



Pia Soronen

Genetics Behind Mood Disorders

Candidate Gene Studies of Bipolar and Major Depressive Disorders



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GENETICS BEHIND MOOD DISORDERS

***CANDIDATE GENE STUDIES OF BIPOLAR
AND MAJOR DEPRESSIVE DISORDERS***

ACADEMIC DISSERTATION

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and

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“It always seems impossible until it's done.”

Nelson Mandela

Rakkaimmilleni

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ABSTRACT

Mood disorders are complex psychiatric disorders that are the leading cause of disability to work in Finland. They include major depressive disorder (MDD) and bipolar disorder (BD). Both are highly heritable, i.e. the variance between individuals is, in MDD partly and in BD mostly, explained by genetic factors. Their specific genetic background, however, remains widely unknown. Previous linkage and association studies have revealed some potential candidate genes and genomic areas that seem to predispose to mood disorders, at least in some populations and subsamples. Recent genome wide association (GWA) studies have revealed some novel susceptibility genes and risk variants as the field has moved into the era of genome-wide genotyping of huge samples sizes from collaborative studies. The risk variants revealed with this approach, however, cover only a small fraction of the total heritability of these diseases. Thus, it is still highly relevant to study samples from more genetically and environmentally homogenous populations, since the amount of risk variants that are enriched in isolated populations is smaller than in more confounded populations. Detailed characterization of the phenotype has also become an important factor in psychiatric genetics, since the psychiatric disease phenotypes themselves are so broad and heterogeneous.

The principal goal of this work was to survey functionally relevant candidate genes for mood disorders and risk variants revealed from the first GWA studies in a Finnish bipolar family sample and in clinical cohorts for mood disorders collected from the metropolitan area of Finland. *The bipolar family sample* comprises 723 individuals (227 affected for BD) collected utilizing nation-wide registers to identify all individuals born between 1940 and 1969 and hospitalized for bipolar disorder type I (BDI) between 1969 and 1991. Part of this sample has been characterized in more detail enabling the search for genetic susceptibility genes for potential endophenotypes of mood disorders. *The clinical cohort sample* is comprised of 272 MDD and 178 BD patients, who were characterized for clinical comorbidities and followed for long term clinical outcome.

In the bipolar family sample, the highly studied variant val66met of *BDNF* was found to be associated with BD with the allelic replication observed in previous association studies. *SLC6A4* allelic variants also showed some association with BD

among males, whilst variants of *P2RX7* associated with both BD and neuropsychiatric test results used to assess executive function. The most statistically significant result demonstrated the association of variants of the *DAOA* gene and the general intellectual function of visuospatial ability. The clinical cohort sample revealed statistically significant association of two functional variants from *P2RX7* gene with familial form of mood disorder, and both variants affected also the clinical outcome of the patients by lengthening the time of illness. Our effort to replicate the results from the first GWA studies in the bipolar family sample revealed five genomic areas that associated with mood disorder also in Finland. Most of these variants also associated with some potential mood-disorder endophenotype, such as neurocognitive trait or seasonality in fluctuation of sleep, social activity, or mood.

In conclusion, the use of detailed characterized samples may provide an advantage to the genetic studies, since it increases the possibility of finding the susceptibility genes behind more homogenous phenotypes. This work has also revealed some potential endophenotypes, or trait features, that might convey the effect of the susceptibility genes to the end diagnosis of mood disorder.

Keywords: mood disorder, major depressive disorder, bipolar disorder, candidate gene, association, BDNF, P2RX7, DAOA, SLC6A4

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TIIVISTELMÄ

Mielialahäiriöt ovat monitekijäisiä psykiatrisia sairauksia, jotka ovat yleisin syy työkyvyttömyyteen Suomessa. Mielialahäiriöihin luetaan vakava masennus sekä kaksisuuntainen mielialahäiriö. Kummatkin näistä ovat vahvasti perinnöllisiä sairauksia, eli geenit vaikuttavat näiden tautien syntyyn. Vakavassa masennuksessa geenit vaikuttavat sairauden syntyyn osittain ja kaksisuuntaisen mielialahäiriön kohdalla suurelta osin. Vaikka näiden sairauksien geneettistä taustaa on tutkittu kauan, vieläkään ei tiedetä mitkä geneettiset tekijät altistavat näille sairauksille. Aiemmat geneettiset kytkentäanalyysit ja assosiaatioanalyysit ovat antaneet viitteitä tiettyjen geenien ja genomialueiden merkityksestä sairauksien synnyssä. Koko genomien sekvensointi ja genotyyppitysmenetelmien kehittyminen ovat mahdollistaneet koko genomien laajuiset assosiaatioanalyysit isoissa, monen tutkimuskeskuksen yhteistyönä keräämissä näyteaineistoissa. Näissä laajoissa aineistossa tehdyissä tutkimuksissa on paljastanut täysin uusia alttiusgeeni ehdokkaita. Ongelmana on kuitenkin ollut se, että paljastuneet riskimuodot kattavat vain pienen osan sairauksien perinnöllisestä taustasta. Tämän vuoksi psykiatrisessa genetiikassa on lisäksi tärkeää tutkia myös geneettisesti homogeenisempia populaatioita, joihin on kasautunut vähäisempi määrä geneettisiä riskitekijöitä. Tärkeäksi tekijäksi on muodostunut myös aineiston tarkka ilmiäsuun karakterisointi, sillä mielialahäiriöt itsessään ovat ilmiäsuuna erittäin heterogeenisiä.

Tämän tutkimuksen tarkoituksena oli tutkia toiminnallisesti tärkeitä mielialahäiriöiden ehdokasgeenejä sekä uusia riskivariantteja jotka on löydetty ensimmäisissä koko genomien laajuisissa assosiaatiotutkimuksissa ja selvittää näiden vaikutus mielialahäiriöön suomalaisissa aineistoissa. *Kaksisuuntaisen mielialahäiriön perheaineisto* koostuu 723 yksilöstä, joista 227 sairastaa kaksisuuntaista mielialahäiriötä ja loput ovat näiden ensimmäisen asteen sukulaisia. Osa tästä aineistosta on tarkemmin karakterisoitu neurokognitiivisten ominaisuuksien ja vuodenaikais- ja vuorokausimuuttujien osalta. Nämä ovat mahdollisia mielialahäiriöiden endofenotyyppisiä, eli ominaisuuksia jotka liittyvät tutkittavaan psykiatriseen sairauteen ja ovat ilmiäsuultaan yksinkertaisempia kuin tutkittava psykiatrisen sairaus. *Klininen kohorttiaineisto* muodostuu 272 vakavaan

masennukseen ja 178 kaksisuuntaiseen mielialahäiriöön sairastuneesta. Näiden potilaiden kliininen ilmiasu on tarkkaan karakterisoitu komorbiditeettihäiriöiden ja sairauden vaikeusasteen osalta.

Aiemmissä tutkimuksissa paljon tutkittu *BDNF* geeni ja sen funktionaalinen variantti val66met assosioitui *perheaineistossa* kaksisuuntaiseen mielialahäiriöön. Samassa aineistossa myös *SLC6A4* geeni assosioitui kaksisuuntaiseen mielialahäiriöön miehillä ja *P2RX7* geeni sekä kaksisuuntaiseen mielialahäiriöön että neurokognitiiviseen testimuuttuun. Perheaineiston vahvin geneettinen löydös oli *DAOA* geenin assosiaatio visuaalis-tilallista kyvykkyyttä mittaavaan neurokognitiiviseen testimuuttuun. *Kohorttiaineistossa* löysimme perheaineiston mielialahäiriön vahvan assosiaation kahteen *P2RX7* geenin aminohappo muuttavaan varianttiin. Kumpikin variantti vaikutti myös kliinisesti lisäten riskialleelia kantavien sairastamisaikaa. *Neljännessä ja viidennessä osatyössä* yritimme toistaa ensimmäisten koko genomien laajuisten assosiaatioanalyysien tuloksia. Tuloksena oli viiden geenin tai genomialueen assosiaatio mielialahäiriöihin myös suomalaisessa aineistossa. Suurin osa näistä assosioitui myös joko johonkin neurokognitiiviseen testimuuttuun tai unen, sosiaalisen aktiivisuuden tai mielialan vuodenaikaisvaihteluun, jotka kaikki ovat mahdollisia mielialahäiriöiden endofenotyyppiä.

Yhteenvetona voidaan todeta, että psykiatrisessa genetiikassa on tärkeää käyttää tarkkaan karakterisoitua aineistoa, sillä se vähentää tutkittavan ilmiasun heterogeenisyyttä ja siten todennäköisyys löytää ilmiasuun vaikuttavia geneettisiä riskitekijöitä kasvaa. Tässä työssä tuli myös ilmi joitain mahdollisia mielialahäiriöiden endofenotyyppiä ja ominaispiirteitä joiden kautta ehdokasgeenien vaikutus sairauteen välittyi.

Avainsanat: Mielialahäiriöt, toistuva vakava masennus, kaksisuuntainen mielialahäiriö, ehdokasgeeni, assosiaatioanalyysi, BDNF, P2RX7, DAOA, SLC6A4

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ABBREVIATIONS

BD	Bipolar Disorder
BDI	Bipolar Disorder type I
BDII	Bipolar Disorder type II
BDNF	Brain derived neurotrophic factor
bp	Base pair
CDCV	Common disease, common variant
CDH7	Cadherin 7, type 2
CIDI	Composite International Diagnostic Interview
CNV	Copy number variant
COWA	Controlled Oral Word Association Test
CVLT	California Verbal Learning Test
DAOA	D-amino acid oxidase activator
DFNB31	Deafness, autosomal recessive 31
DISC1	Disrupted in schizophrenia 1
DNA	Deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual, the fourth edition
GxE	Gene environment interaction
GWAS	Genome wide association study
HADS	Hospital Anxiety and Depression Scale
hME	homogenous Mass Extension
ICD-10	International Classification of Disease version 10
JoBS	Jorvi Bipolar Study
kb	Kilobase
LD	Linkage Disequilibrium
MAOA	Monoamine oxidase A

MDD	Major Depressive Disorder
NOS	Not otherwise specified
PCR	Polymerase chain reaction
PC-VDS	Vantaa Primary Care Depression Study
P2RX7	Purinergic receptor ligand-gated ion-channel 7
QTDT	Quantitative Transmission Disequilibrium test
r-MDD	Recurrent MDD
SCAN	Schedules for Clinical Assessment in Neuropsychiatry
SCID	Structured clinical interview
SD	Standard deviation
SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4
SNP	Single nucleotide polymorphism
SORCS2	Sortilin-related VPS10 domain containing receptor 2
STR	Short tandem repeat
TDT	Transmission disequilibrium test
TPH2	Tryptophan hydroxylase 2
VDS	Vantaa Depression Study
WAIS-R	Wechsler Adult Intelligence Scale – Revised
WHO	World Health Organization
WMS-R	Wechsler Memory Scale-Revised

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals:

- I** **Soronen P**, Palo M, Antila M, Tuulio-Henriksson A, Silander K, Kieseppä T, Loukola A, Lönnqvist J, Peltonen L, Partonen T, Paunio T. Evidence for *BDNF*, *SLC6A4* and *P2RX7* association with bipolar disorder. *Manuscript soon to be submitted*
- II** **Soronen P**, Mantere O, Melartin T, Suominen K, Vuorilehto M, Rytälä H, Arvilommi P, Holma I, Holma M, Jylhä P, Valtonen HM, Haukka J, Isometsä E, Paunio T. *P2RX7* Gene Associates Consistently with Mood Disorder in Three Clinical Cohorts. *American Journal of Medical Genetics, Part B:Neuropsychiatric Genetics* 2011; 156B(4):435-47.
- III** **Soronen P**, Silander K, Antila M, Palo OM, Tuulio-Henriksson A, Kieseppä T, Ellonen P, Wedenoja J, Turunen JA, Pietiläinen OP, Hennah W, Lönnqvist J, Peltonen L, Partonen T, Paunio T (2008) Association of a nonsynonymous variant of *DAOA* with visuospatial ability in a bipolar family sample. *Biol Psychiatry*, 64(5):438-42.
- IV** Ollila HM, **Soronen P**, Silander K, Palo OM, Kieseppä T, Kaunisto MA, Lönnqvist J, Peltonen L, Partonen T, Paunio T. Findings from bipolar disorder genome-wide association studies replicated in a Finnish bipolar family-cohort. *Mol Psychiatry* 2009;14(4):351-3.
- V** **Soronen P**, Ollila HM, Antila M, Silander K, Palo OM, Kieseppä T, Lönnqvist J, Peltonen L, Tuulio-Henriksson A, Partonen T and Paunio T (2009) Replication of GWAS of bipolar disorder: Association of SNPs near *CDH7* with bipolar disorder and visual processing. *Mol Psychiatry* 2010;15(1):4-6.

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Publications I and IV appeared previously in the doctoral thesis of Outi Marika Palo (2010)

1 INTRODUCTION

Mood disorders are common in the population and they are the most prevalent psychiatric disorders. Mood disorders are the leading cause of morbidity, thus their economic impact is huge since they are also the leading cause of disability to work. The high suicide rate also makes them fatal disorders, up to 15 % of people suffering from mood disorders will eventually commit suicide (Guze and Robins 1970). Mood disorders are divided to major depressive disorder (MDD) and bipolar disorder (BD). Common to these two disorders are the fluctuation of mood. In MDD, the fluctuation is unipolar, comprising only depressive episodes of mood. In BD, the fluctuation ranges from extreme elation of mood, mania (BD type I, BDI) or hypomania (BD type II, BDII), to depression. The life-time prevalence of MDD in Finland is 6.5 % (Pirkola et al 2005) and of BD is 0.24 % (Perala et al 2007).

The etiology of mood disorders is highly genetic; heritability estimates in MDD vary from 40 to 50 % (Levinson 2006) and in BD they range as high as 85-93 % (Kieseppä et al 2004; McGuffin et al 2003). The genetic architecture of mood disorders is widely studied, but remains vague. The earliest effort to map genetic factors behind mood disorders utilized linkage technique where linkage peaks were found spread all over the genome for both MDD and BD. The subsequent association analyses later found a wealth of potential candidate genes. The susceptibility genes are based either on functional relevance of the gene, positional mapping of previous linkage findings, or most recently to genome wide association (GWA) studies that scan the whole genome more densely than the genome wide linkage approach. The most promising candidate genes for mood disorder are brain derived neurotrophic factor (*BDNF*), D-amino acid oxidase activator (*DAOA*), disrupted in schizophrenia 1 (*DISC1*), tryptophan hydroxylase 2 (*TPH2*), and solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (*SLC6A4*) (Barnett and Smoller 2009; Levinson 2006; Lohoff 2010), and the genes found in GWA studies, such as *CACNA1C* and *ANKK3* (Alsabban et al 2011).

GWA studies were widely called for to better understand the genetics of psychiatric disorders, but they have failed to yield the depth of results anticipated (Gershon et al 2011). Whilst they have indeed revealed new candidate genes for mood disorders, it is now evident that the high risk variants cannot be found using the huge collaborative samples that are needed for GWA studies, since what is gained with a large sample size (power to the statistical analyses), is lost in heterogeneous phenotypes. Thus, it remains highly relevant to study more homogenous populations with fewer confounding factors and use detailed characterized phenotypes.

2 REVIEW OF THE LITERATURE

2.1 Overview of Mood Disorders

Mood disorders, also known as affective disorders and manic-depressive illnesses, are defined by abnormal fluctuation of mood. They include BD and MDD, which is also known as unipolar disorder, and BD. MDD is characterized by major depressive episodes that affect patients' ability to function. BD patients have had at least one episode of abnormally elevated mood, which can be either hypomania (BDII) that does not affect social or occupational functioning, or mania (BDI) that either affects social and occupational functioning, requires hospitalization, or contains psychotic features (Goodwin and Jamison 2007).

MDD and BD can be seen either as two different disease entities (the categorical view) or as the ends of the same manic-depressive disease spectrum (the spectrum view) (Goodwin and Jamison 2007). Both categorical and spectrum views have supporting findings in the literature (Benazzi 2007). The categorical view is supported by findings such as: gender distribution of MDD and BD differs (female dominance in MDD, equal prevalence in BD) (Angst and Marneros 2001); brain structure abnormalities of MDD are different to those of BD (Kempton et al); and the family distribution of BD and MDD cases differs between mania and depressive patients (Winokur et al 1995). The spectrum view is supported by the fact that in both, the depression is present either with or without hypomania or mania. In addition, the existence of hypomania and the mood continuum from normal to manic, the existence of fluctuation of mood in both disorders (recurrent-MDD, r-MDD and BD), high rate of MDD diagnoses shifting to BD, and similarities in cognitive performance (Benazzi 2007) support the spectrum view of mood disorders.

2.1.1 *Historical aspects*

Primitive societies thought that mental disorders were caused by magical forces, but in ancient Greece they were already seen as symptoms of underlying biological disturbances (Goodwin and Jamison 2007). The first classification categories of mental disorders were defined by Hippocrates (460 – 337 BCE), and included melancholia, mania and paranoia (Angst and Marneros 2001). The first noted suggestion that melancholia and mania are associated is dated back to the year 120 (Goodwin and Jamison 2007) when Arateus wrote that mania is a worsening of

melancholia (Angst and Marneros 2001). In 1854, French psychiatry Jean-Pierre Falret defined bipolar disorder as one disease entity for the first time, which he referred to as “circular disorder” (la folie circulaire), although most clinicians treated mania and melancholia as separate disease entities at that time (Goodwin and Jamison 2007). Melancholia and mania were considered the terms for mental illnesses in a broader sense than they are nowadays: these terms also captured some types of schizophrenia spectrum disorders (Angst and Marneros 2001). It was not until the late 19th century, when Emil Kraepelin segregated schizophrenia (dementia praecox at that time) from manic-depressive illness, and he also stated that all recurrent affective diseases belong to one illness spectrum, manic-depressive illness. This view dominated for decades, until the year 1966, when the opposite views gained ground in the psychiatric field when Jules Angst and Carlo Perris independently published family history data showing that patients suffering mania have a higher rate of mania in their families compared to patients suffering only recurrent depression and that these two disorders have distinct clinical features and course (Angst and Marneros 2001; Perris 1966). Nowadays there are three different diagnostic groups; MDD, BDI, and BDII, where MDD faces the strongest resistance because it is too wide a category, containing a plentitude of different clinical subtypes (Goodwin and Jamison 2007; Winokur 1979)

2.1.2 Diagnostic perspectives

The whole history of psychiatric diagnoses has been full of changes and overlaps in classification systems (Goodwin and Jamison 2007). Nowadays, there are two distinct diagnostic systems: the International Classification of Disease version 10 (ICD-10) (WHO 1992) and the Diagnostic and Statistical Manual, the fourth edition (DSM-IV) (American Psychiatric Association 1994). The ICD-10 is in use in clinical work in Finland, and in most of the world. The DSM-IV is in use in research and also in clinical work in United States. There are some differences between these two classification systems. For example, there are only two core symptoms in DSM-IV major depressive diagnosis (depressed mood and loss of interest or pleasure) and only one is needed for diagnosis. This is contrary to the ICD-10, where also fatigue and loss of energy is one of the core symptoms, and two core symptoms are needed for diagnosis. In addition, ICD-10 major depressive diagnosis requires overall only four symptoms, which is one symptom less compared to DSM-IV. In DSM-IV, BD is split into two main categories: BDI and BDII. In this study, DSM-IV diagnostic criteria are used for patient classification unless otherwise specified. The DSM-IV diagnostic criteria for major depressive episode and mania are summarized in Table 1 and Table 2, respectively.

Table 1. *Diagnostic criteria for Major depressive episode according to DSM-IV (American Psychiatric Association 1994).*

A	Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
	<ol style="list-style-type: none">1. Depressed mood most of the day, nearly every day, as indicated by either subjective report or observation made by others2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day4. Insomnia or hypersomnia nearly every day5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)6. Fatigue or loss of energy nearly every day7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide
B.	The symptoms do not meet criteria for a Mixed Episode.
C.	The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
D.	The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism).
E.	The symptoms are not better accounted for by bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.

Table 2. *Diagnostic criteria for mania episode according to DSM-IV (American Psychiatric Association 1994).*

A.	Distinct period of abnormally and persistently elevated, expansive or irritable mood, lasting at least 1 week (or any duration if hospitalization is necessary)
B.	During the period of mood disturbance, three (or more) of the following symptoms have persisted (four if the mood is only irritable) and have been present to a significant degree:
1.	Inflated self-esteem or grandiosity
2.	Decreased need for sleep (e.g., feels rested after only 3 hours of sleep)
3.	More talkative than usual or pressure to keep talking
4.	Flight of ideas or subjective experience that thoughts are racing
5.	Distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli)
6.	Increase in goal-directed activity (at work, at school, or sexually) or psychomotor agitation
7.	Excessive involvement in pleasurable activities that have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments)
C.	The symptoms do not meet criteria for a Mixed Episode
D.	The mood disturbance is sufficiently severe to cause marked impairment in occupational functioning or in usual social activities or relationships with others, or to necessitate hospitalization to prevent harm to self or others, or there are psychotic features
E.	The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication or other treatment) or a general medical condition (e.g., hyperthyroidism)

MDD is split into two main diagnostic categories, either MDD with single major depressive episode, or recurrent MDD (r-MDD) that requires at least two major depressive episodes in a lifetime. Thus r-MDD contains a wide spectrum of MDD disease types from two mild major depressive episodes to highly recurrent and severe major depressive episodes with psychotic features. BD is similarly divided into two categories: BDI, where the patient must have had at least one episode of mania; and BDII, where patients must have had at least one episode of hypomania in a lifetime.

Some studies argue that 20 to 24% of MDD patients are misdiagnosed BD patients (Hu et al 2012; Muzina et al 2007; Smith et al 2011a; Tafalla et al 2009). The proper diagnosis is crucially important, since the medication of BD with only antidepressant monotherapy could lead to impairment in the course of illness by leading to rapid cycling or turning the mood to mania or hypomania (Altshuler et al 1995; Wehr and Goodwin 1987), lowering the patient's quality of life (Awad et al 2007), and increasing the risk of attempting suicide (Shi et al 2004). The diagnosis of BD in depressive patients is based on retrospective identification of hypomania or mania symptoms, so there might easily be misdiagnoses because of a lack of anamnesis. Because of this difficulty in diagnostics, there are studies that have tried to find clinical differences in depressive symptoms of BD and MDD: in BD there is a greater risk for suicide (Moreno and Andrade 2010; Schaffer et al 2010), more lifetime depressive episodes, earlier age of onset, more substance abuse, more insomnia, decreased concentration, more psychomotor agitation and guilt/worthlessness (Schaffer et al 2010), and higher prevalence of atypical features of depression such as hypersomnia, increase in appetite and/or weight gain (Moreno and Andrade 2010).

There is at least some genetic overlap between MDD and BD, an approximate estimate of the shared genetic variance, i.e. heritability, is about 30 % (McGuffin et al 2003). Family studies show that the shared genetic risk is evident, although there are some discrepancies, for example, probands of BD have an elevated risk for both BD and MDD, whereas probands of MDD have an elevated risk for only MDD (Smoller and Finn 2003).

There is evidence that overlap also exists with other psychiatric disorders, such as schizophrenia and mood disorders, when examining clinical features, including course of illness, brain imaging, molecular neurobiology and genetics (Kempf et al 2005). The symptom of psychosis is the most evident evidence of the overlap of these disorders; in large clinical samples the amount of psychotic cases among MDD was estimated to be 18 %, and among BD it was as high as 52 % (Souery et al 2011). In study by Maier et al., first degree relatives of schizophrenia, BD, and MDD probands were diagnosed to provide evidence for a common genetic

background of schizophrenia and MDD, but not schizophrenia and BD (Maier et al 1993). In contrast, an extensive register study from Sweden showed that relatives of BD probands have an increased risk for schizophrenia, thus implying that there is likely a partly common genetic background for these two disorders (Lichtenstein et al 2009). A common genetic background is further supported by the novel GWA studies that have found a huge amount of common association findings for BD and schizophrenia (Huang et al 2010; Moskvina et al 2009; Purcell et al 2009; Williams et al 2011a; Williams et al 2011b).

2.1.3 Epidemiology

There is a range of prevalence estimations for mood disorders in different populations. In the United States, the life-time prevalence of MDD is 16.2 %, BD-I 1.0 %, and BD-II 1.1 % (Merikangas et al 2007). In general, the estimates of prevalence of MDD varied between 10 and 15 percent (Lohoff 2010) and of BD between 0.8 and 2.6 percent (Kato 2007). In Finland, the prevalence estimates are smaller: 6.5 % for MDD (Pirkola et al 2005) and 0.24 % for BD (Perala et al 2007). The lower prevalence of BD in Finland compared to other populations is hypothesized to result from higher age of onset seen in study of Räsänen et al. (Rasanen et al 1998), but other explanations must exist. There is a clear gender difference in prevalence of MDD with females having twice as high prevalence compared to males (Craddock and Forty 2006; Kessler et al 1994), but this gender difference is not seen in BD cases. The age of onset of MDD is nearly decade later than that of BD; in a large clinical study from Sardinia, the median age of onset was 32 (Tondo et al 2010), and in a smaller Norwegian sample it was 28 years (Oedegaard et al 2009). The mean age of onset in BD, in contrast, has been estimated to be 21 to 23 years (Craddock and Forty 2006; Tondo et al 2010).

2.1.4 Aetiology

Major depressive disorder

The factors that lead to MDD are highly complex, consisting of genetic and environmental factors that are specific to individuals (Sullivan et al 2000). The genetic influence is substantial, the heritability estimates vary from 33 to 50 % (Craddock and Forty 2006; Levinson 2006; Sullivan et al 2000). The risk for a first degree relative of an MDD proband to develop MDD is two to three fold more than individuals from the general population (Levinson 2006). Heritability seems to be

partly distinct between men and women (Kendler et al 2001), and it is higher in early onset (before 30 years) versus late onset (after 30 years) forms of the disease (Lyons et al 1998).

The environmental factors leading to depression include childhood sexual abuse (Kendler et al 2004), stressful life events (Kendler et al 2004), maternal anxiety in late pregnancy (O'Connor et al 2003), and loss of parent (Tennant 1988). Environmental factors seem to be specific to individuals, it has been shown that the common environmental factors affecting heritability is zero (Sullivan et al 2000).

It has been postulated that there are at least four different subtypes of depressive disorders depending of the familial pattern of MDD or co morbidity disorders: 1) sporadic depressive disorder with no familial background; 2) pure depressive disorder with familial loading of MDD; 3) depressive-spectrum disorder with familial loading of MDD, alcoholism or sociopath; 4) bipolar variant (mood disorder spectrum) with familial loading of BD (Winokur et al 1971). Some kind of grouping system would be highly beneficial not only for genetic studies, but also for clinical practice, e.g. for choosing a suitable treatment method, because there is evidence that the groups differ biologically and thus, most probably also, genetically. For example, it has been shown in the late 1970s that familial background has an effect on the activity of the hypothalamic-pituitary-adrenal axis such that the regulation of cortisol secretion was disturbed mostly in the pure depressive-disorder group (Schlesser et al 1979). There are studies that have tried to find the clinical markers that predict higher familial loading and those include cases with early age of onset (Kendler et al 2007; Levinson et al 2003; Weissman et al 1984), cases that meet most of the symptomatic DSM criteria (Kendler et al 2007), and those who suffer recurrences (Kendler et al 2007; Levinson et al 2003).

Bipolar Disorder

The positive family history of affective disorder is the most reliable risk factor for BD. The heritability estimates for BD ranged from 85 (Kiesepää et al 2004) to 93 % (McGuffin et al 2003). The risk for BD of a first degree relative of a BD proband is 9 %, compared to a 1 % risk for the general population (Barnett and Smoller 2009). Relatives of a BD proband are also more vulnerable to other psychiatric disorders, such as MDD and schizophrenia (Craddock and Sklar 2009)

Bipolar disorder, as well as other common psychiatric disorders, have long been considered to be a consequence of common variants (common disease common variant, CD/CV theory), but in light of the current knowledge, it is only partly true. It has recently been estimated, using GWAS data, that around 40 % of heritability of

BD is explained by common variants, i.e. is captured by GWA data (Lee et al 2011b). There has been a widespread debate where the missing heritability lies.

Other suggestive risk factors for BD are obstetric complications, winter/spring birth, stressful life events, traumatic brain injuries, multiple sclerosis, childbirth (Tsuchiya et al 2003), and urban birth place (Mortensen et al 2003). The parent or sibling lost in childhood has shown to be an important risk factor, especially maternal loss before the age of five (Mortensen et al 2003).

2.1.5 Endophenotypes

The term “endophenotype” first emerged in the literature in the year 1966, when an insect geneticist used the word for describing differences in chromosome numbers between distinct insect populations (John and Lewis 1966). Thus the term endophenotype referred to an internal phenotype that was not visible to the naked eye, contrary to the “exophenotype” (external phenotype). The term was later adapted to psychiatric genetics by Gottesman and Shields. They hoped to find schizophrenia endophenotypes that would segregate in a monogenic way among schizophrenics, thus facilitating the search of genetic factors for schizophrenia (Gottesman and Shields 1973). Nowadays, endophenotype is defined as an intermediate component between complex disease and distal susceptibility genes (Gottesman and Gould 2003). The concept assumes that the genetic background of endophenotype is simpler than the genetic background of the disease, not as simple as it was thought in the 1960s and 1970s, but involving fewer genes than the disease phenotype.

The criteria for identification of endophenotypes according to Gottesman & Gould are:

1. In a population, the endophenotype is associated with the illness.
2. The endophenotype is heritable.
3. The endophenotype is state-independent, i.e. it manifest in spite of individuals episode.
4. The endophenotype and illness co-segregate in families.
5. The endophenotype is more frequent in the unaffected family members of probands than in the normal population.

The concept of endophenotype could help researchers to simplify the genetic basis of highly complex phenotypes, such as MDD and BD. These disease entities are

based on clusters of symptoms and clinical characteristics that are highly heterogeneous. The endophenotypes were initially assumed to be more homogeneous with simpler genetic architecture.

Potential endophenotypes for mood disorders include various neuropsychological, biochemical, neuroanatomical, or cognitive traits, such as abnormal regulation of circadian rhythms, response to sleep deprivation, response to cholinergics, deficits in attention, and increase in white matter hyperintensities (Hasler et al 2006; Lenox et al 2002).

Various aspects of cognition and other brain functions are good endophenotype candidates for BD (Glahn et al 2004; Hasler et al 2006; Robinson and Ferrier 2006). The heritability of general intelligence has been most extensively studied and it has been shown to have a strong genetic background (Devlin et al 1997; McClearn et al 1997). There are also data showing that specific cognitive functions, such as executive functioning, verbal ability, attention, spatial and verbal working memory, and processing speed are remarkably heritable (Ando et al 2001; Antila et al 2007b; Fan et al 2001; Finkel et al 1995; Luciano et al 2001). According to many studies, the most promising cognitive function endophenotype for BD seems to be executive functioning, verbal memory, processing speed (Antila et al 2007a; Frantom et al 2008; Glahn et al 2004; Hasler et al 2006; Robinson and Ferrier 2006). Good endophenotypes for psychotic disorders are shown to include executive function and semantic fluency (Hu et al 2011). Novel GWAS findings have revealed common genome-wide significant association findings for BD and schizophrenia (O'Donovan et al 2008), and this same variant has also shown to disturb the connection of brain areas, thus providing a potentially mechanistic endophenotype for psychotic disorders (Esslinger et al 2009). A recent study searched for potential endophenotypes for MDD and it revealed Beck Depression Inventory, bilateral ventral diencephalon volume, and expression levels of the RNF123 transcript (Glahn et al 2012). In addition, new magnetic resonance imaging (MRI) techniques are proposed to be valuable in searching for genuine endophenotypes for MDD (Hasler and Northoff 2011).

Endophenotypes could have a broader use in psychiatry than just as a tool for genetic studies. They could be exploited in diagnostic procedures as well as in the development of new and possibly individually tailored cure, by enabling clinician to subgroup patients into more homogenous groups that might also differ in sensitivity to drugs.

2.2 Genetics of complex diseases

The field of genetics has evolved enormously within the last decade after the complete sequencing of the whole human genome (Lander et al 2001; Venter et al 2001). Before the availability of the whole human genome sequence, genetic research was concentrated on mapping of risk areas by linkage and positional cloning of candidate regions. A number of so-called classical monogenic traits were successfully characterized by linkage studies taking advantage of the Mendelian inheritance model and family structures. However, the more common multifactorial diseases, such as diabetes and psychiatric illnesses, have not been proven to be as successful in genetic vulnerability research (Zondervan and Cardon 2004). New strategies of whole genome associations and sequencing are sought, but current approaches do not yet meet expectations in psychiatric genetics.

2.2.1 Heritability

Heritability is the proportion of phenotypic variance that is due to inherited genetic factors. It varies from zero to one; where zero means that the phenotype is not affected by genes but is rather a consequence of environmental factors alone, and one means that all the variance seen in the phenotype is a result of genes. Thus, heritability is extracted from the total phenotypic variance that is not due to environmental factors. This extraction can be made either by using adoption or twin data. The most optimal are adoption studies, where the phenotypic variance of monozygotic twins adopted to distinct families (= environment) is completely due to the environmental variance. Hence heritability estimates are more accurate in adoption studies, since there are no confounding effects of shared environment. Adoption studies are not that easily available, thus most heritability estimates in complex genetics are established using twin data. Twin studies are based on the assumption that monozygotic and dizygotic twins share the environmental variance, but only monozygotic twins share also all the genetic variance, whereas dizygotic twins share on average half of it. Therefore, if monozygotic twins' phenotype is more similar than dizygotic twins, there is genetic variance, i.e. heritability, affecting the total variance of the phenotype.

Heritability can mean two different concepts. In a broad sense, it refers to all the genetic variance of heritability, i.e. additive, dominance, and epistasis. Dominance variance means the effect of interaction of alleles in a given locus to the phenotype, whilst the epistasis means the effect of interaction of alleles at distinct loci to the phenotype. Additive genetic variance indicates the sum of effects of all loci affecting

the phenotype that does not take into account the interactions, and it also refers to the narrow sense of heritability.

2.2.2 *Human genome*

The following section outlines the historical milestones of defining the human genome in addition to the associated major advances in genetic research. Darwin was the first to introduce the concept of natural selection and inheritance to a wider audience (Darwin 1859). A decade later, Mendel introduced the famous pea crossing studies that demonstrated that there are small hereditary units that can be predicted by statistical rules (Mendel 1866). Then in 1953, the structure of the hereditary unit was exposed when Watson and Crick revealed the structure of deoxyribonucleic acid (DNA) (Watson and Crick 1953) and in 1956 it was established that the human genome constitute of 46 diploid chromosomes (Tjio and Levan 1956). Chromosomes are composed of large DNA molecules and a wide variety of proteins that are attached to the DNA sequence and which function, for example, to tightly package the DNA molecule.

Currently, the human genome is known to be constituted of 3.2×10^9 pairs of nucleotides that are nearly identical between individuals. The estimate of the genetic variance between individuals is 0.1 % (The International HapMap Consortium 2005) and the repertoire of genetic variation is wide. It ranges from single nucleotide polymorphism (SNP) to chromosome number variation and these variations induce the difference between individuals together with recombination and epigenetic differences such as distinct methylation patterns.

The estimates of the amount of protein coding genes in the human genome have varied considerably over the years they have been studied. The first estimate after whole genome sequencing was 30 - 40,000 genes (Lander et al 2001), but in the light of current knowledge, the number of protein coding genes is only around 21,000 (Clamp et al 2007). The concept of “gene” has evolved enormously in tandem with the sequencing of the human genome, and other species’ genomes, has been completed. First, alternative splicing seems to be centrally important in defining the diversity of proteins produces, more so than gene number. Alternative splicing takes place in more than 90 % of genes. Gene duplication is also an important method for defining new proteins, the appearance of completely novel proteins is an extremely rare event. Second, it has been surprising that the vast majority of functional sequence does not encode proteins at all. The protein coding genes encompass only 1.5 % of the genome, but according to the comparison of the human and mouse sequences, the functionally relevant sequence encompass 6 % of

the genome (Waterston et al 2002), thus the protein-centric thinking in search of functional variants behind complex diseases does not cover the whole functionally important variance between individuals. These non-protein coding sequences contain significant roles, including regulatory areas important in embryonic development (Pennacchio et al 2006), but the function of vast majority of them is still unknown. Third, transposons are revealed to be important drivers of evolution. Finally, non-protein coding transcripts are fundamental part of the genome, containing both small non-coding RNAs calling microRNAs that regulate the translational activity of mRNAs and also large RNAs with still mainly unknown functions (Lander 2011).

V a r i a t i o n i n t h e h u m a n g e n o m e

A SNP is a one base substitution in nucleotide sequence and there are both rare and common variants, implicating both different genealogical age of the variant and variants influence to the fitness. Most SNPs are neutral variants whose frequency is a consequence of genetic drift, however a small number are functional variants that have beneficial or harmful effect on the fitness of an individual. The vast majority of SNPs with over 5 % frequency have been discovered and it has been estimated that 95 % of SNPs can be found in public databases (Lander 2011). SNPs are widely used variants in association studies since they are abundant in the genome, they have low mutation rate, ease of genotyping in high-throughput manner, and they are mostly di-allelic which also renders them good variants to use in haplotype maps (more in next chapter). The amount of common SNPs (frequency over 5 %) in the genome is estimated to be 9–10 million (Frazer et al 2007), so there is approximately one SNP per 300 bp.

Microsatellites, also known as short tandem repeats (STR) are another variant type which are traditionally used in genetic analyses. STRs are multiallelic variants that consist of two to 13-base repeats. Di-, tri- or tetra-nucleotide repeats variants are used in linkage studies (Litt and Luty 1989; Weber and May 1989), where the multiallelic marker inheritance, together with the disease or trait studied, is examined in large pedigrees or sibling pairs.

There are also other types of repeat variants in the genome. STR markers belong to the simple sequence repeats that also comprise satellite markers where the repeat sequence is over 500 bp long and minisatellites where the repeat sequence is 14 to 500 bp long. Transposons are also repeat sequences that comprise almost half of the human genome sequence. They are capable to shift positions within and between genomes and they are therefore important players in evolution.

Copy number variants (CNV) are also referred to as repeat variants that are composed of relatively long sequence (over 1 kb) that can contain gene(s) and the number of copy variation ranges from zero to six. There are both common CNVs that are estimated to cover 3 Mb in a typical individual, as well as rare CNVs that are thought to derive from recent mutations (Lander 2011). CNV are found to be important factors inducing genetic variance between individuals and their role in psychiatric disorders are widely studied (Lee et al 2012).

The largest variances in the human genome are chromosomal abnormalities that can be seen by eye at the microscopic level. Whole chromosome deletions and duplications cause syndrome phenotypes such as Down syndrome (21 trisomy) and Klinefelter's syndrome (XXY). Inversions are DNA segments that are rotated in the chromosome and they can be also neutral, i.e. not causing any symptoms to the carrier. Translocations are chromosomal regions that have switched positions from one chromosome to other and those are abundant in cancer cells.

2.2.3 Linkage disequilibrium

Linkage disequilibrium (LD) is a central concept in complex trait genetics. The term LD means that two alleles at different loci do not segregate randomly. These two loci need not be in the same chromosome, although in complex genetics the term is used mainly to examine the genetic distance of two loci within the same chromosome. LD is used when constructing haplotype blocks, which were introduced after the sequencing of the human genome (Daly et al 2001). The haplotype blocks are separated by recombination hot spots, which means that the recombination preferentially happens in a particular area of the sequence and these areas differ between species (Paigen and Petkov 2010). The haplotype blocks enable researchers to do association studies with only a fraction of SNPs and still capture most of the variation in the human genome. This is possible since it has been shown that SNPs in one haploblock comprise haplotypes (that is allele combinations of adjacent loci) that have only a limited amount of diversity. Most of the diversity between individuals can be captured by genotyping only 6 to 8 SNPs within one haplotype block (Gabriel et al 2002); these SNPs are called haplotype tagging SNPs. The tightly linked loci construct haplotype blocks that in humans can span from a few kilobases (kb) to several hundred kb (Wall and Pritchard 2003). The haplotype map of the entire human genome was established in 2005 (The International HapMap Consortium 2005). The haplotype blocks differ between human populations partly due to the allele frequency differences. With this haplotype tagging SNP method, researchers can capture most of the common variation in the

human genome, but the rare variants and possibly also the associations of functional variants can be missed (Terwilliger and Hiekkalinna 2006).

2.2.4 Genetic mapping of complex diseases

Large scale genetic mapping became possible after the use of neutral DNA sequence polymorphisms was proposed in the construction of a genetic map that could be systemically used to hunt the chromosomal regions that transmit together with the disease in a pedigree (Botstein et al 1980). Genetic mapping is based on the variants in the genome that are neutral, i.e. they do not affect the fitness or the phenotype of the individual but they are linked to the causative variant. So the genotype markers define the genomic area that contains a gene variant or other sequence variant that predisposes to a phenotype of interest. The ultimate goal is to find the causative variant that increases the risk of the disease, but in complex disease genetics, these methods have not been as effective as in the case of Mendelian diseases where only one gene is responsible for the phenotype. The genetic mapping of complex diseases use STR and SNP markers, and the methods employed are either linkage or association based mapping.

Linkage studies

The linkage method uses highly variable STR markers (Litt and Luty 1989; Weber and May 1989) to identify genomic areas that are inherited together with the disease (or other phenotype) in a pedigree or in sibling pairs. The method is based on the fact that the chromosomal areas that contain the susceptibility genes are inherited with the disease more often than is expected by chance (Morton 1955). This method would be suitable for complex diseases in which the genetic risk is caused by a relatively small number of genes with relatively large effects. It has been a highly effective method when searching the genetic vulnerability areas for rare, monogenic diseases (Botstein and Risch 2003) such as the Finnish heritage diseases like aspartylglucosaminuria (AGU), but in complex diseases it has not been as successful. This has led to the hypothesis that most complex diseases, like common psychiatric diseases are a consequence of common variants that have relatively small effect on the risk; this is called the “common disease common variant” hypothesis (CDCV) (Reich and Lander 2001).

The linkage method uses either large pedigrees or affected sibling pairs. The linkage analyses are based on the calculations of genetic distance between markers and the predisposing variant. The distance is characterized by recombination fraction (θ ,

theta), that is, the amount of meiotic events of recombination that these two loci are separated by. The recombination fraction (centimorgan) is proportionate to physical distance: one centimorgan corresponds to about one million base pairs on average, although the recombination rate varies greatly between chromosomes (Yu et al 2001).

Association analyses

In association analysis, the allele frequencies of cases and controls are examined and it is proposed that if the allele is more frequent in cases than controls, it associates with the phenotype and it, or some other marker allele that is in LD with the associating allele, increases the risk of phenotype. It has been proposed that association analyses are more suitable for detecting susceptibility genes for complex disorders where single genes confer only modest increased risk for the disease and where the susceptibility variants are thought to be relatively common (Risch and Merikangas 1996). In addition to single locus analyses, haplotypes can also be used in association studies. This enables the examination of larger genomic areas, but it requires that haplotype estimations must be made (Ott et al 2011).

Associating analyses can also be performed with family data. This carries an advantage over case-control data as it avoids control sample bias that can be problematic. In order to avoid the bias in case-control samples, the control sample must be selected carefully, since they must be comparable to cases so that the genetic differences between case and control populations would not bias the association results. There are several programs that analyse the association of family data. Those use either haplotype-relative risk (HRR) or transmission disequilibrium test (TDT) methods to dissect the association signal from family data. Both are based on calculations in which parental alleles are classified as either transmitted or not-transmitted to the affected offspring (Ott 1989). The TDT is a modification of HRR, and it tests linkage in the presence of LD using data from parents and affected offspring. There are several modifications of the TDT method, for example the ability to also use quantitative phenotypes in the analyses (Horvath et al 2001).

Candidate gene studies

Candidate gene studies are usually based on previous linkage findings and subsequent positional cloning of the linkage area and/or *a priori* hypotheses of the importance of the gene function concerning the phenotype of interest. In candidate gene studies, the problem is how to deal with a multiple testing problem and the low effect sizes of associating variants. To obtain statistically significant results, the

sample size request is usually high, since the effects of the associating variants to the complex diseases are from low to modest (Cardon and Bell 2001; Owen et al 1997). A statistical P-value of 0.05 is not strict enough when doing multiple tests in the same study, but the other extreme of Bonferroni corrected P-value is quite conservative, since the tested markers are not independent (there is LD between markers within same genomic area) as they are assumed to be. Publication bias is a problem, since the positive association findings are published more easily than the negative ones.

Genome wide association studies

The availability of the sequence of the whole human genome (Lander et al 2001; Venter et al 2001) and the development of genotyping techniques made it possible to genotype thousands of SNPs throughout the whole genome with a reasonable amount of money and effort. These genome wide association (GWA) studies have taken the field of complex disease genetics to a new level. Now researchers do not need *a priori* hypotheses when conducting association analyses, but the association analyses can be done at the whole genome scale at once. The amount of SNPs in the GWA platforms range from 500,000 to up to 2 million SNPs, and this amount of common variants are estimated to capture about 90 % of the total genetic variation in the population (Lander 2011). The problems with this method are partly the same as in candidate gene studies. Because the susceptibility genes effect sizes are small in complex disorders of psychiatric genetics (1.12-1.20), the required amount of genotyped individuals to obtain genome-wide significant association is estimated to be as high as 8,000 – 20,000 (Cichon et al 2009). The multiple testing problem of GWA studies are dealt with through the agreement that the significance level is 5×10^{-8} , which is equivalent to a Bonferroni correction of one million tests.

Meta analyses

Since there are studies made in different populations with both positive and negative association results, meta-analyses are used to verify the significance of the results in combined analyses of all data sets available to achieve large sample size for statistical analyses (Pearson 1904). The meta-analyses can be performed either by using statistical data from all of the different single studies, or by combining all the original genotypes and data from single studies. The latter is more efficient (de Bakker et al 2008), but harder to achieve. The weakness of meta-analyses is the combination of genetic data of distinct populations, thus population stratification

skews the results. In psychiatric genetics, the wide variety of exact phenotypes used in different studies is also a problem that might falsify the results.

2.3 Genetics of mood disorder

2.3.1 Linkage findings

The linkage peaks of mood disorder are spread all over the genome and also contain few replications and some shared linkage peaks for MDD and BD. Figure 1 summarizes the *genome wide significant* linkage findings of both MDD and BD.

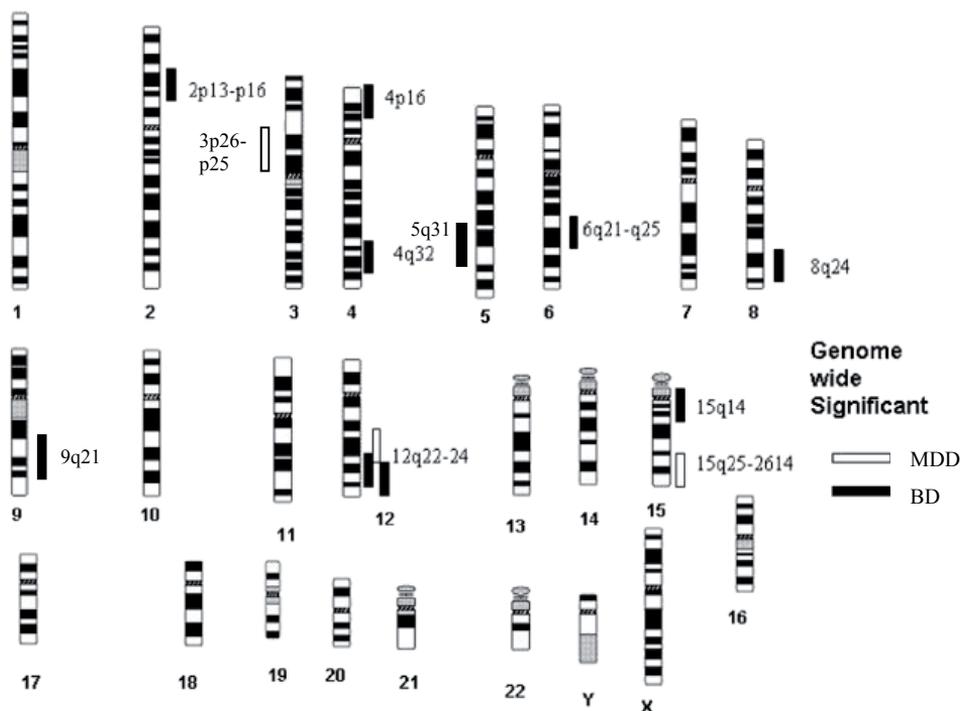


Figure 1. Genome wide significant linkage findings of MDD and BD. The figure is adapted with permission from Craddock and Forty (Craddock and Forty 2006) with modifications and updating.

Linkage of major depressive disorder

Only few genome-wide linkage studies of MDD have been performed with a sufficient number of affected individuals, i.e. over 100 per study, by the end of May 2012 (Abkevich et al 2003; Breen et al 2011; Camp et al 2005; Holmans et al 2007; Holmans et al 2004; Kuo et al 2010; McGuffin et al 2005; Middeldorp et al 2009; Nurnberger et al 2001; Pergadia et al 2011; Schol-Gelok et al 2010; Zubenko et al 2003). Their findings are not consistent with each other and the linkage peaks are spread throughout the genome. In the first linkage study, the collected families contained individuals affected for both MDD and alcoholism (Nurnberger et al 2001). The strongest linkage peak was gained with phenotype MDD or alcoholism and it was located in chromosome 1. The best linkage peak for depression only was located in chromosome 7. Holmans described linkage peak in the 15q25.3-26.2 in an initial analysis of 297 families (Holmans et al 2004) and when they expanded it to 359 families, the linkage peak of 15q was further supported and also suggestive evidence of linkage was observed in chromosomes 8p and 17p (Holmans et al 2007). A study made in a sample of 81 families containing early-onset r-MDD cases (Zubenko et al 2003) contains many confusing points, like the use of many covariates in the analyses, so the comparison of the results to other studies is highly difficult, as summarized in the review article of MDD (Levinson 2006). The highlighted result from this study is the linkage peak in chromosome 2 near the *CREB1* gene.

Studies conducted in large UTAH pedigrees (Abkevich et al 2003; Camp et al 2005) found linkage in different areas depending on the selection of families and phenotype. In Abkevich (Abkevich et al 2003), large pedigrees (N=110) were genotyped and the linkage was analyzed against depressive phenotype containing both recurrent (N=784) and single episode MDD (N=161) as well as BD (N=162) cases. This study revealed strongest linkage on chromosome 12q22-12q23.2, in the same area that has linkage to BD (Cassidy et al 2007; Curtis et al 2003; Ewald et al 2002; McInnis et al 2003a; Morissette et al 1999; Shink et al 2005b). Camp et al selected only families containing at least three r-MDD with early onset (before year 30) excluding BD cases. They also did the analyses combining the anxiety disorder cases. The strongest linkage findings were on chromosomes 3centr, 7p, and 18q. In addition, 18q is a highly replicated linkage finding in BD (Coon et al 1996; Detera-Wadleigh et al 1997; Fallin et al 2004; Freimer et al 1996; Garner et al 2001; McInnis et al 2003b; Schulze et al 2003; Tomas et al 2006).

McGuffin (McGuffin et al 2005) used 497 affected sibling pairs and observed linkage on chromosomes 1p36, 12q23.3-q24.11, and 13q31.1-q31.3. The 12q linkage finding is a replication (Abkevich et al 2003), and as previously mentioned, there are multiple linkage findings for BD in this chromosomal area as well.

In a study of Dutch and Australian pedigrees, strongest linkage was gained on chromosomes 2, 8, and 17. The linkage of chromosome 17 was near highly studied candidate gene of *SLC6A4* (Middeldorp et al 2009), but the association analyses of promoter length polymorphism of *SLC6A4* did not reveal association with depression in either population (Gillespie et al 2005; Middeldorp et al 2007).

In study by Schol-Gelok *et al*, the phenotype of depression was defined by depression subscale of the Hospital Anxiety Depression Scale (HADS-D) and the Center for Epidemiologic Studies Depression Rating Scale (CES-D), so they use the symptoms of depression, not the diagnosis of depression, in the definition. They found significant linkage on chromosomes 2p16.1-15 and suggestive linkage on chromosomes 5p15.33, 11q25, and 19p13.3 (Schol-Gelok et al 2010). Kuo et al. had families ascertained for alcohol dependence families collected from Ireland and used both alcohol dependence and MDD as a phenotype in their analyses. This study did not find genome-wide significant linkage, the strongest linkage peaks were located in the chromosome 4q32.3 (Kuo et al 2010).

Two of the latest linkage studies of MDD revealed linkage on same chromosomal area: 3p25-26 (Breen et al 2011; Pergadia et al 2011). In Breen et al. the linkage was gained with the phenotype of r-MDD with sibling pairs of European origin (N=971). Pergadia et al. had heavy smokers' families collected from Australia and Finland and they found the linkage with the phenotype of MDD in the Australian sample.

Linkage studies of bipolar disorder

Linkage peaks of BD are spread all over the genome, there are linkage findings in every single chromosome with multiple hits within each chromosome (most detailed summarized in Serretti & Mandelli (Serretti and Mandelli 2008). Genome wide linkage studies of BD have been carried out since the beginning of the 90s, and until 2009 there were over 40 genome-wide linkage studies done (Barnett and Smoller 2009). After 2009, at least 7 linkage studies have been published, so the total amount of genome-wide linkage studies of BD is around 50. There are also three published meta-analyses that give distinct results (Badner and Gershon 2002; McQueen et al 2005; Segurado et al 2003). The original linkage studies selected to the meta-analyses are partly overlapping, but the linkage findings in these meta-analyses differ completely. The first meta-analysis used seven studies and found strongest evidence for linkage on 13q and 22q (Badner and Gershon 2002). The largest meta-analysis used 18 studies, but did not find genome-wide significant linkage, although modest linkage was observed on chromosomal regions 9p22.3-21-1, 10q11.21-22.1, and 14q24.1-23.12. The latest meta-analysis used a different approach and combined original data from 11 studies and analyzed it directly

(McQueen et al 2005). They described genome-wide significant linkage on chromosomes 6q21-25 and 8q24. Thus, the traditional genomic approach does not seem to have shed the light on the genetic susceptibility background of BD either.

2.3.2 Candidate gene findings

The selection of candidate genes for association studies of mood disorders are based either on previous linkage findings and/or sophisticated hypotheses of functional genes that could potentially affect the susceptibility of mood disorders. The older ones are mainly based on the relevance of the function of the gene, but more recent candidate genes are based on positional cloning, like in the case of *DAOA* and *P2RX7*. There are numbers of potential candidate genes for both MDD and BD and they are partly overlapping and shared also with other psychiatric disorders such as schizophrenia and anxiety disorder. Table 3 summarizes the previous association findings with mood disorder and related phenotypes of the candidate genes genotyped and analyzed in this thesis.

Table 3. Candidate genes of mood disorder studied in this thesis: the genetic locations and the positive association findings with mood disorder and related phenotypes.

<i>Description</i>	<i>Location</i>	<i>Study</i>	<i>Associations</i>
BDNF, Brain derived neurotrophic factor	11p13	I, II	BD (Liu et al 2008; Lohoff et al 2005; Neves-Pereira et al 2002; Sears et al 2011; Sklar et al 2002), MDD (Licinio et al 2009; Ribeiro et al 2007; Schumacher et al 2005; Sun et al 2011; Verhagen et al 2010), Alcohol dependence –related depression (Su et al 2011), Symptoms of depression (Duncan et al 2009), Treatment response in MDD (Zou et al 2010), Anxiety (Tocchetto et al 2011), Anxiety-related personality (Frustraci et al 2008; Montag et al 2010), Substance related-disorders (Gratacos et al 2007), Psychotic features (Iga et al 2007), Suicidality (Iga et al 2007; Kim et al 2008; Pregelj et al 2011)
CREB1, Cyclic adenosine monophosphate response element-binding protein 1	2q34	I	BD and/or lithium response (Mamdani et al 2008), MDD (Dong et al 2009; Utge et al 2010) Anger expression in MDD cases (Perlis et al 2007a), Treatment-emergent suicidal ideation (Perlis et al 2007b), Treatment resistant in MDD (Serretti et al 2011)
NTRK2, Neurotrophic tyrosine kinase receptor, type 2	9q22.1	I, II	BD (Smith et al 2009), MDD (Dong et al 2009), Alcoholism (Xu et al 2007), Suicidality (Kohli et al 2010)
SLC6A4, Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	17q11-12	I, II	BD (Battersby et al 1996; Cho et al 2005; Hauser et al 2003; Lasky-Su et al 2005), MDD (Battersby et al 1996; Cervilla et al 2006; Goenjian et al 2012; Hauser et al 2003; Hoefgen et al 2005; Lopez-Leon et al 2008; Lotrich and Pollock 2004; Ogilvie et al 1996), Affective disorders (Collier et al 1996; Furlong et al 1998), Anxiety (Lesch et al 1996; Osher et al 2000; Schinka et al 2004; Sen et al 2004), Alcoholism (Feim et al 2005; Lichtermann et al 2000; Sander et al 1997), Suicidality (Campi-Azevedo et al 2003; Gorwood et al 2000;

Hranilovic et al 2003; Lin and Tsai 2004; Lopez de Lara et al 2006; Neves et al 2008; Preuss et al 2001)

TPH2, Tryptophan hydroxylase 2	12q21	I, II	BD (Campos et al 2011; Cichon et al 2008; Harvey et al 2007; Lin et al 2007; Roche and McKeon 2009; Van Den Bogaert et al 2006), MDD (Tsai et al 2009; Van Den Bogaert et al 2006; Zill et al 2004a), Suicidality (Lopez de Lara et al 2007; Zill et al 2004b)
P2RX7, Purinergic receptor P2X, ligand-gated ion channel, 7	12q23-24	I, II	BD (Barden et al 2006; McQuillin et al 2009), MDD (Lucae et al 2006; McQuillin et al 2009), Symptoms of BD (Backlund et al 2011), HADS (Hejjas et al 2009; Nagy et al 2008), Anxiety (Erhardt et al 2007)
DAOA, D-amino acid oxidase activator	13q33	II, III	BD (Detera-Wadleigh and McMahon 2006; Fallin et al 2005; Hattori et al 2003; Muller et al 2011; Prata et al 2008; Schumacher et al 2004), MDD (Rietschel et al 2008)
COMT, Catechol-O-methyltransferase	22q11.21	II, III	BD (Shifman et al 2004; Zhang et al 2009b), MDD (Aberg et al 2011; Massat et al 2005; Nyman et al 2011), Anxiety (Hettema et al 2008; McGrath et al 2004), Alcoholism (Sery et al 2006; Tiihonen et al 1999), Suicidality (Kia-Keating et al 2007; Nedic et al 2010; Ono et al 2004; Schosser et al 2012)
DISC-1, Disrupted in schizophrenia 1	1q42.2	II	BD (Hennah et al 2008; Palo et al 2007; Perlis et al 2008; Schosser et al 2009; Thomson et al 2005), MDD (Harris et al 2010; Schosser et al 2010), Anxiety (Harris et al 2010; Tomppo et al 2009)
MAOA, Monoamine oxidase A	Xp11.3	II	BD (Fan et al 2010; Lim et al 1995; Muller et al 2007; Preisig et al 2000), MDD (Fan et al 2010; Rivera et al 2009; Yu et al 2005; Zhang et al 2010), Anxiety (Tadic et al 2003); Suicidality (Ho et al 2000; Lung et al 2011), Panic disorder (Deckert et al 1999), Alcoholism (Contini et al 2006; Hsu et al 1996; Vanyukov et al 1995), Psychotic symptoms (Gutierrez et al 2004)

Major depressive disorder

As expected, there is no single certain susceptibility gene for MDD showing association in every sample studied. The most studied candidate genes are related to monoamine (serotonin or dopamine) transmission and neuroprotection (Levinson 2006; Lohoff 2010), and in every gene mention, there are positive association findings with both MDD and BD (see Table 3).

Serotonergic genes

One of the most promising systems that are hypothesized to be involved in the susceptibility to MDD is serotonergic transmission, since many antidepressants act on this system. The most highly studied candidate gene of this system is serotonin transporter (*SLC6A4*) and especially its 44-bp repeat polymorphism in the promoter region (HTTLPR), which is also shown to be a functional variant affecting *SLC6A4* expression levels (Lesch et al 1996). The short allele of HTTLPR has been associated with MDD in many studies (Table 3). Two meta-analyses have revealed evidence of short allele association with MDD (Furlong et al 1998; Lopez-Leon et al 2008), although three meta-analyses do not support the evidence of association (Anguelova et al 2003; Lasky-Su et al 2005; Risch et al 2009). This same polymorphism is connected to the vulnerability of environmental factors, i.e. it has been shown to increase the risk of developing MDD in response to stressful life events (Caspi et al 2003). There are both positive and negative replication of this gene environment interaction (GxE) association with MDD (reviewed in (Uher and McGuffin 2010)). There are three meta-analyses done this far, and two of them do not support this GxE significance in MDD (Munafò et al 2009; Risch et al 2009), but the latest one found strong support for the HTTLPR and stress interaction behind MDD susceptibility (Karg et al 2011). One recent study dissected the stressful life-events to childhood sexual abuse and adulthood stressors, and gained synergetic environmental and genetic interaction that increased the risk to develop MDD (Grabe et al 2012). Thus, the genetic and environmental architecture behind mood disorders susceptibility are highly complex.

Widely studied serotonergic genes also include tryptophan hydroxylase 1 and 2 (*TPH1* and *TPH2*) (rate limiting enzymes of serotonin synthesis). *TPH2* was found in 2003, when it was noticed that it is a predominant isoform in the brain, while the other one is expressed mainly in peripheral tissues (Walther and Bader 2003). The brain specific *TPH2* gene was shortly linked to MDD by association analysis (Zill et al 2004a), and this was later replicated (Tsai et al 2009; Van Den Bogaert et al 2006) and positive association was gained also for BD (see Table 3). This gene is also located in the replicated linkage area of 2q.

Monoamine oxidase A (*MAOA*) gene encodes a protein that degrades monoamines, such as serotonin. A widely studied variable number tandem repeat (VNTR) variant in the promoter area of *MAOA* is associated both with MDD and BD, as well as many related phenotypes (Table 3). In recent meta-analyses, some evidence of association with both MDD and BD were found (Fan et al 2010), so it is hypothesized to be potential common candidate gene for mood disorders.

Brain derived neurotrophic factor

Brain derived neurotrophic factor (*BDNF*) is a widely studied candidate gene, both for MDD and BD. Its function makes it a very potential susceptibility gene for mood disorders since it regulates neurogenesis (Huang and Reichardt 2001) and mediates neuronal plasticity (Thoenen 1995). There are many arguments that support the role of *BDNF* behind MDD. Its expression in brain was first shown to be altered by antidepressant treatment in 1995, when it was shown that both chronic antidepressant drugs and electroconvulsive seizure (ECS) treatments increase the expression of *BDNF* in different parts of the rat brain (Nibuya et al 1995). Its expression is decreased by stress (Smith et al 1995) and this reduction is restored by antidepressant treatments (Nibuya et al 1995). The effect of the stress is located mainly to the hippocampus, a brain area that is known to be relevant in depression and emotionality in general (Duman 2004; Groves 2007). Stress promotes cellular death and atrophy, and the same is seen in MDD patients that have shown to have smaller hippocampal volume compared to controls (Sheline et al 1996). The simplified model of stress and antidepressant effect to the *BDNF* function and mood is illustrated in Figure 2 (Groves 2007). The delayed antidepressant effect on mood has also given rise to the theory that brain structure and plasticity dysfunction is the leading cause of depression, and it is a *BDNF* recovery and its effects on brain plasticity that leads to the antidepressant effects of medication. Recent meta-analysis of plasma *BDNF* levels have shown that they are dependent on the state of disease, i.e. the levels are decreased in both depression and mania, but not in euthymia when compared to controls (Fernandes et al 2011). Therefore, there is a wealth of biological evidence that *BDNF* plays a major role in depression and mood disorders.

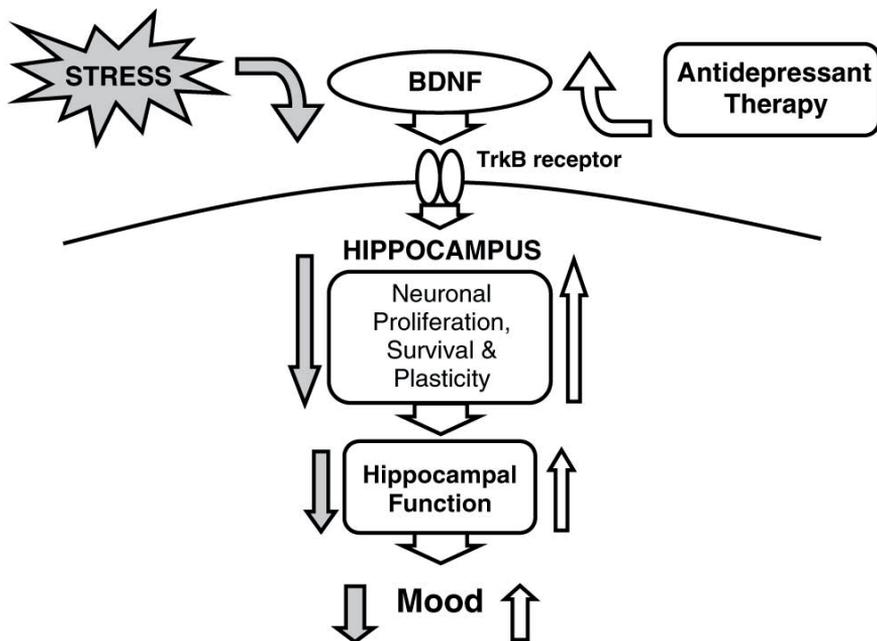


Figure 2. Model of stress and antidepressant effect on BDNF function in hippocampus. *TrkB* is a synonym for *NTRK2*, receptor of BDNF. Figure adapted from review article of Groves with permission (Groves 2007).

The most studied genetic variant within the *BDNF* gene is rs6263, a functional variant that changes amino acid valine to methionine (val66met). The first positive association findings of this val66met variant and BD were made in 2002 (Neves-Pereira et al 2002; Sklar et al 2002), and after that there have been positive replications for both MDD and BD (see Table 3). The meta-analysis of val66met and MDD found supporting evidence of *BDNF* association with MDD among males (Verhagen et al 2010), but the meta-analyses of BD studies did not find evidence for association (Gratacos et al 2007; Kanazawa et al 2007)

The activation of *BDNF* receptor, *NTRK2*, is known to mediate the effects of *BDNF* in the cell, i.e. the plasticity function (Castren and Rantamaki 2010). Thus, the *NTRK2* gene is also a good candidate gene for MDD and there are positive association findings for both MDD and BD, as well as other common co morbid disorders of mood disorders (see Table 3). *CREB1* is also a potential candidate gene, because it enhances the transcription of *BDNF* via a regulatory loop with *BDNF* (Castren 2004). It regulates the transcription of *BDNF* target genes in cells after *NTRK2* activation (Figure 3).

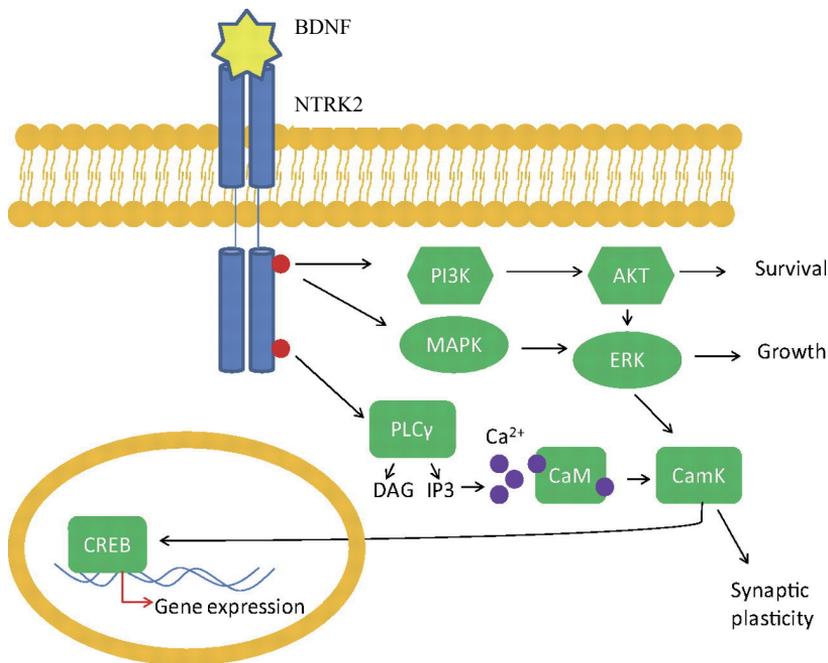


Figure 3. Simplified model of BDNF action in the cell after binding to the receptor NTRK2. Star represents the BDNF and the cell membrane receptor is NTRK2. Adapted from Autry and Monteggia with permission (Autry and Monteggia 2012).

A recent sophisticated study has shown that many environmental factors known to associate with MDD (such as smoking, age, alcohol abuse), together with genetic vulnerability genes (such as *BDNF*), affect disease susceptibility (Wong et al 2012). The interaction of post-traumatic stress and val66met polymorphism of *BDNF* has also been shown to increase the risk for MDD (Rakofsky et al 2012). The *BDNF-CREB1-NTRK2* pathway, together with cognitive and neural intermediate phenotypes, has been shown to increase the risk for depression (Juhász et al 2011).

Meta-analysis and large scale replication

One large meta-analysis has been performed in a large collaborative sample where replication of the candidate gene association results of MDD has been attempted (Lopez-Leon et al 2008) by examining all the polymorphisms that have been analysed in at least three separate studies. Twenty polymorphisms within 18

candidate genes were meta-analysed, these include four genes mention in Table 3 (*BDNF*, *COMT*, *SLC6A4*, and *MAOA*). The only statistically significant association signal among these was gained for *SLC6A4*, the most studied HTTLR marker.

When replication of the candidate gene results of MDD was attempted using GWAS data, only four genes out of the 57 analysed showed any evidence for association (Bosker et al 2011).

Bipolar disorder

The candidate genes of BD overlap widely with the MDD candidate genes - it could be said that all of the high potential candidate genes of MDD are also very good susceptibility candidate genes for BD, and positive association findings exist for both disorders (see Table 3). There is also broad overlap with BD and schizophrenia susceptibility genes, which is based on the phenotypic overlap of psychotic features, among other things.

Purinergic receptor P2X, ligand-gated ion channel, 7 (*P2RX7*)

The *P2RX7* gene is located in linkage locus 12q23-24 of BD (Cassidy et al 2007; Curtis et al 2003; Ewald et al 2002; McInnis et al 2003a; Morissette et al 1999; Shink et al 2005b) and MDD (Abkevich et al 2003; McGuffin et al 2005). The linkage area was further characterized by denser microsatellite genotyping (Shink et al 2005a) and later SNP association studies found that non-synonymous variant rs2230912, also known as Gln460Arg, was most strongly associated with BD (Barden et al 2006) and MDD (Lucae et al 2006). Later association studies have found positive association findings for BD (McQuillin et al 2009), depressive (Hejjas et al 2009; Nagy et al 2008) and BD symptoms (Backlund et al 2011), and anxiety (Erhardt et al 2007), although association studies conducted in large samples have not replicated the association findings for MDD nor BD (Green et al 2009; Grigoriu-Serbanescu et al 2009).

P2RX7 gene encodes a ligand-cated calcium channel that is activated by ATP. It is expressed in a wide variety of tissues and cell types such as antigen presenting immune cells, bone, epidermal cells, and brain. In brain tissue, it has shown to be expressed in astrocytes, microglia cells, and in neurons, and its expression is located in various brain regions, including the hippocampus (Sperlagh et al 2006). The actions of activated *P2RX7* receptor are dependent on ATP exposure time: with short exposure time, calcium channels open and signal transduction, depending on the cell type, proceeds; but with a long exposure time, large pores are formed on the

cell membrane and this eventually leads to the apoptosis of the cell. Thus, the receptor coded by this gene manifests many distinct functions both in brain and other tissues. There might be at least three different mechanisms in brain that lie behind the susceptibility to mood disorders. First, it has shown to promote the release of neurotransmitters, e.g. glutamate in hippocampus (Sperligh et al 2002), thus linking this gene to one of the neurobiological theories (i.e. glutamergic dysfunction) behind mood disorders (Goodwin and Jamison 2007). Second, this gene function in inflammation reactions fits to the inflammation and stress induced theory of depression (Skaper et al 2010). Third, it has also shown to promote neurogenesis (Monif et al 2009) and cell death, thus acting as an important modulator of neuroprotection and neuroplasticity, both important factors behind mood disorders.

D-amino acid oxidase activator locus in 13q34

D-amino acid oxidase activator (*DAOA*) gene was found in 2002 when schizophrenia linkage locus 13q34 was tagged with dense SNP marker map and was found to contain two genes: D-amino acid oxidase (*DAO* also known as *G30*) and *DAOA* (also known as *G72*) both associated with schizophrenia (Chumakov et al 2002). Linkage peaks of BD are also evident in this area, and soon after the first publication, BD was also found to associate with these genes (Hattori et al 2003). Both positive (Table 3) and negative association studies of BD were later published. The meta-analyses found significant association both with schizophrenia and BD, although the associating variants and areas were different between these two disorders (Detera-Wadleigh and McMahon 2006; Muller et al 2011).

DAOA was found to activate *DAO* (Chumakov et al 2002), which in turn is a potent activator of glutamate receptor. The function of this gene, however, has remained vague and functional studies have been controversial (Kvajo et al 2008; Sacchi et al 2011). Kvajo et al. found that one splicing variant of *DAOA* is located in the mitochondria and it seems to enhance the fragmentation of mitochondria and this way promotes the dendrite branching of neurons (Kvajo et al 2008). Functional studies of Sacchi et al. showed that *DAOA* seems to inactivate the *DAO*, not activate as it was previously believed, and thus protect cells from d-serine depletion i.e. it enhances glutamate receptor activity (Sacchi et al 2011). Recent data showed that genetic variation within *DAOA* has an effect on the dopamine turnover in the healthy brain (Andreou et al 2012), thus confirming the *DAOA* function as *DAO* regulator. Regardless of the real function(s) of this gene, it is an interesting gene concerning brain function since it has evolved rapidly in evolution, the open reading

frame of human is twice as long as that of the chimpanzee homologue (Chumakov et al 2002), thus implying that it has an important role in human evolution.

Other psychosis related candidate genes

There are plenty of other candidate genes that were initially discovered in schizophrenia studies, but afterward also linked to BD. These include *DISC1* that was first found in Scottish pedigree, where translocation containing this gene co-segregated with major psychiatric illness such as Schizophrenia, BD, and r-MDD (St Clair et al 1990). There are positive association studies with mood disorders (Table 3), although allelic heterogeneity is evident and negative reports also exist.

Three mainly schizophrenia candidate genes have also gained interest in BD. Neuregulin 1 (*NRG1*) is a strong candidate gene for schizophrenia (Munafò et al 2008; Stefansson et al 2002), but it has also associated with BD (Green et al 2005; Thomson et al 2007), thus it is suggested to be a common susceptibility gene for BD and schizophrenia (Walker et al 2010). Dystrobrevin binding protein 1 (*DTNBP1*) was identified as a schizophrenia candidate gene by systematic linkage disequilibrium mapping of a previously found linkage region 6p (Straub et al 2002). Both negative and positive association findings has been reported since, but only modest evidence of association in meta-analyses has been reported (Li and He 2007). There are also positive association findings with BD (Breen et al 2006; Gaysina et al 2009; Joo et al 2007; Pae et al 2007). V-akt murine thymoma viral oncogene homolog 1 (*AKT1*) functions in actions such as neuronal migration and axon branching and it is associated with schizophrenia (Emamian et al 2004) as well as with BD (Toyota et al 2003). There is also recent evidence that this could be a common susceptibility gene for BD and schizophrenia (Karege et al 2012).

Large scale meta-analyses of candidate gene studies of bipolar disorder

Recent meta-analysis included 487 studies and 33 polymorphisms within 18 genes to the analysis and the GWA data were also included in the analyses (Seifuddin et al 2012). None of the genes exceeded statistically significant P-values, although polymorphisms within *BDNF*, *DAOA*, *DRD4*, and *TPHI* showed nominally significant associations.

2.3.3 Genome wide association studies

In the field of psychiatric genetics, many have been disappointed with the outcome of GWA approaches, since they have not revealed any definite predisposing genetic factors, although they have brought totally new susceptibility candidates that have not even been considered in previous *a priori* studies.

G W A S of major depressive disorder

Altogether seven GWA studies of MDD have been published up to the end of May 2012 (Lewis et al 2010; Muglia et al 2010; Rietschel et al 2010; Shi et al 2011; Shyn et al 2011; Sullivan et al 2009; Wray et al 2012). In the first published GWA study, 1738 MDD cases were genotyped, but no genome-wide significant results were obtained, although 11 of the top 200 association signals were located at the gene piccolo (*PCLO*) (Sullivan et al 2009). The replication sample (N=6079) did not confirm the results, although authors claimed that the reason was a heterogeneous sample. Recent replication efforts have support the role of *PCLO* in the etiology of MDD (Hek et al 2010; Minelli et al 2012), especially among females (Aragam et al 2011).

There have been no genome-wide significant results in any of the subsequent GWA studies, although some interesting potential new candidate genes emerged that warrant mention here. There was suggestive evidence of association with two SNPs in *BICCI* gene ($P < 10^{-6}$) by Lewis (Lewis et al 2010), although the signals were not strengthened when the signal was meta-analyzed with previous GWA data (Muglia et al 2010). In a study by Rietschel et al., nominal association was found with SNPs located upstream of the carboxypeptidase M (CPM) and regulatory area of Homer 1 gene (Rietschel et al 2010). Homer 1 gene seems to be of interest, taking into account the MRI studies that describe the influence that a variant of this gene has on prefrontal activity during executive cognition and anticipation of reward.

In a GWA study conducted by Shi et al., there was evidence of association with a SNP in the mRNA detected in human brain tissue (BC053410) and approximately 75 kb upstream of *DSEL*, and when the data was combined to previous GWAS genotypes, association was seen within the *SP4* gene, a brain-specific transcription factor gene (Shi et al 2011).

When completing a meta-analysis with previous GWA data (Shi et al 2011; Sullivan et al 2009) Shyn found suggestive evidence of association in three different genomic areas: intronic SNPs in *ATP6VIB2* ($P = 6.78 \times 10^{-7}$), *SP4* ($P = 7.68 \times 10^{-7}$), and *GRM7* ($P = 1.11 \times 10^{-6}$) (Shyn et al 2011). Wray also performed meta-analyses

with previous GWAS data (Lewis et al 2010; Sullivan et al 2009) and found suggestive evidence of association with SNPs within adenylate cyclase 3 (*ADCY3*, 2p23.3) and galanin (*GAL*, 11q13.3) (Wray et al 2012).

Two meta-analyses done that included both MDD and BD studies, also explored the genome-wide associations for mood disorders (Liu et al 2011; McMahon et al 2010). In a study by McMahon (McMahon et al 2010), four GWA studies with European ancestry were included in the analyses; one MDD (Boomsma et al 2008) and three BD studies (Sklar et al 2008; Smith et al 2009; WTCCC 2007). This study found shared genome-wide significant association of MDD and BD that was located in the 3p21.1 with smallest P-value gained with the SNP rs2251219. The associating area contains many genes and the LD between markers is strong, thus the exact position of a functional variant is impossible to catch by association approach. The study by Liu (Liu et al 2011) combined MDD (Sullivan et al 2009) and BD (Ferreira et al 2008) GWA data. This study supported the role of *CACNA1C* in the aetiology of mood disorder.

G W A S o f b i p o l a r d i s o r d e r

All GWAS performed for BD thus far (May 2012) are summarized in Table 4. The main findings of these studies are not consistent with each other, although there are replication studies made in smaller samples. The association signal exceeds genome-wide significance level ($P < 5 \times 10^{-8}$) in only one study (Baum et al 2008a), where SNP in *DGKH* associated with BD with the P-value of 1.5×10^{-8} (OR = 1.59). Many of the studies also completed meta-analyses (Table 4) combining previous GWAS data to the analyses. In these additional meta-analyses, some studies exceeded the genome-wide significance level (Ferreira et al 2008). A study by Ferreira (Ferreira et al 2008) used two previous GWAS data sets in the meta-analysis (Sklar et al 2008; WTCCC 2007) reaching altogether 4387 cases. In this study, two SNPs within *ANK3* gene gave genome-wide significant association signals ($P = 9.1 \times 10^{-9}$, OR 1.450 and $P = 1.3 \times 10^{-8}$, OR 1.395).

Table 4. Genome wide association studies of BD completed by the end of May 2012.

Study	Population	Number of p<10 ⁻⁶	Associating areas/genes	Meta-analysis
(WTCCC 2007)	European (2000)	1	16p12	No
(Baum et al 2008a) ^a	European (461+772)	1 ^b	<i>DGKH</i>	No
(Sklar et al 2008)	European (1461)	2	<i>MYO5B, TSPAN8</i>	No
(Ferreira et al 2008)	European (2365)	0		Yes
(Hattori et al 2009)	Japanese (107+395)	0		No
(Scott et al 2009)	European (2076)	2	<i>CPS1, NEK7</i> or <i>ATP6V1G3</i>	Yes
(Smith et al 2009)	European (1001), African (345)	0		No
(Lee et al 2011a)	Chinese (1000)	1	Specificity protein 8 (SP8)	No
(Djurovic et al 2010)	Scandinavian (194+435)	0		No
(Cichon et al 2011)	European (682+2411)	2	<i>NCAN, MAD1L1,</i>	Yes
(Smith et al 2011b)	European (1190+1001)	2	<i>NEK7; MGAT4A</i>	Yes
(Yosifova et al 2011)	Bulgarian (376+122)	0		No

^a Study used a pooled DNA sample in the initial screening

^b P-value exceeded genome-wide significance level

A few meta-analyses have also been performed from the GWA data thus far. Sklar (Sklar et al 2011) combined six GWA studies (Djurovic et al 2010; Ferreira et al 2008; Scott et al 2009; Sklar et al 2008; Smith et al 2009; WTCCC 2007) incorporating altogether 7481 BD cases to the analyses. When the initial data was combined to the replication sample (N = 11 974), genome-wide significant association signal was gained for *CACNA1C* (OR=1.14) and *ODZ4* (OR=0.88) genes.

Overall, genome-wide significant findings are achieved with huge sample sizes and the odds ratios have been small, thus the effects of the variants to the disease susceptibility are only modest.

2.3.4 Other genetic avenues

Large GWA data sets have provided new tools for genetic analyses, such as enabling the study of copy number variants (CNV) in a genome-wide manner. There is evidence that rare singleton deletions could be important factors behind BD (Zhang et al 2009a), although a more recent study reported opposite results showing no excess of CNVs in BD cases compared to controls (Grozeva et al 2010). Hence, although there are studies showing specific alterations among CNVs in BD cases versus controls (Lachman et al 2007; Wilson et al 2006), the overall differences in the number of CNVs continues to be unresolved.

Gene-gene interactions have been thought to play an important role in the aetiology of mood disorders, but the statistical analyses are highly complex and demanding. There are few genome wide interaction analyses done in BD (Abou Jamra et al 2007; Fullerton et al 2012) which are based on linkage studies in large families. The best results that exceeded statistical significance after permutations of these studies were 2q22-q24 - 6q23-q24 and 2q22-q24 - 15q26 (Abou Jamra et al 2007) and 11q23-25-2p15-12, 4q32-35-1p36, 12q23-24-4p16-15, and 20q13-9q21-22 (Fullerton et al 2012).

3 AIMS OF THE STUDY

The principle aim of this investigation was to characterize the genetic background of mood disorders and their endophenotypes in Finnish mood disorder samples, a bipolar family sample and a case-control sample comprising three clinical cohorts. A candidate gene approach was used in studies I, II, and III. In studies IV and V the results from previous GWA studies of bipolar disorder were exploited in an attempt to replicate their findings in the Finnish bipolar family sample.

These aims in detail were:

- 1) To study if so called classical candidate genes of mood disorders explain genetic liability to mood disorders in the bipolar family sample (I) and in the clinical cohorts (II).
- 2) To survey if BD has shared genetic background with MD (II) or with schizophrenia (III).
- 3) To investigate if variants identified by previous GWA studies have impact on genetic aetiology of mood disorders in the Finnish bipolar family sample (IV and V).
- 4) To investigate if some of the potential endophenotypes of mood disorders (cognitive functions, circadian preference and seasonal pattern of mood) mediate the genetic effect of the risk variants (I, III and V).
- 5) To further dissect the effect of the genetic risk variants on comorbidities for mood disorders and on clinical outcome of patients (II).

4 MATERIALS AND METHODS

4.1 Study samples

Two distinct sample sets were used in this thesis. The first one is a bipolar family sample that contains altogether 180 families with 723 genotyped individuals from the whole of Finland (I, III-V). The second one comprises three clinical cohorts for mood disorders, collected from the metropolitan area of Southern Finland (II).

4.1.1 Bipolar family sample

Bipolar family sample was collected by exploiting the National Hospital Discharge Register to identify all individuals who were hospitalized for BDI between 1969 and 1991 in Finland. The register indexes all primary diagnoses for inpatients stays at public and private hospitals. The collection is delimited to individuals who were born between 1940 and 1969. Before the year 1987, the diagnoses were made using the International Classification of Diseases, version 8 (ICD-8) (WHO 1967). After 1987 the diagnoses were made according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, revised third edition (DSM-III-R) (American Psychiatric Association 1987). Two psychiatrists also made independent diagnoses using all available case notes according to DSM-IV diagnostic criteria (American Psychiatric Association 1994) for BDI. Information on first-degree relatives was assembled from the National Population Register. All available records from hospitals, clinics, general practitioners, reports from medico-legal experts and various other sources were examined to provide diagnostic information for the relatives of the probands.

In addition, eight bipolar families were identified among a schizophrenia family sample, which had been collected using the same registers (Hovatta et al 1999). These eight families contain family members with schizoaffective bipolar type disorder. The remaining individuals are from BP twin sample where 32 families were recruited. The Finnish twin cohorts were used to identify twins in which at least one was affected with BD (Kieseppa et al 2004). In these subsamples the diagnoses were made in the same manner as described earlier.

The diagnostic categories that were used in this study were the bipolar spectrum and the psychotic disorder groups. The bipolar spectrum disorder patient group (n=227)

covered: BDI (n=214), BDII (n=5), BD-not-otherwise-specified (BD-NOS) (n=6), and cyclothymia (n=2) cases. The psychotic disorder group includes only individuals with the presence of life-time psychotic symptoms (n=251). This category included patients with the diagnoses of BDI (with intermittent psychotic features) (n=162), schizoaffective disorder (SA) (n=51), psychotic depression (n=15), schizophrenia (n=14), and psychosis NOS (n=9). There was an overlap of 162 individuals between the two categories.

Familial subsample was identified by selecting only those families that had at least two affected individuals in the pedigree. Familiarity was seen in 118 families (66 % of all families) with 543 genotyped individuals, for detailed information see Table 5.

Table 5. *Description of the bipolar family sample.*

	<i>Female</i>	<i>Male</i>	<i>Total</i>	<i>Familial</i>
Affected	170	192	362	258
Bipolar spectrum disorder	109	118	227	173
Psychotic disorders	117	134	251	212
In both categories	83	79	162	127
Other mental disorders	28	36	64	45
Unaffected	208	153	361	241
Neuropsychological subsample	82	77	159	155
Circadian/Seasonal subsample	64	63	127	127
Total genotyped	378	345	723	544

Neuropsychological tests

Neuropsychological test results were used in the genetic analyses in studies I, III, and V. Neuropsychological test pattern was performed for 159 individuals from 65 families. The test pattern used in this study includes subtests from the Wechsler Adult Intelligence Scale – Revised (WAIS-R) (Wechsler 1981) and the Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987), The California Verbal Learning Test (CVLT) (Delis 1987), the interference score from the Stroop Color and Word Test (Golden 1978), and the Controlled Oral Word Association Test (COWAT) (Benton 1989). The tests and the feature they assess are listed in Table 6.

Table 6. *The neuropsychological tests used in the genetic analyses (Studies I, III, and V).*

<i>Neuropsychological test</i>	<i>Assessed feature</i>
<i>WAIS-R</i>	<i>General intellectual functioning</i>
Vocabulary	General ability
Similarities	Verbal abstraction
Digit Symbol	Psychomotor speed
Block Design	Visuospatial ability
<i>WMS-R</i>	<i>Attention and memory</i>
Digit Span forward	Auditory attention
Digit Span backward	Verbal working memory
Visual Span forward	Visual attention
Visual Span backward	Visual working memory
Logical Memory I	Immediate verbal memory
Logical Memory II	Delayed verbal memory
Visual Reproduction I	Immediate visual memory
Visual Reproduction II	Delayed visual memory
<i>CVLT</i>	<i>Verbal learning and memory</i>
Free short delay recall	
Free long delay recall	
Recognition memory	
Retention	
<i>Stroop Color and Word Test</i>	
Interference score	Selective attention and executive functioning
<i>COWAT</i>	Semantic and Phonemic fluency

Circadian preference and seasonality

Two different questionnaire's results were exploited in study V: the results from Quantitative items of the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) (Horne and Ostberg 1976); and six items from of seasonal variation in mood and behavior (Global seasonality score, GSS) from the Seasonal Pattern Assessment Questionnaire (SPAQ) (Rosenthal et al 1987), performed on 127 individuals from 37 families. MEQ test pattern assessed the circadian preference of daily activities like sleep, work, and physical activity. The items and factors of MEQ used in the genetic analyses are summarized in Table 7. The MEQ factors were calculated by making the factor matrix using maximum likelihood principle, and the standard orthogonal varimax rotation was computed in order to examine the degree of correlation among factors using the RELIAB module of the Survo MM program, release 2 (www.survo.fi/mm/english.html). GSS items used in the genetic analyses assess if there is a seasonal variation in 1) sleep duration, 2) social activity, 3) mood, 4) weight, 5) appetite, and 6) energy level.

Table 7. *Quantitative items of the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ).*

Item/Factor	Question/content
#1	"Considering only your own "feeling best" rhythm, at what time would you get up if you were entirely free to plan your day?"
#2	"Considering only your own "feeling best" rhythm, at what time would you go to bed if you were entirely free to plan your evening?"
#10	"At what time in the evening do you feel tired and as result in need of sleep?"
#17	"Suppose that you can choose your own work hours. Assume that you worked a FIVE hour day (including breaks) and that your job was interesting and paid by results. Which FIVE CONSECUTIVE HOURS would you select?"
#18	"At what time of the day do you think that you reach your "feeling best" peak?"
SUM	Sum score of all the 19 items (range=16-86)
Factor 1	Factor loaded mainly by the items #9 "You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 7.0 - 8.0 AM. Bearing in mind nothing else but your own "feeling best" rhythm How do you think you would perform ?", and #17
Factor 2	Factor loaded mainly by the items #7 "During the first half-hour after having woken in the morning, how tired do you feel?", and #4 "Assuming adequate environmental conditions, how easy do you find getting up in the mornings?"
Factor 3	Factor loaded mainly by the items #15 "You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own "feeling best" rhythm which ONE of the following times would you choose?"
Factor 4	Factor loaded mainly by the item #12 "If you went to bed at 11 pm at what level of tiredness would you be?"

4.1.2 Mood disorder cohort

The sample used in study II was gathered from three separate but comparable clinical cohorts that all are collected from psychiatric in- and outpatients from the metropolitan area of southern Finland. Three cohorts are 1) the Jorvi Bipolar Study (JoBS) (Mantere et al 2004; Mantere et al 2008), 2) the Vantaa Depression Study (VDS) (Holma et al 2008; Melartin et al 2002), and 3) the Vantaa Primary Care Depression Study (PC-VDS) (Vuorilehto et al 2005; Vuorilehto et al 2009). All clinical cohorts are collected in collaboration with the Mood Disorder Research Unit of the Department of Mental Health and Alcohol Research Unit of the National Institute for Health and Welfare.

Patient sampling was based on screening for BD (in JoBS), MDD (in VDS), and depressive disorders (in PC-VDS). The target groups were all psychiatric patients aged 18 (in JoBS) or 20 (VDS and PC-VDS) to 59 years and seeking treatment, referred to treatment, or already in treatment. In JoBS, the screening was based on the Mood Disorder Questionnaire and clinical suspicion of BD; in VDS to the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) and Scale for Suicide Ideation, and in the PC-VDS to the Primary care evaluation of mental disorders (PRIME-MD) and Structured clinical interview of DSM-IV axis I disorders, research version, patient edition with psychotic screen (SCID-I/P) by telephone interview. Those that were positively screened were diagnosed using DSM-IV diagnostic criteria. The numbers of the individuals in each cohort and the amount of BD and MDD individuals in the final Mood Disorder Cohort are described in Figure 4.

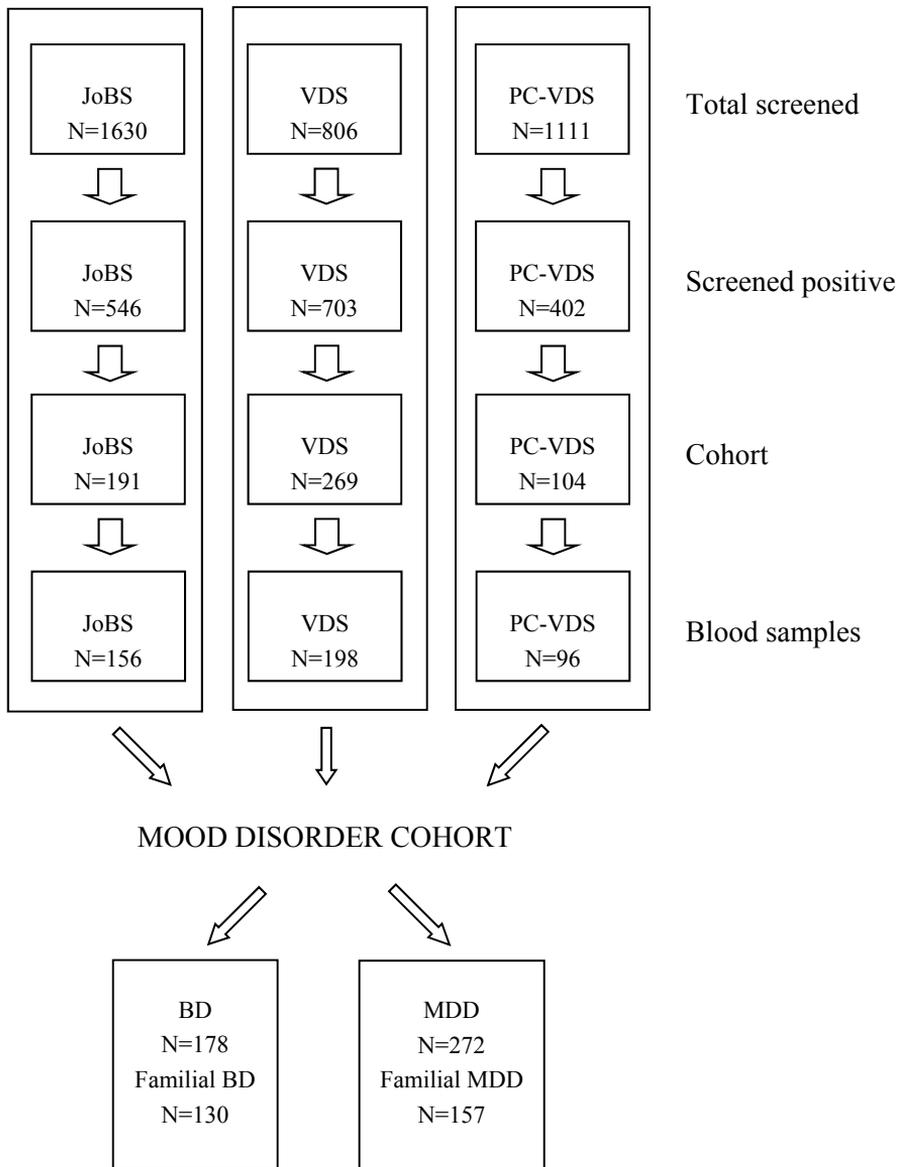


Figure 4. Description of Mood Disorder cohort and its composition from three clinical cohorts.

Most of the genotyped individuals were followed up: 95.5% of JoBS, 93.9 % of VDS, and 97.9 % of PC-VDS. The follow-up median duration was 19, 66, and 62 months in JoBS, VDS, and PC-VDS, respectively. In follow-up patients were interviewed at 6 and 18 months, and at 5 years.

The diagnostic phenotype used in this study was based on the diagnostic interviews. In addition to DSM-IV diagnoses of BD and MDD, co morbid lifetime anxiety disorder and alcohol dependence were used in the analyses, as well as incidence of psychotic symptoms or suicide attempts, age of onset, and time ill.

4.1.3 Control sample

In study II, a control sample of 1322 individuals was derived from the population-based Health 2000 study of Finland (Pirkola et al 2005). The control sample included 708 females and 562 males with no depression or any other psychiatric disorder according to the Composite International Diagnostic Interview (CIDI).

4.2 Laboratory methods and statistical analyses

Table 8 summarized the laboratory methods and the statistical analyses utilized in this study. The methods are described in more detailed in original publications and below.

Table 8. *The laboratory methods used in original publications.*

Method	Reference	Study
<i>Laboratory procedure</i>		
DNA extraction		I-V
Polymerase chain reaction (PCR)	(Kleppe et al 1971)	I-V
Agarose gel electrophoresis		I-V
Allele-specific primer extension on microarrays	(Pastinen et al 2000)	III
Sequenom hME reaction	Sequenom, San Diego, CA, US	I, III
Sequenom iPLEX™ biochemistry	Sequenom, San Diego, CA, US	I, II, IV, V
Electrophoresis, ABI377/3730	Applied Biosystems, Foster City, CA, US	I, II
<i>Analysis program</i>		
SNPSnapper		III
Genemapper	Applied Biosystems, Foster City, CA, US	I, II
Pedcheck	(O'Connell and Weeks 1998)	I, III-V
Haploview	(Barrett et al 2005)	I-III
PLINK	(Purcell et al 2007)	II
HRR	(Terwilliger and Ott 1992)	III
SPSS 14.0	SPSS Inc., Chicago, IL, US	I-III
FBAT	(Horvath et al 2001)	I, III-V
R program	www.R-project.org	II
QTDT	(Abecasis et al 2000)	I, III, V
<i>Statistical method</i>		
ANOVA		III
Logistic regression		II
Pearson Chi-Square		I-V

4.2.1 Marker selection and genotyping

SNP selection was made using public SNP database (dbSNP) (www.ncbi.nlm.gov) (studies I-III) and by taking advantage of the HapMap database to cover the haplotype-tagging SNPs using the cut-off values of 0.2 for minor allele frequency and 0.8 for coefficient of determination (r^2) (I-II). In Studies IV and V, the SNP selection was based on previous GWA findings. The summary of genotyped genes and variants in Studies I-III are listed in table 9.

Table 9. List of studied genes and the number of genotyped variants in studies I - III.

<i>Gene</i>	<i>Location</i>	<i>Number of genotyped variants</i>	<i>Study</i>
<i>AKT1</i>	14q32.32	2	III
<i>BDNF</i>	11p13	10/7 ^a	I, II
<i>COMT</i>	22q11.21	2/11 ^b	II, III
<i>CREB1</i>	2q34	9	I
<i>DAOA</i>	13q34	11/13 ^b	II, III
<i>DISC1</i>	1q42.1	6	II
<i>DTNBP1</i>	6p22.3	14	III
<i>MAOA</i>	Xp11.3	5	II
<i>NRG1</i>	8p12	11	III
<i>NTRK2</i>	9q22.1	15/9 ^a	I, II
<i>P2RX7</i>	12q24	10/8 ^a	I, II
<i>SLC6A4</i>	17q11.2	9/8 ^a	I, II
<i>TPH2</i>	12q21.1	15/9 ^a	I, II

^aNumber of variants genotyped in Study I/II

^bNumber of variants genotyped in Study II/III

Genotyping of *COMT* in Study III was performed by allele-specific primer extension method based on microarrays. Genotyping of other genes in Study III was performed by homogenous Mass Extension (hME) using the MassARRAY System. In Study I, genotyping was performed using both hME and Sequenom iPLEX™ biochemistry. In studies II, IV, and V, Sequenom iPLEX™ biochemistry was used for genotyping. Three microsatellite markers were genotyped in studies I and II, and the genotyping was performed by electrophoresing PCR amplified microsatellite markers on an ABI 3730 Automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA) as described in Utge et al. (Utge et al 2009).

As a quality check for the genotyping, there were duplicates in each sample plate, the family structure were checked by Pedcheck program (studies I, III-V) and deviation from Hardy-Weinberg equilibrium was tested by chi-square test for every marker.

4.2.2 Statistical analyses

The allelic association analyses were done by FBAT (studies I, IV, V), HRR (III), or PLINK (II). Haplotype analyses were made by FBAT (studies I and III) or PLINK (II). Quantitative traits were analyzed with the Quantitative Transmission Disequilibrium Test (QTDT) software package with the polygenic variance component option with total association model assuming no population stratification (studies I, III, V) or by a linear regression model using PLINK (II). In study II, the quantitative trait of time in illness (time ill) was analysed using logistic regression by R program.

5 RESULTS AND DISCUSSION

5.1 Results of candidate gene studies (I-III)

5.1.1 Candidate gene study of bipolar family sample (I)

There are many potential susceptibility genes of BD and MDD. Here, those that seemed to carry the most potential and interest in the era before GWA studies were chosen, at the commencement of the study. This included genes related to neuronal plasticity (*BDNF*, *NTRK2*, and *CREB1*), serotonin neurotransmission (*SLC6A4* and *TPH2*), and inflammation (*P2RX7*). These six functional candidate genes were genotyped and analysed in the Finnish bipolar family sample. The genotyped variants were analysed both against the disease status (against bipolar spectrum disorder and against presence of life-time psychotic symptoms) as well as against neuropsychological test results.

The strongest evidence for association of BD spectrum disorder was gained for *BDNF* with familial subsample and for *SLC6A4* among males (Table 10). The most studied *BDNF* variant rs6265 (Val66Met) associated with the familial BD spectrum with the P-value 0.016, valine being the risk allele. Three variants (rs3794808, rs140700, and rs6354) within *SLC6A4* associated with BD spectrum disorder, strongest among males ($P = 0.047$, 0.007 , and 0.023 respectively). There was also suggestive evidence for association with variant rs2230912 within *P2RX7* gene, and also here the association signal was derived mainly from males ($P = 0.038$).

Haplotype analyses revealed some new evidence of association with all the three genes that associated in the initial two-point analyses. In *BDNF* gene, the 5' flanking region haplotype associated with bipolar spectrum disorder (rs1491850-rs1491851, T-C, $P = 0.004$). Within *SLC6A4*, the strongest haplotype association signal came with the haplotype rs140700-rs6354 (T-G, $P = 0.017$), and the signal strengthened slightly when the analyses were made among males ($P = 0.004$). Several haplotypes within *P2RX7* showed suggestive association signal. The strongest signal was gained with the haplotype rs208290-rs208294-rs208298-rs504677 (G-T-G-C, $P = 0.006$).

In *BDNF*, the same variants that associated with bipolar spectrum disorder, also associated with memory functions. The val66met associated with retention and rs1491850 with immediate verbal memory ($P = 0.0087$ and 0.0081 , respectively). In val66met, the disease associating allele (valine) associated with better performance

in retention. In addition, the haplotype that associated with the disease also associated with retention (T-C of rs1491850-rs1491851; $P=0.0004$). The best association results of neuropsychological tests were gain with the *P2RX7* variants rs504677 and rs1718119 that associated with the semantic fluency ($P = 0.0001$ and 0.0010 , respectively) (Table 10). The same haplotype that associated with the bipolar spectrum disorder, also associated both with semantic and phonemic fluency ($P = 0.006$ and 0.0003 , respectively).

Table 10. The association results of *BDNF*, *SLC6A4* and *P2RX7* variants with *BD* spectrum and neuropsychological test results showing evidence of association with dichotomized traits with P -value < 0.05 and with quantitative traits with P -value < 0.001 .

Variant	BD spectrum				Neuropsychiatric tests			
	All	Familial	Male	Female	Auditory attention (Digit Span Forward)	Immediate verbal memory (Logical Memory I)	Retention	Semantic fluency
BDNF								
rs6265	0.062	0.016	0.203	0.134	0.9546	0.5316	0.0087	0.7037
rs7934165	0.277	0.408	0.967	0.124	0.8204	0.0189	0.0075	0.1382
rs1491850	0.818	0.942	0.412	0.529	0.7781	0.0081	0.0152	0.0722
rs1491851	0.032	0.075	0.433	0.022	0.9231	0.6521	0.2578	0.9783
SLC6A4								
rs7224199	0.076	0.05	0.064	0.545	0.3687	0.0046	0.395	0.3624
rs3794808	0.045	0.041	0.047	0.474	0.7564	0.0114	0.4284	0.4957
rs140700	0.021	0.042	0.007	0.834	0.7383	0.1613	0.5242	0.2609
rs6354	0.083	0.036	0.023	0.973	0.3055	0.1566	0.2261	0.5839
P2RX7								
rs208290	0.755	0.64	0.623	0.935	0.0078	0.71	0.1894	0.6725
rs504677	0.31	0.371	0.235	0.873	0.2468	0.3766	0.5308	0.0001
rs1718119	0.399	0.483	0.342	0.874	0.1039	0.7848	0.3148	0.0010
rs2230912	0.048	0.061	0.038	0.574	0.6253	0.2914	0.6941	0.0575

The main result in this study was the replication of the *BDNF* functional variant val66met association with *BD*, even though the association signal is only modest taking the amount of tests done into account. Although previous studies of *BDNF* have been inconsistent and the meta-analyses have been negative (Gratacos et al

2007; Kanazawa et al 2007), there are family studies that have been more consistent showing association of BD with the valine allele (Liu et al 2008; Neves-Pereira et al 2002; Sears et al 2011; Sklar et al 2002) like in this study. One case-control study also found association of valine allele with BDI and this study used only cases that had positive family history of affective disorders (Lohoff et al 2005), which might explain the contradictory results with the meta-analyses done. So it could be that the *BDNF* is associated with a specific type of BD, since it has been shown that there are plenty of clinical phenotypes that are clustered to the families, like age of onset, life time severity of mania, and presence of psychosis (summarized in Box 2 in Craddock & Sklar (Craddock and Sklar 2009)).

Some encouraging support was seen in the results for the hypothesis that the neuropsychological test data could be a good endophenotype for BD. The risk allele of functional variant val66met of *BDNF* associated with better memory function, which is in line with previous studies where valine allele has been connected with different aspects of better performance in memory function (Egan et al 2003; Goldberg et al 2008; Hariri et al 2003; Ho et al 2006) as well as with better performance in executive function (Lu et al 2012). It has been shown that the valine allele increases depolarization-induced secretion of *BDNF* in brain (Egan et al 2003), thus giving a hint about the mechanism that might be behind this neuropsychiatric association. Population genetics studies have shown that there is positive selection for haplotypes containing the valine allele in the val66met locus (Petryshen et al 2009), thus giving one explanation as to why BD has not vanished in evolution. It has not vanished, because the same allele that predisposes to the disease also gives fitness benefit to the risk allele carrier by enhancing memory and executive functions.

The previous association of *P2RX7* non-synonymous variant rs2230912 and mood disorder was replicated here (Barden et al 2006; Lucae et al 2006; McQuillin et al 2008) (+study II) and this was an allelic replication. For the *P2RX7* gene, the studies carried out in the large cohort samples have also been negative (Green et al 2009; Grigoroiu-Serbanescu et al 2009). This could be due to the fact that *P2RX7* gene contains many functional variants, which potentially could dilute the association findings. This is also seen in our study, where both single variants and larger haplotype associated both with the disease and with potential endophenotypes, that is, executive functions. The associating variants span throughout the gene, the strongest haplotype association spans from the third to eighth intron and the replication variant is located in 14th exon.

The *P2RX7* results also demonstrate the value of using endophenotypes in genetic studies. It seems that the intermediate phenotype by which this gene affects disease susceptibility is different than in the case of *BDNF*. Here, the associating

endophenotype is executive function that has shown to be impaired in euthymic depressive patients (Paelecke-Habermann et al 2005), indicating that this gene might be specific to the depressive symptoms of BD. The depression specificity is further pointed out in study II, where the variants of this gene associate most strongly with familial MDD.

5.1.2 Candidate gene study of mood disorder cohort (II)

In study II, a shared genetic background of MDD and BD was sought in the sample comprising three clinical cohorts. Since the cohorts were also rich in data related to clinical course of mood disorders, the effect of the identified risk variants on the specific co morbidity or long-term clinical outcome was investigated. It was also hypothesized that the genetic loading of the risk alleles would be greater within the cases with a positive family history. The genes examined in II included *BDNF*, its receptor *NTRK2*, serotonin related genes *SLC6A4* and *TPH2*, *P2RX7*, *DAOA*, *COMT*, *DISC1*, and *MAOA*.

Altogether five out of nine studied genes revealed suggestive evidence of association with mood disorder. The strongest signal was gained with two nonsynonymous variants of *P2RX7*, rs208294/His155Tyr (His=C, Tyr=T) and rs2230912/Gln460Arg (Gln=A, Arg=G), in the whole sample (OR=1.26, p=0.003 and OR=1.29, p=0.017, respectively) and specifically in patients with familial mood disorder (OR=1.35, p=0.0013, permutated p=0.064, and OR=1.44, p=0.0031, permutated p=0.173, respectively) (Table 11). The association signals vanished when cases without familial loading were analyzed (p=0.37 and p=0.80, respectively). The risk alleles were T/Tyr in the rs208294 and G/Arg in the rs2230912. Variants rs3794808, VNTR2i, and 5HTTLPR within *SLC6A4* gene were suggestively associated with mood disorder in the whole sample (OR=0.82, p=0.01, OR=1.25, p=0.007, and OR=1.24, p=0.007, respectively) (Table 11). The associating alleles were 9R/10R in the VNTR2i variant and La in the 5HTTLPR variant. Nominal associations were also seen with *NTRK2*, *BDNF*, and *DAOA* variants.

Table 11. Summary of the best association signals (*P*-values) gained with variants within *P2RX7* and *SLC6A4* genes.

	Mood (All)	Mood (Familial)	MDD (All)	MDD (Familial)	Anxiety	Alcoholism	Time in illness
P2RX7							
rs591874	0.0103	0.0026	0.0053	0.0030	0.0008	0.0056	0.5007
rs208290	0.0706	0.0299	0.0507	0.0265	0.0182	0.1426	0.8006
rs208294	0.0033	0.0007	0.0070	0.0023	0.0008	0.0007	0.0683
rs2230912	0.0172	0.0007	0.0217	0.0019	0.0102	0.0016	0.0003
SLC6A4							
rs3794808	0.0115	0.1686	0.0046	0.1012	0.0315	0.0399	0.4453
intron2	0.0066	0.0079	0.0017	0.0056	0.0716	0.0514	0.0136
5HTTLPR	0.0072	0.1396	0.0006	0.0686	0.0659	0.0166	0.4439

Haplotype analyses did not bring any further information to the association findings of *P2RX7* gene. In *SLC6A4* gene, there was slightly stronger association signal with the haplotype spanning from the promoter region to second intron (5HTTLPR-rs4451417-rs6354-VNTR2i haplotype La-C-T-9R/10R; OR=1.31, $p=0.001$, frequency in cases = 0.39, in controls = 0.33).

When the clinical outcome was further analysed, it was noticed that *P2RX7* associated both with BD and MDD, whereas the *SLC6A4* association signal came from MDD cases. The *P2RX7* associated also with co morbid anxiety and alcoholism, psychotic features, and suicide attempts, with the most significant association signal gained with co morbid alcoholism. *P2RX7* risk alleles also associated strongly with more time ill ($p=0.0003$ with rs2230912). This association was also evident when the differences in follow-up periods were taking to account and the age, gender, and the cohort was adjusted (OR=1.71 with 95% CI 1.30-2.26, $p=0.0002$). The effect of the genotypes on the clinical outcome is visualised in Figure 5.

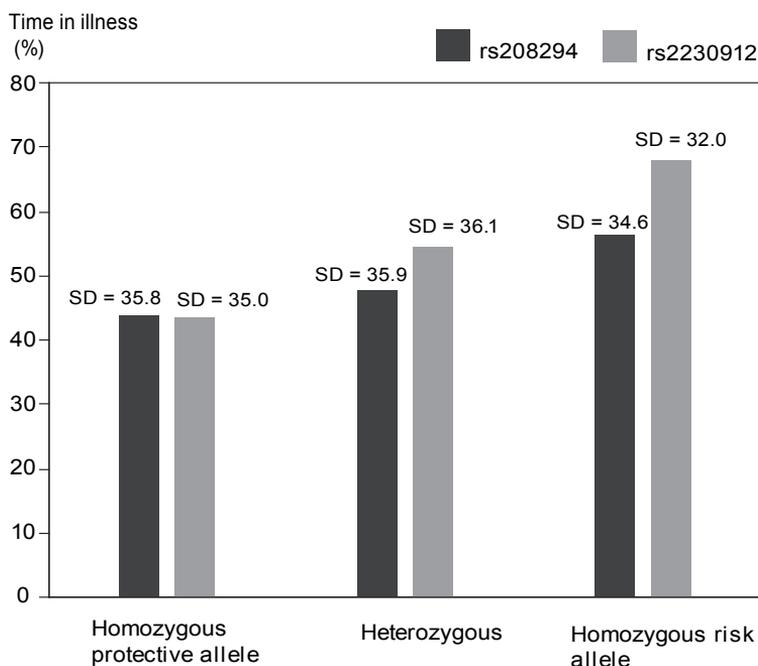


Figure 5. *The influence of P2RX7 genotypes on the clinical outcome relative to the time ill parameter. SD=Standard deviation.*

The most significant finding in this study was the plausible association of the *P2RX7* gene with mood disorder and the risk alleles effects on the clinical outcome, i.e. the risk alleles extend the time in illness within patients. There were two non-synonymous variants that associated in a statistically robust manner and the association was evident in all three cohorts included in this study. Another associating variant was also allelic replication of previous studies, where the rs2230912 / Gln460Arg have associated with BD (Barden et al 2006), MDD (Lucae et al 2006), and anxiety (Erhardt et al 2007). The analyses made in large populations have been negative (Green et al 2009; Grigoriu-Serbanescu et al 2009), but one reason for this discrepancy could be allelic heterogeneity, because there are many non-synonymous variants within this gene, both common and rare (Barden et al 2006; Fuller et al 2009), thus there are many potential causative variants confounding the results in heterogeneous populations. The heterogeneity was also seen in this study, since two variants associated independently, i.e. the variants were not in high LD. This study also implies that this gene has a strong effect, particularly on more serious forms of mood disorders with comorbidities and longer time in illness. In addition, the familiarity was an important factor in the association, since the association signal vanished when only the non-familial cases were included in

the analyses. These factors could also explain why the studies in large populations have been negative (Green et al 2009; Grigoriou-Serbanescu et al 2009), since the MDD cases in these large population studies have not been selected in any way, and in this study it seems that the association signal comes from a familial type of MDD and is most evident within cases that carry a serious form of the disease.

The *P2RX7* gene also associates with co morbidity disorders; most strongly with alcoholism. There is some previous evidence of shared genetic risk factors of alcohol dependence with MDD (Prescott et al 2000) and BD (Johnson et al 2009), and an unpublished finding of *P2RX7* association with alcoholism in Finland (Mantere, submitted 2012).

The exact role of the *P2RX7* gene in the brain remains uncertain and it seems that it might have several distinct functions that can all mediate the effect of this gene on the susceptibility to mood disorders. These functions include its role in inflammation reactions, neurogenesis, neuroplasticity, and neurotransmitter (e.g. glutamate) release (Sperlagh et al 2006). Interestingly, the other associating variant (rs208294/His155Tyr) is a functional variant altering the function of the receptor: the associating Tyr allele increases the receptor activity after ATP activation (Cabrini et al 2005). The same Tyr allele has shown to predispose to higher vulnerability to chronic pain (Sorge et al 2012), thus strengthening further the connection of this variant and depression, since chronic pain is strongly linked to major depressive disorder (Ohayon and Schatzberg 2010). Consequently, the connection between *P2RX7* and mood disorders seems to also be plausible biologically, but plenty of work is still required to explain the exact mechanism(s) behind this connection.

This study strongly implies that *P2RX7* gene is a very important factor in the aetiology of mood disorders, especially of MDD and its familial form. This gene seems to also be important clinically, since the risk alleles predispose to more severe disease form.

5.1.3 Candidate genes of schizophrenia in bipolar disorder (III)

There are some overlapping characteristics as well as common genetic susceptibility genes between BD and schizophrenia. Thus, some potential psychotic disorder candidate genes (*DAOA*, *COMT*, *DTNBPI*, *NRG1* and *AKT1*) were surveyed here in the bipolar family sample, and their association with both bipolar spectrum disorder and psychotic symptoms were assessed. In addition, an assessment was made as to

whether these genes associated with potential endophenotypes of psychiatric disorders, i.e. with neuropsychological traits.

No strong evidence of association between these candidate genes and disease status was shown here. The only nominally significant result from single SNP analyses in the whole study sample were gained with the functional variant of *COMT* rs4680 and BD ($P = 0.046$). When only the familial cases were included in the analyses there was one additional variant in *COMT* (rs165599) showing nominal association with BD ($P = 0.035$) and two variants in *DAOA* (rs701567 and rs778326) showing nominal evidence of association with psychotic disorder ($P = 0.031$ and 0.018 , respectively).

Analyses against neuropsychological test variables revealed robust evidence of association of non-synonymous variant rs2391191/Arg30Lys of *DAOA* with visuospatial ability ($P = 0.000005$), visualized in Figure 6. The Lys allele, previously shown to be a risk variant for schizophrenia (Muller et al 2011), associated with better performance in this cognitive test. The same variant associated also with other traits assessing general intellectual functions as well as several traits assessing attention, memory, learning and, executive performance. *COMT* variant rs165599 also showed evidence of association with several neuropsychological traits, most strongly with the same visuospatial ability as Arg30Lys of *DAOA* ($P = 0.0007$). There was no evidence of interaction of the effect of these two variants to the visuospatial ability, but an additive effect was seen.

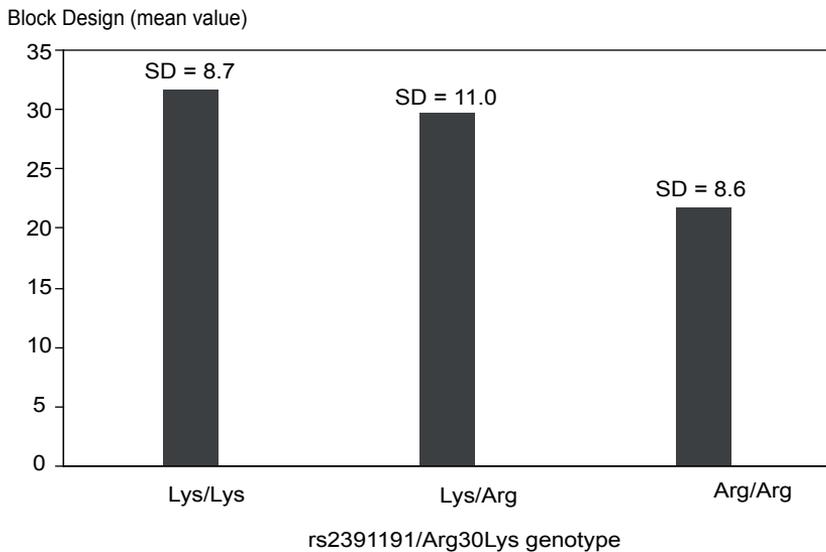


Figure 6. *Effect of the DAOA variant rs2391191/Arg30Lys genotype to the neuropsychiatric test results of Block design assessing visuospatial ability. SD = standard deviation.*

Although there was no strong evidence of association with the disease status, there was convincing evidence of association between the nonsynonymous variant of *DAOA* with general intellectual functioning, most robust with visuospatial ability. The *DAOA* gene is interesting, because it is a primate-specific gene encoding mitochondrial protein that promotes mitochondrial fragmentation and dendritic branching (Kvajo et al 2008) and it is also presumed to function in glutamate signalling (Chumakov et al 2002; Sacchi et al 2011). Mitochondrial dysfunction is hypothesized to be one of the factors affecting susceptibility to psychiatric disorders like BD, MDD, and schizophrenia (Fattal et al 2006; Rezin et al 2009).

DAOA is one of the most robust schizophrenia candidate genes (Muller et al 2011) and its expression is elevated in the schizophrenic brain (Korostishevsky et al 2004). Recent rodent data has also demonstrated that mice expressing human specific *DAOA* gene have several behavioural changes that relate to many psychiatric disorders, like motor-coordination deficits, increased compulsive behaviours, and increased sensitivity to phencyclidine, an antagonist of glutamate (NMDA) receptors (Otte et al 2009). The schizophrenia risk allele of the associating variant, associated here with better performance, has been shown to associate with the reduced

thickness of the cortex of schizophrenic patients (Schultz et al 2011). These and our findings related to *DAOA* effects on intellectual functioning suggest an evolutionary perspective on psychiatric disorders, that act as a gate-away to more plastic brain structure enabling evolution of more complex brains, but at the same time predisposing to psychiatric deficiencies.

This study further attests that the neuropsychiatric test results are useful endophenotypes for psychiatric disorders. Here, two highly potential psychotic disorder candidate genes, *DAOA* and *COMT*, associated strongly with block design assessing visuospatial reasoning within bipolar familial sample. This test defines one aspect of general intellectual functions. Visuospatial reasoning has been shown to be impaired in BD (Tiihonen et al 2005), as well as in healthy siblings of BD patients (Frantom et al 2008). The intelligence quotient has shown to be a high risk factor for schizophrenia and other psychotic disorders (David et al 1997). Thus according to these and our results, the endophenotypes could be partly common between BD, schizophrenia, and other psychotic disorders. There was no evidence here of interaction between *DAOA* and *COMT* that might lead to a cognitive phenotype, although there is a previous study showing interaction of these two genes affecting cognition (Nixon et al 2011).

5.2 Replication of GWAS findings (IV, V)

In studies IV and V, we wanted to survey if the new findings gained from the first three GWA studies (Baum et al 2008a; Sklar et al 2008; WTCCC 2007) show any evidence for association in the Finnish population either for BDI, mood disorder or their potential endophenotypes, that is, neurocognitive abilities or circadian phenotypes like circadian preference of daily activities and seasonal variation of mood and behaviour.

A genotype was performed for the 76 associating variants from the first three GWA studies, as well as from meta-analysis (Baum et al 2008b). Altogether 13 variants showed evidence of association with BDI or mood disorder ($P < 0.05$) (Table 12). The associating variants spanned five distinct genomic areas or genes, including 2p25.1 (nearest gene *LPIN1*), Sortilin-related VPS10 domain containing receptor 2 (*SORCS2*), Deafness, autosomal recessive 31 (*DFNB31*), 18q12.3 (nearest gene Cadherin 7, type 2 (*CDH7*)), and *SLC39A3*. All the associations were modest, P-values ranged from 0.044 to 0.001 (Table 12). Most of the variants that associated with the disease status, also associated with some of the potential endophenotypes, either with circadian phenotypes and/or neuropsychiatric traits. The most significant P-values were gained with *DFNB31* and mood disorder ($P=0.001$) and seasonality of

sleep duration ($P = 0.003$); with *SORCS2* and BDI ($P = 0.004$); and with *CDH7* and visual attention ($P = 0.006$). In *CDH7* gene, the risk allele of BDI associated with increased seasonal variations in social activity and mood ($P = 0.017$ and 0.028 , respectively) and increased visual alertness reflected by improved performance in visual attention ($P = 0.006$).

Table 12. The associations of GWA studies SNPs that replicated in Finnish bipolar family sample with P -value < 0.05 . The quantitative trait associations with P -value lower than 0.001 are shown.

Gene	SNP	BDI	MOOD	Seasonality (N=127)		Neuropsychological
		(N=214)	(N=218)	Sleep length	Weight	traits (N=159)
		P	P			Visual attention
LPIN1	rs4027132	0.042	0.071	0.305	0.053	0.045
SORCS2	rs4411993	0.006	0.014	0.207	0.438	0.741
SORCS2	rs7683874	0.013	0.009	0.046	-	0.179
SORCS2	rs10937823	0.004	0.005	0.046	-	0.168
DFNB31	rs10982256	0.010	0.001	0.005	0.008	0.753
CDH7	rs2850699	0.019	0.028	0.081	0.082	0.008
CDH7	rs2850700	0.012	0.021	0.044	0.09	0.010
CDH7	rs2850701	0.014	0.038	0.043	0.065	0.006
CDH7	rs2658046	0.021	0.078	0.091	0.096	0.009
CDH7	rs976882	0.025	0.039	0.1	0.098	0.006
CDH7	rs12970791	0.044	0.097	0.128	0.162	0.016
CDH7	rs1444067	0.018	0.023	0.073	0.067	0.006
SLC39A3	rs4806874	0.034	0.259	0.583	0.482	0.620

DFNB31 gene, that associated most strongly with broad mood disorder status is a gene that causes sensorineural hearing loss when mutated (Mustapha et al 2002). The gene product called whirlin is shown to be important in the elongation of stereocilia of sensory hair cells (Mburu et al 2003). Besides being expressed in the inner ear, it is also expressed in the developing brain and in the retina (van Wijk et al 2006). In addition, being a causal variant in nonsyndromic hearing loss, it is also responsible for Usher syndrome type II (Ebermann et al 2007) that is characterized by congenital hearing loss and onset of retinitis pigmentosa and thus loss of vision during or after puberty (Smith et al 1994). Thus, it is interesting that the gene associated also with seasonality (e.g. seasonal changes in sleep duration; $P = 0.005$), a phenotype that requires visual information.

SORCS2 gene is highly expressed both in the developing and the mature central nervous system in mice, localised to the dopaminergic nuclei among other places

(Rezgaoui et al 2001). The gene belongs to the Vps10p-D family of proteins that are important in the development of neuronal structures and thus take part in the plasticity of the brain and it is also hypothesized to function as an endocytic receptor and thus as a sorter of neurotransmitter and growth factor receptors (Hermeijer et al 2004). These functions and recent association findings make it interesting as a susceptibility gene for BD.

CDH7 belongs to the cadherin superfamily, which are transmembrane glycoproteins that regulate, among other things, brain development in several ways, including cell sorting, migration, and axon outgrowth (Luo et al 2004). It was discovered in 2000 and shown to be expressed in testis, prostate, and brain, also during development (Kools et al 2000). Chicken and rat homologues of this gene and its expression are widely studied, and it has shown to be expressed in neurons in the visual pathway and retina in chicken (Wohrn et al 1998), and in the developing eye and in the retina in mice (Faulkner-Jones et al 1999). The variants associating here are located in the genomic area 250 kb upstream of the *CDH7* gene. The variants associate with the diagnosis of BDI and with circadian clockwork, seasonal variations, visual attention, and visual working memory. All the endophenotypes that indicate association are processes that are related to neurons using visual information; in circadian and seasonal phenotypes providing information about light-dark transitions, and in WMS-R tests, the ability to manage and remember visual information. This is a second replicated gene of GWA studies that is related to vision and that associates in Finnish sample with mood disorders, thus it implies that the etiology of these disorders could be partly reflected by the ability to adapt to the light-dark transition in northern latitudes.

The strength of GWA data is that they provide new potential susceptibility genes that have not been found in previous linkage and candidate gene studies that are based on researchers own reasoning about genes' likelihood to function behind psychiatric phenotype. When doing GWA studies you need not have any advance hypothesis about the genes' function, instead you can find new surprising genes which were not known beforehand to have important function in brain. A wide variety of human genes are expressed in brain tissue as shown in microarray studies (Ramskold et al 2009; Velculescu et al 1999), although the regulation of expression has shown to be complex (Ramskold et al 2009). So, the GWA studies guide the way of basic brain function studies and this way might lead to new insight that may eventually also guide drug development.

6 CONCLUDING REMARKS AND FUTURE PROSPECTS

Despite the shift of genetic research to the era of large scale GWA studies and availability of the whole human genome sequence, the genetic background of MDD and BD remains largely unknown. Thus, hypothesis-based studies are still valid, in particular when performed in homogenous populations with detailed phenotypic information available for further dissection of the heterogeneous and complex phenotypes to those with genetically simpler architecture. This study implies that some of the classical candidate genes of mood disorders, including variants from *BDNF*, *P2RX7*, and *SLC6A4* have an effect on the disease susceptibility in Finland (Study I and II), and that some psychotic related candidate genes (*DAOA* and *COMT*) have an effect on general intellectual functions that are potential endophenotypes of psychiatric disorders (Study III). It also showed that some of the new GWA study findings for BD associate with BD also in the Finnish population (Study IV and V).

All results that were gained in this thesis were conducted with relatively small samples and thus they should be replicated in bigger samples to ensure that these genes have a role behind mood disorders in Finland. Furthermore, most of the P-values achieved were only nominal when taking into account the number of tests done. We also cannot rule out the role of the genes having negative association results in Finnish mood disorder, since the SNP coverage in large genes such as *NTRK2* were too sparse. In addition, the finding that *DAOA* associates with neurocognitive function should be replicated in a population based sample to establish if there is association in a normal population, or is the association related to the disease phenotype.

This study implies that at least some of the genetic background of MDD and BD might be shared, as the *P2RX7* association was evident in both MDD and BD (Study I and II). Hence, although recurrent MDD and BD are clinically defined as separate illnesses, at least part of their underlying genetic and biological factors are shared as has been also shown in studies on twins and high risk families. This also fits well with the clinical observations on their similar co-morbidity disorders, such as alcoholism, and the shared fluctuation of mood, which is one marker of familial type MDD (Kendler et al 2007; Levinson et al 2003).

This study also showed that familial background of cases has an effect on the findings of the genetic studies. This is plausible since it is likely that the familial

subtype of MDD is more homogenous than sporadic cases of MDD. Consequently, the lack of success of the large GWA studies on MDD might result from selection of cases without taking into account their familial background. Accordingly, in our mood disorder cohort study (II), the association signal of *P2RX7* vanished completely when analyses were performed using cases with no familial loading. The importance of selection of cases was seen also in linkage studies of MDD in a Utah pedigree sample (Abkevich et al 2003; Camp et al 2005), where findings from linkage studies in cases with both r-MDD and single episode MDD, as well as BD cases (Abkevich et al 2003), were completely different from those in cases with early onset r-MDD as well as anxiety disorder cases (Camp et al 2005).

According to the latest GWA studies, it seems that the CDCV theory (common diseases explained by common variants) does not fit to psychiatric disorders as well as originally anticipated, and the issue of “missing heritability” has been a question of interest in the latest reviews (Gershon et al 2011). There are many possible explanations for this discrepancy. One is that rare variants play a more important role in the aetiology of mood disorders than previously thought and these variants might be distinct in different populations and families. That might also explain the huge variety and expansion of linkage peaks throughout the genome in the traditional linkage studies. Another possibility is that the heterogeneity of the genetic component contributes to the aetiology of mood disorders, even within one predisposing gene, which seemed to be the case also for *P2RX7* (Study II). While all of these can be true, the most plausible explanation is that the phenotypes of MDD and BD are too broad, which leads to the confounding results seen in genetic studies, since the different aspects of broad mood disorder phenotypes are affected by distinct genetic susceptibility genes. Thus, analysis of huge sets of samples with thousands of genotyped individuals in future GWA studies might not necessarily lead to the discovery of the missing heritability, but instead to discovery of new low risk alleles. They will definitely reveal important etiological factors for the disease and might help to develop new, more efficient drugs, but they will not tell the whole story of genetics behind mood disorders.

The use of endophenotypes and information on co-morbidity seem to be highly effective in genetic studies of mood disorders. Co-morbid conditions homogenise the case population, and thus enables the search for susceptibility variants also in smaller study samples. GWA data have shown that in order to find genome-wide significant associations for BD (and MDD) thousands of cases are needed in the study population (Chen et al 2011; Sklar et al 2011), but if additional information on the selection of case sample, such as co-morbidity (Kerner et al 2011) or temperament (Greenwood et al 2012) are used, genome-wide significant results might also be obtained with smaller sample sizes (Greenwood et al 2012; Kerner et

al 2011). Thus the complications of heterogeneous phenotype could be overcome by thorough phenotyping of the individuals.

A considerably interesting finding of this study was the evidence that the risk alleles of psychiatric disorders seems to improve the performance in neuropsychiatric tests, such as in tests assessing general intellectual functions and visual attention and memory. This was seen with *BDNF*, *DAOA*, and *CDH7* genes (Studies I, III, and V). It implies that the reasons why psychiatric disorders persist in human populations are partly due to the benefits that the risk alleles give to the carriers by enhancing their cognitive abilities. Thus, it seems that the same neurobiological alterations (e.g. enhanced neuronal plasticity) that improve the risk allele carriers' cognitive functions, predispose the more vulnerable individuals for psychiatric disorders.

This study further confirms the fact that the genetic background of mood disorder is highly polygenic. Even in the quite homogenous Finnish population, the allelic heterogeneity was evident, for example in *P2RX7* association results. This study also illustrates that it is beneficial to use homogenous populations, like the Finnish population, in genetic studies, because it enables the realization of statistical significant P-values with smaller sample size. The downside is that at the same time you could only find certain genetic susceptibility genes that are enriched in that specific population.

One future approach that could lead to the discovery of the hidden causative variants of mood disorder is genome-wide sequencing. This laborious approach is now available and will eventually reveal all the genetic variance between humans, including rare SNPs and CNVs. These future studies might reveal new predisposing variants, also for mood disorders.

The functions of the genes that showed association in this study are still poorly understood. Thus, it would be highly interesting to study the functions of *DAOA*, *P2RX7*, and *CDH7* in brain in more detail. In addition, the effects of associated functional variants of *DAOA* and *P2RX7* would be interesting to survey in cell and animal models to eventually discover how their effects are passed to mood and cognitive functions. Overall, functional studies are crucial in the future, since the new mapping methods will reveal new susceptibility genes whose function in brain will be unknown. Collectively, before any new drug development based on these findings can be made, the exact role of these new candidates in the brain needs to be revealed.

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