

From THE DEPARTMENT OF MOLECULAR MEDICINE AND
SURGERY

Karolinska Institutet, Stockholm, Sweden

**GENETIC ASSOCIATION STUDIES OF
SYMPTOMS, COMORBIDITY AND
OUTCOME IN BIPOLAR DISORDER AND
SCHIZOPHRENIA**

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**Karolinska
Institutet**

Stockholm 2016

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Published by Karolinska Institutet.

Printed by E-print AB 2016

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ISBN 978-91-7676-246-2

GENETIC ASSOCIATION STUDIES OF SYMPTOMS,
COMORBIDITY AND OUTCOME IN BIPOLAR
DISORDER AND SCHIZOPHRENIA
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my beloved family, especially my father who left us too early

ABSTRACT

Schizophrenia and bipolar disorder are complex brain disorders. Research has focused on applying brain research to understand the etiology, as well as clinical research to improve treatment, prognosis and progression. Schizophrenia and bipolar disorder are not lethal in and of themselves, but suicide and the presence of associated physical illnesses are of great concern, since these are the major causes of shortened life in afflicted individuals. In particular, the prevalence of type 2 diabetes and cardiovascular disease are twice as great in schizophrenia and bipolar disorder. By shifting the focus to underlying, sometimes comorbid causes, it is possible to increase knowledge of morbidity and mortality in cardiovascular disease, and thus improve the prognosis and progression for individuals with schizophrenia and bipolar disorder. Another interesting strategy for better understanding such complex disorders is to limit examination to symptoms in order to distinguish the genetics of the symptoms from the disorder itself. Genetic association studies are often used to investigate complex disease. The aim of this thesis was to investigate genetic associations between gene variants and metabolic risk factors in schizophrenia and bipolar disorder patients. An additional aim was to investigate known psychiatric risk genes in the dopamine system and their association to cognitive function.

In Study I, D-amino acid oxidase activator gene (*DAOA*) and catechol-O-methyltransferase gene (*COMT*) were analyzed for allelic association to cognitive dysfunction in bipolar disorder patients. In Studies II-V, common metabolic risk gene variants were analyzed for allelic association to metabolic risk factors in schizophrenia and bipolar disorder patients, and to disorders *per se*. In Study VI, metabolic risk variants were analyzed for possible association to high-sensitive troponin T levels, which is a sensitive biomarker of cardiovascular damage in patients with acute coronary syndrome.

In study I, single nucleotide polymorphisms in D-amino acid oxidase activator gene (*DAOA*) and catechol-O-methyltransferase gene (*COMT*) were associated to cognitive dysfunction in bipolar disorder patients. Data also suggest interaction between these genes. In studies II-V, single nucleotide polymorphisms in common metabolic risk genes: insulin-like growth factor II mRNA binding protein 2 (*IGF2BP2*), neurogenic locus notch homolog 2 (*NOTCH2*), thyroid adenoma associated (*THADA*), wolfram syndrome 1 (*WFS1*), purinergic receptor P2X, ligand-gated ion channel, 7 (*P2RX7*), and melatonin receptor 1B (*MTNR1B*) were associated with increased fasting plasma glucose in schizophrenia. Peroxisome proliferator-activated receptor delta gene (*PPARD*) was associated with schizophrenia independent of glucose levels. Single nucleotide polymorphisms in common metabolic risk genes: calcium/calmodulin-dependent protein kinase kinase 2 (*CAMKK2*), melanoma inhibitory activity family, member 3 (*MIA3*), purinergic receptor P2X, ligand-gated ion channel, 7 gene (*P2RX7*), muscle RAS oncogene homolog gene (*MRAS*), SMAD family member 3 gene (*SMAD3*), peroxisome proliferator-activated receptor delta gene (*PPARD*), melatonin receptor 1B gene (*MTNR1B*), neurogenic locus notch homolog 2 gene (*NOTCH2*), HNF1

homeobox B gene (*HNF1B*) were associated with increased waist circumference in schizophrenia patients. Peroxisome proliferator-activated receptor delta gene (*PPARD*), melatonin receptor 1B gene (*MTNR1B*), neurogenic locus notch homolog 2 gene (*NOTCH2*), and homeobox B gene (*HNF1B*) were associated with schizophrenia irrespective of waist circumference. A genetic overlap between schizophrenia and bipolar disorder was identified through an association between melatonin receptor 1B gene (*MTNR1B*) and increased fasting plasma glucose also in bipolar disorder patients. Neurogenic locus notch homolog 2 gene (*NOTCH2*) was associated to bipolar disorder *per se*. In study VI, melatonin receptor 1B gene (*MTNR1B*) and neurogenic locus notch homolog 2 gene (*NOTCH2*) were associated with high-sensitive troponin T levels in schizophrenia women.

Our genetic findings regarding D-amino acid oxidase activator gene (*DAOA*) and catechol-O-methyltransferase gene (*COMT*) are in line with the dopamine hypothesis of cognitive function. Single nucleotide polymorphisms that increase metabolic risk in the general population are associated with elevated plasma glucose and increased waist circumference among schizophrenia and bipolar disorder patients, as well as with schizophrenia and bipolar disorder *per se*. The melatonin receptor 1B gene (*MTNR1B*) –dependent vulnerability for elevated fasting plasma glucose levels is evident in both schizophrenia and bipolar disorder. Neurogenic locus notch homolog 2 gene (*NOTCH2*) is associated to both to schizophrenia and bipolar disorder type 1 *per se*. These findings may reflect increased metabolic genetic vulnerability in schizophrenia and bipolar disorder patients, as well as common genetics between type 2 diabetes mellitus and these psychiatric disorders. In addition, in women with schizophrenia, there is a possible metabolic genetic component affecting high-sensitive troponin T levels, a biomarker for cardiovascular damage in individuals with acute coronary syndrome (chest pain).

LIST OF SCIENTIFIC PAPERS

- I. **Dzana Sudic Hukic**, Louise Frisé, Lena Backlund, Catharina Lavebratt, Mikael Landén, Lil Träskman-Bendz, Gunnar Edman, Martin Schalling, Urban Ösby. **Cognitive manic symptoms in bipolar disorder associated with polymorphisms in the *DAOA* and *COMT* genes.** PLoS One 2013 Jul 5;8(7):e67450. doi.org/10.1371/journal.pone.0067450
- II. **Dzana Sudic Hukic**, Eric Olsson, Agneta Hildnig, Claes-Göran Östenson, Harvest F Gu, Ewa Ehrenborg, David Erlinge, Gunnar Edman, Martin Schalling, Catharina Lavebratt, and Urban Ösby. **Genes associated with increased fasting glucose in patients with schizophrenia spectrum disorders.** J. Diabetes Metabolism 2015, 6(3):1000512. doi.org/10.4172/2155-6156.1000512
- III. **Dzana Sudic Hukic**, Catharina Lavebratt, Louise Frisé, Lena Backlund, Agneta Hilding, Claes-Göran Östenson, David Erlinge, Ewa Ehrenborg, Martin Schalling, Urban Ösby. **Melatonin receptor 1B gene associated with increased fasting glucose in bipolar disorder.** Psychiatric Genetics 2016, 00:000–000. DOI: 10.1097/YPG.0000000000000131
- IV. **Dzana Sudic Hukic**, Urban Ösby, Eric Olsson, Agneta Hilding, Claes-Göran Östenson, Harvest F Gu, Ewa Ehrenborg, Gunnar Edman, Martin Schalling, Catharina Lavebratt, Louise Frisé. **Genetic variants of increased waist circumference in psychosis.** *Manuscript*
- V. **Dzana Sudic Hukic**, Catharina Lavebratt, Louise Frisé, Lena Backlund, Agneta Hilding, Harvest F Gu, Claes-Göran Östenson, David Erlinge, Ewa Ehrenborg, Martin Schalling, Urban Ösby. **NOTCH2 associated with bipolar disorder.** *Manuscript*
- VI. **Dzana Sudic Hukic**, Catharina Lavebratt, Eric Olsson, Claes-Göran Östenson, Sven V. Eriksson, David Erlinge, Martin Schalling, Urban Ösby. **Troponin T levels associated with genetic variants in NOTCH2 and MTNR1B in women with schizophrenia.** *Manuscript*

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LIST OF ABBREVIATIONS

ABD	Anonymous blood donors
BD	Bipolar disorder
<i>CAMKK2</i>	Calcium/calmodulin-dependent protein kinase kinase 2 gene
<i>COMT</i>	Catechol-O-methyltransferase gene
COMT	Catechol-O-methyltransferase
CMS	Cognitive manic symptoms
CVD	Cardiovascular disease
DA	Dopamine
<i>DAOA</i>	D-amino acid oxidase activator gene
DAOA	D-amino acid oxidase activator
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
FPG	Fasting plasma glucose
FHD	Family history of diabetes
Glu	Glutamate
GWAS	Genom Wide Association Study
<i>HNF1B</i>	HNF1 homeobox B gene
hsTnT	High-sensitive troponin T
IDF	International Diabetes Federation
<i>IGF2BP2</i>	Insulin-like growth factor II mRNA binding protein 2 gene
<i>MIA3</i>	Melanoma inhibitory activity family, member 3 gene
<i>MRAS</i>	Muscle RAS oncogene homolog gene
<i>MTNR1B</i>	Melatonin receptor 1B gene
MTNR1B	Melatonin receptor 1B
NMDAR	N-methyl-D-asparate receptor
NOS	Not otherwise specified
<i>NOTCH2</i>	Neurogenic locus notch homolog 2 gene

PD	Psychiatric disorder
PFC	Prefrontal cortex
<i>PPARD</i>	Peroxisome proliferator-activated receptor delta gene
<i>P2RX7</i>	Purinergic receptor P2X, ligand-gated ion channel, 7 gene
SCZ	Schizophrenia
SDPP	Stockholm Diabetes Prevention Program
SGA	Second-generation antipsychotics
<i>SMAD3</i>	SMAD family member 3 gene
SNP	Single nucleotide polymorphism
SSD	Schizophrenia spectrum disorder
<i>THADA</i>	Thyroid adenoma associated gene
T2DM	Type 2 diabetes mellitus
<i>WFS1</i>	Wolfram syndrome 1 gene

The drawings (adjusted in Format Picture) are interpretations made by my children, after discussing psychiatric disorders.



Deep inside, there is no smile. Emily Hukić, age 7

1 PSYCHIATRIC DISORDERS

1.1 PSYCHIATRIC DISORDERS IN GENERAL

Psychiatric disorder (PD) is considered a major public health problem, rated as one of the top ten disabling conditions worldwide (1). Every fourth individual is or will be affected during some point in their life. In addition, one-third meet the criteria for multiple psychiatric diagnoses (2), and also demonstrate a high presence of other somatic disorders (3-5). PD patients have a higher risk of dying prematurely. Cardiovascular and circulatory diseases are responsible for the highest number of lost life years in PD (5). The cause of PD has not yet been discovered, which is in itself the greatest research challenge – along with the need for better treatment and improved drugs. Moreover, poor attitudes and discrimination towards people with PD still results in social stigma. This stigma is a long-lasting health and social burden that requires more research, resources and public awareness. By generating new knowledge, we may also reduce the stigma.

This thesis focuses on severe psychiatric disorders, particularly schizophrenia (SCZ) and bipolar disorder (BD), as well as related disorders and symptoms. Genetics represents one of the main explanations for why we become mentally ill. All studies in this thesis are based on genetic analysis.

1.2 SCHIZOPHRENIA AND BIPOLAR DISORDER

Schizophrenia (SCZ) and bipolar disorder (BD) are common, severe and essentially lifelong brain disorders. The prevalence in the population for SCZ and BD is about 1% each, irrespective of culture and country variations (6, 7). The disorders usually appear in the late teens or early adulthood (8, 9), although there are cases reported in the early, as well as late years of life.

The classical clinical distinction between these two disorders was based on Emil Kraepelin's theory when he distinguished between manic-depressive disorder and madness. However, both SCZ and BD patients experience mood swings, cognitive impairment and psychotic features - all of which might have fallen into the category of madness according to Kraepelin. Therefore, his clinical division between SCZ and BD is coming under increasing scrutiny as growing genetic and biological evidence identifies overlapping genes and phenotypes.

1.3 CLINICAL SYMPTOMS

1.3.1 Schizophrenia

The word schizophrenia comes from the Greek “σχίζοφ (schizo) φρένια (phrenia) and means split mind. Persons suffering from SCZ have a distorted perception of reality (losing touch with reality) that includes psychotic (or positive) symptoms such as hallucinations (perceptions, hearing or seeing things, without external stimuli) and delusions (they strongly believe in something despite evidence to the contrary) that are present in the acute phase of the disorder and are core features of SCZ. The clinical picture is broad and includes a number of additional symptoms including cognitive impairment typically present in schizophrenia patients, diminished feelings and emotions, as well as loss of interest in daily activities that may persist over time, often becoming chronic.

1.3.2 Bipolar disorder or manic-depressive illness

BD or manic-depressive illness is characterized by disturbed balance in mood. Persons with BD experience depressive and manic episodes. In between episodes, bipolar patients recover from the symptoms and are in a more stable condition. How frequently and how quickly the patients shift between the different episodes depends on the severity and subtype of BD. During manic episodes, persons with BD experience increased self-esteem, reduced sleep, and have a lot of energy and ideas, often with a lack of insight as to the following consequences. On the contrary, depressed episodes include reduced self-esteem and inability to feel pleasure, as well as increased sleep and less energy, and also more serious thoughts of suicide. These may seem like common emotions, feelings and thoughts that we all experience, but for BD sufferers the feelings and emotions are heightened and more tangible, and affect everyday life to a great extent.

1.4 DIAGNOSIS

The diagnosis of SCZ and BD is based on clinical symptoms by observing behavior, feelings, and thoughts. Even today, there are no tests based on genetics, biochemistry, or brain imaging analysis that can assist the diagnosis process.

1.4.1 Schizophrenia

In the Diagnostic and Statistical Manual of Mental Disorders (DSM), which contains classifications and criteria for the diagnosis of psychiatric conditions from the American Psychiatric Association, schizophrenia is classified under the chapter "Schizophrenia Spectrum And Other Psychotic Disorders". The subtypes of schizophrenia are based on the symptoms that dominate (paranoid, disorganized, catatonic, undifferentiated and residual). Patients in this thesis have been diagnosed according to the DSM 4th Edition (DSM-IV) (10). According to DSM-IV, a person needs to fulfill the following diagnostic criteria for the SCZ diagnosis: two (or more) episodes of delusions, hallucinations, disorganized speech,

disorganized or catatonic behavior, and negative symptoms (i.e. affective flattening), each present for a 1-month period (or less if treated with success). Additional criteria involve social dysfunction, duration of the symptoms, and also exclusion of possible use of drugs and alcohol, alternatively endocrine disturbance. Schizophrenia is a spectrum of psychotic disorders rather than a distinct condition. Other psychotic disorders include schizoaffective disorder, delusional disorder and psychosis not otherwise specified (NOS).

1.4.2 Bipolar disorder

BD is classified under the chapter “Mood Disorders” in the DSM-IV (10). Mania or hypomania is a defining criterium for bipolar disorder diagnosis. Criteria for manic episodes include abnormal irritable mood during a period of 1 week (or any period if there is a need for hospitalization), and three (or more) of the following symptoms: inflated self-esteem, decreased need of sleep, talkativeness, racing thoughts, distractibility, increase in goal-directed activity and excessive involvement in activities with a high risk of painful consequences. Additionally, marked deterioration in daily activities and relationships, also exclusion of possible use of drugs and alcohol induced symptoms. During the manic episodes, many patients show cognitive impairment. There is evidence that cognitive impairment may persist over time, even between active episodes (11, 12), thus also affect long-term social functioning.

1.5 ETIOLOGY

The cause of the psychiatric disorders is, to a large extent, unknown. Genetic and environmental risk factors stand out. A multitude of genetic studies have proposed a number of candidate genes. However, the mechanisms by which genetic and environmental factors interact, affect and reflect the neurobiology and symptoms of psychiatric disorders remains to be discovered.

1.5.1 Neurotransmitter hypotheses

Neurotransmitters are chemical substances that transmit signals from one neuron (nerve cell) to another neuron. There are hypotheses built around known abnormal neurotransmission and symptoms in psychiatric disorders. Some sustained hypotheses are listed below and are based on dopamine (DA), and glutamate (Glu) in SCZ. Genetics underlying the signaling of these neurotransmitters is thus of substantial interest.

1.5.1.1 The dopamine hypothesis

The leading hypothesis of SCZ is dysfunctional DA signaling (13, 14). DA is produced in the brain regions called the substantia nigra and ventral tegmental area, and transported via dopaminergic pathways to different regions of the brain. Several studies demonstrate that drugs that induce DA release also induce psychotic symptoms, while, on the contrary, drugs that block DA signaling reduce psychotic symptoms, which is the main evidence suggesting

DA's involvement in the etiology of SCZ (15, 16), and the DA-receptors as targets in the treatment of psychotic symptoms.

The brain region prefrontal cortex (PFC) has also been implicated in the hypothesis of SCZ. In this region, the DA levels are reduced. Studies show reduced prefrontal cerebral blood flow (CBF) in SCZ individuals (17, 18). In addition, DA metabolite levels in cerebrospinal fluid (CSF) correlates with prefrontal CBF (18), indicating low DA in PFC. The PFC is an important region for cognitive function, thus this proposes the involvement of DA in cognitive functioning.

Genes that influence DA are of interest to study. Catechol-O-methyltransferase (COMT) is an enzyme that degrades DA. One functional SNP (*SNP definition see page 28*) of the *COMT* gene has been of special interest. This SNP (Val158Met or rs4680) has been reported to affect the production of the COMT enzyme, thereby regulating the degradation of dopamine. If the "Met" variant is present, the enzyme function is lower and DA level is higher (19). Higher DA level is associated with psychotic symptoms. *COMT* has also been implicated in the neurobiology of cognition. The "Met" variant associated with better performance on working memory in SCZ, and in the general population (more functioning brain) (20), thus also increases the risk for SCZ. Furthermore, *COMT* has been reported to influence the performance on facial emotion recognition in BD (21).

1.5.1.2 The glutamate hypothesis

Glu signaling is important for neurotransmission, plasticity, memory and learning (22). Blocking N-methyl-D-aspartate receptors (NMDAR) is reported to induce schizophrenia-like symptoms (23) and altered NMDA receptors have been associated with reduced memory (24). Lower Glu plasma levels have been detected in SCZ and BD patients at the onset of the disorders, indicating an impaired Glu system at an early stage of the illness (25). High concentration of Glu may induce manic symptoms.

The *DAOA* (D-amino acid oxidase activator or G72) gene is one of the interesting genes related to Glu signaling. The *DAOA* gene codes D-amino acid oxidase activator (DAOA) that may influence the function of D-amino acid oxidase (DAO), which is an enzyme that catalyzes the conversion of D-DOPA into L-DOPA (26) that is a precursor of DA. DAOA also influences the function of D-serine, which activates NMDA receptors (27).

There is also support for combined genetic effects of glutamatergic and dopaminergic pathways on cognitive functioning (28).

1.6 GENETICS OF PSYCHIATRIC DISORDERS IN GENERAL

Genetic differences represent one of the main explanations for why we become mentally ill. SCZ and BD are highly heritable disorders, with heritability estimates as high as 85% (29, 30).

The risk of getting ill increases with shorter genetic distance to the affected person (31). Identical twins share same DNA, thus may share the highest risk of getting ill. If it were only genetics that influenced the development of the disorder, both identical twins would be sick. This is not the case, the genetic penetrance does not alone decide whether we get sick or not.

In addition to genetic variation, environmental factors such as birth complications (32), history of infections (33), drug use (16), and epigenetics (34) may influence susceptibility to psychiatric disorders. Genetics and environmental factors work together in a very complex way, not yet understood.

From large genetic studies, such as Genome Wide Association Studies (GWAS) that examine associations between a large number of common SNPs and diseases, we have learned that a large number of common gene variants are important for the development of SCZ and BD or traits like cognition (polygenic inheritance), although each variant has a small effect size. In addition, we have learned that identified genetic risk variants together explain only a small fraction of the heritability (35). What is more, one gene may also affect more than one disorder (pleiotropy). For example, SCZ and BD patients share substantial genetic risk components (36). Clinically, SCZ and BD are distinct disorders, but the biological distinction does not necessarily follow the clinical division.

Results from genetic studies also lack information of *how* genes affect disorders. As of yet, the relevance of genetic results has not yet been taken into consideration in clinical settings, and such results also still lack clinical value regarding disease onset and outcome.

1.7 TREATMENT

Antipsychotic drugs are the main treatment for SCZ and schizoaffective disorder patients. All antipsychotics act on the dopaminergic system by blocking the release of DA. Clozapine affects a wide range of receptors and has a clinical indication for cases that are resistant to treatment with other antipsychotics. Lithium and other mood stabilizers are the main treatments for BD type 1 patients, often in combination with antipsychotics as complementary therapy. Although there are proposed mechanisms of lithium action via the glutamatergic system (37), its specific function is still unclear. In addition to medications, other measures such as psychosocial interventions are needed. In some cases it can take several years before the correct diagnosis and treatment (right drug and dose) is established. Variations between patients in drug response are common.

1.7.1 Side-effects of antipsychotics

Antipsychotics are known to have harmful effects. First-generation antipsychotics (FGAs) or typical antipsychotics are known to have side effects such as extrapyramidal symptoms (EPS) that are movement disorders, and include muscle contractions, rigidity and irregular movements (38). Although the second-generation antipsychotics (SGAs) have been improved

and offer patients a better experience, the SGAs – in particular clozapine and olanzapine – have contributed to increased cardiovascular disease (CVD) risk, which ultimately has negative effect on the long-term prognosis.



Comorbidity in schizophrenia. Emil Hukić, age 12

2 SCHIZOPHRENIA, BIPOLAR DISORDER & METABOLIC COMORBIDITIES

Mentally ill individuals often have metabolic disorders. There is insufficient treatment of severe mentally ill (SMI) individuals concerning somatic illness, which affects prognosis and outcome in a negative way. Lack of knowledge regarding *how to treat SMI* may result in inadequate communication in health care and greater focus on the primary (mental) diagnosis that shifts attention away from somatic problems. A holistic view of the patient is needed to identify the presence of somatic disorders early on for improved treatment and prognosis of the patient.

One of the major issues in SMI is CVD. Although death from suicide is markedly increased in SCZ and BP, CVD is the leading cause of death in this patient group (3, 4). There are two times more deaths in SCZ and BD patients than the numbers reported for the general population (39). In Sweden, the life expectancy of persons suffering from SCZ is reduced by 15 to 17 years (40). This reduced life expectancy in SCZ is mainly explained by increased CVD comorbidity and mortality. Similar findings have been reported for BD patients (4, 41). Further knowledge about CVD in SCZ and BD is strongly warranted.

2.1 RISK FACTORS FOR CARDIOVASCULAR DISEASE

In addition to risk factors such as age, gender and genetic predisposition (42-44), some risk factors are often emphasized when referring to CVD risks. These are presented below.

2.1.1 Diabetes and metabolic disturbances

Diabetes mellitus (DM) and metabolic disturbances such as increased weight, elevated fasting plasma glucose (FPG), elevated blood pressure, and imbalance in blood lipids, are all established and well known risk factors that increase the risk for CVD. Moreover, smoking, physical inactivity, poor diet and stress are risk factors for DM, especially type 2 diabetes mellitus (T2DM).

T2DM is a major and growing public health concern worldwide for all ages, with a prevalence reported to be around 8.5% in Europe in 2013 (44, 45). T2DM represents the majority of all diabetes cases. It usually manifests in adults when the body's response to insulin becomes impaired (46). The lifetime risk of developing T2DM is over 30%, and higher for women than men. Individuals diagnosed with T2DM have a reduced lifespan (47, 48). Increased levels of FPG relative to age are three times more frequent in patients with psychotic disorders than in population controls (49).

Despite the fact that the global population is aging and these disorders are associated with an aging population, which is also an explanation for increased mortality from CVD (with some

geographical variations), being overweight persists as a major risk component of T2DM (44), and thereby also CVD. SCZ individuals are two times more likely to be overweight than the general population (50). SCZ individuals also have an unhealthier lifestyle including poorer diet and less physical activity (51, 52).

Individuals with a positive family history of T2DM have an increased storage of fat and risk for obesity when compared to individuals without T2DM in the family (42, 43). T2DM is a predictor of increased mortality in SMI individuals (53). Furthermore, SCZ and BD are disorders that have hereditary components in common (36). In this thesis, the increased CVD mortality in SCZ and BD is hypothesized to be an effect, in part, of metabolic risk genes, which might overlap between SCZ and BD, and also with the general population.

2.1.1.1 Etiology of type 2 diabetes

The cause of T2DM is based on insulin resistance (cells in the body no longer respond to insulin). This condition results in increased blood glucose levels, which is a key clinical characteristic of T2DM (46). The oral glucose tolerance test (OGTT) is a clinical T2DM diagnostic test. Another test involves measuring C-peptide in the blood, which is a byproduct during insulin production. One insulin molecule corresponds to one C-peptide molecule, making C-peptide a biomarker of insulin production.

When we eat, our glucose concentration (sugar in the blood) increases, and so does the production of insulin in the pancreas, which helps cells in the body utilize glucose. In addition to the pancreas (where the beta cells produce insulin), the liver has a glucose-related task. In order to maintain glucose homeostasis in the body, the liver absorbs excess glucose in the blood, and stores it as glycogen, (glucose→glycogen = glycogenesis). Once the glucose level is low (i.e. during the night while we sleep), the liver releases glucose reserves, (glycogen→glucose = glycogenolysis) to maintain glucose homeostasis.

The abdominal (stomach) fat is the most harmful kind of fat. Accumulation may contribute to excess fat in the liver, and impair the liver's glucose-related function (54) – causing increased level of FPG in T2DM individuals (55). Waist circumference is a convenient measure of excess abdominal fat (56, 57), and high values indicates metabolic abnormalities, regardless of weight (58-60).

2.1.1.2 The International Diabetes Federation (IDF) criteria

The International Diabetes Federation (IDF) has proposed criteria for reference (cut-off) values that constitute risk factors for T2DM. The IDF criteria are followed in the studies of this thesis. The cut-off level for elevated FPG level is defined as FPG ≥ 5.6 mmol/L, and for increased waist circumference ≥ 80 cm for women and ≥ 94 cm for men.

2.1.2 Antipsychotic and lithium induced metabolic disturbance

Antipsychotic drugs are frequently mentioned in the context of metabolic disturbances, especially increased weight. One possible mechanism behind the weight gain is through up-regulation of histamine receptors (61) since histamine is a neurotransmitter known to increase appetite. There are also indications that antipsychotic medication may affect insulin production directly, without significant weight change (62, 63).

Clozapine and olanzapine are two of the antipsychotics that are most often associated with increased weight. Clozapine treated rats (without significant weight change) had increased expression in the liver of glucose-6-phosphatase (*G6Pase*) which encodes an enzyme that is involved in glycogenesis and glycogenolysis (62). This is in line with the hypothesis that increased weight gain from clozapine may occur through reduced hepatic glucose-related metabolism and increased glucose levels.

In addition, studies of drug free and drug naïve (untreated) patients indicate increased levels of visceral fat deposition (64). What is more, an increased prevalence of T2DM has been reported in drug naïve psychosis patients (64-67), and in healthy relatives of people with SCZ (68), which suggests that there is a genetic predisposition to metabolic disturbances in SCZ patients.

Antipsychotics are used much less in treatment for BD patients compared to SCZ patients, despite the fact that BD patients have a similarly doubled mortality from CVD (4, 41). Lithium has been associated with increased weight, and thereby a risk of metabolic abnormalities (69). However, a differential effects of lithium in obese and non-obese patients has been reported. Patients who are already obese put on more weight, while non-obese patients do not show the same weight gain during lithium treatment (70). This supports the hypothesis that the genetic overlap between SCZ and BD may also include shared metabolic vulnerability beyond drug impact.

2.1.3 Psychiatric disease as a risk factor

Psychosis itself has been reported as an independent CVD risk factor (71). Severity of BD (manic-depressive illness) has been associated with metabolic disturbances (72, 73). Although it is well known that PD increases the risk of T2DM, this correlation might be operating in both directions, T2DM might also increase the risk for PD, which seems to receive less attention. Risk of PD (mood and anxiety disorders) is higher among persons with DM (74). The existence of diabetes doubles the odds for depression (75). SCZ and T2DM share familial risk factor; positive family history of psychosis is associated with positive family history of T2DM in SCZ patients (76), which supports the genetics underlying the increased frequency of T2DM among SCZ patients. There is also a genetic risk region that overlap between SCZ and T2DM (77).

2.2 GENETICS OF TYPE 2 DIABETES

T2DM is a complex chronic disorder with a slow progression and long duration. Both genes and environmental factors contribute to the development of T2DM. The genetic inheritance is supported by genetic studies of identical twins that estimated a concordance rate of 25% for T2DM, and of over 60% for impaired glucose tolerance (78, 79). Genetic findings explain only a small portion of the T2DM heritability.

GWAS have generated several loci containing genes and common SNPs with association to T2DM and impaired glucose traits. Several of the genes and SNPs with associations to metabolic traits presented in this thesis have reached the GWAS level of statistical significance in previously published studies, although the effect sizes for the most GWAS associations are small and limited. There are some proposed mechanisms of genetic action: impaired insulin processing, lower insulin secretion, reduced insulin sensitivity, and defects in early insulin secretion. Most SNPs associated to T2DM indicate a primary defect in beta cells (80).

In this thesis, increased FPG in SCZ and BD patients shows an association to the SNP rs10830963 in the *MTNR1B* gene. The rs10830963 with the top signal to FPG has, in large GWAS, been associated with elevated FPG levels, impaired beta cell function, and T2DM (81-83). This gene codes for melatonin receptor 1B that is expressed in beta cells. T2DM patients tended to have increased expression of this receptor in beta cells (84). The effect of rs10830963 on impaired glucose takes place probably through an initial phase of glucose-stimulated insulin release (82-84), suggesting that *MTNR1B* impairs the early insulin secretion.

Melatonin also regulates the circadian rhythm. There is evidence in the literature for a link between circadian genes and T2DM (85). Moreover, it is not uncommon that PD patients have disturbed sleep.

The metabolic risk gene, *NOTCH2*, presented in this thesis that might be considered as a new finding as it has not been reported previously in association to BD, however it is in need of replication. Notch signaling is a conservative signaling pathway implicated in many cellular functions, although it has been shown to be important for neurogenesis in the adult brain (86). A recent study in a mouse model observe that blocked Notch signaling induced an increase in dopaminergic neurons (87). Moreover, the presence of an altered Notch2 signaling pathway is reported to be associated to suicide victims without PD (88), and additionally it is known that death from suicide is greater in SCZ and BD patients compared to the population (4, 89).

2.3 GENETICS OF CARDIOVASCULAR DISEASE

CVD is a complex disease that involves several heart and blood vessel disorders. Most often mentioned are myocardial infraction and stroke, which are acute events and depend on the loss of blood supply to the heart and brain. The loss of blood supply to the heart and brain is

usually due to a blockage in the vessels due to fat accumulation (lipids). Genetic studies on CVD risks have focused on lipid metabolism (90-92). One of the most known genes is *Apo E* that codes for Apo E protein. Apo E binds to low-density lipoprotein (LDL) and transports it to tissue and cells. Apo E has also been associated with SCZ (93). In one study, SNPs linked to SCZ are reported to associate also with CVD risk factors. The majority of those SNPs are found with lipid levels, suggesting that lipid metabolism might be involved in pathophysiology of SCZ (94).

Regarding the overlap of SNPs between CVD and T2DM, it is small (95).

3 AIMS

The aims of this thesis were to increase the understanding of genetics underlying:

- cognitive symptoms in BD patients
- metabolic risk factors in SSD and BD,
- SSD and BD *per se*
- biomarkers of cardiovascular damage in SCZ patients

3.1 SPECIFIC AIMS

- I. To investigate genetic associations between cognitive manic symptoms during manic episodes in BD type 1 patients and genetic variants in *COMT* and *DAOA*.
- II. To test whether reported genetic metabolic risk variants were associated with 1) elevated FPG in SSD and 2) whether genetic variants were associated with SSD irrespective of glucose levels.
- III. To test whether genetic metabolic risk variants, associated with elevated FPG in patients with SSD, were associated with elevated FPG in BD.
- IV. To investigate whether common genetic metabolic risk variants 1) confer an independent risk factor for increased waist circumference in patients with SSD, and 2) investigate whether the genetic variants were associated with SSD irrespective of waist circumference.
- V. To test whether genetic metabolic risk variants, previously associated with elevated FPG in patients with BD or SSD, were associated with BD regardless of FPG.
- VI. To investigate whether high-sensitive TroponinT levels were associated with common genetic metabolic risk variants reported for SSD patients.



Researcher at work. Emily Hukić, age 7.

4 MATERIAL AND METHODS

4.1 SUBJECTS

Ethical approval was obtained from the Stockholm Regional Ethics Committee separately for patients and control subjects. All participants gave their informed consent to participate.

Subjects in this thesis are from four populations, all mainly from the Stockholm region.

4.1.1 The Swedish study in bipolar disorder

Unrelated patients above the age of 18 years and diagnosed with BD (with life assessment of specific symptoms of mania and depression) were invited to participate. The recruitment of patients was performed in specialized outpatients clinics for affective disorders mainly from Karolinska University Hospital Huddinge.

The module for mania in the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (96) was used to register the DSM-IV manic symptoms: elevated mood, irritability, over-activity, grandiosity, decreased sleep, talkativeness, distractibility, goal-directed behavior, thought disorder, and embarrassing behavior (97). The focus was on patients with the most severe episode of mania. Additional information was obtained from medical records and also from interviews when necessary.

4.1.2 Swedish Study of Metabolic Risks in Psychosis (SMRP)

As part of a general medical examination, unrelated patients with long-term psychotic disorders, especially schizophrenia, treated in specific psychosis outpatient units, were asked to participate in the Swedish Study of Metabolic Risks in Psychosis (SMRP) study. Diagnoses were confirmed according to the DSM-IV (10). Schizophrenia was the most common diagnosis, other diagnosis represented in the patient material were schizoaffective disorder, delusional disorder, psychosis not otherwise specified (NOS), and bipolar disorder (BD).

Patients were asked to answer to various questions including, somatic health, somatic health information in the closely related, smoke and alcohol habits, and employment. Also, psychiatric and somatic assessment was compiled together with/by a doctor, containing information about Global Assessment of Functioning (GAF), Clinical Global Impression (CGI), onset of the disorder and treatment information on drugs and dose, and duration of the treatment. Patients received written instructions to fast overnight before leaving blood for analysis. FPG, blood pressure, body weight, height, and waist circumference were measured.

4.1.3 Stockholm Diabetes Prevention Program (SDPP)

Control individuals were selected from the Stockholm Diabetes Prevention Program (SDPP) (98), comprising 7949 unrelated participants recruited between 1992 and 1998. At inclusion, only subjects without known diabetes were enrolled and half of the subjects had ≥ 1 first-degree relative with known diabetes. A follow-up was performed 9 to 10 years later (2002-2006) and included 5712 subjects (3329 women and 2383 men) (72% of the original participants). At follow-up, 997 individuals (17%) had increased FPG (≥ 5.6 mmol/L) including 289 individuals (5%) who were diagnosed with T2DM during the period between inclusion and follow-up. Data were obtained about weight, height, waist circumference, blood pressure, and FPG both at inclusion and at follow-up.

From the SDPP follow-up sample, control subjects were selected to represent the total SDPP cohort for the genetic association study.

4.1.4 Anonymous blood donors (ABD)

Anonymous blood donors (ABD) were recruited from Karolinska University Hospital in Solna. Information available for the ABD was gender.

4.2 METHODS

4.2.1 DNA preparation

For the patients and controls, venous blood was drawn from each individual. DNA was extracted according to standard procedures.

4.2.2 Genetic variations in humans

The genetic variations in DNA describe and contribute to our uniqueness. Some genetic variation can lead to disease - to examine genetic variations we may achieve better understanding of complex genetic diseases. Genetic variations vary in size and include; duplications, deletions and insertions named copy number variant (CNV). The most common genetic difference ($>1\%$) between two human DNAs is Single Nucleotide Polymorphism (SNP), which is one base change. Every person has two copies of the same position, called alleles (one from each parent) or three possible allele combinations (genotypes) at the specific position or SNP, being homozygous (two same alleles) or heterozygous (two different alleles). The human DNA counts around 3 billion base pairs. SNPs occur every 300 nucleotides on average, so there are about 10 million SNPs. SNPs may be in the gene region (exon or intron) or in between genes (non-coding region or intergenic). SNPs may affect the amino acid formation (non-synonymous), thus may affect the end product of a protein-coding gene (protein) or not affect the amino acid formation (synonymous). In the studies of this thesis,

genetic analyses are based on the investigation of genetic associations between alleles/genotypes and traits (cognitive impairment/increased FPG/increased waist circumference or disorders *per se*). Many SNPs in this thesis are in non-coding region.

Alleles that are located close to each other are basically inherited together, they are said to be in the linkage disequilibrium (LD) (99). Genetic recombination plays a role in how the alleles are organized in the DNA. When genetic recombination occurs between two alleles, the LD between alleles are reduced, and the allele organization is changed.

4.2.2.1 Selection of SNPs

The SNP selection was hypotheses driven based on previously reported findings.

Genetic variants with reported GWAS significance ($p < 5 \times 10^{-8}$) for association with T2DM and/or CVD were selected: rs10923931 (*NOTCH2*), rs7578597 (*THADA*), rs4607103 (*ADMTS9*), rs864745 (*JAZF1*), rs7961581 (*Nearest gene association-TSPAN8/LGR5*), rs12779790 (*Nearest gene association-CDC123*), rs8050136 (*FTO*), rs10811661 and rs4977574 (*CDKN2B*), rs4402960 (*IGF2BP2*), rs1801282 (*PPARG*), rs13266634 (*SLC30A8*), rs7903146 (*TCF7L2*), rs1111875 (*Nearest gene association-HHEX*), rs5219 (*KCNJ11*), rs7754840 and rs7756992 (*CDKAL1*), rs10830963 (*MTNR1B*), rs2237892 (*KCNQ1*), rs2259816 (*HNF1A*), rs9818870 (*MRAS*), rs6922269 (*MTHFDIL*), rs646776 (*CELSR2*), rs17465637 (*MIA3*), rs2943634 (*No gene association*), rs17228212 (*SMAD3*), rs1746048 (*CXCL12*). Other common genetic variants were selected based on relevant associations with T2DM and CVD: rs2251101 (*IDE*), rs10010131 (*WFS1*), rs11037909, rs1113132 and rs3740878 (*EXT2*), rs1800804 (*MTTP*), rs1053049, rs34474204, rs2016520, rs2076167 and rs6902123 (*PPARD*), rs7923837 and rs1544210 (*Nearest gene association-HHEX*), rs3817190 (*CAMKK2*), and rs1718119, rs2230912, and rs3751143 (*P2RX7*), and rs7501939 and rs757210 (*HNF1B*) (Table 1).

4.2.3 Genetic analysis – Genotyping

Genotyping is an analysis used to examine the DNA sequence at, for example specific SNP locations using genetic techniques such as TaqMan and Open Array). In this way we can see whether our patient group differs in frequency of specific SNP alleles or genotypes compared to controls.

4.2.3.1 Genetic techniques and instruments

In this thesis, two instruments have been used for genotyping.

7900HT Fast Real-Time PCR System Instrument using allele-specific TaqMan MGB probes labeled with fluorescent dyes FAM and VIC (Applied Biosystems, Foster City, CA, USA), in accordance with the manufacturer's instructions. Allelic discrimination was performed either

with the ABI PRISM 7900HT SDS and the SDS 2.2.1 program (Applied Biosystems) or with the later version TaqMan Genotype Software (Applied Biosystems, QuantStudio 7 Flex).

The genotyping process was also performed on Open Array Real-Time PCR System Instrument (Applied Biosystems, Foster City, CA, USA). Allelic discrimination was performed with the TaqMan Genotype Software (Applied Biosystems).

Table 1. SNPs tested for genetic association to metabolic risk factors in Study II and Study IV.

Chromosome	Gene	SNP	Allele	Region	
1	<i>CELSR2</i>	RS646776	A/G*	Near Gene-3	
1	<i>MIA3</i>	RS17465637	A/C*	Intron	
1	<i>NOTCH2</i>	RS10923931	G*/T	Intron	
2	<i>No gene association</i>	RS2943634	A*/C	Non coding region	
2	<i>THADA</i>	RS7578597	C/T*	T [Thr] ⇒ A [Ala]	Non-synonymous
3	<i>ADAMTS9-AS2</i>	RS4607103	C*/T	Intron	
3	<i>IGF2BP2</i>	RS4402960	G*/T	Intron	
3	<i>MRAS</i>	RS9818870	C/T*	UTR-3	
3	<i>PPARG</i>	RS1801282	C*/G	P [Pro] ⇒ A [Ala]	Non-synonymous
4	<i>MTTP</i>	RS1800804	C/T*	Intron	
4	<i>WFS1</i>	RS10010131	A/G*	Intron	
6	<i>CDKAL1</i>	RS7754840	C/G*	Intron	
6	<i>CDKAL1</i>	RS7756992	A/G*	Intron	
6	<i>MTHFD1L</i>	RS6922269	A*/G	Intron	
6	<i>PPARD</i>	RS1053049	C*/T	UTR-3	
6	<i>PPARD</i>	RS2016520	A/G*	UTR-5	
6	<i>PPARD</i>	RS2076167	A/G*	N [Asn] ⇒ N [Asn]	Synonymous
6	<i>PPARD</i>	RS34474204	C*/T	Near Gene-5	
6	<i>PPARD</i>	RS6902123	C*/T	Intron	
7	<i>JAZF1</i>	RS864745	A*/G	Intron	
8	<i>SLC30A8</i>	RS13266634	C*/T	R [Arg] ⇒ W [Trp]	Non-synonymous
9	<i>No gene association</i>	RS10811661	C*/T	Non coding region	
9	<i>CDKN2B-AS1</i>	RS4977574	A*/G	Intron	
10	<i>No gene association</i>	RS1111875	A*/G	Non coding region	
10	<i>No gene association</i>	RS12779790	A*/G	Non coding region	
10	<i>No gene association</i>	RS1544210	A/G*	Non coding region	
10	<i>No gene association</i>	RS1746048	C/T*	Non coding region	
10	<i>IDE</i>	RS2251101	C/T*	Near Gene-3	
10	<i>No gene association</i>	RS7923837	A/G*	Non coding region	
10	<i>TCF7L2</i>	RS7903146	C/T*	Non coding region	
11	<i>EXT2</i>	RS11037909	C*/T	Intron	
11	<i>EXT2</i>	RS1113132	C*/G	Intron	
11	<i>EXT2</i>	RS3740878	A*/G	Intron	
11	<i>KCNJ11</i>	RS5219	C*/T	K [Lys] ⇒ E [Glu]	Non-synonymous
11	<i>KCNQ1</i>	RS2237892	C*/T	Intron	
11	<i>MTNR1B</i>	RS10830963	C*/G	Intron	
12	<i>CAMKK2</i>	RS3817190	A/T*	T [Thr] ⇒ S [Ser]	Non-synonymous
12	<i>No gene association</i>	RS7961581	C*/T	Non coding region	
12	<i>HNFL1A</i>	RS2259816	A/C*	Intron	
12	<i>P2RX7</i>	RS1718119	A*/G	A [Ala] ⇒ T [Thr]	Non-synonymous
12	<i>P2RX7</i>	RS2230912	A*/G	Q [Gln] ⇒ R [Arg]	Non-synonymous
12	<i>P2RX7</i>	RS3751143	G/T*	E [Glu] ⇒ A [Ala]	Non-synonymous

15	<i>SMAD3</i>	RS17228212	C/T*	Intron
16	<i>FTO</i>	RS8050136	A*/C	Intron
17	<i>HNF1B</i>	RS7501939	C*/T	Intron
17	<i>HNF1B</i>	RS757210	A*/G	Intron

*Ancestral allele. Data from <http://www.ncbi.nlm.nih.gov>

4.2.4 Statistical program and analysis

The allelic association analyses were performed using PLINK (Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; <http://pngu.mgh.harvard.edu/purcell/plink/>) (100). The level of nominal significance was set to 5 percent (two-tailed). Also, IBM SPSS Statistics version 20 and 23 (IBM Corporation, Armonk, NY, USA) were used. The statistical power was calculated according: <http://pngu.mgh.harvard.edu/Bpurcell/gpc/cc2.html>.

4.2.4.1 Logistic regression analysis

Logistic regression is a statistical test that investigates the relationship between the dependent variable/phenotypes (i.e. elevated/normal FPG) and one or more predictors (i.e. alleles of a SNP). In other words, it allowed us to test the influence of alleles, such as FPG. The probability and effect size were observed.

The measurement of probability (if observed relationship is occurring by chance or not) is given in a p-value. The statistical significance level of probability is $p < 0.05$ – it means that the probability that the observation is due to chance is less than 5%.

The odds ratio (OR) is a measure of how strong is the presence or absence of a character/trait (i.e. allele) between groups (i.e. elevated/normal FPG).

In this thesis, the logistic regression analyzes were adjusted for other metabolic risk factors to avoid any impact on investigated genetic associations.

4.2.4.2 Multiple test corrections

By correcting for the number of tests done in an analysis, it is possible to reduce the incidence of obtaining a positive association due to chance, in addition to reducing the probability of getting false positive (Type I error) results. The more hypotheses there are to test, the higher the probability is of getting a positive result due to chance. In the genetic analyses, many tests are usually made, thus correction for multiple tests is recommended to minimize the incidence of obtaining a positive association due to chance.

4.2.4.2.1 Bonferroni correction

The Bonferroni correction is very strict, with significance level $p < 0.05$, which assumes all tests are independent.

4.2.4.2.2 The false discovery rate (FDR) correction

The false discovery rate (FDR) is defined as the proportion of false positive observations among significant results.

4.2.4.2.3 Max (T) permutation

Max (T) permutation is a less stringent multiple test correction as the permutation preserve the correlational structure between SNPs.

4.2.4.3 Case-case model

In this thesis, both case-case and case-control models have been applied. The case-case model is an analysis within the case group (patients). In genetic analyses use of the case-case model helps to reduce the clinical diversity and environmental differences between disease groups (101). The case-case model may represent a smaller subgroup of the population, thus more biologically correlated and more related to susceptibility genes than the observed population in general (102, 103).

4.3 MATERIAL & METHODS FOR EACH STUDY

4.3.1 Study I

COGNITIVE MANIC SYMPTOMS IN BIPOLAR DISORDER ASSOCIATED WITH POLYMORPHISMS IN THE *DAOA* AND *COMT* GENES

4.3.1.1 Subjects

DNA from all the unrelated BD type I patients recruited were included (n=488). A four factor structure of the manic symptoms was previously reported in this material (109). One factor had high loadings of the cognitive symptoms *talkativeness*, *distractibility*, and *thought disorder* (104). Therefore, patients reported to have all these three symptoms was considered positive for a cognitive manic symptom (CMS) factor, and the other patients not. Of the 488 patients 215 (44%) were positive for all three symptoms, thus showing CMS, 248 (51%) patients did not meet criteria for CMS and were considered as non-CMS. As population controls, 1,044 anonymous blood donors (ABD) were used.

4.3.1.2 Methods

SNPs were selected for genes in the dopamine system, previously reported to influence risk for major psychosis, using the HapMap database (<http://www.hapmap.org>). In the *DAOA*, fifteen SNPs; rs3916967, rs2391191, rs1935062, rs947267, rs778294, rs778326, rs3916971, rs1642681, rs778293, rs1362886, rs778284, rs3918342, rs1421292, rs778308, rs778321 were investigated, and in the *COMT*, four SNPs; rs5993883, rs740601, rs4680, rs165599 were investigated. Hardy Weinberg p-value cut-off was $p \leq 0.05$ for both cases and controls.

4.3.1.3 Statistical analyses

SNPs in the *DAOA* and *COMT* were investigated for allelic association analysis in BD patients in two models: CMS were compared with BD patients with no CMS (case-case model), and BD patients with CMS were compared with ABD (case-control). Associations were corrected for multiple testing by the max (T) permutation in PLINK. Haploblocks, including SNPs allele-wise nominally associated to CMS ($p < 0.05$) or SNPs nearby ($D' > 0.80$), were examined for haplotype distribution difference. Logistic regression was used to test for allele/haplotype associations, adjusting for gender and rs1718119 (*P2RX7*) (the SNPs previously published associations with cognitive deficit) (104). SNPs associated with CMS ($p < 0.05$) were also tested for genotype association using logistic regression in dominant, recessive and codominant models. An allele-by-allele epistasis test was performed between the SNPs rs2391191 in *DAOA* and rs5993883 in *COMT* using logistic regression with a multiplicative interaction term.

4.3.2 Study II

GENES ASSOCIATED WITH INCREASED FASTING GLUCOSE IN PATIENTS WITH SCHIZOPHRENIA SPECTRUM DISORDERS

4.3.2.1 Subjects

652 schizophrenia spectrum disorder (SSD) patients were studied. Of the 652 SSD patients, 52% were men. Mean age was 47 (± 12) years. Mean waist circumference was 97 (± 15) cm for women and 104 (± 15) cm for men. 235 (36%) SSD patients were present smokers. Diabetes in family was reported for 150 (23%) SSD patients. Of those 652 SSD patients, 389 (60%) had normal FPG (< 5.6 mmol/L), and 235 (36%) had increased FPG (≥ 5.6 mmol/L) while 28 (4%) was treated for diabetes. Control subjects were selected from the population based prospective SDPP (98) based on the frequencies of glucose level categories to represent the whole SDPP sample at follow up. For this genetic association study, 494 SDPP control subjects were selected out of which 404 individuals (82%) had normal FPG, 66 (13%) had elevated FPG levels, and 24 (5%) were diagnosed with T2DM.

4.3.2.2 Methods

SNPs tested for allele association are listed in Table 1.

The genotyping process was performed on Open Array Real-Time PCR System Instrument (Applied Biosystems, Foster City, CA, USA). Allelic discrimination was performed with the TaqMan Genotype Software (Applied Biosystems).

4.3.2.3 Statistical analysis

FPG level differences between psychosis diagnoses were investigated using ANOVA (IBM SPSS Statistics 22). Any significant effects were further investigated with Fisher's least significant difference (LSD) post hoc analysis for multiple comparisons.

The cut off level for FPG level was defined according to the IDF criteria. SNPs in the metabolic risk genes were investigated for allelic association analysis to elevated FPG in SSD patients in two models: case-case model - where SSD patients with increased FPG level were compared to SSD patients with normal FPG level, and case-control model - where SSD patients with increased FPG level were compared to control subjects. Logistic regression was used to test for allelic association, adjusted for gender, age, smoking, waist circumference, and family history of diabetes. Associations were corrected for multiple testing by calculating the False discovery rate (FDR) according to the Benjamin Hochberg method. To test the effect of antipsychotic drug - on observed genetic allele associations to elevated FPG in SSD patients - analyses restricted to patients on clozapine (n=62) were performed. The allelic distribution for all SSD patients was compared to SDPP controls, adjusted for gender, age, smoking, waist circumference, family history of diabetes, and FPG level. All calculations were done in PLINK in [BC|SNPmax](#) data management and analysis.

4.3.3 Study III

MELATONIN RECEPTOR 1B GENE ASSOCIATED WITH HYPERGLYCEMIA IN BIPOLAR DISORDER

4.3.3.1 Subjects

453 BD patients, including sub-diagnosis of BD: bipolar disorder type 1 (BD-1) (82%, n=369), bipolar disorder type 2 (BD 2) (13%, n=59), bipolar disorder not otherwise specified (BD NOS) (5%, n=22), and schizoaffective disorder (SCA) (<1%, n=3) were genotyped and had the information on FPG levels. 280 (62%) BD patients had normal FPG (<5.6 mmol/L) and 173 (38%) had increased FPG (≥ 5.6 mmol/L), whereof 7% (n=12) were treated for diabetes. 480 SDPP controls were selected: elevated FPG (≥ 5.6 mmol/L) without T2DM was present in 65 individuals (14%), and 24 (5%) were diagnosed with T2DM, 391 (81%) had normal FPG levels (<5.6 mmol/L).

4.3.3.2 Methods

SNPs in the metabolic risk genes (selected according to the Study II: rs10010131 in wolfram syndrome 1 gene (*WFS1*), rs1718119 in purinergic receptor gene (*P2RX7*), rs4402960 in insulin-like growth factor II mRNA binding protein 2 gene (*IGF2BP2*), rs10923931 in neurogenic locus notch homolog 2 gene (*NOTCH2*) and rs10830963 in melatonin receptor 1B gene (*MTNR1B*) were tested for allelic association analysis to elevated FPG in BD.

The genotyping was performed using TaqMan SNP genotyping assays on an ABI 7900 HT instrument (Applied Biosystems, Foster City, CA, USA). Allelic discrimination was performed with the TaqMan Genotype Software (Applied Biosystems, QuantStudio 7 Flex).

4.3.3.3 Statistical analyses

FPG level differences between BD sub-diagnoses (BD-1, BD-2, BD-NOS and SCA) were investigated using ANOVA, followed by testing distribution normality and the variance homogeneity using the Shapiro-Wilk and the Levene's tests. All analyses were performed in IBM SPSS Statistics 22.

The cut off level for FPG was defined according to the IDF. SNPs in the metabolic risk genes were tested for allelic association analysis to elevated FPG in BD in three models: case-case model - where BD patients with increased FPG level were compared to BD patients with normal FPG; case-control model - where BD patients with increased FPG level were compared to SDPP controls, and control-control model - where SDPP controls with increased FPG were compared to SDPP controls with normal FPG. All analysis were preformed using logistic regression adjusted for gender, age and smoking. P-values were corrected for multiple testing according to Bonferroni (six SNPs). All calculations were performed using PLINK in [BC|SNPmax](#) data management and analysis.

4.3.4 Study IV

GENETIC VARIANTS OF INCREASED WAIST CIRCUMFERENCE IN PSYCHOSIS

4.3.4.1 Subjects

658 SSD patients were included. Schizophrenia was the most common diagnosis (n=356, 54%), schizoaffective disorder in 68 patients (10%), delusional disorder in 41 patients (6%), NOS) in 88 patients (14%), bipolar disorder in 40 patients (6%), and other psychiatric disorders in 65 patients (10%). 534 subjects (81%) had increased waist circumference, 254 subjects (40%) had elevated FPG levels and 153 (23%) were positive for a family history of diabetes. The control sample was 494 SDPP control individuals (see Study II).

4.3.4.2 Methods

SNPs tested for allele association are listed in Table 1.

Single nucleotide polymorphisms (SNPs) were genotyped using an Open Array Real-Time PCR System Instrument (Applied Biosystems, Foster City, CA, USA). Allelic discrimination was performed using TaqMan Genotype Software (Applied Biosystems). Abdominal obesity was measured by increased waist circumference.

4.3.4.3 Statistical analyses

Differences in waist circumference between psychosis diagnoses were analyzed (separately for men and women) in ANOVA in IBM SPSS Statistics 23 (IBM Corporation, USA).

The cutoff level for increased waist circumference was defined according to criteria from the IDF: (≥ 80 cm women and ≥ 94 cm men). SNPs in the metabolic risk genes were tested for allelic association analysis to elevated FPG in BD in three models: case-case model - SSD patients with elevated waist circumference were compared to SSD patients with normal waist circumference; case-control model- SSD patients with elevated waist circumference were compared to SDPP controls, and case*-control model – all* SSD patients were compared to SDPP controls. Logistic regression was used in all analysis adjusted for the age, FPG, family history of diabetes, sex, and smoking. Additionally, the logistic regression model in the case*-control analysis was adjusted for the same factors and also the waist circumference. For multiple testing correction false discovery rate Benjamini & Hochberg (FDR BH) was used. In addition, to test the effect of antipsychotic treatment on nominal allelic associations with increased waist circumference - case-case and case-control analyses were performed where SSD cases were restricted to patients on clozapine (n=62). The allelic association analyses were performed using PLINK in [BC|SNPmax](#) data management and analysis.

4.3.5 Study V

NOTCH2 GENE ASSOCIATED WITH BIPOLAR DISORDER

4.3.5.1 Subjects

453 patients diagnosed with BD, and 480 SDPP controls (*see* Study III) were included.

4.3.5.2 Methods

Genetic variants previously associated with increased FPG among BD patients (Study III) and/or SSD patients (Study II) were selected. The genotyping was performed using TaqMan SNP genotyping assays on an ABI 7900 HT instrument (Applied Biosystems, Foster City, CA, USA). Allelic discrimination was performed with the TaqMan Genotype Software (Applied Biosystems, QuantStudio 7 Flex).

4.3.5.3 Statistical analyses

For nominal SNP-BD associations ($p < 0.05$) differences of BD sub-diagnoses (BD-1, BD-2, BD-NOS and SCA) among genotypes were analyzed using ANOVA using IBM SPSS Statistics 23 (Armonk, New York, USA). SNPs in the metabolic risk genes (Study III) were tested for allelic association analysis to BD - where all BD patients were compared to SDPP controls. The analysis was performed using logistic regression adjusted for gender, age, FPG, and smoking. P-values were corrected for multiple testing according to Bonferroni. All calculations were performed using PLINK in [BC|SNPmax](#) data management and analysis.

4.3.6 Study VI

TROPONIN T LEVELS ASSOCIATED WITH GENETIC VARIANTS IN *NOTCH2* AND *MTNR1B* IN WOMEN WITH SCHIZOPHRENIA

4.3.6.1 Subjects

300 patients with SCZ diagnosis that had serum at the one year follow up were included in this study. The prevalence of a T2DM was 16% (n=18) among men and 11% (n=14) among women.

4.3.6.2 Methods

High sensitive troponin T (hsTnT) levels were measured using electrochemiluminescence immunoassay (ECLIA). The detection level was 5 ng/L and the reference value for hsTnT was ≥ 14 ng/L, (99th percentile).

Genetic variations previously reported in Study II to be associated with elevated FPG in psychosis patients were assessed: rs10010131 in *WFS1*, rs1718119 in *P2RX7*, rs4402960 in *IGF2BP2*, rs10923931 *NOTCH2*, and rs10830963 *MTNR1B*. Additionally, the genetic variation rs12564445 in the gene encoding troponin T (*TNNT2*), previously reported to be associated with hsTnT levels in a GWA study, was genotyped (105). Genotyping was

performed applying Open Array Real-Time PCR System Instrument (Applied Biosystems, Foster City, CA, USA) and TaqMan SNP genotyping assays on an ABI 7900 HT instrument (Applied Biosystems, Foster City, CA, USA). Allelic discrimination was performed using TaqMan Genotype Software (Applied Biosystems, QuantStudio 7 Flex). For the genotyping completion.

4.3.6.3 Statistical analysis

The relationships of hsTnT level distribution in relation to gender, age, FPG levels, smoking and psychosis diagnosis were analyzed, also the distributions of gender, age, and FPG levels in relation to genotypes were analyzed. ANOVA or unadjusted linear regression was applied. FPG level was categorized applying the IDF criteria. The distribution of both hsTnT levels and detectable hsTnT levels (≥ 5 ng/L) among patients with normal FPG levels and increased FPG levels was tested using ANOVA. For testing the association between hsTnT and *TNNT2* rs12564445, ANCOVA was used with and without adjustment for FPG levels. Moreover, to test the distribution of hsTnT levels between genotypes for rs10830963, rs10923931, rs10010131, rs1718119, rs4402960 and rs7578597 an ANCOVA adjusted for FPG and gender was used. A nonparametric Kruskal-Wallis test was performed for the validation of the ANCOVA. Also, linear relationships between hsTnT and genotypes of rs10923931 and rs10830963 were assessed using linear regression adjusted for FPG levels among women. Regression residual distributions were checked for normality. All tests were performed in IBM SPSS Statistics 23 (IBM Corporation, Armonk, NY, USA).

5 MAIN FINDINGS

5.1 STUDY I

COGNITIVE MANIC SYMPTOMS IN BIPOLAR DISORDER ASSOCIATED WITH POLYMORPHISMS IN THE *DAOA* AND *COMT* GENES

CMS in patients with BD type I was associated in the case-case model: allele T in rs3916967 (OR=1.39, p=0.018), and the allele G in rs2391191 (OR=1.33, p = 0.055) in the *DAOA*, and allele G in rs5993883 (OR = 1.37, p=0.025) in *COMT*. In the case-control model: the alleles T (OR=1.28, p=0.029) and G (OR=1.33, p=0.020) in the *DAOA*, and allele G in rs5993883 (OR=1.45, p=0.0017), and allele A in rs165599 (OR=1.34, p=0.014) were nominally associated with CMS in patients with BD type I. The haplotype consisting of the three major alleles TGA from SNPs rs3916967, rs2391191 and rs1935062 was associated with increased risk for CMS in both the case-case (OR=1.38, p=0.029) and the case-control analysis (OR = 1.34, p = 0.0057).

5.2 STUDY II

GENES ASSOCIATED WITH INCREASED FASTING GLUCOSE IN PATIENTS WITH SCHIZOPHRENIA SPECTRUM DISORDERS

The SNPs nominally associated with elevated FPG levels among SSD patients were found in the case-case model: the major allele G of rs4402960 in the *IGF2BP2* (OR=1.39, p=0.019); and in the case-control model: the minor allele T of rs10923931 in the *NOTCH2* (OR=1.84, p=0.011), the major allele T of rs7578597 in *THADA* (OR=1.85, p=0.014), the major allele G of rs10010131 in the *WFS1* (OR=1.43 p=0.010), the major allele G of rs1718119 in the *P2RX7* (OR=1.40, p=0.014), the minor allele G of rs10830963 in the *MTNR1B* (OR=1.51, p=0.0039). Genetic effect sizes were lower, although not statistically significantly, for patients on clozapine with elevated FPG levels compared to all patients. The genetic polymorphism in *PPRAD* was nominally associated to SSD independent of glucose level. None of the relationships survived correction for multiple testing.

5.3 STUDY III

MELATONIN RECEPTOR 1B GENE ASSOCIATED WITH HYPERGLYCEMIA IN BIPOLAR DISORDER

The minor allele G of the rs10830963 in *MTNR1B* was associated with elevated FPG levels among BD patients: in the case-case model ($p=0.016$, $OR=1.49$), although not significant after Bonferroni correction ($BONF=0.066$), in the control-control model ($p=0.0087$, $OR=1.64$, $BONF=0.043$), and case-control ($p=0.00013$, $OR=1.73$, $BONF=0.0005$).

5.4 STUDY IV

GENETIC VARIANTS OF INCREASED WAIST CIRCUMFERENCE IN PSYCHOSIS

The genetic variants associated with increased waist circumference in patients with SSD in the case-case model were: the major allele C of rs17465637 in the *MIA3* ($OR\ 1.43$, $P = 0.027$), the major allele C of rs9818870 *MRAS* ($OR\ 1.47$, $P = 0.042$), the major allele A of rs2230912 in the *P2RX7* ($OR\ 1.45$, $P = 0.038$), the major allele T of rs3817190 in *CAMKK2* ($OR\ 1.56$, $P = 0.0040$), and the minor allele C of rs17228212 in *SMAD3* ($OR\ 1.45$, $P = 0.046$). The genetic variants associated with increased waist circumference in patients with SSD in the case-control model were: the *PPARD*, the minor allele G of rs10830963 in the *MNTR1B* ($OR\ 1.33$, $P = 0.011$), and the minor allele T of rs10923931 in the *NOTCH2* ($OR\ 1.47$, $P = 0.037$). Testing the effect of clozapine treatment on nominal allelic associations with increased waist circumference - the point estimates of ORs were well within the 95% confidence intervals of ORs based on patients irrespectively of pharmacotherapy, except for *NOTCH2*.

The genetic variants nominally associated with SSD per se, irrespectively of waist circumference were: the major allele C of rs34474204 in the *PPARD* ($OR\ 1.92$, $P = 0.0068$), the minor allele G of rs10830963 in the *MNTR1B* ($OR\ 1.32$, $P = 0.012$), the minor allele T of rs10923931 in the *NOTCH2* ($OR\ 1.52$, $P = 0.021$), the major allele C of rs7501939 in the *HNF1B* ($OR\ 1.25$, $P = 0.027$).

5.5 STUDY V

NOTCH2 GENE ASSOCIATED WITH BIPOLAR DISORDER

The minor allele T of the rs10923931 in *NOTCH2* ($p=0.0040$, $OR=1.72$, $BONF=0.024$) was associated with BD. Likewise, associations between the minor allele A of the rs1718119 in *P2RX7* ($p=0.0097$, $OR=0.75$, $BONF=0.058$), and the minor allele C of the rs7578597 in *THADA* ($p=0.023$, $OR=0.64$, $BONF=0.14$) were found.

5.6 STUDY VI

TROPONIN T LEVELS ASSOCIATED WITH GENETIC VARIANTS IN *NOTCH2* AND *MTNR1B* IN WOMEN WITH SCHIZOPHRENIA

Among men (n=146), 73% (n=107) had detectable hsTnT levels (≥ 5 ng/L) and 17% (n=18) had elevated hsTnT levels (≥ 14 ng/L). Among women (n=154), 44% (n=68) had detectable hsTnT levels (≥ 5 ng/L) and 13% (n=9) had elevated hsTnT (≥ 14 ng/L). No differences in hsTnT levels with regard to smoking or psychosis diagnosis were detected. However, hsTnT levels were dependent on age, gender and FPG levels. The association between hsTnT and glucose levels was found also when adjusting for age and gender in a linear regression analysis ($\beta_{\text{standardized}}=0.22$, $p<0.0005$, $R^2=0.32$). In addition, the hsTnT levels and detectable hsTnT levels (≥ 5 ng/L) were higher among patients with FPG level (≥ 5.6 mmol/l) compared to patients with normal FPG level (<5.6 mmol/L) $p=0.001$, $F=11.34$, and $p=0.002$, $F=9.47$ respectively. Difference in hsTnT levels between genotypes of the rs10830963 in *MTNR1B* ($p=0.010$) was detected. The result was verified with Kruskal Wallis test (rs10830963: $p=0.039$) revealing an association of hsTnT level also with *NOTCH2* rs10923931 ($p=0.027$). Moreover, a gender-specific analysis showed that these genetic associations were present only in women (rs10830963: $p=0.026$) and rs10923931: $p=0.011$).

5.7 SUMMARY OF THE FINDINGS

Study I

- Cognitive manic symptoms in patients with bipolar disorder type 1 are associated with *DAOA* and *COMT*.
- Our findings are in line with the hypothesis of dopamine dysregulation contributing to cognitive dysfunction.

Study II - V

- Some SNPs associated with metabolic disorder in an otherwise healthy population are associated with elevated FPG and waist circumference among SSD patients and BD patients.
- Metabolic risk genes associated to increased FPG in SSD patients, differs to a large extent from the metabolic risk genes for increased waist circumference in SSD.
- The *MTNR1B* rs10830963 allele G-dependent vulnerability for elevated FPG levels is shared between SSD and BD.
- Metabolic risk variant *NOTCH2* rs10923931_T is associated with both SSD and BD type 1 *per se*.
- This may reflect:
 - increased metabolic risk allele burden among SSD and BD patients
 - increased metabolic genetic vulnerability in SSD and BD patients
 - shared genetics between T2DM, SSD and BD

Study VI

- hsTnT levels are associated with *MTNR1B* rs10830963 and *NOTCH2* rs10923931 in women with schizophrenia.
- Findings of this study may indicate metabolic genetic influences on hsTnT levels among women with schizophrenia.

6 DISCUSSION AND FUTURE PERSPECTIVES

In this thesis, we provide data suggesting that genetic variants, reported to confer metabolic risk in the general population, are associated with metabolic risk factors in SSD and BD patients. Our findings further suggest that there is a partial overlap of genetic risk factors between SSD, BD type 1 and T2DM.

SCZ and BD are clinically defined disorders. Neither SCZ nor BD have as yet reached such a level of knowledge regarding pathophysiology that they may be termed diseases. Several biological dysfunctions have been identified in these disorders and a number of genes have been associated with disease risk. These findings do not currently explain more than a fraction of the mechanisms behind the disorders. Furthermore, a diversity of clinical symptoms may be related to distinct biological processes effectively complicating the process of identifying the biological and genetic basis of the disorders. Studies using the case-case model of analysis, where specific subgroups of patients are compared to other patients, will reduce clinical diversity, and yield groups that have been exposed to more similar environments both as it relates to psychosocial stress, financial difficulties and in particular similar medication exposures. These similarities may also facilitate the untangling of the biological basis of specific symptoms or complications rather than that of the disorders themselves. This study approach, testing specific hypotheses in samples from selected and clinically well defined patients with long follow-up, is likely to generate more findings of clinical relevance than case-control GWAS using large but more heterogeneous materials with little or no follow-up data. It is possible, that findings from SCZ and BP GWAS are limited because of lack of genetic homogeneity. To a substantial extent SCZ and BD share clinical symptoms, and also show similar comorbidities and mortality in metabolic disorders and CVD. There is also a substantial genetic overlap between SCD and BD (36) that motivates further investigations of the metabolic vulnerability in both SCZ and BD. Other future possible common areas of investigation are the search for biological and genetic factors contributing to the increased suicide risk, especially early in the disorders, common for both SCZ and BD.

Patients in the studies in this thesis were recruited in a population-based way from a few specialized outpatient clinics where emphasis was put on including a large proportion of patients being followed at the respective clinic in order to have a representative sample of outpatient SSD and BD patients in Sweden, mainly from Stockholm County. The patients had been ill for a long time at sampling and those included had a well-defined diagnosis. An advantage with analyzing such a patient sample is that a number of co-morbidities and specific outcomes will have had enough time to present themselves in the sample, and thus will be large enough for analysis, not the least the metabolic diseases which need time to emerge, even though SCZ and BD patients will be affected much earlier than the general population.

The current Swedish population has no strong genetic boundaries, thus the individuals in this study were likely to come from a genetically fairly uniform population (106). Concerning metabolic studies, there was no difference in FPG or waist circumference between the different diagnoses in psychosis patients or sub-diagnosis of BD, thus findings from the present metabolic studies to a large extent are likely to be applicable to a broader spectrum of SSD and BD. Concerning the association between metabolic risk genes and BD *per se*, the clinic for affective disorder from where the vast majority of our BD sample (82%) was recruited, treated more severely ill BD patients (BD type 1). Thus, our results can be interpreted on a population level, but only for the BD type1 patients rather than BD in general.

Regarding the control material, the presence of T2DM in the families was high, about double the rate reported for the general population, which probably impede detection of susceptibility genes for T2DM. This may have contributed to limited detection of allele associations in the case-control analyzes.

The fact that most variants in this thesis had been identified in unbiased GWAS analysis or in functional studies and thus were not random selections mean that many of the findings can be considered as replications of sorts even though the clinical materials may differ in composition. Most SNPs did not survive multiple test corrections. However, the selection of SNPs was based on previous findings, thus our studies were hypothesis-driven at the gene- or SNP-level, effectively constituting replication studies of sorts, although in a different clinical material. For the metabolic studies, the effect sizes were not large, but they were however larger than in the population. A problem for this type of analyses is correction for multiple testing. This is generally required for genetic studies, and as a result it is possible that studies which are actually hypothesis testing are subject to too conservative corrections, resulting in disregarding of actual biological associations. The main weakness of the studies in this thesis would rather be the limited sample size, indicating a possible risk that we were not able to detect differences between the groups that may exist, due to insufficient sample size (low statistical power). *MTNR1B* and *NOTCH2* seem to have the strongest impact in both SSD and BD, given that they hold for the multiple testing correction, and could conceivably be included in a panel for predicting the metabolic risk in SSD and BD. Since we found a genetic overlap between SSD and BD, a analysis of metabolic risk genes in a pooled samples of SSD and BD combined could be justified and be a way to increase sample size.

Medications, in particular antipsychotics, were a limitation across the metabolic studies due to their known side effects to increase the risk for metabolic disorders. To investigate possible impact of antipsychotics on genetic associations to elevated FPG or increased waist circumference, genetic association analysis limited to patients on clozapine treatment was performed, since clozapine treatment is associated with the largest metabolic side effects. Since the clozapine group of patients was small, no significant p-values were expected. We observed a small effect size, thus suggesting a limited impact from clozapine on the observed

genetic associations to increased FPG and increased waist circumference.

For all metabolic studies, replication with larger sample sizes and drug-naïve patients are warranted to validate and confirm associations between metabolic SNPs and metabolic risk factors in SSD and BD patients, as well as association between metabolic SNPs, and SSD and BD type 1 *per se*.

In Study I, the cognitive function was not measured by neuropsychological tests, and there was no information on cognitive function during remission. The CMS factor is a dichotomous categorical variable, extracted from qualitative measurements. Thus, we were not able to analyze cognitive function as a quantitative variable, which would be preferable. There are many unreplicated SNP and haplotype associations in the genetic association literature. These SNP or haplotype analysis results should be taken with caution, as findings need to be replicated. However, the support from the genetic association between CMS and SNPs in *DAOA* gives the haplotype association additional impact. Further analyses should include quantitative evaluation (neuropsychological tests) of cognitive function in BD, both during manic episodes and during remission. Assessment of psychiatric patients with different diagnoses with the same instrument would make it possible to analyze genetic associations with the *DAOA* and *COMT* genes and general cognitive functioning in psychiatry.

The main limitation in Study VI was that this was a cross-sectional study, thus a possible predictive value of hsTnT levels on CVD events and deaths could not be evaluated. Prospective studies would be of interest to investigate if hsTnT may have predictive value of cardiovascular events. If hsTnT levels could be shown to be of predictive value for future CVD events, that might provide new tools for CVD prognosis and prevention in psychosis patients.

As a general conclusion, the studies in this thesis identify several genetic associations covering clinically important aspects of SSD and BD, such as cognitive dysfunction and metabolic risk. , From a GWAS -perspective these studies are performed with a relatively small clinical material but given the stringent selection of genetic variants as well as the method of case-case analysis using well phenotyped patients we argue that the power is sufficient to detect at least more robust risk factors. Although it must be admitted that the hopes for genetic findings relevant to disease risk from the beginning of the GWAS era has not been fulfilled, the prospect of future studies along these lines hold important prospects for clinically relevant future genetic findings, maybe especially focusing on CVD morbidity and mortality, since excess CVD death is the main cause of reduced longevity of life for SCZ and BD patients. Last summer (2015), the United Nations unanimously adopted global development goals that, for the first time, include a commitment to “promote mental health and well-being” and to reduce premature deaths from noncommunicable diseases, including mental disorders, by one-third by 2030. To achieve this ambitious goal for SCZ and BD, the excess mortality in both CVD and suicide will have to be substantially reduced (<https://sustainabledevelopment.un.org/post2015/transformingourworld>).

7 ACKNOWLEDGEMENTS

To my supervisors - infinite thanks for the time I got to grow and develop. My main supervisor, **Dr. Urban Ösby**, psychiatrists and scientist with thousands of projects and collaborations almost literally meant - you are pure energy! You have supported me throughout the years, always available. Meetings with you have been holy - after our meetings, I always felt a relief that things will be just fine. **Professor Martin Schalling**, thank you for giving me the opportunity to start in your lab and realize one of my main goals in life. You get research to sound ultra elegant. You rock science! **Associate professor Catharina Lavebratt**, intelligent and always kind - you have the sense to explain the inexplicable. You've always been a huge help. Thank you! **Dr. Louise Frisé**n, intelligent, kind and woman with style. Thank you for all support during my time as a doctoral student.

Many thanks to a hard core of intelligent co-authors with samples contribution and support while writing manuscripts; **Lena Backlund, Claes-Göran Östenson, Harvest F. Gu, Agneta Hilding, Gunnar Edman, Mikael Landén, Lil Träskman-Bendz, David Erling, Ewa Ehrenborg, Sven V. Eriksson** and **Eric Olsson** (Eric, thanks for the great company to Florence and sunny Italian coffee-time ☺).

It is important to “meet reality of disease” - **Lina Matrinsson** many thanks for arranging meetings with patients. Good luck with your postdoctoral plans – I am absolutely sure that you will reach up to the stars.

A big thank to **Carina Schmidt** and **Inger Römer** for assistance in managing the data and the biological samples. **Carina**, you have been my support in every sense, just to mention our countless conversations on all topics that life may offers.

To my **Neurogenetics unit colleagues** (present and former) that make my work place cozy and loved, a better team at work is difficult to find; **Ida Nilsson, Anna Witasp, Karin Luttröpp, Miao Sun, Ulrika Bergström, Anna-Lee Cöster Jansén, Annika Eriksson, Katarina Gell, Malin Alvehus, Vincent Millischer, Parvin Kumar, Paschalis Efstathopoulos, Charlotte Lindfors, Jeanette Johanssen, Selim Sengul, Björn Johanssen, Louise Nordfors, Yabin Wei, and Philippe Melas** (Yabin aka BinBin and Philippe, I shared one of my the most memorable trips to USA with you. Philippe, a shopping round, and act as a cameraman all around Washington DC was an experience itself ☺. BinBin, thanks for the great company in Boston, and a real nice round to Harvard University with your friends). Also, many thanks to great colleagues from **Epigenetic research group** (especially **Louise Sjöholm, Malin Almgren, Mikael Ringh** who share room, and conversations about important and less important topics ☺), **MS research group**, and **Hematology research group** for a fantastic atmosphere at work.

I would like to express **my great appreciations to all the individuals** who participated and contribute to the research. Also, many thanks for financial support to **Karolinska Institutet, The Swedish Research Council, The Söderström-Königska Foundation, ALF, The Regional Drug and Therapeutic Committee in Stockholm, The Fredrik and Ingrid Thuring's Foundation, Karolinska Institutet's Faculty Funds, The Swedish Council for Working Life and Social Research, The Swedish Diabetes Association, Novo Nordisk Scandinavia, and Centre for Psychiatry Research, Stockholm** (Research, Development and Education) that enabled research projects in this thesis.

To **All my family and friends** spread out in different directions in “former Yugoslavia”, and Sweden – *You are my religion!* **Aunts, uncles, cousins** (especially **Zerina** and **Almir**), **and your lovely families** each of you have a special place in my heart. When needed, you are always there! Love for you is never aging. The youngest cousin, **Kevin-Dinno** who looks up to me, special words to you “You can much more and better than me”. Also, to the youngest and sweetest in the family; **Lorena, Ella, Ines, and Annelie** - I love you girls.

Ambitious, smart, and true friend **Vilda** – when we meet, life pauses for a moment and I feel like nineteen again. We have had much craziness together ☺. Thank you for always being there for me.

Special thanks for *chatting* research during our running routes to my outstanding neighbor, friend, and cancer doctoral student **Belma**. Good luck with your thesis. Also, thank you and your husband for all the dinners and late karaoke nights ☺.

Ksenija and **Mirjana**, my childhood friends, thanks for always being *online* when I need you. It feels that you are always close to me despite the distance. I am happy to have you. Love you girls!

To my role models of humility and elegance - My grandmother and grandfather, **Majka** and **Deda**, thank you for a magical childhood moments, unselfish and endless love, especially during the first years of my life. Softer heart could not raise me and give me a better start in life. First years of life last forever! Grandmother, *Lady Sofija* always elegant in every sense, and as the situation is more challenging you are even more a lady (I hope I have at least a little of your elegance inside of me). Your vision of life is glorious.

To my *one and only* **sister**, you have always your way to snatch up one from stressful situations. "How about a nice bag, dress, travel or turn to the fine SPA", even such a dramatic changes to move and change the country or sell and buy new apartment (nothing is too big to get me in better mood – I love you). All the expensive face creams you contributed must be complementary (I guess) to my genetics, so I aged slower. Thank you for taking care of my look during my PhD time. *Tons of pure love* - to you and your beautiful family **Joakim** and adorable **Alexia!**

To my husband, and my best friend, **Admir**, we go strongly since 1998 (the clock is set on forever). Thank you for your support and endless love, still as strong now as the first day we met - I love you to the moon and back!

My children, **Emil and Emily**, the most precious I have, you have shown the great interest in my work. Thank you for illustrating with me for my thesis. You never stop to inspire me. *Love you my stars!*

Mum, above all, the best grandmother in the world, strong and loving woman. All the time that you spent with Emil and Emily, giving me time for me, helping me get to my goals (without motherly guilt feelings). Your unconditional love and support is never ending. Volim Te (I love you).

We all walk through life the same speed. One minute lasts to all the same. Some may use it - some do not! You taught me to use every breath and sail through this life without a lot of storms. All love to you **Daddy**. Nedostaješ mi Kralju! (I miss you my King).



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