On the importance of inflammation for personality traits and psychiatric morbidity

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ABSTRACT

On the importance of inflammation for personality traits and psychiatric morbidity

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Background: Pro-inflammatory mediators have been implicated in processes that could be both beneficial and toxic to cells in the brain. On the one hand balanced levels of these mediators favour e.g. neurodevelopmental processes, while on the other hand disturbances in this delicate balance, facilitated by activation of microglia and astrocytes for instance, may result in detrimental effects via interference with e.g. neural plasticity. Accumulating reports are linking raised serum levels of pro-inflammatory mediators to patients suffering from psychiatric morbidity and the underlying mechanisms need to be studied. This thesis focuses on four different inflammation-related proteins suggested to be associated with brain function. Firstly, C-reactive protein (CRP) - an acute phase reactant previously correlated with certain personality traits and depression as well as cardiovascular diseases. Secondly, complement factor H (CFH) - an important regulator of the complement cascade that has been implicated in e.g. Alzheimer's disease. Thirdly, the astrocyte-derived protein S100B – the levels of which has been found to be raised in serum of suicide attempters, and of depressed and schizophrenic patients. Fourthly, the receptor for advanced glycation end products (RAGE) - suggested to induce the pro-inflammatory effects of S100B in the brain and implicated in schizophrenia. The aim of this thesis was to i) investigate the possible influence of polymorphisms located in these genes on personality traits in population-based cohorts as well as in suicide attempters, ii) assess whether polymorphisms in *CRP* and *RAGE* increase susceptibility to suicidal behaviour and schizophrenia, respectively, and iii) investigate the level of gene expression of S100B in different brain regions in a genetic rat model of depression (Flinders sensitive line) and examine whether this expression is altered by immune activation. **Results:** The studied polymorphisms were associated with various personality traits in the normal population. The polymorphism +1444C>T located in the *CRP* gene was associated with increased scores of impulsivity both in a population-based cohort and in suicide attempters. The same allele was also found to increase the risk of suicidal behaviour. A polymorphism (Gly82Ser) in *RAGE* was associated with increased scores of the personality trait psychoticism in the normal population and was further associated with increased susceptibility of schizophrenia in patients. In addition, baseline mRNA levels of S100B were up-regulated in several brain regions in the spontaneously depressed rat when compared to control animals. **Conclusions:** The work presented in this thesis supports the hypothesis that inflammatory processes may be of importance for both normal behaviour and psychiatric morbidity. Due to the established connection between low-grade inflammation, cardiovascular diseases and psychiatric disorders, our results may further reflect the possibility that these disorders share a common genetic background.

Key words: inflammation, C-reactive protein, complement factor H, S100B, receptor for advanced end products, polymorphisms, gene expression, personality traits, suicidal behaviour, schizophrenia, depression, Flinders sensitive line

List of original papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. **Petra Suchankova**, Susanne Henningsson, Fariba Baghaei, Roland Rosmond, Göran Holm, Agneta Ekman. Genetic variability within the innate immune system influences personality traits in women. *Genes Brain and Behavior* 2009. **8**:212-7

II. **Petra Suchankova**, Göran Holm, Lil Träskman-Bendz, Lena Brundin, Agneta Ekman. The +1444C>T polymorphism in the *CRP* gene is associated with impulsiveness and suicidal behaviour. *Submitted Manuscript*

III. **Petra Suchankova**, Fariba Baghaei, Roland Rosmond, Göran Holm, Henrik Anckarsäter, Agneta Ekman. Genetic variability within the *S100B* gene influences the personality trait self-directedness. *Psychoneuroendocrinology* 2010. *In press*

IV. **Petra Suchankova**, Jonas Klang, Carin Cavanna, Göran Holm, Staffan Nilsson, Erik Jönsson, Agneta Ekman. Is the Gly82Ser polymorphism in the RAGE gene of relevance for schizophrenia and the personality trait psychoticism? *Submitted Manuscript*

V. **Petra Suchankova**, Staffan Nilsson, Aleksander Mathé, Agneta Ekman. Expression of S100B in a genetic rat model of depression – a pilot study. *Preliminary Manuscript*

CONTENTS

LIST OF ABBREVIATIONS

A	adenine
AMD	age-related macular degeneration
BBB	blood-brain barrier
BDNF	brain derived neurotrophic factor
C	cytosine
cDNA	complementary DNA
CFH	complement factor H
CNS	central nervous system
CRP	C-reactive protein
CSF	cerebrospinal fluid
CVD	cardiovascular disease
DFP	diisopropyl fluorophosphate
DNA	deoxyribonucleic acid
dNTP	deoxribonucleotide triphosphate
FRL	Flinders resistant line
FSL	Flinders sensitive line
FST	forced swim test
G	guanine
GWAS	genome-wide association study
HPA	hypothalamic-pituitary-adrenal
hsCRP	high-sensitivity CRP
HWE	Hardy-Weinberg equilibrium
i.p.	intraperitoneal
IDO	indoleamine 2,3-dioxygenase
IFNγ	interferon gamma
IL	interleukin
iNOS	inducible nitric oxide synthase
KSP	Karolinska Scales of Personality
LD	linkage disequilibrium
LPS	lipopolysaccharide
MDD	major depressive disorders
mRNA	messenger RNA
NCBI	National Centre of Biotechnology Information

NO	nitric oxide
OR	odds ratio
PCR	polymerase chain reaction
PPi	pyrophosphate
PRR	pattern-recognition receptor
qRT-PCR	quantitative real-time PCR
RAGE	receptor for advanced glycation end products
RNA	ribonucleic acid
ROS	reactive oxygen species
SD	standard deviation
SNP	single nucleotide polymorphisms
sRAGE	soluble RAGE
T	thymine
TCI	Temperament and Character Inventory
TLRs	Toll-like receptor
TNF	tumour necrosis factor
UTR	untranslated region
VEGF	vascular endothelial growth factor

PREFACE

The connection between the body and the mind was suggested already by the ancient Greek philosopher and physician Hippocrates (~460–370 BC). By believing that health depends on harmonies between different systems in the body, he saw the importance of determining both behavioural features as well as physical symptoms when examining his patients. Little did he know that it would take over 2000 years before the medical sciences would fully acknowledge his views.

Until recently it was believed that the central nervous system and the immune system are independent of each other, however, with the advent of psychoneuroimmunology it is now the common belief that there is a bidirectional communication between the two, meaning that immunological changes may give rise to behavioural changes and vice versa. The immune system is not only important for defending our bodies against harmful stimuli but it has also been found to affect processes that are important for general brain functions such as neural plasticity and neurodevelopment, in other words processes that essentially lay the ground for how an individual thinks and behaves. Although the immune system is mainly considered to be of beneficial value to the organism there is a dark side to it as an imbalance in this system has the potential of becoming very harmful to most tissues including the brain. However, many of the components of the immune system have been conserved throughout the evolution showing that despite its harmful effects it is of utmost importance for the survival of the organism. This could perhaps explain why various illnesses that are believed to be caused partly by alterations of the immune system such as psychiatric and neurodegenerative disorders, have also survived the evolution; they are simply passed on together with the vital immune system.

Our genes have a powerful influence on our behaviour but still few studies have explored whether the interaction between the immune and nervous system has a genetic background. Since the immune system has been shown to affect a person's behaviour it is possible that such effects can be traced back to various genes involved in this system. The current thesis was fuelled by this notion and presents findings that support the role of inflammation in not only normal behaviour, studied here in the form of personality traits, but also in psychiatric conditions such as suicidal behaviour and schizophrenia.

BACKGROUND

Molecular Genetics

The Central Dogma

Nucleic acids are considered the most important constituents of the living cell. By possessing vital information on the structure and repertoire of proteins made by each cell they control much of an organism's physical development throughout life. Nucleic acids build up genes in the form of deoxyribonucleic acid (DNA) which in humans is organised into 23 pairs (one from the mother paired with one from the father) of chromosomes.

The DNA molecule is composed by monomers consisting of a pentose sugar (2'-deoxiribose) linked to either of four nitrogenous bases: the pyrimidines cytosine (C) or thymine (T) consisting of a single carbon-nitrogen ring and the purines adenine (A) and guanine (G) consisting of double carbon-nitrogen rings.

The true structure of the DNA molecule was discovered by Watson and Crick in the 1950's when they proposed that two polynucleotide strands form a double helix.[1] The two strands were suggested to be held together via hydrogen bonds between the bases and further revealed that A binds only to T and C binds only to G. This in turn led to the assumption that the two strands are complementary, i.e. if the two strands were to be separated they could serve as templates in the synthesis of two new strands applying the base-pairing rule. This turned out to be the core mechanism required for cell division in which the whole genome of the dividing cell is replicated.

The genetic information held by most cells in the nucleus is transformed into proteins in two steps called transcription and translation. First, the DNA code is transcribed into ribonucleic acid (RNA). However, the initial RNA transcript contains both non-coding and coding sequences called introns and exons, respectively, which need to be spliced out. The resulting sequence is called messenger RNA (mRNA) and is transported from the nucleus to the cytoplasm where it serves as a template in the translational process in which a protein is formed.

Genotype and phenotype

The Austrian monk Gregor Mendel discovered over a century ago what would become the basic rules of inheritance by crossing different varieties of pea

plants.[2] Mendel concluded that each studied trait was dependent on two factors that separate and segregate during reproduction. This way the offspring receives one factor from each parent plant. Mendel also found that one of the factors may dominate the other, which meant that in order for the recessive trait to be expressed, the offspring needed to inherit two factors of this kind. To this day, these observations provide the grounds for what is known as Mendel's first law of heredity. The factors are today known as genes and the existence of more than one form of a gene results in different traits or phenotypes in different carriers. Since the offspring receives one set of genes from each parent, each gene is present in two copies (Figure 1). Genetic variations result in the presence of more than on possible outcome at a specific locus. A particular version at one chromosome is called an allele whereas the genotype is the combination of alleles found at the specific locus in the chromosome pairs. If the same allele is found at both chromosomes the individual is said to be homozygous but if they differ the individual is said to be heterozygous. The genotype is thus the combination of two alleles at a genetic locus and the phenotype is the actual trait that is observed. Yet another concept often used in psychiatry is the term endophenotype. An endophenotype is a component that underlies the disease syndrome (i.e. the phenotype). For instance, cognitive deficits seen in schizophrenic patients constitute the endophenotype while schizophrenia is the phenotype. Genetic analysis of complex psychiatric disorders are believed to benefit from the decomposition of such illnesses into their endophenotypic traits as these may better reflect the genetic component.[3]

Most variations found in the DNA have no functional significance, while some will effect the gene function and may in turn give rise to a specific phenotype or disease. Huntington's disease is a good example of a disorder that is influenced by variations in a single gene. However, most traits and diseases have a considerably more complex cause, involving environmental factors as well as genetic variations often in several different genes. The genetic basis for such complex phenotypes are much more difficult to characterise as the pattern of inheritance can be complicated. The degree of complexity depends on numerous factors including the number of genes that are involved, whether there is an interaction between genes that needs to be considered and whether some alleles contribute to the phenotype in an additive manner as opposed to the dominant versus recessive model first proposed by Mendel. Yet another possibility that could further add to the complex hereditary nature of certain diseases are epigenetic changes, i.e. changes in DNA that do not involve the sequence. Briefly, these changes may for example involve chemical modifications in form of methylation of DNA bases or histones which in turn may lead to silencing or activation of the affected gene.

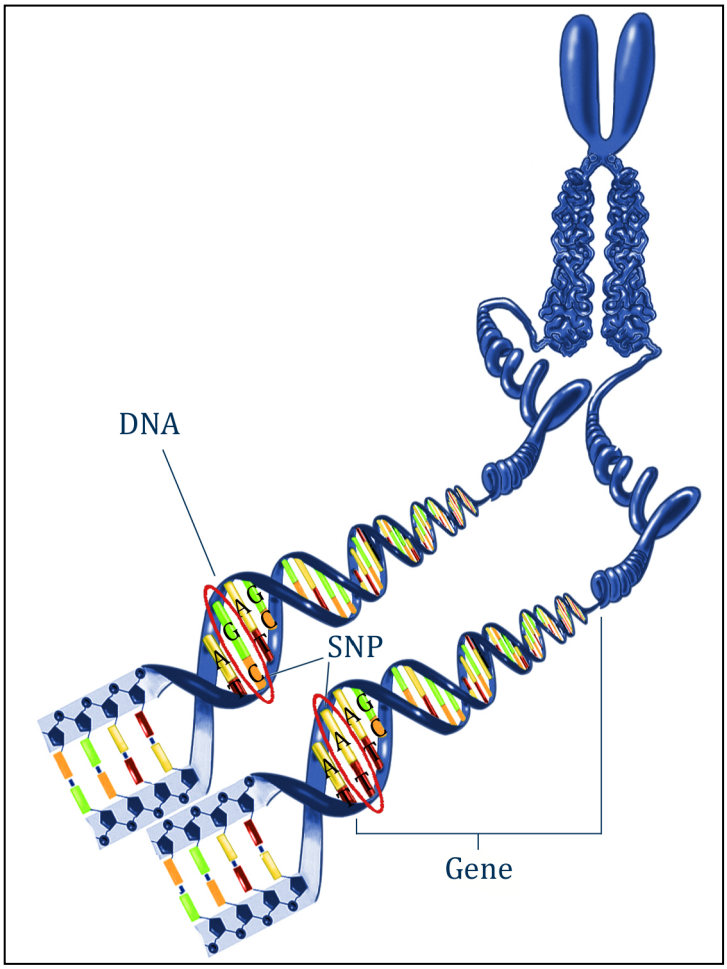


Figure 1 *A chromosome pair where one arm is inherited from the mother and the other from the father. Each parent thereby passes on one copy of every gene to their offspring. In the resting stage, the DNA is densely packed in a supercoiled structure in order to fit into the nucleus of the cell. The uncoiled part of the figure shows the double helix structure of the DNA molecule with the bases in different colours. The circled bases represent a SNP and in this particular case the individual is heterozygote at the locus as the gene inherited from the mother carries a G-allele whilst that of the father carries an A-allele. The term genotype is used to describe both alleles of the template strand and is in this case GA. Adapted from the image gallery of National Institute of General Medical Sciences.*

Genetic variation

Although approximately 99.9% of the DNA sequence is shared by all humans, the resulting 0.1% of genetic variation is what makes each and one of us unique.[4] These variations all stem from mutations such as base-pair substitutions, insertions or deletions of bases, tandem repeats and copy number variations. A genetic locus that displays different forms of alleles in over 1% of the population is termed a polymorphism. Single nucleotide polymorphisms (SNP) are found throughout the genome and are thought to arise through independent mutations that are then spread in the human population by reproduction. With over 14 million validated SNPs reported in the dbSNP database (build

131, April 2010), this is by far the most common genetic variation seen in the human genome.[5, 6]

The effect of a SNP on protein function depends on its location. In exons a base-replacement may lead to a change in amino acid (nonsynonymous), no change (synonymous) or a premature stop codon which alters the length of the protein.[7] SNPs located in introns cannot influence the amino acid sequence but they can nevertheless effect the expression of the gene, especially when located in regulatory regions such as the promoter or enhancer. The promoter region is often found at the 5' untranslated region (UTR) of the gene and acts as a regulator of gene expression by interacting with transcription factors.[8] Enhancers are distal elements that also interact with transcription factors but vary greatly in their location from gene to gene. A polymorphism in either of these regions may thus become functional by influencing the degree by which transcription factors bind to the region and thereby increase/decrease the expression of the gene. A SNP located in an intron may also affect the splicing process. The 3'UTR, located down-stream from the stop-codon of the gene, has also been recognised as an important regulatory region for many genes.[9] Small segments of RNA called microRNA have been found to regulate gene expression by binding to this region.[10] SNPs found in 3'UTR are suggested to influence mRNA localisation, stability and translation efficiency.[7]

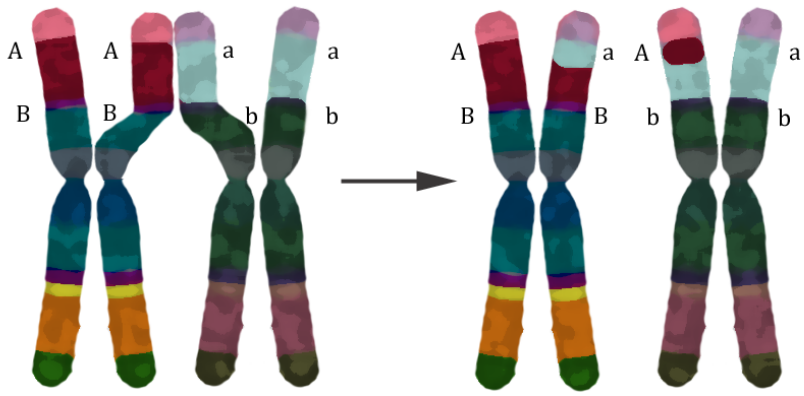


Figure 2 *During meiosis the primary diploid cell duplicates its DNA; 4 homologous chromosomes are formed (left side of the arrow). Crossover between A and B gives rise to two new haplotypes as a and B will be inherited together, as well as A and b (right side of the arrow). A recombination has occurred and the two loci are no longer in strong LD.*

Linkage disequilibrium

Mendel believed that all genes within an organism are inherited independently of one another. However, he was unaware of the existence of chromosomes and the fact that regions close together on the same chromosome are often inherited together. The specific combination of alleles observed on a single chromosome is called a haplotype.[11] In the case of two diallelic loci, A/a and B/b, they are said to be in complete linkage disequilibrium (LD) if one of them

can predict the outcome of the other. In other words, if the first locus is *A* or *a*, the second allele on the same chromosome will be *B* or *b,* respectively (left side of Figure 2). During meiosis (i.e. the formation of haploid cells such as the sperm or egg cell), homologous chromosomes (i.e. chromosomes that pair up) often exchange parts of their DNA with each other in a process called cross-over. This may at times result in recombination, i.e. the formation of new haplotype combinations (right side of Figure 2).

Population genetics and the Hardy-Weinberg equilibrium

The field of population genetics deals with allelic and genotypic frequencies in populations and how these frequencies are affected by certain evolutionary forces, such as natural selection.[2] This field rests upon one simple principle put forward by Hardy and Weinberg in the early 1900s. They stated that genotype and allele frequencies at a certain locus will not change across generations (they are at equilibrium) provided that the population that is being studied is large and randomly mating and that the locus is unaffected by factors such as migration, mutations and natural selection.[12] The Hardy-Weinberg principle further assumes that allele frequencies are the same in women and men. Whether the frequencies of alleles and genotypes deviate from the Hardy-Weinberg equilibrium (HWE) is established by the following multiplication rules.

A polymorphism with two alleles, *A* and *a*, has been genotyped in a population. The *A* and *a* alleles are found to have the respective frequencies of *p* and *q* the sum of which is 1. During random mating, the probability that the offspring will be homozygous for the *AA* allele is determined by the product of the frequency for *A*, i.e. $p \times p = p^2$. The probability of heterozygous offspring is $2pq$ (Table 1). When the population is at HWE the genotype frequency is $p^2 + 2pq + q^2$ and when random mating is assumed this equation will simply equal the product of the $p+q$ from the females and $p+q$ from the males, i.e. $(p+q)^2 = p^2 + 2pq + q^2$.[2]

		Male gametes	
		A (p)	a (q)
Female gametes	A (p)	AA (p^2)	Aa (pq)
	a (q)	Aa (pq)	aa (q^2)

Table 1 *According to the Hardy-Weinberg principle the genotype frequencies of the offspring can be estimated knowing the allele frequencies of the parents by the multiplication of these proportions as shown in the table (reported as genotype and frequency products in brackets).*

Departure from the HWE in a population may be caused by a violation of any of the assumptions mentioned above. However, a highly significant departure from the HWE, calculated by χ^2 test, is most often an indication of an unsuccessful genotyping procedure.[13] In SNP-based association studies, the HWE procedure therefore serves as a quality control method to assure that the genotyping results are reliable.

Genetic Linkage and Association Studies

There are numerous strategies that can be applied when searching for the genetic background to a certain phenotype. In 1913, Sturtevant described after a series of experiments on the fruit fly that certain Mendelian traits (i.e. a trait controlled by a single locus) are inherited together unless recombination occur during meiosis and suggested that these traits are located on the same chromosome.[14] This gave rise to the development of the linkage analysis approach where the same concept is applied to discover genes responsible for diseases in humans. The method involves scanning the entire genome for regularly spaced genetic markers of known location in families affected by a specific Mendelian trait or disease.[15] The aim is to identify chromosome regions where the genetic variation is shared by affected members. These regions can then be further investigated with the objective of finding the responsible gene. Linkage studies are limited in that they require large family based populations of both affected and unaffected members and are not suitable when studying complex traits. Instead, genetic association studies are used to study populations consisting of unrelated subjects. In its simplest form genotype or allele frequencies are compared between affected (cases) and unaffected (controls) individuals in a population sample. However, this approach may also be applied when studying the possible influence of a gene on continuous variables. There are two major strategies within the field of population-based association studies[16] that are discussed below.

The emergence of practical and cost-effective genotyping methods coupled with the hypothesis that common polymorphisms may contribute to susceptibility to common diseases led to a new era in genetic association studies with the development of genome-wide association studies (GWASs).[17] By assessing common SNPs across the whole genome this method aims to identify genes that previously have not been associated with a phenotype or disease. Since the advent of GWAS in 2006, several common SNPs have been linked to common diseases, a good example being the association between a SNP in the gene coding for complement factor H with age-related macular degeneration (AMD).[18] This finding subsequently led to a new biological hypothesis in which the complement system was suggested to be implicated in the pathophysiology of AMD. GWASs have the advantage of not requiring any prior knowledge

of the biological or physiological background to the studied trait or disease. However, the data generated with each GWAS is enormous due to the large number of investigated SNPs and the data analysis is phased with issues such as false positive results (e.g. when not controlling for multiple testing) but also false negative result (e.g. when controlling for multiple testing).

Unlike linkage studies and GWAS the candidate-gene association strategy requires that the investigator has an understanding of the mechanisms that are of importance for the phenotype of interest.[18] This approach is better suited when identifying genes that are expected to have a moderate effect on the susceptibility to a complex trait.[15] Once the candidate gene has been determined, there are several databases and strategies that can be used when deciding which SNPs to include in the association study. The predominant and fastest growing database is the dbSNP established by the National Centre of Biotechnology Information (NCBI) in 1998.[6, 19] New SNPs are added on a regular basis from different sources including the international HapMap project.[11] HapMap (short for haplotype map) aims to identify the common haplotypes found in the human genome by assessing LD between pairs of SNPs. Due to the fact that genotype frequencies vary among people with different ancestry the project focuses on analysing DNA from three different populations: African, Asian and European. The dbSNP and HapMap databases are important short-cuts for any researcher who is about to perform a genetic association study. They provide vital information about the characteristics of a SNP including where in the gene it is located, whether it is nonsynonymous or not and its minor allele frequency. Another important feature of HapMap is its identification of so called tag SNPs. These are SNPs that have been found to be in strong LD with other SNPs. By sticking to tag SNPs the investigator can capture genetic variation across the whole gene without having to genotype all its SNPs.

The innate immune system

Innate and adaptive immunity

The human body is constantly exposed to different disease-causing microorganisms that it needs to protect itself against. In order to do so, it is equipped with a host-defence system that recognises and destroys foreign intruders by possessing the ability to distinguish between self and non-self components. The immune system can be divided into two different branches that interact and complement each other but differ in terms of how they recognise and fend off pathogens. The adaptive immune response is the specific branch of the immune system that relies on the production of antibodies against a certain pathogen. This often results in lifelong immunological memory as the specific antibodies will continue to circulate in the bloodstream even after the pathogen has been cleared. The second branch is the innate immune system which can be seen as the body's first line of defence against common microorganisms as it is effective immediately after recognition of a pathogen.[20] However, the innate immune system lacks memory and is less specific than the adaptive immune system. Innate immunity has been highly conserved at the molecular level throughout the evolution.[21, 22] The word "innate" reflects the fact that the components used for the detection and elimination of the pathogen are encoded in the DNA. The innate immune system is thus inherited and highly important for the organism as it provides the only form of immediate protection against infection, tissue injury and dying cells before e.g. the adaptive system has time to develop with the subsequent production of specific antibodies.

Inflammation: acute and chronic

In humans, macrophages, neutrophils and other phagocytic cells are distributed throughout the body and are central in the innate immune response as they recognise tissue injury and pathogens independent of the adaptive immune system. This is facilitated by specific pattern-recognition receptors (PRRs).[20] The extracellular defence relies on the complement system, which results in the opsonisation and subsequent clearance of the pathogen or damaged cell (see below). The membrane-bound PRRs such as the Toll-like receptors (TLRs) recognise common constituents found on the surface of a wide variety of pathogens as well as apoptotic and necrotic host cells. Regardless of the rout by which harmful stimuli are recognised it leads to the subsequent induction of inflammation, which is also the main focus of this thesis.

Inflammation is an adaptive response that evolved as a means of restoring homeostasis. The inflammatory response is divided mainly into the acute and chronic phase. Acute inflammation is characterised by e.g. heat, vasodilation

and oedema and aims to eliminate the cause of the reaction and restore the tissue by recruiting effector molecules and cells to sites of infection or tissue damage.

Once the harmful stimulus has been detected, usually by resident macrophages and mast cells, an inflammatory cascade is initiated via the secretion of a vast number of inducers and mediators including chemokines, cytokines, histamine, complement factors and acute phase reactants (discussed below). These mediators increase the permeability of nearby vasculature and activate the endothelium of the blood vessel.[23] This facilitates the subsequent migration of plasma proteins and neutrophils from the blood vessel to the extravascular tissue where the infection or injury is taking place.

The neutrophils are then activated - either directly by the pathogen itself or by cytokines produced by resident cells - and start attacking the pathogen by releasing toxic effector molecules stored in granules. However, this attack does not discriminate between pathogen and healthy tissue which is bound to get damage in the process. Once the pathogen has been cleared the restoration of the tissue is initiated via further recruitment of macrophages. An important part of this restoration is the switch in secretion of pro-inflammatory mediators to anti-inflammatory ones that inhibit the recruitment of neutrophils and instead allow monocytes to enter the tissue. These facilitate the removal of dead cells and initiate tissue remodelling.

Chronic inflammation may arise either by the unsuccessful elimination of an irritant by the acute inflammatory response, by autoimmune reactions or by a persistent irritant that is not large enough to elicit an acute inflammatory reaction.[24] The symptoms are not as severe as for the acute inflammation but may still cause tissue damage. As opposed to the acute inflammation in which neutrophils have a central role, the chronic phase is characterised by the infiltration of macrophages and T cells of the adaptive immune system. This is a good example of the cooperation between the innate and adaptive systems as macrophages present the antigen to T cells that start secreting interferon gamma (IFNγ), the key component of the chronic inflammatory response. IFNγ will in turn activate macrophages that start producing reactive oxygen species (ROS), nitric oxide (NO), chemokines and certain metalloproteinases. The chronic inflammatory response will ultimately lead to a series of unfavourable effects for the organism and is suggested to be involved in the onset of diverse disorders such as CVD and cancer.

Inflammatory mediators

The inflammatory response is orchestrated mainly by certain soluble pro- and anti-inflammatory proteins. The following sections will focus on those that are of relevance to the papers included in this thesis.

Pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6 and tumour necrosis factor (TNF) act as messengers between various types of cells in the inflammatory system. They are produced as a response to infection or tissue damage and exert both paracrine and endocrine effects in the body. The paracrine effects include the induction of further cytokine production by nearby cells and the recruitment of neutrophils to the affected tissue. The endocrine effects or systemic effects as they are more often denoted, are the result of the spreading of cytokines via the blood stream to organs in the whole body.

The main systemic effects of these cytokines comprise the induction of fever, sickness behaviour (e.g. loss of appetite, tiredness), metabolic changes, cortisol production (by IL-6) and the synthesis of acute-phase proteins. The latter is a group of proteins synthesised in the liver, whose serum levels have been found to increase by at least 25% during the inflammatory response.[25] C-reactive protein (CRP), studied in paper I and II of this thesis, is the most sensitive of the acute-phase proteins, with levels rising up to 1000-fold in response to an acute inflammation. It binds to phosphocholine found on the surface of damaged cells as well as in bacteria, fungi and parasites. The CRP-ligand complex is able to bind to phagocytic cells as well as to C1q, an important component in the initiation of the complement system (discussed below).

In recent years awareness has been raised for more subtle systemic changes in inflammatory mediators as seen in low-grade inflammation. With the emergence of high-sensitivity methods for measuring low levels of CRP (i.e. hsCRP) in serum, accumulating evidence suggesting that modestly elevated levels of hsCRP, tentatively reflecting low-grade inflammation, are an independent risk factor for cardiovascular disease (CVD) in both women and men.[26-31] Individuals with hsCRP levels higher than 3 mg/L are now believed to be at higher risk of developing CVD.

The complement system

The complement system is one of the most important antimicrobial systems in the body involving approximately 20 soluble plasma proteins that are constantly circulating in the blood stream. These components are activated by cleavage by various proteases in a cascade resulting in i) an amplification of the inflammatory signal, ii) the tagging or opsonisation of bacteria that facili-

tates their recognition by macrophages and iii) the formation of a membrane attack complex that induces lysis of the bacteria.

The complement system is divided into three pathways, the classical, the lectin and the alternative pathway. Without going into detail on the mechanisms underlying these pathways, the principle is the same for all three; however, they differ in terms of activation.

The classical pathway is activated by the binding of the C1q-complex to antibody-antigen complexes, as well as CRP. The lectin pathway recognises certain residues on the surface of the pathogen via the complement component mannose-binding lectin which in turn initiates the cascade. The alternative pathway is a potent reinforcement to the classical and lectin pathways as it is activated by components formed in these cascades, facilitating the subsequent phagocytosis of the cell or bacteria that is under attack. The complement system is a potential threat to many types of host cells that are continuously exposed to the circulating complement factors and raises the need to keep it regulated in order to ensure that host tissue is not damaged. The most abundant complement regulator in plasma is complement factor H (CFH).[32, 33] It binds to polyanions found on the surface of host cells and thereby distinguishes these from potential targets of the complement system. CFH exerts its regulatory effects by inhibiting certain complement enzymes of the alternative pathway present not only on the surface of these cells but also in the fluid surrounding them. CFH has additionally been found to interact with CRP which increases its ability to regulate the complement system. This has been suggested to heighten the protection of host cells during the inflammatory response in which CRP levels are elevated.[34]

The role of inflammation in the brain

Research over the last two decades has shown that there is a complex bidirectional communication between the central nervous system (CNS) and the immune system.

The immune-to-brain signal occurs mainly both by a neural and a humoral pathway that exert their effects in a parallel fashion. In the neural pathway, afferent nerves at the site of inflammation or injury express cytokine receptors that are activated by the release of mainly IL-1, IL-6 and TNF and start signalling the brain via the vagus nerve. This is believed to be the underlying mechanism by which cytokines quickly alert the brain of the classical symptoms of heat and pain that has been produced by the insult.[35] The humoral pathway involves circulating cytokines that interact with TLRs expressed on microglial and macrophage-like cells of the circumventricular organs, i.e. regions of the brain that lack the blood-brain barrier (BBB).[36] Circulating cytokines have also been suggested to signal across the BBB by associating with endothelial cells and perivascular macrophages in the brain vasculature.[37] Signalling to either of the mentioned cells induces them to synthesise inflammatory mediators including cytokines and prostaglandins which are subsequently released inside the brain.

This results in a number of nonspecific symptoms including the onset of fever accompanied by neuroendocrine changes via the activation of the hypothalamic-pituitary-adrenal (HPA) axis and sickness behaviour represented by psychological and behavioural alterations including signs of depression, anorexia and lack of activity.[38] The febrile response is beneficial to the host as it inhibits the pathogen to grow and multiply whilst the sickness behaviour makes up for the energy required to raise the body temperature as the individual's activity decreases. The lack of appetite also serves its purpose since it e.g. restricts important nutrients to fuel the pathogen.[39]

There is also a brain-to-immune connection governed by the efferent vagus nerve that facilitates the bidirectional cross-talk between the two systems. This is often referred to the inflammatory reflex in which the afferent vagus nerve signals the presence of IL-1 to the parasympathetic brainstem region, with the subsequent activation of the efferent vagus nerve that inhibits cytokine synthesis by interacting with cholinergic receptors on macrophages. The nervous system may also dampen the inflammatory response by the HPA axis that works as a physiological feedback loop resulting in the release of anti-inflammatory glucocorticoids.

Cytokine-induced sickness behaviour is a temporary solution to an extreme bodily strain that is fully reversed once the pathogen has been resolved. Accumulating evidence linking abnormal levels of pro-inflammatory mediators to various disorders of the CNS in otherwise healthy individuals has raised awareness for the possibility that i) low-grade or chronic inflammation may influence the brain, ii) inflammation may be induced during such disorders and/or iii) individuals may be genetically predisposed to express increased levels of inflammatory mediators which in turn puts them at higher risk of developing these disorders. However, it has also been established that inflammatory mediators have beneficial effects in the brain when expressed under normal conditions (i.e. in the absence of an inflammatory response). This will be discussed in the following section.

Inflammatory mediators in the healthy brain

Glia

The main populations of cells found in the brain are neurons and non-neuronal cells called glia. Glia are divided into two major families, i) microglia which are considered the macrophages of the CNS and ii) macroglia which are further divided into astrocytes and oligodendrocytes. The latter are responsible for the electrical insulation in the CNS as their membrane wraps around axons and form myelin sheaths.

Astrocytes are large star-shaped cells that are highly abundant in the brain. They are structurally and functionally associated with neurons and the cerebral microvasculature. Astrocytes are unable to generate an action potential; however, they possess the ability to adjust their own electrical properties, which is essential for proper neuronal activity and function.[40] During normal conditions, astrocytes respond to excessive amounts of transmitter in nearby synapses by increasing their intracellular Ca^{2+} levels. This induces the release of various kinds of glial transmitters including classical transmitters, chemokines, cytokines (e.g. TNF), and peptides. In addition, the cytokine-like calcium-binding protein S100B is released.

Microglia comprise approximately 15% of brain cells and are present in a resting phase and an activated phase.[41] At rest, their function is modulated by neuronal activity (i.e. neurotransmitter release that activates receptors expressed by the microglial cell) and astrocytes, resulting in the secretion of (low) levels of IL-1[42] and brain derived neurotrophic factor (BDNF).[41] They are also capable of activating endothelial cells to synthesise various trophic factors such as vascular endothelial growth factor (VEGF), which are important for many functions of the brain.

Neural plasticity

As mentioned above, the inflammatory response is more than just a defence system against pathogens, it is also involved in tissue remodelling in which a specific tissue is altered both morphologically and/or functionally to adapt to the changes imposed by the environment.[41] In both the developing and adult brain, tissue remodelling is an important process by which neural circuits are adapted in a process called neural plasticity that in turn facilitates the brain's ability to learn and form memories.

The aim of neural plasticity is to make neural circuits as perfect and functional as possible. This is achieved by removing unnecessary brain cells, and by inducing the formation as well as elimination of axons, dendrites and synapses. The proposed mechanisms underlying this process involves the modulation of microglia and astrocytes which is mediated by neurotransmitters from axon terminals and their subsequent interaction with receptors expressed on these cells.

In astrocytes, this induces the regulated release of glial transmitters (discussed above) which in turn modulate neuronal excitability and synaptic strength.[40] By releasing low levels of IL-1 (discussed above), the microglial cells signal nearby astrocytes which in turn initiates the production of various compounds important for memory formation and synaptic plasticity including BDNF, TNF and glutamate.[41] Astrocytes have also been suggested to be responsible for the up-regulation of C1q in developing neurons.[43] C1q which is traditionally thought to opsonise pathogens may also tag synapses for elimination. In this vein, it is also possible that regulators of the complement system, such as CFH, protect stronger synapses from pruning.[44] The complement system has also been suggested to be involved in neurogenic and neuroprotective processes[45-47] as well as in the induction of apoptosis and clearance of apoptotic cells.[48]

S100B, a protein produced mainly by astrocytes in the brain, has also been implicated in developmental processes.[49] Nanomolar levels of this protein have been suggested to protect neurons and promote their survival during development as well as to facilitate neurite extension via activation of the receptor for advanced end products (RAGE). These receptors are expressed on various types of cells in the body including neurons, astrocytes and microglia. S100B have also been found to induce inflammation when released at micromolar levels causing toxicity to various cells in the brain (discussed below).

The current knowledge regarding neuro-glia interactions hence indicate that inflammatory cytokines, when produced at low levels, are beneficial in processes important for normal brain functioning. This is a delicate relationship

that is affected during an inflammatory response or when the balance of the components involved is offset in any other way.

Inflammation in mental functions and disorders

Inflammation has been implicated in neurodegenerative diseases such as Alzheimer's (AD) and multiple sclerosis. However, of greater interest for the present thesis are reports indicating that an imbalance in pro-inflammatory mediators may be of importance for normal brain functions on the one hand, and mood disorders, suicidal behaviour and schizophrenia on the other. One suggested mechanism underlying this influence involves peripheral pro-inflammatory cytokines that activate microglia and astrocytes in the nervous system, resulting in a subsequent release of pro-inflammatory cytokines such as IL-1, TNF and IL-6 in the brain.[41] High levels of these mediators are detrimental to the brain as they have been reported to cause e.g. neuronal degeneration and decreased neurogenesis.[50, 51]

Inflammation and personality traits

The concept of personality emerges from the observation that individuals seem to behave consistently over time and when faced with different situations. The personality comprises several different traits that are characteristic for how that person thinks, feels and behaves.

It goes without saying that neurodevelopmental processes of the CNS are of importance for both normal and abnormal behavioural development.[52] The controversial debate regarding 'nature versus nurture' has now come to face the possibility that they do in fact interact, i.e. that external influences exert their effect on an individual's personality by inducing developmental changes in the brain.

The structure of the brain and the way that neurons connect, are determined by developmental processes including cell proliferation, migration, differentiation and apoptosis. Several studies have reported that inter-individual differences in personality traits are influenced by variances in neural activity seen in certain brain regions.[53-56] Given the involvement of the immune system and inflammatory mediators in developmental events (discussed above), it is likely that these have an impact on both normal (e.g. personality traits) and abnormal behaviour (e.g. psychopathology). Support for the possible implication of inflammatory processes in normal behaviour was provided by a study in which serum levels of CRP were positively and negatively correlated to the personality traits harm avoidance and self-directedness, respectively.[57] In line with these findings a recent study found that the personality trait openness to experience was negatively correlated with CRP levels.[58] Needless to say, this

kind of associations could however be the result of certain personality traits being associated with perceived stress, which in turn could influence CRP levels, rather than the result of CRP influencing brain structure or function.

Another interesting aspect to consider is the fact that both elevated serum levels of CRP, Type D personality (characterised by the combination of negative affectivity and social inhibition) and major depressive disorder (MDD) are considered to be important risk factors for the development of CVD.[59] It has therefore been proposed that inflammatory processes are a common denominator for these various traits and diseases. However, it should be noted that increased levels of inflammatory mediators are induced by stress via the HPA axis[60] and it is therefore possible that the elevations seen are the result of extrinsic rather than intrinsic mechanisms.

Personality traits measured by self-report questionnaires have also been found to be heritable and relatively stable over life.[61] Even though numerous genes have been found to influence an individual's personality, there is still much to discover regarding the mechanisms and gentic factors underlying these traits. Although studies involving personality traits are of great use when it comes to investigating the normal range of individual differences, they may also lead to a greater understanding on the mechanisms underlying psychiatric disorders as there is a clear connection between the abnormal range of such traits and psychopathology.

Inflammation in mood disorders and suicidal behaviour

In recent years, inflammation has been implicated in the pathophysiology of MDD after reports showing that inflammatory mediators such as CRP[62-64] and IL-6[65-67] are elevated in serum of these patients. It has also been reported that 30-50% of the patients undergoing cytokine treatment (for the treatment of e.g. cancer or chronic viral infections) develop depressive symptoms as well as suicidal ideation.[68, 69] Interestingly pretreatment with the antidepressant paroxetine did not alleviate the symptoms of fever, anorexia, fatigue and pain, however, it was effective against depression suggesting that sickness behaviour and depression induced by cytokines occur by different mechanisms.[70] In addition, patients undergoing cytokine treatment have been found to have a decrease in plasma levels of tryptophan that in turn correlates (negatively) with severity of depressive symptoms induced by the treatment.[71] A suggested mechanism underlying this observation comprises the enzyme indoleamine 2,3-dioxygenase (IDO) present in many cell types including microglia and which is stimulated by pro-inflammatory cytokines.[72] IDO is responsible for the degradation of tryptophan into kynurenine and quinolinic acid which in turn decreases the bioavailability of this essential amino acid for the synthesis of serotonin. The excess production of quinolinic acid by microglia has also

been reported in chronically depressed patients and is suggested to ultimately lead to the destruction of astrocytes.[60] It is thus possible that the increased cytokine levels seen in patients with MDD decrease serotonergic levels in the brain by inducing IDO as well as causing glial pathology. The latter is supported by various *post mortem* studies of patients with mood disorders in which reduced glial cell density in prefrontal brain regions has been revealed.[73-75]

Recent studies suggest that inflammation may be of specific importance also for suicidal behaviour. Increased levels of pro-inflammatory cytokines have been found both in plasma and cerebrospinal fluid (CSF) of suicide attempters.[76, 77] Moreover, post-mortem examination of brains from suicide victims showed an increased expression of cytokines in the orbitofrontal cortex and accumulation of microglia.[78] Again, however, one should consider the obvious possibility that these changes are secondary to stress, rather than of causal importance for suicide.

Inflammation and schizophrenia

Recent studies have shown that pro-inflammatory cytokines are elevated in serum of schizophrenic patients.[79-85] Schizophrenic patients have also been reported to have increased levels of S100B both in CSF and serum[86-89] and negative symptoms have been found to correlate with increasing S100B serum levels.[88] Although extracellular nanomolar levels of S100B have trophic effects in the brain, micromolar concentrations are suggested to produce toxicity due to apoptosis and necrosis as well as by facilitating the secretion of pro-inflammatory mediators by microglia.[90] These effects are mediated via RAGE expressed and up-regulated on activated microglia. Further support for the possible involvement of inflammation in the pathophysiology of schizophrenia is handed by reports showing that their release from activated microglia is inhibited by certain typical and atypical antipsychotics.[91-96]

AIMS

The overall aims of this thesis are i) to study the influence of genetic variations in inflammation-related genes on personality traits and psychiatric morbidity and ii) to investigate the expression of such a gene in a rat model of depression before and after immune activation.

Specific aims:

- to assess the influence of polymorphisms located in the *CRP* (+1444C>T) and *CFH* (Y402H) gene on personality traits in a female population-based cohort (paper I). The +1444C>T SNP was further investigated in suicide attempters with respect to personality traits and genotype frequencies compared to controls (paper II).

- to assess the influence of two SNPs (2757C>G and 5748C>T) located in the *S100B* gene on personality traits in two population-based cohorts (paper III) as well as to study gene expression of S100B in certain brain regions in a genetic rat model of depression (Flinders sensitive line) and examine whether this expression is altered by immune activation (paper V).

- to assess the influence of a polymorphism in *RAGE* (Gly82Ser) on personality traits and to compare genotype frequencies of this SNP between schizophrenic patients and healthy controls (paper IV). The effect of this SNP on the age of onset of schizophrenia was further investigated (paper IV).

METHODS

Ethics

Paper I-IV comprise human genetic association studies that were approved by the Human Ethics committees at University of Gothenburg (paper I-IV), Lund University (paper II), Karolinska Hospital and Karolinska Institutet (paper IV). All participating individuals gave their written informed consent. The animal study presented in paper V was approved by the Animal Ethics committee at University of Gothenburg.

Human genetic association studies

Subjects

The population-based cohorts

Two population-based cohorts comprising women and men, respectively, were originally recruited for studies on obesity, anthropometrics and cardiovascular risk factors. For detailed description of the recruitment process, the reader is referred to paper I and III as well as to references within these papers. At the time of investigation all women and men were 42 and 51 years old, respectively. No man or woman was excluded from the study due to somatic or psychiatric disease. Blood samples were obtained from 270 women and 247 men for genotyping. Personality trait assessment were conducted using the Temperament and Character Inventory[97] and Karolinska Scales of Personality[98] questionnaire (discussed below).

These cohorts are used to study the influence of certain polymorphisms on personality traits (paper I, III and IV) and serve as controls in an association study (paper II).

The cohort of suicide attempters

The suicide attempters studied in paper II originate from two cohorts. Cohort 1 (n=42, 21 women and 21 men; mean age±SD of 51.0±10.1) comprises a 10-year follow-up study of suicide attempters that were first recruited between the years of 1986 and 1992. The recruitment of subjects in cohort 2 took place between 2005 and 2008, and involved 64 suicide attempters (38 women and 26 men; mean age±SD of 38.1±14.0). Suicide attempters in both cohorts were admitted to the medical intensive care unit of the Lund University Hospital. Within a few days, they were referred to a psychiatric ward of the Lund University Hospital, where they underwent a general physical and psychiatric

examination. Patients in cohort 1 were asked to fill in the Karolinska Scales of Personality questionnaire both when they were first included in the study and at the 10-year follow up.

Suicidal behaviour is studied in paper II.

The schizophrenic cohort

Schizophrenic patients (n=173) were recruited from psychiatric clinics in north-western Stockholm County and assessed for life-time psychiatric diagnosis (DSM-III-R or DSM-IV).[99] 32 patients with an unknown sub-diagnosis or one that was not included among the five sub-classifications of schizophrenia, i.e. 295.1-3, 295.6 and 295.9. were excluded. One patient with ancestors from Sudan was also excluded resulting in a sample of 140 Caucasian patients (87 men and 53 women) with an age (mean±SD) of 44.9±16.7 years. The age of onset of schizophrenia defined as the age at first psychotic symptoms was also available (n=136, 24.2±7.6).

Patients with schizophrenia are studied in paper IV.

The Kungsholmen population

In paper IV, the controls were randomly selected from a longitudinal population-based cohort comprising individuals living in the same region of Sweden as the patients. The Kungsholmen population (n=1090) was first initiated in 1987 and during 12 years thereafter subjects aged 75 years and older and living in the Kungsholmen district of Stockholm were asked to participate. The recruitment and study procedure has been described in detail previously[100] and at www.kungsholmenproject.se. The subjects included in paper IV (n=258) had an age (mean±SD) of 80.5±4.6.

Subjects randomly selected from the Kungsholmen population serve as controls in paper IV.

Personality trait assessment

Personality traits were assessed in paper I-IV using two instruments, the Temperament and Character Inventory (TCI) and the Karolinska Scales of Personality (KSP).

The TCI is a psychometric instrument that was developed to capture both normal and abnormal variation in personality. It is based on a self-administered true/false questionnaire comprising 238 items that have been

translated into Swedish from the original American version.[97, 101] These items form the four temperament dimensions (Table 2): novelty seeking (impulsive vs. reflective), harm avoidance (anxious vs. calm), reward dependence (approval seeking vs. independent), and persistence (steadfast vs. fickle); and the three character dimensions: self-directedness (resourceful vs. helpless), cooperativeness (empathic vs. hostile), and self-transcendence (self-forgetful vs. acquisitive). In this thesis the TCI scores were normalised using a Swedish normative data aimed to represent the general population of Sweden (n=1300, age=20-81).[101]

The KSP inventory consists of a self-report questionnaire that has been widely used in studies involving biological correlates of personality traits.[98, 102, 103] Previous studies have investigated the stability and hereditability different subscales assessed by the KSP questionnaire and found that: i) there are non-significant differences between mean scores in these subscales when tested at two different occasions, indicating the stability of the test and ii) that several of its factors are partly heritable.[102, 104] KSP is based on 135 items forming 15 subscales that can further be classified into four factors covering different dimensions of temperament (Table 2): extraversion (comprising the subscales impulsiveness and monotony avoidance), neuroticism (comprising the subscales somatic anxiety, muscular tension, psychic anxiety, psychasthenia, inhibition of aggression, guilt and socialisation), psychoticism (comprising the subscales detachment and suspicion) and non-conformity (comprising the subscales verbal aggression, indirect aggression, irritability and social desirability).[105] In paper I and IV the KSP factors and subscales are standardised to adjust for age and sex using normative data to have a mean±SD of 50±10, i.e. T-scores.

TCI	**TCI dimensions**	**KSP factor**	**KSP subscales**
Temperament	novelty seeking	Extraversion	impulsiveness
	harm avoidance		monotony avoidance
	reward dependence	Neuroticism	somatic anxiety
	persistence		muscular tension
Character	self-directedness		psychic anxiety
	cooperativeness		psychasthenia
	self-transcendence		inhibition of aggression
			guilt
			socialisation
		Psychoticism	detachment
			suspicion
		Non-conformity	verbal aggression
			indirect aggression
			irritability
			social desirability

Table 2 TCI dimensions and KSP factor and subscales.

Genetic analyses

Polymerase chain reaction

The polymerase chain reaction (PCR) is a technique frequently used in molecular genetics in which a specific fragment of DNA is copied and amplified into thousands or even millions of copies. DNA polymerase is the driving force behind DNA replication as it facilitates the synthesis of new strands of DNA by copying a single-stranded template in the 5'→3' direction. The PCR technique utilises this ability in combination with two short and target-specific oligonucleotides (i.e. single-stranded DNA) called primers. The primers are designed to fit the borders of the target region and make the PCR specific. In order for the reaction to take place the sample needs to be exposed to cycles of temperature changes:

1. double-stranded DNA is separated by heat denaturation at 95°C and the primers are able to access their target region
2. the temperature is lowered to the optimal temperature (depending on the primers used) during which the primers anneal to their respective target sequence
3. DNA polymerase (HotStarTaq, Qiagen, Hilden, Germany) is activated at 72°C and uses the primer-bound DNA segments as starting point and initiates the synthesis of a copy of the target DNA region by adding dNTPs (deoxribonucleotide triphosphate; freely available in the sample) in the correct order

This cycle is repeated up to 44 times and for each cycle the number of copies of the target region is doubled leading to an exponential increase of PCR product.

Genotyping

In paper I-IV human genomic DNA is extracted from blood samples using the QIAamp DNA Blood Mini Kit (Qiagen). Genotyping of SNPs is conducted both on-site by Pyrosequencing and at facilities abroad using KASPar (paper II) and Sequenom (paper III) technology. For detailed information on the KASPar and Sequenom methods, the reader is referred to www.kbioscience.co.uk and [106] , respectively. In Pyrosequencing the DNA sequence is determined by detecting the release of pyrophosphate (PPi) that occur when a nucleotide is incorporated by DNA polymerase.[107] The procedure involves the following steps:

1. Amplification of the DNA-sequence by PCR in which one of the primers is biotinylated*
2. Isolation of the biotinylated sequence of the PCR-product
3. Incubation of the biotin-tagged, single-stranded DNA fragment with a sequence primer**
4. Enzyme and substrate are added to each well in the pyrosequencer
5. dNTPs are added one at a time in a specific dispensation order
6. Successful incorporation of dNTP is accompanied by a light-signal that is presented in a Pyrogram (see Figure 3)

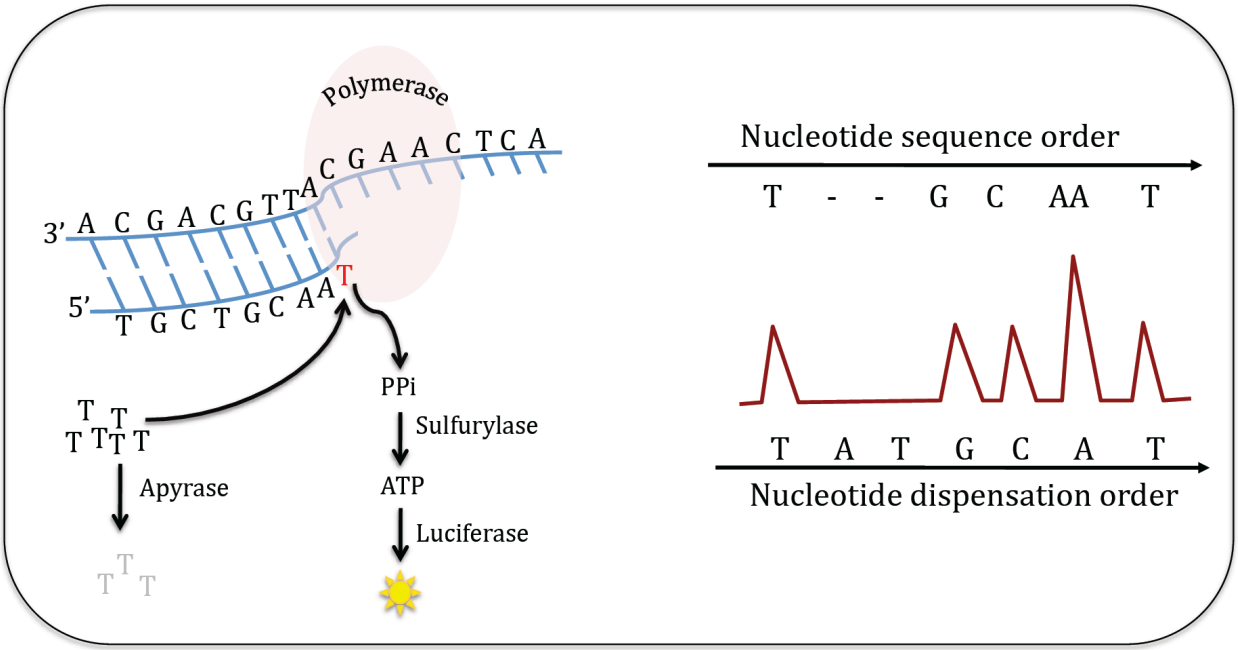


Figure 3 *The Pyrosequencing reaction showing the train of events occurring after the addition of dNTP (in this case dTTP): release of PPi, PPi is converted by sulfurylase into ATP which in turn is converted by luciferase into a light-signal that is detected in real-time. After each cycle, unbound dNTPs as well as ATP are degraded by apyrase before the addition of the next dNTP. The results of the genotyping are reported in the Pyrogram, each peak representing a nucleotide in the sequence that is analysed. The height of the peaks is proportionate to the number of bases that have been incorporated.*

* A biotin molecule is added to the 5'-end of one of the primers. The biotin molecule has the advantage that it binds to streptavidin. The fragment containing the primer-biotin complex can thereby be isolated from the rest of the PCR-product using streptavidin-coated Sepharose beads.

** The sequence primer is designed to anneal to the template in close proximity of the SNP that is being genotyped.

Animal studies

Flinders sensitive and resistant line

The Flinders sensitive line (FSL) rat originate from a breeding attempt to develop a rat-strain that was genetically resistant to the anticholinesterase agent diisopropyl fluorophosphate (DFP).[108] This rat strain would serve in research investigating the mechanisms underlying the development of tolerance towards this pesticide, however, instead a rat-line genetically sensitive to this agent was developed. The FSL rat is now a widely used animal model of depression as these genetically predisposed animals were found to share some behavioural characteristics with depressed individuals including reduced appetite, reduced psychomotor function, increased REM sleep, learning difficultied and increased immobility when exposed to stressors.[109, 110] As in the case of patients with MDD,[66, 111, 112] it has been suggested that FSL have immune abnormalities[110] as well as increased serum levels of pro-inflammatory cytokines.[113] Flinders resistant line (FRL) rats are used as control animals in paper V. This line was bred along side the FSL and was found to be more resistant to DFP than FSL.

FSL and FRL rats are studied in paper V.

Treatment with lipopolysaccharide

Lipopolysaccharide (LPS) is an endotoxin that is a major constituent of the outer membrane of Gram-negative bacteria. LPS administration in rodents has been found to induce an inflammatory response as well as sickness behaviour, characterised by anhedonia (e.g. reduced exploratory activity and social investigation), increased sleep and decreased food intake.[114] The animals in paper V are injected intraperitoneally (i.p.) with LPS *(Escherichia coli* 055:B5, Sigma) (0.5 mg/kg body weight) dissolved in 0.9% sodium chloride (saline vehicle). Control animals are injected with vehicle alone.

The effect of a peripherally induced inflammation on gene expression in the brain of FSL and FRL rats is studied in paper V.

Forced swim test

A modified version of the forced swim test (FST) originally developed by Porsolt and colleagues[115] is used in this thesis. This is a behavioural test applied to rats or mice to assess the antidepressant effect of different drugs.

The model is based on the observation that animals after being placed in a cylinder filled with water will initially try to escape but will ultimately develop an immobile posture.[116] This immobility is believed to reflect either the animals behavioural despair or the incapability to cope with stressful stimuli.

FSL rats have been shown to have higher immobility scores than their respective controls FRL, which is believed to reflect increased behavioural despair.[115] Treatment with antidepressant drugs prior to the FST has been found to increase the escape-oriented behaviour resulting in a decrease in time the animal spends immobile.[117] This has been observed in various rat strains including the FSL.[109] However, the original FST failed to reliably detect the effect of selective serotonin reuptake inhibitors, which raised the need for a modification of the model to increase the sensitivity. This was achieved by increasing the water depth from 15-18 cm to 30 cm and using a time sampling technique in which the investigator distinguishes between three different behaviours (climbing, swimming and immobility) and scores are given to the predominant behaviour every 5 s of the test. In this thesis a transparent acrylic cylinder (diameter 24 cm, height 60 cm, water depth 45 cm) is used which is rinsed and filled with fresh water (25±1 °C) in between each animal being tested. The FST is conducted in two parts, the first exposures to the water tank of 15 min serves the purpose of familiarising the animals to the test and is administered one day before the experimental day. The second part of the procedure is conducted 6 hours following an i.p. LPS or vehicle and lasts for 10 min. The FST was recorded using a camcorder that was set in the same horizontal level as the water tank . The animals were dried with a towel and placed in a cage below an infrared lamp for approximately 10 min following each swim.

The effect of LPS on performance in the FST is studied in paper V.

Quantitative real-time PCR

In paper V, each animal was sacrificed by decapitation 1 h and 20 min after the FST, i.e. 7 h and 20 min after the LPS administration. The brain is removed from the scull and the hippocampus, striatum, amygdala, hypothalamus and prefrontal cortex were carefully dissected out immediately and submerged in RNA stabilisation reagent (Qiagen).

Gene expression can be quantified by measuring the amount of mRNA in a given tissue sample and this is done using a quantitative real-time PCR (qRT-PCR). The steps in this procedure are as follow:

1. RNA isolation from the extracted tissue using the RNeasy Lipid Tissue Mini Kit (Qiagen).

2. Reverse transcription PCR in which RNA is transcribed into complementary DNA (cDNA)*
3. qRT-PCR is performed in a thermocycler using fluorescent probe sequences (TaqMan probes) that are activated during hydrolysis.[118, 119]

In this thesis a custom made 384-well TaqMan® micro fluidic card (Applied Biosystems, Foster City, CA, USA) is used and the reaction is performed in a 7900HT Sequence Detection System (Applied Biosystems). The fluorescent intensity detected in real-time by the apparatus is proportional to the amount of PCR product and is plotted against the cycle number. The resulting curve shows a baseline at which the PCR product is low, an exponential and finally a plateau phase.[119]

In relative quantification of gene expression the aim is to quantify differences in expression of a target gene between different samples. In order to do so a threshold common to all samples is set and the cycle number for each sample at threshold, C_T, is determined. The threshold should hit all curves in the exponential phase as this is the most accurate place to take a measurement of the amplicon. The C_T value is dependent on the amount of mRNA present in the sample; lower C_T values indicating higher mRNA levels. C_T values are obtained from all samples, however, before these can be compared they need to be normalised against the expression level of at least one endogenous control gene using the following formula:

$$\Delta C_T = C_{T\ \text{target gene}} - C_{T\ \text{endogenous control gene}}$$

It is important that the endogenous control is not affected by the studied parameters and that it is equally expressed in all samples. This normalisation will eliminate the uncertainty component caused by having different starting amount of each sample. However, to minimise this uncertainty further the RNA concentration is determined prior to the RT-PCR using a NanoDrop (Thermo Scientific, Wilmington, DE, USA).

The fold change between treated and untreated animals was calculated using the Comparative C_T method for relative quantification[120]:

$$\Delta\Delta C_T = C_{T\ \text{target gene of treated animal}} - C_{T\ \text{target gene of untreated animal}}$$

* RNA is easily degraded by ribonucleases which makes it very unstable and for this reason it must be transcribed into cDNA before proceeding with the qRT-PCR. cDNA differs from genomic DNA in that it is does not contain any non-coding segments as these have been spliced out in the mRNA template.

$2^{-\Delta\Delta C_T}$ = fold change

A fold change of 4 means that the treated animal has a 4-fold increase in expression of the target gene as compared to the untreated sample. When the treatment has resulted in reduced expression of the target gene the fold change is <1. The fold change reduction may then be calculated by taking the negative inverse of $2^{-\Delta\Delta C_T}$.[120]

Gene expression using the qRT-PCR method is used in paper V.

Genetic Statistics

Statistical analysis

The studied polymorphisms in paper I-IV were analysed in relation to personality traits using either independent samples t-test or linear regression. Independent samples t-test is only used when there are no more than two groups to compare and no covariates to control for whilst linear regression is used otherwise.

In paper II and IV genotype frequencies are compared between cases and healthy controls. This is done using the non-parametric χ^2 test. The risk of developing the studied behaviour or disorder if carrying a certain allele was presented with odds ratios (OR) calculated using the following equation:

$$OR = \left(\frac{risk}{1-risk}\right)_{cases} \Big/ \left(\frac{risk}{1-risk}\right)_{controls}$$

in which risk is the frequency of the allele associated with the disease. Departure from the HWE is calculated for all populations using χ^2 test to assure that the genotyping results are reliable.

In paper V, gene expression differences in two different rat-lines that were each treated with LPS or vehicle were compared between groups using a two-way analysis of variance. In this analysis the two independent variables are rat-line and treatment while gene expression levels serve as the dependent variable.

Power analysis

A general aim in genetic association studies is, e.g., to test whether a certain genetic variation has an effect on a studied trait. The investigator has two hypotheses to test: i) that the genotype has no affect on the trait (i.e. the null hy-

pothesis), and ii) that the genotype has an effect on the trait. In the present thesis the null hypothesis is rejected if a statistical significance level of $\alpha<0.05$ is demonstrated. This is to limit the risk of making a Type 1 error, i.e. rejecting the null hypothesis when it is in fact true.

It is also a risk of erroneously accepting the null hypothesis when there is an actual difference between the genotypes; and this is referred to as a Type 2 error. A power analysis may be performed to measure the probability of avoiding a Type 2 error by a given statistical test.[121] Increased power indicates a decreased risk of making a Type 2 error. The power of a test depends on the type of statistical test (parametric tests generally having higher power than non-parametric tests), sample size (increased power with larger sample sizes), effect size (i.e. the influence of the independent variable on the dependent variable), variability (increased power with lower variability) and the α-level set by the investigator .

*Power analysis are made in paper III and IV using the software G*power (version 3.1.2).*

Adjustment for multiple tests

The risk of making a Type I error increase with the number of statistical tests that are made. In order to minimise this risk it has become more or less the norm for the researcher to make adjustments for multiple testing. There are several methods that can be applied; however, in this thesis adjustments are made using the Bonferroni principle. This principle is based on the observation that even when the null hypothesis is true, a significant difference will still be seen by chance once in 20 trials.[122] In order to control for this possibility the p-value is adjusted by simply multiplying it with the number of tests that were performed. Although acceptable when searching for significant associations without pre-established hypotheses, the Bonferroni adjustment method has been criticised.[123] Not only does it increase the risk of a Type II error but it does not take into account that researchers may often have an a priori hypothesis that is supported by previous findings which in turn should decrease the probability of making a Type I error.

Bonferroni adjustments were made in paper III and IV.

Linkage disequilibrium

In paper III, two SNPs in the same gene are analysed. As these SNPs are located in close proximity to each other, it is necessary to investigate whether the two

loci are dependent of each other, i.e. whether they are in LD or not. LD can be calculated by deducting the product of allele frequencies from the observed frequency of that haplotype[124]:

$$D = x_1 - p_1 q_1$$

in which x_1 is the frequency of the haplotype A_1B_1, p_1q_1 is the expected frequency of this haplotype as p_1 is the frequency of A_1, and q_1 is the frequency of B_1. D is standardised using the following equation:

$$D' = \frac{D}{D_{\max}}$$

where $D_{\max}$ is the maximum disequilibrium possible for the given allele frequencies.[125] The closer D' is to 1 the more dependent are the two loci of each other. The correlation coefficient, r^2, is another measure used to describe LD:[124]

$$r^2 = \frac{D^2}{p_1 p_2 q_1 q_2}$$

If D'=1 and r^2=1, then one allele at a specific locus will always be inherited with a certain allele at the second locus and the allele-frequencies are thus the same for both loci. A D'=1 and r^2<1 indicates that there is LD between the two loci, however, knowing one of the polymorphisms will not reliably determine the other.[124]

RESULTS AND DISCUSSION

Paper I. Genetic variability within the innate immune system influences personality traits in women

As discussed above, raised levels of inflammation markers have been associated with several mental disorders;[83, 111, 126-129] however, studies regarding the relationship between inflammation or the immune system and various aspects of normal human behaviour are not numerous.

In paper I, the possible influence of two SNPs found in the candidate genes *CRP* and *CFH* on personality traits in middle-aged women (n=270) is investigated. The genes and SNPs were selected after reviewing previously published articles. The +1444C>T (rs1130864) polymorphism located in the 3'UTR of the *CRP* gene has been associated with serum levels of CRP[130-132] and the non-synonymous Y402H (1277T>C, rs1061170) polymorphism located in exon 9 of *CFH* appears to be both functional and an established risk factor of AMD. The personality traits were assessed by KSP (see above).

In this study we report that the +1444T allele in *CRP* is associated with higher scores of impulsivity, monotony avoidance and social desirability in women. Interestingly, this is the same allele that has been linked to increased serum levels of CRP. Taken previous findings demonstrating increased levels of CRP in personality disorders[133] and in women with low levels of positive wellbeing into consideration,[134] it may be suggested that the studied polymorphism influences CRP levels that via unknown mechanisms influence brain functions. Of further interest is the fact that higher scores of the personality trait novelty seeking, relating to a person's impulsivity, extravagance and the tendency to respond strongly to novelty and actively avoid monotony,[135, 136] have been associated with depression,[137, 138] a disorder shown to be characterized by elevated serum levels of CRP in several studies.[57, 139]

The 402H allele (i.e. 1277C) in *CFH* was associated with higher scores of the personality trait verbal aggression and also with lower scores in social desirability, i.e. the same trait that was significantly associated with the +1444C>T polymorphism. The 402H allele has repeatedly been associated with AMD and has recently been linked to AD,[140] CVD[141] and stroke.[142] The amino acid substitution caused by the 402H is located at the site where CRP binds to CFH,[143] increasing the ability of CFH to regulate the alternative pathway of the complement system. Consistent with this mechanism, the 402H allele of the Y402H polymorphism has been associated with reduced binding of CRP to CFH[143, 144]

and to increased levels of CRP[141, 145] as well as IL-6,[141] indicating that the 402H does indeed influence pro-inflammatory mechanisms.

How the studied polymorphisms actually influence personality traits remains to be disclosed. Whereas effects of polymorphisms in genes related to neurotransmission on personality traits have been reported by others;[146, 147] genes regulating inflammation have previously not been studied in this context. As discussed above, the immune system is known to be highly interlinked with the central nervous system,[84, 148] and activation of certain components of the immune system may thereby affect neuroendocrine and neurotransmitter processes.[111] The complement system has also been suggested to be involved in neurogenesis[47, 149] as well as in the elimination of synapses in the CNS.[150] With this in mind coupled with the strong hereditary component underlying an individuals personality traits, it is not farfetched to suggest that genetic variations in genes related to the innate immune system may have an impact on these.

Our findings may shed further light upon previous observations regarding personality traits and disorders associated with inflammation such as depression and CVD. Various personality traits, including anger, have been reported to predict morbidity in both depression and CVD.[151-153] The association between verbal aggression and the *CFH* genotype suggests that the relationship between personality traits and CVD may not only be a direct causal one, but partly due to the fact that genes related to inflammation could be independently associated with both personality and risk for CVD. Likewise, the recognized co-morbidity between depression and CVD[154-156] may be partly explained by a genetic vulnerability within the innate immune system concomitantly influencing risk for depression and risk for CVD, rather than by the mental stress of depression solely inducing a peripheral inflammation leading to CVD.

In paper I, the CRP +1444T allele was associated with higher scores of impulsivity, monotony avoidance and social desirability in a population-based cohort of middle-aged women. In the same cohort, the CFH allele 402H was associated with higher scores of verbal aggression and lower scores of social desirability.

Paper II. The +1444C>T polymorphism in the CRP gene is associated with impulsivity and suicidal behaviour

Twin studies have shown that suicidal behaviour is partly attributable to genetic factors.[157, 158] However, molecular studies have so far focused on genes of importance for monoaminergic transmission[159, 160] where e.g. genes coding for

the serotonin transporter[161] and catechol-O-methyltransferase[162] have been suggested to be of relevance. Research on genetic variations in immune-related genes and suicide is hitherto sparse.

Prompted by our finding in paper I, where the +1444C>T SNP located in the *CRP* gene was found to be associated with impulsivity, a personality trait that is an important risk factor for suicidal behaviour,[163] we wanted to investigate whether this polymorphism predisposes for suicidal behaviour. We expected the same allele linked to increased scores of the KSP trait impulsivity (i.e. +1444T) to be more frequent in suicide attempters (n=98) when compared to controls (n=516). As one of the patient cohorts had been tested with respect to KSP shortly after the suicide attempt, we also explored if this polymorphism was associated with impulsivity scores also in suicidal patients.

The +1444T allele was not only found to increase the susceptibility for suicidal behaviour (see Figure 4a), but the presence of this allele in patients that were assessed by KSP was also associated with significantly higher scores in impulsivity when compared to +1444CC carriers (see Figure 4b).

How do we interpret these results? The suggested involvement of inflammation in mechanisms important for neural plasticity and the development of the CNS (see above) gives us reason to believe that a genetic variation in the gene coding for the acute phase reactant such as CRP may influence the balance of neuroglia interactions by e.g. increased expression of the protein. However, these interpretations should be regarded as preliminary as the underlying mechanism regarding the potential influence of peripherally expressed CRP on brain functions needs to be established; also, it is not known whether CRP is expressed in the brain in the absence of an ongoing infection/inflammation.

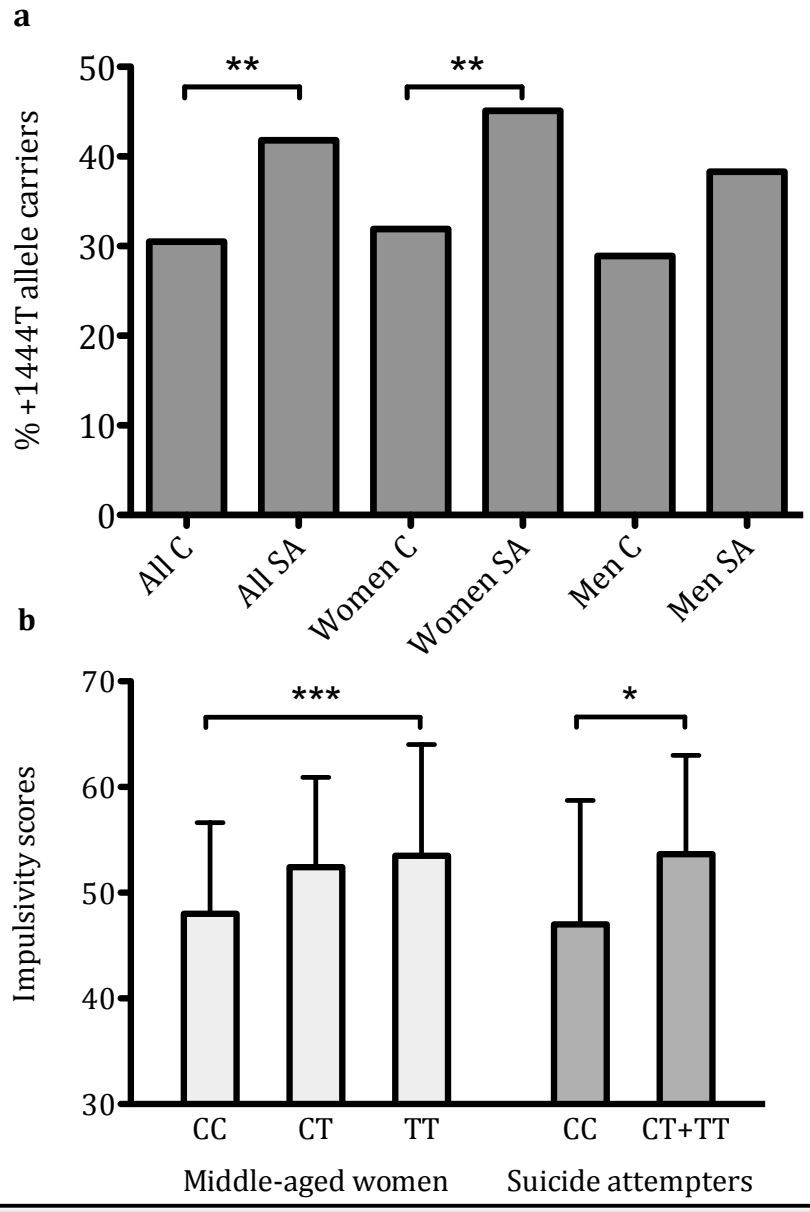
Further support for this hypothesis is the suggested role of neurodevelopmental abnormalities in both personality disorders and psychopathy[164] as it is possible that also the risk of suicidal behaviour is at least partially established during the development of the brain. Secondly, peripheral inflammation has been reported to inhibit the synthesis of serotonin in the brain[72] and CSF levels of cytokines in suicide attempters have been shown to correlate with monoamine metabolites.[76] It is thus possible that the studied SNP affects the reactivity of the inflammatory response which in turn may alter monoamine neurotransmission and thereby generate psychiatric symptoms in susceptible individuals.

Several studies have previously shown that impulsivity, together with psychiatric morbidity, is a major determinant for suicide attempts.[165-167] In light of the results in paper I and II, we suggest that carriers of the CRP +1444T allele,

which was associated with increased impulsivity scores both in a normal population and in suicide attempters, may be at increased risk of developing suicidal behaviour.

In paper II, the +1444T allele was found to predispose to suicidal behaviour and was again associated with impulsivity scores this time in suicide attempters.

Figure 4.a) *Distribution of the +1444C>T allele in controls (C) and suicide attempters (SA).* ***b)*** *mean±SD of impulsivity scores assessed by KSP in middle-aged women (paper I) and suicide attempters (paper II). Due to the small sample size, +1444CT and TT carriers were merged in the patient cohort.*
** p≤0.05; ** p≤0.01; *** p≤0.001*



Paper III. Genetic variability within the S100B gene influences the personality trait self-directedness

During brain injury as well as peripheral immune response, microglia and astrocytes in the brain are activated resulting in elevated levels of S100B as well as a disrupted BBB. This in turn leads to the subsequent diffusion of S100B into the blood stream where it serves as a serum biomarker of the severity of brain injury. During the past decade, several studies have reported elevated serum levels of S100B in a number of psychiatric disorders such as major depression,[168] suicidal behaviour[169] and schizophrenia.[88]

In paper III, we sought to investigate whether two polymorphisms in the *S100B* gene, 2757C>G and 5748C>T, influence personality traits assessed by TCI in middle-aged women (n=270) and men (n=247). The two SNPs have previously been reported to constitute a haplotype that was found to be more frequent in schizophrenic patients when compared to healthy controls.[170]

The 2757C>G polymorphism was found to significantly influence self-directedness scores in men and women (Figure 5), however, in the latter cohort this significance did not survive Bonferroni correction. In addition, men homozygote for the 5748C had significantly lower scores as compared to the 5748CT/TT group in self-directedness.

The personality trait self-directedness relates to a person's ability to adapt to a situation in a way that corresponds with concepts of one's own behaviour, evaluation of their consequences and personal goals. High scores of this trait are indicative of, e.g., high self-esteem and responsibility, whereas low scores are highly predictive of a personality characterised by lack of internal control, self-acceptance and a clear personal identity. Low scores are reported in several psychiatric diseases including depression[171] and schizophrenia.[172] Self-directedness thus forms on overall estimate of mental health in relation to oneself.

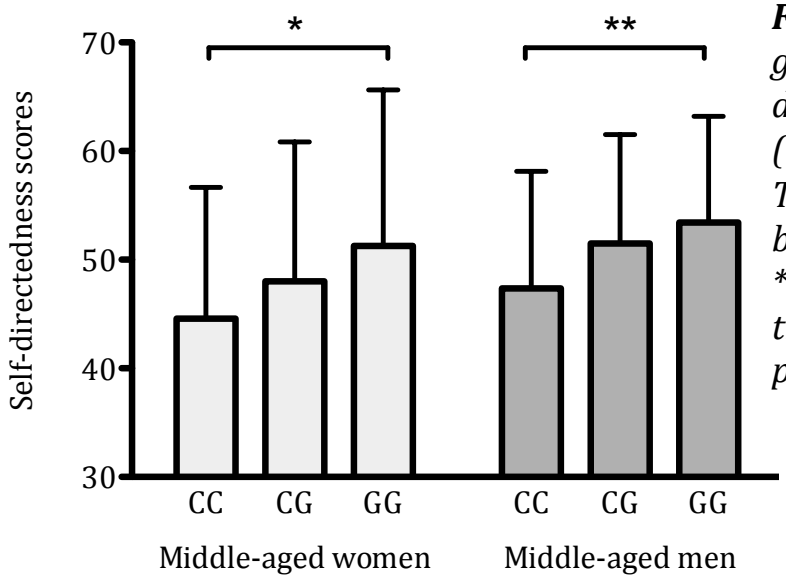


Figure 5. *The 2757C>G genotype and Self-directedness scores (mean±SD) assessed by TCI in two population-based cohorts. * p≤0.05; ** p≤0.01 before correction was made for multiple testing.*

With respect to previous findings we draw the following conclusions. Firstly, S100B appears to be of relevance not only for brain dysfunction such as mood disorders and schizophrenia[88, 173, 174] but also seems to influence normal human behaviour. Secondly, the fact that the two SNPs are associated with self-directedness and have been identified as a susceptibility haplotype for schizophrenia[170] indicates that these phenomena may partially share the same genetic origin.

S100B has been found to inhibit phosphorylation of synaptic proteins in a Ca^{2+}-dependent manner and may thus be involved in signal transduction.[88] The protein has also been reported to interact with the dopamine D_2-receptor, an interaction that was found to result in an enhanced receptor signalling.[175] Given the well-established influence of neurotransmitters on an individual's personality,[176] one could speculate on the possibility that influence of variations in *S100B* on personality is secondary to an influence on brain neurotransmission.

Moreover, it has been suggested that extracellular S100B mediates both its neurotrophic and toxic effects by interacting with RAGE. At micromolar levels, S100B has been proposed to stimulate RAGE on neurons, leading to an overproduction of reactive oxygen species ultimately resulting in apoptosis while stimulation of RAGE found on astrocytes up-regulates the transcription factor NF-κB that in turn results in the transcription of several pro-inflammatory cytokines.[49] This may in part explain the findings in the present study as inflam-

matory processes have been suggested to be of relevance for normal brain development and also for brain functions in healthy adults.[177]

With the present findings in mind, it is possible that the raised S100B levels observed in patients with various psychiatric diseases is not merely a consequence of illness, but may in fact indicate that astrocytic activation with the subsequent release of S100B in the brain is of importance in the mechanisms underlying such diseases.

In paper III, two SNPs located in the S100B gene were associated with self-directedness scores in middle-aged men. One of the SNPs, 2757C>G, was also associated with self-directedness in middle-aged women (same direction as in men), however, this finding did not remain significant after correction for multiple testing.

Paper IV. Is the Gly82Ser polymorphism in the RAGE gene of relevance for schizophrenia and the personality trait psychoticism?

Prompted by our findings in paper III, we next studied if a functional SNP (Gly82Ser) in the gene coding for the S100B receptor RAGE affects personality traits in these population-based cohorts of middle-aged women (n=270) and men (n=247). This non-synonymous polymorphism is located in the ligand binding domain of RAGE and the Gly/Ser genotype (very few Caucasians are homozygotes for the Ser allele) has previously been linked to i) increased pro-inflammatory induction followed by the interaction of e.g. S100B with RAGE[178] and ii) decreased plasma levels of soluble RAGE (sRAGE).[179] sRAGE is a spliced form of the receptor secreted in plasma that competes with cell-bound RAGE by binding to the same ligands but lacking the signalling ability.[180] sRAGE was recently suggested to regulate the detrimental effects of S100B seen in schizophrenic patients.[174] With these findings in mind we performed a case-control study, using randomly chosen controls from the Kungsholmen population (n=258) and schizophrenic patients (n=138), in order to investigate whether the Gly82Ser SNP predisposes to schizophrenia.

We found that the 82Ser allele was associated with higher scores in psychoticism and its subscales detachment and suspicion (compared to Gly homozygotes) when the two cohorts were merged (Figure 6a). Moreover, the case-control study revealed that the 82Ser allele increased the susceptibility to schizophrenia and was potentially associated with an earlier age of onset in male patients (Figure 6b-c).

There is a consistency between the different findings within this study as well as with previously published results. Firstly, the 82Ser allele was associated with increased scores in such personality traits that are featured among the symptoms of schizophrenia.[176, 181, 182] The same allele was found to predispose to schizophrenia and to affect the age of onset in men. This gives us reason to believe that the Gly82Ser SNP influences normal variability in the personality which in turn, along with additional risk factors, renders certain individuals susceptible to psychopathology. Secondly, two independent studies showed an association between the 82Ser allele and the risk of developing Alzheimer's disease.[183, 184] Interestingly, cognitive deficits[185] and neuronal atrophy[186] are suggested to be prevalent also in patients with schizophrenia and it is thus possible that the Gly82Ser polymorphism is a common denominator for cognitive impairment.

As mentioned above, neurobiological factors in the developing brain are of relevance for the personality of an individual[56] and schizophrenia is suggested to be a neurodevelopmental disorder.[187] Our results may thus indicate that the influence of the studied polymorphism on mental functions is occurring early in life. In addition, the current finding with regards to age of onset and genotype provides further support for this hypothesis as it is likely that a variant that causes susceptibility to schizophrenia via neurodevolopmental processes also speeds up the onset of disease.

In light of the present literature on RAGE in conjunction with the current findings we hypothesise i) that carriers of the 82Ser allele have increased RAGE-signalling leading to increased secretion of inflammatory mediators which may influence mental brain functions through either developmental processes and/or neurotoxicity and ii) that the elevated serum levels of S100B seen in psychiatric patients may signify the involvement of the RAGE:S100B axis in the aetiology of psychiatric disorders as oppose to merely being elevated as a consequence of such disorders.

In paper IV, the 82Ser allele was associated with increased factor scores of psychoticism in a merged sample of middle-aged men and women. The same allele was also found to increase the risk of schizophrenia and lower the age of onset of the disease in men.

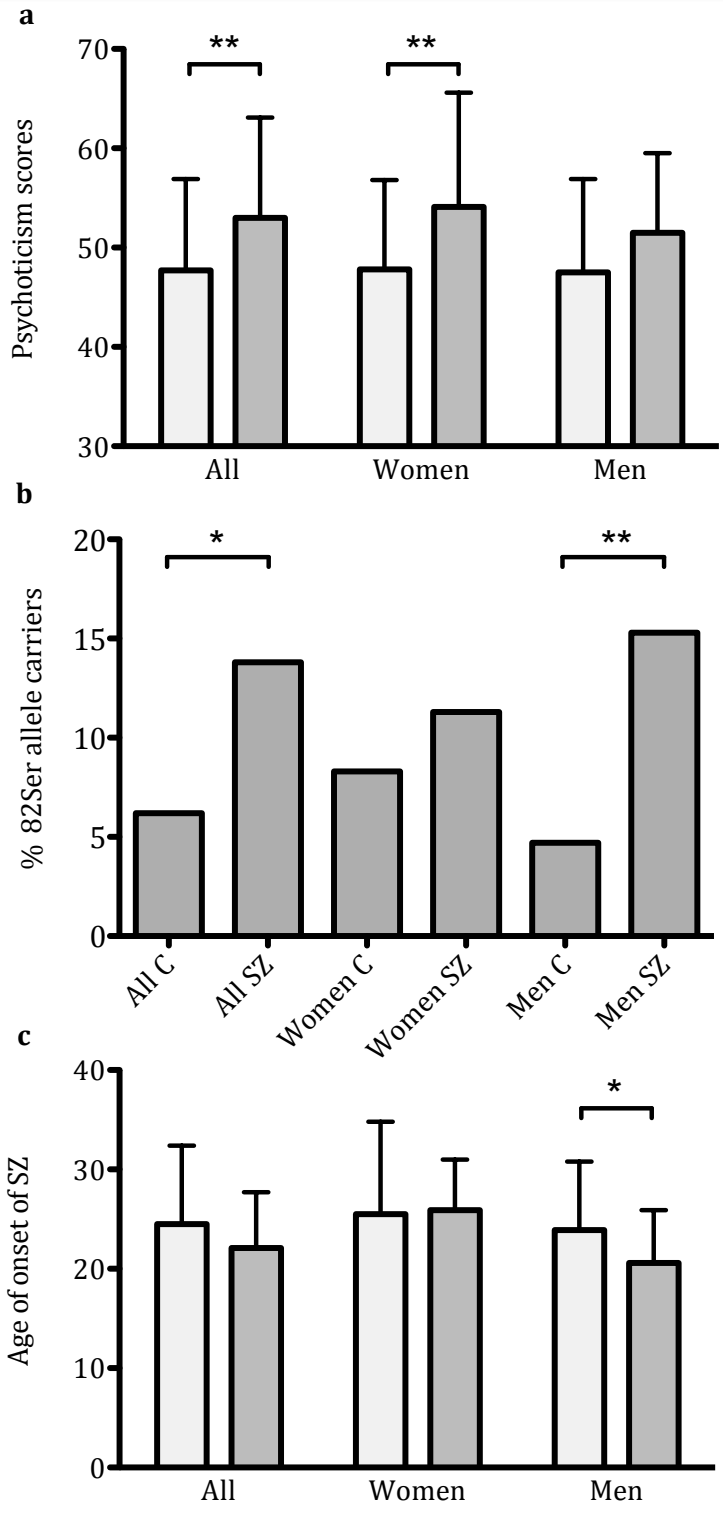


Figure 6 a *Gly82Ser genotype (light=82Gly/Gly, dark=82Gly/Ser+Ser/Ser in all diagrams) and psychoticism scores in two population-based cohorts of women and men (mean±SD).* ***b*** *Frequencies of controls (C) and schizophrenic patients (SZ) carrying the 82Ser allele.* ***c*** *Age of onset of schizophrenia in the different genotype groups. * p≤0.05; ** p≤0.01*

Paper V. Expression of S100B in a genetic rat model of depression – a pilot study

LPS has been shown to induce depressive-like behaviour in animals via mechanisms involving the innate immune system.[188] The effect of LPS on neither S100B expression in the brain nor on FSL rats has been established. The aim of paper V was therefore to investigate whether these spontaneously depressed animals differ in terms of S100B expression in various regions of the brain both at baseline and following an intraperitoneal (i.p.) injection of LPS using FRL animals as controls. We further aim to investigate whether FSL are more sensitive to the depressive-like effects of LPS by testing all animals in the FST.

We found that S100B expression was significantly increased at baseline in the amygdala, hippocampus, prefrontal cortex and striatum of the FSL rats when compared to the FRL rats. An effect of LPS treatment was observed only in the hypothalamus of both rat lines with a down regulation of S100B following treatment.

The animals were also tested in a behavioural test, the FST, which showed that FSL rats were more prone to climbing behaviour and spent less time swimming compared to the control rats. LPS treatment had no significant effect on the behavioural outcome in the FST for neither FRL nor FSL. However, we did observe a tendency towards higher immobility scores in both the FRL LPS and FSL LPS group (data not shown). We believe that this tendency may benefit from adding more animals to the study in the future.

Our findings regarding S100B expression are in line with a recent animal study where S100B levels were increased in serum of two rat models of depression.[189] In contrast to our results, they did not find S100B gene expression to be elevated in neither the striatum nor the hippocampus and instead found down-regulated expression in the prefrontal cortex. However, the depression models used in that study consisted of olfactory bulbectomy and chronic unpredictable stress applied on male Sprague-Dawley rats. The discrepancy from our results may thus be due to the fact that the current study used a genetic animal model of depression as opposed to an induced depression model.

The mechanisms underlying the association between animal depression models and S100B have not been established. One can speculate on, in accordance to psychiatric disorders in humans, that S100B may be of relevance for depressive-like behaviour. However, whether the supposed raised levels in brain of the glial protein S100B presented here are part of the aetiology or just a consequence of depressant behaviour needs to be established. Interestingly,

glial pathology is implicated in mood disorders[190] - this theory stems from various *post mortem* studies of patients with mood disorders in which reduced glial cell density in prefrontal brain regions has been revealed.[73-75]

The neuroprotective role of S100B must also be taken into consideration as elevated serum levels of S100B have been found to correlate positively with treatment outcome of antidepressants in patients with MDD.[191] In this vein, one could suggest the possibility that S100B is up-regulated in the brain of FSL animals as a compensatory mechanism induced by e.g. neural damage or stress.

The cause of the down regulation of S100B in hypothalamus after LPS treatment is not obvious. It is possible that the expression of S100B is differently affected by immune activation in different parts of the brain. Due to the low number of animals used in this study further investigations need to be performed before any conclusions can be drawn.

The FSL rats compared to the controls did not behave as expected by previous reports where they have been shown to spend more time immobile;[110] however, a low number of animals were used in this pilot study and the difference in immobility scores between the two lines did not reach the level of significance. Instead we found a significant increase in climbing scores and significantly decrease in swimming scores in the FSL rats when compared with FRL.

Preliminary investigations that are not reported in paper V show a positive correlation between S100B and inducible nitric oxide synthase (iNOS) expression ($p=0.032$) in the studied animals. In fact, mRNA expression of iNOS was detectable in 24 samples of the FSL LPS group compared to only nine samples of the FRL LPS group [χ^2-test: $p=0.00007$]. iNOS is not expressed in the brain under normal conditions, however, high doses of S100B in combination with LPS have been found to induce the synthesis of the enzyme by microglia.[192, 193] This leads to the subsequent production and release of high levels of NO by these cells which in turn have been suggested to cause neuronal death. [194] It is thus likely that the increased expression of S100B seen in FSL rats represents detrimental levels of the protein as they coincide with an up-regulation of iNOS following LPS treatment. These findings further support the notion that the spontaneously depressed FSL is susceptible to neuroinflammatory responses due to a genetic predisposition.

The findings in paper V indicate that the cellular changes seen in mood disorders in terms of glial pathology may stem from a genetic predisposition to elevated S100B expression.

In paper V, increased gene expression at baseline of S100B is reported in several brain regions of FSL when compared to FRL.

CONCLUDING REMARKS

The immune system is vital for the survival of any given organism and evidently so as many of its components have been conserved throughout the evolution. It is becoming increasingly clear that immune-related processes, namely inflammation, are not only involved in defending the host against harmful stimuli but also that they are involved in mechanisms vital for the brain like e.g. neural plasticity.

As is the case of most systems influencing the brain there is a tight regulation of cytokine expression in brain; however, the beneficial effects of these mediators on brain function may become detrimental when the delicate balance is disarranged.

Hitherto, studies regarding the immune system and brain functions have mainly focused on psychological and environmental stimuli. The investigations undertaken in this thesis aimed to go further and study the possible influence of genetic variations in immune-related genes on personality traits and psychiatric morbidity.

Genetic variability in the studied genes was found to be associated with personality traits. Even though numerous other genes implicated in e.g. neurotransmission are of importance for the personality of an individual it is not farfetched to suggest that our findings may at least partly illuminate the established relationship between personality traits and inflammatory disorders such as cardiovascular diseases and psychiatric disorders.

In addition, we found that genetic variations in inflammatory genes made individuals susceptible to psychiatric illness. Therefore we suggest that the increased serum levels of pro-inflammatory mediators seen in patients with psychiatric disorders are not merely the result of mental stress but may also reflect a genetic vulnerability towards increased expression of these mediators.

The current thesis further reports that certain genetic variations found to be more common in psychiatric illness were also associated with personality traits (in population-based cohorts) that constitute common features of that particular illness. That is, these personality traits and psychopathological conditions appear to partially share the same genetic background.

It is of utmost importance to define animal models that can be used when studying the influence of inflammation not only on behaviour but also on cellular mechanisms in the brain. We used a genetic animal model of depression

(FSL) and found that these animals have increased expression of an inflammation-related gene at baseline in various parts of the brain when compared to control animals. This model may therefore be suitable for studying the pathological mechanisms linking inflammatory processes to MDD.

Finally, the results in this thesis support the suggested importance of the immune system for psychiatric disorders and may lead to the development of new treatment strategies for these disorders.

SAMMANFATTNING PÅ SVENSKA

Immunförvaret har i huvuduppgift att försvara en organism mot angrepp som exempelvis uppstår när den utsätts för bakterier, virus eller vävnadsskada och är därför avgörande för organismens överlevnad. Detta återspeglas i det faktum att många av de aktörer som medverkar i immunförsvaret har bevarats genom evolutionen och återfinns i de mest primitiva av arter. De senaste årtiondenas forskning har visat att immunrelaterade processer, särskilt inflammatoriska sådana, även har andra uppgifter än att skydda mot diverse angrepp. Det är visat att kroppsegna ämnen som stimulerar inflammation, så kallade cytokiner, styr olika processer i hjärnan som är viktiga för exempelvis inlärning och neuroplasticitet (d.v.s. hjärnans förmåga att omorganisera sig i respons till förändringar i miljön).

Precis som är fallet för kroppens övriga system är mängden cytokiner som bildas i hjärnan högst kontrollerad. Cytokin-medierade effekter kan å ena sidan vara fördelaktiga för hjärnan när de produceras i måttliga mängder men när dessa mängder ökar som exempelvis vid sjukdom kan det å andra sidan leda till att hjärnans olika celler tar skada. Det har i tidigare studier visats att psykiatriska sjukdomar är förknippade med en ökad kroppslig låg-gradig inflammation, dock är det inte känt hur denna inflammation uppstår. Det skulle kunna vara så att den stress som upplevs vid en psykisk sjukdom leder till aktivering av kroppens immunsystem men det kan också vara så att inflammation i kroppen leder till att hjärnan drabbas på ett sådant sätt att mental ohälsa uppstår.

Hittills har studier som undersökt immunförsvarets effekter i hjärnan huvudsakligen fokuserat på hur miljö- och psykologiska faktorer påverkar dessa förhållanden. Arbetet i denna avhandling har som syfte att gå steget längre och studera huruvida genetiska variationer i inflammationsrelaterade gener påverkar en människas personlighetsdrag samt huruvida dessa kan leda till ökad risk för psykisk sjukdom.

Här presenteras att variationer i de inflammationsgener vi undersökt påverkar personlighetsdrag som i sin tur tidigare ansetts vara relevanta för psykisk sjukdom. Även om flertalet andra typer av gener har visats vara av betydelse för personligheten så är det möjligt att våra fynd till viss del belyser de redan fastställda sambanden mellan personlighetsdrag och andra sjukdomar kopplade till inflammation såsom kardiovaskulära och psykiska sjukdomar.

Vidare presenteras att genetiska variationer i två av de studerade generna ökar risken för psykisk sjukdom. Detta skulle kunna vara orsaken till varför

man tidigare visat förhöjda eller förändrade nivåer av dessa proteiner (d.v.s. de proteiner som kodas av dessa två gener) hos psykiskt sjuka patienter snarare än den traditionella åsikten att själva sjukdomen leder till ökade/förändrade nivåer.

För att kunna studera de bakomliggande mekanismerna som leder till att inflammation påverkar beteende krävs att en lämplig djurmodell utvecklas. I den här avhandlingen använde vi oss av en genetiskt framavlad råtta som visar tecken på depression. Vi fann att denna stam hade högre genuttryck av ett inflammationsrelaterad protein i flera delar av hjärnan när dessa jämfördes med normala djur. Detta tyder på att denna djurmodell kan vara lämplig vid framtida studier som ämnar undersöka de patologiska mekanismer som kopplar inflammation till depression.

Slutligen styrker resultaten i den här avhandlingen immunförsvarets föreslagna roll i psykiatriska sjukdomar och kan stå till grund för vidare studier som förhoppningsvis kan leda till utvecklingen av nya behandlingsmöjligheter.

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