From the DEPARTMENT OF CLINICAL NEUROSCIENCE
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MOLECULAR GENETIC STUDIES ON CEREBROSPINAL FLUID MONOAMINE METABOLITES IN PSYCHOTIC PATIENTS AND HEALTHY INDIVIDUALS

Dimitrios Andreou

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Molecular genetic studies on cerebrospinal fluid monoamine metabolites in psychotic patients and healthy individuals

THESIS FOR DOCTORAL DEGREE (Ph.D.)

by

Dimitrios Andreou

Principal Supervisor:
Associate professor Erik Jönsson
Karolinska Institutet, Stockholm, Sweden
Department of Clinical Neuroscience
Division of Psychiatry

Co-supervisor:
Professor Ingrid Agartz
University of Oslo, Oslo, Norway
Department of Clinical Medicine
Diakonhjemmet Hospital, Oslo, Norway
Department of Psychiatric Research
Karolinska Institutet, Stockholm, Sweden
Department of Clinical Neuroscience

Opponent:
Professor emeritus Jayanti Chotai
Umeå University, Umeå, Sweden
Department of Clinical Sciences
Division of Psychiatry

Examination Board:
Associate professor Catharina Lavebratt
Karolinska Institutet, Stockholm, Sweden
Department of Molecular Medicine and Surgery
Neurogenetics Unit
Karolinska University Hospital, Solna, Sweden
Center for Molecular Medicine

Professor emerita Lil Träskman-Bendz
Lund University, Lund, Sweden
Department of Clinical Sciences
Division of Psychiatry

Professor emeritus Leif Lindström
Uppsala University, Uppsala, Sweden
Department of Neuroscience
Division of Psychiatry

ABSTRACT

Dopamine, serotonin and noradrenaline are the major monoamines in the human central nervous system (CNS) and following their basic pathways they are degraded to their major metabolites homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG), respectively. The cerebrospinal fluid (CSF) concentrations of the three monoamine metabolites (MM) are considered to reflect the monoamine turnover rates in the CNS, are under genetic influence and have been associated with schizophrenia.

In the first part of the thesis (Studies I, II and III), 132 healthy individuals (78 men and 54 women) were included, whereas in the second part of the thesis (Studies IV and V) 74 psychotic patients (45 men and 29 women) participated. CSF samples were drawn by lumbar puncture and genomic DNA was extracted from whole blood samples for genotyping.

In the first part of the thesis, we have searched for association between single nucleotide polymorphisms (SNPs) in the tryptophan hydroxylase 1 (*TPH1*), dystrobrevin binding protein 1 (*DTNBP1*) and D-amino acid oxidase activator (*DAOA*) genes and the CSF MM concentrations in healthy individuals in order to shed further light to the understanding of the effect of the genes on CSF MM in humans. One *TPH1* SNP and one *DTNBP1* SNP were found to be significantly associated with both CSF 5-HIAA and HVA, giving evidence for association between the genes and dopamine and serotonin turnover rates in CNS. Two *DAOA* SNPs were significantly associated with CSF HVA concentrations, proposing that the *DAOA* gene is implicated in dopaminergic mechanisms.

In the second part of the thesis, we searched for association between genes implicated in dopamine, serotonin and noradrenalin metabolism (Study IV) and glutamate-related genes (Study V) and CSF MM concentrations in psychotic patients. Several nominal associations and one significant association between *MAOB* and MHPG in men (Study IV) were found, suggesting that CSF MM concentrations can be considered as psychosis intermediate phenotypes in previously reported associations between gene variants and the disorder.

Keywords: Cerebrospinal fluid (CSF), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylglycol (MHPG), psychiatric genetics, single nucleotide polymorphisms (SNPs), schizophrenia, psychosis, intermediate phenotypes, healthy individuals

LIST OF SCIENTIFIC PAPERS

I. Andreou D., Saetre P., Werge T., Andreassen O.A., Agartz I., Sedvall G.C., Hall H., Terenius L., Jönsson E.G. Tryptophan hydroxylase gene 1 (*TPH1*) variants associated with cerebrospinal fluid 5-hydroxyindole acetic acid and homovanillic acid concentrations in healthy volunteers. Psychiatry Research 2010:180;63-67.

II. Andreou D., Saetre P., Kähler A.K., Werge T., Andreassen O.A., Agartz I., Sedvall G.C., Hall H., Terenius L., Jönsson E.G. Dystrobrevin-binding protein 1 gene (*DTNBP1*) variants associated with cerebrospinal fluid homovanillic acid and 5-hydroxyindoleacetic acid concentrations in healthy volunteers. European Neuropsychopharmacology 2011:21;700-704.

III. Andreou D., Saetre P., Werge T., Andreassen O.A., Agartz I., Sedvall G.C., Hall H., Terenius L., Jönsson E.G. D-amino acid oxidase activator gene (*DAOA*) variation affects cerebrospinal fluid homovanillic acid concentrations in healthy Caucasians. European Archives of Psychiatry and Clinical Neurosciences 2012:262;549-556.

IV. Andreou D., Söderman E., Axelsson T., Sedvall G.C., Terenius L., Agartz I., Jönsson E.G. Polymorphisms in genes implicated in dopamine, serotonin and noradrenalin metabolism suggest association with cerebrospinal fluid monoamine metabolite concentrations in psychosis. Behavioral and Brain Functions 2014:10;26.

V. Andreou D., Söderman E., Axelsson T., Sedvall G.C., Terenius L., Agartz I., Jönsson E.G. Cerebrospinal fluid monoamine metabolite concentrations as intermediate phenotypes between glutamate-related genes and psychosis. Submitted manuscript.

CONTENTS

LIST OF ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
CNS	Central nervous system
CNVs	Copy number variants
CSF	Cerebrospinal fluid
D-DOPA	D-3,4-dihydroxyphenylalanine
DNA	Deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorder
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, Third Edition - Revised
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
GWAS	Genome Wide Association Studies
HVA	Homovanillic acid
ICD	International Classification of Diseases
ICD-10	International Classification of Diseases, Tenth Revision
LD	Linkage disequilibrium
L-DOPA	L-3,4-dihydroxyphenylalanine
MHPG	3-methoxy-4-hydroxyphenylglycol
MM	Monoamine metabolites
mRNA	Messenger RNA
NMDARs	N-methyl-D-aspartate receptors
NOS	Not otherwise specified

SD	Standard deviation
SNP	Single nucleotide polymorphism
SPIR	Swedish psychiatric inpatient register

Investigated genes:

BDNF	brain-derived neurotrophic factor
COMT	catechol-O-methyltransferase
DAO	D-amino acid oxidase
DAOA	D-amino acid oxidase activator
DBH	dopamine beta-hydroxylase
DDC	DOPA decarboxylase
DISC1	disrupted in schizophrenia 1
DTNBP1	dystrobrevin binding protein 1
GRIN1	glutamate receptor, ionotropic, N-methyl-D-aspartate, subunit 1
GRIN2B	glutamate receptor, ionotropic, N-methyl-D-aspartate, subunit 2B
IDO1	indoleamine 2,3-dioxygenase 1
KMO	kynurenine 3-monooxygenase
MAOA	monoamine oxidase A
MAOB	monoamine oxidase B
TDO2	tryptophan 2,3-dioxygenase
TH	tyrosine hydroxylase
TPH1	tryptophan hydroxylase 1
TPH2	tryptophan hydroxylase 2

1 INTRODUCTION

The present thesis focuses on the association between genetics and the monoamines, dopamine, serotonin and noradrenaline in the central nervous system (CNS), aiming to shed further light to the hypothesis that gene variants affect the monoamine systems in different populations. Dopamine, serotonin and noradrenaline are the major monoamines in the human CNS and following their basic biochemical pathways they are degraded to their major metabolites homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG), respectively (Figure 1). Throughout the thesis, the cerebrospinal fluid (CSF) concentrations of HVA, 5-HIAA and MHPG have been used as the outcomes. The population of interest in the first part of the thesis is healthy subjects, whereas the second part of the thesis focuses on psychotic patients.

1.1 DOPAMINE, SEROTONIN AND NORADRENALINE

The neurotransmitters dopamine, serotonin and noradrenaline are the major monoamines in CNS. Dopamine and noradrenalin are also referred to as catecholamines. The prefrontal cortex, implicated in higher executive processes, is interconnected with several cortical and subcortical structures, including the brainstem monoamine nuclei (Hensler et al., 2013).

The three monoamines, often viewed in isolation, modulate specific brain functions. Dopamine is involved in cognition as well as motivation and reward (Tzschentke, 2001; Vallone et al., 2000). Serotonin (5-hydroxytryptamine) is implicated in the regulation of mood, sleep and appetite as well as in perception and several cognitive processes, whereas noradrenaline (norephinephrine) modulates arousal, concentration and attention as well as learning and memory (Hensler et al., 2013; Ressler and Nemeroff, 1999; Saxena, 1995).

Several lines of evidence from more recent neuroanatomical and neurochemical studies have given evidence for significant functional interactions and anatomical interconnections between the three monoamine systems in CNS. These interacting systems regulate brain functions, such as cognition, emotion,

motor function and coordination. Their dysregulation has been associated with numerous mental and other disorders, including schizophrenia (Hensler et al., 2013).

1.2 CEREBROSPINAL FLUID MONOAMINE METABOLITES

1.2.1 CSF monoamine metabolites and monoamine turnover rates in central nervous system

HVA, 5-HIAA and MHPG are the major degradation products of dopamine, serotonin and noradrenaline, respectively (Figure 1). The concentrations of the three monoamine metabolites (MM) can be measured in the CSF and they are considered to represent, mainly HVA and 5-HIAA, indirect indexes of the monoamine turnover rates in the CNS. Concentrations of HVA and 5-HIAA in ventricular, cisternal, and lumbar CSF show a craniocaudal gradient (Moir et al., 1970; Nordin et al., 1982; Wiesel, 1975). Postmortem human studies have shown that CSF HVA and 5-HIAA reflect brain HVA and 5-HIAA concentrations (Knott et al., 1989; Stanley et al., 1985; Wester et al., 1990). CSF HVA concentrations correlate with nigrostriatal dopamine function in Parkinson's disease (Ishibashi et al., 2010). The results suggest that a significant proportion of the lumbar HVA and 5-HIAA derives from the brain. In addition, there is a well established positive correlation between CSF 5-HIAA and HVA, mainly in healthy individuals (Hsiao et al., 1993).

On the other hand, CSF MHPG has been reported to mainly derive from noradrenergic nerves in the spinal cord and the blood compartment (Kopin et al., 1983; Post et al., 1973). However, a more recent study have shown a significantly positive correlation between CSF MHPG and MHPG concentrations in hypothalamus, temporal cortex, and pons in human autopsy cases (Wester et al., 1990), suggesting that CSF MHPG can also be used for the purpose of the thesis.

1.2.2 CSF monoamine metabolites and genetics

In humans, CSF MHPG is under major genetic influence, whereas CSF 5-HIAA and HVA are under both genetic and environmental influence (Oxenstierna et al., 1986). Studies in other primates also indicate that CSF concentrations of the MM are partially under genetic influence (Higley et al., 1993; Rogers et al., 2004).

1.2.3 CSF monoamine metabolites and schizophrenia

Schizophrenia has been associated with monoamine metabolite concentrations, mainly HVA. HVA concentrations have been reported to be significantly lower in drug-free patients with schizophrenia when compared to controls (Bjerkenstedt et al., 1985; Lindstrom, 1985; Wieselgren and Lindstrom, 1998). Both quetiapine and olanzapine administration have been associated with a significant increase in CSF HVA (Nikisch et al., 2010; Scheepers et al., 2001). Elevated MHPG concentrations have been shown in patients with psychosis (Hsiao et al., 1993). Moreover, untreated psychotic patients showed substantially lower correlation between HVA and 5-HIAA concentrations relative to controls. This correlation, which is almost linear in healthy controls, was normalized after treatment (Hsiao et al., 1993).

1.3 PSYCHOSIS

1.3.1 DSM and ICD

There are two main systems of classification for psychiatric diagnoses, the Diagnostic and Statistical Manual of Mental Disorders (DSM) developed by the American Psychiatric Association and the International Classification of Diseases (ICD) developed by the World Health Organization.

In Studies IV and V, the patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Third Edition - Revised (DSM-III-R) after a diagnostic interview (Spitzer et al., 1988) and according to DSM-III-

R (American Psychiatric Association, 1987) and the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association, 1995) after retrieving data from medical records. The final diagnoses were the ICD (8th, 9th or 10th revisions) hospital discharge diagnoses from the Swedish psychiatric inpatient register (SPIR).

1.3.2 Schizophrenia and related disorders

A classification of the psychotic disorders according to the latest DSM version, i.e. the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American Psychiatric Association, 2013) and the latest ICD version (ICD-10) (World Health Organisation, 1992) is illustrated. The criteria for schizophrenia, according to DSM-5 as well as the main differences relative to the previous versions are also given.

In DSM-5, the following disorders are listed as schizophrenia spectrum and other psychotic disorders: schizotypal personality disorder, delusional disorder, brief psychotic disorder, schizophreniform disorder, schizophrenia, schizoaffective disorder, substance/medication-induced psychotic disorder, psychotic disorder due to another medical condition, catatonia, other specified schizophrenia spectrum and other psychotic disorder and unspecified schizophrenia spectrum and other psychotic disorder. In ICD-10 the following disorders are listed as schizophrenia and schizotypal and delusional disorders: Schizophrenia (including other schizophrenia, corresponding to DSM-IV schizophreniform disorder), schizotypal disorder, persistent delusional disorders, acute and transient psychotic disorders, induced delusional disorder, schizoaffective disorders, other nonorganic psychotic disorders and unspecified nonorganic psychosis.

Briefly, delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior and negative symptoms (at least two of these symptoms and at least one of the first three have to be present) during one month or less if successfully treated, characterize schizophrenia according to DSM-5 (Criterion

A). Moreover, the level of functioning in major areas is markedly below the level before the onset of the disorder and continuous signs persist for at least six months, including prodromal and residual periods. Schizoaffective disorder, depressive and bipolar disorder with psychotic features, substance/medication-induced psychotic disorder and psychotic disorder due to another medical condition have been ruled out. In the case of autism or a communication disorder, an additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are present.

In DSM-5, two Criterion A symptoms are required for a schizophrenia diagnosis, whereas in DSM-IV, only one Criterion A symptom is required in the case of bizarre delusions or auditory hallucinations consisting of a voice commenting on the patient's thoughts or behavior, or voices conversing with each other. In DSM-IV Criterion A, there is no requirement for at least one of the following symptoms: delusions, hallucinations, and disorganized speech. The DSM-IV, schizophrenia subtypes are eliminated in DSM-5. In DSM-III-R, the required duration for active phase symptoms is one week or less if successfully treated, whereas in ICD-10, DSM-IV and DSM-5 the required duration is one month.

1.4 GENETICS

Genetic research uses numerous study designs, investigating families or unrelated individuals. Family studies with family history or family study approach have been used, searching for familial aggregation. Moreover, numerous twin and adoption studies have been conducted, where the researcher is trying to explain the familial aggregation, differentiating environmental and genetic factors. Linkage studies (model-based and model-free linkage analysis) have been extensively used in order to localize the chromosomal region, in which the relevant genetic variant is located.

Another study design is the association analysis with unrelated individuals, searching for associations between specific gene variants and a phenotype. In the investigation of complex diseases, association studies have been mostly

used as they have a greater power and genetic resolution relative to linkage studies (Risch and Merikangas, 1996). Due to the possibility of population stratification, an alternative analysis of association within families has also been used (Laird and Lange, 2006). Genetic association studies search for association between specific gene variants, candidate genes, chromosomal regions or the whole genome and a phenotype.

Two major classes of human genome variants have been identified, i.e. structural variants, mainly attributable to insertions, deletions, block substitutions, inversions and copy number variants (CNVs) and single nucleotide variants (Frazer et al., 2009; Sachidanandam et al., 2001). Single nucleotide polymorphisms (SNPs) are variants in only one base, which is substituted with another and are the most abundant form of variation in human deoxyribonucleic acid (DNA), occurring on average every 1-2 kb (Sachidanandam et al., 2001).

Genome Wide Association Studies (GWAS) have revolutionized the field of genetic research, as SNPs covering the whole genome are investigated for association with the phenotype of interest, without a priori biological hypotheses. If an association is found between a SNP and a phenotype, this can be attributable to a direct association, reflecting a causal association between the SNP and the phenotype or to an indirect association due to the fact that the associated SNP is in strong linkage disequilibrium (LD) with another genetic locus, which affects the phenotype. LD describes the deviation from independence between the alleles at two or more genetic loci.

As both common and rare variants are likely to contribute to the genetic architecture of complex disorders, such as the psychiatric disorders, recent advances in next-generation sequencing have made possible the discovery of rare variants, using whole-exome sequencing, i.e. sequencing of the 1% of the human genome encoding proteins, or whole-genome sequencing (Ezewudo and Zwick, 2013).

The epigenetic information, superimposed upon the DNA sequence, is also a focus of current research. It is mainly stored as DNA methylation (mainly methylation of cytosines in cytosine/guanine dinucleotides) and histone modifications and it is determined by genetic and environmental factors (Bernstein et al., 2007). There is an increasing interest in exploring how

epigenetic factors are implicated in disease aetiology and epigenetic studies, including epigenome-wide association studies, create new opportunities for the understanding of complex disesases (Rakyan et al., 2011).

In the present thesis, SNPs in different genes have been used as genetic markers and have been investigated for association with our outcomes of interest, i.e. the CSF MM concentrations in different populations. The SNPs selected in our studies were either candidate SNPs, previously reported to be associated with schizophrenia, other mental disorders, monoamine metabolite concentrations or having other known functionality, or tag-SNPs, selected to cover the investigated genes with an r^2 threshold of 0.8. Tag-SNPs capture surrounding SNP information because of the presence of LD and serve therefore as proxies for correlated SNPs that are not genotyped.

1.5 HEALTHY SUBJECTS AND OUR HYPOTHESES IN THE FIRST PART OF THE THESIS (STUDIES I-III)

In the first part of the thesis, we searched for association between markers in the tryptophan hydroxylase 1 (*TPH1)*, dystrobrevin binding protein 1 (*DTNBP1)* and D-amino acid oxidase activator (*DAOA)* genes and CSF HVA, MHPG and 5-HIAA in healthy individuals. Our main interest was to test the hypothesis that *TPH1*, *DTNBP1* and *DAOA* gene variations affect the dopamine, serotonin and noradrenaline turnover rates in healthy humans. The selection of the three genes was not at random, but was based on the fact that these genes are expected to influence the monoamine systems due to biochemical or functional associations. Associations between gene variants and different biological markers are generally of great interest as they shed further light to the understanding of gene functionality in healthy humans, in other words the effect of genetics on physiology. As the selection of the genes and SNPs was also based on their associations with schizophrenia, positive results may indirectly suggest that disturbed monoamine metabolism is a mechanism behind the previously reported associations between gene variants and psychosis in humans. This question is directly addressed in the second part of the thesis, where psychotic patients participate. To our knowledge, the studies

of the present thesis are the first studies searching for association between *DTNBP1* and *DAOA* gene variants and CSF MM in healthy subjects, whereas in the case of *TPH1*, there are previous studies searching for association between some of our included SNPs and CSF MM with partially discordant results. These *TPH1* studies and their results are illustrated in the discussion of Study I.

1.6 PSYCHOSIS AND OUR HYPOTHESES IN THE SECOND PART OF THE THESIS (STUDIES IV-V)

Schizophrenia is a mental disorder with substantial morbidity and mortality, affecting around 1% of the world's population (Millier et al., 2014; Tandon et al., 2008). The disorder has a high heritability, considered to be up to 80% (Sullivan et al., 2012). Two of the most influential theories concerning the pathophysiology of the disorder implicate dopamine and glutamate dysfunctions, followed by other theories implicating the serotonergic, cholinergic, and GABAergic systems (Howes et al., 2015; Laruelle, 2014).

Several lines of evidence, including pharmacological, post-mortem and in vivo imaging studies, support the classical dopamine hypothesis of schizophrenia (Howes et al., 2015). The most robust evidence comes probably from pharmacological studies showing the efficacy of antipsychotics to block dopamine D2 receptors in a dose-dependent manner as well as the psychotogenic effect of dopamine enhancing substances (Laruelle, 2014). It has been suggested that positive psychotic symptoms are associated with excessive stimulation of dopamine D2 receptors in subcortical brain regions, mainly striatum, whereas negative and cognitive psychotic symptoms may be due to a lack of stimulation of dopamine D1 receptors in prefrontal cortex (Laruelle, 2014).

Glutamate, deriving from glucose and glutamine, is the major excitatory neurotransmitter in CNS and is implicated in several neural processes, such as neuronal development and plasticity (Goff and Coyle, 2001). Several lines of evidence, including CSF studies (Kim et al., 1980), studies on glutamate antagonists that can induce or exacerbate psychotic symptoms (Krystal et al.,

1994; Lahti et al., 1995), as well as pathological studies (Mueller and Meador-Woodruff, 2004; Nudmamud-Thanoi and Reynolds, 2004) have given evidence for an implication of the glutamate system in the pathophysiology of schizophrenia. Multiple interactions and associations have been identified between the glutamatergic system and dopamine, serotonin and noradrenaline (Feldman and Weidenfeld, 2004; Missale et al., 2006).

Several monoamine-related and glutamate-related genes have been reported to be associated with schizophrenia in independent studies, however the results have been ambiguous and difficult to replicate in subsequent studies. A recent GWAS of more than 100 000 individuals showed genome wide significant associations between the dopamine D2 receptor gene and a number of genes related to glutamatergic neurotransmission and schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

In the second part of the thesis (Studies IV and V), we have searched for association between genes related to monoamine metabolism (Study IV) and glutamate-related genes (Study V) and CSF MM concentrations in psychosis. To our knowledge, there are no previous studies searching for association between gene variants and CSF MM in psychotic patients.

The approach used in this part of the thesis, i.e. the investigation of possible associations between gene variants and psychosis intermediate phenotypes, is a powerful approach that can lead to a better understanding of the associations between genotypes and phenotypes, as well as to more robust results (Bilder et al., 2009; Freimer and Sabatti, 2003). Intermediate phenotypes are mechanism-related manifestations of a complex phenotype, in this case the highly complex psychotic disorder (Goldman and Ducci, 2007). An endophenotype meets specific criteria, i.e. association with the disorder, heritability and state-independence. Moreover, within families, the endophenotype and the disorder co-segregate. The endophenotype is found in nonaffected family members at a higher rate relative to general population (Gottesman and Gould, 2003). The term intermediate phenotype is used by many authors for traits that have not been formally shown to meet the criteria for endophenotypes, whereas other authors use the two terms interchangeably (Goldman and Ducci, 2007; Gottesman and Gould, 2003). Biochemical, endocrinological, neuroanatomical

neurophysiological and neuropsychological measures have been used as putative endophenotypes/intermediate phenotypes (Gottesman and Gould, 2003).

As MM concentrations have been reported to be heritable in human and other primates (Higley et al., 1993; Oxenstierna et al., 1986; Rogers et al., 2004) and to some extent psychosis-related (Bjerkenstedt et al., 1985; Lindström, 1985; Wieselgren and Lindstrom, 1998), but do not formally fulfill all the endophenotype-related criteria required, the terms intermediate steps and intermediate phenotypes have been used in Studies IV and V, to characterize the monoamine metabolite concentrations relative to psychosis.

1.7 INVESTIGATED GENES

1.7.1 Genes encoding enzymes implicated in monoamine metabolism (Studies I and IV)

The following genes encode the major enzymes involved in dopamine, serotonin and noradrenaline pathways (Figure 1). Due to this direct biochemical connection, functional variations in the selected genes are expected to affect the major degradation products of the monoamine pathways, i.e. HVA, 5-HIAA and MHPG.

The *TPH1* and tryptophan hydroxylase 2 (*TPH2*) genes are located on chromosome 11p15.3–p14 and 12q21.1, respectively. The two genes encode the two active isoforms, TPH1 and TPH2, of the enzyme TPH. TPH catalyzes the first and rate-limiting step in the serotonin metabolism, converting tryptophan to 5-hydroxytryptophan (Cooper and Melcer, 1961). Both isoforms are expressed in CNS, with the TPH2 messenger RNA (mRNA) preferentially expressed in the brain, whereas TPH1 preferentially expressed in pineal gland and the periphery as well as during later developmental brain stages (Nakamura et al., 2006; Walther et al., 2003).

The tyrosine hydroxylase gene (*TH*) is located on chromosome 11p15.5. TH catalyzes the rate-limiting step in the biosynthesis of catecholamines, i.e. the conversion of L-tyrosine to 3,4-dihydroxy-L-phenylalanine (L-DOPA).

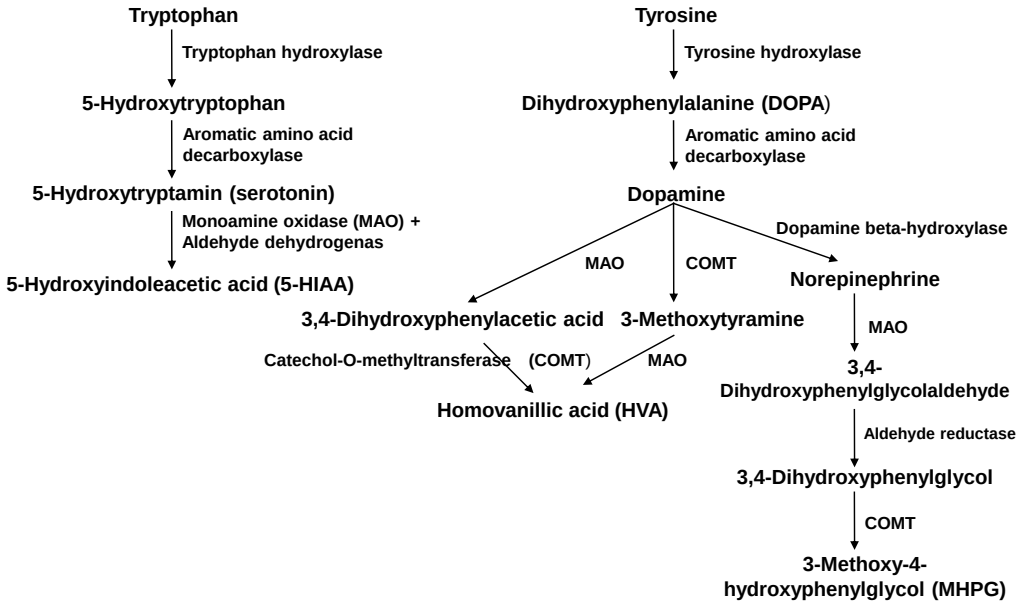

Figure 1. Dopamine, serotonin and noradrenaline pathways.

The DOPA decarboxylase gene (*DDC*) is located on chromosome 7p12.1. DDC, also known as aromatic L-amino acid decarboxylase, is implicated in both serotonin and catecholamine metabolism, catalyzing the decarboxylation of L-DOPA to dopamine and 5-hydroxytryptophan to serotonin. The dopamine beta-hydroxylase gene (*DBH*) is located on chromosome 9q34. DBH, localised in the vesicles of catecholaminergic cells, is involved in the metabolism of the catecholamines, catalyzing the hydroxylation of dopamine to noradrenaline (Kemper et al., 1987).

The catechol-O-methyltransferase gene (*COMT*) is located on chromosome 22q11.2. COMT catalyzes the inactivation of both dopamine and noradrenaline and thus regulates the degradation of the catecholamines, being the most important regulator of the prefrontal dopamine function (Sagud et al., 2010). The monoamine oxidase A (*MAOA*) and monoamine oxidase B (*MAOB*) genes are closely linked on chromosome Xp11.23. *MAOA* and *MAOB* encodes the two enzyme forms, i.e. MAOA and MAOB, respectively. MAOA and MAOB are located in the outer mitochondrial membrane, with MAOA preferentially oxidizing serotonin and noradrenaline and MAOB oxidizing dopamine (Shih et al., 1999).

TPH1, *TH*, *DBH*, *COMT*, *MAOA* and *MAOB* gene variants have been associated with schizophrenia in individual studies with positive meta-analyses for *TPH1* and *COMT* (www.szgene.org). *TPH2* has been associated with the severity of psychotic symptoms in schizophrenia (Zhang et al., 2011), whereas *DDC* has been associated with the age of disease onset in psychotic men (Børglum et al., 2001).

1.7.2 Glutamate-related genes (Study V), *DTNBP1* (Study II) and *DAOA* (Study III)

The glutamate receptor, ionotropic, N-methyl-D-aspartate, subunit 1 (*GRIN1*) and glutamate receptor, ionotropic, N-methyl-D-aspartate, subunit 2B (*GRIN2B*) genes are located on chromosomes 9q34.3 and 12p12, respectively and encode different subunits of the N-methyl-D-aspartate receptors (NMDARs), which are glutamate receptors implicated in memory, neurodevelopment and learning (Hirasawa et al., 2003; Riedel et al., 2003).

The *DAOA* gene is located on chromosome 13q34, whereas the D-amino acid oxidase gene (*DAO*) is located on chromosome 12q24. The product of *DAOA*, the DAOA protein, has been found in various brain regions (Chumakov et al., 2002) and modulates the function of DAO, an enzyme catalyzing the oxidative deamination of D-amino acids, including D-3,4-dihydroxyphenylalanine (D-DOPA) and D-serine (Kawazoe et al., 2007; Wu et al., 2006). D-DOPA is converted to L-DOPA (Kawazoe et al., 2007; Wu et al., 2006), a precursor of

dopamine, whereas D-serine is implicated in glutamatergic neurotransmission (Mothet et al., 2000). The biochemical and functional connections of DAOA and DAO with the MM are illustrated in Figure 2.

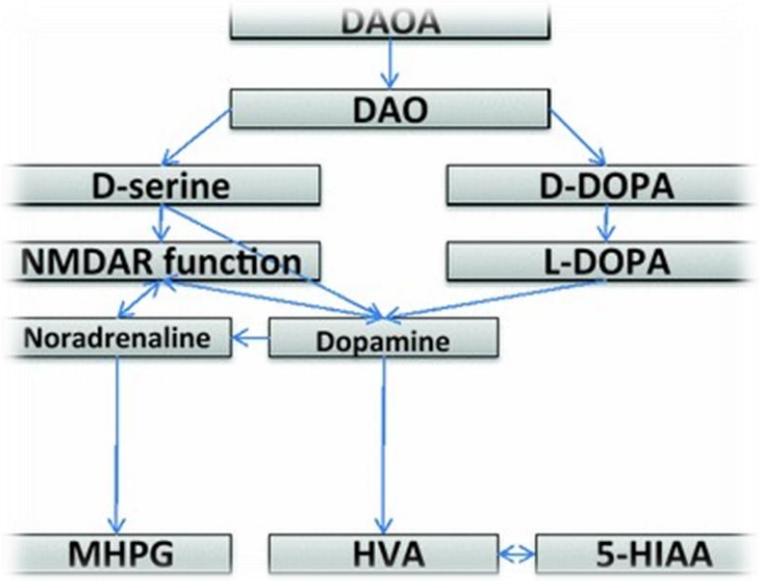

Figure 2. Biochemical and functional connections between D-amino acid oxidase activator and cerebrospinal fluid monoamine metabolites.

The disrupted in schizophrenia 1 gene (*DISC1*) is located on chromosome 1q42 and encodes DISC1, a protein implicated in neurodevelopment, neural plasticity and migration (Thomson et al., 2013). It modulates serine racemase, an enzyme affecting glutamate neurotransmission (Snyder and Gao, 2013).

The brain-derived neurotrophic factor gene (*BDNF*) is located on chromosome 11p13. Its product, BDNF, is a neurotrophic factor with critical function for neural development, survival and regeneration (Balaratnasingam and Janca, 2012; Nurjono et al., 2012). BDNF is implicated in the glutamatergic and monoaminergic neurotransmission (Nurjono et al., 2012).

The *DTNBP1* gene is located on chromosome 6p22.3. *DTNBP1* encodes dysbindin, a protein implicated in dopaminergic and glutamatergic

neurotransmission (Talbot et al., 2004; Weickert et al., 2004). Dysbindin is a component of protein complexes in CNS, involved in synaptic structure and signaling, as well as in neurodevelopment (Benson et al., 2001; Ghiani et al., 2010).

The indoleamine 2,3-dioxygenase 1 (*IDO1)*, tryptophan 2,3-dioxygenase (*TDO2)* and kynurenine 3-monooxygenase (*KMO*) genes are located on chromosomes 8p12-p11, 4q31-q32 and 1q42-q44, respectively and encode enzymes implicated in the kynurenine pathway of the tryptophan degradation (Myint and Kim, 2014; Schwarcz et al., 2012). A number of neuroactive metabolites deriving from this pathway, including the glutamate receptor antagonist kynurenic acid (Schwarcz et al., 2012), are involved in glutamatergic and monoaminergic neurotransmissions (Myint and Kim, 2014).

GRIN1, *GRIN2B*, *DAOA*, *DAO*, *DISC1*, *BDNF* and *DTNBP1* and *KMO* gene variants have been associated with schizophrenia in individual studies with *DAOA* showing evidence for association in two meta-analyses (www.szgene.org). *DAOA* has also been associated with antipsychotic treatment response (Pae et al., 2010) and the progression of prodromal psychotic syndromes to psychosis (Mossner et al., 2010).

2 AIMS

The overall objective of the thesis was to investigate the associations between relevant gene variants and the dopamine, serotonin and noradrenaline turnover rates in CNS, as reflected by the CSF concentrations of HVA, 5-HIAA and MHPG in psychotic patients and healthy individuals.
The specific aims were:

- To investigate the associations between *TPH1*, *DTNBP1* and *DAOA* gene variants and the CSF concentrations of HVA, 5-HIAA and MHPG in healthy controls (Studies I-III).

- To evaluate the effect of genes encoding enzymes implicated in monoamine metabolism and glutamate-related genes on the CSF concentrations of HVA, 5-HIAA and MHPG in psychotic patients (Studies IV-V).

- To evaluate if previously reported associations between gene variants and schizophrenia can be mediated by dopaminergic, serotonergic or noradrenergic mechanisms, as reflected by the CSF concentrations of HVA, 5-HIAA and MHPG in psychotic patients (Studies IV-V).

3 METHODS

3.1 SUBJECTS

3.1.1 Studies I-III

In the first part of the thesis, 132 unrelated healthy Caucasians (78 men and 54 women) were included. They were mostly students and hospital stuff or individuals drawn from a population register from Stockholm county council. The subjects had previously participated in studies regarding CSF and heritability factors (Oxenstierna et al., 1986; Sedvall et al., 1980; Wiesel et al., 1982), or studies of CSF circulation (Oxenstierna et al., 1996).

Sixty six out of the 132 healthy subjects had previously participated in studies, searching for association between gene variation and CSF monoamine metabolite concentrations (Jönsson et al., 1997; Jönsson et al., 1996; Jönsson et al., 1998). Moreover, about 90 of the subjects participated in further similar studies (Annerbrink et al., 2010; Damberg et al., 2004; Jönsson et al., 2004; Jönsson et al., 2000). More recently, all individuals (n=132) were included in a study, where the author of the thesis was also co-author, searching for association between *BDNF* SNPs and CSF HVA, 5-HIAA and MHPG concentrations (Jönsson et al., 2008).

At the first diagnostic interview, when CSF samples were drawn, all participants were found to be healthy, i.e. the absence of psychiatric morbidity was ascertained. The subjects' mean age ± standard deviation (SD) was 27 ± 9 years at lumbar puncture. All participants were drug-free, with the exception of 22 female participants, who used oral contraceptives.

Eight to 20 years after the first interview, subjects were re-interviewed to re-assess psychiatric morbidity (Jönsson et al., 2004; Jönsson et al., 2008) and whole blood was drawn for DNA extraction and genotyping. At this second interview, a structured interview was performed by psychiatrists to assess psychiatric (Spitzer et al., 1986) and somatic morbidity as well as the presence of psychiatric or nervous system disorders among relatives. If the subjects have been in contact with inpatient or outpatient psychiatric department, the records

were obtained and examined for diagnosis. Forty three of the subjects were found to fulfill criteria for a number of DSM-III-R psychiatric lifetime diagnoses: major depression and simple phobia (n=2), bipolar disorder, alcohol dependence and cannabis dependence (n=1), alcohol dependence and cannabis dependence (n=1), social phobia and simple phobia (n=1), depressive disorder not otherwise specified (NOS) and simple phobia (n=1), major depression (n=4), depressive disorder NOS (n=3), bipolar disorder (n=2), psychosis NOS (n=1), panic disorder (n=1), simple phobia (n=1), obsessive compulsive disorder (n=1), anxiety disorder NOS (n=1), somatoform disorder NOS (n=1), alcohol dependence (n=6), alcohol abuse (n=6), cannabis abuse (n=1), stuttering (n=1), bulimia nervosa (n=1), functional enuresis (n=2), adjustment disorder NOS (n=1), unspecified mental disorder (n=4).

3.1.2 Studies IV-V

In the second part of the thesis, patients with psychotic disorder were recruited among inpatients at four psychiatric university clinics in Stockholm County between 1973 and 1987 and were asked to participate in pharmacological or biological research projects (Bjerkenstedt et al., 1977; Härnryd et al., 1984; Oxenstierna et al., 1996; Wode-Helgodt et al., 1977). The participants were observed for at least 48 hours without any antipsychotic medication and CSF samples (12.5 ml) were drawn.

Three to 34 years after the first investigation, patients were asked to participate in genetic research studies and whole blood was drawn for genotyping. Patients were asked to participate in a diagnostic structured interview (Spitzer et al., 1988) and permit the researchers to retrieve their medical records. Available records were scrutinized by researchers to obtain a life-time diagnosis according to DSM-III-R and DSM-IV. In 2010, hospital discharge diagnoses were also obtained from the SPIR, a register covering all inpatient hospitalizations in Sweden since 1973. For each hospitalization the diagnosis was recorded according to the ICD, 8th, 9th or 10th revisions. The majority of the participants had experienced several hospitalizations, but only one diagnosis was given per participant, following a diagnostic hierarchy (Ekholm et al., 2005; Vares et al., 2006). The final diagnoses were based on the SPIR, as

it was not possible to retrieve all medical records and several of the patients were not willing to participate in a diagnostic interview.

Seventy-four psychotic patients (45 men and 29 women) participated in the present study. The mean age of disease onset ± SD was 27.6 ± 7.8 years, whereas the patients' mean age ± SD was 30.4 ± 7.2 years at lumbar puncture. Twenty-six of the patients were treated with antipsychotics at the time of lumbar puncture, whereas thirty-sex patients were free from antipsychotic medication since three weeks or longer. Sixty-four patients were diagnosed with schizophrenia spectrum disorder (schizophrenia n=60, schizoaffective disorder n=4) and ten with other psychosis (psychosis NOS n=7, delusional disorder n=1, bipolar disorder n=1, alcohol induced psychotic disorder n=1).

Analyses in healthy controls were conducted for SNPs that were nominally associated with monoamine metabolite concentrations in psychotic patients, in order to evaluate whether the effects of the associated SNPs were restricted to patients with psychosis. CSF samples were drawn by lumbar puncture from 111 unrelated healthy Caucasians. Eight to 20 years after the first investigation, the subjects were interviewed to re-assess the absence of psychiatric morbidity (Jönsson et al., 2004) and whole blood was drawn for genotyping.

3.2 CEREBROSPINAL FLUID SAMPLES

CSF samples of 12.5 ml were drawn by lumbar puncture with the participants, both healthy subjects and psychotic patients, in a sitting or recumbent position. CSF was sampled between 8 and 9 a.m, after at least 8 hours of supervised bed-rest and absence of food intake or smoking. 5-HIAA, HVA, and MHPG concentrations were measured by mass fragmentography with deuterium-labeled internal standards (Swahn et al., 1976). Back-length was defined as the distance between the external occipital protuberance and the point of needle insertion.

3.3 GENOTYPING

3.3.1 Studies I-III

Genomic DNA was extracted from whole blood samples (Geijer et al., 1994). The investigated SNPs, selected due to previously reported association with schizophrenia, were genotyped at the SNP Technology Platform at Uppsala University and Uppsala University Hospital, Sweden (http://www.genotyping.se), using the Illumina BeadStation 500GX and the 1536-plex Illumina Golden Gate assay (Illumina Inc., San Diego, CA, USA).

Five *TPH1* SNPs, i.e.rs4537731, rs211105, rs1800532, rs1799913 and rs7933505 were genotyped in Study I. Eleven *DTNBP1* SNPs, i.e. rs12524251, rs760666, rs2619539, rs3213207, rs1011313, rs2619528, rs2619522, rs1018381, rs909706, rs2743852 and rs2619538, were genotyped in Study II and four *DAOA* SNPs, i.e. rs2391191, rs778294, rs3918342 and rs1421292 were genotyped in Study III.

3.3.2 Studies IV-V

Genomic DNA was extracted from whole blood samples (Geijer et al., 1994). Candidate SNPs, previously associated with schizophrenia, other mental disorders, enzyme function or CSF monoamine metabolite concentrations, and tag-SNPs selected using HapMap to cover the investigated genes with an r^2 threshold of 0.8, were genotyped. The genotyping was performed using the Illumina BeadStation 500GX and the 768-plex Illumina Golden Gate assay (Illumina Inc., San Diego, CA, USA (Fan et al., 2003).

In study IV, 119 SNPs in eight genes, i.e. *TPH1* (n=10), *TPH2* (n=21), *TH* (n=10), *DDC* (n=24), *DBH* (n=25), *COMT* (n=16), *MAOA* (n=6) and *MAOB* (n=7) were genotyped. In study V, 238 SNPs in ten genes, i.e. *GRIN1* (n=10), *GRIN2B* (n=5), *DAOA* (n=19), *DAO* (n=11), *DISC1* (n=122), *BDNF* (n=10), *DTNBP1* (n=26), *KMO* (n=25), *IDO1* (n=3) and *TDO2* (n=7) were genotyped.

3.4 STATISTICAL ANALYSIS

3.4.1 Studies I-III

In the first part of the thesis (Studies I-III), the associations between SNPs and CSF MM concentrations were tested with a general linear model (Proc GLM, SAS/STAT software, version 9.1.3, SAS institute Inc., Cary, NC, USA), where concentration was modeled as a linear function of the allele count (0,1,2).

The selection of covariates in the final analyses was based on a preliminary analysis of the effect of potentially important confounders, i.e. back-length, weight, gender, age at lumbar puncture, and presence of a lifetime psychiatric diagnosis, on CSF monoamine metabolite concentrations, excluding the SNPs. CSF 5-HIAA and HVA concentrations were influenced by back-length and presence of a lifetime psychiatric diagnosis, whereas CSF MHPG concentrations were influenced by back-length and gender.

Hardy–Weinberg equilibrium (HWE) was tested using Fisher's exact test as implemented in PEDSTATS (Wigginton and Abecasis, 2005). The normal distribution of residuals was tested with the Anderson–Darling test, and residuals were approximately normally distributed after a square root transformation in the case of 5-HIAA and HVA and a logarithmic transformation in the case of MHPG.

Correction for multiple testing was done through randomly permuting the marker genotypes among individuals and recalculating p-values for each permuted data set (1000 permuted data sets). The corrected p-value was then calculated as the fraction of permutated data sets where the minimum p-value was equal to, or smaller than, the observed p-value.

Haploview 4.0 was used for calculation and virtualization of LD and for haplotype block estimation (Barrett et al., 2005). In Study I, the haplotype analysis was performed with UNPHASED (Dudbridge, 2008) , using all five SNPs and covariates as described above.

3.4.2 Studies IV-V

In the second part of the study (Studies IV and V), the associations between SNPs and CSF MM concentrations were tested with multiple linear regression using STATA 12.1 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP), where concentration was modeled as a linear function of the allele count (0,1,2).

In the case of healthy controls, three covariates were included in the analysis, i.e. back-length, gender and age at lumbar puncture, whereas in the case of psychotic patients two more binary covariates were used, i.e. the diagnosis (schizophrenia spectrum psychosis or other psychosis) and the use of antipsychotics. Antipsychotic treatment was considered as present if the patient had taken antipsychotics during a three-week period prior to the lumbar puncture.

HWE was tested using exact significance as implemented in STATA 12.1. Normality of residuals was checked graphically with STATA 12.1. Adjustments for multiple testing were performed using Bonferroni correction taking into account the total number of tests conducted in each study. In Study IV, adjustment for multiple testing was also performed using a less conservative correction, taking into account the number of tests conducted, restricted to the candidate SNPs.

3.5 ETHICAL APPROVAL

The studies of the present thesis were conducted in accordance with the Declaration of Helsinki and the project was approved by the Regional Research Ethics Committee in Stockholm (dnr 2009/1403-31/1) and the Ethics Committee of the Karolinska University Hospital (dnr 94-036). Written informed consent was obtained from all the participating subjects.

4 RESULTS

4.1 STUDY I

One out of the five investigated *TPH1* SNPs, i.e. rs4537731, was found to be nominally associated with both CSF 5-HIAA and HVA (uncorrected p-values 0.0028 and 0.0016, respectively) in healthy subjects (Figure 3). Both associations remained significant after correction for multiple testing (p-values 0.026 and 0.029, respectively).

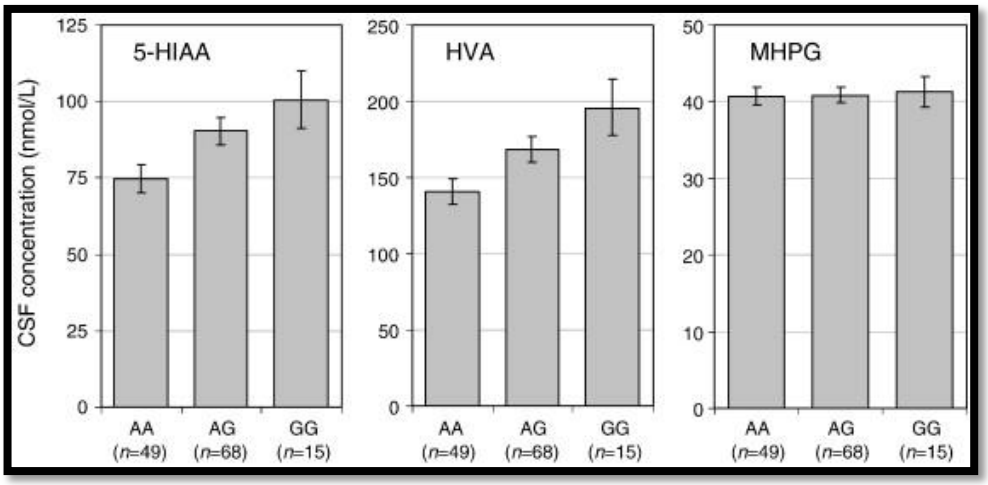

Figure 3. Cerebrospinal fluid (CSF) 5-hydroxyindole acetic acid (5-HIAA), homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in control individuals as a function of the number rs4537731 G-alleles (p=0.003, 0.002 and 0.82 for 5-HIAA, HVA and MHPG, respectively). Least square means and standard errors are given.

After the single marker association analysis, a haplotype association analysis was also carried out. Five haplotypes were segregating in the population and a significant overall association was found between the *TPH1* haplotypes and both CSF 5-HIAA and HVA concentrations (p-values 0.012 and 0.013, respectively). The concentrations of both 5-HIAA and HVA were found to be positively associated with the GTCCG haplotype and negatively associated with the ATCCG haplotype. No significant association between the *TPH1* SNPs or haplotypes and CSF MHPG concentrations was found.

4.2 STUDY II

Three of the eleven investigated *DTNBP1* SNPs, i.e. rs2619538, rs760666, and rs909706, were found to be nominally associated with CSF HVA concentrations in healthy subjects. After correction for multiple testing, only the association between rs2619538 and HVA remained significant (corrected p-value 0.01). HVA mean concentration decreased with 26 nmol/L with each copy of the minor allele (A) (Figure 4).

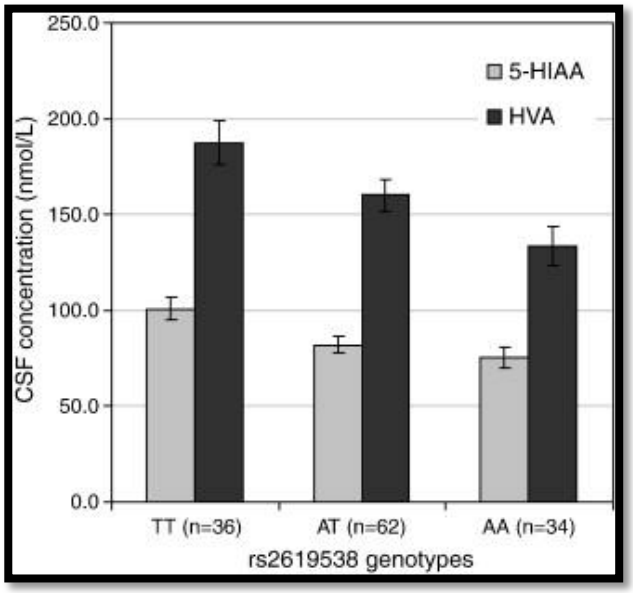

Figure 4. Cerebrospinal fluid (CSF) 5-hydroxyindole acetic acid (5-HIAA) and homovanillic acid (HVA) concentrations in control individuals as a function of the number rs2619538 A-alleles (p=0.04 and p=0.01 for 5-HIAA and HVA, respectively). Least square means and standard errors are given.

Two of the eleven investigated *DTNBP1* SNPs, i.e. rs2619538 and rs760666, were nominally associated with CSF 5-HIAA concentrations in healthy Caucasians. After correction for multiple testing, only the association of rs2619538 remained significant (corrected p-value 0.04). 5-HIAA mean concentration decreased with 13 nmol/L with each copy of the minor allele (A) (Figure 4).

4.3 STUDY III

Two of the four investigated *DAOA* SNPs, i.e. rs3918342 (Figure 5) and rs1421292, were significantly associated with CSF HVA concentrations in healthy Caucasians, with corrected p-values 0.013 and 0.043, respectively. The two SNPs were in almost complete LD in Scandinavian population and rs1421292 explained no additional variation in HVA concentration on top of that explained by rs3918342. Rs3918342 was also found to be nominally associated with CSF 5-HIAA concentrations.

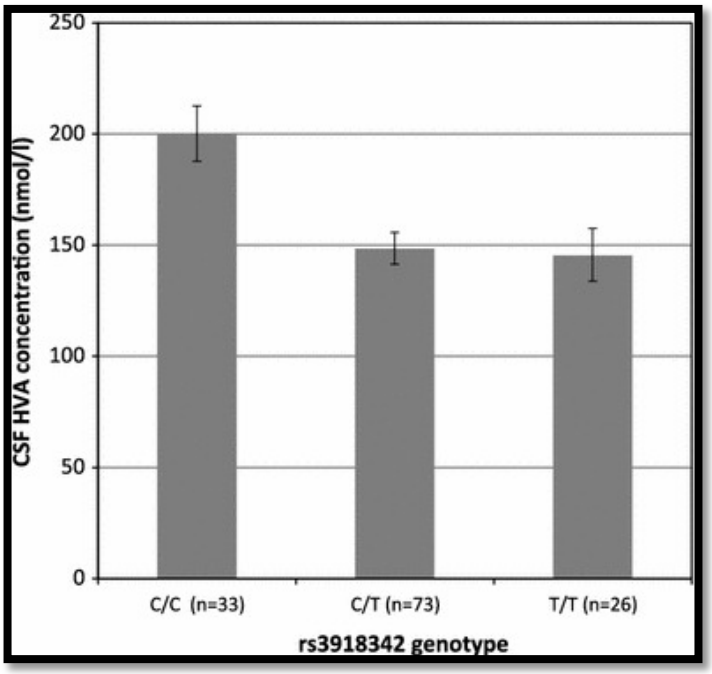

Figure 5. Cerebrospinal fluid (CSF) homovanillic acid (HVA) concentration in healthy subjects as a function of the number rs3918342 T-alleles (corrected p-value=0.013). Least square means and standard errors are given.

Regarding rs3918342, C homozygotes (C/C) had 50 nmol/l higher HVA mean concentration relative to both C/T and T/T carriers, whereas no difference on HVA mean concentration was detected between subjects carrying the C/T and T/T genotypes, suggesting a dominant pattern with the T allele as the dominant allele. As expected, a decrease of the uncorrected p-value from 0.0016 to 0.0001 was observed, when a dominant model of segregation was applied.

4.4 STUDY IV

Twelve, 12 and 18 of the 119 investigated SNPs were found to be nominally associated with CSF HVA, 5-HIAA and MHPG concentrations, respectively, in psychotic patients. The nominally associated SNPs are illustrated in Tables 1-3.

When correction for multiple testing was performed applying a conservative Bonferroni correction, taking into account the total number of tests conducted ($\alpha = 0.05/396 = 1.26 \times 10^{-4}$), no associations remained significant. However, a correction for multiple testing taking into account the number of tests conducted restricted to the 28 candidate SNPs was also performed ($\alpha = 0.05/90 = 5.56 \times 10^{-4}$). In this case, one association remained significant, i.e. the association between the candidate *MAOB* SNP rs5905512 and CSF MHPG concentrations in psychotic men. Moreover, there were 42 nominal associations, which exceeded the expected number (20) of nominal associations.

Separate tests were performed in healthy controls, investigating whether the 42 nominal associations were also present in this subject group. The analyses revealed that 41 out of 42 associations were restricted to patients with psychosis (Tables 1-3).

Table 1. SNPs nominally associated with CSF HVA concentrations in psychotic patients.

		Patients with psychosis (n=74; 45 men, 29 women)			**Healthy controls (n=111; 63 men, 48 women)**		
HVA mean (S.D.)		178.6 (79.3) nmol/l			167.5 (68.4) nmol/l		
Gene	**SNP**	**MAF(%)**	**HWE**	***P***	**MAF(%)**	**HWE**	***P***
DDC	rs11238133	36	0.211	0.004	41	1.000	0.757
DDC	rs6951648	19	0.273	0.005	17	0.735	0.297
DDC	rs10499696	11	0.186	0.009	9	0.594	0.681
DDC	rs921451	42	0.018	0.011	43	0.564	0.661
DDC	rs9942686	21	0.723	0.017	23	1.000	0.305
TH	rs10770141	33	0.608	0.023	40	1.000	0.433
TPH1	rs211105	31	0.785	0.029	25	0.449	0.292
TH	rs10840491	16	0.680	0.035	12	0.640	0.433
DDC	rs6593011	15	0.652	0.038	13	0.208	0.959
COMT	rs165774	32	0.003	0.043	30	0.258	0.650
TPH2	rs1872824	34	0.606	0.047	33	0.830	0.685
TH	rs10840489	18	1.000	0.048	14	1.000	0.357

Minor allele frequencies (MAF), p-values of testing for Hardy–Weinberg equilibrium (HWE) and p-values (*P*) from multiple linear regressions of single nucleotide polymorphisms (SNPs) nominally associated with homovanillic acid (HVA) concentrations in the cerebrospinal fluid of psychotic patients and the corresponding association statistics among healthy controls.

Table 2. SNPs nominally associated with CSF 5-HIAA concentrations in psychotic patients.

		Patients with psychosis (n=74; 45 men, 29 women)			**Healthy controls (n=111; 63 men, 48 women)**		
5-HIAA mean (S.D.)		93.1 (34) nmol/l			90.8 (36.2) nmol/l		
Gene	**SNP**	**MAF(%)**	**HWE**	***P***	**MAF(%)**	**HWE**	***P***
MAOB(women)	rs2311013	3	1.000	0.009	4	1.000	0.866
MAOB(women)	rs1181252	3	1.000	0.009	4	1.000	0.053
DDC	rs9942686	21	0.723	0.009	23	1.000	0.069
DDC	rs11238133	36	0.211	0.012	41	1.000	0.516
DBH	rs1611118	7	1.000	0.014	7	0.397	0.895
DDC	rs11238131	30	0.784	0.019	32	0.826	0.829
MAOA (men)	rs5906957	31		0.027	17		0.089
DDC	rs11575535	4	1.000	0.035	4	1.000	0.302
TPH1	rs1799913	42	0.096	0.039	41	0.334	0.330
DBH	rs6271	3	1.000	0.047	5	1.000	0.528
MAOA (men)	rs4301558	36		0.048	20		0.123
MAOA (men)	rs3027396	36		0.048	21		0.299

Minor allele frequencies (MAF), p-values of testing for Hardy–Weinberg equilibrium (HWE) and p-values (*P*) from multiple linear regressions of single nucleotide polymorphisms (SNPs) nominally associated with 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the cerebrospinal fluid of psychotic patients and the corresponding association statistics among healthy controls. *MAOA* and *MAOB* are located on chromosome X and the analyses were therefore conducted separately for men and women.

Table 3. SNPs nominally associated with CSF MHPG concentrations in psychotic patients.

		Patients with psychosis (n=74; 45 men, 29 women)			**Healthy controls (n=111; 63 men, 48 women)**		
MHPG mean (S.D.)		43.3 (9.3) nmol/l			41.7 (8.1) nmol/l		
Gene	**SNP**	**MAF(%)**	**HWE**	***P***	**MAF(%)**	**HWE**	***P***
MAOB(men)	rs5905512	47		0.0004	50		0.479
MAOB (men)	rs1799836	38		0.0008	44		0.483
DDC	rs11575535	4	1.000	0.005	4	1.000	0.126
TPH1	rs4537731	44	0.475	0.007	40	0.047	0.646
DDC	rs6592961	28	0.400	0.008	29	0.104	0.855
TPH1	rs7933505	41	0.148	0.010	41	0.334	0.342
DDC	rs7809758	40	0.811	0.013	38	0.159	0.956
TPH1	rs7122118	43	0.239	0.014	40	0.029	0.821
DBH	rs6271	3	1.000	0.017	5	1.000	0.340
TPH1	rs1799913	42	0.096	0.017	41	0.334	0.342
TPH1	rs684302	42	0.232	0.017	41	0.171	0.397
DDC	rs9942686	21	0.723	0.018	23	1.000	0.189
MAOB (men)	rs6651806	24		0.021	32		0.328
DDC	rs17133853	11	1.000	0.033	9	0.569	0.436
DBH	rs1611115	21	1.000	0.035	14	1.000	0.791
DBH	rs3025388	18	0.445	0.047	18	0.517	0.064
TPH1	rs12292915	44	0.161	0.047	43	0.052	0.066
TPH1	rs211105	31	0.785	0.049	25	0.449	0.033

Minor allele frequencies (MAF), p-values of testing for Hardy–Weinberg equilibrium (HWE) and p-values (*P*) from multiple linear regressions of single nucleotide polymorphisms (SNPs) nominally associated with 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in the cerebrospinal fluid of psychotic patients and the corresponding association statistics among healthy controls. *MAOA* and *MAOB* are located on chromosome X and the analyses were therefore conducted separately for men and women.

4.5 STUDY V

In psychotic patients, 13, 16 and nine of the 238 genotyped SNPs were nominally associated with CSF HVA, 5-HIAA and MHPG concentrations, respectively. The associated SNPs are illustrated in Tables 4-6. None of the nominal associations remained significant after correction for multiple testing. In order to investigate whether the 38 nominal associations were restricted in psychosis, separate tests were conducted in healthy controls. The analyses showed that five associations were also present in healthy subjects, whereas the other 33 associations were restricted to patients with psychosis (Tables 4-6).

Table 4. Minor allele frequencies (MAF), p-values for Hardy–Weinberg equilibrium tests (HWE) and p-values (*P*) from nominal associations between single nucleotide polymorphisms (SNPs) and cerebrospinal fluid homovanillic acid (HVA) concentrations in psychotic patients. Corresponding association statistics among healthy controls.

		Patients with psychosis (n=74; 45 men, 29 women)			**Healthy controls (n=111; 63 men, 48 women)**		
HVA mean (S.D.)		178.6 (79.3) nmol/l			167.5 (68.4) nmol/l		
Gene	**SNP**	**MAF(%)**	**HWE**	***P***	**MAF(%)**	**HWE**	***P***
DISC1	rs12046794	11	0.590	0.001	9	0.173	0.435
DISC1	rs1934909	14	1.000	0.007	14	0.123	0.964
DISC1	rs10158776	2	1.000	0.013	1	1.000	0.970
IDO1	rs6991530	14	1.000	0.015	14	1.000	0.290
DAO	rs17041020	7	1.000	0.021	5	1.000	0.040
DISC1	rs823162	7	0.042	0.022	5	1.000	0.402
DISC1	rs4325116	39	0.462	0.034	37	0.838	0.904
DISC1	rs4385690	18	1.000	0.038	24	0.203	0.717
DAOA	rs1421292	43	0.344	0.040	42	0.561	0.006
DISC1	rs1322783	12	0.277	0.043	15	0.280	0.878
DAOA	rs3916971	47	0.066	0.045	47	0.849	0.009
DISC1	rs16854967	14	0.609	0.049	14	0.468	0.925
DAO	rs2070586	14	1.000	0.049	19	0.355	0.790

Table 5. Minor allele frequencies (MAF), p-values for Hardy–Weinberg equilibrium tests (HWE) and p-values (*P*) from nominal associations between single nucleotide polymorphisms (SNPs) and cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA) concentrations in psychotic patients. Corresponding association statistics among healthy controls.

		Patients with psychosis (n=74; 45 men, 29 women)			**Healthy controls (n=111; 63 men, 48 women)**		
5-HIAA mean (S.D.)		93.1 (34) nmol/l			90.8 (36.2) nmol/l		
Gene	**SNP**	**MAF(%)**	**HWE**	***P***	**MAF(%)**	**HWE**	***P***
DAOA	rs3918342	47	0.647	0.002	46	0.703	0.072
DISC1	rs1934909	14	1.000	0.010	14	0.123	0.400
DAOA	rs1421292	43	0.344	0.010	42	0.561	0.085
DISC1	rs1331056	45	0.154	0.012	40	0.844	0.131
DAOA	rs3916971	47	0.066	0.018	47	0.849	0.093
GRIN1	rs28489906	47	0.100	0.025	48	1.000	0.500
KMO	rs1932441	29	0.395	0.026	36	0.680	0.093
IDO1	rs6991530	14	1.000	0.028	14	1.000	0.194
DISC1	rs2806465	50	0.062	0.033	45	0.443	0.033
KMO	rs12410855	36	0.079	0.034	36	0.681	0.946
DAOA	rs778293	43	0.237	0.034	42	1.000	0.049
DISC1	rs12046794	11	0.590	0.035	8	0.484	0.365
DISC1	rs17820909	10	1.000	0.036	6	1.000	0.597
DTNBP1	rs12525702	9	0.438	0.040	8	0.115	0.250
DTNBP1	rs3829893	11	0.031	0.042	10	0.321	0.656
DISC1	rs9726024	34	0.606	0.048	34	0.403	0.158

Table 6. Minor allele frequencies (MAF), p-values for Hardy–Weinberg equilibrium tests (HWE) and p-values (*P*) from nominal associations between single nucleotide polymorphisms (SNPs) and cerebrospinal fluid 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in psychotic patients. Corresponding association statistics among healthy controls.

		Patients with psychosis (n=74; 45 men, 29 women)			**Healthy controls (n=111; 63 men, 48 women)**		
MHPG mean (S.D.)		43.3 (9.3) nmol/l			41.7 (8.1) nmol/l		
Gene	**SNP**	**MAF(%)**	**HWE**	***P***	**MAF(%)**	**HWE**	***P***
KMO	rs2275163	39	0.808	0.003	35	0.290	0.965
KMO	rs4660103	33	1.000	0.003	29	0.242	0.558
KMO	rs6677357	28	1.000	0.005	26	0.622	0.877
DISC1	rs1934909	14	1.000	0.015	14	0.123	0.574
KMO	rs12138459	27	0.769	0.024	28	0.485	0.938
DISC1	rs2812385	42	0.474	0.026	28	1.000	0.775
DAOA	rs1570709	22	0.165	0.026	22	0.096	0.447
IDO1	rs7010461	35	0.619	0.028	29	0.167	0.952
KMO	rs2050516	47	0.647	0.044	40	0.691	0.714

5 DISCUSSION

5.1 METHODOLOGICAL CONSIDERATIONS

A candidate SNP approach was used in the first part of the thesis resulting in a limited number of statistical tests conducted and significant associations in all studies (Studies I-III), even after a correction for multiple testing. The limitation of this approach is the fact that other possibly relevant SNPs relative to MM are not included. This is not an issue in the second part of the thesis (Studies IV-V), as both candidate SNPs and tag-SNPs were genotyped. A limitation of this approach is the limited power to detect true associations, after a correction for multiple testing, due the relatively small number of both patients and controls, relative to the large number of tests conducted. In other words, there is an increased risk of false negative results (type II errors) and that could explain the fact that no significant associations were found in Studies IV or V, applying a conservative Bonferroni correction.

When a less conservative approach was applied, taking into account the total number of tests conducted restricted to the candidate SNPs tested, the association between one candidate SNP, the intronic *MAOB* rs5905512, and MHPG concentrations in psychotic men remained significant (Study IV). Another approach is to consider that the genes selected in the second part of the thesis, mainly in Study IV, are highly likely to have an effect on CSF MM. Giving such a strong a priori hypothesis, it may be argued that a correction for multiple testing is not necessary (Rothman, 2014). Therefore, all the nominal associations are illustrated in Tables 1-6 and the most relevant nominal results are discussed.

5.2 ANTIPSYCHOTICS

A general issue in all studies regarding psychosis concerns the medication effects on the associations under investigation. In the second part of the thesis, where psychotic patients participated, we performed preliminary analyses in order to evaluate the effect of antipsychotics on CSF MM concentrations. Mean CSF MHPG concentration was significantly lower in patients who were under

treatment compared to antipsychotic-free patients, whereas mean CSF HVA and 5-HIAA concentrations were not found to be associated with antipsychotic treatment. As our independent variables, the SNPs, are not expected to be associated with the antipsychotic treatment and moreover the use of antipsychotics was included as a covariate in the multiple linear regressions, the antipsychotic treatment should not confound our analyses.

5.3 STUDY I

One *TPH1* SNP, rs4537731, located upstream the *TPH1* gene, was found to be significantly associated with both CSF 5-HIAA and HVA in healthy subjects. As TPH is the rate-limiting enzyme of the serotonin synthesis, *TPH1* gene variation is expected to affect the major serotonin metabolite 5-HIAA. The association between *TPH1* and the major dopamine metabolite HVA is also expected, due to the well-established relationship between 5-HIAA and HVA in CSF (Hsiao et al., 1993; Ågren et al., 1986). Finally, the lack of association between *TPH1* gene variants and MHPG is also expected, as TPH is not implicated in norepinephrine synthesis (Figure 1). The present result is not in agreement with Galfalvy et al. (2009), who did not find any association between the rs4537731 polymorphism and MM concentrations in patients with major depression (Galfalvy et al., 2009).

In the present study, the *TPH1* rs4537731 A-allele was associated with decreased CSF 5-HIAA and HVA concentrations. The rs4537731 A-allele has been previously associated with suicide attempts among violent offenders and arsonists (Rotondo et al., 1999), and the AA-genotype with suicide attempts among patients with major depression (Galfalvy et al., 2009). We could therefore suggest that the associations between A-allele/AA-genotype and a higher risk for suicide attempts may be mediated by disturbed serotonin and dopamine turnover rates in CNS, reflected by decreased CSF 5-HIAA and HVA concentrations, respectively. However, we need to be cautious, as our results come from analyses of healthy subjects and moreover other studies, including a meta-analysis, have not found evidence for association between rs4537731 and suicidal behavior (Li and He, 2006).

The best studied *TPH1* SNP, regarding CSF monoamine metabolite concentrations, is the intronic rs1799913. In the present study, no association was found between this SNP or another intronic *TPH1* rs1800532, in almost complete LD with rs1799913 in Caucasians, and MM CSF concentrations. Thus, we have not been able to replicate previously reported associations between rs1799913 and CSF 5-HIAA in healthy controls (Jönsson et al., 1997) and violent offenders (Nielsen et al., 1994). However, the lack of association, found in the present study, is in agreement with a study of Nielsen et al. (1998), who also failed to replicate the results of Jönsson et al. (1997) and Nielsen et al. (1994), reporting lack of association between rs1799913 and CSF 5-HIAA in violent and non-violent offenders as well as in healthy controls (Nielsen et al., 1998). Our results are also in agreement with a more recent association study, which failed to find any association between rs1800532 polymorphism and MM concentrations in patients with major depression or bipolar disorder (Galfalvy et al., 2009).

The divergent results regarding *TPH1* SNPs and CSF MM concentrations may be explained by the non-inclusion of back-length as a covariate in all studies, as well as by the fact that populations with different characteristics regarding ethnicity and psychiatric profile were analyzed.

5.4 STUDY II

One *DTNBP1* SNP, rs2619538, was significantly associated with CSF HVA concentrations in healthy subjects. Rs2619538, located 1.5 kb upstream the only identified promoter region of the *DTNBP1* gene (Pedrosa et al., 2009), has been associated with schizophrenia (Riley et al., 2009; Williams et al., 2004) as well as with negative and manic symptoms in psychotic patients (Corvin et al., 2008).

The present result supports the notion that *DTNBP1* gene variation significantly affects the dopamine system, as reflected by the CSF HVA concentrations, in healthy individuals. This effect is possibly mediated by the BLOC-1 complex (Iizuka et al., 2007) and is in accordance with animal studies showing association between the *DTNBP1* gene and its product dysbindin and

the dopamine system. The most robust evidence of association between *DTNBP1* and the dopamine system is the significant reduction of dopamine content in different brain regions in Sandy mice (Murotani et al., 2007). Sandy mice have a large deletion in the *DTNBP1* gene, resulting in essentially total loss of dysbindin (Li et al., 2003).

Rs2619538 was also found to be significantly associated with CSF 5-HIAA in healthy individuals. Taking into account the lack of association between the almost total loss of dysbindin and a serotonin response in animal studies (Nagai et al., 2010), we conclude that this association may be due to the correlation between CSF 5-HIAA and HVA, which is among the most well-established in human CSF research (Geracioti et al., 1998). A direct association between *DTNBP1* and the serotonin system, despite lack of associations in animal studies, may also be considered.

5.5 STUDY III

Two *DAOA* SNPs in almost complete LD, rs3918342 and rs1421292, were significantly associated with CSF HVA concentrations in healthy subjects. As rs1421292 explained no additional variation in HVA concentration on top of that explained by rs3918342, only rs3918342 is discussed.

The TT genotype of the *DAOA* rs3918342 has been reported to be associated with attention and memory deficits in schizophrenia (Goldberg et al., 2006), decreased hippocampal activation and increased prefrontal activation in individuals at high genetic risk for schizophrenia (Hall et al., 2008) and with temporal lobe and amygdala gray matter reduction in bipolar disorder (Zuliani et al., 2009). In the present study, the TT genotype was associated with significantly lower HVA concentrations (Figure 5), suggesting that a disturbed dopamine turnover rate in CNS, reflected by decreased CSF HVA concentrations, may be a possible intermediate mechanism in the previously reported associations. A substantial limitation of this suggestion is the fact that the results of the present study concern healthy individuals and not patients with schizophrenia or bipolar disorder.

5.6 STUDY IV

MAOB rs5905512, located in intron 1, was one of the candidate SNPs selected due to its reported association with schizophrenia. The association between *MAOB* rs5905512 and CSF MHPG concentration in psychotic men showed the lowest p-value (uncorrected p-value 0.0004) in the present study. Moreover, this association remained statistically significant, taking into account the total number of tests conducted restricted to the candidate SNPs tested. Rs5905512 was not found to be associated with CSF MM concentrations in women with psychosis, healthy men or healthy women. A literature search showed that two independent studies have searched for association between *MAOB* rs5905512 and schizophrenia (www.szgene.org). The first study reported a significant association between this SNP and schizophrenia only in men (Carrera et al., 2009), which is in accordance with our result, whereas the second study did not find any associations in either gender (Mas et al., 2009). The present result suggest that noradrenaline turnover rates, as reflected by the CSF MHPG concentrations, can be considered as an intermediate phenotype in the association between *MAOB* rs5905512 and psychosis in men.

Some of the nominally associations illustrated in Tables 1-3 are further described, due to previously reported associations between the SNPs and schizophrenia. All these associations are restricted to psychosis, as they were not present in healthy controls.

Two *DBH* SNPs, i.e. rs1611115 and rs6271, were associated with MHPG concentrations and rs6271 was also associated with 5-HIAA concentrations in psychotic patients. Rs1611115 is located upstream of *DBH* and accounts for 31% to 52% of the variance of plasma DBH activity in different populations (Zabetian et al., 2001), whereas the nonsynonymous exonic rs6271 independently accounts for additional variance (Tang et al., 2005). Moreover, rs6271 has been associated with schizophrenia as well as with bipolar disorder (Ates et al., 2013; Kukshal et al., 2013). Taken together, the results suggest that noradrenergic and serotonergic mechanisms may mediate the previously reported association between this exonic *DBH* SNP and psychosis.

TPH1 rs1799913, reported to be associated with schizophrenia in single studies and a meta-analysis (www.szgene.org), was in the present study associated

with both CSF 5-HIAA and MHPG, proposing that the previously reported associations with schizophrenia may be mediated by altered serotonin and noradrenalin turnover rates.

The intronic *MAOB* rs1799836 was found to be nominally associated with MHPG concentrations in men with psychosis (uncorrected p-value 0.001). This *MAOB* SNP has been reported to be associated with schizophrenia in women (Gasso et al., 2008), as well as with altered enzyme activity in vitro and in vivo (Balciuniene et al., 2002; Costa-Mallen et al., 2005).

The intronic *COMT* rs165774 was nominally associated with HVA concentrations in psychosis. Rs165774 showed departure from HWE in psychotic patients but not in healthy controls. As rs165774 has been reported to be significantly associated with schizophrenia (Voisey et al., 2012), the present result suggests that dopaminergic mechanisms may be an intermediate step between the gene variant and the disorder.

5.7 STUDY V

An extensive literature search has shown that a number of the psychosis associated SNPs, shown in Tables 4-6, have been ascribed some functionality or association with schizophrenia or other mental disorders.

DAOA rs3918342 has been associated with schizophrenia as well as with bipolar disorder in independent studies (Bass et al., 2009; Korostishevsky et al., 2004; Ma et al., 2009; Schumacher et al., 2004). *DAOA* rs778293 has also been associated with schizophrenia (Korostishevsky et al., 2004; Ma et al., 2006; Ma et al., 2009). In the present study, both rs3918342 and rs778293 were found to be associated with 5-HIAA concentrations in psychotic patients. Rs778293 was also found to be associated with 5-HIAA in controls. Rs3918342, located 28 Kb downstream of the *DAOA* gene, showed the lowest p-value (0.002) in association with 5-HIAA concentrations in psychosis and moreover this association was restricted in psychotic patients. We can therefore conclude that a disturbed serotonin turnover rate in CNS may be an

intermediate phenotype in the previously reported association between rs3918342 and schizophrenia.

DAOA rs1421292 and rs3916971, located 40 kbp and four kbp downstream of the *DAOA* gene, respectively, have been associated with schizophrenia (Korostishevsky et al., 2004; Schumacher et al., 2004). Our results showed that both SNPs were associated with CSF HVA and 5-HIAA in patients with psychosis. Both SNPs were also associated with HVA in healthy controls, whereas the associations with 5-HIAA were absent in controls, suggesting that serotonergic mechanisms may be intermediate steps in the associations between *DAOA* rs1421292 and rs3916971 and psychosis.

DAOA rs1570709, associated with MHPG concentrations in psychotic patients only, has been previously reported to be associated with schizophrenia (Opgen-Rhein et al., 2008). Taken together, our results support the notion that noradrenergic mechanisms may modulate the association between rs1570709 and psychosis.

The intronic *DAO* SNP rs2070586, associated with HVA concentrations in psychotic patients only, has been reported to be associated with schizophrenia (Kim et al., 2010), proposing that the association between the SNP and psychosis may be due to dopaminergic mechanisms.

The intronic *KMO* SNP rs2275163 showed the lowest p-value (0.003) in association with MHPG in psychosis and moreover this association was not present in healthy subjects. As the rs2275163 has been associated with schizophrenia (Aoyama et al., 2006), our result suggests that noradrenergic neurotransmission may play a role in this reported association between the *KMO* SNP and the disorder.

6 CONCLUSIONS

In healthy individuals, *TPH1* and *DTNBP*1 gene variants were significantly associated with both dopaminergic and serotonergic neurotransmission, as reflected by the CSF HVA and 5-HIAA concentrations, respectively. Moreover, *DAOA* SNPs were significantly associated with CSF HVA in healthy individuals, suggesting that the *DAOA* gene has an effect on dopamine turnover rate in CNS.

In psychotic patients, nominal associations were found between SNPs in genes encoding enzymes implicated in the monoamine pathways and the CSF concentrations of HVA, 5-HIAA and MHPG. The number of the nominal associations exceeded the expected number and moreover all but one associations were absent in healthy controls. A candidate *MAOB* SNP, previously reported to be associated with schizophrenia in men, was found to be significantly associated with MHPG concentrations in men with psychosis, correcting for multiple testing taking into account the number of candidate SNPs included in the study. Some of the nominally associated SNPs in *DBH*, *TPH1*, *MAOB* and *COMT* have also been reported to be associated with schizophrenia. The present results suggest that altered monoamine turnover rates in CNS may represent intermediate steps in the associations between genes related to monoamine metabolism and psychosis.

In psychotic patients, we have also found nominal associations between SNPs in glutamate–related genes and the CSF concentrations of HVA, 5-HIAA and MHPG. The majority of the nominal associations were absent in healthy individuals. Some of the nominally associated SNPs in *DAOA*, *DAO* and *KMO* have been previously reported to be associated with schizophrenia. The results suggest that CSF monoamine metabolite concentrations may represent intermediate phenotypes in the associations between glutamate-related genes and psychosis.

7 FUTURE RESEARCH

In the present thesis, we were able to find significant and nominal associations between gene variants and CSF MM in healthy individuals and psychotic patients. Our findings need replication in independent studies using the candidate gene approach. GWAS are also a subject of future research, where without a priori hypothesis the whole human genome can be investigated for associations with CSF MM. To our knowledge, there are no GWAS studies conducted, searching for association between SNPs and MM in psychosis. Given a large sample, a GWAS study could shed further light revealing genes not being included in candidate gene studies, due to absence of known functionality relative to dopamine, serotonin and noradrenaline neurotransmission.

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