	577	203	82	165	47	0.60	0.58	0.92	0.88	0 (0%)	0.823	0.022	1.01	0.87
rs10995190	22	270	908	5	61	228	0.16	0.13	0.86	0.91	0 (0%)	0.818	0.793	1.00	1.01
rs11249433	411	553	206	80	147	67	0.40	0.43	1.09	1.30	30 (2%)	0.395	0.980	1.02	1.00
rs13387042	299	576	292	62	135	97	0.51	0.51	1.14	1.28	33 (2%)	0.640	0.224	1.01	1.07
rs10931936	624	502	68	164	107	22	0.72	0.73	0.96	1.05	7 (<1%)	0.045	0.503	0.95	1.03

gg, gG, GG, genotype combinations with reference to Table 1 allele; (0) for controls; (1) cases; EAF is the expected risk allele frequency (COGS); RAF is the observed risk allele frequency; EOR is the expected per-allele odds ratio (COGS); OR is the point estimate from these data; HWE is tested where Ph is the p-value from a test on the number of homozygotes, where the observed number of homozygotes divided by expected is also given.

Table S2: Summary of SNPs genotyped, BRCA1 only. Those included in SNP3 highlighted in bold.

SNP	gg(0)	gG(0)	GG(0)	gg(1)	gG(1)	GG(1)	EAF	RAF	EOR	OR	Fail	Ph ctl	Ph case	O/E ctl	O/E case
rs614367	144	42	10	47	29	3	0.15	0.18	1.00	1.45	0 (0%)	0.079	0.758	1.07	0.97
rs704010	66	96	34	29	37	12	0.38	0.41	1.00	0.89	1 (<1%)	0.987	0.885	0.99	1.00
rs713588	38	92	65	12	44	23	0.44	0.43	1.00	1.00	1 (<1%)	0.554	0.283	1.04	0.87
rs889312	97	73	24	43	32	4	0.28	0.29	1.00	0.76	2 (1%)	0.117	0.700	1.09	0.96
rs909116	48	100	48	21	42	16	0.51	0.51	1.00	1.14	0 (0%)	0.830	0.627	0.98	0.93
rs1011970	139	48	9	64	14	1	0.17	0.15	1.00	0.59	0 (0%)	0.238	0.821	1.05	1.01
rs1156287	18	75	102	1	37	40	0.28	0.27	1.00	0.83	2 (1%)	0.476	0.093	1.04	0.84
rs1562430	41	83	72	17	36	25	0.57	0.57	1.00	0.90	1 (<1%)	0.062	0.484	1.12	1.07
rs2981579	58	96	41	31	34	14	0.40	0.44	1.00	0.78	1 (<1%)	0.857	0.348	1.01	1.09
rs3757318	184	12	0	62	16	1	0.07	0.05	1.20	4.14	0 (0%)	0.996	0.922	1.00	1.00
rs3803662	115	67	13	42	33	4	0.29	0.24	1.05	1.12	1 (<1%)	0.522	0.616	1.03	0.95
rs4973768	50	96	50	23	37	19	0.47	0.49	1.00	0.91	0 (0%)	0.721	0.515	1.02	1.06
rs8009944	95	77	23	38	37	3	0.74	0.70	1.00	1.21	2 (1%)	0.265	0.217	1.07	0.88
rs9790879	74	87	34	28	35	16	0.40	0.41	1.00	1.11	1 (<1%)	0.323	0.358	1.06	1.09
rs10995190	3	48	145	2	19	58	0.16	0.14	1.00	1.07	0 (0%)	0.864	0.780	0.99	1.01
rs11249433	60	106	27	34	34	11	0.40	0.40	1.00	0.76	3 (1%)	0.089	0.551	0.88	1.05
rs13387042	53	91	52	13	46	19	0.52	0.51	1.01	1.18	1 (<1%)	0.284	0.127	1.07	0.82
rs10931936	94	82	14	40	34	4	0.72	0.72	1.00	1.11	7 (3%)	0.620	0.512	0.97	0.93

Table S3: Summary of SNPs genotyped, BRCA2 only. Those included in SNP12 highlighted in bold.

SNP	gg(0)	gG(0)	GG(0)	gg(1)	gG(1)	GG(1)	EAF	RAF	EOR	OR	Fail	Ph ctl	Ph case	O/E ctl	O/E case
rs614367	147	55	2	54	23	1	0.14	0.15	1.08	1.14	0 (0%)	0.508	0.688	0.97	0.96
rs704010	65	105	34	28	38	12	0.38	0.42	1.01	0.89	0 (0%)	0.496	0.973	0.95	0.98
rs713588	37	99	68	16	43	19	0.44	0.44	1.00	1.27	0 (0%)	0.873	0.421	1.01	0.90
rs889312	105	89	10	36	33	8	0.29	0.28	1.04	1.31	1 (<1%)	0.217	0.838	0.93	1.01
rs909116	51	100	53	17	41	20	0.51	0.51	1.10	1.06	0 (0%)	0.727	0.724	1.02	0.95
rs1011970	137	61	6	52	25	1	0.17	0.18	1.03	0.96	0 (0%)	0.923	0.578	0.99	0.95
rs1156287	10	76	118	7	31	40	0.28	0.25	1.02	1.32	0 (0%)	0.756	0.731	0.98	1.02
rs1562430	32	93	79	12	39	26	0.57	0.61	1.02	0.90	1 (<1%)	0.567	0.771	1.03	0.96
rs2981579	70	104	30	24	39	15	0.40	0.41	1.28	1.19	0 (0%)	0.447	0.996	0.94	0.99
rs3757318	176	28	0	69	9	0	0.07	0.07	1.15	0.82	0 (0%)	0.748	0.956	0.99	0.99
rs3803662	131	65	8	44	31	3	0.27	0.21	1.24	1.26	0 (0%)	0.957	0.587	1.00	0.94
rs4973768	63	82	59	20	42	16	0.47	0.49	1.00	0.94	0 (0%)	0.004	0.556	1.20	0.92
rs8009944	113	72	19	47	28	3	0.74	0.74	1.00	1.30	0 (0%)	0.204	0.819	1.07	0.97
rs9790879	69	106	29	26	41	11	0.40	0.40	1.07	1.01	0 (0%)	0.298	0.505	0.93	0.91
rs10995190	3	52	149	1	17	60	0.16	0.14	0.94	0.83	0 (0%)	0.766	0.980	0.99	0.99
rs11249433	74	98	32	32	36	7	0.40	0.38	1.00	0.75	3 (1%)	0.981	0.613	1.00	0.94
rs13387042	54	97	49	16	36	23	0.52	0.50	1.01	1.26	7 (2%)	0.627	0.700	1.03	1.03
rs10931936	108	75	21	42	33	2	0.72	0.73	1.00	1.26	1 (<1%)	0.201	0.299	1.07	0.90

Table S4: Results excluding those from the EMBRACE project in *BRCA1/2* groups. The odds ratio (OR) is between the 75 and 25 quantile of each predictor in controls.

	Control	Case	IQR	IQR-OR	OR CI	LR-CHI	AUC	AUC CI
SNP3 (BRCA1)	8	21	0.95 - 0.98	1.14	0.85 - 1.80	0.7	0.57	0.35 - 0.79
SNP13 (BRCA2)	12	27	0.74 - 1.28	1.72	0.49 - 6.47	0.7	0.57	0.36 - 0.79
SNP18 (BRCA1)	8	21	0.79 - 1.03	0.98	0.55 - 1.84	0.0	0.49	0.25 - 0.73
SNP18 (BRCA2)	12	27	0.75 - 1.30	1.45	0.46 - 4.69	0.4	0.53	0.31 - 0.75