Genetic regulation of the host response to cardiac surgery and cardiopulmonary bypass

E.M.SVOREN MBBS, FRCA, EDIC.

Statement of originality

I, Eduardo M Svoren, 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 accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis.

I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university.

Signature:

Date:

Contributors

I am very grateful to the research nurses, Ms Alice Purdy and Eli McAlees, for their collaboration in patient recruitment and blood sampling.

Both genome-wide expression array and genotyping arrays (Illumina R™) were processed at the Wellcome Trust Sanger Institute, Cambridge.

Peter Hamburg and Emma Davenport collaborated in the eQTL analysis.

Acknowledgments

To Professor Charles Hinds whose support and encouragement made possible what I had considered for many years a dream. From the first discussion that I had with him over undertaking research for the accomplishment of this thesis, several years passed by and my personal circumstances changed, including obtaining a completion certificate in Anaesthesia and becoming consultant in neuro-anaesthesia and neuro-critical care. This did not happen without hurdles and the occasional disappointment. However, Professor Hinds always had words of encouragement and some good old-fashioned advice, both invaluable in achieving the completion of this chapter of my life.

I would like to thank Dr Julian Knight who patiently tolerated my lack of laboratory experience and whose intellectual input through stimulating informal discussions, corrections and advice provided me with the tools and the right frame of mind to accomplish this investigation. Peter Hamburg and Emma Davencroft collaborated in the eQTL analysis. I couldn't have progressed with the analysis without their help.

To Sandra Sims for her help in "how to do things" and many late afternoon conversations over a cup of coffee.To Leona for her positive outlook.

Finally, to Juanita and Aliyah for keeping my sanity and pushing me forward.

Awards and funding

Intensive Care Society (Young Investigator Award) (£10,000)

HCA International. Bench fees (£19,500)

Collaborative Work University College of London (UCL). Genotyping cost (£20,000)

To the memory
of Professor
Nelly Coarasa.

Abstract

There is significant variation between individual patients in the magnitude and pattern of their systemic response to cardiac surgery. Poor outcomes in these patients have been associated with a dysfunctional host response. This thesis seeks to define such variability at the level of gene expression by sequential analysis of transcription before and after surgery for a low risk group of patients undergoing elective cardiac surgery and cardiopulmonary bypass (CPB) patients using expression microarray profiling. To that aim, we analysed sequential global gene expression patterns in circulating peripheral blood leukocytes. We also investigated the role of DNA sequence variation in modulating the observed changes in gene expression. This approach allowed us to identify important genetic modulators and novel biological pathways and gain new insights into the mechanisms that regulate the host response to surgery.

Background:

Despite advances in surgical, anaesthetic and cardio-protective techniques, cardiac surgery continues to be associated with a considerable risk of postoperative complications and significant mortality (2% to 8%)[4-6]. Moreover, patients undergoing elective cardiac surgery are increasingly elderly, with more severe cardiac disease and multiple co-morbidities[8]. Much of this morbidity and mortality is related to a dysfunctional host inflammation that can predispose to nosocomial infection and multiorgan failure in as many as 10% of cases, of which up to 40% can be fatal [9]. Specific complications which are often attributable, at least in part to a dysfunctional inflammatory response, include acute lung injury and acute respiratory distress syndrome (ARDS) in 1 to 2% of patients, renal impairment (8%), cognitive impairment (up to 65%), cerebrovascular accident (1.5 to 5.2%) and myocardial injury (7 to 15%)[10-13].

The host response to cardiac surgery is triggered by a number of distinct events, including the initiation of cardiopulmonary bypass (CPB) during which blood is exposed to foreign material, ischaemia/reperfusion of vital organs such as the heart and lungs, the re-warming period and surgical tissue injury[2]. This response involves the triggering of innate immune pathways, a systemic inflammatory response and immune compromise that can predispose to infectious complications and organ failures[14]. The ability to successfully regulate the propagation and resolution of this inflammatory response is now considered to be one of the principal determinants of individual patient outcome following cardiac surgery[15, 16] and involves an orchestrated pattern of changes in gene expression that is designed to protect the host and promote recovery[17]. Current understanding predicts that individual differences in this response are mediated by DNA sequence variation and modulation of gene expression by epigenetic changes.

The evolution of the host response is orchestrated by multiple genes, the activity of which must be sequentially regulated in a coordinated manner to produce a reaction commensurate with the trigger. Differences in gene expression between individuals have been shown to be heritable and to be regulated to a significant degree by underlying genetic diversity at the DNA sequence level. Expression quantitative trait studies involving genome-wide expression profiling have demonstrated that genes involved in immunity and inflammation show particularly strong evidence of modulation by genetic diversity[18]. Recent advances in our understanding of gene function have highlighted the importance of a diverse array of regulatory mechanisms, including cis and trans effects[19].

Aims and objective of this research:

My hypothesis is that individual differences in the host response to cardiac surgery, as reflected by temporal changes in gene expression, are modulated by DNA sequence variation. I will be using the insult of cardiac surgery as a “probe” to unmask large effect sizes of genes that would not be detectable by other means.

The aims of this proposal, therefore, are:

1. To define sequential, genome-wide patterns of differential gene expression and their individual variability post cardiac surgery.
2. To identify important, novel biological pathways involved in the host response to cardiac surgery.
3. To define the role of DNA sequence variation in modulating gene expression following cardiac surgery.

Figure and Tables

AF: atrial fibrillation
ALI: acute lung injury
ANOVA: analysis of variance
ARDS: acute distress respiratory syndrome
ARF: acute renal failure
BAN: biological association network analysis
BIND: biomolecular interaction network
CPB: cardiopulmonary bypass
COPD: cognitive post-operative dysfunction
CRF: clinical records form
CRP: c reactive protein
e-QTL: expression quantitative trait loci
HIV: human immunodeficiency virus
ICU: intensive care unit
ICS: Intensive Care Society
DNA: deoxyribonucleic acid
ELISA: enzyme-linked immunosorbent assay
GEWA: genome-wide expression array
GO: gene ontology
PCA: principal component analysis
LPS: lipopolysaccharide
MINT: molecular interaction
MODS: multiorgan dysfunction syndrome
PCR: polymerase chain reaction
RNA: ribonucleic acid
SIRS: systemic inflammatory response
SNP: single nucleotide polymorphism
RIN: RNA integrity
TEE: transthoracic echocardiography
WTCHG: Wellcome Trust Centre for Human Genetics

General outline of the thesis:

Chapter 1: Cardiac surgery

Review of the host responses to cardiac surgery and CPB

Chapter 2: Genomics in sepsis and cardiac surgery

Review of the evidence of DNA sequence variation in cardiac surgery and sepsis

Gene expression investigation (experimental design results and discussion)

Chapter 3: Expression quantitative trait eQTL

Introduction to eQTL

eQTL investigation (experimental design, results and discussion)

Chapter- 1. Cardiac Surgery

Surgical procedures consume a significant proportion of the costs and human resources of health care systems worldwide. In England, 4.2 million operations are carried out every year, of which 30.000 are cardiac procedures (Royal College of Surgeons, database). The NHS annual budget allocated to work in operating theatres in England and Wales is over £1 billion, while in the US 40 million people undergo surgical interventions every year, at a cost of $450 billion [20].

It is expected that by 2020 the number of surgical interventions will increase by 25% and given that the population is ageing, the number of cardiovascular, renal and cerebro-vascular complications are predicted to increase as much as 100% during the same period [21].

Advances in pre-operative risk assessment and peri-operative management may serve not only to identify patients at higher risk of peri-operative events but also to improve outcome. Moreover, targeting interventions to those patients may reduce harm and ensure that resources are used cost-effectively.

This Thesis will cover

1. Main pathophysiological host responses after cardiac surgery and its main systemic associated complications.
2. Review of previous evidence: genetic variants association studies in cardiac surgery, sepsis and critical illness.
3. Study of gene expression profile in a cohort of patients undergoing elective cardiac surgery and cardiopulmonary bypass (CBP).
4. Expression quantitative trait (eQTL) study in a cohort of patients undergoing elective cardiac surgery and cardiopulmonary bypass (CBP).

1-1 Elective cardiac surgery as a model of the host response to injury

Cardiac surgery triggers a strong and diverse physiological response that involves the immune system, coagulation cascades and multiple biological systems required for tissue repair and maintenance of homeostasis. Furthermore, cardiac surgery is associated with the highest incidence of postoperative morbidity of any elective surgical procedure [7].

Elective surgical procedures provide a useful opportunity to investigate the human response to injury. Furthermore, there are unique features of cardiac surgery that make this procedure a particularly suitable model of the host response to tissue injury including:

1-Surgery can be viewed as an experimentally induced injury; there is a clear starting and finishing point. Pre-insult samples can be obtained.

2-The response to injury is vigorous and generalized.

3- The degree of injury can be graded (length of surgery, duration of Cardiopulmonary Bypass (CPB) and/or aortic cross clamp time).

4- Post-operative complications are well understood and easily recognised.

Finally, human responses to surgery and sepsis are strikingly similar, so that advances in understanding as a consequences of investigations in cardiac surgery patients are potentially applicable to the host response to sepsis[20].

1-2 The host response to cardiac surgery

The host response to surgery, trauma and inflammatory diseases such as pancreatitis is clinically similar to that induced by invasive infections. This observation has lead led to the concept of the “Systemic Inflammatory Response Syndrome”(SIRS)[22], the aetiology of which may be infectious or non-infectious (Table 1)Mortality rates in patients who appear to have sepsis but have negative cultures are similar mortality to those seen when infection is proven[23] and SIRS may lead to complications such as acute lung injury (ALI)/ARDS, shock, renal impairment and multiple organ dysfunction syndrome (MODS).The principal triggers of the inflammatory response after cardiac surgery are surgical trauma, blood loss and hypothermia, as well as activation of hematopoietic cells (polymorphonuclear cells and monocytes) and vascular endothelial cells by the passage of blood through the cardiopulmonary bypass circuit [24]. Furthermore, splanchnic hypoperfusion during CPB is common and the presence of Ecoli bacteremia detected by (PCR) (Polymerase Chain Reaction) has been shown to correlate with gastro-intestinal mucosal hypoperfusion in cardiac surgery patients[25]. Metabolically sepsis, trauma and surgery induce a catabolic response characterised by muscle breakdown and a negative nitrogen balance. Hyperglycemia is common. .Pro-inflammatory cytokines such as IL-6 are released by monocytes and plasma levels of IL6 correlate with mortality and length of stay in the intensive care unit (ICU) after paediatric cardiac surgery[26]. The use of tight glycemic control in critically ill and cardiac surgery patients has been shown to reduce mortality up about one third [27], although later trials failed to confirm these findings[28]. Moreover, the pathophysiological disturbances induced by sepsis may have long lasting effects on mortality and survival, increasing the risk of death even 5 years after the event[29].

The host response to cardiac surgery and cardiopulmonary bypass mirrors the reactions elicited by infectious pathogens, trauma and exposure to foreign bodies The tissue injury caused by cardiac surgery triggers a robust inflammatory response that involves an intricate cellular network and a complex humoral component. The exposure of blood to the surface of the extracorporeal circuit activates the complement cascade through the alternate pathway acting on C3 and generating anaphylotoxins C3a , C5a and C3b that in turn activates the common complement pathway. Contact activates Factor XII, generating thrombin, and promoting clot formation, plasmin activation and fibrinolysis. In addition, tissue factor liberated from damaged endothelial cells activates plasmin . Complement activation[2] and tissue factor [21] stimulate the production of cytokines that are released into the circulation by monocytes/macrophages, lymphocytes and/or endothelial cell and are responsible for the propagation and regulation of the inflammatory response. Cytokines may stimulate inflammation (TNF-alpha, IL-1β and IL6) or have an anti-inflammatory role (IL-10, IL1 -ra). Moreover, an anti-inflammatory response defined by a high IL10/TNF mRNA ratio, has been found to be an independent predictor of an uncomplicated outcome in cardiac surgery[30] .

The reported plasma levels of cytokines after cardiac surgery have been variable, perhaps in part because of the heterogeneity of the procedures and/or the anaesthetic and surgical techniques. Furthermore, plasma levels may not be a reliable representation of cytokine activity, as many cytokines bind to receptors and exert their action locally. Nevertheless, Roth-Isigkeit et al studied a group of 20 males undergoing CABG and showed that the distribution of allelic variants at the promoter regions of IL-6 and TNF correlate significantly with plasma cytokine levels[31]. The concept of individual variability in the host response to an inflammatory

stimulus is supported by a study of monocytes collected from 60 healthy volunteers which identified high and low responders to lipopolysaccharide (LPS) as determined by TNF gene expression [32]. Cardiac surgery may be complicated by varying degrees of systemic inflammatory response, leading to multiple organ dysfunction (MODS), the mortality of which may be as high as 40%[9]. The pathogenesis of postoperative complications is not completely understood but preoperative co-morbidity, surgical complications and genetic predisposition are contributing factors, often in combination. Specific aspects of the inflammatory response to cardiac surgery may affect specific organs, resulting in organ dysfunction. Genetic predisposition may also in part explain this variability.

There is evidence that cardiac surgery and cardiopulmonary bypass precipitate not only a pro-inflammatory response but also a transient period of immunosupression that develops during or after a SIRS [33]. There is also up-regulation of immune cell receptors that "prime" immune cells so that they are readily available to invade peripheral tissues and amplify inflammation in response to a second insult such us a nosocomial infection or persistent hypo-perfusion. This second immunological "hit" can trigger organ damage leading to MODS and death[14, 34].

1- 4 Renal Dysfunction

Many of patients after cardiac surgery develop some degree of renal impairment as measured by discreet elevation of plasma creatinine levels during the post-operative period[6]. Renal dysfunction as defined by an increase in plasma creatinine levels of >1mg/dl occurs in approximately 8% of patients and renal failure requiring dialysis in 1 to 3%[6]. Those patients who develop renal dysfunction have a 20 fold increase in

mortality risk, as well as prolonged ITU and hospital stay[35, 36]. In addition, a proportion of those who develop renal impairment are not discharged to their homes but to intermediate care facilities [36].

During cardiac surgery there is a decrease in renal blood flow of between 25% and 75%, and because of the narrow balance between oxygen delivery and metabolic demands, the kidneys are particularly vulnerable to hypoxia/ischaemia at low partial pressures of oxygen[15, 36]. In addition cytokines and endotoxin released after cardiac surgery have been implicated in the aetiology of renal dysfunction. Although age, diabetes, black ethnicity, and preoperative serum creatinine have been consistently identified as being associated with high risk of renal impairment after cardiac surgery, these risk factors failed to predict the magnitude of increase in creatinine in the majority cases.

1-5 Neurological dysfunction

Postoperative cognitive dysfunction (POCD) after cardiovascular surgery manifests as memory loss and impaired concentration during the post-operative period. POCD has been identified in up to 80% of patients 7 days after cardiac surgery and in between 10% and 60% 3 months after cardiac surgery [37].

The high incidence of POCD after cardiac surgery has been traditionally considered to be the consequence of microembolism generated by the manipulation, cross clamp and cannulation of the aorta. However, similar levels of cognitive decline were detected at 12 months follow up in a group of 281 patients when randomized to “off

pump" or "on pump" cardiac surgery[38]. Moreover, recent data collected from patients undergoing total hip replacement (n:162) and cardiac surgery (n:281) using eight neuropsychological tests failed to find significant differences in the incidence of POCD independently of the operation and the anaesthetic technique employed (16% for both groups at 3 months)[39].

It has been hypothesized that the inflammatory response triggered by surgery, together with high levels of stress hormones (glucocorticoids are known to affect cognition) contribute to the development of POCD[37]. Platelet activation during CPB promotes platelet aggregation and fibrin deposition, local thrombosis and thromboembolism have all been postulated to be contribution factors[40] .

1-6 Post-operative bleeding

Major bleeding occurs in 10% of patients after cardiac surgery[41] and one in three bleeding patients will require surgical re-exploration [42] The mortality of patients undergoing CABG who develop coagulopathic bleeding is increased five times[43]. Tissue factor is one of the initiating molecules responsible for the activation of coagulation during CPB [44]. Tissue factor is expressed by monocytes and endothelium and is contained in alpha granules[7]. Recombinant IL-6 and Il-8 have been shown to increase the levels of Tissue Factor m-RNA in monocytes harvested from healthy volunteers[45], highlighting the close relationship between coagulation and inflammation. Furthermore, pro-inflammatary cytokines activate endothelial cells and neutrophils to express cell adhesion molecules: E- selectin (endothelial cells)

and L-selectin (in leukocytes) , thereby facilitating the transmigartion of leukocytes to areas of injury or infection[7].

1-7 Atrial Fibrillation (AF)

AF is the most common complication associated with cardiac surgery, with an incidence of 27% to 40%. Cost and length of stay are increased and AF is associated with a higher risk of stroke, renal and infectious complications.

The pathogenesis of AF is still not well understood but inflammation has been considered to be one of the main triggers for new onset AF. High levels of CRP have been associated with an increased risk of postoperative AF after CABG (OR :4.6) both when CPB was used and following off pump surgery (n:73 with CPB, 79 off-pump CPB)[46]. Atriotomy (in a canine model) was found to increase the inhomogeneity of atrial conduction predisposing to induced AF (burst pacing). An increase in the activity of tissue myeloperoxidase as a marker of inflammation was also observed. Interestingly the preoperative administration of methyprednisolone prevented the inflammation and conduction abnormalities[47].

1-8 Lung dysfunction

Around 25% of patients develop temporary respiratory impairment during the first week after cardiac surgery. Acute Respiratory Distress Syndrome (ARDS) develops

in only 1 % to 2 % of patients but is associated with a high mortality (15% to 50%). Even though the pathogenic mechanisms that cause ARDS have not been completely elucidated recognised contributing factors include the use of extracorporeal circulation, hypothermia, hemodynamic instability , lung-ischemia-reperfusion and transfusions of blood and blood products. The SIRS triggered by CPB and surgical trauma increases capillary permeability and predisposes to pulmonary oedema. In addition, pro-inflammatory cytokines (TNF-alpha, IL-1, IL-2,IL-6,IL-8) and endotoxin arising from the gastrointestinal tract encourage the entrapment of inflammatory cells in the pulmonary capillaries. In short, the action of proteolytic enzymes originating from the inflammatory cells, combined with endothelial swelling and increased capillary permeability lead to interstitial pulmonary oedema and respiratory dysfunction[24, 48].

Chapter - 2. Genomics in sepsis and cardiac surgery:

2-1 Introduction

The fundamental rules of inheritance were established in the middle of the19th century by the Austrian Monk Gregor Mendel who studied the hereditary transmission of 7 traits in peas. He observed that each trait (allelic variant of a single gene, for example colour) was inherited unchanged from each parent and transmitted individually. Mendel enunciated three laws of inheritance;

1- The law of segregation: Each individual carries a pair of alleles for each particular trait. Each parent passes a copy (allele) to its offspring.

2-The law of independent assortment: Separate traits are passed independently of one another from parent to offspring.

3- The law of dominance: Some alleles are dominant and others are recessive; an organism with at least one dominant allele will display the effect of the dominant allele.

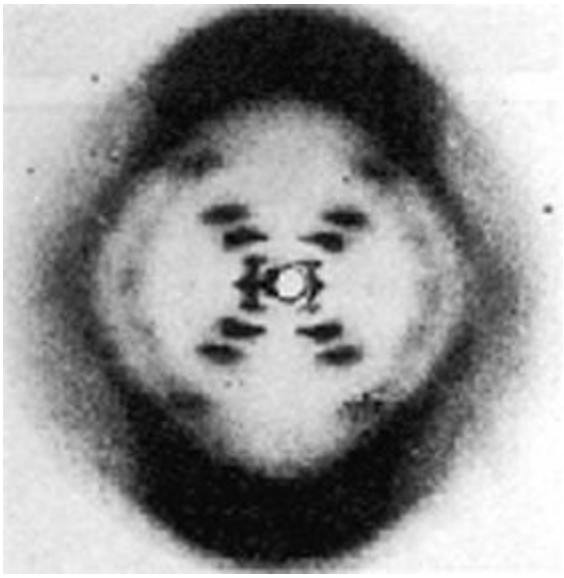
The value of his work remained unrecognized until the 20th century. In 1903 Sutton[49] proposed that the genes were contained in chromosomes, while Avery in 1944[50] first demonstrated that deoxyribonucleic acid was the main component of genetic material. The definitive helicoidal double helix DNA structure was defined by Watson and Crick in 1953[51], prompted by Rosalind Franklin's X crystallographic studies (Figure 1). During the following decade the dynamic function of DNA was revealed by elucidating the process of transcription and translation. Frederick Sanger

in 1977 described Chain DNA Sequencing [52] making it possible to read DNA nucleotide sequences. Kary Bank Mullis developed the Polymerase Chain Reaction(PCR) [53] allowing amplification of very small amounts of DNA .This laboratory technique rapidly became a standard tool for microbiological detection and diagnosis. The completion of the first sequencing and mapping of the entire human genome (2003) paved the way for the rapid expansion of Genomic Medicine. Today human genomic research aims not only to identify the genes that cause disease but to understand how the interaction of multiple gene networks with environmental factors contributes to disease pathogenesis. There are good reasons to hope that this recently opened chapter in medicine will lead to the discovery of novel therapeutic targets as well as new biomarkers for early diagnosis, risk stratification and finally effective treatment of many diseases

2-2 Basic principles:

Human beings are multi-cellular eukaryotic organisms the principal component of their nuclei being DNA. DNA is packed with lysine and arginine rich proteins called histones named H1, H2A, H2B, H3 and H4 that form together a complex structure called chromatin. The activity of histones is influenced by the addition of methyl or acetyl groups and such histone modifications are important for regulating transcription and DNA repair .[54]During mitosis chromatin aggregates into chromosomes. Each chromosome consist of two strands of chromatids that are linked in the middle (centromere) acquiring the shape of a cross. Figure 1

Figure 2-1: Fibre diagram of deoxypentose nucleic acid by Watson, J.D. and Crick, F.H.C., Nature 1953[51]



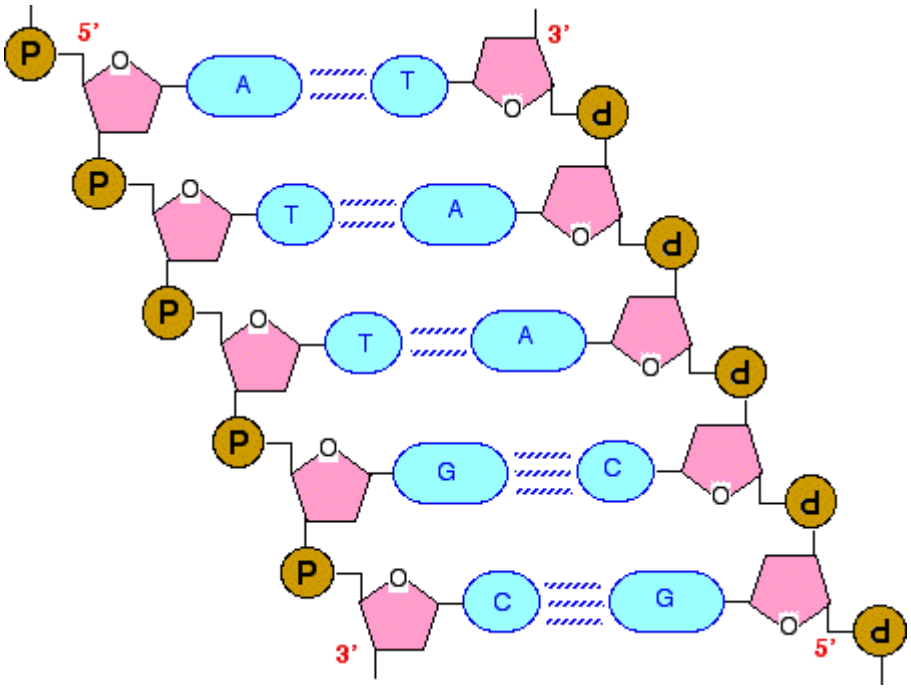
Humans possess 22 pairs of chromosomes, the 23rd pair are called the sex chromosomes and consist of two X in females and one X and and a small Y chromosome in males.

DNA contains four nucleotide bases: Adenine(A), Thymine(T), Cytosine(C) and Guanine(G) that link together in pairs (AT-CG) to form a helical double stranded structure. Each gene is a sequence of DNA that codes for a protein. An allele is a sequence variation in a gene that arises at the same site on a chromosome. The transmission of the information contained in the DNA to mRNA is called transcription. Translation is the process by which a protein is synthesized. The sugar (ribose)

phosphate DNA backbone gives a direction to the DNA chain with the 5’ pentose carbon being the beginning and the 3’ the end. Transcription of DNA into mRNA always occurs in the same direction from 5’ to 3’. Figure 2

Figure 2-2: DNA structure

The basic repeating units of DNA are nucleotides. Each nucleotide compromise a five carbon sugar, which is linked to a nitrogenous base; adenine (A), guanine (G), and thymine(T) or cytosine(C) and phoshodiester group (P) from carbon 3’ of a sugar to carbon atom 5’. Two strand of DNA are held together by hydrogen bonds between base pairs. A binds to T and C to G given rise to a DNA double helix structure.



The m-RNA is generated thorough the action of three RNA polymerases:

RNA Pol I transcribes the r-RNA genes

RNA Pol II transcribes the protein coding genes. This enzyme interacts with the promoter region, its actions are “fine-tuned” by other proteins

RNA Pol III trancribes the t-RNA genes.

The primary RNA transcript unit is the precursor of the m-RNA and incorporates the 5’untranslated region(UTR) , the exons(coding DNA) , the introns (intervening sequences) and the 3 UTR. (Figure 3). The precursor RNA undergoes several modifications before leaving the nucleous as m-RNA:

Capping: addition of a nucleotide cap

Polyadenylation: addition of adenosine residues

Alternative splicing: The introns are removed and the exons are bound together.

Only 1 % of the human genome encodes proteins. To elucidate the function of the remaining “non-coding regions” is the main objective of investigators participating in the ENCODE project [55][56].

A mutation is a nucleotide substitution that occurs in less than 1% of the population. Mutations that are seen in more than 1% of the population are called Single Nucleotide Polimorphisms(SNP). SNPs are the most common DNA sequence variation; more than 10 million have been identified. SNPs can involve substitution, deletion or addition of single bases. Other types of variants are called structural variants, which include copy number variants (loss or gain), chromosomal rearrangement (translocation, inversion or uniparental disomy) and numerical variants (cellular genomes are polyploidy or aneuploidy).

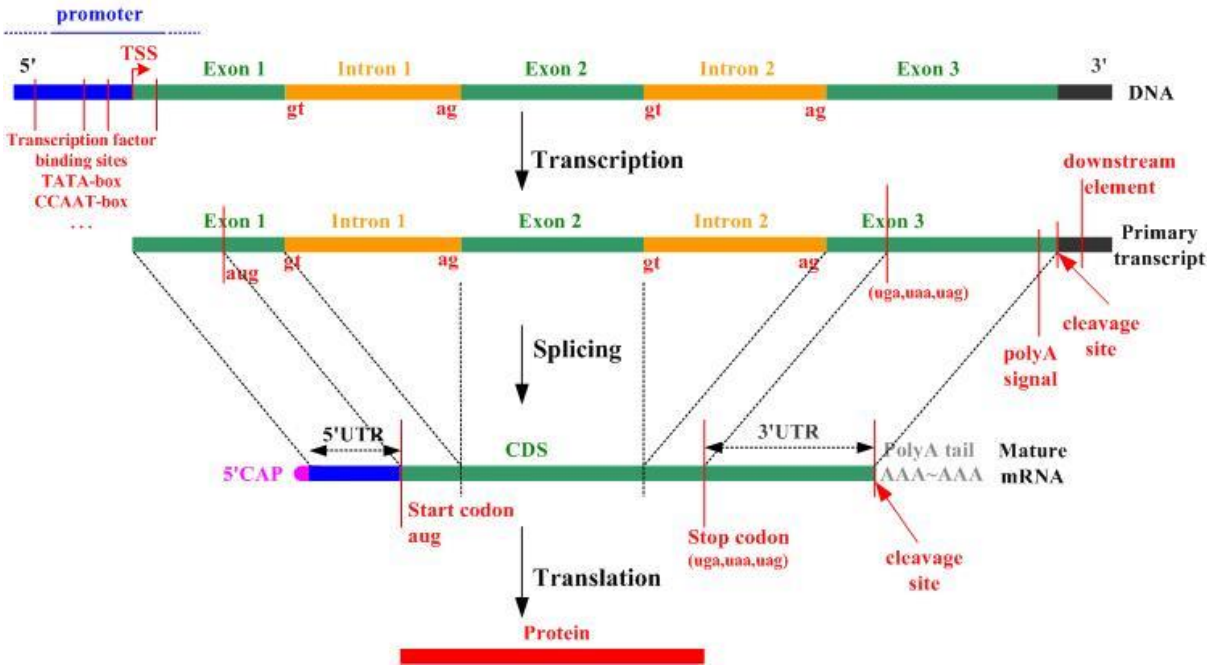


Figure 2-3: Transcription and Translation

Coding regions (exons) are interrupted by noncoding regions(introns). During transcription the entire gene is copied into a pre-m RNA which includes exons and introns. During the process of RNA splicing, introns are removed and exons joined to form a contiguous coding sequence. This “mature” m RNA is ready for translation. In

translation, messeneger RNA (m RNA) produced by transcriptions from DNA is decoded by a ribosome to produce a specific amino acid chain or polypeptide.

The rediscovery of Mendelian concepts during the 20th century encouraged the search for individual alleles with a clear phenotypic effect. This approach has proved extremely successful in revealing the molecular bases of some inherited diseases such as Haemoglobinopathies (e.g Sickle cells disease, Thalassemias).

Recent advances, however, suggest that the classical distinction between monogenic diseases associated with high penetrance and oligogenic or multi-genic diseases (in which the influence of the environment is more marked) is no longer so clear.

For example, phenylketonuria (PKU) is caused by a lack of the enzyme phenylalanine hydroxylase (PAH) that converts phenylalanine to tyrosine leading to an abnormal accumulation of phenylalanine, high plasma levels of which cause neurological damage. Although inheritance of this disease follows Mendelian rules, the resulting phenotype is variable, highlighting the importance of the environment and other genetic factors in this disease.

Cystic fibrosis follows an autosomal-recessive pattern of inheritance. Unfortunately, mapping of the CFTR gene (cystic fibrosis transmembrane conductance receptor regulator) in 1989 did not enable clinicians to predict the outcome of these patients. Patients who are heterozygous for this mutation may develop rhinosinusitis and some suffer from milder forms of CF without carrying CFTR mutants[57].

Many genetic disorders that were previously considered to be monogenic are increasingly found to be associated with mutations in a number of different loci. These findings have encouraged the development of new statistical tools such as

Linkage Analysis; This strategy has only proved successful in Mendellian diseases where a major genetic locus is known to be the causative region[58].

The availability of low-cost, high throughput technologies for genotyping hundreds of thousands of genes has made it possible to perform genome wide association studies (GWAS). These studies focussed on SNPs have advanced our knowledge of polygenic disease responsible such as diabetes, prostatic cancer and breast cancer. GWAS have been useful in identifying common variants contributing to the inheritance of common disease, Most of them with modest or low penetrance [59]. In addition, the biological function and clinical significance of many of the SNPs identified still remained to be demonstrated. Furthermore, many of the loci identified by GWAS are located outside coding regions in areas previously known as “junk DNA” or “gene deserts” but now know many of these areas code for micro RNA etc. [60]. One of the main current hypothesis is that polygenic common diseases are greater extend being caused by low frequency variants with intermediate penetrance. These ideas have encouraged the application of new tools aimed at elucidating the ways in which DNA sequence variation can influence phenotype variation, through performing GWAS meta-analysis as well as functional analysis integrating gene expression with genotype mapping[61] . Finally in vitro and in vivo (model organism) experiments are increasingly used to validate the biological function of the allelic variant detected [62].

2-3 DNA sequence variation and complications after cardiac surgery

A number of candidate gene studies have been performed in the hope of finding a link between the possession of single allelic variants and specific postoperative outcomes. Most papers published in the last 10 years have based their primary hypothesis on extrapolation of data relating to predisposition to cardiovascular disease. Consequently, the most frequently investigated genes have been those involved with lipid metabolism, apoproteins, coagulation and inflammatory mediators.

2-3-1 Renal Dysfunction

Genetic predisposition may in part explain individual variability in susceptibility to renal impairment following cardiac surgery. Brull et al[26] studied 127 patients after CABG and found that two IL -6 promoter region polymorphisms -572C and -174CC were associated with higher postoperative plasma levels of IL6 .In another study possession of the IL-6 -167allelic variant (in 117 patients having CABG) was associated with higher levels of IL-6, an increased incidence of renal impairment, and greater use of diuretics and “low dose” dopamine[63]. However a larger study (1671 patients undergoing CABG) assessing 12 candidate polymorphisms failed to confirm the association between renal impairment and IL-6 -167 but found that the

combination of Il-6 -572(OR 20.04) and angiotensinogen (AGT) -842 was associated with a 121 % increase in the mean peak rise in creatinine[64].

ApoE is a protein that is involved in lipoprotein metabolism. The APOE e allele has been associated with a higher risk of coronary disease and thoraco-abdominal arteriosclerosis, which is a predictor of renal failure after cardiac surgery. It is assumed that the manipulation of the aorta during cannulation and cross clamping detaches atheromatous plaques that embolise to the kidneys. Patients that lack the APO e allele have a lower risk of renal impairment (OR -0.13) after cardiac surgery, independently of the same of ascending aortic atheroma as measured by transesophageal echocardiogram(TEE)[65].

Postoperative fluid overload is associated with increased incidence of acute kidney injury, cardiorespiratory dysfunction and mortality. A large study including 1026 patients undergoing elective cardiac surgery were genotype for 31 SNPs associated previously with inflammation and poor outcomes to cardiac surgery found a protective positive association of the allele rs12917707 of the uromodulin gene and fluid overload (which known function influence Henle's loop cell surface events and renal reabsorption of Na and K). However, the relative effect of this finding of this results, after adjusting clinical variables, was very modest (OR, 1.15)[66].

Renal blood flow represents 20% of the cardiac output and oxygen extraction is 79% (the highest of any organ in the body). Blood supply is tightly controlled by neuro-hormonal and vasomotor renal and extra-renal mechanisms. Any alteration in the normal renal microcirculation due to haemodynamic instability or release of stress related hormones (catecholamines, serotonin) may expose the kidneys to a high risk of hypoxic/ischaemic injury.

Nitric oxide (NO) is an endogenous vasodilator that is synthetized by a constitutive NO synthetase (c-NOS), while an inducible NOS (i-NOS) is upregulated by cytokines including TNF. Pathologically increased synthesis of NO may contribute to vasodilatation and hypotension after CPB which may in turn lead to acute kidney injury. Inotropes and/or vasopressors are often required to maintain blood pressure and ensure adequate perfusion of vital organs. There is evidence that vascular responsiveness may be influenced by genetic variability. A study of 68 patients undergoing CABG or valve surgery showed that patients possessing the 894T allele in the endothelial e-NOS gene were more responsive to increasing doses of phenylephrine[67], while Yates et al found that the combination of e-NOS 894T and AGTR1(angiotensine II receptor type I) was associated with the development of renal dysfunction[6].

Finally, a meta-analysis of three GWAS studies [68](validation cohort of 873 non-emergent CABG and two replications studies totalling 380 patients undergoing elective cardiac surgery) revealed that two genetic regions; GRM7/LMCD-AS1; p= 2.49 × 10-11 and the Bardel-Bredl syndrome 9 gene (BBS9); p=6.51 × 10-08 were associated with the percentage of variation of plasma level of creatinine during the postoperative period with. Even though BBS syndrome includes renal structure abnormalities, there is no evidence of the possible functional mechanism behinds this association.

2-3-2 Neurological dysfunction

Several published studies have investigated the influence of candidate genes involved in inflammation, thrombosis and antigen presentation on the incidence of POCD after cardiac surgery.

Plasminogen activator inhibitor -1 (a negative regulator of fibrinolytic activity) polymorphism 4G/4G(OR 3.1) has been associated with an increased risk of postoperative encephalopathy and cognitive impairment in a group of 260 patients undergoing CABG surgery. Supporting the role of thrombosis in the pathophysiology of POCD a link between cognitive decline and a polymorphism of platelet GPIIIa (glycoprotein constituent of the platelet integrin receptor) was found by Mathews et al, although this finding was based on the mini-mental status examinations (MMSE), a method that may underestimate the true incidence of POCD[69 70].

Tardiff et al[71] found an association between APOE ε(neuronal repair gene) and POCD in 63 patients undergoing CABG, although Silbert et al [72]in a larger study employing neurpsychological testing 3 and 12 months after CABG, failed to corroborate this initial finding. Moreover, Gaynor et al[73] found a positive association between ε2(not ε4) and a reduced psychomotor development index one year after cardiac surgery in children .

The PEGASUS (Perioperative Genetics and Safety Outcome Study) study investigated genetic influences on the risk of POCD and included 443 European American patients undergoing CABG using CPB. They analysed a panel of 37 polymorphisms in candidate genes in pathways known to be involved in POCD based on previous literature .They found that possession of two alleles; CRP

1059G/C(OR 0.37) and SELP1087G/A(OR O.51) conferred an absolute risk reduction of POCD of 20.6% for CRP1059 and 15.2%for SELP .In addition these allelic variants were associated with a reduction in plasma levels of CRP and platelet activation (P- selectin mediated) in patients homozygous for the minor allele when compared with heterozygotos or homozygtes for the major allele[74]. The PEGASUS investigators failed to confirm the previously described associations between APOE, PAI and GPIIIa polymorphisms and the risk of POCD.

Two genes (from a set of 26) related to inflammatory pathways have been associated with an increased risk of stroke (0R 3.3) in 1635 patients after cardiac surgery [CRP (3'UTR1846) and IL6 (-174 G/C)] . Surprisingly no genes associated with thrombosis/coagulation were found to increase the risk of stroke. Moreover, the IL-6 (-174 G/C) polymorphism previously found by the same group of investigators to be a risk factor for POCD was not associated with an increased risk of stroke. This could be explained by the pathophysiological differences between POCD and stroke or could be related to the extra challenge of establishing the diagnosis of POCD in comparison with the diagnosis of stroke [75].

2-3-3 Lung dysfunction

It is considered that lung dysfunction after cardiopulmonary results from a combination of the systemic inflammatory response and mechanical stresses affecting the lung [24].

Tomasdottir et al[76] studied 95 consecutive, unselected patients undergoing elective cardiac surgery. They found that patients homozygous for the TNF-alpha allele TNFB2 had significantly higher plasma concentrations of TNF-alpha and IL-6 and an

increased incidence of pulmonary dysfunction (OR 5.21 (95% CI, 1.49-18.3)). Grunenfelder et al[52] studied the role of the apoprotein E (ApoE) allelic variant e4 (APOE*E4), a SNP associated with higher levels of cholesterol and an increased incidence of coronary disease, in the inflammatory responses to cardiac surgery. They found in a small group of 38 patients undergoing CABG that carriers of the combination of APOE*E4/TNFB*A329G (homozygotes and heterozygotes) had higher levels of TNF-alpha and IL-8 and that mechanical ventilation was prolonged in these patients (773+/- 234 vs 1427+/- 841 minutes).

These two investigations are both limited by the small number of patients studied but they do support a link between individual genetic variants, the severity of the systemic inflammatory response and impaired oxygenation in the post-operative period

2-3-4 Postoperative Bleeding

Welsby et at[43] investigated the role of inflammatory genes in bleeding after cardiac surgery. They used a candidate gene approach selecting 17 polymorphisms in 7 genes (TNF, Interleukin 1β and 6, interleukin 1 receptor antagonist, intercellular adhesion molecule-1(ICAM-1), P- selectin and endothelial leucocyte adhesion molecule-1(E-selectin). The possession of an E-selectin polymorphism was associated with an increased activated partial thromboplastin time (APTT) and bleeding at 4 and 12 hours after non-emergency CABG in 759 patients, although this difference did not translate into differences in the number of red blood cells (RBC) received by any of the groups (2 each). Carriers of the minor alleles did, however, receive more haemostatic products (40%versus 31%). Further Welsby et al [77], using

the same database (780 patients undergoing CABG with CPB recruited between 2000 to 2002 by the PEGASUS group) tested allelic variants in coagulation proteins and found in a multi-variate analysis that seven polymorphisms correlated with the risk of bleeding as determined by chest tube output 12 hours after CABG: ITGA2 (OR -1.25), GP1BA (-0.22), TF (OR -0.03), TFP1 (OR -0.05), F2 (OR 0.38), ACE (OR 0.15). Furthermore, a mathematical predictive model combining these allelic variants with clinical predictors (after multivariate analysis) doubled the ability to predict bleeding in comparison with clinical predictors alone. This study failed to confirm the previously reported association between Factor V Leiden (FVL) and a reduced risk of bleeding in patients undergoing cardiac surgery(CABG, valve operations and other cardiac procedures).In this study Donahue et al[78] found that 46.2% of FVL positive in comparison with 28.3%negative did not receive any blood products(n:517). This failure to replicate findings could be explained by the different percentage of patients with FVL between the two studies (0.02% to 0.05 % respectively). Moreover, in the study by Weslby et al all patients received prophylactic antifibrinolytic treatment (ε-aminocaproic) in comparison with more inconsistent usage of ἐ- aminocaproic and aprotinin in Donahue's paper.

2-3-5 Atrial fibrillation (AF)

A genome wide (Illumina Hap 300 chip (316,515 SNPs) association study of 550 patients of European/Chinese ancestry (4476 controls) presenting with ambulatory AF found a positive association between carriers of three variants in chromosome 4q25 and the risk of AF(rs2200733;OR 1.75, rs 2220427;OR1.75 and rs2634075;OR

1.6 P values here). Body et al in a prospective study of 959 patients undergoing CABG with or without valve surgery found that, of 51 SNPs that described 98.2% variation of the locus (chromosome 4q25) seven (including the 3 SNPs previously found in the ambulatory group) were associated with the occurrence of AF in the post-operative period : rs4626276;OR 2.1, rs2634073;OR1.57, rs2634071:OR1.58, rs2129982;OR;1.75, rs2200733;OR;2.14,rs13143308;OR;1.75, rs2220427;OR;2.17.Furthermore a validation cohort confirmed the association between 3 of the SNPs reported as associated with AF in a further 494 patients[79]. The SNPs identified have no known biological function but they are located 90kb from PITX2 (a gene involved in atrial embryogenesis). The authors of this study speculated that SNPs, regulate the transcription of genes responsible for the electrical susceptibility of atrial tissue to develop AF.

An Italian group studied 110 patients undergoing CABG and found that patients who carried the IL-6 -174 promoter gene variants GG or GC had an increased risk of AF in the post-operative period (OR 3.25). Adding credence to the role of the inflammatory response in the pathophysiology of AF, the plasma levels of IL-6 were significantly higher in those who developed AF ($p<0.001$).However, CRP plasma levels were not significantly different[80].

Other investigators have not, however, corroborated these findings. In addition IL-6 -174 is in strong linkage disequilibrium with of AnTn (-303), another predictor of IL-6 plasma levels after CABG**[81].**

2-3-6 Adverse myocardial outcomes and graft patency after cardiac surgery

As with other investigations of adverse outcomes after cardiac surgery, biological pathways associated with oxidative stress, inflammation and thrombosis have been the focus of several studies relating to graft patency and myocardial events after cardiac surgery. Inflammation is associated with the pathogenesis of atherosclerosis and coronary heart disease. There is evidence of a significant degree of heritability for inflammatory markers; heritability (h2) for CRP, for example is 0.40 and for WBC is 0.35 [82].

An excessive inflammatory response induced by cardiac surgery and CPB has been implicated in the pathogenesis of postoperative myocardial infarction(PMI). Podgoreanu et[83] al tested the association between 48 SNPs in 23 genes (previously reported as being involved in inflammation and ischaemia-reperfusion injury) and the incidence of PMI, these investigators found that 3 polymorphisms increased the risk of PMI after CABG; IL6-572 (OR:2.47) and adhesion molecules ICAM1 Lys469Glu (OR:1.88) and E-selectin SELE 98G>T (OR:0.16).Interestingly, IL-6 plasma levels have been linked with prolonged hospitalization[26]. Several authors have also shown that another variant of e-selectin (Ser128Arg) can affect the adhesion of leukocytes to the endothelium and contribute to the pathogenesis of artherosclerosis[84, 85].

The function of haptoglobin (Hp) is to bind free haemoglobin (Hb) during physiological destruction of erythrocytes, thereby preventing the accumulation of free Hb and reducing oxidative stress is reduced [86] .Several Hp polymorphisms have been described (Hp1-1,Hp-2-2 and Hp 2-1). Haptoglobin Hp2-2 contributes to hypertension and has been associated with atherosclerosis[87]. Hp 2-2 has also been found to be a predictor of previous MI before CABG (OR:1.78) and of reduced graft patency over time (p<0.05% OR: nr)[88].

Another group of investigators studied 765 Flemish individuals undergoing CABG and found that carriers of the DD genotype of the angiotensin I converting enzyme (ACE) gene, had a higher mortality at 2 years after surgery (0%genotype ACE II, 11.2% ACE ID and 14.1% ACE DD p <0.05)[89].The pathophysiological explanation for this association has not been established, but the DD genotype is associated with a two-fold increase in the enzymatic activity of ACE and conveys an increased risk of coronary disease and myocardial infarction in patients without classic risk factors for coronary disease. In addition, local concentrations of ACE in endothelial and intimal layers of vein grafts has been associated with a differential response to angiotensin I and II in an experimental graft model[90]

Hyperhomocysteinemia is an independent risk factor for vascular disease including coronary disease (0R:3.2) [91].A mutation has been reported in the enzyme methylenetetrahydrofolate reductase that intervenes as a methyl donor in the conversion of homocysteine to methionine (C677C — T) . Individuals that are homozygous for the alleles TT have high plasma levels of homocysteine. Botto et al[92] followed up (6.9±0.3 months) a group of 159 patients after re-vascularisation (72 after percutaneous transluminal coronary angioplasty (PTCA) and 87 after CABG).Individuals with the TT genotype had a higher incidence of major cardiac

events; recurrent angina, nonfatal MI ,revascularization , heart failure and cardiac death (OR :2.8).Interestingly hypercysteinemia has been associated with DNA methylation and lipid deposition in the aorta of mice [93].

After CABG, angina recurs in up to 10% of patients, mainly due to graft failure. Angiographic studies have revealed that after one year 15 to 30% of grafts are stenosed and approximately 50% are not patent 10 years post-surgery[94]. Progression of atherosclerosis and thrombus formation are considered to be the main factors that lead to graft occlusion. Platelet glycoprotein IIb/IIIa is a complex receptor for fibrinogen and von Willebrand factor and carries the human platelet antigen (HPA)1. HPA-1B codes for a leucine/proline substitution, is a hereditary factor for platelet thrombogenicity and is strongly associated with coronary thrombosis (OR: 6.2 for patients with coronary disease <60 years old)[95].In a population of 261 patients after CABG only the possession of the polymorphism HPA-1b after logistic regression analysis was predictive of major complications (MI, bypass occlusion or death) [96]with an OR of 4.7.

Other investigators studied the development of postoperative ventricular dysfunction (VnD) after surgery. In a meta-analysis based on one validation study and two replication studies by Fox et al [97](188 cases of VnD and 1200 controls), the authors found three allelic variant in chromosome three associated with an increased in the incidence of VnD: rs17691914 (SNP located near cell death 6 interacting protein gene), rs17061085 (located within the intron of MARVEL, a transmembrane domain associated with heart failure), rs12279572 reside near histidine triad (FHIT) which expression has been found to be decreased in LVEF. One of the limitations of these impressive findings is that the definition of VnD is based only on clinical variables; inotropes requirements or ventricular support after CABG, the other is that the

positive association (which carries a considerable effect; OR; ≥ 2.1) lack of µsupportive evidence about the functional role of the SNPs studied.

2-4 DNA sequence variation and sepsis

Sorensen et al [98] in a landmark paper studied 960 children adopted early in life . These authors showed that the risk of premature death (<50 years old) as a result of infection had a stronger heritable component (OR:4.5) than cardiovascular and cerebrovascular disease (OR:1.19).

Over the course of evolution infectious diseases may have selected for protective variations in the areas of the genome associated with human immunity, explaining the large number of polymorphisms in the human leukocyte antigen (HLA) region, for example. It is worth noting that selective genetic pressure is only exerted when the disease affects mortality and morbidity before the reproductive age [99].

There is strong evidence of susceptibility to some specific infectious diseases such as leprosy, tuberculosis, malaria and HIV amongst others .Lessons learnt from these studies may be applicable and help to unravel the key genes affecting susceptibility to sepsis and septic shock in critically ill patients.

2- 4- 1 Malaria

One of the first genetic associations to be investigated was prompted by the observation that there was a similar geographical distribution for haemoglobinopathies and the presence of *plasmodium falciparum*[100] .

HbS is a consequence of an aminoacid substitution ,from glutamic acid to valine in the β globulin chain caused by a mutation in chromosome 11p15.5.The HBS allele is found particularly in large areas of sub-Saharan Africa, as well as in parts of the Middle East and India[99]. Homozygotes (HbSS) suffer from sickle cell disease but heterozygotes are usually asymptomatic. The heterozygous state (HbAS) confers >90 % protection against severe and lethal malaria and 50% protection against mild attacks [101].There are several theories to explain the mechanisms by which HbS results in protection, including an increase in the clearance of infected red blood cells by the spleen and a reduction in the growth of the parasite inside affected red cells[102]. Another structural variant of haemoglobin associated with malaria infection is hemoglobin C (glutamic acid to lysine aminoacid substitution). Less common than HbS, HbC is found in several parts of West Africa, but the protective effect of heterozygosity (HbAC) is only 30% in comparison with >90% in homozygotes [101].

The malaria parasite binds to the erythrocyte surface prior to invading red blood cells. The Duffy blood group antigen is a genetic variant encoded by the gene DARC also known as FY. A substitution from glycine to asparagine at aminoacid position 44 inhibits DARC expression on erythrocytes. Those individuals who are Duffy negative (lack the antigen) are immune to Plasmodium Vivax[99, 100].

Glucose 6 phosphate dehydrogenase (G6PD) deficiency impairs the capacity of red blood cells to deal with oxidative stress, this deficiency confers in homozygous males and heterozygous females protection against malaria, presumably by favouring the elimination of red blood cells at an early stage of infection [102].

Finally, a number of associations have been reported between malaria and polymorphisms of host receptors for cytoadherence; endothelium antigen CD36, intercellular adhesion molecules ICAM-1, CD31 (platelet –endothelial cell adhesion

molecule), cytokine polymorphisms (TNF, IL-4, IL-10) and antigen recognition molecules; HLA-B53, HLA-DR.

2- 4 -2 Leprosy

Leprosy is a chronic infection caused by *Mycobacterium leprae ,* an intracellular bacillus that affects macrophages and Schwan cells. Humans are the only reservoir of the disease.

One of the unusual characteristics of leprosy is that notwithstanding its transmissibility, 90 % of individuals exposed to M.leprae do not acquire the disease. One of the factors that contributes to the risk of contracting leprosy is genetic susceptibility [103].

In a study of 197 Vietnamese families a region overlapping the 5' region adjacent to PARK2 and the co-regulated gene PACRG was found to be associated with leprosy. Individuals carrying at least one haplotype with both risk alleles(PARK2_e01(-2599)-T and rs1040079-C) have an increased risk of leprosy (OR 5.28). These findings were corroborated in an independent sample of 586 unrelated Brazilian unrelated cases. In a "meta-SNP" combining the information at SNPs PARK2_e01(-2599) and rs1040079 of both studies the PARK2/PACRG 5' region was associated with increased susceptibility to leprosy(OR:2.21)[104].PARK2/PACRG are expressed in macrophages and Schwan cells. PARK2 is a ubiquitination eligase involved in the proteasome complex (ubiquitin-mediated proteolysis). Ubiquitination is a regulatory process for immune proteins, including those involved in the TLR signalling pathway [105]. Interestingly, in Salmonella typhi (typhoid fever) and Salmonella paratyphi

(paratyphoid fever) , a proteasome mediated protein degradation process has been also implicated in the host response [103].

The same strategy used in the above studies was used to investigate the region that includes the HLA class III and class II genes. Lymphotoxin-alpha LTA+80 was found to be associated with leprosy in 104 family cases (OR:2.11). In another study of 209 Brazilian cases of leprosy (192 controls), however, there was no association between LTA SNPs and leprosy. Nevertheless further analysis in which data from 298 Vietnamese family samples were combined with the Brazilian group confirmed the association of LTA-80 and the risk of leprosy, but only when the younger cases were considered (OR:5.63)[106]. These results appear to implicate the LTA+80 variant in the early onset of leprosy.

2-4-3 Human immune-deficiency virus (HIV)

HIV infected patients can present three patterns of disease evolution 1: slow progression. 2: AIDS develops between 3 to 10 years after seroconversion. 3: rapid progression; AIDS develops in 2 -3 years. 3: “elite control” where individuals have undetectable viral loads for more than 10 years without any clinical manifestations of immunosuppression [107].

An improved understanding of the genetic and immunological mechanisms underlying “natural resistance” against HIV is one focus of research aimed at developing the first generation of vaccines against HIV [108].

In the same manner that plasmodium vivax uses the Duffy antigen to access red blood cells, the HIV virus uses the chemokine receptors CCR5 and CXCR4 to invade immune cells[108]. Individuals carrying the 32-p deletion of the chemokine (C-C motif) receptor 5 (CCR5) gene have a lower risk of acquiring HIV infection . Homozygotes for the deletion are almost immune to HIV while heterozygotes show a 2 to 4 years delay in progression to AIDS [109]. Homozygotes or heterozygotes for the chemokine CCR2-V641 that results from a change from G to A at position 190 present with a 2 to 4 year delay in progression to AIDS. Conversely other polymorphisms of CCR5 have been associated with rapid progression to AIDS(CCR5P1, CCR5-249A)[110].HLA genes play a key role in the immune response. Heterozygocity at HLA class loci HLA-A, HLA-B and HLA-C is associated with resistance to progression to AIDS. Other variants of genes coding for cytokines have been implicated in the immune response to HIV (TNF-alpha, Interferon alpha receptor 1,IL-4,IL-10, Mannose-binding lectin and Killer Immunoglobulin-like receptor)[108, 110].

It is expected that in the not very distant future genome wide analysis of patients affected with HIV will be used not only as a prognostic tool but also to guide treatment, Advances in our knowledge of the mechanisms that the HIV virus uses to evade the host immune response are currently being used to develop an effective HIV vaccine[108, 111].

2-5 DNA sequence variation and sepsis in the critically ill patient

The traditional approach to studying genetic variants associated with susceptibility to sepsis has been based on case-control studies in which the incidence of selected SNPs in the septic population is compared with that seen in matched unrelated control groups. A similar study design has been used in candidate gene association studies investigating the influence of DNA sequence variations on outcome. Most of the selected candidate gene loci have been involved with innate immunity, cytokines (pro and anti-inflammatory) and thrombosis/coagulation[112].

2-5-1 Cytokines

One of the key events in the host response to infections is the production by the endothelium, macrophages and leucocytes of pro and anti-inflammatory cytokines (e.g. TNF-alpha, IL-1, IL6, IL1-ra, IL-10) that activate the cellular and humoral networks that are responsible for eliminating invading micro-organisms. Although an oversimplification the conventional view has been that an imbalance between inflammation/anti-inflammation may lead to uncontrolled inflammation with shock and organ injury or immunosuppression with recurrent sepsis, multi-organ dysfunction and death.

2-5-2 Tumour Necrosis Factor

In experimental models administration of TNF induces shock that mimics the response to endotoxin [113]. In addition TNF can activate nitric oxide synthase,

increasing the production of nitric oxide, a mediator of shock and hypotension in septic patients[114].

Higher plasma levels of TNF-alpha have been associated with mortality in patients with infection. Two polymorphisms at position -308 of the promoter region of TNF have been investigated in relation to sepsis outcome. TNF1 (G allele; guanine at position-308) and TNF2 (A allele; adenine at position -308). An early study of 80 patients with severe sepsis failed to establish an association between TNF2 and sepsis outcome[115]. However, Mira et al [116] in a case control study found that the TNF2 allele was more common in patients with septic shock than in healthy controls (39% vs 18%) and that the probability of death was increased in those with the TNF2 allele (OR:3.7). Similarly a Taiwanese case-control study of 112 postoperative critically ill patients confirmed the association of TNF2 with an increased risk of death in patients presenting with septic shock, although patients that were carriers of the TNF2 allele were not more likely to develop sepsis or a higher mortality[117]. One of the limitations of these studies was that the patients' haplotype was not known and only a small number of SNPs were analysed. Gordon et al [118] investigated a larger cohort (n; 213) of patients with severe sepsis or septic shock but failed to find any association between a number of TNF and TNF receptor polymorphisms or their haplotypes and the risk of developing sepsis, illness severity or outcome. Subsequently Menges et al[119] reinvigorated the debate about the role of TNF polymorphisms in sepsis. They studied 159 patients admitted with severe trauma of whom 72 developed sepsis and found that the TNF A allele(rs1800629) was associated with the development of sepsis(OR:7.14) and a fatal outcome (OR: 7.65). Sepsis susceptibility and the risk of death are thought to be affected by interactions between multiple genes and the environment, the extremely high odds

ratio reported in some studies are very unusual for what is considered to be a polygenic disease. Patients with trauma have a severe systemic inflammatory response that It is very difficult to differentiate from sepsis based on microbiological cultures alone[120]. In addition, a link between the TNF -308 allele and an increase in the transcription of TNF has not been consistently demostrated[121, 122].

2-5-3 Interleukin-1

IL-1a and IL-1b are pro-inflammatory gene products while IL-1ra (IL-1 receptor antagonist) is an anti-inflammatory acute phase protein that binds to but does not activate the IL-1 receptor [112].In addition IL-1ra allelic variants regulate the production of IL-1b[123]. In a study of 93 patients the development of severe sepsis was associated with the possession of the IL-1raA2 (plasma levels of IL-1ra were not measured), although no survival difference was detected[124] , Contrary to these findings, Ma et al [125] found a positive association between IL-1raA2 and an increased risk of death in 60 patients with severe sepsis (plasma levels of this molecule were not measured), while a similar study in 78 patients with severe sepsis showed that patients homozygous for IL-1raA2 had a higher risk of death (OR: 6.47).Interestingly, PBMC collected from patients that carried the IL-1raA2 allele produced less IL-1raA2 after LPS stimulation [126]. Disappointingly, in a randomised, controlled trial the administration of recombinant interleukin -1 receptor antagonist to patients with sepsis was associated with increased mortality[127].

2-5-4 Interleukin 6

In a landmark paper Roger Bone et al [128] studied 97 patients with severe sepsis and showed that plasma levels of IL-6 were a better predictor of outcome than were TNFα and IL1β.

A polymorphism (G to C) in the promoter region of IL-6 (-174) has been associated with increased production of IL-6 in an ex vivo study in healthy adults[129]. In a German case control study that included 326 critically ill patients (hospitalized for major abdominal , cardiothoracic, head or trauma surgery) of whom 50 developed sepsis, the investigators failed to associate this IL-6 (-174 G/C) polymorphism with increased susceptibility to sepsis or survival rates, although they found that GG homozygotes were represented more frequently amongst the non survivors. Interestingly, these investigators confirmed Bone et al's finding of an association between IL-6 levels and an increased risk of death. The lack of association between (-174 G/C) polymorphisms and IL-6 production was replicated in an ex vivo study in trauma patients[130]. These conflicting findings between ex-vivo studies have been attributed to differences in the assays used to measure IL-6 production[112].

2-5-5 Interferon-γ (INF-γ)

INF-γ is produced by natural killer cells, its main actions being to promote T cell differentiation, up-regulate the major histocompatibility complex(HLA) class 1 and 2 ,

and increase the production of enzymes in monocytes responsible for the respiratory burst, INF-Ɣ also regulates IL-12 [131] .

Stassen et al[132] studied the expression of HLA –DR (major histocompatibility complex class II receptor) in a group of patients (n= 61) after trauma (ISS: 16) of whom 30 became septic Patients homozygous for the D allele (DD) were more susceptible to sepsis (OR: 2.09). Furthermore, in a case control study of 24 patients undergoing elective laparotomy and 21 patients suffering severe sepsis, a postoperative reduction in HLA-DR expression (measured by fluorescent-labelled monoclonal antibody) was associated with a higher risk of sepsis, while in the surgical patients reduced expression of HLA-DR was linked to higher mortality ($P<0.05$).In addition, after ex vivo incubation of monocytes extracted from the non-surgical infected group with LPS, the monocytes from the surviving patients expressed more HLA-DR than those from the non-survivor group[133].

2-5-6 Toll-like receptors (TLRs)

The innate immune system is the first line of defence against invasion by microorganisms. Monocytes-macrophages, granulocytes and dendritic cells possess surface receptors that recognise bacteria, viruses and fungi, triggering the initial cascade of the immune response [134]. There are at least ten TLR receptors, that are activated selectively by different pathogen associated molecular patterns (PAMPs) ;

TLR-2 (gram positive bacteria and fungi), TLR-4(gram negative bacteria and some viruses), TLR-5(flagellae of legionella pneumophilia).

Binding of LPS to TLR-4 activates the transcription factors nuclear factor-κB and activator protein 1. A missense mutation has been described (Asp299Gly and Thr399lle)affecting TLR-4 .Arbour et al [135] found in 83 patients that individuals homozygous or heterozygous for the two mutations (n:10) were hyporesponsive to inhaled LPS in comparison to volunteers with the wild variant. This was the first evidence that allelic variation of TLR-4 in humans may affect the immune response to endotoxin and opened the avenue for many other investigations. A further investigation by the same authors in a case control study of 91 patients with septic shock found that the possession of the Asp299Gly mutant was associated with a higher incidence of septic shock and gram negative infections , although there were no significant differences in disease severity (SAPS-2), multiorgan dysfunction (OSF score) or mortality [136]. Contrary to this positive finding a British study including 1047 patients with microbiologically proven meningococcal disease failed to show any difference between patients who survived and those who died (n:86) in terms of possession of the 299 variant (OR:1.55; 95% CI, 0.70-3.44).These investigators highlight the need to investigate TLR-2 and other TLR-4 co-factors in order to have a better understanding of the roles of TLR in innate immunity [137]. Furthermore , Shalhub et al [138]in a group of trauma patients (n:598) 25% of whom developed severe sepsis or septic shock used haplotype tagging to demonstrate that the TLR-4 299 variant was linked to a common haplotype associated with a decreased risk of complicated sepsis (OR:0.2).

The TLR-2 Arg753 variant has been found to be responsible for hypo-responsiveness to bacterial challenge in vitro and was more common in patients

suffering from septic shock induced by staphylococcal infection (9% versus 3% in a healthy control group)[139]. However, the fact that only 2 out of 22 patients presenting with gram positive septic shock were carriers of the mutation seriously limits the validity of this finding.

Mal (Ser180 leu,TIRAPrs8177374) is a polymorphism in a protein involved in of LPS signalling that is located downstream of Toll-like receptors 2 and 4. This polymorphism is more common in the European population and seems to have spread in West-Eurasia under evolutionary pressure. Using an in vivo model in healthy volunteers it was found that carriers of this variation inTIRAP/Mal produced more proinflammatory cytokines when exposed to LPS. The authors hypothesised that this polymorphism may have a protective effect in sepsis[140]. Furthermore, two SNPs have been described that differentially influence susceptibility to sepsis. TIRAP/Mal SNP re7932766 has been associated with an increased risk of meningeal tuberculosis in Asian patients[141] whereas TIRAP/Mal SNP (Ser180 leuco TIRAP rs8177374) has been shown to have a protective effect in pneumococcal pneumonia[142] A further study investigated the possession of TLR4 (299 SNP) and TIRAP/Mal (Ser 180 leu) in three groups of patients; 375 complicated surgical patients, 159 patients with ventilator associated pneumonia(VAP) and 415 patients after uncomplicated cardiac surgery. In the surgical group homozygotes for the TIRAP/Mal polymorphism were at an increased risk of sepsis (OR:7.3) while the possession of both SNPs (TIRAP/Mal and TR-4 299) was associated with an odds ratio of 5.5 for sepsis risk. In the group of patients with pneumonia the combination of TLR-4 and TIRAP/Mal SNPs was not associated with lower levels of cytokines whereas cytokine levels were lower in patients who were carriers of only one SNP. In patients after cardiac surgery no differences were found between any genotype

combination and cytokine levels (TNF-alpha, IL-6, IL-10, IL-8).This study suggests that lower cytokine levels are associated with an increased risk of developing sepsis after surgical interventions[143]. These findings contradict the observations made by Ferweda et al[140], whose results suggest a negative interaction between TLR-4 and TIRAP/Mal SNPs and plasma cytokine levels .

2-5-7 Mannose-Binding lectin

Mannose-binding lectin (MBL)is an opsonic that binds to carbohydrates on the surface of micro-organism, promoting bacterial elimination through activation of the complement system. MBL plays an important role in innate immune defence[112].
The MBL gene has point mutations within the exon 1 at codon 52, 54 or 57 (variants B,C and D) while the wild allele is called A[144].
Individuals homozygous for any of the mutations have been found to have lower plasma levels of MBL(normal >1000 µg/l) and present with recurrent, unusual severe infections throughout life[145].
The presence of MBL polymorphisms has been associated with lower plasma levels of MBL before chemotherapy in children (n:100) and adults(n;54) suffering from haematological malignancies and with a more prolonged median duration of febrile neutropenic episodes[146, 147].Furthermore, in a group of 266 children , the proportion of patients presenting with meningococcal meningitis was higher in the group with mutations than in healthy controls (OR:6.5)[148].
Gordon et at[144] in a multicentre study including 174 patients with severe sepsis or septic shock confirmed that MBL allelic variants are more frequent in patients with

sepsis and that lower plasma levels of MBL (<1000 µg/l) are associated with higher mortality. However these investigators failed to corroborate the hypothesis that in patients with MBL mutations (A/O or O/O) the level of MBL was different between survivors and non-survivors.

2-5-8 Cell wall recognition molecules: BPI, LPB and CD14

Permeability increasing protein (BPI) is a protein present in the granules of neutrophils. It binds to the lipid A portion of LPS and inhibits LPS induced responses, BPI is cytotoxic for Gram-negative bacteria and promotes phagocytosis. Three polymorphisms have been identified with an incidence of >1%; (G545>C) Tag is a silent mutation, BPI (A645 >G)216 corresponds to a lys216 amino acid exchange for Glutamine and BPI Pstl (T for C) intron 5.

Lipopolysaccharide binding protein (LBP) is a plasma protein produced by the liver in increased amounts during the acute phase response .LBP binds to LPS and jointly with CD14(LPS –receptor found on the cell surface of macrophages and monocytes) induces macrophage activation. Two polymorphic nucleotide exchanges of LBP have been investigated (Cys98 to Gly) and (Leu436 to pro)[112].

CD14 is a glycoprotein expressed on the cell surface of macrophages, monocytes and polymorphonuclear leukocytes. It is a co-receptor for LPS, peptidoglycan and lipoteichoic acid. CD14 is also present in a soluble form that mediates the activation of cells that do not express the CD14 receptor, such as endothelial and epithelial

cells. A polymorphism within the promoter region that consists of an exchange of C to T at position -159 has been described. Individuals who carry the T allele have higher levels of CD14s [149].

A case control study of 345 patients aged 0-19 years old presenting with SIRS ,severe sepsis or septic shock investigated the presence of two BPI polymorphisms BPI (G45>C) Taq and BPI (A645>G)216. These investigators found a significant difference in the presence of BPI Taq and sepsis in all groups (SIRS ;OR:2.07, severe sepsis;OR:1.59 and MODS;OR:3.04).Interestingly, all the non survivors(14 patients;4.1%) were homozygous for the SNP Tag (GG)[150] .

Contrary to this finding, a similar study of 204 adult septic cases with a higher overall mortality than the previous study (47%) found no difference in the possession the BPI genotype between patients and controls, although further analysis revealed that male patients who were carriers of the minor allele of LBP Gly98 were at higher risk of sepsis, The protective effect of female hormones (post-pubertal) might help to explain the differences between findings in adults and children. On the other hand one could speculate that the significant difference in mortality (4% versus 47%) between the two studies at least in part, may explain the discrepancy in findings.[151]

Gibot et al [152] investigated the influence of CD14 polymorphisms on sepsis susceptibility and mortality. These investigators studied just 90 patients with septic shock; individuals homozygous for C-159T had an increased risk of death (OR:5.30). However, a German case control study of 204 patients with severe sepsis failed to corroborate this finding [153] .One of the limitations of these studies is the lack of quantification of CD14 .In addition CD14 expression levels (ex vivo measured by flow cytometry in monocytes) has not been found to differ in patients with severe sepsis or septic shock in comparison with healthy volunteers[154].

2-5-9 Immunoglobulin Receptors

Fc receptors for immunoglobulins (IgG, IgA and IgM) facilitate the process of phagocytosis and play an essential role in the host response to invading microorganisms.

There are three principal FcRs for IgG (Fcγ receptors Ia, IIA, IIIa and IIIb). Three classes of polymorphisms have been described. FcγRIIa is expressed by granulocytes and mononuclear phagocytes, a single point mutation has been reported in exon 4 at amino acid 131; H(histidine) to R (arginine). Neutrophils homozygous or heterozygous for this mutation showed reduced phagocytic activity. Another mutation in FcγRIIIa at aminoacid 158 Fenylalanine (F) for Valine (V) affects the binding of FcγRIIIa to its effectors; IgG1, IgG3 and IgG4. Two variants of FcγRIIIb carry 4 substitutions (FcRIIIb-NA1 and FcγRIIIb-NA2).

Although Neisseria menigitidis nasopharyngeal colonization is frequent and has been reported in up to 10% of the population, the incidence of invasive Meningoccal disease (MD) is only approximately 1-3 cases /100.000 person a year. Airway colonization elicits the production of specific antibodies (IgG, IgA and IgM) that protect individuals from invasive infection. Consequently, several investigators have examined the role of Fcγ receptor polymorphisms in predisposition to invasive MD. In children (n:98) an association was found between carriers of RIIaR131 and the incidence of meningococcal disease (OR:2.9) as well as the development of severe disease (OR:4.8)[155]. In addition, Van der Pol et al [156]studied 50 survivors of MD and 183 first degree relatives of survivors and non survivors; they confirmed the observation that RIIaR131 was more common in patients with meningitis than in 239

healthy control subjects. In addition the frequency of the FcγRIIa/IIIa/IIIb combination RR-FF-NA2/2 was nearly 3 fold higher in relatives of patients than in controls (0R:2.6).

2-5-10 Heat shock proteins

The heat shock protein 70 (HSP70) family of proteins have an immunomodulatory role. Synthesis of heat shock proteins is stimulated by infection and cellular stress (heat, ischaemia/reperfusion and inflammation). They exert several cytoprotective effects including stabilization and repair of denatured protein structures. In addition, HSP70 gene expression exerts an anti- inflammatory effect by inhibiting the translocation of NF-κβ into the nucleus. Surprisingly secreted in the extracellular space HSP70 acts as a pro-inflammatory cytokine, increasing the production of TNF and IL-6[157-159]. Furthermore, HSP70 is, in evolutionary terms, part of an ancient mechanism of cellular preservation, believed to be present in all living organisms (bacteria, plants, animals and humans beings). Interestingly, all eukaryotic organisms exhibit a remarkable aminoacid sequence similarity (60% to 78%) for HSP70. Located in chromosome 6 within the major histocompatibility complex, a highly polymorphic area involved with antigen recognition. it is not surprising that HSP70 allelic variants have been the focus of several studies investigating sepsis susceptibility in humans. Three polymorphisms of HSP70 genes have been identified HSPA1A, HSPA1B and HSPA1L. In a prospective cohort of 434 patients presenting with Community Acquired Pneumonia (204 black patients and 139 white patients) the possession of the genotype HSP701- B1267 AA was associated with a trend toward higher mortality (p=0.06) and in the group of patients with septic shock (30

patients , mortality: 40%) the HSP701-B1267 AA genotype was found to increase significantly the mortality risk (OR: 3.5)[160]. However striking these results are, HSP70-B1267 A is a silent mutation and unlikely to be biologically responsible for the observed difference in septic shock mortality. Investigators from the same group studied genetic variants in the promoter region of HSPA1A and HSPA1B in 100 healthy individuals and measured m-RNA production (HSPA1A and HSPA1B) using PCR in stimulated monocytes (E.coli LPS) collected from 36 selected individuals. Two SNPs HSPA1A-27G>C and HSPA1A-372A>C were found to be in linkage disequilibrium with HSPA1B1267A>G but this variant was not linked to increased m-RNA production. Interestingly, another variant HSPA1B-179C>T, also in linkage disequilibrium with the others, was associated with an increase in HSPA1A and HSPA1B's m-RNA synthesis [158]. Further research into the functional role of the haplotype HSPA1B1267A>G: HSPA1B-179 was performed using an ex vivo experiment with peripheral blood monocytes (PBMC) isolated from 31 healthy volunteers. After stimulation (killed streptococcus pneumonia and E.coli bacteria) individuals with the haplotype HSPA1B-179:HSPA1B267 A had decreased expression of HSP-70 of m-RNA and protein, as well as more elevated levels of m-RNA for TNF. These findings suggest that polymorphisms of HSP70 may have an impact on the phenotypic variation observed in patients presenting with severe sepsis and septic shock [157].

2-5-11 Plasminogen activator inhibitor-1

Plasminogen activator inhibitor (PAI) is an acute phase reactant synthesised by the liver, endothelial cells and platelets. PAI reacts with plasminogen activators as well

as with protein C. Reduced fibrinolytic activity has been associated with coronary disease. The polymorphism (4G/4G) in the PAI-1 promoter has been associated with reduced PAI-1activity and an increased risk of myocardial infarction in young patients (OR 2.15) [161].

Microvascular thrombosis is frequently found in septic patients who develop multiorgan failure and PAI-1 activity has been associated with poor outcome both in sepsis and severe trauma[112, 162]. Consequently, several authors have investigated a possible link between carriers of PAI-1 polymorphism and outcome in sepsis. Coagulopathy is a common feature in meningococcal disease the pathogenesis of which is considered to be in part the result of an imbalance between pro-coagulant and anticoagulant activity. In a group of 133 children with meningococcal sepsis the 4G/4G genotype was associated with higher PAI-1 activity and a higher mortality (OR: 2.0)[163]. Furthermore, Westendorp et al[164] examined 50 patients who survived meningococcal infection, together with 183 first degree relatives and 131 controls. Amongst the 50 patients who survived carriers of the 4G/4G genotype had an increased risk of developing septic shock (OR: 4.8). Moreover the 4G/4G genotype was more common in first degree relatives of patients who presented with severe disease. Based on these observations the authors calculated that patients whose relatives were carriers of the 4G/4G allele have a higher risk of developing septic shock (OR: 5.9). However important these findings are, meningococcal disease has a very distinctive presentation and is usually associated with disseminated intravascular coagulation. Consequently, these results may not be applicable to patients presenting with severe sepsis or septic shock caused by other micro-organisms. A more recent study including 319 patients (74% had sepsis, 19% had severe sepsis and 7% had septic shock) with documented gram negative

bacteraemia (powered >80% to detect a 1.5 fold increase of relative risk of death for the heterozygous forms) failed to corroborate any correlation between mortality and possession of PAI-1alleles [165].

2-5-12 Protein C

Sepsis triggers an imbalance between procoagulant and fibrinolytic pathways .LPS induced up-regulation of tissue factor in endothelial cells activates coagulation Endotoxin exposure can precipitate thrombosis in the micro circulation that may lead to areas of hypoperfusion, tissue necrosis and ultimately may be partially responsible for the development of multiple organ dysfunction[166]. The Protein C pathway is one of the major regulators of the responses observed in sepsis. During activation of the coagulation cascade thrombin reacts with thrombomodulin to form a complex that transforms protein C to activated protein C(a-PC). Activated Protein C (a-PC) exerts anti-thrombotic, profibrinolytic, anti-inflammatory and anti-apoptotic activities, as well as stabilising the endothelial barrier[167] . In sepsis protein C deficiency is common (>85%) and plasma levels correlate inversely with outcome, independently of the infecting micro-organisms[168] .

Two haplotypes in the promoter region of protein C have been described (-1641 A/G and -1654 C/T).-1641G/-1654C and are associated with decreased plasma levels of protein C and an increased risk of thrombotic events [169].

In a case control study of 288 children presenting with meningococcal infection, patients younger than one year old who carried the genotype CG-CG had a higher

risk of developing severe sepsis (3.43). However the positive association between allele CG and sepsis severity was only present in patients younger than 2 years old. In addition plasma levels of protein C were not measured to confirm the functional role of these variants [170].

Furthermore, In 240 Asian patients Chen et al [171] found that the haplotype -1641A/-1654 C was associated with fatal outcome in patients suffering from severe sepsis(OR:1.74). Interestingly, the C allele of protein C 673(in linkage disequilibrium with the haplotype A/C) was also found to be linked to higher mortality in a group of patients of East Asian ancestry living in the USA [172]. In a prospective candidate gene association study including just 62 Caucasian patients only the genotype -1641A was associated with decreased 28 day survival and more renal , neurological and hepatic dysfunction, as well as higher plasma levels of IL-6[169].

The haplotype -1641 G/-1654C is associated with lower levels of Protein C and this haplotype has been associated with worse outcome in severe sepsis. These conflicting findings and the lack of measurement of plasma levels of Protein C or other surrogates of the coagulation pathway (fibrinogen or dimer D) in any of the 4 studies described above, unfortunately fails to advance our understanding of the functional role of genetic variants in of protein C. They also fail to answer the important question about which patients would benefit most from activated Protein C administration.

2-5-13 Angiotensin-converting enzyme (ACE)

Angiotensin I converting enzyme plays a key role in blood pressure control generating angiotensin II (AT)-II from (AT)-1a potent vasopressor. In addition, ACE degrades bradykinin, a vasodilator peptide involved in inflammatory responses. The ACE gene has a restriction fragment length polymorphism consisting of the presence (insertion I) or absence (deletion D) of a repeat sequence in intron16. In 35 healthy volunteers those who carried the DD genotype had a mean increase in plasma levels of angiotensin II of 75% and 39% in T-lymphocytes[173]. ACE activity may be involved in the pathogenesis of ARDS through mechanisms involved in the control of vascular tone and permeability as well as fibroblastic activity[174]. In addition, one British group found that the DD genotype was associated with susceptibility to ARDS [174].

Due to the widespread actions of ACE, several investigators have explored the association of I/D polymorphism with sepsis. In a case control study of 212 patients with severe sepsis Villar et al[175] failed to find a link between ACE polymorphisms and severe sepsis susceptibility or mortality, including in patients with sepsis-induced ARDS.

2-6 Gene expression

2-6-1 Introduction

Mendel's conclusion that allelic variants are inherited independently and unchanged was based on the study of discrete traits encoded by single genes with definitive, easily recognised phenotypic effects. Mendelian genetic cannot, however explain complex and quantitative traits [57].

In 1932 Haldane introduced the concept that genes "accelerate or retard " other genes or groups of genes and that the activity of genes can also be affected by environmental factors[176]. The question as to how multiple genes communicate between themselves and respond to environmental factors to shape phenotypic variation remains to be answered. Improved understanding of this intricate process will be invaluable to advance our understanding of the pathogenesis of complex and multi-factorial diseases [177].

The availability of low-cost high throughput technologies for genotyping hundreds of thousands of genes have made genome wide association studies (GWAS) possible. However, the biological meaning of genetic signals associated with very high levels of significance remains unclear[60] These findings have prompted the application of new methodologies aiming to elucidate how DNA polymorphisms can affect phenotypic variation. The development of microarrays in the mid 1990's made it

possible to perform genome wide expression arrays, treating transcript abundance as a quantitative trait and allowing statistical analysis of multiple mRNA transcripts.[178].

2-6-2 Gene-expression in sepsis:

The host response to sepsis involves the interaction of multiple mechanisms related to the innate and adaptative immune systems. Cytokine based studies have been at the centre of research into the pathogenesis of sepsis. The conventional view of the inflammatory process has been that pro-inflammatory mechanisms predominate initially, whilst later inflammatory pathways are responsible for controlling inflammation and re- establishing homeostasis. However, the lack of success of trials using interventions aimed at pro-inflammatory mediators, the considerable variability of cytokine plasma levels observed in patients with sepsis and the increase body of evidence suggesting that immune stimulation and downregulation occurs simultaneously has stimulated investigators to use genomic studies to discover other mechanisms/pathways that might contribute to the pathogenesis of sepsis[179].

Response to endotoxin

Several investigators have administered endotoxin to healthy human subjects in order to define gene expression profiles in sepsis. Gene expression was determined in whole blood using a global gene expression array of >44.000 probe sets by Calvano et al[17]. After administration of endotoxin to 4 individuals (there were 4

controls) 3714 genes showed a change in signal intensity, more than half (1857) being down-regulated. A small number of probe sets showed up-regulation by 2 hours and the remaining genes were induced at 4-9 hours. Expression of all genes returned to baseline by 24 hours. Network analysis (Ingenuity Pathways analysis) of the main interactomes revealed an initial up-regulation of pro-inflammatory cytokines and chemokines (TNF, IL1A, IL1B,IL8,CXCL10) followed by increased expression of several members of the nuclear factor/relA transcription factor complex (NFKB1, NFKB2, RELA and RELB). 4-6 hours after endotoxin injection a number of transcription factors involved in the innate immune response were over-expressed (STAT genes), c-AMP–response element binding protein (CREB) as well as genes that limit the inflammatory response (IL1RAP, IL1R2, IL10 and TNFRSF1A). In addition, the authors identified suppression at the transcription level of regions associated with mitochondrial energy production and protein synthesis.

Another study investigating gene expression profiles after administration of endotoxins to healthy volunteers (8 individuals compared to 4 controls) showed a similar pattern in whole blood and in mononuclear cells. The peak change in mononuclear gene expression occurred 6 hours after endotoxin administration (439 genes up-regulated and 428 down-regulated). Endotoxin administration up-regulated genes associated with pathogen recognition and immune response (complement receptors, bactericidal/permeability-increasing protein BPI and leukocyte immunoglobulin receptors), chemokines (IL1RA, IL17R, IL15R, CCR2, CCR1,CCR7), microbicidal oxidase and oxidative stress genes and S100 calgranulins. The main down-regulated genes were T and B cell associated (CD48, CD43, CD7, CD3E, CD3D,CD4,CD3Z,CD3G ,TNFRSF7), major histocompatibilty complex class 2, apoptosis (caspase 8, Fas-induced apoptosis) and Natural Killer

inhibitory receptors (KIR2DL3,KIR3DL1), the latter binds human leukocyte antigen (HLA) class 1 [180] .

Interestingly, both studies of changes in gene expression after endotoxin challenge revealed a widespread response of the transcriptome involving very diverse genes that returned to baseline by 24 hours.

Gene expression profiles to diagnose and predict sepsis

Using a microarray containing 340 genes implicated in inflammation Prucha et al [181] identified in 8 patients with severe sepsis (compare to a group of 4 patients who underwent spinal surgery), a gene profile that included 50 genes (19 up-regulated and 31 down-regulated) with a positive predictive value of 98% for the diagnosis of sepsis.

Since then, several investigators have used peripheral blood leukocyte transcription profiles for the diagnosis of sepsis or infectious complications in critically ill patients. Using a global gene expression array (56, 613 probes sets) Cobb et al[182] found that a panel of 1837 genes could predict the onset of VAP in a group of 158 blunt trauma patients however with low (57%) sensitivity and specificity (69%). More importantly the transcript model was associated with 90% negative predictive value for pneumonia. Amongst the genes associated with pneumonia several are implicated in the host response to infection: NAIP activates caspase-1 and leads to IL-1B secretion and apoptosis, CEACAM8 regulates recruitment of adhesion to endothelial cells and α-Defensins are antimicrobial peptides stored in neutrophil granules.

Using a genome wide microarray containing 54.613 probes, Johnson et al [183, 184] identified a unique gene expression profile in patients 0 to 48 hours before the development of clinical sepsis that differed from the profile seen in non-infected patients (45 patients with non-infectious SIRS and 45 before development of infected

SIRS). Of the 459 genes differentially expressed in those who developed sepsis, 65 were down regulated and 394 up regulated .Pathways analysis using recognised annotations (KEGG and Biocarta) identified 10 inflammatory pathways implicated in sepsis that could be separated into 4 domains: 1-Innate Immunity :characterized by the up-regulation of TLR-2, TLR-4 and TLR-5 as well as the MAPK and IRAK pathways , this last being implicated in the activation of NF-κβ leading to cytokine production.2-Cytokine receptor up-regulation of the IL-1 receptor family and the IL-22 receptor pathways with involvement of SOCS3. The latter decreases pro-inflammatory signalling and inhibits the expression of MHC II molecules. 3-T cell differentiation (dependent on the STAT signalling pathways) and evidence of upregulation of caspase –dependent and independent pro-apoptotic activity, as well as anti-apoptotic mechanisms. 4- protein synthesis regulation.

Tang et al[185] have also explored the use of global expression signatures in the diagnosis of sepsis. Gene- expression profiling was performed in neutrophils obtained from 44 patients with retrospectively confirmed sepsis “training set” and 50 prospectively enrolled patients with suspected sepsis “validation set”. A set of 50 genes (35 genes up-regulated and 15 down-regulated) were associated with a predictive accuracy for sepsis of 91% and 88% for the training and validation sets respectively. Hierarchical clustering of the group of genes comprising the signature profile revealed 3 groupings. 1-The inflammatory cluster was, perhaps surprisingly, down-regulated. The reduction in the expression of genes implicated in systemic inflammation suggests that infection inhibits the neutrophil associated inflammatory response. 2- genes implicated in immune regulation were predominantly repressed. 3- Genes involved in mitochondrial function such as oxidative phosphorylation and ATP synthesis were downregulated. Inhibition of mitochondrial activity is considered

to be a physiological adaptation to ensure cell survival by reducing oxygen consumption[186].

A similar study by the same investigators aimed at identifying gene signatures implicated in sepsis in circulating peripheral blood mononuclear cells (PBMC) rather than neutrophils. A similar pattern of sepsis-related immunosuppression was seen. Seventy patients were recruited in whom the diagnosis of sepsis or SIRS was established at the end of the patient's hospital stay (35 "training set "and "35 validation set"). A molecular signature of sepsis that included 138 genes was identified (105 up-regulated and 33 down-regulated) with a predictive accuracy 91% and 80 % in the training and validation sets, respectively. Interestingly, there was no difference in the genes differentially expressed in relation to the causative micro-organism (gram positive or negative sepsis). These findings suggest that the host response to bacterial infection is non-specific. Hierarchical clustering of the genes comprising the signature profile revealed three groups. 1-Pro-apoptotic genes were up-regulated, for example CARD12 (caspase recruitment domain family). Furthermore, anti-apoptotic genes such as BCL2 were down-regulated. 2-Genes associated with the activation of lymphocytes were upregulated. 3 and 4- These clusters consisted of genes implicated in the inflammatory and immune response the expression of which was reduced[187].

It must be recognised that the sepsis related –immunosuppression described above is based on evidence obtained from peripheral blood immune cells, and may not be a reflection of the activity of monocytes and neutrophils resident in other organs [188].

Gene expression profiles over time and in relation to outcome

In order to study leukocyte gene expression profiling during recovery of septic shock Payen et al [189] measured gene expression in leukocytes (monocytes, immature

neutrophils and lymphocytes) obtained from 17 septic shock patients (14 survivors 3 deaths) at 0,1,7 and 28 days using a microarray containing genes implicated in the inflammatory process (340 genes) . Hierarchical clustering showed that decreased expression over time of S100A8 and S100A12 was associated with survival (Friedman test, p=0.001). S100A8 and S100A12 m-RNA encode calcium binding proteins (calgranulins). S100A8 is a pro-inflammatory protein concentrated in granulocytic granules and is secreted by immune cells in response to inflammation and LPS administration. In addition, S100 proteins are responsible for a prothrombotic response in endothelial cells. Another relevant finding of this study is that CD74 (a major histocompatibility complex (MHC) class II associated protein) was down-regulated during the initial stages of sepsis compared to later in the evolution of the disease. This observation is in accordance with previous evidence showing reduced HLA-DR expression during early sepsis [190, 191].

Another transcriptional analysis of peripheral blood cells collected from 38 patients (25 survivors and 13 non survivors) presenting with septic shock corroborated the decrease in expression of HLA-DR in all patients, but showed that this downregulation was more pronounced in non-survivors as measured by means of flow cytometry of monocytes. Employing an array that interrogated 14.500 genes these authors identified a group of 28 up-regulated genes whose expression discriminated between survivors and non-survivors with a sensitivity of 100% and a specificity of 86%. Survival was associated with the restoration of immune function. In survivors there was an increased expression of genes implicated in the modulation of T cell activity; Toll like receptor signalling pathways, chemokine CX3CR1 (known to play an important role in lymphocytic cytotoxicity) and IL-2RB (involved in T cell proliferation and activation)[192].

One of the limitations of the studies described above is that the investigators have used arrays containing a limited number of genes. Wong et al [193] used a global microarray that interrogated the whole genome (54, 681gene probes) and described genome level expression profiles in 42 children presenting with septic shock (33 survivors and 9 non-survivors). Cluster analysis of the genes most differentially expressed in non-survivors versus survivors revealed a list of 34 genes that were up-regulated and 27 genes that were down-regulated. Interestingly two isoforms of metallothionein (MT) showed increased expression in non-survivors.MT are intracellular binding proteins that are capable of binding zinc avidly. Conversely, the authors demonstrated a reduction in the plasma levels of zinc in non-survivors. Zinc metabolism has been associated with immunity; furthermore MT null mice were protected (in comparison with wild-type controls) against the lethal effects of TNF administration [194].

Wong et al [195, 196] showed the utility of pre-defined genome wide expression sub-classes. Using K means clustering analysis of leukocytes obtained from patients with septic shock (98 derivation group and 82 validation group), the authors were able to identify 3 expression mosaics; A, B and C (each containing 100 genes) .Visual inspection by clinicians of the graphic composite mosaic for the sub-classes A, B and C in comparison with a computer generated classification resulted in a high level of agreement between the allocation to subclasses. The odds ratio for mortality for subclass A vs subclass B was 1.5 and vs subclass C 2.2. Analysis of the biologically relevant genes for each subclass, revealed that the majority of the genes that corresponded to adaptive immunity and glucocorticoid receptor signalling are repressed in subclass A in comparison with subclasses B and C.

These exciting advances may have profound implications for the future management of patients with sepsis and several experts are formulating the following question: Is genomic profiling on the threshold of becoming a diagnostic/treatment tool in the care of critically ill patients? It is probably too early, to give a definitive answer to this question. However, it is likely that an increasing understanding of genomics will become a necessary aspect of the training of future clinicians.

2-6-3 Gene expression studies in cardiovascular surgery:

Very few of studies have investigated changes in gene expression in response to cardiac surgery (see Table 1). Analysis of genome-wide expression among a group of 42 patients undergoing CPB revealed that the differential expression of gene pathways involved in the inflammatory response was associated with an increased risk, and greater severity of neurocognitive decline (42). This study clearly illustrates the potential power of this approach but did not define the role of genetic diversity in modulating the individual patient responses.

All of these studies analysed gene expression in peripheral blood leukocytes in small numbers of patients, and only Ramlawi and colleages interrogated the whole genome (>40.000 probes). In two of the studies the first sample was obtained after, rather than before induction of anaesthesia (i.e. no “baseline” sample) and the period of investigation did not exceed 6 hours after surgery, with the exception of 8 patients in the study by Tomic and colleages in whom samples were obtained 24 hrs after surgery (19). Finally the lack of genotyping in these studies means that the role of DNA sequence variation in modulating gene expression, and consequently individual

patient responses, could not be ascertained. Nevertheless, all three studies clearly demonstrate that genes involved in cell adhesion, regulation of inflammation and apoptosis are the most differentially expressed after cardiac surgery and CPB. These findings enrich and challenge our traditional understanding of the stress response to surgery that has been based on literature focussing on the determination of plasma levels of selected inflammatory cytokines at various time-points after surgery.

There remains a need to more precisely define sequential patterns of global gene expression following cardiac surgery and to understand the mechanisms by which DNA sequence variation modulate disease severity. At the same time important advances in molecular biology have opened several doors to advancing our understanding. High throughput genotyping, for example has facilitated new approaches to investigating individual genetic variability and to elucidating how DNA sequence variation can affect the way patients react to insults.

Table 1

Studies investigating gene expression in response to cardiac surgery

Authors	Type of Surgery Number of Patients	Time of sampling	Number of genes studied	Results
Seeburger et al[197]	CABG N:12 Comparison heparin&protein coated CPB circuits	After Induction 6 hours post-op	22.283	More genes up-regulated and down-regulated in Heparin coated circuit
Tomic et al[34]	CABG ON/OFF CBP N: 8/5	After Induction During CPB 24 hrs post-op	4868	CPB associated with more up-regulation of adhesion, apoptotic and cell communication signalling pathways
Ramlawi et al[198]	CABG-VALVES-COMBINED N:42	After Induction 4 Hrs post-op	≥40.000	Neuro-cognitive deficit associated with up-regulation of apoptotic and coagulation pathways

2-6-4 Sequential gene expression profiles following elective cardiac surgery involving cardiopulmonary bypass (CPB)

There is increasing evidence that changes in leukocyte activation are an important cause of organ dysfunction after cardiac surgery [34], but the key molecular pathways involved are not well understood. In this context gene expression profiling using genome-wide microarrays of mRNA transcribed from leukocytes has the potential to shed light on the complex pathophysiological mechanisms, regulatory functions and gene to gene interactions that are involved in the response of leucocytes to cardiac surgery and CPB.

I set out to define these biological pathways at the level of gene expression by sequential analysis of transcription before and after surgery using global expression microarray profiling. I analysed gene expression in circulating peripheral blood leukocytes, collected with a leukodepletion filter system that can be used at the bedside and minimises delay in sample processing.

2-6-5 Methods: Experimental details and design of the investigation

1. Patients were recruited at St Bartholomew's Hospital. Following written informed consent, adult patients presenting for first time, elective open cardiac surgery for coronary artery bypass grafting (CABG), valve replacement or repair requiring CPB were recruited. Exclusion criteria included: lack of capacity for informed consent, immunocompromised (HIV positive, neutropenic or regular therapy with immunosuppressive drugs including steroids), emergency operation, malignancy and pregnancy.

Approval for this study was obtained from the East London and the City Ethics Committee.

2. The anaesthetic technique was standardised. Patients were premedicated with an oral benzodiazepine (temazepam) on the morning of surgery. Anaesthesia was induced with fentanyl and thiopentone, muscle relaxation was achieved with rocuronium or vecuronium. Following tracheal intubation, anaesthesia was maintained with isoflurane and fentanyl before CPB and with a continuous infusion of propofol during and after CPB. Patients were mechanically ventilated with oxygen enriched air. Anticoagulation was achieved with heparin sodium (initial bolus 3mg/kg) and supplemented as necessary to maintain an activated clotting time more than 480 s during CPB. Moderate hypothermia 32-34ºC was achieved using a heat exchanger in the bypass circuit. Non pulsatile flow was maintained at 2.3 to 2.6 l/min per square metre. Glyceryl trinitrate, metaraminol and phentolamine were titrated against blood

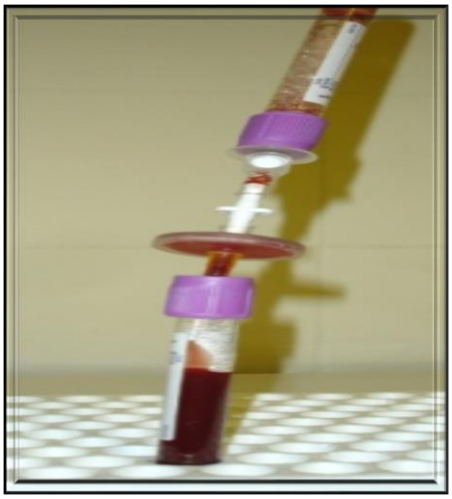
pressure as required. At the end of surgery, heparin was antagonized with protamine to return the activated clotting time to baseline. Patients were returned to the intensive care unit (ICU) for postoperative care.

3. A comprehensive clinical report form (CRF) was completed for each enrolled subject that included differential white cell count, indices of postoperative organ dysfunction and infectious complications. Date of hospital discharge and 28 day mortality was recorded.

4- Samples were obtained before induction of anaesthesia, after aortic cross-clamp release, on admission to intensive care, on the first postoperative day and on day three following surgery. 8 ml of whole blood was collected at each time point and after centrifugation at 1600 G at 4°C for 10 minutes, plasma and buffy coat was collected.

6 ml of whole blood was drawn into a Vacutainer through a leukoLOCK filter that captures leukocytes as illustrated in the **Figure 2-2** below. The filter was flushed with phosphate buffered saline (PBS) (2.5ml) to eliminate red blood cells and RNA later (2.5ml) solution to stabilize the RNA. Filters and plasma, together with the buffy coat for later DNA extraction, were stored at −80ºC.

Figure 2-2: LeukoLOCK capture filter – 6ml sample of blood is passed through a LeukoLOCK filter, which captures the total leukocyte population



5. RNA and DNA extraction and processing were carried out by me at the Wellcome Trust Centre for Human Genetics (WTCHG) in Oxford under the supervision of Dr Julian Knight.

RNA was prepared using the LeukoLOCK ™ Total RNA Isolation Kit (Ambion); proteinase k was used to degrade cellular proteins and bead capture was used to bind the RNA during washing and subsequent Turbo DNase treatment.

RNA concentration was measured by the absorbance of 260nm wavelength light employing a NanoDrop 1000A spectrometer **figure 2-3**, using aliquots of 1ul of purified RNA . The calculation of concentration was based on the Beer-Lambert law: A=E*B*C (A:absorbance, E:wavelength dependent molar absorptive coefficient ,C:analyte concentration, B:path length). The ratio of absorbance at 260nm and 280nm is used as a measure of RNA purity (an index >2 is indicative of pure RNA).

RNA integrity (RIN) was assessed using the Agilent 2100 bionalyser

Figure 2-3: NanoDrop 1000 – A measurement. Features displayed:

Sample ID

Concentration: based on absorbance at 260 nm

A-260: absorbance at 260 nm

A-280: absorbance at 280 nm

A-260/A280 ratio

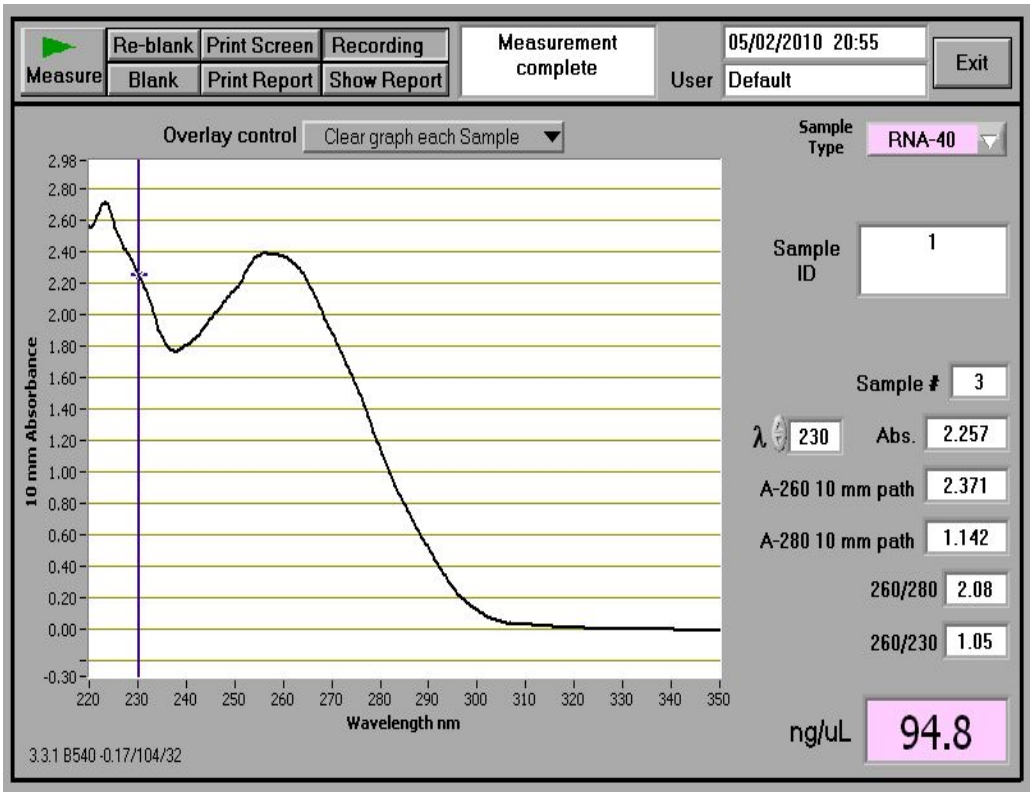
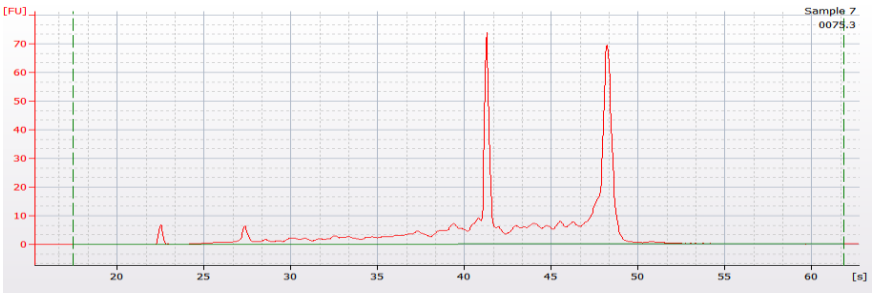


Figure 2- 4. An algorithm is used to analyse the entire electrophoretic trace of the RNA sample, including the presence or absence of degradation products (range 0-10; higher values >7RIN indicate intact RNA). The ratio of ribosomal RNA bands 18S and 28S ratio is also used (>1 indicates integrity)



5-Gene expression profiles were generated for samples taken at baseline, immediately postoperatively and 24 hours after surgery by hybridisation of RNA to Illumina Bead array (HumanHT-12). This arrays targets more than 25,440 annotated genes with more than 48,000 probes

6-Data analysis was carried out using GeneSpring v 11.05(Agilent technologies, Santa Clara US) using the illumina workflow analysis. Our experimental set up aimed to detect differentially expressed genes before and 24 hours after cardiac surgery. The main covariate used for the analysis of differentially expressed gene was sampling time; before and after 24 cardiac surgery

Normalization was achieved by dividing each measurement by the 70th percentile of all measurements in the array. Quality control involved unsupervised analysis and Filtering by flag values and samples. In addition to the analysis of individual genes, an analysis was performed based on a pre-selected group of gene sets that are involved in common biological processes : Pathways and Gene Ontology were performed to obtain more information at the functional level and for hypothesis generation .

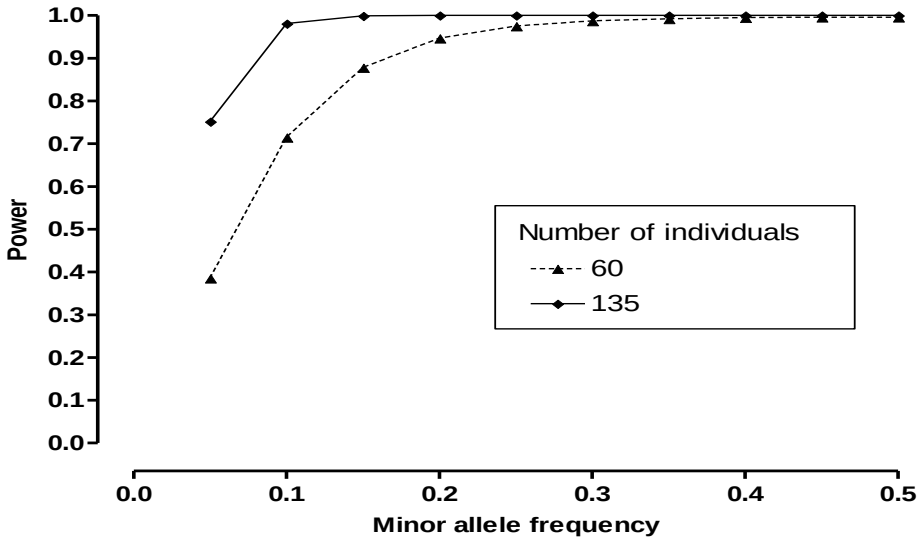
I used Gene Spring© to generate networks and regulatory pathways based on the information contained in the literature (Medline) and accurate databases BIND (Biomolecular interaction network) and MINT (molecular interaction). In order to identify significant interactions between differentially expressed entities, we filtered genes according to categories of binding, metabolism, protein modification and regulation. Finally the entities without direct connections were removed before generating the figures displayed in the results section.

7-Statistical analysis was carried out by selecting the genes that displayed a P-value of <0.05 corrected by false discovery rate (FDR) using Benjamini Hochberg correction. FDR is one way to conceptualise the rate of type 1 errors in null hypothesis testing when multiple testing. This is achieved in Benjamini Hochberg by the ratio of the hypothesis at a significant level by the number of entities tested. The output of this analysis was filtered by fold expression. Thus, lists of genes differentially expressed at least twofold (q <0.05 after correction for multiple testing) were generated for each time-point**.**

Power calculations are shown in **Figure 3-4.** This demonstrates that 60 individuals is the minimum number required to detect a 1.5-fold difference in expression with a

false discovery rate of 0.01 while 135 patients would allow detection of such differences even when minor allele frequencies are low

Figure 3-4: Power calculation. The lower bound of power to detect a 1.5-fold difference in expression with a false discovery rate of 0.01 was calculated for 60 or 135 unrelated individuals. A classical t-test approach was used, incorporating the minor allele frequency and unequal sample sizes in each genotype group, as a result of allele frequency. The population variance (SD 0.424) was calculated based on published datasets[199]. (Power calculations were performed by Dr Jennifer Taylor, Bioinformatics and Statistical Genetics Group at the Wellcome Trust Centre for Human Genetics.)



2-6-5 Results:

The median age of the subjects was 69 years (range 43-81).Total number of subjects; 63 .CPB time ; 108(97-119).Aortic clamp time 87(75-99).See **table 1**. Post-operative complications were : Atrial Fibrillation 16 patients (25%), infection 6 patients (9.5%) ,respiratory failure requiring invasive or non-invasive respiratory support 5 patients (7%),confusion 2 patients (3.1%).The median lenght of stay(LOS) in hospital was 5 days (3-16) of which a median of 1 day(1-4) was spent in ITU .

Of the 95 genes differentially regulated 24 hours after cardiac surgery 80 were found to be up-regulated and 15 were down-regulated (table 2 and 3). A heat map generated using hierarchical clustering is shown to illustrate these changes in gene expression (GeneSpring) **Figure 2-5**.

We grouped the 80 up-regulated genes into functional groups according to the information available through literature searches (Medline, Online Mendelian Inheritance in Man database) 1-Regulation of apoptosis(*TLR5, IL4R, MAPK14, ZDHHC19, ATP9A, DYSF, PLSCR1*), 2-Mitochondrial function (*TPSO, HK3, PGS1,*

PFKFB3) 3- Adhesion molecules (*CD177, RETN, ANNEXIN3,ILR4,VNN1*) 4-Epigenetics (*HISTPH2BB, CDK5RAP2, TDRD9*),5-Microcirculation(*ARG1,METTL7B*, *CD163,HP*), 6-Glucose metabolism(*GYL1,UGCG,MGAM,OPLAH,SC37A3*),7-Leukocyte receptors (*LILRA5,LILRA6,FCGR1B,IL18RAP,IL18R1*),8-Complement (*CR1,C1QB*) and 9-Estrogen receptor(*GPER)****Table 2,*** **Figure 2-6 and 2-7**.

Of the 15 down-regulated genes, two are interferon induced proteins (*IFIT1 and IFIT44L),* both being implicated in the clearance of pathogens as part of the innate immune response. Other down-regulated genes were associated with leukocyte trafficking and chemotaxis (*GPR56, CCR3*). The majority of down-regulated genes (7 out of 16) are implicated in the activation of T lymphocytes (CD4 , CD8 ,γδ) and Natural Killer cells *(EOMOS, GNLY, FGFBP2, GZMK,FCER1A,PRSS33*) **Table 3, Figures 2-6 and 2-7.**

DEMOGRAPHICS	
Age	69 (43-81)
Sex (M/F)	47/16
PROCEDURES	
CABG	38
Valv repl/repair	14
Combined	11
COMORBIDITIES	
Prior MI	8
Diabetes	14
HTA	19
COPD	3
LVEF>30	63
OPERATIVE DATA	
CPT time	108 (97-119)
Cross Clamp time	87 (75-99)
N patients req RBC	20
Deaths	0

Table 1: Demographics

Table 2: Significant by up-regulated genes after cardiac surgery (>2-fold change, q<0.05)

Gene Symbol	Corrected p-value	Definition
C19orf59	5.72415E-16	Homo sapiens chromosome 19 open reading frame 59
RETN	5.72415E-16	Homo sapiens resistin (RETN), mRNA.
TLR5	1.84444E-15	Homo sapiens toll-like receptor 5 (TLR5), mRNA.
TSPO	1.84444E-15	Homo sapiens translocator protein (18kDa) (TSPO), transcript variant
LILRA5	5.81439E-15	Homo sapiens leukocyte immunoglobulin-like receptor, subfamily A
IL4R	6.00336E-15	Homo sapiens interleukin 4 receptor (IL4R), transcript variant
ANXA3	1.59058E-14	Homo sapiens annexin A3 (ANXA3), mRNA.
HP	2.15181E-14	Homo sapiens haptoglobin (HP), mRNA.
HK3	2.18525E-14	Homo sapiens hexokinase 3 (white cell) (HK3)
C5orf32	2.21979E-14	Homo sapiens chromosome 5 open reading frame 32
LILRA5	2.21979E-14	Homo sapiens leukocyte immunoglobulin-like receptor, subfamily A
PGS1	3.18618E-14	Homo sapiens phosphatidylglycerophosphate synthase 1
GYG1	4.9167E-14	Homo sapiens glycogenin 1 (GYG1), mRNA.
ZDHHC19	5.33388E-14	Homo sapiens zinc finger, DHHC-type containing 19
LILRA6	5.4222E-14	Homo sapiens leukocyte immunoglobulin-like receptor, subfamily A
UPP1	2.23105E-13	Homo sapiens uridine phosphorylase 1 (UPP1), transcript variant
ALPL	3.60899E-13	Homo sapiens alkaline phosphatase, liver/bone/kidney (ALPL)
DYSF	3.80787E-13	Homo sapiens dysferlin, limb girdle muscular dystrophy 2B
LMNB1	4.24313E-13	Homo sapiens lamin B1 (LMNB1), mRNA.
PFKFB3	4.45655E-13	Homo sapiens 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase
MAPK14	7.03465E-13	Homo sapiens mitogen-activated protein kinase 14 (MAPK14)
RGL4	7.45685E-13	Homo sapiens ral guanine nucleotide dissociation stimulator
METTL7B	7.96494E-13	Homo sapiens methyltransferase like 7B (METTL7B)
PLSCR1	9.72622E-13	Homo sapiens phospholipid scramblase 1 (PLSCR1)
CR1	1.05145E-12	Homo sapiens complement component (3b/4b) receptor 1
CD177	1.11551E-12	Homo sapiens CD177 molecule (CD177), mRNA.
S100A12	1.18023E-12	Homo sapiens S100 calcium binding protein A12 (S100A12), mRNA.
CYP1B1	1.19942E-12	Homo sapiens cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1), mRNA.
GADD45A	1.3174E-12	Homo sapiens growth arrest and DNA-damage-inducible.
UGCG	1.42211E-12	Homo sapiens UDP-glucose ceramide glucosyltransferase
GPER	1.90687E-12	Homo sapiens G protein-coupled estrogen receptor 1 (GPER)
SOCS3	2.0375E-12	Homo sapiens suppressor of cytokine signaling 3
ATP9A	3.7798E-12	Homo sapiens ATPase, class II, type 9A (ATP9A), mRNA.
HIST1H2BD	3.85193E-12	Homo sapiens histone cluster 1, H2bd (HIST1H2BD)
CDK5RAP2	4.04209E-12	Homo sapiens CDK5 regulatory subunit associated protein 2 (CDK5RAP2)
LOC648984	4.04209E-12	PREDICTED Homo sapiens similar to Baculoviral IAP repeat.
PFKFB3	4.29083E-12	Homo sapiens 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase

CR1	4.81582E-12	Homo sapiens complement component (3b/4b) receptor 1
MGAM	5.08185E-12	Homo sapiens maltase-glucoamylase (alpha-glucosidase)
TDRD9	5.3819E-12	Homo sapiens tudor domain containing 9 (TDRD9)
OPLAH	5.53517E-12	Homo sapiens 5-oxoprolinase (ATP-hydrolysing)
MMP9	5.85406E-12	Homo sapiens matrix metallopeptidase 9
GPER	7.73973E-12	Homo sapiens G protein-coupled estrogen receptor 1 (GPER)
F5	1.44309E-11	Homo sapiens coagulation factor V (proaccelerin, labile factor)
CD177	1.60141E-11	Homo sapiens CD177 molecule (CD177), mRNA.
TDRD9	1.6578E-11	Homo sapiens tudor domain containing 9 (TDRD9),
GADD45A	1.68232E-11	Homo sapiens growth arrest and DNA-damage-inducible, alpha
PPARG	1.73218E-11	Homo sapiens peroxisome proliferator-activated receptor gamma (PPARG)
CA4	1.76482E-11	Homo sapiens carbonic anhydrase IV (CA4), mRNA.
LOC100170939	2.19151E-11	Homo sapiens glucuronidase, (LOC100170939)
CST7	2.28599E-11	Homo sapiens cystatin F (leukocystatin) (CST7), mRNA.
C1QB	3.54476E-11	Homo sapiens complement component 1, q subcomponent, B chain
FCGR1B	3.67399E-11	Homo sapiens Fc fragment of IgG, high affinity Ib, receptor (CD64) (FCGR1B)
LOC153561	3.72328E-11	Homo sapiens hypothetical protein LOC153561
LOC642103	4.40724E-11	PREDICTED: Homo sapiens similar to Maltase-glucoamylase, intestinal (
IRAK3	6.97223E-11	Homo sapiens interleukin-1 receptor-associated kinase 3 (IRAK3)
CES1	1.16432E-10	Homo sapiens carboxylesterase 1 (monocyte/macrophage serine esterase 1)
FCAR	1.58255E-10	Homo sapiens Fc fragment of IgA, receptor for (FCAR), transcript
CDK5RAP2	1.89858E-10	Homo sapiens CDK5 regulatory subunit associated protein 2 (CDK5RAP2)
PGLYRP1	1.90602E-10	Homo sapiens peptidoglycan recognition protein 1 (PGLYRP1)
RNASE2	2.0339E-10	Homo sapiens ribonuclease, RNase A family, 2
OSM	2.09299E-10	Homo sapiens oncostatin M (OSM), mRNA.
GPR84	2.22834E-10	Homo sapiens G protein-coupled receptor 84 (GPR84), mRNA.
LOC728519	2.24904E-10	PREDICTED: Homo sapiens similar to Baculoviral IAP repeat-containing protein 1 (Neuronal apoptosis inhibitory protein) (LOC728519), mRNA.
FCAR	5.06518E-10	Homo sapiens Fc fragment of IgA, receptor for (FCAR), transcript variant 10, mRNA.
SLC37A3	7.11227E-10	Homo sapiens solute carrier family 37 (glycerol-3-phosphate transporter), member 3 (SLC37A3), transcript variant 2, mRNA.
MERTK	1.26984E-09	Homo sapiens c-mer proto-oncogene tyrosine kinase (MERTK), mRNA.
LOC100130904	2.02304E-09	PREDICTED: Homo sapiens misc_RNA (LOC100130904), miscRNA.
VNN1	4.08378E-09	Homo sapiens vanin 1 (VNN1), mRNA.
ORM1	6.0905E-09	Homo sapiens orosomucoid 1 (ORM1), mRNA.
ARG1	6.19529E-09	Homo sapiens arginase, liver (ARG1), mRNA.
IL18RAP	7.67715E-09	Homo sapiens interleukin 18 receptor accessory protein (IL18RAP), mRNA.
CCR3	1.15839E-08	Homo sapiens chemokine (C-C motif) receptor 3 (CCR3), transcript variant 2, mRNA.
FCGR1B	1.22552E-08	Homo sapiens Fc fragment of IgG, high affinity Ib, receptor (CD64) (FCGR1B), transcript variant 1, mRNA.
CLEC4D	1.99724E-08	Homo sapiens C-type lectin domain family 4, member D (CLEC4D), mRNA.
HPGD	3.45641E-08	Homo sapiens hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD), mRNA.

PFKFB2	3.03439E-07	Homo sapiens 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2), transcript variant 2, mRNA.
CD163	5.67315E-07	Homo sapiens CD163 molecule (CD163), transcript variant 2, mRNA.
CD163	5.71855E-07	Homo sapiens CD163 molecule (CD163), transcript variant 2, mRNA.
IL18R1	1.82709E-06	Homo sapiens interleukin 18 receptor 1 (IL18R1), mRNA.

Table 3: Significant downregulated genes after cardiac surgery (< 2 fold change, q<0.05)

Gene Symbol	Corrected p-value	Definition
GPR56	2.50E-13	Homo sapiens G protein-coupled receptor 56 (GPR56), transcript variant 2, mRNA.
S1PR5	4.00E-13	Homo sapiens sphingosine-1-phosphate receptor 5 (S1PR5), mRNA.
GPR56	9.70E-13	Homo sapiens G protein-coupled receptor 56 (GPR56), transcript variant 3, mRNA.
EOMES	3.30E-12	Homo sapiens eomesodermin homolog (Xenopus laevis) (EOMES), mRNA.
GNLY	9.00E-12	Homo sapiens granulysin (GNLY), transcript variant 519, mRNA.
FGFBP2	1.20E-11	Homo sapiens fibroblast growth factor binding protein 2 (FGFBP2), mRNA.
GNLY	2.30E-10	Homo sapiens granulysin (GNLY), transcript variant NKG5, mRNA.
GZMK	2.70E-10	Homo sapiens granzyme K (granzyme 3; tryptase II) (GZMK), mRNA.
PRSS33	5.10E-10	Homo sapiens protease, serine, 33 (PRSS33), mRNA.
FCER1A	7.10E-10	Homo sapiens Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide (FCER1A), mRNA.
GZMH	3.00E-09	Homo sapiens granzyme H (cathepsin G-like 2, protein h-CCPX) (GZMH), mRNA.
CCR3	1.10E-08	Homo sapiens chemokine (C-C motif) receptor 3 (CCR3), transcript variant 2, mRNA.
CLC	2.80E-08	Homo sapiens Charcot-Leyden crystal protein (CLC), mRNA.
IFIT1	2.40E-07	Homo sapiens interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), transcript variant 2, mRNA.
IFI44L	1.16E-05	Homo sapiens interferon-induced protein 44-like (IFI44L), mRNA.

See Appendix 1 for further exploration of genes above

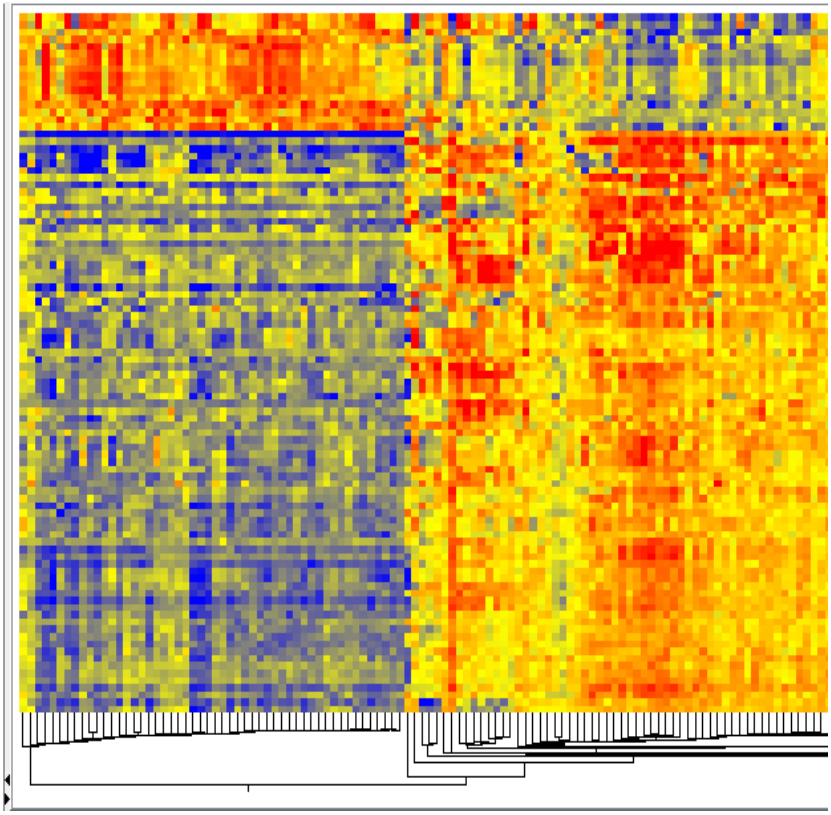


Figure 2-5: Hierarchical clustering of gene expression matrices; We observe a graphical representation of the normalized signal intensity where the individuals' values contained in the matrix are represented in colours. Gene expression experiments produced large amount of data. One of the methods to summarize unsupervised data to extract information from the biological process investigated is to build a gene expression matrix. Hierarchical clustering was performed using GeneSpring X10.5. Each row represents a single gene and each column represents an individual. Left: Baseline measurements Right: 24 hours after cardiac surgery. Red: Indicates over-expression of genes and blue under-expression of genes. The branches of the dendrogram represent the degree of closeness of genes
We can observe that the sampling time (before and 24 hour after surgery) is clear determinant of the gene expression investigated.

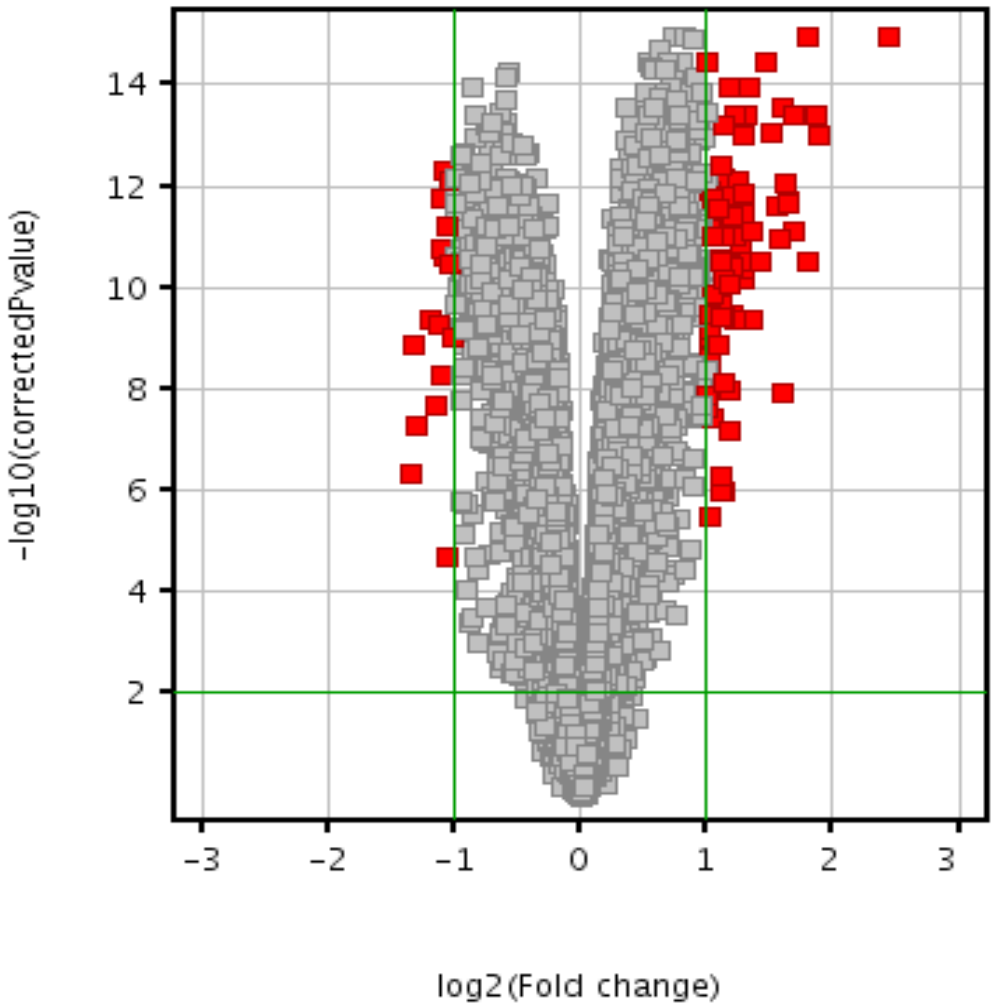
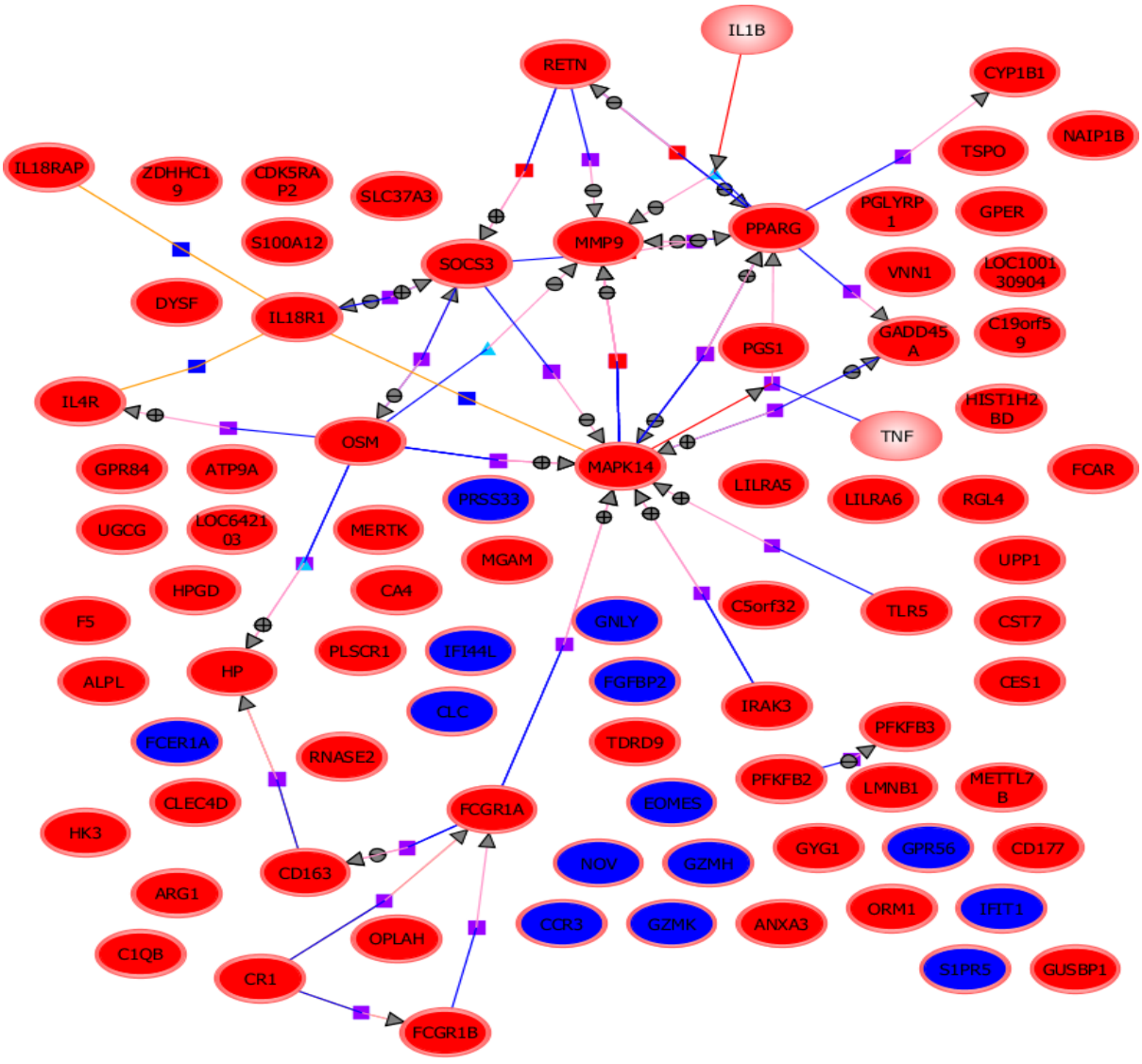


Figure 2-6:Volcano plot: Y axis shows statistical significance (log10 (p-value).
X axis shows fold change (log2 fold change) .On the right hand side (red squares) are displayed the genes that are significantly up-regulated. On the left hand side (red squares) are displayed the genes that are significantly down-regulated. Centre (gray squares) are displayed the rest of genes

Figure 2-7: Graphical representations of genes that are differentially up or down regulated by 2-fold change. Up-regulated genes are red, down-regulated genes. We observed that most differentially expressed genes after cardiac surgery are upregulated. Multiple and complex interactions link MAPK14, PPARG and MMP9.

Interactions are shown: blue square = binding, light blue diamond = metabolism, purple square = regulation, red square = expression, pink = protein modification, ± stimulation or inhibition.; see entities and relations diagram in **Figure 2-9**



Gene Ontology:

To further explore the functional relevance of the genes that are differentially 24 hours over-expressed after cardiac surgery and the associated pathways, I employed gene ontology (GO) and pathways analysis using GeneSpring. GO is a controlled vocabulary that describes gene products' properties. There are three groups: 1- Cellular component (structural part of a cell); 2- Molecular function (activity of a cell product); 3- Biological process (molecular process involved). Enrichment p value is calculated for each GO term. The closer the p value to zero indicates the less likelihood that the genes of your interest fell into any category, just by chance.

The GO analysis demonstrated that the domains Molecular Function (42%) and Biological Process (57%) contain GO terms satisfying a corrected p value <0.05. Those terms, significantly enriched, correspond to molecular functions implicated in molecular, signal transducer activity and receptor activity (immunoglobulin receptor activity). Within the biological process branch the main GO terms are associated with the innate immune response and response to stimulus (defence, stress, wounding and inflammatory responses).

Figure 2-8: GO analysis. GO accession is the numerical identifier and GO term is the name given to each entity in the ontology

GO ACCESSION	GO Term
GO:0006955	immune response
GO:0045087|GO:0002226	innate immune response
GO:0050896|GO:0051869	response to stimulus
GO:0002376	immune system process
GO:0006952|GO:0002217|...	defense response
GO:0006950	response to stress
GO:0009611|GO:0002245	response to wounding
GO:0019865	immunoglobulin binding
GO:0004872|GO:0019041	receptor activity
GO:0006954	inflammatory response
GO:0002526	acute inflammatory response
GO:0060089	molecular transducer activity
GO:0004871|GO:0005062|...	signal transducer activity
GO:0019763|GO:0016489	immunoglobulin receptor activity

Network and regulator analysis

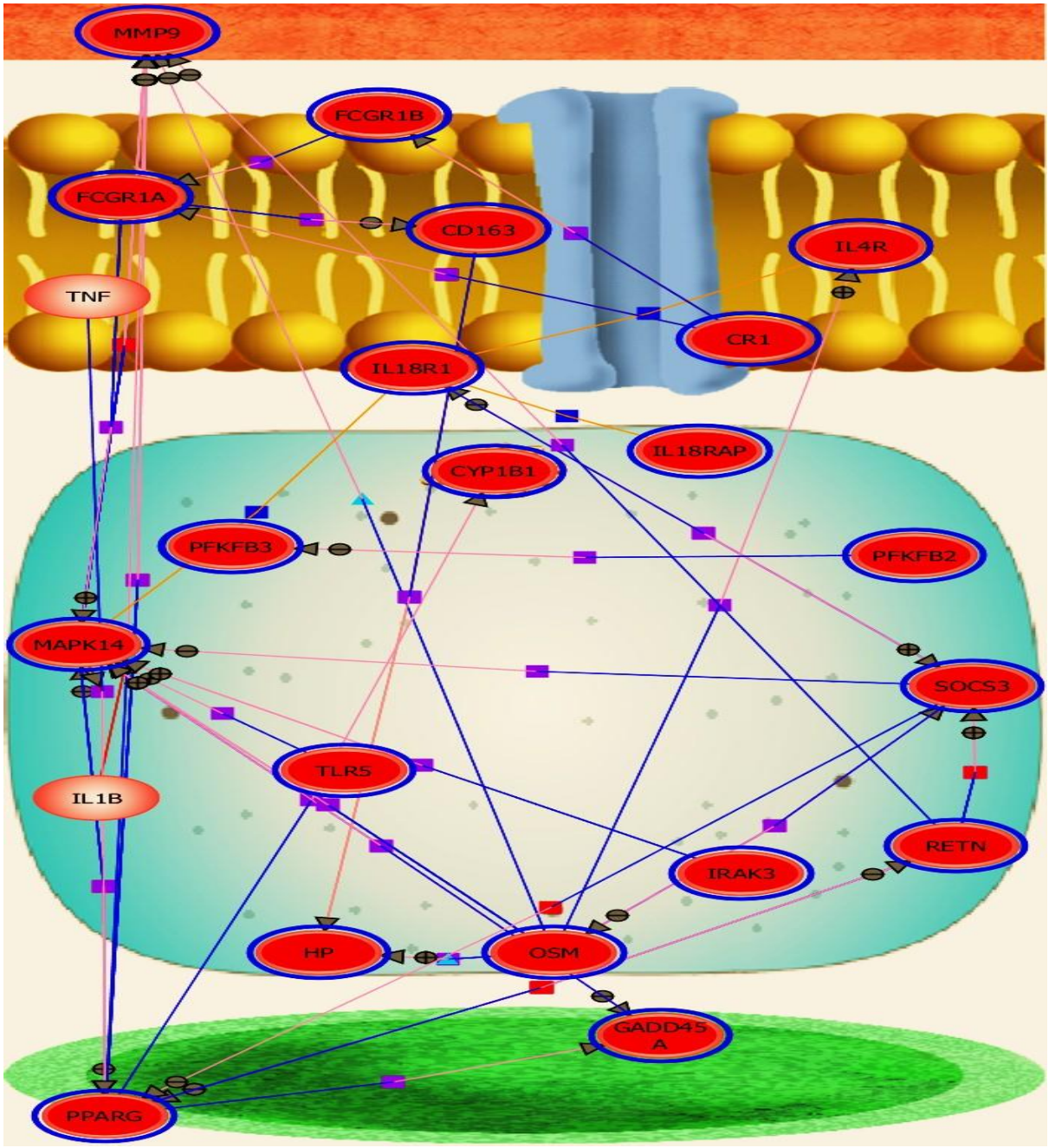
The biological association network analysis (BAN) was used to generate a diagram of all the differentially expressed genes arranged by groups of nodes with different interactions. Finally, genes are coloured according to expression levels (red for over-expressed and blue for under-expressed) **Figure 2-9**.

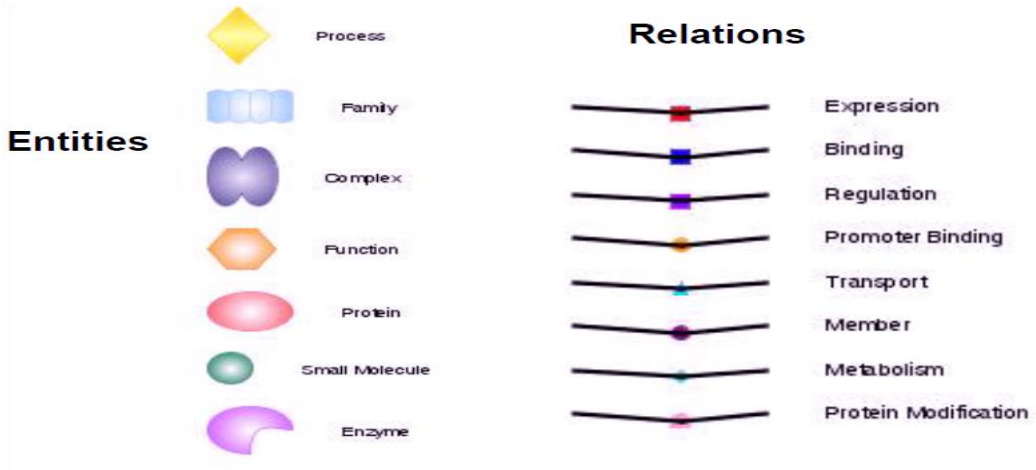
The BAN revealed several nodes with high density inter-connectivity 24 hours after cardiac surgery. The DNA damage inducible/repair genes *GADD45A* and *PPARG* molecular pathway that represses the transcription activity of multiple inflammatory genes are prominent in the nuclei.

In the cytosol, the key gene nodes are *MAPK14* (cytokine-suppressive anti-inflammatory drug-binding protein 2), which is activated by *TLR5* and *OSM* (tyrosine phosphorylation of STAT 1 protein). In addition, we found key regulators of glucose metabolism to be important; *RETN* (known to increase the resistance to insulin), and PFKFB3 *and PFKFB2* (phosphofructo-2-kinase/fructose-2,6-biphophatse 2 and 3). Furthermore *RETN* acts to suppress the activity of *SOC3* and *PPARG*.

At the plasma membrane, the main network node gene is *MMP9* (matrix metalloproteinase), the key connection for which is *MAPK14*. Other gene connections are with receptors for immunoglobulins *FRCGR1B* and *FRCGR1A* (CD64 Fc fragment of IgG), *CR1*(complement receptor 3b/4b), *IL4R*(regulator of IgE production) and *IL18R1*(interleukin 18 receptor).

Figure 2-9: Biological network analysis of differentially expressed genes (p >0.05 after multiple comparison correction), by setting advance filters, we selected genes that present a direct level of interaction (binding, regulation expression or metabolism); blue square = binding, light blue diamond = metabolism, purple square = regulation, red square = expression, pink = protein modification, ± stimulation or inhibition. Genes are shaped and colour coded. The level of expression was added; red for over-expression and blue for under-expression (see entities and relations diagram).


MMP9
FCGR1B
FCGR1A
CD163
IL4R
TNF
CR1
IL18R1
IL18RAP
CYP1B1
PFKFB3
PFKFB2
MAPK14
SOCS3
TLR5
IL1B
RETN
IRAK3
HP
OSM
GADD45A
PPARG



2-6-7 Discussion

Previous gene expression studies have found that genes related to cell signalling, immune response cell adhesion and apoptosis were significantly altered by cardiac surgery using cardiopulmonary bypass[34, 200]. This study of changes in global gene expression in response to uncomplicated cardiac surgery and CPB confirms the importance of similar groups of genes, but further analysis of the host response to cardiac surgery and its regulation reveals a novel and more complete picture of the biological mechanisms involved.

Using global gene expression arrays, I found that the key biological pathways involved in the host response to cardiac surgery include those related to the regulation of apoptosis, mitochondrial biogenesis and immune responses. Genes involved in epigenetic regulation seem to be important. The majority of significantly down-regulated genes are likely to contribute to the immune compromise known to occur in response to cardiac surgery and CPB [14]. Network analysis revealed PPARγ, MAPK14 and MMP9 as key regulators of the innate immune response in this setting.

The host response to cardiac surgery is triggered by a number of distinct events, including the initiation of CPB(during which blood is exposed to foreign material) ischaemia/reperfusion of vital organs such as the heart and lungs, the re-warming period and surgical tissue injury [2]. The interplay of all these factors triggers a host response that involves activation of innate immune pathways, a systemic inflammatory response and immune compromise that can predispose to infectious complications and organ failures[14]. The ability to successfully regulate the propagation and resolution of this host response is now considered to be one of the principal determinants of individual patient outcome following cardiac surgery[15, 16] and involves an orchestrated pattern of changes in gene expression that is designed to protect the host and promote recovery[17]. The principal cause of death in patients undergoing cardiac surgery is sepsis and MODS[3].

Several studies in which immune cells obtained from patients after cardiac surgery have been exposed to mitogens [201] or LPS [14] have found evidence of an impaired immune response, suggesting that immune paresis may be an important feature of the response to CPB. Apoptosis may contribute to this immune dysfunction by inducing T cell anergy, whilst uptake of apoptotic cells by macrophages and dendritic cells has been shown to induce immune tolerance [202 203, 204]. Indeed, post-mortem examination of patients with sepsis and MODS has revealed histological evidence of extensive immune cell apoptosis [205, 206] Moreover, the severity of circulating lymphocyte apoptosis has been associated with poor outcomes and intestinal tissue samples taken from patients after road traffic accidents have shown severe reversible epithelial and lymphocyte apoptosis[207]. Interesting, evidence suggest that sepsis may induce an state of immunosuppression characterised by apoptosis of

immune cells lymphoid cell loss , and decreased capacity of T cells to fight and eliminate pathogens[208] . Moreover agents currently being investigated for the treatment of sepsis related immune suppression (IL-7, IL-15 and Granulocyte macrophage colony-stimulating factor) enhance T-cell activity and decreased sepsis induced apoptosis[209]

In addition, cellular stress caused by ischaemia /reperfusion and tissue damage are known triggers of biological pathways that can induce mitochondrial dysfunction and thereby lead to cellular death.

I observed down-regulation of genes involved in leucocyte activation (T cell cytotoxity and tissue damage) and up-regulation of those involved in mitochondrial function and regulation of apoptosis. These findings suggest that these pathways may play a key role in the evolution of the response to cardiac surgery and may be important in determining whether patients regain immune homeostasis and recover uneventfully or “immune dysfunction” supervenes, increasing the susceptibility of patients to nosocomial infections and/or MODS.

Up-regulation of cell surface markers that function to facilitate adhesion and tissue invasion have been documented using flow cytometry[14] and RNA hybridisation[34] in post cardiac surgery patients. We also found up-regulation of T cell receptors gene expression that was more pronounced immediately after surgery (CD3D, CD36, CD8, CD2) .This up-regulation of T cell receptors has been previously considered as evidence of “priming” of immune cells that will then be readily available to invade peripheral tissue in the event of a secondary insult (hypotension, infection or re-operation) , perhaps thereby triggering the cascade of events that can lead to MODS or death[14, 34].

BAN analysis indicated that *MAPK14*, and its interaction with T cell receptors and *GADD45* is an important regulator of the host response to cardiac surgery. These genes form part of the alternative *MAPK14* activation pathway in T cells. Activation of *MAPK14* is triggered by environmental stress, pro-inflammatory cytokines and *TNF. MAPK14* plays a crucial role in pro-inflammatory signalling cascades and is considered an attractive molecular target for novel treatments for inflammatory diseases. *GADD45* is a cellular stress response gene, that in the presence of antigen receptor T cell activation, represses the activity of *MAPK14*, probably acting as a negative feedback mechanism of the immune response[210, 211].

As well as MAPK14, I also identified *PPARγ* and *MMP9* as important biological network regulators during the host response to cardiac surgery and CPB. Singer et al[212] have shown that up-regulation of PPARγC1(a key factor in maintenance of mitochondrial biogenesis) was associated with survival in a group of sixteen critically ill patients presenting with sepsis and MODS. PPARγ binds to PPARγC1 and intensifies its biological activity. Moreover, MAPK14 is a known activator of PPARγ[213, 214]. In addition, a recent association has been demonstrated between the activity of MMP8 (which normally up-regulates MMP9) and sepsis survival in children[215]. We could speculate that the response of survivors to sepsis may mirror the response to uncomplicated major surgery.

Traditionally the response to cardiac surgery has been described as an acute, predominantly pro-inflammatory reaction, characterized by increased plasma levels of pro-inflammatory cytokines (TNF-alpha, Interleukin 6 and Interleukin 8), to some extent counterbalanced by a parallel antinflammatory response aimed at controlling and curtailing the inflammatory process [216] . Increased levels of some pro-inflammatory cytokines have been correlated with increased morbidity[217], whilst

other investigators have shown that a higher ratio of IL10/TNFa is predictive of an uncomplicated postoperative course [216].

Interestingly, using a global gene expression array none of the genes encoding cytokines traditionally associated with the inflammatory host response to surgery (TNFα, IL-1, IL-4, IL-6, IL8 and IL-10) were significantly differentially expressed 24hrs after cardiac surgery. Moreover, there was no evidence of a sequential pro-inflammatory response followed by an anti-inflammatory phase. These findings are in agreement with a recent systematic review of genome-wide expression profiling in human sepsis[179]. There are two possible explanations for these observations. Firstly, considerable variability in plasma cytokine levels has been reported after cardiac surgery [31] and in sepsis[179]. Secondly, changes in gene expression in circulating leucocytes may not reflect those in local tissue resident leukocytes[218] or other cells/organs.

Given the well-recognised limitations of sepsis studies based on measurement of plasma levels cytokine a number of investigators have attempted to use genome-wide transcriptional studies in order to characterise signatures gene expression profiles of sepsis.(principal published investigations have been reviewed in detail in chapter 2). In these studies, most investigators nearly exclusively report the genes and pathways up-regulated or down regulated in the septic group [183, 219].. Consequently the transcription profile of patients presenting with “sterile SIRS ” reported to be as high as 70% patients with SIRS in ITU is not explored in detail[220]. Moreover the list of genes differentially regulated in this group is generally not reported[181, 184].

A systematic review of gene-expression profiling in 12 cohorts of 784 individuals diagnosed with sepsis showed that activation of genes associated with the host

response were variable and in consistent . Moreover there was no evidence at the transcriptional level of a biphasic response to sepsis (a pro-inflammatory response followed by an anti- inflammatory reaction) [179]. These investigators also found that the activation of pattern recognition receptors such as Toll receptors and CD14 jointly with the activation of pathways that included mitogen activated protein kinase (MAPK), janus kinase (JAK) and activator of transcription protein (STAT) was common in most of the studies reviewed. Recently Maslove et al[221] used hierarchical clustering of gene expression profiles from neutrophils taken from 126 patients with SIRS and infection to identify two subtypes of gene expression patterns . One was associated with significantly more differential expression of genes linked to the inflammatory response and Toll receptor signalling pathway. In addition different expression signatures were identified according to the pharmacological agents that patients had received (e.g vasopressin, norepinephrine or Protein C). These findings may in part explain the intermittent findings of many of these studies.

The objective of the study reported in this chapter was characterize in detail of the gene expression profile of the host response to major surgery. Interestingly my results showed that the genes and signalling pathways activated after cardiac surgery are very similar to the ones detected in patient with sepsis[222].

2-6-8 Limitations:

One of the limitations of our investigation is that we analysed RNA extracted from a mixed population of peripheral blood leucocytes. Nevertheless, the separation of white cells into different cellular subsets, particularly when collecting a large number of samples in an acute clinical environment is technically challenging and the process of separation may in itself stimulate changes in gene expression, as well as inducing RNA degradation[187].

One of the potential confounders in our study is the lack of mathematical corrections for differential cell counts. To our knowledge, no preceding studies to our investigation into gene expression in cardiac surgery employed statistical corrections of cell counts [34, 197, 200]. Different statistical strategies to deal with difficulties arising from investigations in peripheral blood cells and gene expressions signatures have been developed over last decade[219, 222, 223] . We acknowledge that differential cell counts and cell-type specific gene expression should be considered in future studies[224] . Nevertheless, we still hope that our description of the global leukocytes transcriptome after cardiac surgery can contribute to shed some light into the complex pathophysiology of the host response to cardiac surgery

Interestingly, Tang et al[187, 225, 226] demonstrated that genetic signatures obtained from PBMC or neutrophils provide similar diagnostic power in sepsis.

Another limitation of the present study is the lack of tissue sampling or information at the proteomic level. Moreover, epigenetic and post-transcriptional modifications were not analysed.

2-6-9 Conclusions

Functional genomics has the potential to identify key biological pathways and genetic modulators involved in the propagation and resolution of the host response to injury. I believe that the investigation of the normal host response to low risk cardiac surgery is an essential first step in achieving these objectives. To our knowledge this study is the largest cohort of adult patients undergoing cardiac surgery in whom global gene expression profiling (>48000 genes) has been undertaken.

The pathogenesis of host response to cardiac surgery and CPB is complex, not completed understood and deserves further investigation

It involves the simulation activation of genes linked to a pro- inflammatory and anti-inflammatory response.

The regulation of apoptosis is one of the key biological pathways

We have shown that genes associated with mitochondrial biogenesis are relevant. Furthermore, the observed down regulation of genes related to leukocyte trafficking/chemotaxis and activation of lymphocytes and NK cells, as well as interferon induced proteins, is likely to contribute to the immune compromise that follows cardiac surgery. Network analysis revealed PPARγ, MAPK14 and MMP9 as key regulators of the innate immune response in these patients.

These findings will provide a valuable comparator group for functional genomics studies in sepsis, and will be informative in the study of other groups of patients presenting with inflammatory diseases and/or trauma related tissue injury.

Chapter- 3. Expression Quantitative Trait eQTL

3-1 Introduction

Most of the genetic association studies investigating disease traits have identified SNPs that are in non-coding regions. The current challenge is to understand how these genetic variants exert an effect. It is increasingly recognised that these SNPs regulate gene activity at the transcription level[227]. Investigating the genetic determinants that affect gene expression can help to understand how genetic variability acts at the functional level[228, 229].

One type of variant that influences gene expression is called expression quantitative trait loci (eQTL). eQTLs are regions of DNA sequence variants that influence the expression level of one or more genes. To carry out an eQTL analysis two types of data are analysed. First, each individual of the population investigated is genotyped (using SNPs microarrays). Second, the expression of each gene is measured using RNA microarrays. Statistical analysis determines which genetic variants are linked to a significant different level of expression within two populations or within one population (before and after experimental exposure to a stimuli). In other words

eQTL can provide a link between genetic variants in a particular chromosomic region and the biological process they affect[228].

If eQTLs are located within 1Mb of the genes they influence, they are called “cis eQTLs”. If they affect genes located at a longer distance they are called “trans eQTLs”[229]. Cis-acting SNPs can act to change transcription of local genes, post-transcriptional, by affecting message stability or affecting the targets for messenger RNA processing or decay. Trans-polymorphism can act on different genes most commonly with transcription factors or more indirectly by altering the transcription of a number of genes activated by an environmental factor (e.g. diet, endotoxins).

In 2003 Schadt et al[230] reported analysis of 56 individuals from four CEPH (Centre d’Etude du Polymorphisme Humain) reference families (CEPH/Utah pedigrees). These investigations showed that 29% of the differentially expressed genes had a genetic detectable component. These were the first genome-wide eQTLs carried out in humans. The high level of genes whose gene expression had a hereditable component stimulated investigators to see eQTL as a useful tool to interrogate the genome in order to unravel genetic networks that underlie the pathogenesis of disease and/or new therapeutic targets.

The capacity of eQTLs for identifying statistics associations between regions of the genome and expression of genes has opened a new road map where hits detected in genome-wide studies are enriched for eQTL and vice versa.

One example of this approach is Moffat et al[231] who investigated the influence of genetic variants on the risk of asthma: after characterising 317,000 SNPs in DNA obtained from 994 patients with childhood onset asthma they identified several explanatory genes on chromosome 17q21 ($p < 10^{-12}$). This finding was then independently replicated in two cohorts of British (3,301) and German (2,301)

patients[232]. Gene expression analysis in EBS transformed lymphoblastoids cells showed that within or near the 206kb region on chromosome 17 q21 one SNP (rs7216389) was significantly ($p < 10^{-22}$) associated with the transcript of the gene ORDMDL3; ORMDL3 encodes transmembrane proteins. Barret et al[233] performed a meta-analysis of three studies of patients with inflammatory bowel disease (3,230 cases and 4,829 controls) and confirmed 11 previously reported loci including replication of the well-recognised association: with NOD2[234] and IBD5[235], as well as discovering 21 new loci. Some of these markers are found on chromosome 5. Interestingly an eQTL showed that one of the implicated SNPs (rs4495224) increased the expression of PTGER4 (prostaglandin E receptor) in transformed lymphoblastoid cells. Given knockout mice for EP4 develop ulcerative colitis after dextran sodium sulphate treatment this gene is a clear candidate for further investigation[236]. Barret's meta-analysis adds additional information in relation to the functional aspect of the IBD5 region; eQTL analysis associates this region with the expression of ORMDL3 (lod 20), establishing a novel link between the pathophysiology of asthma[231] and Crohn's disease[233].

It is becoming increasingly clear that variability on gene expression is not only determined by DNA variation but affected by individual characteristics such as sex, age and clinical setting. There is a need to fill an important gap that links pathophysiology of diseases with human regulatory variants. One remarkable example is Musunuru et al's study[237]: high plasma levels of low-density lipoprotein cholesterol (LDL-C) are associated with an increased risk of myocardial infarction (MI).

GWAs for plasma lipoproteins traits have identified a number of SNPs associated with plasma levels of LDL-C. These SNPs lie on chromosome 1p13. Europeans who

are homozygous for the major alleles of these SNPs have a 40% increased risk of MI. A common SNP at the 1p13 locus (rs12740374) located in the 3' untranslated region of a gene alters a predicted binding site for a transcriptor factor, the minor allele creating the site and the major allele disrupting it. The minor allele (associated with a decreased risk of myocardial infarction) increases the expression of sortilin (SORT1), preferentially expressed in the liver. Over-expression studies in mouse liver subsequently showed that the minor allele results in over-expression of SORT1 and high levels of sortilin are associated with lower levels of LDL-C.

Nevertheless of significant advance, eQTL studies still pose a significant challenge. The abundance of transcript is a quantitative phenotype in which thousands of traits allow a very rich depiction of the biological landscape of the process interrogated. However, the number of traits to be analysed has become a formidable statistical and interpretative challenge.

Unless a study has a large number of individuals only e-QTLs with the largest effect can be detected. In addition, more than two eQTLs are needed to explain one trait (due to the limited size effect of one individual eQTL on genetic variation). Trans e-QTLs need to cover markers over the whole genome increasing the number of comparisons consequently increasing the risk of false-positive results. To overcome this problem, a more stringent threshold should be applied to reach the same level of significance. Cis-eQTL by affecting local transcription have better resolution and are less statistically cumbersome.

Studies based on transformed cell lines have the advantage that only one type of cell is investigated (mostly lymphoblastoids cell lines) increasing the reproducibility and power of the studies. However, cell manipulation for immortalisation and prolonged culture may introduce artefacts into the observed gene expression patterns, perhaps

caused by epigenetic changes and consequently may alter cellular phenotypes. As a result, findings obtained from such studies may not accurately reflect the in vivo changes induced by the disease processes being investigated[178, 238].

Furthermore, regulatory DNA elements (e.g. enhancers, promoters, silencers or insulators) can be cell context specific and dependent on the cell type[239] and cellular activation[240].

To advance our understanding of how genetic variability affects the way patients responded to cardiac surgery we performed an eQTL analysis in our cohort of patients undergoing cardiac surgery.

3-2 Methods

For this eQTL study samples obtained before induction of anaesthesia and on day one following surgery were analysed (see Chapter 2 for full experimental details and study design).

3-2-1 Genotyping

DNA was purified from buffy coat and isolated using a QiAcube machine (Qiagen™). Picogreen quantification and normalisation of the DNA concentration was carried out. Genotyping of the 63 samples was performed in the High Throughput Genomics facilities at the WTCHG on Illumina Infinium Human OmniExpressed-12v1 beadchips using the Infinium HD Ultra protocol. These beadchips capture a large proportion of variability by screening samples for ~400.000 single nucleotides polymorphisms (SNP). A MAF ≥ 5% was used to minimise the risk of type 1 error (false positive) and type 2 error (false negative).

We use Plink toolset [241] for the analysis of genotypes data.

Given the large number of SNPs included in the analysis, even a low genotype error rate can result in a high level of false negative and false positive associations. Quality control assessment of the data was assessed prior to statistical analysis. We carried out the following procedures to identify and remove poorly genotyped markers or individuals[242].

1. Sample mix up and plating; 2 patients were removed.
2. Low quality of DNA, missing genotypes were determined (threshold 0.05 %); 6 patients removed
3. Plates effects were evaluated using Principal Component Analysis.
4. Population stratification. Identification of individual with discordant ancestry were identified using multidimensional scaling (MDS) based on 1000 genomes population ancestry [243]; 6 patients removed.
5. Identification of duplicates was carried out by genetic distribution metric to identified related to samples duplicates (DST); 3 patients removed.
6. Call rate: markers with call rate of less than 95% were removed.
7. Deviation from Hardy Weinberg Equilibrium (HWE) was assessed .

This quality control process resulted in the removal of 17 subjects out 63. This resulted in a group of 46 patients consisting of 35 males and 11 females with a median age of 69 (41-97) years.

3-2-2 Data analysis

As explained in the introduction, the goal of an eQTL analysis is to identify SNPs that affect the expression levels of known genes. To achieve this computationally

demanding task we used R package Matrix eQTL[244], that tests associations between each SNP and transcripts using an ANOVA model. Matrix eQTL is able to compute 38.4 billion operations per second. We performed a cis regulatory gene analysis at this stage, testing genes –SNP pairs within 1 million base pairs of each other. After analysis of each SNP, Matrix calculates the FDR (≤0.05) only for the genes that passed the standard accepted threshold of established significance (10e-6; this cut-off has been selected as per Matrix software [244] and was supervised by Dr Peter Humburg, statistician at the Wellcome Trust for Human Genetics, Oxford University). The calculations are based on Benjamini Hochberg algorithms.

We included major principal components (PCs) of the gene expression data as covariates in the eQTL analysis. Mapping an increasing number of cis eQTL allowed us to detect the maximum number of probes that had cis eQTLs. No others clinical covariates were analysed.

To check for results errors in our eQTLs results due to unaccounted covariates, population stratification, plate effects or model misspecification. We built a quantile-quantile (Q-Q) plot. Matrix eQTL Q-Q plots graphic the distribution of p-values, even in those below the significance threshold p-value

http://www.bios.unc.edu/research/genomic_software/Matrix_eQTL/runit.html.

Figure 3-1.

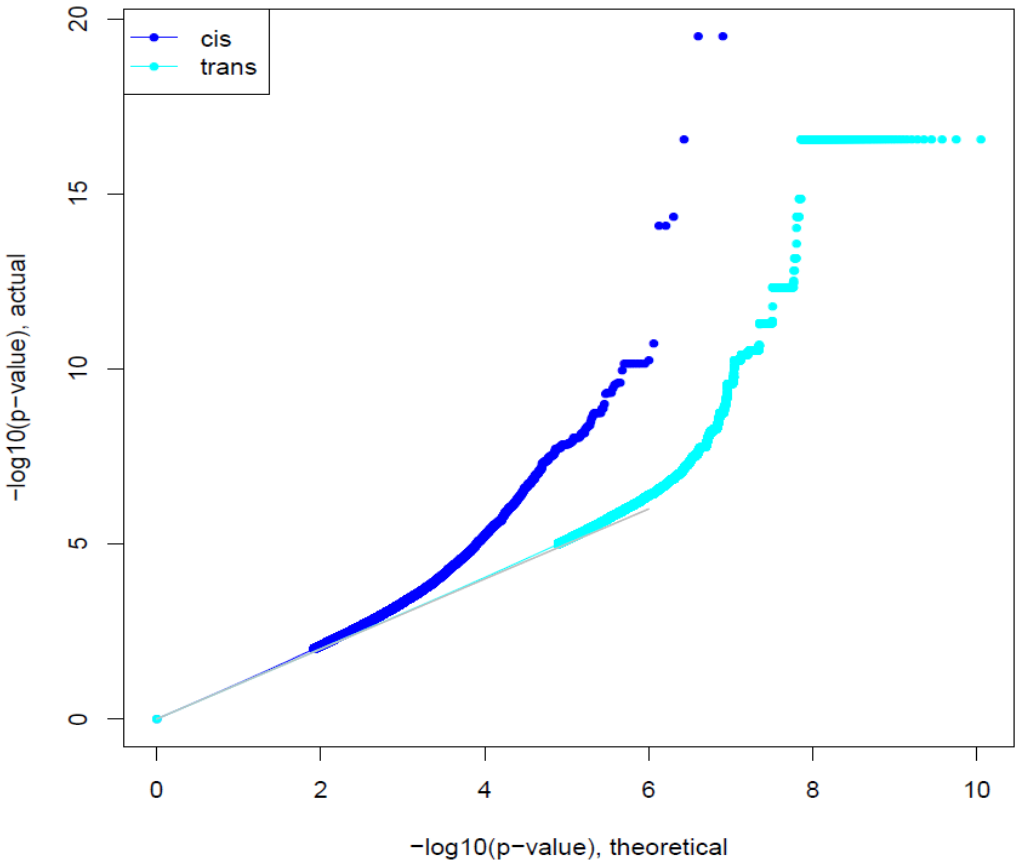
Specific SNPs associated with cardiac surgery were analysed using RegulomeDB[245]. This online database gathers annotated high-throughput data from the ENCODE Project consortium and other sources. By intersecting eQTL associated regions with RegulomeDB we looked for evidence to support the functional role of our findings. To assist with the interpretation of results RegulomeDB results are grouped according to a score that ranges from 1a to 6 according to the evidence available

regarding how confident it is possible to establish that a variant lies in a functional location. Variants that are known eQTL for genes and have been associated with expression belong to Category 1; on the other hand, eQTL SNPs not associated with functional annotation are Category 6.

We used the National Institute of Health (NIH) online catalogue of SNPs-trait associations from published genome-wide association studies in order to identify already reported underlying functionality at the trait/disease-associated loci http://www.genome.gov/gwastudies[246].

eQTLs obtained were further analysed to identify networks, bio functions and canonical pathways of interest using Ingenuity Pathway Analysis (IPA, Ingenuity Systems; http://www.ingenuity.com). We adjusted the software to detect, direct and indirect relationships of variants enriched for s only observed in humans. This software develops networks and functions from target genes. In addition, we used IPA-Tox – a tool within IPA that provides a toxic genomic analysis based on peer-reviewed toxicological databases http://www.ingenuity.com/library/pdf/tox. IPA-Tox identifies a subset of genes which are activated by a biological insult or a pharmacological compound. This toxic-genomics data-set may help to predict potential cellular damage or biochemical alterations that may occurs in host after exposure to a chemical or biological trigger . By linking our results to specific sets of toxicological data, we aimed to identify a subset of genes that might be predictive of organ damage. We downloaded our eQTLs results into the Genotype-Tissue Expression (GTEx) browser (database of eQTL detected in multiple tissues**)** http://www.gtexportal.org/home/ . No matching references were found.

Figure 3-1: Q-Q plot for all p-values



3- 3 Results

We found 659 cis eQTLs associated with elective cardiac surgery and CPB **(Table 3-1).**

We found that a group of our enriched eQTLs had high RegulomeDB scores (1a to 1d) (**Table 3-2**).

According to the information available in RegulomeDB and other online databases (OMIN, ENCODE) we classify them according to their cellular function in two groups: cis eQTLs involved in the immune process and cis eQTLs involved in cellular activity and senescence.

Immune response-related eQTLs

RS11663049 main motif are the transcriptor factors NF- kappa B and E2F-1:DP-2. NF-κB belongs to a family of transcription factors that have a primordial role in inflammation and the innate immune response. **RS12145700** motif is USF. Degradation of USF has been associated with suppression of the immune host response. **RS693235** acts on the transcription factor RREB1 that acts as an HLA-6 promoter[247]. **RS4766497** main motif is NF-Y major histocompatibility class II promoter[248]. **RS3768324** motifs are the transcription factors FL-1 and GABPA, ELK1 and SAP-1A[248]. GAPB activates genes related to apoptosis (BCL2). **RS226503** main motif is the transcriptor factor OLF-1. OLF-1 is involved in the regulation of pre-B and early B cell lines. **RS1362126** main motif is IKAROS, a zinc finger transcriptor factor. The main gene affected by this SNP is HLA–A (major histocompatibility complex, class 1). **RS226503** main motif is the transcriptor factor OLF-1. OLF-1 is involved in the regulation of pre-B and early B cell lines. **RS4766497** main motif is NF-Y major histocompatibility class II promoter[248]. **RS1362126** main motif is IKAROS a zinc finger transcriptor factor. The main gene affected by this SNP is HLA–A (major histocompatibility complex, class 1). **RS540** main motif is FOXP1 and TBP. FOXP1 is the transcription factor that plays a critical role in monocyte differentiation and macrophage function.

Cellular and senescence related eQTLs

RS3007030 main motif is POU5F1 involved in reprogramming pluripotency stem cells[247]. **RS1025196** acts on the transcription factors FREAC-3, OCT-1, FOXA2. FREAC induce the expression of stress response and apoptosis genes. **RS3754109** acts on the transcription factors SIX-1, SIX-2, SIX-4[249]. Overactivity of SIX-1 is associated with breast cancer invasiveness.

RS13230744 main motif is MTF-1, a metal-regulatory transcription factor that is involved in heavy metal detoxification and free radical scavenging. **RS540** main motif is FOXP1 and TBP. FOXP1 is the transcription factor that plays a critical role in monocyte differentiation and macrophage function.

RS9630856 main motif are ERR and RORA. ERR plays a role in the regulation of cellular energy. **RS6102** main motif is NANOG, a homeobox transcription factor that is involved in cellular differentiation. **RS575** main motif are zinc finger protein transcription factors ZIC1, ZIC2, ZIC3. These transcription factors exert their modulatory effect in the vicinity of any sequence to which a protein domain binds. **RS4668936** main motif is the zinc finger ZBT3. The gene affected by this SNP is putative RNA helicase that by altering RNA can influence translation, initiation and splicing. **RS160404** main motif is EWSR1-FLI, a putative tumour suppressor.

Cis Matrix e-QTL Results		
	SNPs	Genes
Before Cardiac Surgery	758	285
24 hours post Cardiac Surgery	783	247
Only after Cardiac Surgery	659	218

Table 3-1: Cis regulated SNPs (independent) and genes, before surgery, 24 hours after surgery. We found 659 cis eQTLs after cardiac surgery (P > 1+06) out of the 758 present before surgery. It is remarkable the significantly changes of eQTLs pre-and post-cardiac surgery. We could hypothesise that the reason to explain the change observed is that before elective low risk cardiac surgery patients are clinically stable and no receiving any acute medical intervention. On the contrary, cardiac surgery and CPB is characterised by a massive induction of cytokines[1], activation of coagulation and haematopoietic cells[2, 3]. Furthermore, cardiac surgery is associated with the highest incidence of post-operative morbidity of any elective surgical procedure [7]

Table 3-2: Matrix e-QTL. List of SNPs only present 24 hours after cardiac surgery. SNPs are ordered according to P- values

Gene ID	dbSNP ID	p-value	Regulome DB Score
CEP192	rs11663049	1.2 E-06	1a
NDUFS5	rs3768324	2.4 E-08	1a
CCDC23	rs12145700	3.0 E-08	1a
SF1	rs693235	3.0 E-08	1b
GPN3	rs4766497	3.2 E-09	1b
PPIL5	rs3007030	4.6 E-06	1b
SERPINB10	rs1025196	7.9 E-07	1b
TTF2	rs3754109	2.8 E-08	1b
FAM118A	rs226503	9.2 E-08	1b
HLA-F	rs1362126	2.8 E-06	1b
GATS	rs13230744	2.6 E-06	1b
C5orf35	rs540	2.4 E-07	1d
SERPINB10	rs9630856	1.0 E-09	1d
SERPINB10	rs6102	1.8 E-10	1d
SARS2	rs575	3.0 E-08	1d
DDX1	rs4668936	3.0 E-08	1d
CTNNA1	rs160404	6.8 E-07	1d
ZFAND2A	rs2949170	2.2 E-07	1d
TMM5	rs4621903	1.6 E-06	1d

After R Matrix analysis, a list of the top regulatory 20 SNPs 24 hours after surgery according their level of significance are shown in **Table 3-3**. Most of the genes listed are involved in processes activated as a result of insults that trigger the host innate immune responses and some regulate responses to viral infection through the action of interferon. OAS1, APOBEC3B and ClEC4C are involved in the proliferation of immune cells through the activation of complement.

C4BPA codes for the C4B-binding protein (C4BP) that takes part in the coagulation/fibrinolysis cascade (It acts by inhibiting the activated protein C pathway. **See Appendix 2 for** further analysis of SNPs associated with cardiac surgery.

We tested the most significant SNPs against the level of gene expression measured by probe signal intensity. Correlation between genotype and expression level are indicated by each boxplot. The level of significance is due to the population minor allele frequency of each SNP **Figure 3-2**. To explore the possibility that genes near BLM may be functionally relevant we used a LocusZoom. This web-based software displays the area of genome surrounding BLM and gives an indication of the extent of linkage disequilibrium, recombination patterns and the position of other genes in the region **Figure 3-3**.

We interrogated the RegulomeDB database in order to explore the current knowledge associated with the most significant cis eQTLs in relation with the p-value (Matrix eQTL FDR <0.05). The RegulomeDB score for the top ten cis eQTLs ranges from 1F to 6 **Table 3-3**. We could divide them into two groups:

1. Cis eQTLs involving the immune response

RS10774671, rs2660, rs4767030 cis eQTL for *OAS1* (SNPs rs3177979 and rs474189 did not have available evidence in RegulomeID database). The OAS1 gene is a key component of the innate immune response to viruses.

RS4766497 regulates gene GPN3 and acts on the transcriptor factor NF-Y.

The CCAAT box is a widespread element found in promoters and enhancers. NF-Y is a heterodimer binding factor that binds with CCAAT in major histocompatibility (MHC) class 2 promoters.

This gene affects the action of ATP2A2, an ATPase located in the sarcoplasmic reticula of muscle cells involved in the pumping outside the cytosol CA (2+). Mutations of this gene have been associated with Darier syndrome, characterised by keratosis follicularis.

RS11687089 cis eQTL of ADCY3 allelic variants of ADCY3 have been found to be associated with inflammatory diseases (ulcerative colitis and Crohn's disease)[250, 251].

2. Cis eQTLs involved in cell division and senescence

RS933489 cis eQTL of PAQR6. This gene regulates the activity of KIAA0907, an oncogene associated with lung tumorogenesis[252, 253].

RS4767030 cis regulation of gene BLM. Stavropoulos et al[254] found that BLM facilitates the amplification of telomeres through ALT (alternative lengthening of telomeres).

SNP	symbol	gene	beta	t-stat	p-value	FDR
rs3813948	C4BPA	2000128	5.034494	18.04706	5.46E-19	2.21E-12
rs11120211	C4BPA	2000128	5.034494	18.04706	5.46E-19	2.21E-12
rs11826261	GLYATL2	270170	4.122478	17.52127	1.36E-18	3.65E-12
rs3026681	APOBEC3B	3170068	4.814469	15.50214	5.56E-17	1.12E-10
rs389480	BLM	2450717	1.38009	12.12277	6.79E-14	1.10E-07
rs7130826	PIK3C2A	5270379	-0.85616	-10.8255	1.47E-12	1.70E-06
rs7949405	PIK3C2A	5270379	-0.85616	-10.8255	1.47E-12	1.70E-06
rs12310416	CLEC4C	5960136	1.368433	10.39172	4.32E-12	4.36E-06
rs11150882	C17orf97	4640161	1.565518	10.19953	7.02E-12	6.29E-06
rs7143764	CHURC1	6370612	1.158741	10.09107	9.24E-12	7.46E-06
rs6488608	CLEC4C	5960136	1.314545	9.566246	3.59E-11	2.63E-05
rs11150882	C17orf97	2640142	1.307159	9.444538	4.94E-11	3.32E-05
rs872914	PF4V1	1410278	1.648202	9.370048	6.01E-11	3.46E-05
rs941758	PF4V1	1410278	1.648202	9.370048	6.01E-11	3.46E-05
rs12310416	CLEC4C	130743	1.294303	9.304611	7.15E-11	3.85E-05
rs10774671	OAS1	3450180	-1.19397	-9.1543	1.07E-10	4.53E-05
rs3177979	OAS1	3450180	-1.19397	-9.1543	1.07E-10	4.53E-05
rs2660	OAS1	3450180	-1.19397	-9.1543	1.07E-10	4.53E-05
rs4767030	OAS1	3450180	-1.19397	-9.1543	1.07E-10	4.53E-05
rs6060676	CPNE1	4570500	-0.5406	-9.0961	1.25E-10	4.90E-05

Table 3-3: Top enriched eQTLs. SNPs ordered

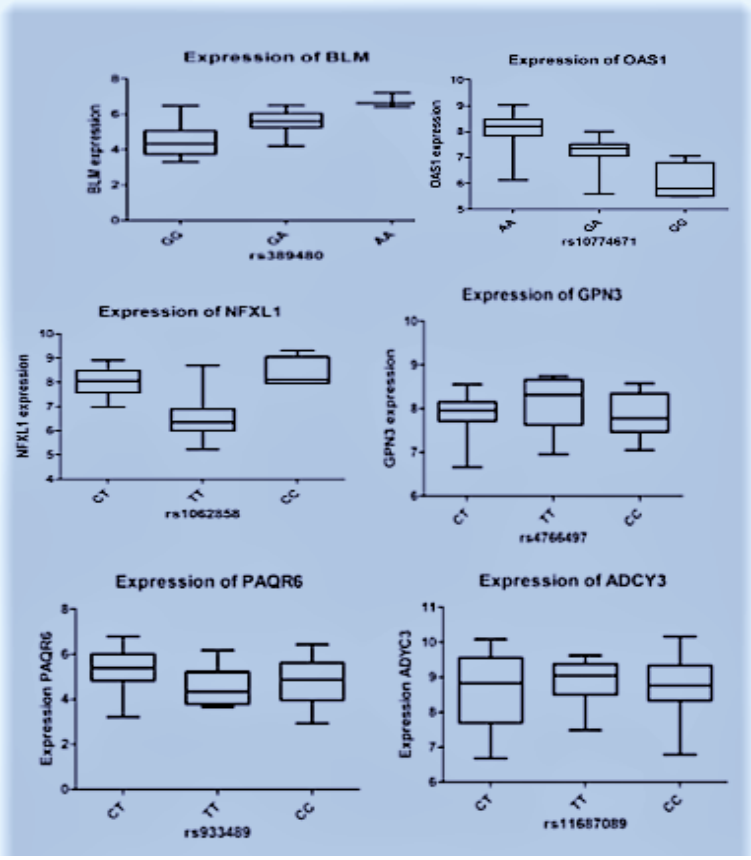
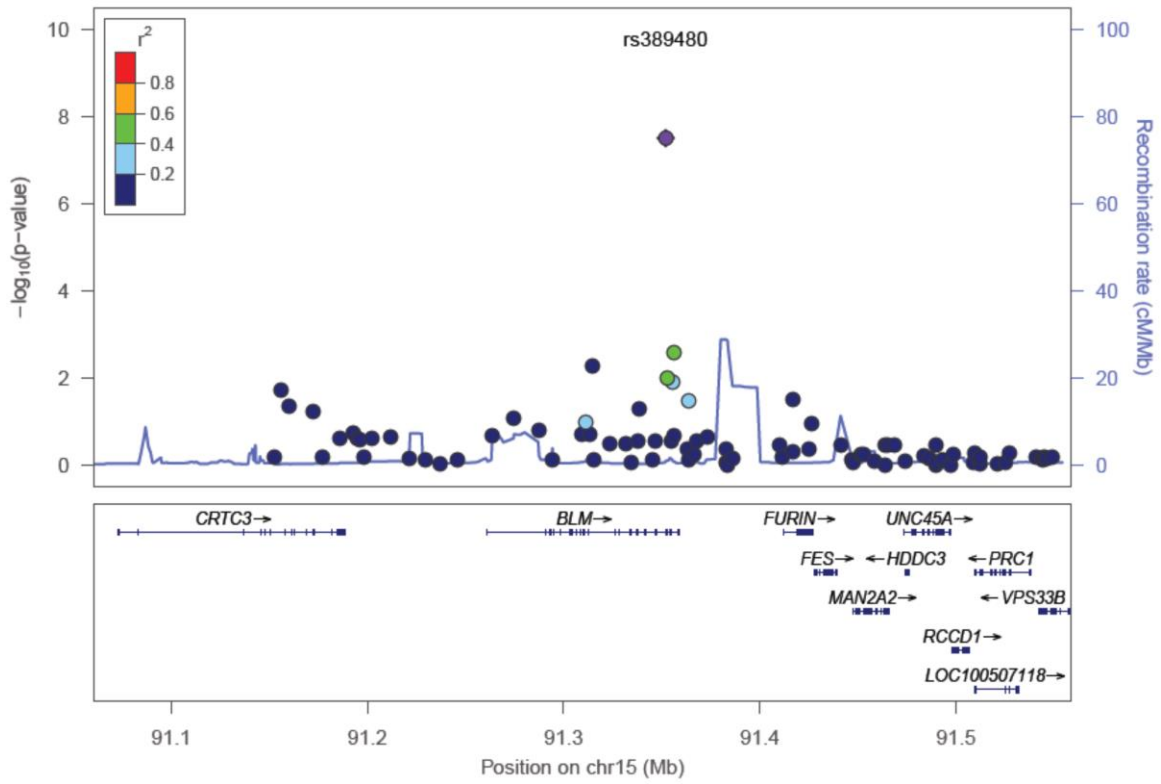


Figure 3-2: Allelic expression of top enriched eQTL: BLM, OAS1, NFXL1, GPN3, PAQR6 and ADCY3. X axis represents expression signal for each gene; p-value <0.0001. GPN3 P-value 0.19.

Figure 3-3



RS39480 is associated with the expression of BLM P 3.06 E-08. This plot shows association p values on the –log 10 scale on the vertical axis and the chromosome position (Chr 15) on the horizontal axis. We show BLM and the gene names and size of the flanking region. RS389480 is displayed by a blue diamond. Each circle represents an SNP with the colour indicating linkage disequilibrium (LD) with RS389480. Estimated recombination rates from Hap Map are shown.

Table 3.3. Matrix eQTL. List of SNPs only 24 hours after cardiac surgery. SNPs are ordered as per p-values.

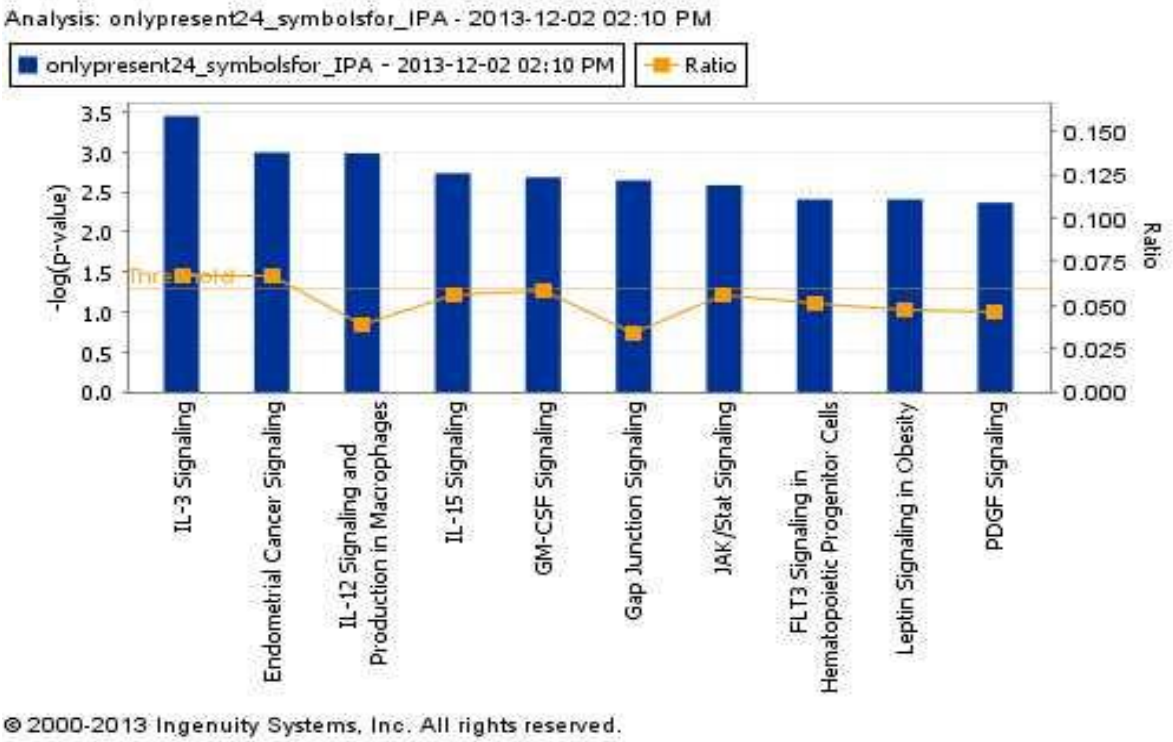
Top SNPs ordered according P value			
Cis regulated genes 24 after cardiac surgery only: cis e-QTL			
Gene ID	dbSNP ID	p-value	Regulome DB Score
BLM	rs389480	6.7 E-14	4
OAS1	rs10774671	1.0 E-10	6
OAS1	rs3177979	1.0 E-10	NIL
OAS1	rs2660	1.0 E-10	6
OAS1	rs4767030	1.0 E-10	6
NFXL1	rs1062858	1.8 E-10	7
GPN3	rs4766497	1.8 E-10	1B
OAS1	rs3741981	1.8 E-10	NIL
PAQR6	rs933489	2,0 E-10	5
ADCY3	rs11687089	2.0 E-7	1F

3-5 Pathways present only 24 hours after cardiac surgery

Matrix eQTL results were submitted to the Ingenuity Pathway Analysis program (IPA, see Methods). We found canonical pathways associated with our results **Figure 3-3**. **See Appendix 3** for further analysis. We detected that the top ten enriched pathways had several eQTLs in common. We constructed three subgroups that share four cis eQTLs.

Figure 3-4: Pathways enriched for cis eQTL – 659 only present 24 hours after cardiac surgery. The Y axis shows the –log (p value threshold is <0.05) for the enrichment of each pathway. The p value is calculated using a right tailed Fisher exact test by considering: 1. The number of focus genes that participate in the process; 2. The total number of genes that are known to be associated with that process in the selected pathway reference set. The enrichment p value indicates the likelihood that the genes detected fell into a pathway category, just by chance.

First group: Jak/Stat signalling, IL-2 signalling, IL-3 signalling, IL-15 signalling, FLT3 signalling. **Figure 3-4**



Second group: IL-3 signalling, RANK and GM-CSF signalling and T cells receptor is signalling. **Figure 3-5**

Third group: GAP junction signalling, leptin signalling in obesity, melanocyte development signalling. **Figure 3-6**

PI3K (RS 51330 p 2.4 E-06), Mek1/2 (RS 12611262 p 4.5 E-07), ERK1/2 (RS 12373078 p1.5 E-06) are present in all pathways groups. Other genes that showed enriched eQTLs are AKT (RS 10787735 p1.5 E-09), RAS (RS 10993 p 4.4 E-06), STAT6 (RS 4559 p1.2 E-06), AC (RS 9964798 p 6.5 E-10).

The first group of pathways. **Figure 3-5**

The activation of granulocyte-macrophage colony-stimulating factor (GM-CSF) activates three cascades[255]:

1. A small GTP binding protein (RAS) triggers the activation of mitogen activated protein kinases (MAPK) including MEK1/2 and ERK1/2, resulting in an increase in cell survival, proliferation and cell differentiation.

2. JAK/STAT signalling.

3. Activation of PIK3 and AKT.

2 and 3 cascades lead to an increase of cellular survival and cell proliferation.

The second group of pathways. Figure 3-6

The second group of pathways are activated by the action of interleukins, GMF-CSF and interferon after engaging on their respective membranous receptor through two cascades:

1. The activity nucleotide exchange factor (m SOS), RAS and the mitogen-activate kinases (MAPK) ERK ½ and MEK1/2 trigger the activation of NF-κB, CEBP and JUN (having a proliferative and haematopoietic effect).

2. JAK/STAT signalling pathway that activates the nuclear transcription of pro-inflammatory genes (IL-6 induce) and BCLXL (gene that regulates cellular proliferation)[256].

The third group of pathways. **Figure 3-7**

The third group integrates different intracellular mechanisms activated by leptins. Leptin levels increase after acute inflammation. Leptin acts via transmembrane receptors that are similar to other cytokines (IL-2, IL-3, IL-4 and GM-CSF). Binding to its receptors results in the activation of inflammatory signalling pathways via JAK/STAT or phosphorylation (PI3K). Leptin as well as other cytokines, growth factors and stressors stimulates the protein kinase JNK (c-Jun N-terminal kinase).

Figure 3-5: First group pathways

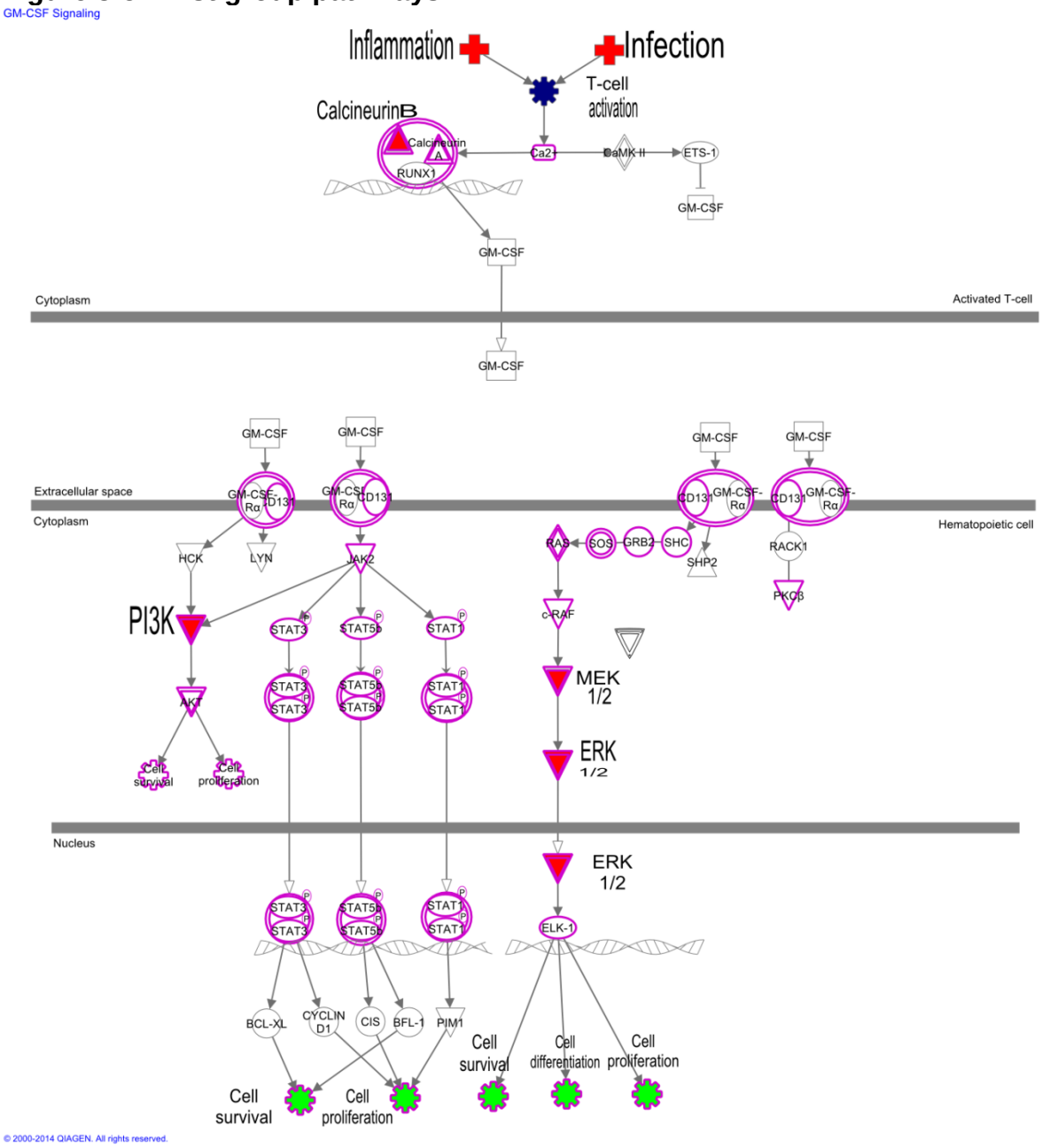


Figure 3-6: Second group pathways

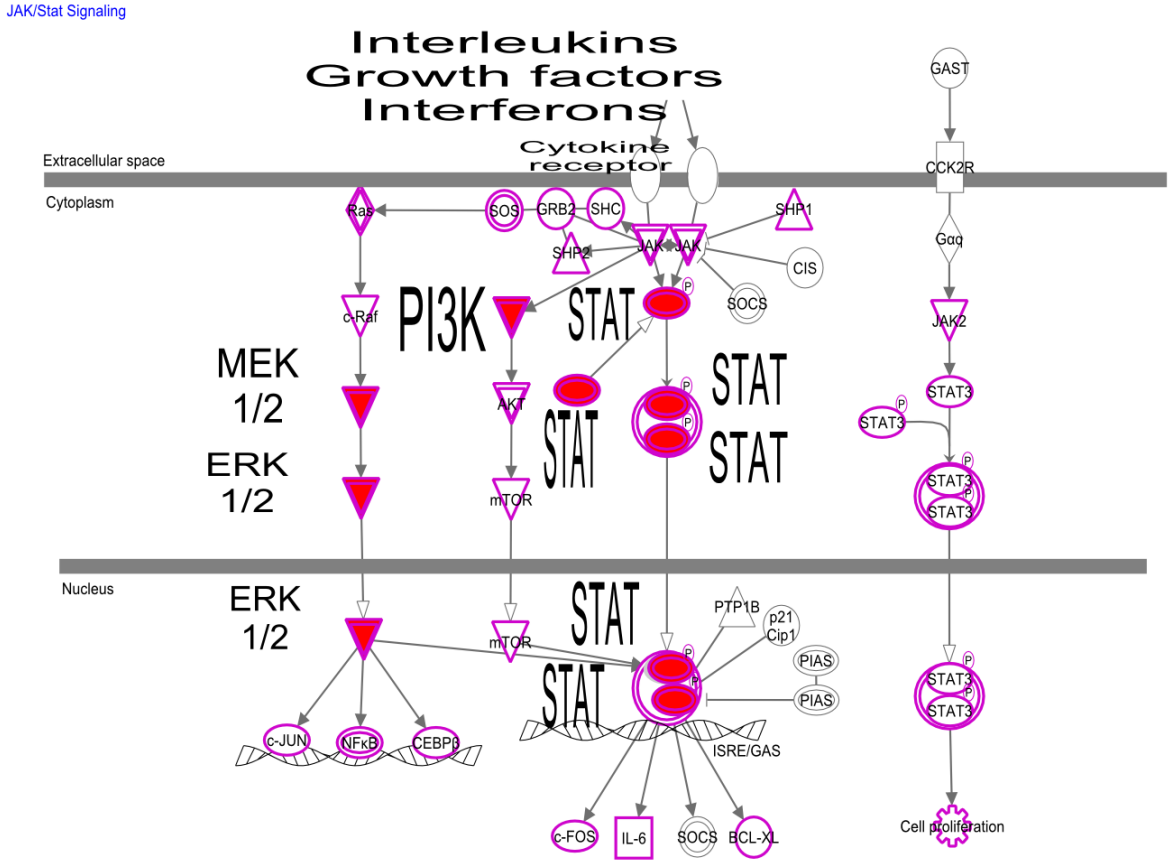
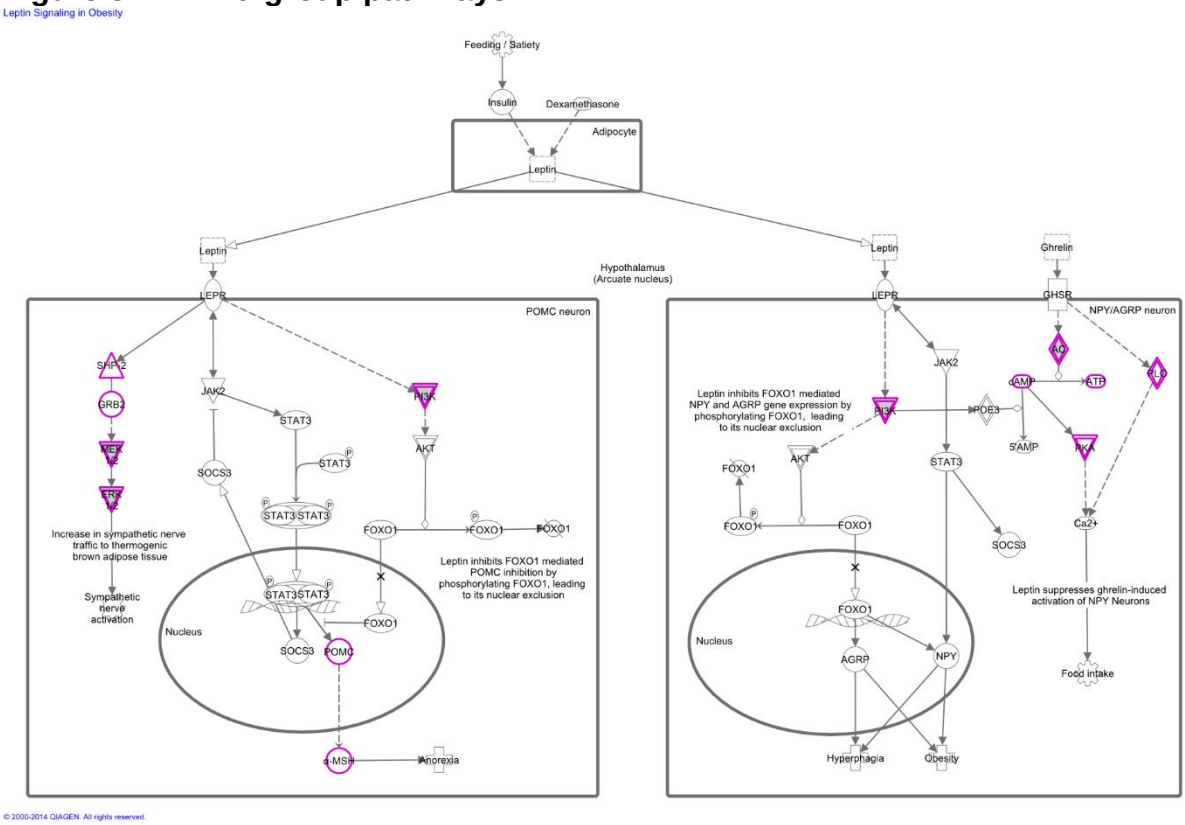


Figure 3-7: Third group pathways.



3-5 Toxic genomic analysis

To complement our analysis we submitted the results obtained by matrix-eQTL to IPA toxico-genomic database in order to explore the presence of potential genetic biomarkers. We identified three functional areas which enriched genes for eQTLs.

A: Clinical chemistry and haematology

Increased level of Alkaline Phosphatase; MAPK3 (RS 12373078, p 1.5 E-06). Increased level of haematocrit: JMJD6 (RS 8067445, p 5.3 E 08) and increased levels of red blood cells: EIF2AK1(RS 4724770, p 3.2 E-06)

B: Nephrotoxicity

Genes expressed in renal tubular cells: PLEKHA1 (RS 12765679, p 3.3 E-06), TRIM 27 (RS 2015436, p 4.5 E-08), genes involved in the physiopathology of the immune response in renal failure: GSTT1 (RS 6734622, p 3.2 E-07), TNFRSF1A (RS 7955850, p 2.0 E-06) and mitochondrial cytopathy: SARS2 (RS 79025271, p 1.5 E-09).

C: Cardiotoxicity

Genes that control immune and inflammatory responses: STAT6 (RS 4559, p 1.2 E-06), TNFRF1A (RS 795580, p 2.01 E-06), PP3R1 (RS 6734622, p 3.2 E-07). Other genes in this group are linked to thyroid function: DIO3 (RS 17100339, 5.73 E-07) and intercellular junction CTNNA1 (RS 3111602, 7.9 E-07). **See Appendix 4** for further analysis

3-6 Discussion

In this eQTL study we focused on answering two questions:

1. Which are the most relevant regulatory SNPs involved in the host response to elective cardiac surgery in a population of European ancestry?

2. What is the clinical relevance of the regulatory SNPs detected?

In this study we identified 569 SNPs (10.E-06) that are significantly associated with the cis regulation of 218 genes, 24 hours after cardiac surgery.

Detailed analysis of the top SNPs using different online databases and catalogues revealed a complex picture. Other investigators have found that genes linked to the immune response, apoptosis and coagulation cascades are involved in the host response to cardiac surgery. In this context, we confirmed that many of the SNPs significantly associated with the response to cardiac surgery are linked to genes involved with the same biological processes. Interestingly, we detected SNPs affecting the regulation of genes not conventionally associated with the host response to cardiac surgery such as telomere activity, epigenetic modifications, cell programming and mitochondrial function. Interpretation of these findings and moreover their clinical relevance remains elusive.

Unsurprisingly the pathways associated most strongly with the post-operative response to surgery, at least in a mixed population of leukocytes, are related to the innate immune response and inflammation.

We found that many genes up-regulated in response to surgery have eQTL (*MMP9, MAPK14* and *PPARY*).

Analysis of the top ten pathways associated with the host response 24 hours after cardiac surgery reveals that all pathways share three genes (ERK 1/2, MEK 1/2 and PIK3). ERK and MEK have been linked with the pathogenesis of infections caused by Yersinia Pestis and Bacillus Anthracis respectively. The concept that multiple genes located in different loci as part of a functionally linked common pathway, has proved successful in identifying genes involved in tumorigenesis or tumour progression in pancreatic, brain cancer and metabolic disease such as obesity-related traits[257-260]. Interestingly tox functions linked our dataset with clinical biochemistry and genes previously associated with cardiotoxity and nephrotoxity (see Appendix 4).

We evaluated the potential for mapping by comparing the top SNPs for BLM gene (rs389480) by performing a relation association plot. The differential LD pattern (r2=0.6-0.8) may prove useful to identify and prioritise SNPs for functional evaluation see **Figure 3-2**.

This study demonstrates the feasibility of carrying out functional molecular studies in the context of critical illness and major surgery. Furthermore, it contributes to our understanding of the host response to acute physiological challenges. However, there are inevitably a number of limitations. One resides in the technology being used since the current resolution of eQTLs limits their ability to detect all regulatory variants[229]. Interpretation of the results of this study should be viewed cautiously in view of the small sample investigated and consequently the low statistical power to detect eQTLs for threshold MAF of 5% [261]. However, we hope that by employing a rigorous process of selection and following a stringent quality control analysis we have guaranteed a homogenous population within the limitations of a clinical study. Another challenge to be overcome is how to extrapolate findings resulting from the analysis of cells harvested from one specific tissue (in this case circulating leukocytes). Gene regulation is complex and tissue specific[229]. Cells from different tissues share common ancestral features, but later developmental cell variation can have significant functional effects[229]. Gene expression comparisons between human osteoblast (Hobs) and lymphoblastoid cell lines (LCLs) showed that genes associated with cell survival and cellular metabolism such as protein ubiquitination, glucocortocoid receptor signalling, PI3/AKT signalling, oxidative phosphorylation and purine/pyrimidine metabolism are commonly expressed in both cell types, constituting 43% of the total genes expressed. A large number of genes are not only

expressed in both cells but they seem to be regulated by the same SNPs acting independently in different tissues.

The potential value of studying tissue specific functional diversity has been demonstrated by the characterisation of a brain specific splicing factor called Nova2. The activation of Nova 2 induces the synthesis of different isoforms of proteins affecting brain synapses. In Nova 2 knockout mice splicing changes represent 6.6% of the total splicing in relation to immune cells. Interestingly Nova-regulated RNA levels are the same in knockout mice as in the wild type, suggesting that NOVA acts mainly by affecting the quality of synapses regulation[262].

The above examples illustrate the necessity not only of studying genetic diversity by analysing the different allelic variants involved but complementing these findings with proteome and metabolomics analysis.

3-7 Conclusions

This is to our knowledge the first study of expression quantitative trait loci (eQTL) in the context of cardiac surgery. Our aim was to reveal how the function of the genome interrogated through the use of whole genome expression array is affected by DNA sequence variation.

We were able to identify some of the key SNPs, genes and pathways involved in the host response to cardiac surgery and CPB. As well as the expected predominance of genes/pathways associated with inflammation and innate immune responses, pathways previously implicated in cancer pathogenesis were also identified. Associated network functions showed a predominance of processes involved in cell to cell signalling, cellular integrity and DNA repair. Top disease and biological

function associated with the gene variant detected are predominantly related to disorders of the gastrointestinal, haematological and renal systems.

Hence, we believe the results of this study may serve as a platform from which to develop a wider understanding of the biological processes involved in the host response to cardiac surgery, allowing the identification of potential biomarkers of organ damage and finally facilitating the development of novel therapeutic approaches.

Context of the research, final remarks and added value:

Adults require cardiac surgery using CPB relieve life-threatening coronary disease and for treatment of severe valvular heart disease. Surgery and CBP trigger a systemic inflammatory response[22,2], the severity of which is an important determinant of outcome[9].

Genetics variants have been associated with the host response to surgery and the development of complications. However, candidates' gene associated studies are frequently inconsistent and failure in replicating their results is common[63, 64, 71, 72, 80]. Several investigators have attempted to explain the functional role of the association detected, many times without exploring the role that genetic diversity plays in modulating the host response to cardiac surgery [34, 197, 198].

This investigation tried to fill a need to complement genotyping with functional analysis to understand the mechanisms by which genetic variation affects host responses to cardiac surgery.

This is to our knowledge the first study to investigate regulatory eQTLs variants in the context of cardiac surgery.

We first investigated the transcriptome response of 63 Caucasian patients undergoing elective cardiac surgery requiring CPB. The anaesthetic technique was standardised. We obtained whole blood samples before and 24 hours after surgery for RNA and DNA isolation.

Traditionally the host response to cardiac surgery has been described as an inflammatory response characterised by increased plasma levels of proinflammatory cytokines[2]. We found a transcriptome response that showed a different response pattern.

We identified 90 genes that were differentially expressed 24 hours after cardiac surgery (FC >2, FDR <0.05), with 80 upregulated and 15 downregulated. The upregulated genes are majority related to immune response including T receptors and downregulation of T cells and natural killers. Gene ontology enriched terms ($p < 0.05$) after cardiac surgery corresponded to the immune response and response to stimulus (defence, stress and inflammatory pathways). Network analysis suggests a complex interaction between key mediators of the immune response (MMPK14, PPARγ and MMP9).

We then identified 659 cis-acting eQTLs (FDR <0.05) that significantly enriched for canonical pathways, IL-3, JAK/Stat signalling and GM-CSF signalling that are

involved cell proliferation, survival and differentiation. In addition, Leptin signalling pathways (most commonly known for its role in feeding/satiety behaviour) that is linked to the inflammation response mediated via activation of JAK/STAT or PIK3.

One of the principal limitations of this study is the small sample size; findings should be taken with caution. However, results obtained by our work are currently being used by colleagues at the Wellcome Trust for Human Genetics as a “control group” for the investigation of the host response of patients to sepsis. Provision acceptance: Blue 201608-1685C.R1 “Shared and distinct aspects of the sepsis transcriptomic response to faecal peritonitis and pneumonia” – American Journal of Respiratory and Critical Care Medicine (AJRCCM).

Appendix 1

Gene expression study

1-1 Genes significantly upregulated 24 hours after cardiac surgery (>2fold change ,p-value <0.05)

RETN: Resistin (corrected p :5.72 E-16) is a signaling molecule traditonaly known for increasing the resistance to insulin in peripheral tissues that, has been implicated in the pathophysiology of type 2 diabetes and obesity.However more recent investigations have linked resistin with inflammation .Expressed in circulating monocytesresistin up-regulates adhesion molecules , chemokines and down regulates TRAF3 (tumor necrosis factor receptor associated factor-3).High plasma levels of resistin have been associated with an increased risk of heart failure in a population with documented coronary disease(N:980 OR : 2.06) [263] .

TLR5: (corrected p:1.8 E-15) Toll-like receptor 5 belongs to a family of genes (TLR1 to TLR10) associated with molecular pattern recognition. Flagellin, present in Gram-negative and Gram-possitive bacteria lead to activation of TLR5 triggering NF-κB and TNFα production. Interesting TLR5 signaling induce anti-inflammatory and anti-apoptotic as well as antimicrobial molecules including NOD2/CARD15

(genetic polymophisms of these genes have been associated with Crohn's disease) [264] .

TSPO: (corrected p value 1.84 E-15)Translocator protein 18-kd is located in the outer mitochondrial membrane and is part of the mitochondrial transition pore (MPTP).TPSO mitochondrial tranport activity is dependant on its interation with Heat Shock Proteins (mainly HSP 70).The best known function of TSPO is the transport of cholesterol through mitochondrial membranes which is one of the key steps in steroidogenesis.TPSO has a particular role regulating the opening of the MPTP and has been implicated in apoptosis necrotic cell death and cellular proliferation. Expressed in immune cells TPSO is up-regulated by inflammation and plays a role in immune regulation [265] [266] .

LILRA5 : (corrected p 5.8 E-15) called leukocyte immunoglobulin-like receptors 9 is expressed by natural killer cells where it inhibits cytotoxic responses against cells expressing major histocompatibility complex (MHC) on the surface of moncytes LILRA5 induce the liberation of cytokines:IL1B,Tκalpha and IL6.LIRA5 acts as an immunological regulator that influence modify the host response determing its major components at the cellular or humoral level [267] .

IL4R: Interleukin 4 receptor regulates the productin of Ig-E Polymophisms of IL4-R have been associated with atopy and slow progression of HIV as discussed previously. More interesting theIL4/IL4R SYSTEM has been associated with the Tyrosine kinase Syk Activated in PMN is involved in phagocytosis,increase neutrhophil adhesion capacity and delay apoptosis [268] .

ANNEXIN A3: (corrected p 1.5 E-14) is a of calcium-dependent phospholipid binding protein.ANXA3 is better known as a tissue marker of prostatic cancer and reduced expression of this protein has been associated with a bad prognosis in such cases[269]

. ANXA3 has beeen implicated in neutrophil degranulation , leukocyte trafficking and lymphocyte migration [270].

HP: (corrected p 2.1 E-14) secreted by the endoplasmic reticulum the main function of haptoglobin main is to bind haemoglobin, forming the complex HP-HB that is subsequently removed from the circulation by monocytes and macrophages, thereby preventing the oxidative harm associated with free haemoglobin.In addition, free haemoglobin reduces intravascular nitric oxide (NO) bioavailability and may contribute to microcirculatory dysfunction after cardiac or major vascular surgery.Interestently haemolysis increases the levels of arginase 1 converting L-arginine (substrate of NO) to ornthine , in that way reducing the production of new NO[271, 272].

HK3: (corrected p 2.1 E-14)HK3 belong to the family of hexokinases. These are glycolytic enzymes that are implicated in the first step of glucose metabolism .They function as a link between mitchondrial ATP synthesis and glucose metabolism.In addition hexokinases are known to be overexpressed in malignant tumours .Recent evidence from cell culture experiments has suggested that HK2 could be a key regulator of apoptosis via changes in mithocondrial function [273] .

PGS1 (corrected p 3.1 E-14) phosphatidylglycerophosphate synthetase function is the biosynthesis of anionic phospholipids and cardiolipin has been decribed in the mithocondria and in the endoplasmic reticulum. In a hamster model investigators transfecting cells with a defective PGS1 gene induced a reduction in the synthesis of phospholipid in additon to mitochondrial morphological and functional abnormalities [274] .

GYG1: (corrected p 4.9 E-14) Glycogenin is involved in the synthesis from glucose.

ZDHHC19: (corrected p 5.33 E-14) zinc finger DHHC-type containing 19 belongto the family of finger proteins, These are involved in multiple mechanisms including regulation of apoptosis, lipid binding,DNA recognition and RNA packaging.ZDHHC19 has been found to be one of the 15 genes most significantly upregulated(corrected p <0.001) after infection with mycobacterium bovis in cattle [275] .

LILRA6: (corrected p value 5.4 E-14) belongs to a group of leukocyte receptors expressed in monocytes and granulocytes and known to exhibit a high degree of polymorphism. Upregulated LILRA6 receptors act to inhibit NF-κB and dendritc cells, inducing anergy and immunosupression [276] [277].

UPP1: (corrected p value 2.23 E-13) Uridine phosphorylase 1 catalyses the phosphorylation of thymidine or deoxyribose to ribose or deoxyribose increased expression off UPP1has been found in several type of tumoural cells, expression of UPP1 is controlled by oncogenes,and tumor supressant genes .UPP1 expression is increased by inflammatory cyokines :TNFα a IL-1 and Interferon δ [278].

ALPL: (corrected p 3.6 E-13) Alkaline phosphates are membrane glycoproteins that hydrolyse monophosphate esters .There are 4 well decribed alkaline phosphatases :intestinal , placental , placental-like and liver/bone/kidney In addition ALPL has been found to be expressed in B lymphocytes . In children with recurrent tonsilitis it has been postulated that B lymphocytes of diseased tonsils are less able to responde to bacterial antigens . Interesting, levels of alkaline phosphate are a marker of B cell capacity of proliferation upon antigen stimulation and can be used as a marker of organ immune competence in children with tonsilitis[279] .

DYSF : (corrected p 3.8 E-13) this encode a skeletal muscle protein that plays a role in calcium-mediated membrane fusion events. Several mutations of this gene have been implicated in mucular dystrophies and myopathies.The endoplamic

reticulum(ER) is a quality sensor of cytoplasmic protein , aberrant proteins has been are extracted and by the ER –associated degradation(ERAD) system of authophagy/lysosome. Dysferin plays a role in the vesicular membrane that is subsequently degraded by the ubiquinin/proteasome ERAD system. Dysferin mutant (L1341P) is not properly degraded trigering ER stress cell death pathway through c-Jun N-terminal kinase (JNK) and caspase -12 [280] .

S1PR5: (corrected p 4.0 E -14) Lysosphingolipid sphingosine 1- phosphate (S1P)is expressed in mature CD56 human NK cells.Natural killer (NK) cells are a subpopulation of lymphocytes involved anti-tumour survillance and constitute part of the innate immune response to viruses , bacteria and parasites . Emigration of Natural killers cells from Lymph nodes and bone marrow is stimulated by the expression of S1PR5 probably promoted by T-bet (containing trancription factor required for Th1 cell development)[281].Investigators have shown in mice that S1PR5 is responsible for NK cell trafficking in inflammatory situations ;interestenting this mechanism of migration is independent of chemokines [282] .

LMNB1: (corrected p 4.2 E -13) ,Lamins are a major component of the nuclear lamina which covers the nuclei of eukaryotic cells .Lamina B is present in T cells , in a member of the interphase nuclear lamina family and plays a role in mantaining the shape and mechanical integrity of the nucleus . A mutacion of lamina B causes the Pelger-Huet anomalycharactherized by abnormal nuclear shape and chromatin abnormalities in granulocytes [283].In addition, Malhas et al[284] demostrated using an in vitro model that lamin B1 negative cells have high levels of reactive oxygen species (ROS) and are more suceptible to oxidative stress.

PFKFB3: (corrected p 4.45 E-13) 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 regulates the concentration of fructose 2-6-biphosphate and is a

key activator of the glucolysis.In astrocytes, PFKFB3 facilitates the maintenance of mitochondrial membrane potential by increasing the production of glucolytically-generated ATP, thereby preventing apoptotic cell death [285].

MAPK14: (corrected p 7.03 E-13) , mitogen-activated protein kinase 14 catalyzes the phosphorylation of serines and threonines and is activated in response to cellular stress and LPS stimulation .MAPK14 expression is rapidly increased after treatment with IL-1, lipopolysaccharide (LPS) and heat shock stress.The JNK/c-Jun pathway regulates cell proliferation and cell death, MAPK14 in haematopoeitic cells seems to supress JNK kinases.In addition,MAPK14 modulates the expression of inflammatory cytokines TNF-alpha, IL-6 and IL12 . Inhibitors of MAPK14 are being developped to treat chronic inflammatory conditions[211, 286].

RGL4 :(corrected p 7.4 E-13) Ral guanine nucleotide dissociation stimulator-like 4 initially identified like as oncongene and is expressed in human T-cell malignacies [287]

METTL7B:(corrected p 7.9 E-13) Methyltranferase like b7. Grape seed proanthocyanidins (GSPE) contained in grapes and wine have been reported to promote health by acting as anti-oxidant, antimicrobial and neuroprotective agents.Grape and wine exert a cardiprotective effect through increasing HDL, decreasing the level of endothelial oxidation and promoting vasorelaxation.Interestently, METTL7B has been found as one of the putative genes differentially upregulated in rats fed with a high fat diet (HFD) when compared with rats fed with HFD+GPSE [288] **.**

PLSCR1: (corrected p value9.72 E-13) Phospholipid Scramblases are activated by an increase in the intracellular Ca++ concentration caused by of cellular injury or apoptosis. Their function is to alter the normal structure of the plasma membrane by increasing the expossure of phosphatidylserine (PS) .This action not only

facilitates the phagocytosis of damaged cells by circulating macrophages but triggers the activation of the blood coagulation cascade.In addition , PLSCR1 integrate the family of genes stimulated by Interferon that require JAK/STAT signalling and which bilogical functions are antiviral, immunomodulatory and anti-proliferative [289].

CR1: (corrected p 1.05 E-12) complement component receptor 1is expressed in eosinophils, monocytes , macrophages ,B-lymphocytes,CD4 T cells and dendritic cells. It binds the C3b/C4b opsonized immune complex that is engulfed by macrophages and other phagocytes. Polymorphisms of CR1 have been associated to SLE and other autoimmune disoders, as well as to suceptibility to malaria and disease progression in HIV patients.Cytokines(TNF-alpha, TNF-B, IL-4,IFN-G) increase plasma levels of CR1while IL-4 and IL-10 supress CR1 expression. In SLE immune complexes reduce the level of CR1.Interestenting, recombinant s-CR1 is being investigated as a novel treatment in autoimmune and inflammatory disorders[290].

CD177: (corrected p 1.11 E-12) glycoprotein expressed on the suface of neutrophils. After neutrophil activation CD177 is translocated from secretory vesicles and specific granules to the plasma membrane .Up-regulated by bacterial infections . The main function of CD177 is to facilitate adhesion of neutrophils to the endothelialium [291].

Other genes significantly upregulated 24 hours after Cardiac Surgery:

S100A12: (corrected p 1.18 E-12) this calgranulin related protein is abundant in neutrophils and infiltrating granulocytes ,after engaging with its receptor RAGE on endothelial, phagocytes or lymphocytes trigger activation.

CYP1B1: (corrected p 1.19 E -12) ; cytochrome P450, subfamily 1, CYP1B1 belong to the P450 cytochrome family (reposible for phase 1 oxidation).However , Cyp1b1 is not expressed in the liver, but is expressed by most tumour cells and on monocytes .It is considered a target for antigen-specific cytotoxic T-cells [292] .

GADD45A: (corrected p 1.31 E-12), DNA damage–inducible gene . Induced by enviromental stress GADD45A activates MAPK14.In addition ,GADD45 is a negative regulator of T cell proliferation.Studies in animal models have shown that lack of GADD45 is associated increase vulnerability to autoimmune disorders.

UGCG: (corrected p 1.4 E-12), UDP-glucose ceramide glucosyltransferase catalyses the first step in the synthesis of glycosphingholipids which are a component of plasma membrane

GPER: (corrected p 1.9 E-12), G protein–coupled estrogen receptor.Estrogens are known to have an anti-atherogenic effect on blood vessels and to exert a protective effect against vascular injury. The GPER gene is expressed in B and T cells and in peripheral monocytes and plays a role in the adaptative immune response [293] .

SOCS3: (corrected p 2.03 E-12) ,supressor of cytokine signaling 3, predominantely expressed by T-helper acts as a negative regulator of cytokines(JAK/STAT3 pathway)

ATP9A: (corrected p 3.77 E-12), ATP ase ,class 2.In eukaryotic plasma mebranes contain different lipids such as phosphodylserine(PS) and phosphatidylethanolamine distritributed in an asymetric structure.Exposure of PS on the outer side of the membranes as occurs durig cytokinesis lead to apoptosis and blood coagullation. ATPases are located in different areas of the cells ,their function is to translocate phospholipids .ATP9A has been recently found distributed thoroghout the cytoplasm as well as in Golgi-like structures in Hella [294] .

HIST1H2BD: (corrected p 3.8 E-12), histone gene cluster family member B,histones are reponsible for the nucleosome structure and epigenetic regulation

CDK5RAP2: (corrected p 4.02 E-12), regulatory subunit–associated protein.This gene encodes a protein that form the form pericentriolar matrix scaffold.

MGAM: (corrected p 5.08 E-12), maltase-glucoamylase ,codes for an enzyme invoved in the breakdown of sugar.

TDRD9: (corrected p 5.38 E-12), tudor domain containing 9, is a key factor in the biogenesis of chromatoid bodies which are thought to regulate translation and RNA decay [295] .

OPLAH: (corrected p 5.5 E-12), 5-@oxoprolinase(ATP-HYDRLYZING);catalyzes the hydrolysis of pyroglutamate to L-gutamate and ATP to ADP.

MMP9: (corrected p 5.3 E-12), matrix metalloproteinase 9,is a type IV collagenase belonging to the group of zinc metalloproteinases that degrade extracellular collagen.Neuthrophils, macrophages and dendritic cells secrete MMP9 in variable amounts .It has been show that MMP9 controls trafficking of inflammatory cells and T-cells in response to allergens .Several MMP9 SNPs have been associated with an increased incidence of asthma and COPD[296].

F5: (corrected p 1.4 E-11), factor V leiden, is a key protein in the coagulation pathway and is a cofactor for the conversion of prothrombin to thrombin by factor Xa.

PPARG: (corrected p 1.73 E-11), peroxime proliferator-activated receptor-gamma acts as a nuclear co-repressor of NF-κB.

CA4: (corrected p 1.76 E-11), carbonic anhydrase IV,belongs to a family of zinc metalloenzymes that catalyse the hydration of CO2 to HC03 . Located in the plasma membrane this gene is up-regulated by metabolic acidosis.

CST7: (corrected p 2.2 E-11) ,cystatin 7 secreted by TH1, TH2 and natural killer cells is an immune regulator by inhibiting cysteine proteases

C1QB: (corrected p 3.54 E -11),complement componen 1, the complement sytem is has more than 20 plasma proteins that contribute to the clearance of microbes by binding opsonins and anaphylotoxins .C1QB is produced by macrophages and dendritic cells and contributes to the clearance of apoptotic cells . C1 QB plays a key role in the pathogenesis of SLE and its production is increased by IFNγ.

FCGR1B:(corrected p 3.6 E-11), Fc Fragment of IgG ,high affinity Ib is encoded by three genes that share over 98% of sequence homology located within chromosme 1.

IRAK3: (corrected p 6.9 E-11), interleukin 1 receptor –associated kinase 3,IRAK is induced after TLR stimulation and negatively regulates TLR signalling, playing a key role centre of the innate immune self-regulation.

CES1: (corrected p E -10) ,carboxylesterase 1monocyte esterase is implicated in the metabolism of multiple drugs .In addition, deficiency of CES1 is associated with impaired lytic monocyte activity[297] .

FCAR: (corrected p 1.58 E-10) , Fc fragment of IgA, is present in monocytes,neutrophils,macrphages and eosinophils and mediates phagocytosis , antibody dependant cytotoxicity and stimulates od release of inflammatory mediators

PGLYRP1: (corrected p1.9 E-10), peptidoglycan recognition protein1, PGLYRP1 binds peptidoglycan and gram positive bacteria resulting in membrane depolarization and inhibition of DNA and RNA synthesis, leading to bacterial death.

RNASE2:(corrected p 1.9 E-10) ,eosinophil-derived neurotoxin(EDN), encodes an eosinophil granule protein. The RNASE2 gene has been found to be upregulated in peripheral blood mononuclear cells taken from patients presenting with inflammatory

bowel disease and rheumathoid arthritis [298] . In addition ,EDN is a chemotatic antimicrobial protein that acts on dendritic cells [299] .

OSM: (corrected p 2.03 E-10), OSM is ubiquous in many tissues , It has been identidfied as an effector of cell death induced by tyrosine kinase (trk) in the nervous system [300] .

GPR84: (corrected p 2.2 E -10) ,G protein–coupled receptor 84, plays a role in cell-cell communication

SLC37A3: (corrected p 7.11 E-10), belongs to a family of 4 genes that act as transmembrane sugar transport.

MERTK: (corrected p 1.2 E-10), a tyrosine kinase protooncogene, expressed in blood monocytes acts as a feedback inhibitor of TLR and cytokine triggered inflammatory responses.

VNN1: (corrected p 4.08 E -09) , vanin 1 facilitate adhesion of peripheral lymphocytes.

ORM1: (corrected p 6.09 E -09) ,orosomucoid 1, alpha 1 acid glycoprotein

ARG1: (corrected p 6.19 E -09), arginase 1 induction has been found to lead to T cell dysfunction and nitric oxide production thereby increasing the suceptibility to infections [301]

IL18RAP: (corrected p 7.67 E -09),interleukin 18 receptor accesory ptrotein, is expressed in peripheral blood leukocytes .Coexpression of IL18RAP and IL18R1 are required for NF-κB and JNK activation.

FCGR1B: (corrected p 1.2 E -08), high affinity receptor for Immunoglobulin G.

CLEC4D: (corrected p 1.99 E -08) ,C-type lectin domain family 4,expressed on the surface of macrophages are implicated in microbial recognitiona and cell interaction

HPGD: (corrected p 3.4 E-08), hydroxyprostaglandin dehydrogenase is the main enzyme involved in prostagladin degradation.

PFKFB2: (corrected p 3.03 E-07), fructose2-6 bisphospate controls glycolysis in all eukaryotic cells

CD163: (corrected p 5.71 E-07), macrophage antigen that mediates endocytosis of haptoglobin –hemoglobin complexes

IL18R1: (corrected p 1.8 E -06), IL-18 receptor activates NF-κB.

1-2 Genes significally downregulated 24 hours after cardiac surgery (>2 fold change, p-value < 0.05).

GPR56 trancript variant 2 and 3 (p; 2.5 E-13 and 9.7 E- 13): GPR56 belongs to the family of G protein –coupled receptors (GPRs) that exhibit several transmembrane domains(LNB-7TM) and are found in a wide range of tissue. Preferentially expressed in neuronal progenitor cells allelic variant of this GPR56 have been associated with polymicrogytia .Downregulation of GPR56 has been show to reduce tumor progression and metastasis in melanoma cell lines.Interestently LNB-7TM protein has been reported to play a role in cell-cell or matrix cell adhesion and leukocyte trafficking [302, 303].

EOMOS (p; 3.34 E -12) belongs to the T-box family of transcription factors . Eomes regulate the expression of a group of cells involved in Type 1 immunity. Eomes expressed in CD8+ T cells might regulate cytotoxic actions of these cells.

Itlekofer et al[304] demostrated in an experimental model of Eomes knockout mice infected with lymphocytic choriomeningitis virus (LCMV) that CD8 cells collected from infected mice had a reduced cytotoxic capacity and a significantly reduced IFN-γ expression.Not only might this lead to a reduction in the clearance of viruses but in addition, the authors made the striking observationthat 1 week after infection EOMOS knockout mice started to lose weight and developed extensive mutliorgan dysfunction. Postmorten histology revealed generalized neuthrophil infiltration and anatomical destruction of several organs.

GNLY trancription variants 519 and NKG5 (corrected p;9.0 E-12 and p; 2.35 E-10)This gene encodes for granulysin(saposin-like protein) that is present in the cytotoxic granules of T and Natural killer cells. Granulosyn exerts an antimicrobial effect against intracellular pathogens such as Listeria monocytogenes and Mycobacterium tuberculosis.In addition, GNLY expression has been recently found to increase after acute myocardial infartion (6 fold) and GNLY lymphocytes, probably attracted by chemokines and interleukins, lead to apoptotic rather than necrotic cell death at the site of infarction[305]

FGFBP2 (corrected p; 1.2 E -11) killer –specific secretory protein, Ksp37. In peripheral blood Ksp37 is secreted by Th1-type CD4 , CD8 , γδ and CD16 NK cells. Ksp37 is commonly expressed in cytotoxic lymphocytes .Ogawa et al [306] studying patients with infectious mononucleosis suggest that plasma levels of Ksp37 may be of clinical value for monitoring cytotoxic lymphocytic activity .

GZMK granzyme H and K (corrected p;2.75 E-10 and 3.05 E-9) are serine proteases found in the granules of cytotoxic T lymphocytes and natural killer (NK). They exert a significant role against tumours, viruses and transplant tissue.Granzyme K(GrK) has been implicated in caspase-independent apoptosis, characterized by mitochondrial

damage an increase in intracellular reactive oxygen species (ROS) and single stranded DNA .Using PBMCs isolated from patients with multiple sclerosis Jiang et al [307]demostrated that GrK plays a role in the immunorgulation of the immune response including immune memory.

FCER1A (P corrected;5.18 E-10)Immunoglobulin E receptor, high-affinity, of mast cells. This Ig E receptor allow allergens to stimulate mast cells triggering inflammatory and hypersensitivy responses (type 1 and 2), that charactherise allergic reactions as well as asthma and hay fever.High affinity IgE receptors are expressed in mast and basophil cells. Investigators have shown in a rodent model that the inhibition of FCER1A by a chimeric protein (GE2) reduced mast cell degranulation/recruitment [308].

PRSS33: (corrected p ; 5.18 E -10) (EOS) Protease serine 33 has been detected in many tissues including leucocytes and macrophages.Photeases belong to a family of proteins that are implicated in polypeptides post-translation .Protease serine 33 has a role in macrophage-related functions . The definitve role of PRSS33 and the stimuli that release EOS has not been fully elucidated [309].

CLC: (corrected p; 2.85 E -8) Charcot-Leyden crystal protein or Galectin are hexagonal bipyramodal crystals found in secretions and tissues in relation to allergic conditions or parasitic infections [310].CLC/galectin-10 belongs to the lectin family .Galectin10 is considered a marker of eosinophil activity .In addition 8 SNPs in the promoter region of CLC are associated with an increased risk of allergic rhinitis(OR of minor alleles 5.9-7.8) [311].

CCR3 or CKR3: (corrected p; 1.15 E-8)Chemokine ,CC motif ,receptor . Chemokines are characterised according to their aminoacid sequences CC(cyteine-

cysteine) or CXC(cysteine-X-cysteine) . Included among the CC family are MIP-1-alpha, Rantes, ,MCP3 and eotaxin.

There are three CC receptors CKR1/CKR2/CKR3, the latter being this last is expressed in eosinophils, T helper lymphocytes , basophils and mast cells. CCR3 can be constitutively expressed or temporarily induced by specific cytokines.The activation of CCR3 leads to the degranulation of eosinophils that result in the release of reactive oxygen species and cytotoxic molecules at sites of inflammation.In basophils, CCR3 activation results in the liberation of histamine and leukotrienes [312] .

IFIT1: (corrected p;2.4 E-7) Interferon-induced protein with tetratricopeptide repeats 1is considered to be a mediator of negative-feedback regulation of virus-triggered induction of type I IFNs . Another function has been recently attribuited to IFIT, similar to the leucitine –rich repeat that confer pattern recognition to the Toll- like receptors,IFIT tetratricopeptide repeats have nucleic acid-binding ability.It is this capacity that confers the ability of IFIT to clear pathogens ,playing a key role in the interferon induced innate immune nextwork [313].

IFIT44L (corrected p: 1.16 E-05) Interferon-induced protein 44-like gene encodes a protein that has antiviral activity against hepatitis C.In addition other functions of interferon induced protein have been revealed recently. C-reactive protein (CRP) is an acute phase protein the levels of which rise in acute and chronic inflammation, as well as in bacterial, viral and fungal infection .CRP presents in two forms (m) CRP on the cell membrane of activated platelets and (p) CRP in the plasma. Deposition of (m)-CRP has been proposed as a link between CRP and inflammation.Interferon-responsive genes are up-regulated by (m)CRPand are to be innvolved in the pathophysiology of localized inflammation e.g artherosclerosis [314].

nt 2 and 3 (p; 2.5 E-13 and 9.7 E- 13): GPR56 belongs to the family of G protein – coupled receptors (GPRs) that exhibit several transmembrane domains(LNB-7TM) and are found in a wide range of tissue. Preferentially expressed in neuronal progenitor cells allelic variant of this GPR56 have been associated with polymicrogytia .Downregulation of GPR56 has been show to reduce tumor progression and metastasis in melanoma cell lines.Interestently LNB-7TM protein has been reported to play a role in cell-cell or matrix cell adhesion and leukocyte trafficking [302, 303].

EOMOS (p; 3.34 E -12) belongs to the T-box family of transcription factors . Eomes regulate the expression of a group of cells involved in Type 1 immunity. Eomes expressed in CD8+ T cells might regulate cytotoxic actions of these cells. Itlekofer et al[304] demostrated in an experimental model of Eomes knockout mice infected with lymphocytic choriomeningitis virus (LCMV) that CD8 cells collected from infected mice had a reduced cytotoxic capacity and a significantly reduced IFN-γ expression.Not only might this lead to a reduction in the clearance of viruses but in addition, the authors made the striking observationthat 1 week after infection EOMOS knockout mice started to lose weight and developed extensive mutliorgan dysfunction. Postmorten hisstology revealed generalized neuthrophil infiltration and anatomical destruction of several organs.

GNLY trancription variants 519 and NKG5 (corrected p;9.0 E-12 and p; 2.35 E-10)This gene encodes for granulysin(saposin-like protein) that is present in the cytotoxic granules of T and Natural killer cells. Granulosyn exerts an antimicrobial effect against intracellular pathogens such as Listeria monocytogenes and Mycobacterium tuberculosis.In addition, GNLY expression has been recently found

to increase after acute myocardial infartion (6 fold) and GNLY lymphocytes, probably attracted by chemokines and interleukins, lead to apoptotic rather than necrotic cell death at the site of infarction[305]

FGFBP2 (corrected p; 1.2 E -11) killer –specific secretory protein, Ksp37. In peripheral blood Ksp37 is secreted by Th1-type CD4 , CD8 , γδ and CD16 NK cells. Ksp37 is commonly expressed in cytotoxic lymphocytes .Ogawa et al [306] studying patients with infectious mononucleosis suggest that plasma levels of Ksp37 may be of clinical value for monitoring cytotoxic lymphocytic activity .

GZMK granzyme H and K (corrected p;2.75 E-10 and 3.05 E-9) are serine proteases found in the granules of cytotoxic T lymphocytes and natural killer (NK). They exert a significant role against tumours, viruses and transplant tissue.Granzyme K(GrK) has been implicated in caspase-independent apoptosis, characterized by mitochondrial damage an increase in intracellular reactive oxygen species (ROS) and single stranded DNA .Using PBMCs isolated from patients with multiple sclerosis Jiang et al [307]demostrated that GrK plays a role in the immunorgulation of the immune response including immune memory.

FCER1A (P corrected;5.18 E-10)Immunoglobulin E receptor, high-affinity, of mast cells. This Ig E receptor allow allergens to stimulate mast cells triggering inflammatory and hypersensitivy responses (type 1 and 2), that charactherise allergic reactions as well as asthma and hay fever.High affinity IgE receptors are expressed in mast and basophil cells. Investigators have shown in a rodent model that the inhibition of FCER1A by a chimeric protein (GE2) reduced mast cell degranulation/recruitment [308].

PRSS33: (corrected p ; 5.18 E -10) (EOS) Protease serine 33 has been detected in many tissues including leucocytes and macrophages.Photeases belong to a family of

proteins that are implicated in polypeptides post-translation .Protease serine 33 has a role in macrophage-related functions . The definitve role of PRSS33 and the stimuli that release EOS has not been fully elucidated [309].

CLC: (corrected p; 2.85 E -8) Charcot-Leyden crystal protein or Galectin are hexagonal bipyramodal crystals found in secretions and tissues in relation to allergic conditions or parasitic infections [310].CLC/galectin-10 belongs to the lectin family .Galectin10 is considered a marker of eosinophil activity .In addition 8 SNPs in the promoter region of CLC are associated with an increased risk of allergic rhinitis(OR of minor alleles 5.9-7.8) [311].

CCR3 or CKR3: (corrected p; 1.15 E-8)Chemokine ,CC motif ,receptor . Chemokines are characterised according to their aminoacid sequences CC(cyteine-cysteine) or CXC(cysteine-X-cysteine) . Included among the CC family are MIP-1-alpha, Rantes, ,MCP3 and eotaxin.

There are three CC receptors CKR1/CKR2/CKR3, the latter being this last is expressed in eosinophils, T helper lymphocytes , basophils and mast cells. CCR3 can be constitutively expressed or temporarily induced by specific cytokines.The activation of CCR3 leads to the degranulation of eosinophils that result in the release of reactive oxygen species and cytotoxic molecules at sites of inflammation.In basophils, CCR3 activation results in the liberation of histamine and leukotrienes [312] .

IFIT1: (corrected p;2.4 E-7) Interferon-induced protein with tetratricopeptide repeats 1is considered to be a mediator of negative-feedback regulation of virus-triggered induction of type I IFNs . Another function has been recently attribuited to IFIT, similar to the leucitine –rich repeat that confer pattern recognition to the Toll- like receptors,IFIT tetratricopeptide repeats have nucleic acid-binding ability.It is this

capacity that confers the ability of IFIT to clear pathogens ,playing a key role in the interferon induced innate immune nextwork [313].

IFIT44L (corrected p: 1.16 E-05) Interferon-induced protein 44-like gene encodes a protein that has antiviral activity against hepatitis C.In addition other functions of interferon induced protein have been revealed recently. C-reactive protein (CRP) is an acute phase protein the levels of which rise in acute and chronic inflammation, as well as in bacterial, viral and fungal infection .CRP presents in two forms (m) CRP on the cell membrane of activated platelets and (p) CRP in the plasma. Deposition of (m)-CRP has been proposed as a link between CRP and inflammation.Interferon-responsive genes are up-regulated by (m)CRPand are to be innvolved in the pathophysiology of localized inflammation e.g artherosclerosis [314].

Appendix 2.

Further analysis of SNPs associated with cardiac surgery

2-1 We explore all the SNPs using RegulomeDB. The top SNPs according to RegulomeDB scoring scheme are shown in the table 3 below.

Gene ID	dbSNP ID	p-value	Regulome DB Score
CEP192	rs11663049	1.2 E-06	1a
NDUFS5	rs3768324	2.4 E-08	1a
CCDC23	rs12145700	3.0 E-08	1a
SF1	rs693235	3.0 E-08	1b
GPN3	rs4766497	3.2 E-09	1b
PPIL5	rs3007030	4.6 E-06	1b
SERPINB10	rs1025196	7.9 E-07	1b
TTF2	rs3754109	2.8 E-08	1b
FAM118A	rs226503	9.2 E-08	1b
HLA-F	rs1362126	2.8 E-06	1b
GATS	rs13230744	2.6 E-06	1b
C5orf35	rs540	2.4 E-07	1d
SERPINB10	rs9630856	1.0 E-09	1d
SERPINB10	rs6102	1.8 E-10	1d
SARS2	rs575	3.0 E-08	1d
DDX1	rs4668936	3.0 E-08	1d
CTNNA1	rs160404	6.8 E-07	1d
ZFAND2A	rs2949170	2.2 E-07	1d
TMM5	rs4621903	1.6 E-06	1d

Table 3: Matrix e-QTL. List of SNPs **only** 24 after cardiac surgery. SNPs ordered according to Regulome DB Score.

RS 11663049 regulates two motifs (short sequence of DNA) the transcriptions factors NF-κB and E2F-1: DP-2 . 2 An eQTL has found SNPs of this gene to be

associated with heart disease susceptibility[249]. Protein binding between this SNP and NF-κB has been documented by the investigators of the ENCODE project.

NF-κB belongs to a family of transcription factors that have a primordial role in inflammation and the innate immune response. Activation of NF-κB can be mediated through Toll –like receptors(TLR), Interleukin-1, tumor necrosis factor (TNFα), Lipopolysacchrides (LPS) and Lymphotoxin B-receptor. In addition activation occurs through IKK after genotoxic stress (this has been called the atypical activation pathway).

NF-κB regulates the activity of thousands of genes. The most recognised effect of this transduction factor is a proinflammatory response characterized by the activation of cytokines such as TNFα, IL-1,IL-6 and IL-8 as well as up-regulation of adhesion molecules that facilitate the migration of leukocytes to inflammatory sites. Furthermore, NF-κB and STAT3 (another key inflammatory transcription factor) regulate other genes, and have a synergistic anti-apoptotic action. This relationship between inflammation and apoptosis is increasingly the subject of investigations that seek to clarify not only aspects of the inflammatory host responses but the potential link between the pathogenesis of cancer and inflammation . Experimental compounds that target NF-kappa B are being developed as potential therapies[315].

RS 3768324 cis regulated motifs are the transcription factors FL-1 and GABPA, ELK1 and SAP-1A[248]. FLI1 is a proto-oncogene present in haematopoietic and endothelial cells. GABPA; intervene in the regulation of mitochondrial enzymes. ELK1; activates genes involved in gene differentiation.

This SNPs is NDUFS5; the multi-subunit NADH; ubiquinone oxireductase is the first enzyme complex in the electron transport chain of the mitochondria.

Yu et al [316]have confirmed the binding of rs3768324 to GABPA in mice cells. These authors have found that GABPA is not only essential for the maintenance and differentiation of lymphopoiesis stem/progenitors' cells but it actions regulating different genes. GAPB activates genes related to apoptosis (BCL2), as well as transcritption factors essential for cells survival (Zfx, Etv6) and cell renewal (Foxo3) . GABPA exerts direct/indirect regulation of genes involved in DNA repair (ATM), telomere maintenance (TERF2) and epigenetics modifications; including DNA methylation (DNMT1) and Histone acetylation (ep300, MYST4).

RS12145700 regulates the motif USF. Degradation of USF has been associated with suppression of the immune host response.

RS 693235 cis regulate the transcription factor RREB1that acts as an HLA-6 promoter. The gene whose expression is cis regulated by this SNPs is splicing factor 1 SF1; a zinc protein that regulates cellular RNA metabolism[247].

RS4766497regulates the motif NF-Y major histocompatibility class II promoter[248]. The gene affected by this SNP ID ATP2A2 this ATPase acts as a slow Ca transporting system. A mutation of ATP2A2 has been found to contribute to the abnormal transport of intracellular Ca and has been associated with hypertension.

RS3007030 regulates the motif POU5F1 involved in reprogramming pluripotent stem cells.[247].

RS1025196 regulates the transcriptions factors FREAC-3, OCT-1, FOXA2. FREAC to induce the expression of stress response and apoptosis genes. OCT-1 interacts with immunoglobin heavy chain promoters. Mutations of FOXA2 have been involved in cholestatic and pancreatic disease and alterations in insulin production. The gene affected by this SNP is SERPINB10 that expresses a serine protease inhibitor that regulates proteases during haematopoiesis.

RS3754109 regulates the transcription factors SIX-1, SIX-2, SIX-4 [249]. Overactivity of SIX-1 is associated breast with cancer invasiveness. SIX2 and 4 have been described as being involved in the development of the eye. This SNP affects the expression of TTF2 a transcriptor termination factor that participates in the control of t lymphocytes gene expression by acting on the transcription elongation.

RS 226503 regulates the transcription factor OLF-1. OLF-1 is involved in the regulation of pre B and early B cell lines. This gene affects the gene FLJ20639 that forms a complex with heat shock protein 90 that is involved in protein folding[317].

RS 1362126 regulates the motif named IKAROS, a zinc finger transcriptor factor. The main gene affected by this SNP is HLA –A (Major histocompatibility complex, class 1). MHC class1 plays a crucial role in the antigen presentation and initiation of T cell mediated immune responses

RS 13230744 regulates the motif is MTF-1, a metal-regulatory transcription factor that is involved in heavy metal detoxification and free radical scavenging. The gene affected by this SNP is GATS, a glycine N-acyl transferase. That transfers acyl groups in the mitochondria. The functions of this gene have been mainly described in the liver where lt has been shown to be downregulated in hepatitis.

RS 540 regulates the motif FOXP1 and TBP. FOXP1 is a transcription factor that plays a critical role in monocyte differentiation and macrophage function. TBP is a DNA-binding subunit involved in the expression of protein-encoding genes. The gene regulated by this SNP is MRPL28; a component of the large subunit of the mitochondrial ribosome encoded in the nuclear genome.

RS 9630856 regulates the motif ERR and RORA. ERR plays a role in the regulation of cellular energy. RORA is a member of the steroid hormone receptor present in peripheral leukocytes. The gene affected by this SNP is SERPINB10.

RS 6102 regulates the motif NANOG, a homeobox transcription factor that is involved in cellular differentiation.

RS 575 regulates the motifs are zinger finger protein transcription factors ZIC1, ZIC2, ZIC3. These transcription factors exert their modulatory effect in the vicinity of any sequence to which a protein domain binds. The gene affected by this SNP is SARS2 a mitochondrial seryl-t RNA synthetase 2 , which provide serine aminocetylation to mitochondrial T-RNA . Mutations of this gene have been associated with alterations in the mitochondrial translation system that lead to derangements in the synthesis of mitochondrial proteins and cellular energy supply

RS 4668936 regulates the motif zinc finger ZBT3.The gene affected by this SNP is a putative RNA helicase that by altering RNA can influence translation, initiation and splicing.

RS 160404 regulates the motif EWSR1-FLI, a putative tumour suppressor. The gene affected by this SNP is CTNNAI, a cadherin associated protein that plays a role in cell cycle regulation.

RS 2949170 regulates the motifs MAFB, transcription factors that contribute to differentiation and function of, monocyte and macrophages. Another transcription factor influenced by this SNP is V-ETS, an oncogene that is involved in the regulation of telomerase activity. The gene affected by this SNP is ZFAND2A, a gene that encodes for a zinc finger protein involved in the proteosomal adaptation to environmental stress.

Appendix 3

3-1 Network analysis

Analysis of associated network functions showed that the top five eQTLs relate to cell signalling, cell to cell communication and different aspects of cellular integrity including DNA replication, recombination and repair.

Table 3-1 see below shows the top networks and the number of genes from our dataset that belong to each network.

Associated Network functions	Molecules
Cancer, Cell Cycle, Reproductive System and Function	43
Cell Morrphology, Cellular Development, Embryonic and function	39
Protein Synthesis, Dermatological Development and Conditions, infectious Disease	35
Cell-To-Cell Signaling and Interaction , Hematological System Development and Function, Inflammatory Response	27
Cell Cycle, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair	26

5 Top functional Networks according to the number of genes

3-2 Top disease and biofunctions

Top diseases and bio functions associated with each gene are divided into three groups .**Table 3-2**.

A: Diseases and disorders B: Molecular and cellular functions C: Physiological system and development and function

Top Disease and Bio Functions

Disease and Disorders	p_value	Molecules
Gastrointestinal Disease	1.0E-03-4.7E-02	23
Hepatic System Disease	1.0E-03-4.7E-02	11
Infectious Disease	1.0E-03-2.9E-02	13
Inflammatory Disease	1.0E-0.3-3.8E-02	13
Inflammatory Response	1.0E-3-4.7E-02	17
Molecular and Cellular Functions		
Cellular Development	1.2E-03-4.7E-02	24
Protein Synthesis	3.2E-0.3-4.3E-0.2	9
Cell Cycle	3.9E-0.3-4.7E-0.2	13
Cell-To-Cell Signaling and Interaction	3.9E-0.3-4.7E-02	13
Cellular Assembly and Organization	3.9E-0.3-4.7E-0.2	12
Physiological System Development and Function		
Digestive System Development and Function	1.0E-03-4.7E-0.2	8
Hepatic System Development and Function	1.0E-03-4.7E-0.2	9
Organ Development	1.0E-03-4.7E-0.2	9
Hematological System Development and Function	1.2E-03-4.7E-02	21
Hematopoiesis	1.2E-03-4.3E-02	18

A. Diseases and disorders

Most genes that belong to this group are linked to gastrointestinal and hepatic diseases. Within this group genes associated with inflammatory disorders have been found to be associated with an increased susceptibility to inflammatory bowel disease.

Some of the most relevant genes form this category.

ADK: adenosine kinase. This gene plays a role in the inflammatory process. Mutations of this gene have been associated with liver dysfunction [318].

GAA: glucoside alpha is a liposomal enzyme involved in the degradation of glycogen within cellular vacuoles. Mutations of this gene have been associated with glycogen storage diseases.

LGALS9: lectin, galactoside-binding soluble 9 is derived from T cells and is a potent chemo-attractant [319].

PPP3R1: protein phosphatase s regulatory subunit B . This gene increases tumorogenesis, potentially by acting through senescence associated mechanisms [320].

ROGDI: encodes a leucine zipper protein expressed in many cell including mononuclear cells. This gene is involved in intracellular transport and cells division[321].

APC: encodes a protein associated with tumour suppression and cell adhesion[322] .

NOD2: nucleotide-binding oligomerization domain protein 2. NOD expression activates NFKB . NOD activation in Crohn's disease has been associated with the inability of the bowel to clear pathogens [323].

STAT6: signal transducer and activator of transcription 6. STAT6 is involved in the physiological action of leptin. This gene mediates immune signalling in response to cytokines and to viral infection at the level of the endoplasmic reticulum (chen et al 2011). STAT6 activity has been implicated in the pathogenesis of non-atopic asthma[324].

TNFRSF1A: tumour necrosis factor receptor superfamily.TNFRSF1A polymorphisms have been associated with coronary artery disease[325] and multiple sclerosis [326] .

IRF5: Interferon regulatory factor 5 is a regulator of Interferon activity. Polymorphisms of these genes have been linked with the pathogenesis of inflammatory bowel disorders, rheumathoid arthritis and primary biliary cirrhosis[327].

CLEC2B: C-type lectin domain family 2 member B. This gene is a myeloid specific activating receptor that binds NKp80 on natural killer (NK) cell, acting as a cross talking molecule between myeloid cells and NK cells[328] .

C3AR1: complement 3a receptor-1 present in neutrophils. Activation of this gene is implicated in the pathogenesis of inflammatory disease through de-granulation, smooth muscle contraction, arachidonic acid metabolism and cytokine release [329]

B. Molecular and cellular functions.

This category includes genes that play roles in intracellular signalling, cell to cell communication and development. Genes idenfied by eQTL that belong to this group include APC, C3AR1, IRF5, LGALS9, STAT6 and TNFRSF1A. Other genes that take part in this group are described below.

BCR: breakpoint cluster region.This gene encodes a phophodiesterase associated with serine/threonine kinase. BCR is involved in translocation 9,22 known as the Philadelphia chromosome. BCR is involved in the regulation of RAC mediated superoxide production by the NADPH-oxidase system in leukocytes. BCR null mice exposed to endotoxin develop severe septic shock and injury in diverse organs caused by enhanced neutrophil activity [330].

JMJD6: jumonji domain-containing protein 6. JMJD6 is a phosphatydil serine receptor that enables B and T cells to recognize and engulf apopototic cells in a phosphatidylserine-serine dependant manner.

MAP2K2: mitogen-activated protein kinase 2. MAP2K2 activates ERK, JNK, p38 and the NFKappa B signalling pathway [331].

PLEKHA1: pleckstrin homology domain containing protein family A. PLEKHA1 interacts with phosphodylisitol 3, 4 biphosphate. Polymorphisms in the critical region 10q 26 are associated with a 5 fold increase in susceptibility to age-related maculopathy [332].

RALBP1: associated EPS domain –containing protein 2. RALBP1 plays a role in receptor mediated endocytosis.

RASGRP4: ras guanyl nucleotide releasing protein 4. RASGRP4 encodes transduction signals through RAS; a superfamily of small GTP binding proteins.

SEMA4A: semaphoring 4 A. SEMA4A is a member of a group of soluble and transmembrane proteins that enhance priming of antigen-specific T cells. Polymorphisms of this gene have been associated with an increased susceptibility to retinitis pigmentosa and cone-rod dystrophy[333] .

SNAI3: snail drosophila homolog zinc finger protein.

LGALS2: lectin galactose-binding signalling, galactin 2. Polymorphisms of LGALS2 have been associated with an increased susceptibility to myocardial infarction.

GSTT1: gluthatione s-tranferase theta-1. GSTT1 acts as a transferase of potential toxins.

CEP152: centrosome protein that act as a major micro-tube organizer centre that influences cell shape, polarity and motility.

CIB1: calcium and integrin binding protein. CIB1 takes part in the adhesion process between megakaryocytes. CIB1 activity has been linked to cardiac hypertrophy in response to pressure overload [334].

RALBP1: associated EPS domain-containing protein 2. It intervenes in receptor mediated endocytosis[335].

CTNNA1: catenin alpha 1. CTNNA1 integrate the adhesion complex, This gene acts as a convector that anchors the e-cadherin to cytoskeletal actin bundles.

C. Physiological system development and function.

Genes involved in these functions are: APC, BCR, C3AR1, IRF5, LGALS9, MAPK2, MAPK3, PLEKA1, RALBP1, RASGRP4, SNAI3, MAPK3, CIB1, BLM, SARS2 already described above. In addition, EIF2AK1 is a major kinase that is activated by heme deficiency and other stimuli to promote haematopoiesis.

3-3 Canonical pathways

Table 3-3: Top 5 pathways 24hours after surgery

Name	P-value	Ratio
IL-3 Signaling	6.2E -04	(5/75)
Endometrial Cancer Signaling	1.50E-03	(4/60)
IL- 12 Signaling and production in Macrophages	1.94E-03	(6/157)
Role of Pattern Recognition Receptor in Recognition of Bacteria and Virus	2.40E-03	(5/109)
IL-15 Signaling	2.80E-03	(4/72)

Top Canonical Pathways. Ratio :number of molecules in the data set/number of molecules in the pathway.

IL-3 signaling pathway: IL-3 is produced by activated T cells in response to mitogens or antigens. IL-3 receptor recruits JAK2 tyrosine kinase, resulting in the activation of multiple STATs (STAT1, STAT3, STAT5 and STAT6). Furthermore, IL-3 activates signal transduction proteins such as RAS and PIK3. These activate ERK, P38 and JNK. IL-3 receptor activation mediates the activity of the BCL2 family that promotes cell survival. Interestingly, the IL-3 pathway regulates the glycolytic cycle and in BAF cells has been found to decrease glucose uptake and lead to lactate production.

IL- 12 signaling and production in macrophages: IL -12 is a pro-inflammatory cytokine produced by macrophages or activated T cells. IL-12 signaling induces the synthesis of interferon gamma (IFN-γ) that promotes the differention of T helper cells. In addition, IL-12 stimulates the production of nitric oxide that facilitates bacterial clearance. Moreover, IL12p40 or IL1-1280 monomers induce the production of tumour necrosis factor through the activation of the mitogen activated protein kinase (MAPK) and NF kappa B. At the same time IFN-Kβ up-regulates the production of IL-12p40 that in turn is inhibited by two nuclear receptors; peroxime proliferator activator receptor gamma (PPARγ) and retinoid X receptor (RXR) [336].

IL-15 signaling: IL- 15 signaling is mediated through a transmembrane heterodimer receptor complex (that contains some subunits shared by IL-2) . IL-15 stimulates JAK/STAT and NF-κB that act on NK cells and T cells to control proliferation, survival and enhance phagocytosis, thereby, playing an important role in the host inflammatory responses.

Role of Pattern recognition receptors (PRRs) in recognition of bacteria and viruses: PRRs participate in the innate immune response, recognizing bacterial cell-wall components, such as peptidoglycan and lipopolysaccharide.

PRRs are divided into three groups: 1-Toll like receptors (TLRs) activate pro-inflammatory pathways through TRAF6 and Myd88 adaptors and NF-κB.

2- Extracellular PRRs: take part in the process of phagocytosis and apoptosis triggered by the exposure of immune cells to the surface of virus and bacteria. 3-Cytoplasmic PRRs: These comprise several mediators of pro-inflammatory cytokines (NODs, NALPs and PKRs).

Endometrial Cancer Signalling: The most common histological form of endometrial cancer is adenoncarcionoma. They exist in two forms, type 1 (oestrogen and progesterone related) and Type 2 (unrelated to hormonal stimuli), those lacking or having scarce oestrogen and progesterone.

Type 1 carcinomas are associated with alteration of the PTENK-Ras genes and type 2 with mutations of the p53 gene.

Appendix 4

4-1 Tox-Integrated Pathways Analysis

This analysis is based on an integrated literature review of toxicological lists and toxicological functions . This method is mainly used in the pharmacological industry to evaluate potential toxicity of new pharmacological compounds. However, by virtue of examination of annotated pathways and gene to gene interactions integrated with clinical chemistry databases, this type of analysis has the potential to contribute to the identification possible biomarkers, generate hypothesise and link genes to the pathogenesis of specific organ injuries, such as liver cholestasis, kidney injury or cardiac damage.

Top Toxonomics List:

Top Tox List. Number of molecules in the data set/number of molecules in the pathway

Name	p-value	Ratio
Mechanism of Gene Regulation by Peroxime Proliferators via PPARα	1.36E-02	(4/95)
Xenobiotic Metabolism Signaling	5.54E-02	(7/352)
Increases Liver Nephritis	5.71E-02	(2/40)
Increases Liver Hepatitis	7.50E-02	(2/47)
NRF2-mediated Oxidative Stress Response	7.72E-02	(5/234)

PPAR signalling:

This pathway involves a family of peroxime proliferator-activated receptors that act as transcriptional regulators linked to genes that regulate the plasma levels of lipids. In addition, PPARs are involved in fatty acid metabolism, peroxisome proliferation, colon and hepatocarcinoma. PPAR activity is inhibited by TNF and IL-1 through activated NFKB and ERK , this last in response to growth factors.

NRF2-mediated oxidative stress response:

Severe oxidative stress activated by the accumulation of peroxides and free radicals can activate apoptosis and necrosis. The main molecular agent of the antioxidant response is the nuclear factor-erythroid 2-related factor 2 (Nrf2). Nrf2 circulates in an inactive form in the cytosol, being activated upon responses to cellular stress by protein kinase C and MAPK. Nrf2 travels to the nucleus and subsequently binds to antioxidant response elements (ARE). This process results in the activation of detoxifying and antioxidant enzymes such as gluthatione S-transferase, cytochrome P450, NADPH, quinone oxireductase , heme oxygenase and superoxide dismutase.

Xenobiotic Metabolism Signaling:

Xenobiotics include endogens or foreign compounds that have the particularity of activate cellular stress responses leading to cellular apoptosis and necrosis. This category includes a group of metabolizing enzymes or XME that by chemical transforming xenobiotic compounds facilitate their elimination, thereby minimizing or preventing cellular damage. They are divided into three groups Phase 1 enzymes (CYP, ALDH, FMO) introduce a polar moiety into the xenobiotics. Phase 2 enzymes (UGT, GST, SULT) by means of conjugation with diverse hydrophilic compounds (glucuronic acid, sulphate). Phase 3 enzymes (MDR, OATP2 and MPR) facilitate the transport of xenobiotics to the extracellular space. In addition, there are constitutive XME (CAR, PRX, AHR) enzymes. These defence molecules are normally inactive until stimulated by cellular stress mediated by MAPK activation of NRF2 and MAF. Interestingly, the activation of these enzymes is potentiated by their own substrates leading to enhanced cell survival.

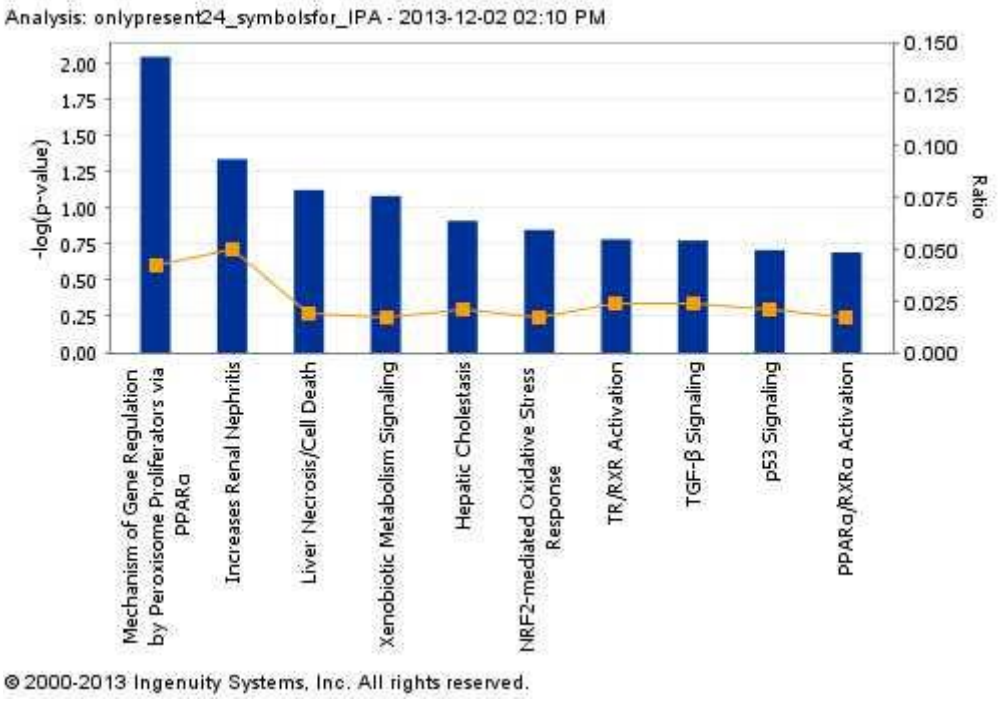


Figure 4-1 : Top tox pathways enriched for cis eQTL of the 218 genes only present 24 hours after cardiac surgery. The Y axis show the –log (pvalue) for the enrichment of each pathway. The ratio is calculated from the number of genes in the dataset divided by the number of genes in the pathway

Top toxicogenomic functions: Figure 4-1

Clinical chemistry and Haematology:

Increased Level of Alkaline:

MAPK3**:** is a mitogen-activated protein kinase that regulates ERK and JNK in addition, to NF-κB exerting anti-apoptotic actions, after being cleaved by caspases, however MAPK3 can have an anti-apoptotic effect.

Increased level of haematocrit:

JMJD6 is a histone arginine demethylase that requires the presence of Fe (++), 2 oxoglutamate and ascorbate. Interestingly, the fact that hypoxia induced factor (HIF) hydroxylase acts as a sensor of tissue hypoxia in virtue of its substrates or cofactors raises the possibility that demethylation could be regulated by metabolic or environmental changes [337].

Increased levels of red blood cells:

EIF2AK1: this gene is activated by heme deficiency in reticulocytes and its activity is triggered by nitric oxide as part of the coordination and regulation of the innate immune response by this molecule[338] .

Nephrotoxity:

SARS2: mutations in the Seryl-t RNA gene result in decreased aminoacetylation, affecting the synthesis of mitochondrial proteins and energy supply. In children polymorphisms of this gene are linked with an un-characterized multystemic mitochondrial cytopathy that leads to renal failure[339].

AGA: Deficiency of the enzyme Glucosylaparaginase leads to the accumulation of aspartylglucosamine within lysosomes. Mutations of apartylglucosamidase are associated with aspartylglycosamanuria [340].

GSTT1: This is a calmodulin regulated protein phosphate also called calcineurin B. Inhibition of calcineurin/nuclear factor of activated T cells is associated with nephrotoxicity in transplanted kidneys. The pathogenesis of this process is unclear but is perhaps linked to the calcineurin inhibitor induced fibrogenic activity of NOX2 , in association with other genetic factors predisposing to renal failure[341].

TNFRSF1A**:** TNF and TNFRSF1A mediate activation of β, C-Jun NH2 terminal kinase and mitogen activated kinase family (MAPKK) and death domain. TNFRSF1A is a receptor involved in the pathogenesis of glomerulonephritis and a negative correlation between the TNFR1 m-RNA and creatinine has been found 24 and 48 after burns injury[342].

PLEKHA1: in a recent GWAS investigating the associating between the possession of certain single nucleotide polymorphism in a population of European ancestry and glomerular filtration rate. PLEKHA1, a gene expressed in renal tubular cells was

significantly associated with renal impairment. Moreover an eQTL showed cis regulation of PLEKHA1 was associated with expression of this gene in blood and lymphocytes [343].

Trim27: This molecule integrates a group of RET finger proteins which activate several intracellular pathways such as Ras, extracellular signed regulated kinase, p38 mitogen activated protein kinase and the c-Jun N-terminal kinase pathway. In addition, RET induces GZPF1, a protein expressed in tubular cells of adult kidneys. Tubular expression of GZPF1 correlates inversely with creatinine levels in a number of chronic inflammatory kidney disorders as well to acute tubular necrosis. GZPF1 is regarded as a future candidate biomarker for renal proximal tubular damage [344].

Cardiotoxicity:

CTNNA1**:** cadherin-associated alpha. Desmosomes at the intercellular junction include molecules of the cadherin family. Polymorphisms of these genes are suspected as being involved in the pathogenesis of certain arrhythmias [345].

DIO3**:** deiodinase iodothyronine type 3 catalyses the conversion of T4 to T3 and to inactive metabolites. Heart failure is associated with T3 signalling disturbances. In rodents, re-expression of D3 has been found in pathological ventricular hypertrophy. Interestingly, whilst hypoxia induces D3 signalling [346].

STAT6: This molecule is a transducer and transcriptor induced by IL-13 and whose effectors are P and E selectin. The activation of STAT6 is considered as evidence of the inflammatory character of the atherosclerotic process that leads to coronary disease [347, 348].

TNFRF1A**:** TNF and TNF receptors activate signalling pathways that control inflammatory, immune and stress responses, in addition to host defence and apoptosis. In a group of 28 elderly patients suffering from heart failure, TNFRF1A was one of 22 genes differentially expressed in peripheral mononuclear cells (PBMC) in comparison with age matched healthy controls. Interestingly, differentially expressed genes linked with inflammation were still present in the recovery phase[349]

.

PPP3R1**:** Protein phosphate 3 is a calcineurin. Calcineurins are implicated in the pathogenesis of myocardial remodelling after infarction[350].

JMJD6**:**This gene acts as a phosphatidylserine receptor (PSR) that when transfected into B and T lymphocytes, enable them to detect and phagocytose apoptotic cells .In rodents, PSRs have been identified as one of the key molecules in the pathogenesis of cardiac development and congenital heart malformations [351].

CRELD1**:** This gene encodes a matricellular protein considered a putative cell adhesion molecule. Allelic variants of this gene have been linked to atrioventricular septal defect [352].

Table 4-1: Top toxicogenomic functions. The right hand side of the table shows the number of genes in the dataset that associates with each function.

TOP TOX FUNCTIONS		
Assay Clinical Chemistry and Haematology	**p-value**	Molecules
Name		
Increased Levels of Alkaline Phosphatase	4.89E-0.1-4.8E-01	1
Increased Levels of hematocrit	6.1E-01-6.1E-01	1
Increased Levels of Red Blood Cells	6.1E-01-6.1E-01	1
Cardiotoxity		
Name		
Cardiac Damage	9.6E-03-9.6-03	1
Cardiac Fibrosis	9.6E-03-3.8E-02	2
Congenital Heart Anomaly	9.6E-03-4.3E-01	2
Pulmonary Hypertension	9.6E-03-3.0E-01	2
Cardiac Inflammation	6.2E-02-6.22E-02	2
Hepatotoxicity		
Name		
Liver Inflammation/Hepatitis	1.0E-03-2.6E-01	7
Liver Necrosis/Cell Death	1.0E-02-1.4E-01	5
Liver Damage	1.8E-02-3.3E-0.1	2
Liver Hyperplasia/Hyperproliferation	2.8E-0.2-1.0E00	6
Liver Steatosis	2.8E-02-2.1E-01	5
Nephrotoxicity		
Name		
Kidney Failure	9.6E-03-4.0E-01	5
Glomerular Injury	7.8E-02-7.8E-02	2
Renal Fibrosis	7.86E-02-7.8-02	2
Renal Necrosis/Cell Death	1.0E-01-1.0E-01	5
Renal Atrophy	1.9E-0.1-1.9E-01	1

1. Hirai S. Systemic inflammatory response syndrome after cardiac surgery under cardiopulmonary bypass. Ann Thorac Cardiovasc Surg 2003;9:365-70.
2. Miller BE, Levy JH. The inflammatory response to cardiopulmonary bypass. J Cardiothorac Vasc Anesth 1997;11:355-66.
3. Laffey J, Boylan J, Cheng D. The systemic inflammatory response to cardiac surgery. Anaesthesiology 2002;97:215-52.
4. Filsoufi F, Rahmanian PB, Castillo JG, Silvay G, Carpentier A, Adams DH. Predictors and early and late outcomes of dialysis-dependent patients in contemporary cardiac surgery. J Cardiothorac Vasc Anesth 2008;22:522-9.
5. Bridgewater B, Grayson AD, Brooks N, et al. Has the publication of cardiac surgery outcome data been associated with changes in practice in northwest England: an analysis of 25,730 patients undergoing CABG surgery under 30 surgeons over eight years. Heart 2007;93:744-8.
6. Yates RB, Stafford-Smith M. The genetic determinants of renal impairment following cardiac surgery. Semin Cardiothorac Vasc Anesth 2006;10:314-26.
7. Fox AA, Shernan SK, Body SC. Predictive genomics of adverse events after cardiac surgery. Semin Cardiothorac Vasc Anesth 2004;8:297-315.
8. Taggart DP. Thomas B. Ferguson Lecture. Coronary artery bypass grafting is still the best treatment for multivessel and left main disease, but patients need to know. Ann Thorac Surg 2006;82:1966-75.
9. Laffey JG, Boylan JF, Cheng DC. The systemic inflammatory response to cardiac surgery: implications for the anesthesiologist. Anesthesiology 2002;97:215-52.
10. Rady MY, Ryan T, Starr NJ. Early onset of acute pulmonary dysfunction after cardiovascular surgery: risk factors and clinical outcome. Crit Care Med 1997;25:1831-9.
11. Yates RB, Stafford-Smith M. The genetic determinants of renal impairment following cardiac surgery. Seminars in Cardiothoracic and Vacular Anaesthesia 2006:314-26.
12. Asimakopoulos G, Smith P L, Ratnatunga C P, Taylor K M. Lung Injury and Acute Respiratory Distress Syndrome after cardiopulmonary bypass. Ann Thoracic Surgery 1999;68:1107-15.
13. Lan Gao RT, Dominique Gauvin, Lamia B. Othmen, Yang Wang, Gilbert Blaise. Postoperative Cognitive Dysfunction After Cardiac Surgery. Chest 2005:3664-70.
14. Hadley JS, Wang JE, Michaels LC, et al. Alterations in inflammatory capacity and TLR expression on monocytes and neutrophils after cardiopulmonary bypass. Shock 2007;27:466-73.
15. Herskowitz A, Mangano DT. Inflammatory cascade. A final common pathway for perioperative injury? Anesthesiology 1996;85:957-60.
16. Royston D. The inflammatory response and extracorporeal circulation. J Cardiothorac Vasc Anesth 1997;11:341-54.
17. Calvano SE, Xiao W, Richards DR, et al. A network-based analysis of systemic inflammation in humans. Nature 2005;437:1032-7.
18. Calvano S, Xiao W, Richards D, Felciano R M, Baker H V, Cho R J, Chen R O, Brownstein B H, Cobb P J, Tompkins R G, Lowry S F and the inflammation and host response to injury large scale collaborative research program. A network-based analysis of systemic inflammation in humans. Nature 2005;437:1032-7.
19. consortium TEP. Identification and analysis of functional elements in 1% of the human genome by ENCODE pilot project. Nature 2007;447:799-816.
20. Schwinn DA, Podgoreanu M. Pharmacogenomics and end-organ susceptibility to injury in the perioperative period. Best Pract Res Clin Anaesthesiol 2008;22:23-37.
21. Podgoreanu MV, Schwinn DA. New paradigms in cardiovascular medicine: emerging technologies and practices: perioperative genomics. J Am Coll Cardiol 2005;46:1965-77.

22. Bone RC, Sibbald WJ, Sprung CL. The ACCP-SCCM consensus conference on sepsis and organ failure. Chest 1992;101:1481-3.
23. Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP. The natural history of the systemic inflammatory response syndrome (SIRS). A prospective study. JAMA 1995;273:117-23.
24. Rinder C. Cellular inflammatory response and clinical outcome in cardiac surgery. Curr Opin Anaesthesiol 2006;19:65-8.
25. Rossi M, Sganga G, Mazzone M, et al. Cardiopulmonary bypass in man: role of the intestine in a self-limiting inflammatory response with demonstrable bacterial translocation. Ann Thorac Surg 2004;77:612-8.
26. Brull DJ, Montgomery HE, Sanders J, et al. Interleukin-6 gene -174g>c and -572g>c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler Thromb Vasc Biol 2001;21:1458-63.
27. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med 2001;345:1359-67.
28. Marik PE, Preiser JC. Toward understanding tight glycemic control in the ICU: a systematic review and metaanalysis. Chest;137:544-51.
29. Quartin AA, Schein RM, Kett DH, Peduzzi PN. Magnitude and duration of the effect of sepsis on survival. Department of Veterans Affairs Systemic Sepsis Cooperative Studies Group. JAMA 1997;277:1058-63.
30. Duggan E, Caraher E, Gately K, et al. Tumor necrosis factor-alpha and interleukin-10 gene expression in peripheral blood mononuclear cells after cardiac surgery. Crit Care Med 2006;34:2134-9.
31. Roth-Isigkeit A, Hasselbach L, Ocklitz E, et al. Inter-individual differences in cytokine release in patients undergoing cardiac surgery with cardiopulmonary bypass. Clin Exp Immunol 2001;125:80-8.
32. Schraut W, Wendelgass P, Calzada-Wack JC, Frankenberger M, Ziegler-Heitbrock HW. TNF gene expression in monocytes of low and high responder individuals. Cytokine 1997;9:206-11.
33. Gessler P, Pretre R, Burki C, Rousson V, Frey B, Nadal D. Monocyte function-associated antigen expression during and after pediatric cardiac surgery. J Thorac Cardiovasc Surg 2005;130:54-60.
34. Tomic V, Russwurm S, Moller E, et al. Transcriptomic and proteomic patterns of systemic inflammation in on-pump and off-pump coronary artery bypass grafting. Circulation 2005;112:2912-20.
35. Conlon PJ, Procop GW, Fowler V, Eloubeidi MA, Smith SR, Sexton DJ. Predictors of prognosis and risk of acute renal failure in patients with Rocky Mountain spotted fever. Am J Med 1996;101:621-6.
36. Mangano CM, Diamondstone LS, Ramsay JG, Aggarwal A, Herskowitz A, Mangano DT. Renal dysfunction after myocardial revascularization: risk factors, adverse outcomes, and hospital resource utilization. The Multicenter Study of Perioperative Ischemia Research Group. Ann Intern Med 1998;128:194-203.
37. Funder KS, Steinmetz J, Rasmussen LS. Cognitive dysfunction after cardiovascular surgery. Minerva Anestesiol 2009;75:329-32.
38. Van Dijk D, Jansen EW, Hijman R, et al. Cognitive outcome after off-pump and on-pump coronary artery bypass graft surgery: a randomized trial. JAMA 2002;287:1405-12.
39. Evered L, Scott DA, Silbert B, Maruff P. Postoperative cognitive dysfunction is independent of type of surgery and anesthetic. Anesth Analg;112:1179-85.
40. Weerasinghe A, Taylor KM. The platelet in cardiopulmonary bypass. Ann Thorac Surg 1998;66:2145-52.
41. Karkouti K, Wijeysundera DN, Yau TM, et al. The independent association of massive blood loss with mortality in cardiac surgery. Transfusion 2004;44:1453-62.

42. Hall TS, Brevetti GR, Skoultchi AJ, Sines JC, Gregory P, Spotnitz AJ. Re-exploration for hemorrhage following open heart surgery differentiation on the causes of bleeding and the impact on patient outcomes. Ann Thorac Cardiovasc Surg 2001;7:352-7.
43. Welsby IJ, Podgoreanu MV, Phillips-Bute B, et al. Association of the 98T ELAM-1 polymorphism with increased bleeding after cardiac surgery. J Cardiothorac Vasc Anesth;24:427-33.
44. De Somer F, Van Belleghem Y, Caes F, et al. Tissue factor as the main activator of the coagulation system during cardiopulmonary bypass. J Thorac Cardiovasc Surg 2002;123:951-8.
45. Neumann FJ, Ott I, Marx N, et al. Effect of human recombinant interleukin-6 and interleukin-8 on monocyte procoagulant activity. Arterioscler Thromb Vasc Biol 1997;17:3399-405.
46. Lo B, Fijnheer R, Nierich AP, Bruins P, Kalkman CJ. C-reactive protein is a risk indicator for atrial fibrillation after myocardial revascularization. Ann Thorac Surg 2005;79:1530-5.
47. Ishii Y, Schuessler RB, Gaynor SL, et al. Inflammation of atrium after cardiac surgery is associated with inhomogeneity of atrial conduction and atrial fibrillation. Circulation 2005;111:2881-8.
48. Apostolakis E, Filos KS, Koletsis E, Dougenis D. Lung dysfunction following cardiopulmonary bypass. J Card Surg;25:47-55.
49. Hegreness M, Meselson M. What did Sutton see? Thirty years of confusion over the chromosomal basis of Mendelism. Genetics 2007;176:1939-44.
50. Hotchkiss RD. Oswald T. Avery: 1877-1955. Genetics 1965;51:1-10.
51. Watson JD, Crick FH. Genetical implications of the structure of deoxyribonucleic acid. Nature 1953;171:964-7.
52. Sanger F, Air GM, Barrell BG, et al. Nucleotide sequence of bacteriophage phi X174 DNA. Nature 1977;265:687-95.
53. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988;239:487-91.
54. Zhou W, Wang X, Rosenfeld MG. Histone H2A ubiquitination in transcriptional regulation and DNA damage repair. Int J Biochem Cell Biol 2009;41:12-5.
55. Birney E, Stamatoyannopoulos JA, Dutta A, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007;447:799-816.
56. An integrated encyclopedia of DNA elements in the human genome. Nature;489:57-74.
57. Badano JL, Katsanis N. Beyond Mendel: an evolving view of human genetic disease transmission. Nat Rev Genet 2002;3:779-89.
58. Risch NJ. Searching for genetic determinants in the new millennium. Nature 2000;405:847-56.
59. McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 2008;9:356-69.
60. Gilad Y, Rifkin SA, Pritchard JK. Revealing the architecture of gene regulation: the promise of eQTL studies. Trends Genet 2008;24:408-15.
61. Evangelou E, Ioannidis JP. Meta-analysis methods for genome-wide association studies and beyond. Nat Rev Genet;14:379-89.
62. Ioannidis JP, Thomas G, Daly MJ. Validating, augmenting and refining genome-wide association signals. Nat Rev Genet 2009;10:318-29.
63. Gaudino M, Di Castelnuovo A, Zamparelli R, et al. Genetic control of postoperative systemic inflammatory reaction and pulmonary and renal complications after coronary artery surgery. J Thorac Cardiovasc Surg 2003;126:1107-12.
64. Stafford-Smith M, Podgoreanu M, Swaminathan M, et al. Association of genetic polymorphisms with risk of renal injury after coronary bypass graft surgery. Am J Kidney Dis 2005;45:519-30.
65. MacKensen GB, Swaminathan M, Ti LK, et al. Preliminary report on the interaction of apolipoprotein E polymorphism with aortic atherosclerosis and acute nephropathy after CABG. Ann Thorac Surg 2004;78:520-6.

66. Enger TB, Pleym H, Stenseth R, Wahba A, Videm V. Genetic and clinical risk factors for fluid overload following open-heart surgery. Acta Anaesthesiol Scand;58:539-48.
67. Philip I, Plantefeve G, Vuillaumier-Barrot S, et al. G894T polymorphism in the endothelial nitric oxide synthase gene is associated with an enhanced vascular responsiveness to phenylephrine. Circulation 1999;99:3096-8.
68. Stafford-Smith M, Li YJ, Mathew JP, et al. Genome-wide association study of acute kidney injury after coronary bypass graft surgery identifies susceptibility loci. Kidney Int;88:823-32.
69. Grocott HP. Perioperative genomics and neurologic outcome: we can't change who we are. Can J Anaesth 2009;56:562-6.
70. Mathew JP, Rinder CS, Howe JG, et al. Platelet PlA2 polymorphism enhances risk of neurocognitive decline after cardiopulmonary bypass. Multicenter Study of Perioperative Ischemia (McSPI) Research Group. Ann Thorac Surg 2001;71:663-6.
71. Tardiff BE, Newman MF, Saunders AM, et al. Preliminary report of a genetic basis for cognitive decline after cardiac operations. The Neurologic Outcome Research Group of the Duke Heart Center. Ann Thorac Surg 1997;64:715-20.
72. Silbert BS, Evered LA, Scott DA, Cowie TF. The apolipoprotein E epsilon4 allele is not associated with cognitive dysfunction in cardiac surgery. Ann Thorac Surg 2008;86:841-7.
73. Gaynor JW, Gerdes M, Zackai EH, et al. Apolipoprotein E genotype and neurodevelopmental sequelae of infant cardiac surgery. J Thorac Cardiovasc Surg 2003;126:1736-45.
74. Mathew JP, Podgoreanu MV, Grocott HP, et al. Genetic variants in P-selectin and C-reactive protein influence susceptibility to cognitive decline after cardiac surgery. J Am Coll Cardiol 2007;49:1934-42.
75. Grocott HP, White WD, Morris RW, et al. Genetic polymorphisms and the risk of stroke after cardiac surgery. Stroke 2005;36:1854-8.
76. Tomasdottir H, Hjartarson H, Ricksten A, Wasslavik C, Bengtsson A, Ricksten SE. Tumor necrosis factor gene polymorphism is associated with enhanced systemic inflammatory response and increased cardiopulmonary morbidity after cardiac surgery. Anesth Analg 2003;97:944-9, table of contents.
77. Welsby IJ, Podgoreanu MV, Phillips-Bute B, et al. Genetic factors contribute to bleeding after cardiac surgery. J Thromb Haemost 2005;3:1206-12.
78. Donahue BS, Gailani D, Higgins MS, Drinkwater DC, George AL, Jr. Factor V Leiden protects against blood loss and transfusion after cardiac surgery. Circulation 2003;107:1003-8.
79. Body SC, Collard CD, Shernan SK, et al. Variation in the 4q25 chromosomal locus predicts atrial fibrillation after coronary artery bypass graft surgery. Circ Cardiovasc Genet 2009;2:499-506.
80. Gaudino M, Andreotti F, Zamparelli R, et al. The -174G/C interleukin-6 polymorphism influences postoperative interleukin-6 levels and postoperative atrial fibrillation. Is atrial fibrillation an inflammatory complication? Circulation 2003;108 Suppl 1:II195-9.
81. Kelberman D, Fife M, Rockman MV, Brull DJ, Woo P, Humphries SE. Analysis of common IL-6 promoter SNP variants and the AnTn tract in humans and primates and effects on plasma IL-6 levels following coronary artery bypass graft surgery. Biochim Biophys Acta 2004;1688:160-7.
82. Pankow JS, Folsom AR, Cushman M, et al. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. Atherosclerosis 2001;154:681-9.
83. Podgoreanu MV, White WD, Morris RW, et al. Inflammatory gene polymorphisms and risk of postoperative myocardial infarction after cardiac surgery. Circulation 2006;114:I275-81.
84. Yoshida M, Takano Y, Sasaoka T, Izumi T, Kimura A. E-selectin polymorphism associated with myocardial infarction causes enhanced leukocyte-endothelial interactions under flow conditions. Arterioscler Thromb Vasc Biol 2003;23:783-8.
85. Wenzel K, Baumann G, Felix SB. The homozygous combination of Leu125Val and Ser563Asn polymorphisms in the PECAM1 (CD31) gene is associated with early severe coronary heart disease. Hum Mutat 1999;14:545.

86. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 1996;42:1589-600.
87. Delanghe JR, Duprez DA, De Buyzere ML, et al. Haptoglobin polymorphism and complications in established essential arterial hypertension. J Hypertens 1993;11:861-7.
88. Delanghe J, Cambier B, Langlois M, et al. Haptoglobin polymorphism, a genetic risk factor in coronary artery bypass surgery. Atherosclerosis 1997;132:215-9.
89. Volzke H, Engel J, Kleine V, et al. Angiotensin I-converting enzyme insertion/deletion polymorphism and cardiac mortality and morbidity after coronary artery bypass graft surgery. Chest 2002;122:31-6.
90. O'Donohoe MK, Davies MG, Radic ZS, Mikat EM, Hagen PO. Increased concentrations of angiotensin-converting enzyme in the intimal hyperplasia of experimental vein grafts. J Cardiovasc Pharmacol 1994;23:594-601.
91. Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 1991;324:1149-55.
92. Botto N, Andreassi MG, Rizza A, et al. C677T polymorphism of the methylenetetrahydrofolate reductase gene is a risk factor of adverse events after coronary revascularization. Int J Cardiol 2004;96:341-5.
93. Chen Z, Karaplis AC, Ackerman SL, et al. Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. Hum Mol Genet 2001;10:433-43.
94. Fuster V, Vorchheimer DA. Prevention of atherosclerosis in coronary-artery bypass grafts. N Engl J Med 1997;336:212-3.
95. Weiss EJ, Bray PF, Tayback M, et al. A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med 1996;334:1090-4.
96. Zotz RB, Klein M, Dauben HP, Moser C, Gams E, Scharf RE. Prospective analysis after coronary-artery bypass grafting: platelet GP IIIa polymorphism (HPA-1b/PIA2) is a risk factor for bypass occlusion, myocardial infarction, and death. Thromb Haemost 2000;83:404-7.
97. Fox AA, Pretorius M, Liu KY, et al. Genome-wide assessment for genetic variants associated with ventricular dysfunction after primary coronary artery bypass graft surgery. PLoS One;6:e24593.
98. Sorensen TI, Nielsen GG, Andersen PK, Teasdale TW. Genetic and environmental influences on premature death in adult adoptees. N Engl J Med 1988;318:727-32.
99. Knight JC. Human genetic diversity : functional consequences for health and disease. Oxford ; New York: Oxford University Press; 2009.
100. Cooke GS, Hill AV. Genetics of susceptibility to human infectious disease. Nat Rev Genet 2001;2:967-77.
101. Williams TN. Human red blood cell polymorphisms and malaria. Curr Opin Microbiol 2006;9:388-94.
102. Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria. Am J Hum Genet 2005;77:171-92.
103. Alter A, Alcais A, Abel L, Schurr E. Leprosy as a genetic model for susceptibility to common infectious diseases. Hum Genet 2008;123:227-35.
104. Mira MT, Alcais A, Nguyen VT, et al. Susceptibility to leprosy is associated with PARK2 and PACRG. Nature 2004;427:636-40.
105. Alcais A, Mira M, Casanova JL, Schurr E, Abel L. Genetic dissection of immunity in leprosy. Curr Opin Immunol 2005;17:44-8.
106. Alcais A, Alter A, Antoni G, et al. Stepwise replication identifies a low-producing lymphotoxin-alpha allele as a major risk factor for early-onset leprosy. Nat Genet 2007;39:517-22.
107. Chatterjee K. Host genetic factors in susceptibility to HIV-1 infection and progression to AIDS. J Genet;89:109-16.
108. Kaur G, Mehra N. Genetic determinants of HIV-1 infection and progression to AIDS: susceptibility to HIV infection. Tissue Antigens 2009;73:289-301.

109. Samson M, Libert F, Doranz BJ, et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 1996;382:722-5.
110. Carrington M, Nelson G, O'Brien SJ. Considering genetic profiles in functional studies of immune responsiveness to HIV-1. Immunol Lett 2001;79:131-40.
111. Garcia-Arriaza J, Najera JL, Gomez CE, et al. A Candidate HIV/AIDS Vaccine (MVA-B) Lacking Vaccinia Virus Gene C6L Enhances Memory HIV-1-Specific T-Cell Responses. PLoS One;6:e24244.
112. Lin MT, Albertson TE. Genomic polymorphisms in sepsis. Crit Care Med 2004;32:569-79.
113. Strieter RM, Kunkel SL, Bone RC. Role of tumor necrosis factor-alpha in disease states and inflammation. Crit Care Med 1993;21:S447-63.
114. Cobb JP, Danner RL. Nitric oxide and septic shock. JAMA 1996;275:1192-6.
115. Stuber F, Petersen M, Bokelmann F, Schade U. A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-alpha concentrations and outcome of patients with severe sepsis. Crit Care Med 1996;24:381-4.
116. Mira JP, Cariou A, Grall F, et al. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. JAMA 1999;282:561-8.
117. Tang GJ, Huang SL, Yien HW, et al. Tumor necrosis factor gene polymorphism and septic shock in surgical infection. Crit Care Med 2000;28:2733-6.
118. Gordon AC, Lagan AL, Aganna E, et al. TNF and TNFR polymorphisms in severe sepsis and septic shock: a prospective multicentre study. Genes Immun 2004;5:631-40.
119. Menges T, Konig IR, Hossain H, et al. Sepsis syndrome and death in trauma patients are associated with variation in the gene encoding tumor necrosis factor. Crit Care Med 2008;36:1456-62, e1-6.
120. Gordon A, Knight JC, Hinds CJ. Genes and sepsis: how tight is the fit? Crit Care Med 2008;36:1652-4.
121. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 1997;94:3195-9.
122. Brinkman BM, Zuijdeest D, Kaijzel EL, Breedveld FC, Verweij CL. Relevance of the tumor necrosis factor alpha (TNF alpha) -308 promoter polymorphism in TNF alpha gene regulation. J Inflamm 1995;46:32-41.
123. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. Scand J Immunol 1998;47:195-8.
124. Fang XM, Schroder S, Hoeft A, Stuber F. Comparison of two polymorphisms of the interleukin-1 gene family: interleukin-1 receptor antagonist polymorphism contributes to susceptibility to severe sepsis. Crit Care Med 1999;27:1330-4.
125. Ma P, Chen D, Pan J, Du B. [Genomic polymorphism within interleukin-1 family cytokines influences the outcome of septic patients]. Zhonghua Yi Xue Za Zhi 2002;82:1237-41.
126. Arnalich F, Lopez-Maderuelo D, Codoceo R, et al. Interleukin-1 receptor antagonist gene polymorphism and mortality in patients with severe sepsis. Clin Exp Immunol 2002;127:331-6.
127. Opal SM, Fisher CJ, Jr., Dhainaut JF, et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. Crit Care Med 1997;25:1115-24.
128. Casey LC, Balk RA, Bone RC. Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. Ann Intern Med 1993;119:771-8.
129. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369-76.
130. Heesen M, Bloemeke B, Heussen N, Kunz D. Can the interleukin-6 response to endotoxin be predicted? Studies of the influence of a promoter polymorphism of the interleukin-6 gene, gender, the density of the endotoxin receptor CD14, and inflammatory cytokines. Crit Care Med 2002;30:664-9.

131. Boehm U, Klamp T, Groot M, Howard JC. Cellular responses to interferon-gamma. Annu Rev Immunol 1997;15:749-95.
132. Stassen NA, Leslie-Norfleet LA, Robertson AM, Eichenberger MR, Polk HC, Jr. Interferon-gamma gene polymorphisms and the development of sepsis in patients with trauma. Surgery 2002;132:289-92.
133. Cheadle WG, Hershman MJ, Wellhausen SR, Polk HC, Jr. HLA-DR antigen expression on peripheral blood monocytes correlates with surgical infection. Am J Surg 1991;161:639-45.
134. Joram N, Lopez E, Texereau J, Mira JP. [Genetic polymorphisms and infections]. Med Mal Infect 2006;36:314-21.
135. Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet 2000;25:187-91.
136. Lorenz E, Mira JP, Frees KL, Schwartz DA. Relevance of mutations in the TLR4 receptor in patients with gram-negative septic shock. Arch Intern Med 2002;162:1028-32.
137. Read RC, Pullin J, Gregory S, et al. A functional polymorphism of toll-like receptor 4 is not associated with likelihood or severity of meningococcal disease. J Infect Dis 2001;184:640-2.
138. Shalhub S, Junker CE, Imahara SD, Mindrinos MN, Dissanaike S, O'Keefe GE. Variation in the TLR4 gene influences the risk of organ failure and shock posttrauma: a cohort study. J Trauma 2009;66:115-22; discussion 22-3.
139. Lorenz E, Mira JP, Cornish KL, Arbour NC, Schwartz DA. A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. Infect Immun 2000;68:6398-401.
140. Ferwerda B, Alonso S, Banahan K, et al. Functional and genetic evidence that the Mal/TIRAP allele variant 180L has been selected by providing protection against septic shock. Proc Natl Acad Sci U S A 2009;106:10272-7.
141. Hawn TR, Verbon A, Janer M, Zhao LP, Beutler B, Aderem A. Toll-like receptor 4 polymorphisms are associated with resistance to Legionnaires' disease. Proc Natl Acad Sci U S A 2005;102:2487-9.
142. Khor CC, Chapman SJ, Vannberg FO, et al. A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. Nat Genet 2007;39:523-8.
143. Kumpf O, Giamarellos-Bourboulis EJ, Koch A, et al. Influence of genetic variations in TLR4 and TIRAP/Mal on the course of sepsis and pneumonia and cytokine release: an observational study in three cohorts. Crit Care;14:R103.
144. Gordon AC, Waheed U, Hansen TK, et al. Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence, and outcome. Shock 2006;25:88-93.
145. Summerfield JA, Ryder S, Sumiya M, et al. Mannose binding protein gene mutations associated with unusual and severe infections in adults. Lancet 1995;345:886-9.
146. Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. Lancet 2001;358:614-8.
147. Peterslund NA, Koch C, Jensenius JC, Thiel S. Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. Lancet 2001;358:637-8.
148. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Meningococcal Research Group. Lancet 1999;353:1049-53.
149. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. Am J Respir Cell Mol Biol 1999;20:976-83.
150. Michalek J, Svetlikova P, Fedora M, et al. Bactericidal permeability increasing protein gene variants in children with sepsis. Intensive Care Med 2007;33:2158-64.

151. Hubacek JA, Stuber F, Frohlich D, et al. Gene variants of the bactericidal/permeability increasing protein and lipopolysaccharide binding protein in sepsis patients: gender-specific genetic predisposition to sepsis. Crit Care Med 2001;29:557-61.
152. Gibot S, Cariou A, Drouet L, Rossignol M, Ripoll L. Association between a genomic polymorphism within the CD14 locus and septic shock susceptibility and mortality rate. Crit Care Med 2002;30:969-73.
153. Hubacek JA, Stuber F, Frohlich D, et al. The common functional C(-159)T polymorphism within the promoter region of the lipopolysaccharide receptor CD14 is not associated with sepsis development or mortality. Genes Immun 2000;1:405-7.
154. Astiz M, Saha D, Lustbader D, Lin R, Rackow E. Monocyte response to bacterial toxins, expression of cell surface receptors, and release of anti-inflammatory cytokines during sepsis. J Lab Clin Med 1996;128:594-600.
155. Platonov AE, Shipulin GA, Vershinina IV, Dankert J, van de Winkel JG, Kuijper EJ. Association of human Fc gamma RIIa (CD32) polymorphism with susceptibility to and severity of meningococcal disease. Clin Infect Dis 1998;27:746-50.
156. van der Pol WL, Huizinga TW, Vidarsson G, et al. Relevance of Fcgamma receptor and interleukin-10 polymorphisms for meningococcal disease. J Infect Dis 2001;184:1548-55.
157. Kee C, Cheong KY, Pham K, Waterer GW, Temple SE. Genetic variation in heat shock protein 70 is associated with septic shock: narrowing the association to a specific haplotype. Int J Immunogenet 2008;35:465-73.
158. Temple SE, Cheong KY, Ardlie KG, Sayer D, Waterer GW. The septic shock associated HSPA1B1267 polymorphism influences production of HSPA1A and HSPA1B. Intensive Care Med 2004;30:1761-7.
159. Bruemmer-Smith S, Stuber F, Schroeder S. Protective functions of intracellular heat-shock protein (HSP) 70-expression in patients with severe sepsis. Intensive Care Med 2001;27:1835-41.
160. Waterer GW, ElBahlawan L, Quasney MW, Zhang Q, Kessler LA, Wunderink RG. Heat shock protein 70-2+1267 AA homozygotes have an increased risk of septic shock in adults with community-acquired pneumonia. Crit Care Med 2003;31:1367-72.
161. Eriksson P, Kallin B, van 't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci U S A 1995;92:1851-5.
162. Menges T, Hermans PW, Little SG, et al. Plasminogen-activator-inhibitor-1 4G/5G promoter polymorphism and prognosis of severely injured patients. Lancet 2001;357:1096-7.
163. Hermans PW, Hibberd ML, Booy R, et al. 4G/5G promoter polymorphism in the plasminogen-activator-inhibitor-1 gene and outcome of meningococcal disease. Meningococcal Research Group. Lancet 1999;354:556-60.
164. Westendorp RG, Hottenga JJ, Slagboom PE. Variation in plasminogen-activator-inhibitor-1 gene and risk of meningococcal septic shock. Lancet 1999;354:561-3.
165. Jessen KM, Lindboe SB, Petersen AL, Eugen-Olsen J, Benfield T. Common TNF-alpha, IL-1 beta, PAI-1, uPA, CD14 and TLR4 polymorphisms are not associated with disease severity or outcome from Gram negative sepsis. BMC Infect Dis 2007;7:108.
166. Russell JA. Management of sepsis. N Engl J Med 2006;355:1699-713.
167. Mosnier LO, Zlokovic BV, Griffin JH. The cytoprotective protein C pathway. Blood 2007;109:3161-72.
168. Fisher CJ, Jr., Yan SB. Protein C levels as a prognostic indicator of outcome in sepsis and related diseases. Crit Care Med 2000;28:S49-56.
169. Walley KR, Russell JA. Protein C -1641 AA is associated with decreased survival and more organ dysfunction in severe sepsis. Crit Care Med 2007;35:12-7.
170. Binder A, Endler G, Rieger S, et al. Protein C promoter polymorphisms associate with sepsis in children with systemic meningococcemia. Hum Genet 2007;122:183-90.

171. Chen QX, Wu SJ, Wang HH, et al. Protein C -1641A/-1654C haplotype is associated with organ dysfunction and the fatal outcome of severe sepsis in Chinese Han population. Hum Genet 2008;123:281-7.
172. Russell JA, Wellman H, Walley KR. Protein C rs2069912 C allele is associated with increased mortality from severe sepsis in North Americans of East Asian ancestry. Hum Genet 2008;123:661-3.
173. Costerousse O, Allegrini J, Lopez M, Alhenc-Gelas F. Angiotensin I-converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes. Biochem J 1993;290 (Pt 1):33-40.
174. Marshall RP, Webb S, Bellingan GJ, et al. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. Am J Respir Crit Care Med 2002;166:646-50.
175. Villar J, Flores C, Perez-Mendez L, et al. Angiotensin-converting enzyme insertion/deletion polymorphism is not associated with susceptibility and outcome in sepsis and acute respiratory distress syndrome. Intensive Care Med 2008;34:488-95.
176. Haldane JBS. The Time of Action of Genes, and Its Bearing on some Evolutionary Problems. The American Naturalist 1932;66:5-24.
177. Rockman MV, Kruglyak L. Genetics of global gene expression. Nat Rev Genet 2006;7:862-72.
178. Goring HH, Curran JE, Johnson MP, et al. Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. Nat Genet 2007;39:1208-16.
179. Tang BM, Huang SJ, McLean AS. Genome-wide transcription profiling of human sepsis: a systematic review. Crit Care;14:R237.
180. Talwar S, Munson PJ, Barb J, et al. Gene expression profiles of peripheral blood leukocytes after endotoxin challenge in humans. Physiol Genomics 2006;25:203-15.
181. Prucha M, Ruryk A, Boriss H, et al. Expression profiling: toward an application in sepsis diagnostics. Shock 2004;22:29-33.
182. Cobb JP, Moore EE, Hayden DL, et al. Validation of the riboleukogram to detect ventilator-associated pneumonia after severe injury. Ann Surg 2009;250:531-9.
183. Johnson SB, Lissauer M, Bochicchio GV, Moore R, Cross AS, Scalea TM. Gene expression profiles differentiate between sterile SIRS and early sepsis. Ann Surg 2007;245:611-21.
184. Lissauer ME, Johnson SB, Bochicchio GV, et al. Differential expression of toll-like receptor genes: sepsis compared with sterile inflammation 1 day before sepsis diagnosis. Shock 2009;31:238-44.
185. Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. Lancet 2007;370:657-66.
186. Singer M, De Santis V, Vitale D, Jeffcoate W. Multiorgan failure is an adaptive, endocrine-mediated, metabolic response to overwhelming systemic inflammation. Lancet 2004;364:545-8.
187. Tang BM, McLean AS, Dawes IW, Huang SJ, Lin RC. Gene-expression profiling of peripheral blood mononuclear cells in sepsis. Crit Care Med 2009;37:882-8.
188. Giroir BP, Johnson JH, Brown T, Allen GL, Beutler B. The tissue distribution of tumor necrosis factor biosynthesis during endotoxemia. J Clin Invest 1992;90:693-8.
189. Payen D, Lukaszewicz AC, Belikova I, et al. Gene profiling in human blood leucocytes during recovery from septic shock. Intensive Care Med 2008;34:1371-6.
190. Pugin J. Profiling genes in the ICU: are we there yet? Intensive Care Med 2008;34:1357-9.
191. Monneret G, Finck ME, Venet F, et al. The anti-inflammatory response dominates after septic shock: association of low monocyte HLA-DR expression and high interleukin-10 concentration. Immunol Lett 2004;95:193-8.
192. Pachot A, Lepape A, Vey S, Bienvenu J, Mougin B, Monneret G. Systemic transcriptional analysis in survivor and non-survivor septic shock patients: a preliminary study. Immunol Lett 2006;106:63-71.

193. Wong HR, Shanley TP, Sakthivel B, et al. Genome-level expression profiles in pediatric septic shock indicate a role for altered zinc homeostasis in poor outcome. Physiol Genomics 2007;30:146-55.
194. Waelput W, Broekaert D, Vandekerckhove J, Brouckaert P, Tavernier J, Libert C. A mediator role for metallothionein in tumor necrosis factor-induced lethal shock. J Exp Med 2001;194:1617-24.
195. Wong HR, Cvijanovich NZ, Allen GL, et al. Validation of a gene expression-based subclassification strategy for pediatric septic shock. Crit Care Med;39:2511-7.
196. Wong HR, Wheeler DS, Tegtmeyer K, et al. Toward a clinically feasible gene expression-based subclassification strategy for septic shock: proof of concept. Crit Care Med;38:1955-61.
197. Seeburger J, Hoffmann J, Wendel HP, Ziemer G, Aebert H. Gene expression changes in leukocytes during cardiopulmonary bypass are dependent on circuit coating. Circulation 2005;112:I224-8.
198. Ramlawi B, Otu H, Rudolph J R , Mieno S, Kohane I S, Can H, Libermann T A, Marcantonia E R, Bianchi C, Sellke F W. Genomic expression pathways associated with brain injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg 2007:996-1005.
199. Cheung VG, Conlin LK, Weber TM, et al. Natural variation in human gene expression assessed in lymphoblastoid cells. Nat Genet 2003;33:422-5.
200. Ramlawi B, Otu H, Rudolph JL, et al. Genomic expression pathways associated with brain injury after cardiopulmonary bypass. J Thorac Cardiovasc Surg 2007;134:996-1005.
201. Naldini A, Borrelli E, Cesari S, Giomarelli P, Toscano M. In vitro cytokine production and T-cell proliferation in patients undergoing cardiopulmonary by-pass. Cytokine 1995;7:165-70.
202. Albert ML. Death-defying immunity: do apoptotic cells influence antigen processing and presentation? Nat Rev Immunol 2004;4:223-31.
203. Hotchkiss RS, Osmon SB, Chang KC, Wagner TH, Coopersmith CM, Karl IE. Accelerated lymphocyte death in sepsis occurs by both the death receptor and mitochondrial pathways. J Immunol 2005;174:5110-8.
204. Griffith TS, Yu X, Herndon JM, Green DR, Ferguson TA. CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. Immunity 1996;5:7-16.
205. Hotchkiss RS, Strasser A, McDunn JE, Swanson PE. Cell death. N Engl J Med 2009;361:1570-83.
206. Hotchkiss RS, Swanson PE, Freeman BD, et al. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. Crit Care Med 1999;27:1230-51.
207. Hotchkiss RS, Schmieg RE, Jr., Swanson PE, et al. Rapid onset of intestinal epithelial and lymphocyte apoptotic cell death in patients with trauma and shock. Crit Care Med 2000;28:3207-17.
208. Hotchkiss RS, Coopersmith CM, McDunn JE, Ferguson TA. The sepsis seesaw: tilting toward immunosuppression. Nat Med 2009;15:496-7.
209. Hutchins NA, Unsinger J, Hotchkiss RS, Ayala A. The new normal: immunomodulatory agents against sepsis immune suppression. Trends Mol Med.
210. Ashwell JD. The many paths to p38 mitogen-activated protein kinase activation in the immune system. Nat Rev Immunol 2006;6:532-40.
211. Karin M, Lawrence T, Nizet V. Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. Cell 2006;124:823-35.
212. Carre JE, Orban JC, Re L, et al. Survival in critical illness is associated with early activation of mitochondrial biogenesis. Am J Respir Crit Care Med;182:745-51.
213. Burgermeister E, Seger R. MAPK kinases as nucleo-cytoplasmic shuttles for PPARgamma. Cell Cycle 2007;6:1539-48.
214. Gersemann M, Wehkamp J, Stange E. Innate immune dysfunction in inflammatory bowel disease. J Intern Med.
215. Solan PD, Dunsmore KE, Denenberg AG, Odoms K, Zingarelli B, Wong HR. A novel role for matrix metalloproteinase-8 in sepsis*. Crit Care Med;40:379-87.

216. Dehoux MS, Hernot S, Asehnoune K, et al. Cardiopulmonary bypass decreases cytokine production in lipopolysaccharide-stimulated whole blood cells: roles of interleukin-10 and the extracorporeal circuit. Crit Care Med 2000;28:1721-7.
217. Gueret G, Lion F, Guriec N, et al. Acute renal dysfunction after cardiac surgery with cardiopulmonary bypass is associated with plasmatic IL6 increase. Cytokine 2009;45:92-8.
218. Tang BM, Craig JC, Eslick GD, Seppelt I, McLean AS. Use of corticosteroids in acute lung injury and acute respiratory distress syndrome: a systematic review and meta-analysis. Crit Care Med 2009;37:1594-603.
219. Parnell GP, Tang BM, Nalos M, et al. Identifying key regulatory genes in the whole blood of septic patients to monitor underlying immune dysfunctions. Shock;40:166-74.
220. Sprung CL, Sakr Y, Vincent JL, et al. An evaluation of systemic inflammatory response syndrome signs in the Sepsis Occurrence In Acutely Ill Patients (SOAP) study. Intensive Care Med 2006;32:421-7.
221. Maslove DM, Tang BM, McLean AS. Identification of sepsis subtypes in critically ill adults using gene expression profiling. Crit Care;16:R183.
222. Maslove DM, Wong HR. Gene expression profiling in sepsis: timing, tissue, and translational considerations. Trends Mol Med.
223. Whitney AR, Diehn M, Popper SJ, et al. Individuality and variation in gene expression patterns in human blood. Proc Natl Acad Sci U S A 2003;100:1896-901.
224. Palmer C, Diehn M, Alizadeh AA, Brown PO. Cell-type specific gene expression profiles of leukocytes in human peripheral blood. BMC Genomics 2006;7:115.
225. Tang BM, McLean AS, Dawes IW, Huang SJ, Cowley MJ, Lin RC. Gene-expression profiling of gram-positive and gram-negative sepsis in critically ill patients. Crit Care Med 2008;36:1125-8.
226. Tang BM, McLean AS, Dawes IW, Huang SJ, Lin RC. The use of gene-expression profiling to identify candidate genes in human sepsis. Am J Respir Crit Care Med 2007;176:676-84.
227. Schadt EE, Molony C, Chudin E, et al. Mapping the genetic architecture of gene expression in human liver. PLoS Biol 2008;6:e107.
228. Albert FW, Kruglyak L. The role of regulatory variation in complex traits and disease. Nat Rev Genet;16:197-212.
229. Montgomery SB, Dermitzakis ET. From expression QTLs to personalized transcriptomics. Nat Rev Genet;12:277-82.
230. Schadt EE, Monks SA, Drake TA, et al. Genetics of gene expression surveyed in maize, mouse and man. Nature 2003;422:297-302.
231. Moffatt MF, Kabesch M, Liang L, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature 2007;448:470-3.
232. Aubert B, Boutigny D, De Bonis I, et al. Measurement of CP-violating asymmetries in B0 decays to CP eigenstates. Phys Rev Lett 2001;86:2515-22.
233. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 2008;40:955-62.
234. Hugot JP, Laurent-Puig P, Gower-Rousseau C, et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature 1996;379:821-3.
235. Rioux JD, Daly MJ, Silverberg MS, et al. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. Nat Genet 2001;29:223-8.
236. Kabashima K, Saji T, Murata T, et al. The prostaglandin receptor EP4 suppresses colitis, mucosal damage and CD4 cell activation in the gut. J Clin Invest 2002;109:883-93.
237. Musunuru K, Strong A, Frank-Kamenetsky M, et al. From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus. Nature;466:714-9.
238. Kwan T, Grundberg E, Koka V, et al. Tissue effect on genetic control of transcript isoform variation. PLoS Genet 2009;5:e1000608.
239. Dimas AS, Deutsch S, Stranger BE, et al. Common regulatory variation impacts gene expression in a cell type-dependent manner. Science 2009;325:1246-50.

240. Fairfax BP, Humburg P, Makino S, et al. Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. Science;343:1246949.
241. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-75.
242. Zeggini E, Morris AP. Analysis of complex disease association studies : a practical guide. 1st ed. London ; Burlington, MA: Academic Press/Elsevier.
243. Abecasis GR, Auton A, Brooks LD, et al. An integrated map of genetic variation from 1,092 human genomes. Nature;491:56-65.
244. Shabalin AA. Matrix eQTL: ultra fast eQTL analysis via large matrix operations. Bioinformatics;28:1353-8.
245. Boyle AP, Hong EL, Hariharan M, et al. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res;22:1790-7.
246. Hindorff LA, Sethupathy P, Junkins HA, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A 2009;106:9362-7.
247. Stranger BE, Nica AC, Forrest MS, et al. Population genomics of human gene expression. Nat Genet 2007;39:1217-24.
248. Montgomery SB, Sammeth M, Gutierrez-Arcelus M, et al. Transcriptome genetics using second generation sequencing in a Caucasian population. Nature;464:773-7.
249. Zeller T, Wild P, Szymczak S, et al. Genetics and beyond--the transcriptome of human monocytes and disease susceptibility. PLoS One;5:e10693.
250. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature;491:119-24.
251. Speliotes EK, Willer CJ, Berndt SI, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet;42:937-48.
252. Mei YP, Liao JP, Shen J, et al. Small nucleolar RNA 42 acts as an oncogene in lung tumorigenesis. Oncogene;31:2794-804.
253. Kamatani Y, Matsuda K, Okada Y, et al. Genome-wide association study of hematological and biochemical traits in a Japanese population. Nat Genet;42:210-5.
254. Stavropoulos DJ, Bradshaw PS, Li X, et al. The Bloom syndrome helicase BLM interacts with TRF2 in ALT cells and promotes telomeric DNA synthesis. Hum Mol Genet 2002;11:3135-44.
255. Seger R, Krebs EG. The MAPK signaling cascade. FASEB J 1995;9:726-35.
256. Murray PJ. The JAK-STAT signaling pathway: input and output integration. J Immunol 2007;178:2623-9.
257. Nica AC, Dermitzakis ET. Expression quantitative trait loci: present and future. Philos Trans R Soc Lond B Biol Sci;368:20120362.
258. Chen Y, Zhu J, Lum PY, et al. Variations in DNA elucidate molecular networks that cause disease. Nature 2008;452:429-35.
259. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801-6.
260. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807-12.
261. The Genotype-Tissue Expression (GTEx) project. Nat Genet;45:580-5.
262. Ule J, Ule A, Spencer J, et al. Nova regulates brain-specific splicing to shape the synapse. Nat Genet 2005;37:844-52.
263. Zhang MH, Na B, Schiller NB, Whooley MA. Association of resistin with heart failure and mortality in patients with stable coronary heart disease: data from the heart and soul study. J Card Fail;17:24-30.
264. Buchholz BM, Bauer AJ. Membrane TLR signaling mechanisms in the gastrointestinal tract during sepsis. Neurogastroenterol Motil;22:232-45.

265. Rone MB, Liu J, Blonder J, et al. Targeting and insertion of the cholesterol-binding translocator protein into the outer mitochondrial membrane. Biochemistry 2009;48:6909-20.
266. Scarf AM, Kassiou M. The translocator protein. J Nucl Med;52:677-80.
267. Mitchell A, Rentero C, Endoh Y, et al. LILRA5 is expressed by synovial tissue macrophages in rheumatoid arthritis, selectively induces pro-inflammatory cytokines and IL-10 and is regulated by TNF-alpha, IL-10 and IFN-gamma. Eur J Immunol 2008;38:3459-73.
268. Ennaciri J, Girard D. IL-4R(alpha), a new member that associates with Syk kinase: implication in IL-4-induced human neutrophil functions. J Immunol 2009;183:5261-9.
269. Kollermann J, Schlomm T, Bang H, et al. Expression and prognostic relevance of annexin A3 in prostate cancer. Eur Urol 2008;54:1314-23.
270. Ernst JD, Hoye E, Blackwood RA, Jaye D. Purification and characterization of an abundant cytosolic protein from human neutrophils that promotes Ca2(+)-dependent aggregation of isolated specific granules. J Clin Invest 1990;85:1065-71.
271. Vermeulen Windsant IC, Hanssen SJ, Buurman WA, Jacobs MJ. Cardiovascular surgery and organ damage: time to reconsider the role of hemolysis. J Thorac Cardiovasc Surg;142:1-11.
272. Sertorio JT, Lacchini R, Amaral LM, et al. Haptoglobin polymorphism affects nitric oxide bioavailability in preeclampsia. J Hum Hypertens;27:349-54.
273. Dendele B, Tekpli X, Sergent O, et al. Identification of the couple GSK3alpha/c-Myc as a new regulator of hexokinase II in benzo[a]pyrene-induced apoptosis. Toxicol In Vitro;26:94-101.
274. Kawasaki K, Kuge O, Chang SC, et al. Isolation of a chinese hamster ovary (CHO) cDNA encoding phosphatidylglycerophosphate (PGP) synthase, expression of which corrects the mitochondrial abnormalities of a PGP synthase-defective mutant of CHO-K1 cells. J Biol Chem 1999;274:1828-34.
275. Meade KG, Gormley E, Doyle MB, et al. Innate gene repression associated with Mycobacterium bovis infection in cattle: toward a gene signature of disease. BMC Genomics 2007;8:400.
276. Pfistershammer K, Lawitschka A, Klauser C, et al. Allogeneic disparities in immunoglobulin-like transcript 5 induce potent antibody responses in hematopoietic stem cell transplant recipients. Blood 2009;114:2323-32.
277. Suciu-Foca N, Cortesini R. Central role of ILT3 in the T suppressor cell cascade. Cell Immunol 2007;248:59-67.
278. Yan R, Wan L, Pizzorno G, Cao D. Uridine phosphorylase in breast cancer: a new prognostic factor? Front Biosci 2006;11:2759-66.
279. Jesic S, Stojiljkovic L, Stosic S, Nesic V, Milovanovic J, Jotic A. Enzymatic study of tonsil tissue alkaline and acid phosphatase in children with recurrent tonsillitis and tonsil hypertrophy. Int J Pediatr Otorhinolaryngol;74:82-6.
280. Fujita E, Kouroku Y, Isoai A, et al. Two endoplasmic reticulum-associated degradation (ERAD) systems for the novel variant of the mutant dysferlin: ubiquitin/proteasome ERAD(I) and autophagy/lysosome ERAD(II). Hum Mol Genet 2007;16:618-29.
281. Jenne CN, Enders A, Rivera R, et al. T-bet-dependent S1P5 expression in NK cells promotes egress from lymph nodes and bone marrow. J Exp Med 2009;206:2469-81.
282. Walzer T, Chiossone L, Chaix J, et al. Natural killer cell trafficking in vivo requires a dedicated sphingosine 1-phosphate receptor. Nat Immunol 2007;8:1337-44.
283. Chi YH, Chen ZJ, Jeang KT. The nuclear envelopathies and human diseases. J Biomed Sci 2009;16:96.
284. Malhas AN, Lee CF, Vaux DJ. Lamin B1 controls oxidative stress responses via Oct-1. J Cell Biol 2009;184:45-55.
285. Bolanos JP, Almeida A, Moncada S. Glycolysis: a bioenergetic or a survival pathway? Trends Biochem Sci;35:145-9.
286. Hui L, Bakiri L, Stepniak E, Wagner EF. p38alpha: a suppressor of cell proliferation and tumorigenesis. Cell Cycle 2007;6:2429-33.

287. Martello LA, Pellicer A. Biochemical and biological analyses of Rgr RalGEF oncogene. Methods Enzymol 2006;407:115-28.
288. Baiges I, Palmfeldt J, Blade C, Gregersen N, Arola L. Lipogenesis is decreased by grape seed proanthocyanidins according to liver proteomics of rats fed a high fat diet. Mol Cell Proteomics;9:1499-513.
289. Sahu SK, Gummadi SN, Manoj N, Aradhyam GK. Phospholipid scramblases: an overview. Arch Biochem Biophys 2007;462:103-14.
290. Khera R, Das N. Complement Receptor 1: disease associations and therapeutic implications. Mol Immunol 2009;46:761-72.
291. Bux J, Stroncek D. Human neutrophil antigens. Transfusion 2002;42:1523.
292. Gribben JG, Ryan DP, Boyajian R, et al. Unexpected association between induction of immunity to the universal tumor antigen CYP1B1 and response to next therapy. Clin Cancer Res 2005;11:4430-6.
293. Olde B, Leeb-Lundberg LM. GPR30/GPER1: searching for a role in estrogen physiology. Trends Endocrinol Metab 2009;20:409-16.
294. Takatsu H, Baba K, Shima T, et al. ATP9B, a P4-ATPase (a putative aminophospholipid translocase), localizes to the trans-Golgi network in a CDC50 protein-independent manner. J Biol Chem;286:38159-67.
295. Tanaka T, Hosokawa M, Vagin VV, et al. Tudor domain containing 7 (Tdrd7) is essential for dynamic ribonucleoprotein (RNP) remodeling of chromatoid bodies during spermatogenesis. Proc Natl Acad Sci U S A;108:10579-84.
296. Sampsonas F, Kaparianos A, Lykouras D, Karkoulias K, Spiropoulos K. DNA sequence variations of metalloproteinases: their role in asthma and COPD. Postgrad Med J 2007;83:244-50.
297. Markey GM. Carboxylesterase 1 (Ces1): from monocyte marker to major player. J Clin Pathol;64:107-9.
298. Bovin LF, Brynskov J, Hegedus L, Jess T, Nielsen CH, Bendtzen K. Gene expression profiling in autoimmune diseases: chronic inflammation or disease specific patterns? Autoimmunity 2007;40:191-201.
299. Yang D, Rosenberg HF, Chen Q, Dyer KD, Kurosaka K, Oppenheim JJ. Eosinophil-derived neurotoxin (EDN), an antimicrobial protein with chemotactic activities for dendritic cells. Blood 2003;102:3396-403.
300. Harel L, Costa B, Fainzilber M. On the death Trk. Dev Neurobiol;70:298-303.
301. Zhu X, Herrera G, Ochoa JB. Immunosupression and infection after major surgery: a nutritional deficiency. Crit Care Clin;26:491-500, ix.
302. Leemans JC, te Velde AA, Florquin S, et al. The epidermal growth factor-seven transmembrane (EGF-TM7) receptor CD97 is required for neutrophil migration and host defense. J Immunol 2004;172:1125-31.
303. Xu L, Begum S, Hearn JD, Hynes RO. GPR56, an atypical G protein-coupled receptor, binds tissue transglutaminase, TG2, and inhibits melanoma tumor growth and metastasis. Proc Natl Acad Sci U S A 2006;103:9023-8.
304. Intlekofer AM, Banerjee A, Takemoto N, et al. Anomalous type 17 response to viral infection by CD8+ T cells lacking T-bet and eomesodermin. Science 2008;321:408-11.
305. Persic V, Ruzic A, Miletic B, et al. Granulysin Expression in Lymphocytes that Populate the Peripheral Blood and the Myocardium After an Acute Coronary Event. Scand J Immunol.
306. Ogawa K, Tanaka K, Ishii A, et al. A novel serum protein that is selectively produced by cytotoxic lymphocytes. J Immunol 2001;166:6404-12.
307. Jiang W, Chai NR, Maric D, Bielekova B. Unexpected role for granzyme K in CD56bright NK cell-mediated immunoregulation of multiple sclerosis. J Immunol;187:781-90.
308. Zhu D, Kepley CL, Zhang M, Zhang K, Saxon A. A novel human immunoglobulin Fc gamma Fc epsilon bifunctional fusion protein inhibits Fc epsilon RI-mediated degranulation. Nat Med 2002;8:518-21.

309. Chen C, Darrow AL, Qi JS, D'Andrea MR, Andrade-Gordon P. A novel serine protease predominately expressed in macrophages. Biochem J 2003;374:97-107.
310. Grigoriadis G, Anderson MA, Whitehead S. Charcot-Leyden crystals. Intern Med J;40:792.
311. Bryborn M, Hallden C, Sall T, Cardell LO. CLC- a novel susceptibility gene for allergic rhinitis? Allergy;65:220-8.
312. Willems LI, Ijzerman AP. Small molecule antagonists for chemokine CCR3 receptors. Med Res Rev;30:778-817.
313. Pichlmair A, Lassnig C, Eberle CA, et al. IFIT1 is an antiviral protein that recognizes 5'-triphosphate RNA. Nat Immunol;12:624-30.
314. Ahrens I, Domeij H, Eisenhardt SU, et al. Opposing effects of monomeric and pentameric C-reactive protein on endothelial progenitor cells. Basic Res Cardiol;106:879-95.
315. Hoesel B, Schmid JA. The complexity of NF-kappaB signaling in inflammation and cancer. Mol Cancer;12:86.
316. Yu S, Cui K, Jothi R, et al. GABP controls a critical transcription regulatory module that is essential for maintenance and differentiation of hematopoietic stem/progenitor cells. Blood;117:2166-78.
317. Veyrieras JB, Kudaravalli S, Kim SY, et al. High-resolution mapping of expression-QTLs yields insight into human gene regulation. PLoS Genet 2008;4:e1000214.
318. Spychala J, Datta NS, Takabayashi K, et al. Cloning of human adenosine kinase cDNA: sequence similarity to microbial ribokinases and fructokinases. Proc Natl Acad Sci U S A 1996;93:1232-7.
319. Matsumoto R, Matsumoto H, Seki M, et al. Human ecalectin, a variant of human galectin-9, is a novel eosinophil chemoattractant produced by T lymphocytes. J Biol Chem 1998;273:16976-84.
320. Wu X, Nguyen BC, Dziunycz P, et al. Opposing roles for calcineurin and ATF3 in squamous skin cancer. Nature;465:368-72.
321. Schossig A, Wolf NI, Fischer C, et al. Mutations in ROGDI Cause Kohlschutter-Tonz Syndrome. Am J Hum Genet;90:701-7.
322. Rubinfeld B, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P. Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. Science 1996;272:1023-6.
323. van Heel DA, Ghosh S, Butler M, et al. Muramyl dipeptide and toll-like receptor sensitivity in NOD2-associated Crohn's disease. Lancet 2005;365:1794-6.
324. Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC. Defective STAT signaling by the leptin receptor in diabetic mice. Proc Natl Acad Sci U S A 1996;93:6231-5.
325. Zhang JY, Green CL, Tao S, Khavari PA. NF-kappaB RelA opposes epidermal proliferation driven by TNFR1 and JNK. Genes Dev 2004;18:17-22.
326. Gregory AP, Dendrou CA, Attfield KE, et al. TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in multiple sclerosis. Nature;488:508-11.
327. Sigurdsson S, Goring HH, Kristjansdottir G, et al. Comprehensive evaluation of the genetic variants of interferon regulatory factor 5 (IRF5) reveals a novel 5 bp length polymorphism as strong risk factor for systemic lupus erythematosus. Hum Mol Genet 2008;17:872-81.
328. Welte S, Kuttruff S, Waldhauer I, Steinle A. Mutual activation of natural killer cells and monocytes mediated by NKp80-AICL interaction. Nat Immunol 2006;7:1334-42.
329. Ames RS, Li Y, Sarau HM, et al. Molecular cloning and characterization of the human anaphylatoxin C3a receptor. J Biol Chem 1996;271:20231-4.
330. Voncken JW, van Schaick H, Kaartinen V, et al. Increased neutrophil respiratory burst in bcr-null mutants. Cell 1995;80:719-28.
331. Orth K, Palmer LE, Bao ZQ, et al. Inhibition of the mitogen-activated protein kinase kinase superfamily by a Yersinia effector. Science 1999;285:1920-3.
332. Weeks DE, Conley YP, Tsai HJ, et al. Age-related maculopathy: a genomewide scan with continued evidence of susceptibility loci within the 1q31, 10q26, and 17q25 regions. Am J Hum Genet 2004;75:174-89.

333. Abid A, Ismail M, Mehdi SQ, Khaliq S. Identification of novel mutations in the SEMA4A gene associated with retinal degenerative diseases. J Med Genet 2006;43:378-81.
334. Heineke J, Auger-Messier M, Correll RN, et al. CIB1 is a regulator of pathological cardiac hypertrophy. Nat Med;16:872-9.
335. Kariya K, Koyama S, Nakashima S, Oshiro T, Morinaka K, Kikuchi A. Regulation of complex formation of POB1/epsin/adaptor protein complex 2 by mitotic phosphorylation. J Biol Chem 2000;275:18399-406.
336. Feng GJ, Goodridge HS, Harnett MM, et al. Extracellular signal-related kinase (ERK) and p38 mitogen-activated protein (MAP) kinases differentially regulate the lipopolysaccharide-mediated induction of inducible nitric oxide synthase and IL-12 in macrophages: Leishmania phosphoglycans subvert macrophage IL-12 production by targeting ERK MAP kinase. J Immunol 1999;163:6403-12.
337. Chang B, Chen Y, Zhao Y, Bruick RK. JMJD6 is a histone arginine demethylase. Science 2007;318:444-7.
338. Tong L, Heim RA, Wu S. Nitric oxide: a regulator of eukaryotic initiation factor 2 kinases. Free Radic Biol Med;50:1717-25.
339. Belostotsky R, Ben-Shalom E, Rinat C, et al. Mutations in the mitochondrial seryl-tRNA synthetase cause hyperuricemia, pulmonary hypertension, renal failure in infancy and alkalosis, HUPRA syndrome. Am J Hum Genet;88:193-200.
340. Park H, Vettese MB, Fensom AH, Fisher KJ, Aronson NN, Jr. Characterization of three alleles causing aspartylglycosaminuria: two from a British family and one from an American patient. Biochem J 1993;290 (Pt 3):735-41.
341. Datta SK, Kumar V, Pathak R, et al. Association of glutathione S-transferase M1 and T1 gene polymorphism with oxidative stress in diabetic and nondiabetic chronic kidney disease. Ren Fail;32:1189-95.
342. Speeckaert MM, Speeckaert R, Laute M, Vanholder R, Delanghe JR. Tumor necrosis factor receptors: biology and therapeutic potential in kidney diseases. Am J Nephrol;36:261-70.
343. Chasman DI, Fuchsberger C, Pattaro C, et al. Integration of genome-wide association studies with biological knowledge identifies six novel genes related to kidney function. Hum Mol Genet;21:5329-43.
344. Saito S, Murakumo Y, Tsuzuki T, et al. Analysis of glial cell line-derived neurotrophic factor-inducible zinc finger protein 1 expression in human diseased kidney. Hum Pathol;42:848-58.
345. Campuzano O, Alcalde M, Berne P, et al. Genetic testing of candidate genes in arrhythmogenic right ventricular cardiomyopathy/dysplasia. Eur J Med Genet;55:225-34.
346. Pol CJ, Muller A, Simonides WS. Cardiomyocyte-specific inactivation of thyroid hormone in pathologic ventricular hypertrophy: an adaptative response or part of the problem? Heart Fail Rev;15:133-42.
347. Satterthwaite G, Francis SE, Suvarna K, et al. Differential gene expression in coronary arteries from patients presenting with ischemic heart disease: further evidence for the inflammatory basis of atherosclerosis. Am Heart J 2005;150:488-99.
348. Booz GW, Day JN, Baker KM. Interplay between the cardiac renin angiotensin system and JAK-STAT signaling: role in cardiac hypertrophy, ischemia/reperfusion dysfunction, and heart failure. J Mol Cell Cardiol 2002;34:1443-53.
349. Vo TK, de Saint-Hubert M, Morrhaye G, et al. Transcriptomic biomarkers of the response of hospitalized geriatric patients admitted with heart failure. Comparison to hospitalized geriatric patients with infectious diseases or hip fracture. Mech Ageing Dev;132:131-9.
350. Polovlkova OG, Makeeva OA, Lezhnev AA, et al. [Expression profile of calcineurin pathway genes in myocardium tissues in relation to ischemic heart remodeling in humans]. Mol Biol (Mosk);47:433-40.
351. Schneider JE, Bose J, Bamforth SD, et al. Identification of cardiac malformations in mice lacking Ptdsr using a novel high-throughput magnetic resonance imaging technique. BMC Dev Biol 2004;4:16.

352. Ghosh P, Bhaumik P, Ghosh S, et al. Polymorphic haplotypes of CRELD1 differentially predispose Down syndrome and euploids individuals to atrioventricular septal defect. Am J Med Genet A;158A:2843-8.