Susceptibility genes for bipolar disorder, with focus on pleiotropy and amygdala activity

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2 LIST OF STUDIES

Study I

Tesli M, Kähler AK, Andreassen BK, Werge T, Mors O, Mellerup E, Koefoed P, Melle I, Morken G, Wirgenes KV, Andreassen OA, Djurovic S. No association between *DGKH* and bipolar disorder in a Scandinavian case-control sample. *Psychiatr Genet. 2009 Oct;19(5):269-72.*

Study II

Tesli M, Athanasiu L, Mattingsdal M, Kähler AK, Gustafsson O, Andreassen BK, Werge T, Hansen T, Mors O, Mellerup E, Koefoed P, Jönsson EG, Agartz I, Melle I, Morken G, Djurovic S Andreassen OA. Association analysis of *PALB2* and *BRCA2* in bipolar disorder and schizophrenia in a Scandinavian case-control sample. *Am J Med Genet B Neuropsychiatr Genet. 2010 Oct 5;153B(7):1276-82.*

Study III

Tesli M, Koefoed P, Athanasiu L, Mattingsdal M, Gustafsson O, Agartz I, Rimol LM, Brown AA, Wirgenes KV, Smorr LL, Kähler AK, Werge T, Mors O, Mellerup E, Jönsson EG, Melle I, Morken G, Djurovic S, Andreassen OA. Association analysis of *ANK3* gene variants in Nordic bipolar disorder and schizophrenia case-control samples. *Am J Med Genet B Neuropsychiatr Genet. 2011 Dec;156B(8):969-74.*

Study IV

Tesli M, Skatun KC, Ousdal OT, Brown AA, O, Thoresen C, Agartz I, Melle I, Djurovic S, Jensen J, Andreassen OA. *CACNA1C* risk variant and amygdala activity in bipolar disorder, schizophrenia and healthy controls.
Submitted to Bipolar Disorders.

2 ABBREVIATIONS

ANK3	ankyrin 3, node of Ranvier (ankyrin G)
BRAIN	Bipolar Research And Innovation Network, Norway
BRCA2	breast cancer 2, early onset
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit
CIDI	Composite International Diagnostic Interview
CNV	Copy number variation
DGKH	diacylglycerol kinase, eta
DNA	Deoxyribonucleic acid
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision
FWE	Family-Wise Error
GAF	Global Assessment of Functioning Scale – Split version
GWAS	Genome Wide Association Study
HWE	Hardy-Weinberg equilibrium
ICD-10	International classification of diseases, 10th revision
IDS	Inventory of Depressive Symptoms
LD	Linkage disequilibrium
MAF	Minor allele frequency
MRI	Magnetic resonance imaging
OPCRIT	Operational Criteria Checklist for Psychotic Illness and Affective Illness
OR	Odds ratio
PALB2	partner and localizer of BRCA2
PANSS	Positive and Negative Syndrome Scale

PGC	Psychiatric GWAS Consortium
RDC	Research Diagnostic Criteria
ROI	Region of interest
SADS-L	Schedule for Affective Disorders and Schizophrenia Lifetime Version
SCAN	Schedules for Clinical Assessment in Neuropsychiatry
SCID-1	Structured Clinical Interview for DSM-IV Axis I Disorders
SCOPE	Scandinavian Collaboration on Psychiatric Etiology
SNP	Single nucleotide polymorphism
SPM	Statistical Parametric Mapping
TOP	Thematically Organized Psychosis
WASI	Wechsler Abbreviated Scale of Intelligence
YMRS	Young Mania Rating Scale

3 ABSTRACT

Bipolar disorder (BD) is a common and highly heritable disorder, but few susceptibility genes have been identified and the underlying biological mechanisms remain poorly understood. Epidemiological and molecular genetic studies have provided evidence for genetic overlap between BD and other psychiatric disorders, including schizophrenia (SZ). However, we lack knowledge on which genetic variants and pathophysiological processes are involved in this overlap, and which are confined to one diagnostic category or mechanism. Recent large genome-wide association (GWA) studies have detected several novel candidate risk variants for BD. In order to confirm these findings, they should be replicated in independent samples, and their diagnostic specificity as well as neurobiological effects must be determined.

Among these recently identified genetic variants are single nucleotide polymorphisms (SNPs) in the genes *DGKH*, *PALB2*, *ANK3* and *CACNA1C*. We genotyped 37 *DGKH* SNPs, one *PALB2* SNP, three *ANK3* SNPs and one *CACNA1C* SNP in Nordic BD and SZ case-control samples. SZ cases were included for the purpose of testing for genetic overlap, and ten SNPs in the gene *BRCA2* were also genotyped, as this gene is functionally related to *PALB2*. To test the hypothesis of increased amygdala activity as a potential genetically conditioned underlying mechanism for BD, we measured amygdala activity in a subsample of Norwegian individuals genotyped for a *CACNA1C* risk variant with a functional magnetic resonance imaging (fMRI) negative faces paradigm.

We confirmed the association between the previously identified *PALB2* SNP and BD in a meta-analysis, including our Nordic samples and international replication samples, and identified one new candidate risk SNP for BD in *BRCA2*. There was no significant association between these SNPs and SZ.

We also replicated the association between two *ANK3* SNPs and BD, but found no evidence for genetic overlap with SZ.

There was no significant association between the *DGKH* SNPs, including one previously identified variant, and BD in our Danish and Norwegian samples.

Carriers of the *CACNA1C* risk allele were found to have increased activity in the left amygdala, with indications of a more pronounced effect in BD cases than in SZ cases and healthy controls.

Taken together, these findings further support that *PALB2* and *ANK3* are BD risk genes, and indicate that *BRCA2* might be of interest for further investigation. As both *PALB2* and *BRCA2* are involved in DNA repair, this mechanism could potentially be related to the development of BD. Although we found no evidence for *DGKH*, further studies are needed to finally determine the role of *DGKH* in BD susceptibility. Our findings also support the hypothesis that increased amygdala activity is a mechanism underpinning the clinical phenotype of BD, and that this mechanism might be conditioned by the *CACNA1C* risk variant. Our findings also implicate ion channelopathy as a putative pathophysiological process in BD, taken into consideration that both *ANK3* and *CACNA1C* encode for proteins related to ion channel functioning. Furthermore, the current findings indicate a partial genetic overlap between BD and SZ, as some of the variants investigated in this study were found to be more specific for BD than SZ, while other were associated with the same biological mechanism, although more prominent in BD than SZ.

4 INTRODUCTION

4.1 Bipolar disorder

4.1.1 History of bipolar disorder

Although states of depression and exaltation were described already in the pre-Hippocratic era, Hippocrates (460–337 BC) was probably the first to give a systematic description of mania and melancholia. The hypothesis that these two conditions were manifestations of the same disease was first proposed by Aretaeus of Cappadocia, a Greek physician of the 1st century AD (Angst and Marneros, 2001). Aretaeus also suggested that mania and melancholia had the same etiology, namely a disturbance of the function of the brain and some other organs. The hypothesis of mania and depression being part of the same disease was re-introduced in 19th century France with the publications of Falret (1851) and Baillarger (1854), describing, respectively, the clinical pictures of "folie circulaire" and "folie a` double forme" (Haustgen and Akiskal, 2006). In 1899, the German psychiatrist Emil Kraepelin unified all types of affective disorders into "manic-depressive insanity", which he considered to be a separate diagnostic entity from "dementia praecox" (Angst and Marneros, 2001), the precursor to the current concept of schizophrenia. "Manic-depressive insanity" became split anew by the Wernicke-Kleist-Leonhard school into "unipolar disorder" and "bipolar disorder" (Kleist, 1953). Further evidence for the distinction between unipolar and bipolar disorders was given in the 1960s with publications from Jules Angst, Carlo Perris, and George Winokur, who independently showed that there exist clinical, familial and course characteristics validating this distinction (Angst and Marneros, 2001). In 1976, Dunner and co-workers distinguished depressions with hypomania (bipolar II) from those with mania (bipolar I) (Dunner et al., 1976). The currently most commonly used descriptions of bipolar

disorder for clinical and scientific purposes are those given in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) (First and Tasman, 2004), including bipolar I disorder, bipolar II disorder, cyclothymic disorder and bipolar disorder not otherwise specified (NOS), and in the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10), wherein the diagnostic category bipolar affective disorder is included (http://www.who.int/classifications/icd/en).

However, new and broader diagnostic systems, often termed 'bipolar spectrum disorders' are being discussed. One model suggests several distinct entities, e.g. bipolar disorder type III (hypomania in association with antidepressant treatments) and IV (depressions superimposed on hyperthymic temperament) in addition to I and II (Akiskal, 2007), whereas other researchers propose a multi-factor continuum model (Phelps et al., 2008).

Common for the above-mentioned models of bipolar disorder, is that the diagnostic categories are based on clinical symptomatology, and not on underlying etiological factors. Therefore, new neurobiological and genetic findings in bipolar disorder and other psychiatric illnesses could provide the basis for a new diagnostic system. This new model could move away from the current clinical entities and towards dimensions/categories based on knowledge on genetic risk factors and the underlying structures and functions of the brain (Craddock and Owen, 2010).

4.1.2 Clinical picture, course, outcome and treatment

According to the DSM-IV, bipolar I disorder (BDI) is characterized by the presence of at least one manic or mixed episode, whereas the criteria for bipolar II disorder (BDII) is at least one major depressive episode and one episode of hypomania. Patients with cyclothymic disorder suffer from recurrent episodes of hypomania and dysthymia, although only one episode of

hypomania is required to fulfill the diagnostic criteria of cyclothymia. Psychotic symptoms may be present in both mania and major depression, but these symptoms must be related to and co-occur with the manic or depressive episode.

Mania is defined as "a distinct period of abnormally and persistently elevated, expansive, or irritable mood", in addition to a list of related symptoms. Hypomania is less severe than mania, and can be distinguished from the latter by the absence of marked impairment in occupational functioning, usual social activities, relationships with others, the necessity for hospitalization, as well as psychotic features. Furthermore, a manic episode must last for at least 7 days, whereas a time-period of 4 days is sufficient for an episode to be regarded as hypomania. A major depressive episode is defined as a discrete episode of persistent and pervasive emotional depression with a set of related symptoms, lasting for a period of at least 2 weeks (First and Tasman, 2004).

Lifetime (and 12-month) prevalences have been estimated to be 1.0% (0.6%) for BDI, 1.1% (0.8%) for BDII, and 2.4% (1.4%) for subthreshold BD in the USA (N = 9282) (Merikangas et al., 2007). In an international study comprising 61392 adults in 11 countries, the prevalences were slightly lower. The lifetime (and 12-month) prevalences were found to be 0.6% (0.4%) for BDI, 0.4% (0.3 %) for BDII, 1.4% (0.8 %) for subthreshold BD, and 2.4% (1.5 %) when added together. Furthermore, three-quarters of patients with the above-mentioned diagnoses had at least 1 other disorder, of which anxiety disorders were the most common conditions (Merikangas et al., 2011). The authors of this study also reported that the severity, impact, and patterns of comorbidity were remarkably similar in different countries across the globe, despite some variation in prevalence rates.

Most recent studies have found age at onset of BD to be in adolescence and young adulthood (Leboyer et al., 2005;Perlis et al., 2004). In a study comprising 2839 patients with BD, the median age at onset was 17.5 years and the average age at onset was 19.8 years

(Kupfer et al., 2002). A study of Norwegian in-patients diagnosed with BD (N = 146) found the mean age at first affective episode to be 20.2 years (Morken et al., 2009), while a study of Norwegian out-patients with BD (N = 225) reported the average age at onset to be 22.8 years (Larsson et al., 2010). Early age of onset in BD has been found to be associated with long delay to first treatment, more episodes, comorbidities and rapid cycling, as well more severe mania, depression, and fewer days well (Leverich et al., 2007).

With regards to clinical course and outcome, the McLean/Harvard cohort I (N = 75) found that 28 % of the BD patients were episode-free and 28 % had experienced >3 relapses after four years follow-up (Tohen et al., 1990). As for the functional outcome in this study, 19 % of the patients in this study were unable to live independently and 28 % were unable to study or work after four years (Tohen et al, 1990). Another prospective cohort study (N = 186) found that 82 % of BD patients experienced relapse 7 years after recovery. The corresponding number for those whose index episode had been followed by at least 3 years without symptoms was 69.9 %. For those who were treated with lithium prophylaxis, 70% experienced relapse within 5 years of recovery (Coryell et al., 1995). Among the reported poor prognostic factors for BD are substance abuse (Nolen et al., 2004;Weiss, 2004), family history of substance abuse (Nolen et al., 2004), rapid cycling (Dittmann et al., 2002;Nolen et al., 2004), poor occupational functioning at study entry (Nolen et al., 2004), and past comorbid attention-deficit disorder (Nierenberg et al., 2005).

Patients with BD have been found to have an increased mortality ratio compared to the general population. Suicide and cardiovascular disease are two of the most important factors. A Danish study assessing all patients admitted to a psychiatric hospital from 1973 to 1993 (N = 54103) found standard mortality ratio (SMR) to be significantly increased compared to the general population (1.8 for women and 2.2 for men). For the BD patients included in this study, SMR for suicide was 20.3 for women and for 18.1 men (Hoyer et al., 2000). A

Norwegian study found the prevalence of cardiovascular risk factors in patients suffering from BD to be approximately twice that of the general population, and not significantly different from the prevalence in SZ (Birkenaes et al., 2007).

Cognitive dysfunction in patients with BD is well documented, and there is evidence for cognitive impairment also in the euthymic phase (Robinson et al., 2006). One recent study found the severity of neurocognitive deficits in patients suffering from BD and SZ to be dependent on history of psychosis rather than diagnostic category and subtype (Simonsen et al., 2011). The same research group found patients with BD I to perform significantly poorer on certain neurocognitive measures than those suffering from BD II (Simonsen et al., 2008).

The pharmacological treatment of BD usually comprises Lithium, anticonvulsants, typical and atypical antipsychotics, antidepressants and benzodiazepines. Treatment recommendations are can be divided into three categories based on phases of the illness, i.e. the acute, continuation and maintenance phase. The acute phase can be further divided into those predominated by mania/hypomania and depression. Randomized clinical trials (RCTs) with sufficient power (> 0.8) to detect statistically significant differences ($P < 0.05$) (category A evidence) have found that 8 dopamine-blocking agents (Olanzapine, Ziprasidone, Quetiapine, Risperidone, Haloperidol, Aripiprazole Paliperidone and Asenapine) and 3 non-dopamine-blocking agents (Lithium, Valproate and Carbamazepine) have an effect on mania. For depression, Lamotrigine, Olanzapine and Quetiapine monotherapy meet the same empirical criteria as listed above, as well as combination therapy with Olanzapine and Fluoxetine (Sachs et al., 2011). With regards to the maintenance phase, Lithium, Valproate, Olanzapine, Ziprasidone, Quetiapine, Risperidone, Aripiprazole, Lamotrigine were found to prevent recurrence of acute episodes significantly (Sachs et al., 2011).

Among the non-pharmacological treatments, Electro-convulsive therapy (ECT) has a documented effect on depressive as well as manic episodes in patients with BD (Taylor,

2007). Several kinds of psychotherapy have been investigated in intervention studies in BD, including psychoeducation, cognitive-behavioural, interpersonal and social rhythm and psychoanalytic therapy (Jones, 2004). Individual cognitive-behavioural therapy (CBT) has been shown to reduce symptoms and risk of relapse and improve social functioning (Jones, 2004), whilst pscychoeducation has been associated with increased knowledge of the disorder, improvement of adherence and reduced risk of relapse (Rouget and Aubry, 2007). However, the goal of these forms of treatment is to teach the patient how to live with the disorder rather than to gain insight. Moreover, these approaches are designed to be used as adjuvant therapy to medication (da Costa et al., 2010;Rouget and Aubry, 2007).

4.1.3 Etiology and pathophysiology

The hypothesis that certain psychiatric illnesses are inherited brain disorders was already proposed by the Swiss physician Paracelsus in 1520, and the 'hereditary taint' of manic-depressive illness was estimated to be about 80 % by the German psychiatrist Emil Kraepelin in 1899 (Goodwin and Jamison, 2007). These hypotheses have to a large degree been confirmed by more recent studies. Family, twin and adoption studies from the 20th century have provided evidence that BD is a highly heritable disorder (Smoller and Finn, 2003). While the studies conducted in the first half of this century often included both unipolar and bipolar cases, studies undertaken after 1960 have investigated the familial clustering and heritability of BD specifically. Whereas the family studies have shown that BD has a tendency to run in families, the twin studies suggest that this phenomenon is due to genetic rather than environmental factors (Smoller and Finn, 2003). In a meta-analysis of family studies comprising more than six thousand BD relatives, the weighted summary morbid risk estimate for probands was 8.7 (Smoller and Finn, 2003). This estimate is in line with the results from a recent Swedish epidemiological study of more than nine million individuals,

including over thirty-five thousand BD patients and forty thousand SZ patients, which reported that first-degree relatives of probands with BD had a relative risk of 7.9 for BD. Interestingly, these probands also had significantly increased relative risk for SZ, and vice versa (Lichtenstein et al., 2009).

Twin studies comparing concordance rates for monozygotic (MZ) and dizygotic (DZ) twin pairs have made it possible to assess the heritability of BD more accurately than family studies, as MZ twin pairs share 100 % of their genetic variation, whilst DZ twin pairs share 50 %. Thus, assuming shared environment for MZ and DZ twin pairs, any differences in concordance rates for a given disease between MZ and DZ twin pairs, is likely to result from more genetic similarity of the MZ than DZ twin pairs. There are several ways to estimate heritability based on twin studies. One of the simplest methods is 'Holzinger's heritability', which calculates the heritability with this formula: (Concordance rate in MZ twins – concordance rate in DZ twins) divided by (100 – concordance rate in DZ twins) (Goodwin and Jamison, 2007). Based on several twin studies, the heritability for BD has been estimated to be in the range of 60–85%, and the same studies provide little evidence that shared environment plays a large role (Barnett and Smoller, 2009). However, the concordance rates for MZ twins has never been found to be 100 %, which means that environmental and/or epigenetic factors probably increases the risk for developing BD in individuals with high risk genetic variants.

Adoption studies might distinguish environmental from genetic factors when comparing the rates of BD in biological and adoptive family members. Unfortunately, there are few such studies for BD (Smoller and Finn, 2003). One adoption study (N = 299 parents) found the frequency of affective illness (comprising bipolar, unipolar, schizoaffective, and cyclothymic disorders) to be significantly higher in the biological parents (32%) than in the adoptive parents (12%) of BD cases (Mendlewicz and Rainer, 1977), a finding which adds to

the evidence gained from family and twin studies that genetic factors are important in the development of BD.

Despite the high heritability estimates for BD, few susceptibility genes have been unambiguously identified and the neurobiological mechanisms remain poorly understood (Nothen et al., 2010). Nevertheless, there are interesting findings on different neurobiological and clinical levels in BD, which we will discuss briefly in the following section.

Post mortem studies have shown structural abnormalities in the brains of BD patients. There is evidence of decreased neuron and glia density and decreased size of neurons in frontal and subcortical areas. It has been hypothesized that this may result from increased apoptosis and oxidative stress in BD (Gigante et al., 2011), but mitochondrial dysfunction (Kato, 2008), excitotoxicity and neuroinflammation (Rao et al., 2010) and genetically influenced abnormalities in synaptic and neuronal plasticity (Schloesser et al., 2008) are also suggested as putative underlying mechanisms.

Several imaging studies have reported structural and functional brain abnormalities in BD patients. A recent large structural magnetic resonance imaging (sMRI) study (N = 139 BD cases, 173 SZ spectrum cases and 207 healthy controls) found no cortical thinning in subjects with BD compared with healthy controls, but a subgroup of patients with BDI were found to have cortical thinning in the frontal lobes, superior temporal and temporo-parietal regions. Furthermore, subcortical volume reductions for BD subjects were reported bilaterally in the hippocampus, in the left cerebellar cortex, the left thalamus, the right nucleus accumbens and the brainstem. Additionally, the authors found substantial ventricular enlargements (Rimol et al., 2010). A recent meta-analysis of functional magnetic resonance imaging (fMRI) studies reported over-activation of amygdala, thalamus and striatum, as well as less consistent evidence for increased activation in the dorsolateral and ventrolateral cortex (Cerullo et al., 2009). Another MRI finding in the brain possibly related to pathophysiological processes in

BD is hyperintensities. In a meta-analysis of 27 studies, hyperintensities were significantly more prevalent in BD subjects than healthy controls, with the most pronounced findings in adolescents and children. These hyperintensities were most often localized in the deep white matter and subcortical grey matter. However, there was no significant difference between hyperintensities observed in BD, and those found in unipolar depression and SZ. And the role of these hyperintensities in BD remains elusive (Beyer et al., 2009). Furthermore, medication status has been shown to influence the structure and function of certain brain regions. For example, the use of lithium has been associated with volumetric increase in the medial temporal lobe and anterior cingulate gyrus (Emsell and McDonald, 2009) and with increased total grey-matter volume (Moore et al., 2000).

An animal study found the administration of lithium to be associated with increased Neuropeptide Y in the hippocampus and striatum and decreased corticotropine-releasing hormone in maternally deprived rats (Husum and Mathe, 2002), a finding which might be interpreted as an indication that lithium counteracts the physiological effects on a state similar to clinical depression.

Among other noteworthy findings from animal studies on psychopharmacological drugs, are the blocking effect of fluoxetine, an SSRI (selective serotonin reuptake inhibitor), on the downregulation of cell proliferation in mice resulting from inescapable shock in a learned helplessness model of depression (Malberg and Duman, 2003). Lithium, carbamazepine and valproic acid have been found to inhibit the collapse of sensory neuron growth and increase growth area, possibly by inositol depletion (Williams et al., 2002). Moreover, the protein p11, which interacts with the serotonin 1B receptor 5-hydroxytryptamine (5-HT_{1B}) receptor, is reported to be increased in rodent brains with the use of ECT and antidepressants, and to be decreased in an animal model of depression and in brain tissue from depressed patients. p11 knockout mice exhibit a depression-like phenotype

and have reduced responsiveness to 5-HT_{1B} receptor agonists and reduced behavioral reactions to an antidepressant (Svenningsson et al., 2006).

As neurotransmittors are involved in the pharmacological treatment of BD, it has been hypothesized that dysfunctions in neurotransmission may be part of the neurobiological mechanisms. A particular focus has been put on glutamatergic abnormalities. A review article reported findings from magnetic resonance spectroscopy (MRS) studies on glutamate and glutamine. The authors found a consistent pattern of reduced level of mixture signal from glutamate and glutamine (Glx) in MDD and elevations of Glx in BD. Additionally, there was evidence of a reduced glutamine/glutamate ratio in depression and an elevated glutamine/glutamate ratio in mania. This might, according to the authors, result from reduced glutamate conversion to glutamine by glial cells (Yuksel and Ongur, 2010).

Glutamate abnormalities have also been hypothesized to play a role in certain types of epilepsy (Eid et al., 2008). Further evidence supporting common pathophysiological mechanisms in BD and epilepsy include comorbidity between epilepsy and mood disorders, the episodic pattern of both diseases, and the fact that the kindling phenomenon and modifications in neurotransmitters, voltage-gated ion channels and second-messenger systems have been reported for both disorders (Mula, 2010).

The episodic outburst of manic/hypomanic and depressive episodes in BD have led to research on possible trigger mechanisms. There is some evidence that abnormal catecholamine levels, up-regulation of neurotrophic and neuroplastic factors, hypothalamic-pituitary-adrenal axis (HPA) hyperactivity, circadian rhythms and tricyclic antidepressants might trigger mood switches in BD (Salvadore et al., 2010).

As for dopamine and serotonin synaptic transmission, in vivo imaging of synaptic function in BD patients have yielded inconsistent results (Nikolaus et al., 2009).

Abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis have been studied in mood disorders, with consistent evidence for elevated cortisol and corticotrophin releasing hormone (CRH) levels and hypofunction of the glucocorticoid receptor (GR) in depression (Zunszain et al., 2011). However, there is more inconsistency in the corresponding features in BD, although increased response to dexamethasone-suppression test in BD cases compared to healthy controls has been reported (Watson et al., 2004). Furthermore, it is unknown whether these observed HPA abnormalities represent an underlying factor involved in the pathophysiology of the disorder or whether it is a result of disease-related processes.

As for the potential environmental risk factors, a kindling hypothesis has been proposed, in which stressful life events trigger initial episodes, with successive episodes becoming less dependent of stressors and may occur autonomously (Post, 1992). Childhood trauma has been suggested as a possible environmental risk factor in BD. In a cohort study comprising 100 patients with BD, histories of severe childhood abuse were identified in 51%, a finding which indicates that childhood abuse might increase the risk of developing BD in individuals with genetic susceptibility for this disorder (Goldberg and Garno, 2005).

4.2 Human genetics

The DNA double-helix structure was discovered in 1953, by Francis Crick and James D. Watson, and in 1957 Crick presented the central dogma of molecular biology, describing the relationship between DNA, RNA, and proteins. In February 2001, the publicly funded International Human Genome Sequencing Consortium (IHGSC) (Lander et al., 2001) and the private company Celera Genomics (Venter et al., 2001) each reported the first draft sequences of the human genome. Two years later a more comprehensive version was made available by the IHGSC, containing 2.85 billion nucleotides and covering ~99% of the euchromatic genome (International Human Genome Sequencing Consortium, 2004). On the basis of these

investigations, it has been estimated that the human genome encodes 20,000-25,000 protein-coding genes and that the total length of the euchromatic genome covered by coding exons is ~1.2% (International Human Genome Sequencing Consortium, 2004).

Human to human genetic variation is estimated to be only about 0.2%, in other words, human genetic similarity is ~99.8 %. Of these 0.2 % differences, approximately 0.12 % have been found to be structural variants and 0.08 % single nucleotide variants (Sebat, 2007). Structural variants can be further classified as inversions, insertion-deletions and Copy Number Variants (CNVs), whereas single nucleotide variants with a minor allele frequency (MAF) above 1% are referred to as single nucleotide polymorphisms (SNPs). The total number of SNPs in the human genome has been estimated to be about 11 millions (Kruglyak and Nickerson, 2001).

Newly developed methods, like the DNA microarray (DNA chip) technology, have enabled the investigation of up to 1 million genetic variants at a steadily lower consumption of time and financial expenses. When testing for association between a certain SNP and a disease, one can either use a direct or indirect approach. A direct approach hypothesizes that the actual SNP is the disease-causing variant, for example by altering the protein structure or the expression of the protein, whereas an indirect approach aims at maximizing the actual SNPs` ability to collect information form surrounding SNPs, of which one or more may be disease-causing.

A SNP with a high ability to capture information from other SNPs is called a 'tagSNP'. In order to gain information on other SNPs, the actual SNP and the other SNP must be in strong Linkage Disequilibrium (LD). LD denotes the phenomenon of two or more alleles at different loci having a stronger association than what we could expect to occur if they were randomly combined. A high LD usually means that the two SNPs are localized within the same haplotype block (the SNPs are inherited together, no recombination has

occurred between them). The degree of LD is expressed by either D' ($0 \leq D' \leq 1$) or by r^2 ($0 \leq r^2 \leq 1$). If $D'/r^2 = 1$, the two SNPs are considered to be in complete/perfect LD, while if $D'/r^2 = 0$, the two loci are totally independent of each other. On the basis of LD, tagSNPs can be used as proxies for other SNPs that are not genotyped. The International HapMap Project is an available database for assessing LD between SNPs and picking tagSNPs (www.hapmap.org). The DNA samples for the HapMap come from 270 individuals from four populations: the Yoruba people in Ibadan, Nigeria (30 trios), Japanese in Tokyo (45 unrelated individuals), Han Chinese in Beijing (45 unrelated individuals), and from U.S. (residents with ancestry from Northern and Western Europe, collected by the Centre d'Etude du Polymorphisme Humain (CEPH)) (30 trios). More than 1.1 million SNPs were genotyped in phase I of the project (Thorisson et al., 2005), phase II included over 3.1 million SNPs (Frazer et al., 2007), and phase III characterized 1.6 million SNPs in 1,184 individuals from 11 populations (Altshuler et al., 2010).

In 2008 the '1000 Genomes Project' was launched, a project which aims at acquiring accurate haplotype information on all forms of DNA polymorphism in several human populations. By combining low-coverage whole-genome sequencing, array-based genotyping and deep targeted sequencing in 2500 individuals from five large regions of the world (Europe, East Asia, South Asia, West Africa and the Americas), the goal is to gain information on 95% of variants with an allele frequency of 1% or higher. The pilot phase of this project has already been released (The 1000 Genomes Project Consortium, 2010).

4.3 Bipolar disorder and genetics

Although heritability estimates of BD are high, no risk genes or genetic regions have been firmly established for this disorder. Epidemiological studies have shown that BD is a complex illness with many risk variants involved (Craddock et al., 1995). A 'common disease-common

variant' (CDCV) model has been assumed, in which several common variants (SNPs), each conferring a small risk, must interact to give rise to the disorder (Barnett and Smoller, 2009). But rare variants (CNVs) with larger effect sizes, as well as multiple rare variants with small effect sizes have also been hypothesized to be implied in the genetic susceptibility for BD (Lee et al., 2012) (Figure 1).

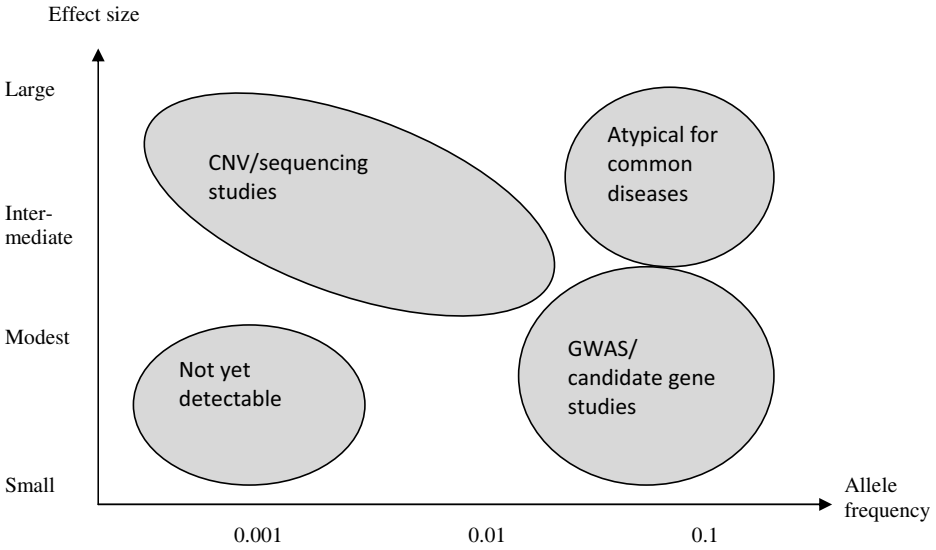

Figure 1. Effect size and allele frequency determine which study approach is best suited for detecting risk genetic variants (modified after Owen et al., 2009).

4.4 Study design and results

Susceptibility genes for BD have been sought with three major approaches (Serretti and Mandelli, 2008):

- Functional candidate approach

- Positional candidate approach
- Genome-wide association (GWA) studies

4.4.1 Functional candidate approach

The functional candidate approach is based on a priori hypotheses and knowledge on physiological alterations of the disease, pharmacological studies and animal models (Serretti and Mandelli, 2008). Selected candidate genes/genetic variants are genotyped in cases and controls, and statistical tests undertaken to assess whether these genetic variants are overrepresented in cases. If a statistical significant association is found, this increases the likelihood of the actual genetic variant being a disease-causing variant. The early candidate gene studies had a particular interest in the serotonin, dopamine, and noradrenaline neurotransmitter systems, as these are involved in the pharmacological treatment of BD. Several studies investigated genes encoding catechol-O-methyltransferase (COMT), monoamine oxidase A (MAOA) and the serotonin transporter (5HTT), but with inconsistent results. The same holds for studies on genes involved in circadian rhythm and BD (Craddock and Sklar, 2009). Due to the clinical, and proposed genetic, overlap between BD and schizophrenia (SZ), several studies have assessed the potential involvement of SZ risk genes in BD, including disrupted in schizophrenia (DISC1), the G72/G30 locus (D-amino acid oxidase activator [DAOA]), Neuregulin 1 (NRG1) and brain derived neurotrophic factor (BDNF). However, both positive and negative findings have been reported, as is the case with several other candidate genes, including those encoding dystrobrevin binding protein 1 (DTNBP1), tryptophan hydroxylase 2 (TPH2), dopamine receptor D4 (DRD4) and solute carrier family 6 (neurotransmitter transporter, dopamine), member 3 (SLC6A3) (Craddock and Sklar, 2009).

4.4.2 Positional candidate approach

The positional candidate approach is based on findings from linkage studies and selection of genes located in regions associated with BD. Linkage studies investigate which regions are co-inherited with the disease by comparing unaffected and affected family members with respect to genetic markers across the genome. Many linkage studies on BD have been conducted, suggesting several areas of the genome being co-inherited with the disorder, but again without consistent replications between studies (Barnett and Smoller, 2009). This lack of robust findings indicates that no single gene or genetic region has a major effect on increasing the risk for developing BD.

4.4.3 Genome-wide association (GWA) studies

In the recent couple of years, several GWA studies have been performed in BD case-control samples. GWA studies can investigate millions of markers across the genome, without an a priori hypothesis. Such an 'agnostic' approach seems meaningful for BD, as no pathophysiological pathways have been identified. Thus, GWA studies, if successfully conducted and interpreted, could be hypothesis-generating for new potential neurobiological mechanisms as well as for drug development. However, with such a high number of genetic variants, GWA studies impose major statistical challenges with respect to multiple testing correction. Since 2007, several GWA studies on BD have been published, with larger samples and more significant findings than experienced before in psychiatric genetics, although the effect sizes have been found to be very low for each variant. The largest published BD GWA studies at the time when the studies in this thesis were planned and performed, includes the Wellcome Trust Case-Control Consortium (WTCCC) study (N = 1868 cases and 2938 controls of British descent), finding strong association between a SNP in *PALB2* (partner and localizer of *BRCA2*) and BD (WTCCC 2007), a German/American study (N = 1233 cases and

1439 controls) reporting association between a SNP in *DGKH* (diacylglycerol kinase eta) and BD (Baum et al., 2008), an American/British study (N = 1461 BDI cases and 2008 controls in discovery sample) with *MYO5B* (myosin5B) and *TSPAN8* (tetraspanin-8) as the top hits (Sklar et al., 2008), and a large collaborative study (N = 4387 cases and 6209 controls of European descent) identifying *ANK3* (ankyrin3) and *CACNA1C* (alpha 1C subunit of the L-type voltage-gated calcium channel) as BD candidate genes (Ferreira et al., 2008). Of these findings, *ANK3* and *CACNA1C* have been replicated (Sklar et al., 2008;Lee et al., 2011;Schulze et al., 2009;Scott et al., 2009;Smith et al., 2009).

Furthermore, during the current PhD work a large multi-center international GWA study (Psychiatric GWAS Consortium (PGC)) has been initiated for psychiatric disorders, including autism (AUT), Attention Deficit Disorder (ADD), BD, major depressive disorder (MDD) and SZ (PGC 2009).

An overview on available BD GWA studies can be found at (http://www.genome.gov/GWAStudies).

Taken together, molecular genetic studies have shown that BD is a polygenic disorder where each variant has a small effect for developing the disorder. Due to small effect sizes, large numbers of cases and controls are needed to detect true risk variants.

Recent GWA studies have yielded more consistency with regards to highly significant findings, but we still lack knowledge on the potential multitude of genetic risk variants, pathophysiological pathways, genetic and gene-environment interactions for BD. Further, in contrast to SZ, few rare structural variants (CNVs) have been identified for BD (Lee et al., 2012).

4.5 Pleiotropy and bipolar disorder

'Pleiotropy' denotes the phenomenon of one gene (or one genetic variant) affecting multiple traits (Wagner and Zhang, 2011). A pleiotropic effect has been hypothesized for genetic variants conferring risk to severe psychiatric disorders, including BD and SZ, and recent evidence seems to support this hypothesis. Epidemiological studies have found overlap in heritability between BD and SZ (Lichtenstein et al., 2009), and molecular genetic studies have identified overlapping risk variants for these two disorders (Moskvina et al., 2009). A recent report found that six out of eight of the most robustly associated loci for BD and SZ, were significantly associated with both disorders (Williams et al., 2011). There is also evidence for genetic overlap between BD and major depressive disorder (MDD). In a meta-analysis combining and comparing results from a GWAS on BD (N = 4387 BD cases and 6209 controls) (Ferreira et al., 2008) with a GWAS on MDD (N = 1695 MDD cases and 1761 controls) (Sullivan et al., 2009), variants in *CACNA1C* attained genome-wide significant association in the combined sample (Liu et al., 2010).

However, the concept of pleiotropy is particularly challenging when investigating psychiatric disorders, as there are no quantifiable bio-markers unambiguously distinguishing one psychiatric phenotype from another. Thus, the finding of common risk genes for BD, SZ and MDD might as well be interpreted as an evidence for floating diagnostic borders, and the proposed support for the pleiotropy hypothesis could result from a flawed diagnostic system rather than from multiple distinct effects of each genetic variant.

It is interesting in this respect that Emil Kraepelin, although suggesting that manic-depressive illness and dementia praecox were separate diagnostic entities, also expressed doubt about this distinction himself: "No expert will deny that cases which cannot be classified safely are disturbingly frequent… We will have to get used to the idea that all signs

are insufficient to delineate manic–depressive insanity from schizophrenia… and that overlap occurs'' (quoted in Angst, 2002).

Several models challenging the current DSM-IV and ICD-10 criteria for severe psychiatric diagnostic categories have been proposed, including the pre-kraepelinian 'unitary psychosis' model, two or more overlapping categories and a floating continuum model (Craddock et al., 2009).

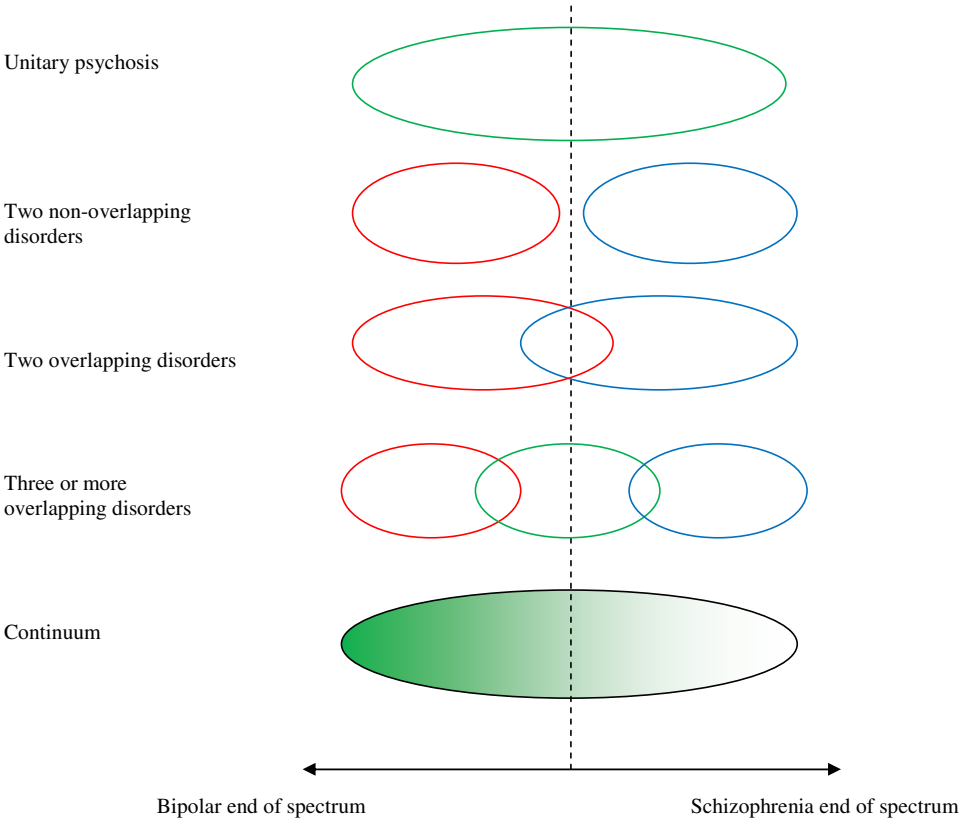

Figure 2. Possible models describing the relationship between bipolar disorder and schizophrenia (modified after Craddock et al., 2009)

To identify the best-fitting of these models, genetic cross-disorder and endophenotype studies could prove helpful. Hopefully, such studies might answer the questions of which, if any, genetic variants confer risk solely to BD, which increase the risk of developing broader spectrums of diagnostic categories, and by which mechanisms.

4.6 Endophenotypes and subphenotypes

An endophenotype is defined as 'a measurable component unseen by the unaided eye along the pathway between disease and distal genotype' (Gottesman and Gould, 2003). Furthermore, an endophenotype must fulfill certain criteria:

1. The endophenotype is associated with illness in the population.
2. The endophenotype is heritable.
3. The endophenotype is primarily state-independent (manifests in an individual whether or not illness is active).
4. Within families, endophenotype and illness co-segregate.

In psychiatry an endophenotype may be neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological (Gottesman and Gould, 2003).

In BD many attempts have been performed to identify an endophenotype which lies closer to the genetic variation than the clinical phenotype does. These markers include mRNA expression, brain structure observed with sMRI, brain function assessed with fMRI, histological anomalies investigated with postmortem studies, neurotransmission with Positron Emission Tomography (PET), neurocognition validated with neurocognitive testing,

personality traits assessed with testing, electromagnetic activity with electroencephalography (EEG) and other biomarkers extracted from blood samples.

As no neurobiological mechanisms or risk genes are firmly identified for BD, this is also true for endophenotypes. But the endophenotype strategy is still an emerging field in psychiatric genetics, which will hopefully develop as we gain more insight form genetic association studies and studies on neurobiological aspects of BD and psychiatric disorders in general. One approach in endophenotype studies is to look for association between previously identified genetic variants and putative endophenotypic markers for the disease. An example of this is a study investigating the possible association between variants in the *CACNA1C* gene previously identified from BD GWAS studies, and brain volume as assessed with structural MRI, finding evidence of altered brainstem volume (Franke et al., 2010). Another endophenotype approach is to look for association between genetic variants and an endophenotypic marker previously identified for the disease. A hypothetical example of this is using amygdala activation as a phenotype in a GWA study, to identify genetic variants associated with abnormal amygdala activation.

A subphenotype is a symptom or group of symptoms which constitutes a small spectrum of the total diagnostic phenotype, for example BD with psychosis (Lett et al., 2011). Considering the fact that our current diagnostic systems are based solely on a pattern of symptoms rather than on an underlying etiology, it is plausible that genetic variants encode proteins that in turn give rise to symptoms not easily fitting into the categories of the psychiatric nosology. Attempts at identifying such subphenotypes have been and are being performed, either with a case-control or a case-case design, but like the rest of molecular genetic studies on BD – with few, or none, robust findings.

To summarize, no endophenotypes or subphenotypes have been firmly identified for BD. But new findings form major BD GWA case-control studies enable the investigation of

association between recently identified genetic risk variants and potential sub- and endophenotypes.

4.7 Amygdala activity in bipolar disorder

Emotional dysregulation is part of the BD phenotype, manifesting as mania, hypomania and depression. Thus, it has been hypothesized that abnormal activity in the limbic system is involved in BD pathophysiology (DelBello et al., 2004). In particular, amygdala has been studied extensively as a potential endophenotype.

The amygdalae are complexes of grey matter situated in the medial temporal lobes and considered part of the limbic system. Three major groups of nuclei have been identified, with projections to various cortical and subcortical structures. Animal studies, clinical observations and imaging studies have shown that amygdala plays a role in memory and processing of emotions, including negative and positive conditioning (Baxter and Murray, 2002;Johansen et al., 2011). More recently, it has been hypothesized that amygdala is a 'detector of relevance' in a broad category of biologically relevant stimuli, and that, through evolution, social relevant events have become the predominant area of amygdala`s domain of influence (Sander et al., 2003).

Volumetric studies have yielded inconsistent results with respect to altered amygdala size in BD (Kempton et al., 2008). One recent mega-analysis including 321 patients with BD I and 442 healthy control subjects, found amygdala volume to be increased in patients treated with lithium compared to controls and patients not treated with lithium (Hallahan et al., 2011), whereas another large study with 139 BD patients and 207 healthy controls found no change in amygdala volume in BD (Rimol et al., 2010).

As for the amygdala activity in BD, a meta-analysis comprising 65 fMRI studies of 1074 healthy volunteers and 1040 BD cases, found evidence for amygdala over-activation in euthymic BD patients compared with healthy controls (Chen et al., 2011). This over-activation was observed mainly during emotional, and not during cognitive, paradigms. Decreased frontal activity was observed during both emotional and cognitive paradigms.

However, it is unclear whether this abnormal activation is a genetically conditioned pathophysiological mechanism in BD, or an effect of other disease-related processes. Molecular genetic studies could play an important role in addressing this question.

4.8 Aims of the thesis

The overall aim of the thesis was to gain insight in the molecular genetic basis of BD, by investigating for association between candidate genes and disease phenotypes in Nordic case-control samples. In study I – III, we investigated the potential association between previously identified BD candidate risk genetic variants and BD, in our case-controls samples. The genetic variants studied were all selected on the basis of findings from large recent GWA studies on BD. As GWA studies are hypothesis-free in terms of gene functions and mechanisms, it was of particular interest that some of the genes found to be strongly associated with BD in these studies, are known to be expressed in the brain, where their expressed proteins have functions related to ion channel functioning (*ANK3, CACNA1C*) and to a lithium sensitive phosphatidyl inositol pathway (*DGKH*). On the basis of this statistical as well as pathophysiological evidence, it would increase the likelihood of these variants being truly involved in the pathophysiological processes of BD, if these findings could be replicated in our independent Nordic population.

The secondary aims of the thesis were:

1. To assess the potential pleiotropic effect of these genetic variants
2. To investigate if a BD risk variant increases amygdala activity, and to explore the potential diagnostic specificity of this effect.

The aim of Study I was to investigate the association between genetic variants in diacylglycerol kinase eta (*DGKH*) and BD in a Scandinavian BD case-control sample.

The aim of Study II was to confirm the association between a genetic variant in *PALB2* (partner and localizer of BRCA2) and BD in a Scandinavian case-control sample, and to test for potential genetic overlap with SZ. We also wanted to assess the potential association between variants in the functionally related gene *BRCA2* (breast cancer 2, early onset) and BD and SZ.

The aim of Study III was to corroborate the association between genetic variants in ankyrin 3 (*ANK3*) and BD and to test for genetic overlap with SZ in Nordic case-control samples.

The aim of Study IV was to assess the potential effect of *CACNA1C* SNP rs1006737 on amygdala activity, and to determine the diagnostic specificity of this effect in Norwegian BD and SZ cases and healthy controls.

5 MATERIAL AND METHODS

5.1 Study samples

5.1.1 SCOPE bipolar disorder and schizophrenia case-control samples

The SCOPE (Scandinavian Collaboration on Psychiatric Etiology) sample used in the studies in this dissertation comprises individuals included in the Norwegian TOP (Thematically Organized Psychosis) study, the Norwegian BRAIN (Bipolar Research And Innovation Network, Norway) study, the Swedish Human Brain Informatics (HUBIN) study and the Danish Psychiatric Biobank.

The Norwegian TOP sample

The TOP study is an ongoing translational research study recruiting patients and healthy control subjects from several hospitals in the Oslo area. To be included in the TOP study, patients have to be between 18 and 65 years old, speak a Scandinavian language and fulfill the diagnostic criteria for a SZ spectrum or BD spectrum disorder according to DSM-IV. Individuals with serious brain damage or developmental disorder are excluded. Diagnostic assessment is performed by trained psychologists, psychiatrists or MDs specializing in psychiatry, of whom all participate in diagnostic meetings supervised by professors in psychiatry. The studies in this dissertation include patients with bipolar I disorder (BDI), bipolar II disorder (BDII), bipolar disorder not otherwise specified (BD NOS), schizophrenia (SZ), schizoaffective disorder (SZA), schizophreniform disorder (SZF) and persistent delusional disorder according to DSM-IV using the Structural Clinical Interview for DSM-IV (SCID) (Spitzer et al., 1992). Reliability of the diagnostic assessment in the TOP study has

been tested, and the overall agreement for the DSM-IV diagnostic categories tested was 82 % and the overall Kappa 0.77 (95 % CI: 0.60-0.94).

Clinical, neurocognitive and psychosocial assessment was undertaken during an initial interview. In this interview information on length of education, age of onset, number of relapses, medication status, alcohol and illegal substance abuse was obtained. Patients included in the studies in this thesis were assessed clinically with Young Mania Rating Scale (YMRS) (Young et al., 1978), Inventory of Depressive Symptoms (IDS) (Rush et al., 1996), Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), Global Assessment of Functioning – symptom score (GAF-S) and Global Assessment of Functioning – function score (GAF-F) (First and Tasman, 2004), and neurocognitively with the Wechsler Abbreviated Scale of Intelligence (WASI) (Brager-Larsen et al., 2001). Alcohol abuse and use of illegal substances during the last six months was measured with the Evaluating Substance Abuse in Persons with Severe Mental Disorders scale (Drake, 1996). On the day of fMRI scanning, patients underwent an abbreviated re-interview including YMRS, IDS and PANSS.

The healthy control subjects were randomly recruited from the same catchment area as the patients, were born in Norway and between 18 and 60 years old. All healthy controls underwent an initial interview where demographic and clinical information was obtained. Exclusion criteria were head injury, mental retardation, neurological disorder, a history of medical problems potentially interfering with brain function and illegal drug use. Additionally, control subjects were screened with the Primary Care Evaluation of Mental Disorders (Spitzer et al., 1994), and excluded if they or any close relatives had a lifetime history of a severe psychiatric disorder (SZ, BD, MDD).

The Norwegian BRAIN sample

The Bipolar Research and Innovation Network (BRAIN) study is a Norwegian collaborative study including patients with BD from throughout Norway. To be eligible for the study, patients have to be above 18 years old and fulfill the criteria for BD I, BD II, or BD NOS disorder according to the DSM-IV criteria. Diagnoses were assessed with the SCID-1 for the DSM-IV (Spitzer et al., 1992) or the Mini-International Neuropsychiatric Interview (MINI-Plus Version 4.4) (Sheehan et al., 1998). The patients were diagnosed by trained psychiatrists who held regular meetings to increase their inter-rater diagnostic reliability. By June 2009, the BRAIN study comprised a total of 252 patients (Schoeyen et al., 2011).

The Danish sample

The Danish patients were recruited from all over Denmark, and were diagnosed with either ICD-10 clinical assessment, Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (Wing et al., 1990) interview fulfilling a best estimate diagnosis according to ICD-10-DCR or DSM-IV, or with the Operational Criteria Checklist for Psychotic Illness and Affective Illness (OPCRIT) semi-structured interview (McGuffin et al., 1991). The studies included in this thesis comprised Danish patients diagnosed with bipolar affective disorder F31, manic episode, F30, schizophrenia F20, schizotypal personality disorder F21, persistent delusional disorder F22 and schizoaffective disorder F25 according to ICD-10, bipolar I disorder according to DSM-IV, and bipolar disorder, mania with psychosis and bipolar with psychosis according to the OPCRIT classification system.

The Danish control subjects were either recruited randomly from 15000 individuals from the Danish Blood Donor Corps in the Copenhagen area, or selected and screened for psychiatric disease for inclusion in a previous study (Mellerup et al., 2001).

The Swedish sample

The Swedish patients were selected from psychiatric hospitals in the Stockholm area, and were diagnosed with schizophrenia, schizoaffective disorder or schizophreniform disorder, according to DSM-III-R/DSM-IV criteria using medical record reviews and clinical interviews.

The Swedish control subjects were either selected from a group of individuals who had participated in previous biological psychiatric research at the Karolinska Institute or recruited from a population register in the Stockholm area. All the controls were of Caucasian origin (86 % Swedish, 6 % Finnish, 8 % European) and none suffered from schizophrenia.

5.1.2 Replication samples

Icelandic bipolar disorder and schizophrenia case–control samples

Patients and controls were recruited from all over Iceland. 316 of the BD patients were diagnoses according to Research Diagnostic Criteria (RDC) (Spitzer et al., 1978) using the Schedule for Affective Disorders and Schizophrenia Lifetime Version (SADS-L) (Spitzer, 1977). The remaining BD patients were recruited through a genetic study of anxiety and depression (Thorgeirsson et al., 2003) and had been diagnostically assessed with the Composite International Diagnostic Interview (CIDI) (Peters and Andrews, 1995;Wittchen et al., 1996). The Icelandic SZ patients were diagnosed in accordance with the Research Diagnostic Criteria (RDC) (Spitzer, Endicott, and Robins, 1978) using the SADS-L (Spitzer, 1977). The 11491 control subjects were recruited as a part of various genetic programs at deCODE genetics and were not tested for psychiatric disorders.

WTCCC bipolar disorder case–control sample

The Wellcome Trust Case Control Consortium (WTCCC) BD sample consisted of 1868 cases and 2938 controls, all from a British population. The patients were diagnosed with bipolar I

disorder, schizoaffective disorder bipolar type, bipolar II disorder or manic disorder according to Research Diagnostic Criteria (Spitzer et al., 1978) (WTCCC 2007).

STEP-UCL/ED-DUB-STEP2 bipolar disorder case–control sample

The STEP-UCL/ED-DUB-STEP2 BD sample (N = 2558 cases and 3274 controls of European descent) consisted of the STEP-UCL BD sample (N = 1460/2007) and the ED-DUB-STEP2 BD sample (N = 1098/1267), and is described in details elsewhere (Ferreira et al., 2008;Sklar et al., 2008).

The studies in this dissertation used information from the following samples:

SCOPE bipolar disorder and schizophrenia case-control samples:

Study I: 594 BD cases and 1421 healthy controls

Study II: 686 BD cases, 781 SZ cases and 2839 healthy controls

Study III: 854 BD cases, 1073 SZ cases and 2919 healthy controls

Study IV: 66 BD cases, 61 SZ cases and 123 healthy controls

Replication samples:

Icelandic bipolar disorder and schizophrenia case–control samples:

Study II: 435 BD cases and 11491 controls

Study III: 435 BD cases, 651 SZ cases and 11491 controls

WTCCC bipolar disorder case–control sample:

Study II: 1868 BD cases and 2938 controls

STEP-UCL/ED-DUB-STEP2 bipolar disorder case–control sample:

Study II: 2558 BD cases and 3274 controls

5.2 The genes studied

DGKH

Diacylglycerol kinase eta (DGKH) was selected on the basis on a prior finding from a GWAS reporting strong association between three SNPs in this gene and BD (Baum et al., 2008). *DGKH* is located on chromosome 13 and is encoding DGKH, a member of the diacylglycerol enzyme family. DGKH is involved in regulating the intracellular concentrations of diacylglycerol and phosphatidic acid, and has been shown to be a key protein in the lithium-sensitive phosphatidyl inositol pathway (Berridge, 1989). Based on this, it has been hypothesized that *DGKH* plays a role in lithium response and affect regulation, an assumption that still awaits verification (Zeng et al., 2010).

PALB2

PALB2 (partner and localizer of BRCA2) was selected due to the evidence of strong association between a *PALB2* SNP and BD reported from a GWAS from 2007 (WTCCC 2007). *PALB2* is located on chromosome 16 and encodes for PALB2, a protein which binds to and co-localizes with BRCA2 (breast cancer 2, early onset) in the cell nucleus and promotes its stable localization in cellular structures like chromatin and nuclear matrix (Xia et al., 2006). Mutations in *PALB2* have been found to increase the risk for breast cancer (Rahman et al., 2007).

BRCA2

BRCA2 was selected because of its function, which is closely related to that of *PALB2*. *BRCA2* is located on chromosome 13 and encodes for BRCA2, a protein involved in DNA repair. A dysfunction in BRCA2 leads to an increased risk of developing certain forms of cancer, like breast cancer, as well as Fanconi's anaemia (Tutt and Ashworth, 2002). Furthermore, and of particular relevance for our study, a recent report found *BRCA2* to be of importance for normal neurogenesis in mice (Frappart et al., 2007).

ANK3

Anyrin3 (*ANK3*) was investigated on the basis of several GWA and candidate gene studies finding significant association between SNPs in *ANK3* and BD (Ferreira et al., 2008;Lee et al., 2010;Schulze et al., 2009;Scott et al., 2009;Smith et al., 2009;Takata et al., 2011). *ANK3* is located on chromosome 10 and is expressed in the central and peripheral nervous system. Its encoded protein Ankyrin G has been shown to link membrane proteins to the cytoskeleton, and to be involved in the clustering of potassium channels and voltage-gated sodium channels at initial segments of the axon and for action potential firing (Kordeli et al., 1995;Pan et al., 2006;Zhou et al., 1998).

CACNA1C

L-type voltage-gated calcium channel gene (*CACNA1C*) has also been found to be associated with BD in several association studies (Ferreira et al., 2008;Sklar et al., 2008). *CACNA1C* is located on chromosome 12 and encodes an alpha-1 subunit of a voltage-dependent calcium ($Ca_v1.2$) channel. These channels have a variety of functions, including excitation-contraction coupling, endocrine secretion and regulation of neuronal Ca^{2+} transients, enzyme activity and transcription. Mutations in *CACNA1C* have been shown to give rise to Timothy Syndrome, a

lethal condition consisting of symptoms like cardiac arrhythmia, autism and mental retardation (Splawski et al., 2004).

5.3 Genotyping technologies

GoldenGate® Assay

GoldenGate 1536plex assay (Illumina Inc., San Diego, California, USA) uses a discriminatory DNA polymerase and ligase to investigate 1536 tagSNPs simultaneously. 36 tagSNPs in *DGKH* and 10 tagSNPs in *BRCA2* were genotyped using the GoldenGate 1536plex assay on Illumina BeadStation 500GX at the SNP Technology Platform, Uppsala University, Sweden (www.genotyping.se), approved according to a quality system based on the international SS-EN ISO/IEC 17025 standard and accredited by the Swedish accreditation agency SWEDAC.

TaqMan®SNP Genotyping Assay

The TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA) technology makes uses of a hydrolysis probe which binds to a target site on the genome. This probe is cleaved by the 5' exonuclease activity of the enzyme Taq Polymerase, resulting in an increase in fluorescence intensity. One SNP in *DGKH,* one *PALB2 SNP,* one *BRCA2* SNP and two *ANK3* SNPs were genotyped with single-plex TaqMan SNP Genotyping Assay, at the Department of Medical Genetics Oslo University Hospital.

Affymetrix Genome-Wide Human SNP Array 6.0

Affymetrix Genome-Wide Human SNP Array 6.0 is a DNA microarray technology which enables the detection of 906600 SNPs on the entire genome simultaneously. In contrast to the Illumina chip, the Affymetrix chip is not based entirely on LD structure and tagSNPs. For a subset of the Norwegian sample, genotype data for one *ANK3* SNP and one *CACNA1C* SNP

was extracted from a Norwegian GWAS data set comprising patients with BD and SZ, as well as healthy controls (Athanasiu et al., 2010;Djurovic et al., 2010). Individuals in this GWAS study were genotyped with Affymetrix Genome-Wide Human SNP Array 6.0 at Department of Medical Genetics Oslo University Hospital.

5.4 fMRI analyses

A negative faces matching paradigm was used to measure amygdala reactivity in the subjects included in study IV (Hariri et al., 2002). In this paradigm, the participants selected which of two stimuli (either human faces expressing anger or fear, or geometrical shapes) matched a target stimulus. The subjects completed 4 blocks of the faces matching task, where each block consisted of 6 emotion-specific face trios from a standard set of facial affect pictures (Tottenham et al., 2009). Between these 6 blocks, participants completed 5 blocks of matching geometrical shapes (sensorimotor control task). E-prime software (version 1.0 Psychology Software Tools, Inc, Pittsburgh, PA, USA) controlled the presentations of the stimuli using VisualSystem (NordicNeuroLab, Bergen, Norway). Responses were made using ResponseGrips (NordicNeuroLab, Bergen, Norway).

MRI scans were acquired on a 1.5 T Siemens Magnetom Sonata scanner (Siemens Medical Solutions, Erlangen, Germany) supplied with a standard head coil.

fMRI volumes were preprocessed and analyzed with Statistical Parametric Mapping (SPM2) (http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB7.1 (The Mathworks Inc, Natick, Massachusetts). Individual contrast images were created by subtracting "figures" from "faces". Contrast images for each subject were entered into a random effects statistical model. These data were analyzed with a region of interest (ROI) approach and a pre-defined

anatomical mask (bilateral amygdala) derived from the Wake Forest University PickAtlas for SPM2 (Maldjian et al., 2003).

5.5 Statistics

All SNPs investigated in study I-III in this dissertation were tested for deviation from Hardy-Weinberg equilibrium (HWE) in the controls. In the second and third study, we used the exact X^2 test implemented in PLINK (version1.07;http://pngu.mgh.harvard.edu/purcell/plink), whilst in the first study we used the exact test implemented in the genetics package in R (www.r-project.org).

In the first study, genotype-based association tests were calculated by the MAX-test (Freidlin et al., 2002), a test using the maximal test statistic for the recessive, dominant, and additive inheritance pattern implemented by different weights in the Cochrane–Armitage trend test. For each SNP, 10000 permutations were made to evaluate the underlying null distribution, and thus leading to corresponding P values. As a previous report found no indication of ethnic admixture in the controls included in the SCOPE samples (estimated overall gene-based fixation index (FST) = 0.00071) (Kähler et al., 2008), we did not correct for potential population stratification in this study.

In the second and third study, allele tests were undertaken in each subsample, in addition to genotypic, trend, dominant and recessive models in the second study as implemented in the function “Model” in PLINK. In the SCOPE sample, Cochran–Mantel–Haenszel (CMH) tests were performed in the second and third study, using case-control sample origin as the stratification factor. The heterogeneity of the sample specific odds ratios (ORs) was evaluated with the Breslow–Day test. For the total combined samples in the second study, the P value was assessed with Fisher`s combined probability test. The meta-analysis function in PLINK was used for the meta-analysis in the third study. Pairwise SNP_SNP

interaction analysis (PLINK) was performed for the two most significant SNPs in the second study.

Multiple testing correction in study I was performed with the Sidak approach, where the effective number of statistical tests was estimated by the Moskvina approach (Moskvina and Schmidt, 2008). This approach takes into consideration the dependency between the loci (i.e. the corresponding test statistics). In study II, gene-wise Bonferroni correction was used for the ten *BRCA* SNPs, and in study III we made use of Bonferroni correction for the three *ANK3* SNPs.

The statistical software used in study I-III in this dissertation were the genetics package in R (www.r-project.org) in study I and PLINK (version1.07;http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell et al., 2007) in study II and III.

The analyses in the fourth study were performed with the software program Statistical Parametric Mapping (SPM2) (http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB7.1 (The Mathworks Inc, Natick, Massachusetts). As multiple testing correction we applied Family-Wise Error (FWE) within the respective region of interest (ROI).

6 SUMMARY OF RESULTS

Study I

No association between DGKH and bipolar disorder in a Scandinavian case-control sample

37 *DGKH* SNPs were genotyped in 594 BD cases and 1421 healthy controls of Danish and Norwegian origin. We found no significant association between these SNPs and BD after multiple-testing correction. Specifically, we found no significant association between the SNP rs1012053 previously identified in a GWA study and BD (Nominal P = 0.76).

These findings make it less likely that *DGKH* variants are involved in BD pathophysiology.

Study II

Association analysis of PALB2 and BRCA2 in bipolar disorder and schizophrenia in a Scandinavian case-control sample

The *PALB2* SNP rs420259 was genotyped in a sample of 686 BD cases, 781 SZ cases and 2839 healthy controls. The ten *BRCA2* tagSNPs were genotyped in a subsample of 554 BD cases and 1419 healthy controls, of which the most significant SNP (rs9567552) was genotyped in the remaining subjects of the total Scandinavian case-control sample. The *BRCA2* SNP rs9567552 attained a nominal P-value of 0.00043 and the *PALB2* SNP rs420259 a nominal P-value of 0.025. When we combined the latter result with results from an Icelandic BD case-control sample (N = 435 BD cases and 11491 controls), the WTCCC BD sample (N = 1868 BD cases and 2938 controls) and the STEP-UCL/ED-DUB-STEP2 study (N = 2558 BD cases and 3274 controls) in a meta-analysis, we calculated a P-value of 1.2×10^{-5} for association between *PALB2* SNP rs420259 and BD (N = 5547 BD and 20241 controls). None

of the SNPs were nominally significantly associated with SZ in our Scandinavian sample (N = 781 SZ cases and 2839 healthy controls).

These findings suggest that genetic variants in *PALB2* and *BRCA2* may increase the risk of developing symptoms which are more prevalent in the BD spectrum than in the SZ spectrum of the proposed continuum of severe psychiatric disorders. Furthermore, it opens the possibility for altered DNA repair related to neurogenesis in BD pathophysiology.

Study III

Association analysis of ANK3 gene variants in Nordic bipolar disorder and schizophrenia case-control samples

We genotyped the three *ANK3* SNPs rs10994336, rs1938526 and rs9804190 in a Scandinavian case-control sample (N = 854 BD cases, 1073 SZ cases and 2919 healthy controls). When adding the results from this sample to the results from an Icelandic case-control sample (N = 435 BD cases, 651 SZ cases and 11491 controls), we found rs10994336 and rs9804190 to be nominally significantly associated with BD. Nominal P was 0.015/0.018 (fixed/random effect) for rs10994336 (Bonferroni corrected P = 0.044/0.053) and 0.023 for rs9804190 (Bonferroni corrected P = 0.069) in this combined Nordic sample (N = 1289 BD cases and 14105 controls). None of the SNPs were significantly associated with SZ.

Our findings suggest allelic heterogeneity for *ANK3* in BD and ion channelopathy as a potential underlying neurobiological mechanism.

Study IV

CACNA1C risk variant and amygdala activity in bipolar disorder, schizophrenia and healthy controls

We investigated if carriers of a *CACNA1C* risk allele had altered amygdala activation during a negative faces matching paradigm in a sample of 66 BD cases, 61 SZ cases and 123 healthy controls, all of Norwegian origin. In the total sample (N = 250), we found enhanced activity in the left amygdala in risk allele carriers (x = -24, y = -2, z = -14; Z = 3.47) (FWE P = 0.026). There was also a significant association for increased activity in the left amygdala in the BD group (x = -24, y = 0, z = -14; Z = 3.35) (FWE P = 0.041), but not in the SZ or healthy control group when analyzing group wise.

The current findings strengthen the hypotheses of *CACNA1C* involvement in BD and ion channelopathy and increased amygdala activity as pathophysiological mechanisms.

Overall summary

In all the studies in this thesis we investigated genetic variants which were strongly associated with BD in previous GWA studies. Of these variants, we replicated the association between a *PALB2* SNP and BD and detected a novel association between *BRCA2* and BD, found borderline significant association between two *ANK3* SNPs and BD, and no association between a *DGKH* SNP and BD in our Nordic BD sample. In our fourth study, we found carriers of a *CACNA1C* risk allele to have increased amygdala activity, most pronounced in the BD subgroup.

Taken together, these findings make it more likely that genetic variants in *PALB2* and *ANK3* increase the risk of developing BD, while it decreases the probability that *DGKH* is related to the disorder. Furthermore, in strengthens the hypothesis that ion channelopathy is involved in BD pathophysiology, as both *ANK3* and *CACNA1C* have functions related to ion channel regulation, and that amygdala over-activation may be an underlying mechanism for BD.

7 DISCUSSION

7.1 Discussion of results

The main findings in the studies in this thesis were significant associations between genetic variants in *PALB2*, *BRCA2*, *ANK3* and BD, no association between *DGKH* and BD, and amygdala over-activation in carriers of a *CACNA1C* risk allele, most pronounced in BD.

Since the studies in this thesis were planned and undertaken, the afore-mentioned international multicenter PGC study was published, comprising 11974 BD cases and 51792 controls (Ripke et al., 2011;Sklar et al., 2011). As this is the largest case-control association study in psychiatric genetics, with more statistical power than previous studies, the results in this thesis must be interpreted in the perspective from the PGC mega-analysis, as well as from other major studies.

In the first article in this dissertation, we found no association between SNPs in *DGKH* and BD. The SNP formerly found associated with BD (rs1012053) (Baum et al., 2008), obtained a P-value of 0.70 in our study, with OR in opposite direction. Two other studies have reported negative findings for *DGKH* SNPs and BD (Ollila et al., 2009;Yosifova et al., 2009), whereas one Sardinian study (Squassina et al., 2009) and one Chinese study (Zeng et al., 2011) found association between *DGKH* haplotype blocks and BD. In the PGC BD mega-analysis, the P-value for rs1012053 was 0.0087 (p = 0.26 for SZ, but OR in opposite direction) (Sklar et al., 2011). Taken together, there are inconclusive results for *DGKH* SNPs and BD, but considering the low statistical power for some of these studies and the biological rationale, this is nevertheless a gene which warrants further investigation in BD.

In study II, *PALB2* SNP rs420259 was associated with BD in the meta-analysis (N = 5547/20241), with a nominal P-value of 1.2×10^{-5}, but not with SZ. Our meta-analysis

included the WTCCC sample, where the primary association between this *PALB2* SNP and BD was detected (WTCCC 2007). A Finnish family-based BD association study (N = 723) (Ollila et al., 2009) found no association for this SNP, but the statistical power of this study was smaller than in our study and the WTCCC study. Further, our results for *PALB2* are in line with corresponding P-values from the PGC study is (P = 0.006 for BD and 0.052 for SZ; ORs in the same direction as in our study) (Sklar et al., 2011), and indicates that this SNP might increase the risk of developing BD, but not SZ to the same degree. Nonetheless, this SNP did not reach genome-wide significance in the PGC BD sample, and the OR was lower than in our study (1.07 vs 1.19 for risk allele A). This might indicate that the true effect size is lower than first reported (winner's curse), or that carriers of the risk allele constitute a subgroup of BD patients. As for our association between *BRCA2* SNP rs9567552 and BD, (Nominal P = 0.00043) in the Scandinavian sample (N = 686 BD cases and 2839 healthy controls), the corresponding P-value in the PGC study was 0.11 with OR in the opposite direction, and there are no available results for the SZ sample. To best of our knowledge, no other studies have investigated this genetic variant in psychiatric disorders. Thus, in the perspective of the present findings, it is less likely that this SNP confers susceptibility to BD, at least not to the entire BD phenotype and in all populations.

The two *ANK3* SNPs rs10994336 and rs9804190 were nominally significantly associated with BD in our combined Nordic sample (N = 854 BD cases and 2919 healthy controls), but only borderline significantly associated after correcting for multiple testing. These results are in line with previous findings on *ANK3* and BD, with regards to statistical power and effect size. Since the primary evidence of association between *ANK3* SNPs and BD (Ferreira et al., 2008), several studies have found rs10994336 (Schulze et al., 2009;Scott et al., 2009), rs9804190 (Schulze et al., 2009) and rs1938526 (Lee et al., 2011;Smith et al., 2009;Takata et al., 2011) to be associated with BD. One study found another *ANK3*

SNP (rs10761482) to be associated with SZ (Athanasiu et al., 2010). Results from the PGC study support these findings ($P = 7.96 \times 10^{-6}$ for rs9804190 in the BD sample and 0.065 in the SZ sample; both ORs in the same direction as in our sample; no results available for rs10994336 and rs1938526). On this basis, *ANK3* is one of the most consistent findings among the proposed risk genes for BD. Moreover, the *ANK3* variants studied in this thesis do not seem to increase the risk for SZ to the same degree as for BD.

As for *CACNA1C*, several reports have found rs1006737 to be associated with BD (Ferreira et al., 2008;Sklar et al., 2008), SZ (Green et al., 2010;Nyegaard et al., 2010) and MDD (Green et al., 2010). In the PGC study, the same allele was associated with both BD ($P = 1.73 \times 10^{-5}$) and SZ ($P = 1.17 \times 10^{-6}$). Thus, there is robust evidence for overlap at the clinical phenotype level for this genetic variant. With regards to brain activity, two fMRI studies reported enhanced amygdala activity for carriers of the rs1006737 risk allele (Jogia et al., 2011;Wessa et al., 2010), but neither of these studies determined the potential diagnostic specificity of this association. In study IV in this thesis, having the *CACNA1C* risk allele, was associated with significantly enhanced activity in the left amygdala in the total sample (N = 250), and in the BD group (N = 66), but not in the SZ or healthy control group when analyzing the groups separately. This suggests that the currently studied risk allele has an effect on amygdala activation in all individuals, which is more pronounced in BD subjects.

7.1.1 *PALB2*, *BRCA2*, DNA repair and neurogenesis

In study II, we replicated the association between the *PALB2* SNP rs420259 and BD, which was previously reported in a GWA study from 2007 (WTCCC 2007). Additionally, we found a novel association between *BRCA2* SNP rs9567552 and BD. The lack of significant

associations between these two SNPs and SZ indicates no genetic overlap between BD and SZ for these SNPs.

The encoded proteins from *BRCA2* and *PALB2* are functionally related in DNA repair mechanism, and variants in both genes have been shown to increase the risk for developing breast cancer and other forms of cancer (Rahman et al., 2007; Tutt and Ashworth, 2002). A recent study showed that *BRCA2* is important for normal neurogenesis in mice, particularly in the cerebellum (Frappart et al., 2007). There is evidence for cerebellar involvement in emotional processing, and reduced cerebellar volume and activity has been observed in BD (Bolbecker et al., 2009;Konarski et al., 2005). Further support comes from a Diffusion Tension Imaging study finding reduced cerebellar gray matter density in pediatric BD (James et al., 2011). On this basis, it seems possible that genetic variants in *BRCA2*, and the functionally related gene *PALB2*, may modulate the neurogenesis in certain brain regions, like the cerebellum, whose activity in turn might give rise to emotional dysregulation, manifesting as mania or depression.

With regards to the relationship between severe psychiatric disorders and cancer, it has been hypothesized that individuals suffering from SZ have lower incidence rates of cancer than healthy individuals. A meta-analysis comprising studies of cancer incidence in SZ cases and their first-degree relatives compared with general population samples, reported that the overall cancer incidence in SZ patients was not significantly increased. They found slightly increased lung cancer incidence, which was reduced after adjusting for smoking prevalence, whereas the incidence of other forms of cancer unrelated to smoking was reduced in SZ patients. Breast cancer was more prevalent in female patients, and the total cancer incidence in relatives was significantly reduced (Catts et al., 2008). As for BD, fewer studies on cancer incidence are published, but of the few reports available, one reported significantly enhanced cancer risk in BD subjects in both genders, and a trend-significantly increased risk for breast

cancer in females (Barchana et al., 2008). Several possible explanations of these relations have been proposed; one suggests that neuroleptics have a protective effect against cancer in SZ patients (Mortensen, 1994), another indicates that the increased prolactin level resulting from neuroleptics increases the risk of breast cancer (Grinshpoon et al., 2005), and a third hypothesis proposes that the relation between severe psychiatric disorders and cancer may result from the underlying genetic variation (Catts and Catts, 2000).

From this perspective, it was interesting to explore how our *PALB2* and *BRCA2* candidate risk variants for BD might relate to breast cancer. The *BRCA2* SNP rs9567552 has, to our knowledge, not been studied in breast cancer, but the *PALB2* SNP rs420259 investigated in our study, is in LD (r^2 = 1.0 based on the Chinese HapMap population; r^2 = 0.84 in CEU), with the *PALB2* SNP rs249954, which was found to be associated with breast cancer (G/A, A risk allele) in a Chinese study (Chen et al., 2008). The G allele of rs249954 is in LD with A of rs420259, according to www.hapmap.org. Hence, the risk allele for breast cancer is in LD with the protective allele for BD. There are several ways to interpret this phenomenon, taking into consideration that we do not know which SNP is causatively associated with these two diseases. One possible explanation is that the same SNP is protective for one phenotype and confers risk to the other, in which case the increased incidence of breast cancer in BD could be independent of these SNPs. Another interpretation is allelic heterogeneity at the same locus in different populations, which could imply that the risk allele for BD/breast cancer in Europeans is the protective allele in the Chinese population and vice versa (Hennah et al., 2009). A third possibility is false positive findings, which might very well be the case with *BRCA2,* in the light of the recent PGC findings.

BRCA2 and *PALB2* variants have been shown to increase the risk for developing breast cancer, ovarian cancer and other cancer types (Rahman et al., 2007;Tutt and Ashworth, 2002;Wooster et al., 1995). In this respect, it is important to mention that the SNPs selected

for study II in this thesis were chosen on the basis of one previous report on BD (*PALB2*) (WTCCC 2007), and on tagSNPs from the Goldengate assay (*BRCA2*), without any prior knowledge on how these variants might relate to cancer incidence. Furthermore, the known genetic risk variants for breast and ovarian cancer are most often rare deleterious mutations, and not common SNPs (Pylkas et al., 2008). A recent review article concluded that with the current knowledge on breast cancer risk genes, we lack evidence for justification of screening for common low penetrance genetic variants (Ripperger et al., 2009). Hence, it is unlikely that the SNPs genotyped in our study could have any significant predictive value for diagnostic assessment of cancer.

7.1.2 Ion channelopathy in bipolar disorder

Several GWA and candidate gene association studies have found association between SNPs in *CACNA1C* and *ANK3* and BD (Ferreira et al., 2008;Schulze et al., 2009;Sklar et al., 2011). Study III in this thesis adds evidence on diagnosis level to these findings, while study IV explores further a potential underlying neurobiological mechanism.

Interestingly, both *CACNA1C* and *ANK3* encode for proteins known to be involved in ion channel regulation. *CACNA1C* encodes for an alpha-1 subunit of a voltage-dependent calcium ($Ca_v1.2$) channel, and *ANK3*`s encoded protein Ankyrin G has been shown to be important for clustering of potassium channels and voltage-gated sodium channels at axon initial segments (Jenkins and Bennett, 2001). Based on these findings, it has been hypothesized that ion channelopathy may be an etiological factor in BD.

Channelopathies are mutations that change the function of ion channels in such a way that they give rise to clinical syndromes, including cardiac arrhythmias and neurological disorders like ataxia, epilepsy, migraine and headache (Kullmann, 2010). Until recently, there has been little evidence for implication in psychiatric disorders, and the associations found

between *ANK3* and *CACNA1C* and BD shed new light on this tentative pathophysiological mechanism. *CACNA1C* mutations have been found to lead to Timothy syndrome, a rare and lethal disorder consisting of somatic symptoms like cardiac arrhythmia, dysmorphic facial features, syndactyly digits, dysregulation of the immune and secretory systems, and psychiatric symptoms like autism and cognitive disability (Splawski et al., 2004). In patients with Timothy syndrome mutations, there is an impaired $Ca_v1.2$ channel inactivation, and a sustained influx of calcium into the myocytes during action potential, resulting in longer QT intervals (Bidaud and Lory, 2011). Alas, Timothy syndrome mutations lead to a gain of function of $Ca_v1.2$ channels, whereas in another inherited calcium channelopathy syndrome, the Brugada syndrome, mutations result in a loss of function of $Ca_v1.2$ (Bidaud and Lory, 2011). A recent study on Timothy syndrome investigated cortical neuronal precursor cells and neurons from induced pluripotent stem cells in subjects with Timothy syndrome. The authors reported that these cells had defective calcium signaling and activity-dependent gene expression. Further, these neurons displayed abnormal differentiation, abnormal expression of tyrosine hydroxylase and increased production of norepinephrine and dopamine. Intriguingly, these features proved to be reversed by roscovitine, a cyclin-dependent kinase inhibitor and atypical L-type-channel blocker (Pasca et al., 2011).

To the best of our knowledge, the effect of the risk allele A in the *CACNA1C* SNP rs1006737 on its expressed protein is not known. But as rs1006737 is situated in one of the introns of *CACNA1C,* it is probably regulating the expression of the protein rather than changing its structure, as is the case with Timothy syndrome, in which the causative de novo mutations are located in one of the exons (Splawski et al., 2005). If we consider a sliding-scale model of a spectrum of psychiatric disorders, with autism in the neurodevelopmental end and MDD in the affective end (Craddock and Owen, 2010), we could speculate that polymorphisms regulating the expression of the *CACNA1C* gene might confer risk to less

severe conditions (like BD and SZ) than those observed resulting from Timothy syndrome mutations. Further, this spectrum might not be confined to psychiatric disorders. It is possible that channelopathies predispose to a broad group of neuropsychiatric disorders, including epilepsy, migraine, ataxia, autism, SZ, BD and MDD. Interestingly, some neurological disorders caused by channelopathies (epilepsy, migraine, dyskinesia) are paroxystic in nature, like BD (Ryan and Ptacek, 2010). Moreover, the PGC study found the *CACNA1C* SNP rs1006737 to be associated with both BD ($P = 1.7 \times 10^{-5}$; OR = 1.11) and SZ ($P = 1.2 \times 10^{-6}$; OR = 1.11) (Ripke et al., 2011), at similar significance levels and with the same effect sizes. Hence, the different clinical manifestations could be conditioned by different gene-gene interactions, epigenetic factors and gene-environment interactions. In line with this proposed model, the findings from our fourth study suggest that this *CACNA1C* risk allele increases amygdala activity in all individuals, but with a more prominent effect in BD subjects, possibly due to other genetic and environmental risk factors.

With regards to the pharmacological implications of the association between *CACNA1C* and BD, the calcium channel blocker Verapamil has been shown to have an effect on cardiac arrhythmia in Timothy syndrome (Bidaud and Lory, 2011). Calcium channel blockers have also been studied in mood and substance abuse disorders (Casamassima et al., 2010). Some promising early results have been reported for dihydropyridine-based blockers that bind specifically to $Ca_v1.2$ channels in the treatment of BD, but larger studies are needed (Keers et al., 2009). The above-mentioned drug roscovitine should also be taken into consideration for further investigation (Pasca et al., 2011). However, as long as the effect of the *CACNA1C* BD risk variants on protein structure and expression level remains unknown, it will be difficult to develop specific pharmacological treatments based on the channelopathy theory.

As for *ANK3* and the channelopathy hypothesis for severe psychiatric disorders, recent studies have yielded interesting results. One animal study showed that axons of ankyrinG-deficient mice develop resembling features with dendrites, and that ankyrinG therefore is important for maintaining appropriate axo-dendritic polarity in vivo (Sobotzik et al., 2009). This finding is in accordance with a cell study reporting that silencing of the expression of ankyrinG dismantles the axonal initial segment and gives axons similar characteristics as dendrites (Hedstrom et al., 2008). As the effect of the *ANK3* polymorphisms associated with BD on the expressed protein is still unknown, two recent studies are of interest in this respect. An animal study found *ANK3* and calcium channel subunits to be downregulated in the mouse brain in response to lithium (McQuillin et al., 2007). As lithium is a potent mood-stabilizing drug, this finding might be interpreted as an evidence for increased expression of ankyrinG in BD cases. This seems slightly contradictory to the findings from a recent translational study, in which *ANK3* mRNA expression levels were lower in SZ subjects than in healthy controls and *ANK3* expression was lower in carriers of the *ANK3* SNP rs9804190 C allele (Roussos et al., 2012). However, the pathophysiological mechanisms by which *ANK3* SNPs are related to BD and SZ might differ, mRNA expression levels could vary from one brain region to another, and the effect of lithium on BD might be unrelated to the impact of *ANK3* risk alleles.

To summarize, it remains unclear whether the polymorphisms in *CACNA1C* and *ANK3* found to be associated with BD result in hyper- or hypofunction of the expressed proteins. Future studies investigating mRNA levels and other potential endophenotypes could address this question and add to the growing body of knowledge on the putative role of channelopathies in BD.

7.1.3 Increased amygdala activity - an endophenotype for BD?

In the fourth study in this thesis, we found increased activity in carriers of the *CACNA1C* SNP rs1006737 risk allele in the left amygdala in the overall sample, with a more pronounced effect in the BD subsample than in the SZ and healthy control subsamples.

In order to assess whether increased amygdala activity is an endophenotype for BD, and to what extent our findings add to prior knowledge on this subject, we should go back to the criteria from the introductory section in this thesis, and see if they are fulfilled by the current findings.

1. The endophenotype is associated with illness in the population.

As stated in the introduction, a recent meta-analysis (N = 1040 BD cases and 1074 healthy controls) reported increased amygdala activity in BD subjects compared to healthy controls during emotional paradigms (Chen et al., 2011). However, the studies included in this meta-analysis are rather small, and the findings are not entirely consistent from study to study. Larger studies with both emotional and cognitive paradigms are needed to address this question properly.

2. The endophenotype is heritable.

To the best of the author's knowledge, no studies have explicitly investigated the heritability of amygdala activity or volume, neither in terms of epidemiology nor molecular genetics. But at least two imaging genetics studies have investigated the influence of genetic variants on amygdala activity. Both studies used the *CACNA1C* SNP rs1006737 as independent variable and amygdala activity as dependent variable. The first study found increased activation in the

right amygdala in a reward paradigm for healthy carriers of the risk allele (N = 64) (Wessa et al., 2010), whereas the latter found increased activation in the right amygdala in carriers of the risk allele during a fear-face paradigm (N = 41 euthymic BD cases, 25 relatives and 50 healthy controls) (Jogia et al., 2011).

Our study is substantially larger than these studies, and comprises SZ cases in addition to BD cases and healthy controls. Our findings confirmed the association between this genetic variant and increased amygdala activity, and provided new evidence for a more pronounced effect in patients suffering from BD.

Based on these three studies, is seems like carriers of this *CACNA1C* SNP have an enhanced amygdala activation, but more studies are needed before concluding on diagnostic specificity for BD. It is also unclear whether the increased amygdala activation pattern is unilateral or bilateral. The two previous studies reported over-activation only in the right amygdala, whilst we found a stronger effect in the left amygdala. However, there were trend-significant associations also in the right amygdala in our total sample (x = 26, y = 0, z = -16, Z = 2.65, cluster-size = 32) and in the BD subgroup (x = 22, y = 0, z = -20, Z = 2.54, cluster-size = 61). This might indicate bilateral increased amygdala activation in AA/AG carriers, not reaching significance level due to low statistical power in these three studies. The above-mentioned meta-analysis reported higher activation in the left medial temporal structures (parahippocampal gyrus, hippocampus and amygdala) in BD in six studies, and lower activation in similar regions in the right hemisphere in two studies (Chen et al., 2011). However, this discrepancy might result from different inclusion criteria, low statistical power and different paradigms. A potential problem when performing fMRI meta-analyses and replication studies is the various paradigms used by different research groups. We used a negative faces matching paradigm in our study, while the other studies used a fairly similar fear-face paradigm (Jogia et al., 2011) and a reward paradigm (Wessa et al., 2010). Although

all these paradigms have been validated for measuring amygdala activity, this activation might be the end-result of different mechanisms, like positive/ negative conditioning and relevance detection (Ousdal et al., 2008;Sander et al., 2003).

Further, the abnormal amygdala activation reported in BD cases could be a cause to as well as effect of pathological processes related to the disorder. However, the fact that a known risk genetic variant for BD is associated with increased amygdala activity in both healthy controls and BD cases, strengthens the hypothesis that increased amygdala activity is a genetically conditioned biological cause to the BD clinical phenotype.

With regards to the variation in amygdala activity explained by the *CACNA1C* risk SNP, it seems rather unlikely that a substantial part of this could be determined by one SNP, taken into consideration the polygeneity in BD and the low effect size for each single variant. On the other hand, as the genotypic variation probably lies closer to amygdala activity than to the clinical phenotype, the effect size for each variant may be larger and the polygeneity smaller at the brain activity level. To obtain more information on the heritability of amygdala activity, larger GWA studies and candidate gene studies should be performed, with well-defined and validated paradigms. Findings from such studies should be compared with those from BD studies, to assess the genetic co-heritability.

3. The endophenotype is primarily state-independent (manifests in an individual whether or not illness is active).

In the afore-mentioned meta-analysis, increased amygdala activity was reported in euthymic BD cases compared to healthy controls (Chen et al., 2011). Thus, this suggested endophenotype seems to be state-independent. To further investigate this state/trait-

relationship, one should compare euthymic subjects with individuals in manic/hypomanic and depressed state, in different emotional and cognitive paradigms, as well as in resting-state.

4. Within families, endophenotype and illness co-segregate.

One study (N = 20 BD I cases, 20 unaffected 1st degree relatives, 20 healthy controls) reported increased activation in the left amygdala in patients and relatives in response to intensively happy faces (Surguladze et al., 2010). But these findings need to be confirmed in larger studies and further investigated with other paradigms.

Taken together, there is evidence for increased amygdala activity in euthymic BD cases compared to healthy controls, and one genetic variant in *CACNA1C* has been shown to increase the amygdala activity in the general population, possibly with a more prominent effect in BD. However, we still lack knowledge on the overall heritability of amygdala activity, the relationship between amygdala heritability and the heritability of BD, on familiar co-segregation of phenotype and potential endophenotype, and on the relation between state and trait activity. Moreover, the activation patterns between amygdala and other parts of the brain should also be investigated further in BD.

7.1.4 Pleiotropic effects for bipolar disorder risk variants?

In study II, III and IV in this thesis, we investigated genetic variants previously associated with BD, in patients diagnosed with SZ and BD. We did not find evidence for overlap at diagnosis level in study II and III, and the results from study IV indicated a general effect for the *CACNA1C* risk SNP independent of diagnostic category, with a possibly stronger effect in BD cases.

In the introductory section of this thesis we outlined several hypothesized models describing the relationship between severe psychiatric disorders, hereunder the pre-kraepelinian unitary psychosis model, the kraepelinian disctinction between manic depressive illness and dementia praecox, two or more overlapping categories and a floating continuum (figure 2) (Craddock et al., 2009). Of these models, the unitary psychosis model and the kraepelinian dichotomy could probably be rejected on the basis of recent studies. In the above-mentioned Swedish epidemiological study comprising 9 million subjects, first-degree relatives of BD cases were found to have 7.9 % risk for BD and 3.9 % risk for SZ (Lichtenstein et al., 2009). This is a clear evidence for a partial overlap between BD and SZ, excluding the unitary psychosis model as well as the dichotomous model. Recent molecular genetic studies have provided evidence for at least three common genetic risk variants for BD and SZ (*CACNA1C*, *ANK3* and the *ITIH3-ITIH4* region) (Ripke et al., 2011), but also genetic variants more specific to SZ than BD, like CNVs (Stefansson et al., 2008) and the extended major histocompatibility complex (MHC, 6p21.32-p22.1) (Ripke et al., 2011;Stefansson et al., 2009). Additionally, one recent study reported a different polygenic score for SZ risk variants in subjects diagnosed with schizoaffective bipolar disorder (N = 277) than those with BD (N = 1552). However, they found no significant difference between psychotic and non-psychotic BD (Hamshere et al., 2011). This finding is in line with a recent subphenotype GWA analysis (N = 2836 BD cases and 2744 controls), in which no SNP obtained genome-wide significance level for age at onset or psychotic symptoms (Belmonte et al., 2011).

The hypothesis of partial overlap between BD and SZ is further supported by imaging and neurocognitive studies. In a brain volumetric study, patients diagnosed with BD and SZ were found to have smaller subcortical volumes than healthy controls, and BD I and SZ cases had smaller volumes in prefrontal regions. For both findings, there was in general a more pronounced effect in SZ than BD (Rimol et al., 2010). On the neurocognitive level, there is

evidence for impairment in individuals with SZ as well as BD, and this impairment seems to be dependent on the number of psychotic episodes rather than on diagnostic category (Simonsen et al., 2011). Furthermore, neurocognitive dysfunction is found to be more severe in BD I than in BD II cases (Simonsen et al., 2008).

In a recent article, the authors suggested a continuum model of severe psychiatric disorders with an inverse sliding gradient of affective and neurodevelopmental pathology, MDD in the affective end, moving on to BD, schizoaffective disorder, SZ, autism, and mental retardation in the neurodevelopmental end. In this model, there is overlapping heritability crossing the current diagnostic boundaries, with structural genetic variants more prevalent in the neurodevelopmental end and single genetic variants conferring risk to different parts of, or to the whole, diagnostic spectrum (Craddock and Owen, 2010). The authors suggest that it is time to leave the Kraepelinian categories, and create a new model, either with dimensions, multiple overlapping categories, or both, which lie closer to the underlying biological findings, and at the same time closer to the every-day clinical experience of inter-individual symptomatological heterogeneity. However, in order to create such a model and implement it in research and clinical practice, more studies on the molecular genetic level as well as on all possible endophenotype levels of severe psychiatric disorders are needed.

7.2 Methodology – strengths and limitations

7.2.1 GWAS versus candidate gene approach

One of the major advantages of GWA studies is that they are hypothesis-free, i.e. the results from these studies are not biased by a priori hypotheses of the researchers. On the other hand, when investigating up to one million genetic variants, the risk of getting false positive

findings before multiple testing correction as well as false negative findings after multiple testing correction is substantially increased compared to candidate gene studies. As for candidate gene studies, a major strength is the reduced need for multiple testing correction and the resulting reduced number of false negative findings. But the limited possibility for screening a vast number of variants across the entire genome makes this approach inefficient in the search for novel and unexpected risk variants, and dependent on the already existing hypotheses for potential risk genes. A general problem in psychiatric genetics, for both candidate gene and GWA studies, has been low statistical power, a phenomenon which until recently resulted in a lack of consistency between studies (Craddock et al., 2009;Porteous, 2008). This phenomenon is most likely due to the polygenic nature of severe psychiatric disorders, and the low effect size of each single risk variant. However, the most recent multicenter GWA studies of BD (Ferreira et al., 2008;Sklar et al., 2011) have shown a larger degree of consistency, which implies that in order to obtain a level of adequate statistical power, several thousands of subjects are probably needed. Furthermore, to address the problem with false positive findings, it has shown to be important with replication of previous results in the field of psychiatric genetics.

The studies included in this thesis have used the advantage of highly significant findings from previous well-powered BD GWA studies (WTCCC 2007;Baum et al., 2008;Ferreira et al., 2008). We have explored potential biological pathways for these variants, in order to test novel hypotheses, such as genetic overlap with SZ and increased amygdala activity as a BD endophenotype. One of the strengths of this approach is that the hypotheses are based on empirical evidence as well as on a biological rationale. One might say that this approach re-introduces the importance of candidate gene studies as a post-GWAS research method. One of the disadvantages with this approach is the reduced the possibility for discovering new genetic variants.

7.2.2 Materials - clinical phenotypes and population stratification

One disadvantage with using psychiatric diagnostic categories like BD and SZ as phenotypes, is their symptomatological definitions. The lack of biomarkers in the diagnostic assessment for these disorders might give rise to heterogeneity at the phenotypic level, which could reduce the statistical power to detect risk variants. However, high heritability and cross-heritability estimates for BD and SZ (Lichtenstein et al., 2009) imply common and partially shared underlying mechanisms for these disorders.

A further limitation in this regard is the different diagnostic assessment tools used in the studies in this thesis, as we have used DSM-IV for the Norwegian TOP sample and the Swedish sample, and ICD-10 and the OPCRIT system for the Danish sample. Additionally, cases included in the Icelandic replication sample were assessed with RDC and CIDI. But a Danish study from 2006 showed high diagnostic agreement of SZ across the ICD-10 and DSM systems (pairwise concordance rate (CR) > 0.70, $\kappa > 0.70$) (Jakobsen et al., 2006). To correct for the potential phenotypic discrepancies between our samples, we applied the Cochrane-Mantel-Haenszel (CMH) method using "sample origin" as stratification factor in our statistical analyses in study II and III. Also, phenotypic heterogeneity would most likely increase the rate of type II errors, and not type I errors. In this regard, our studies are probably more susceptible to false negative than false positive findings.

The CMH method was also used for correcting for potential genetic differences across the Scandinavian samples in study II and III, and between the Scandinavian, Icelandic and British WTCCC samples in the meta-analysis in study II. Admixture of populations with different genetic architectures, if not corrected for, might lead to type I as well as type II errors. As for potential population stratification between our Scandinavian samples, a study from 2008 found no evidence for this (Kähler et al., 2008). Furthermore, a study of more than

2500 individuals from 23 European subpopulations, using Affymetrix GeneChip 500K genotype data, reported continent-wide correlation between geographic and genetic distance and little genetic differentiation between the subpopulations. Moreover, northern European populations showed less heterozygosity and smaller mean LD than southern European populations (Lao et al., 2008).

7.2.3 Statistical power and multiple testing correction

Statistical power is the probability that a test will reject a false null hypothesis, and is dependent upon several factors. The Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/) uses the following criteria for determining the statistical power of a given case-control association study:

- Risk allele frequency
- Disease prevalence
- Relative risk for homozygous and heterozygous carriers of the risk allele
- LD between the investigated marker and the true risk allele
- Number of cases
- Control/case ratio
- Selected/unselected controls

Statistical power is highest when the risk allele frequency equals 0.50, the D' for LD between the investigated and true risk marker is 1 and the markers are equally frequent. Furthermore, statistical power increases with increasing number of cases and controls, if the controls are selected, and with an allelic statistical model.

Of the studies included in this thesis, we performed statistical power analyses only in study III. We used the above-mentioned Genetic Power Calculator to calculate the power to detect P = 0.05 and number of cases needed for statistical power of 80%. We used a 10:1 control/case ratio, and effect sizes were based on ORs for the respective SNPs reported in previous studies (Ferreira et al., 2008;Schulze et al., 2009). For the three *ANK3* SNPs investigated (rs9804190, rs10994336 and rs1938526), the respective power estimates were 0.9727, 0.9993 and 0.9985, and the numbers of cases needed for statistical power of 80% were 677, 386 and 420. The discrepancies were most likely due to differences in previously reported ORs, which were, respectively, 1.21, 1.45 and 1.395. However, these ORs might have been slightly inflated, as the corresponding OR for rs9804190 in the PGC study was 1.17, and taken into consideration the fact that the first association between a marker and disease tend to report higher effect sizes than replication studies, a phenomenon termed 'winner's curse' (Sklar et al., 2011). Thus, our statistical power in study III might have been overestimated, as a consequence of overestimated ORs.

Due to the low effect sizes in psychiatric genetics, it has been estimated that in order to detect risk variants at a genome-wide level in case-control studies, even more subjects are needed than those included in PGC BD mega-analysis, which might also be underpowered (Sklar et al., 2011). In this perspective, investigating a phenotype with substantially higher effect size would require a much smaller number of subjects. Endophenotype analyses are suggested as potentially promising in this respect, as genetic risk variants are closer to endophenotypes than to the clinical phenotypes. Thus, our fourth study might have had higher statistical power than the former three, although there is no available output in terms of effect size for this study. We also lack information on the nature of the relationship between fMRI activation (in this case in the amygdala) and the clinical BD phenotype; reported abnormalities may result from clinical features as well as from genetic variation. Further, the

CACNA1C risk variant in study IV was selected from a GWAS investigating the BD clinical phenotype, and it is uncertain whether the most significant variants from such a study are more specific at phenoptype or endophenotype level.

As for multiple testing correction, which represents a particular challenge in GWA studies, this was addressed with Bonferroni correction in study II and III, and with the above-mentioned MAX method in study I. In study IV, Family-wise error for the respective ROI (amygdalae) was applied as part of the SPM2 software.

7.3 Implications and future research

The overall implication of the findings in this dissertation is the support of the understanding of BD as a highly heritable and polygenic disorder, where each variant has a small effect, and must interact with other variants in order to give rise to the disease. Moreover, the current works support that most previous studies are probably underpowered, of which reason several thousands of cases and controls are needed to detect true risk variants for BD. Of the same reason, replication of former findings is necessary to identify risk variants with a high level of certainty. In this regard, GWA studies might be hypothesis-generating and provide a basis for further more focused candidate gene studies, investigating specific pathways and potential endophenotypes. Further, despite the identification of several BD genetic risk variants in recent association studies, the explained variance of these variants is small (Sklar et al., 2011). The reason for this is most likely that many other common and rare variants each confer a small increase of risk for developing BD. Hence, even larger genetic association studies are warranted to explain a substantial part of the observed clinical variance.

As we still lack knowledge on the pathophysiological mechanisms underlying BD, several potential pathways have been explored. In this thesis we investigated *DGKH* and the lithium-sensitive phosphatidyl inositol pathway, *PALB2*, *BRCA2* and a DNA repair and

impaired neurogenesis hypothesis, in addition to *ANK3*, *CACNA1C* and the ion channelopathy hypothesis. In the light of the findings in this thesis and recent major GWA studies, *ANK3* and *CACNA1C* are likely to be true BD susceptibility genes, and ion channelopathy is the most probable of these pathways to increase the risk for BD. Our findings also suggest that genetic variants related to ion channelopathy may increase amygdala reactivity, which in turn might be a neurologic mechanism underpinning the clinical symptoms observed and experienced in BD. With regards to genetic overlap between BD and SZ, the studies in this thesis support the hypothesis of a partial overlap, where some genetic variants increase the risk for one diagnostic category or biological mechanism, while others confer susceptibility to a broad spectrum of neuropsychiatric disorders and potentially underlying processes.

In order to gain more knowledge on the genetic susceptibility and endophenotypes in BD, future studies should explore the genetic association with clinical phenotypes as well as with biological markers related to clinical symptoms. Well-powered case-control and case-case association studies and specific diagnostic categorization might determine which genetic variants and pathways are associated with broad phenotypes and which are causatively involved in more specific phenotypic features. Larger endophenotype studies could provide information on how these associations at clinical phenotype level might be explained neurobiologically. The ultimate psychiatric translational study should have the size of the largest current GWA studies, with detailed information on all levels, including DNA, mRNA, protein structure and function, intra- and intercellular mechanisms, brain development, volume and function, neurocognitive functioning, environmental stressors, and detailed clinical information based on structured interviews and observation. This merits thorough description of each subject included in each study, as well as close national and international collaboration and sharing of data.

8 CONCLUSIONS

BD is a highly heritable and polygenic disorder, but few susceptibility genes have been identified, probably due to the small effect size of each variant. In order to obtain sufficient statistical power in genetic association studies, thousands of cases and controls are needed. Additionally, replication studies must be performed to confirm previous findings. Recent GWA studies have identified genetic variants which have generated novel hypotheses, which are suitable for further investigation in focused candidate gene and endophenotype studies. The findings in this thesis make it less likely that genetic variants in *DGKH* are involved in BD pathophysiology, while it increases the possibility that *PALB2, BRCA2* and impaired neurogenesis may play a role, and that *ANK3, CACNA1C* and ion channelopathy, possibly through increased amygdala activation, might underlie BD symptomatology. Furthermore, these findings support a partial genetic overlap between BD and SZ.

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TITLE PAGE

CACNA1C risk variant and amygdala activity in bipolar disorder, schizophrenia and healthy controls

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ABSTRACT

Objectives

Several genetic studies have implicated the *CACNA1C* SNP rs1006737 in bipolar disorder (BD) pathology. This polymorphism was recently found associated with increased amygdala activity in a combined sample of healthy controls and patients with BD. We performed a functional Magnetic Resonance Imaging (fMRI) study in a sample of BD and schizophrenia (SZ) cases and healthy controls to test for altered amygdala activity in carriers of the rs1006737 risk allele (AA/AG), and to investigate if there were differences across the subgroups.

Methods

Rs1006737 was genotyped in 250 individuals (N = 66 BD, 61 SZ and 123 healthy controls), all of Norwegian ethnicity, who underwent an fMRI negative faces matching task. Statistical tests were performed with standard fMRI tools, with a model correcting for sex, age, diagnostic category and medication status in the total sample, and then in each subgroup.

Results

In the total sample, carriers of the risk allele had increased activation in the left amygdala (x = -24, y = -2, z = -14; Z = 3.47), (Family-wise error (FWE) P = 0.026). Subgroup-analyses showed that this effect was significant in the BD group (x = -24, y = 0, z = -14; Z = 3.35) (FWE P = 0.041), but not in the other subgroups.

Conclusions

These results strengthen the hypothesis that *CACNA1C* SNP rs1006737 is involved in amygdala activity during emotional processing. This effect seems most prominent in BD, which further supports that the *CACNA1C* effect on amygdala activity is of importance for BD pathophysiology.

KEY WORDS: ***CACNA1C*****, Bipolar disorder, Schizophrenia, fMRI, Amygdala**

INTRODUCTION

Despite the high heritability estimates for bipolar disorder (BD), the molecular genetic and neurobiological mechanisms for this disorder remain poorly understood. However, recent large genome-wide association (GWA) studies with substantially more statistical power than former psychiatric genetic studies have provided evidence for new and promising candidate genes (1). One of the most consistent findings is related to *CACNA1C* (calcium channel, voltage-dependent, L type, alpha 1C subunit) gene variants. The *CACNA1C* SNP rs1006737 was first found to be associated with BD in a combined analysis of two GWA study datasets ($P = 3.15 \times 10^{-6}$) (2). When adding a third sample, this *CACNA1C* SNP was significantly associated with BD in the combined sample of 4,387 BD cases and 6,209 healthy controls ($P = 7.0 \times 10^{-8}$, OR = 1.181) (3). Subsequent studies also reported association between *CACNA1C* and schizophrenia (SZ) (4,5) and major depressive disorder (MDD) (4,6), thereby supporting the hypothesis of genetic overlap between these severe psychiatric disorders.

CACNA1C is located on chromosome 10 and encodes an alpha-1 subunit of a voltage-dependent calcium ($Ca_v1.2$) channel. $Ca_v1.2$ channels can be found in cardiac smooth muscle, neuronal and endocrine cells, where they have a variety of functions including excitation-contraction coupling, endocrine secretion and regulation of neuronal Ca^{2+} transients, enzyme activity and transcription (7,8). Mutations in *CACNA1C* have been found to lead to Timothy syndrome, a lethal disorder consisting of somatic symptoms like cardiac arrhythmia, and psychiatric symptoms like autism and cognitive disability (9).

Emotional dysregulation is part of the clinical phenotype of BD and SZ, manifesting as mood swings as well as affect flattening. Neurobiological correlates of emotional dysregulation have been reported for both these disorders, in structural Magnetic Resonance Imaging (sMRI) and functional MRI (fMRI) analyses measuring the volume and activity of the limbic system. In particular, the amygdala has been extensively studied in BD. In a recent

mega-analysis of 321 patients with bipolar I disorder and 442 healthy controls, amygdala volume was found to be greater in patients treated with lithium compared to controls and patients not treated with lithium (10). A meta-analysis comprising 65 fMRI studies of 1074 healthy volunteers and 1040 BD cases, found evidence for amygdala over-activation in euthymic BD patients compared with healthy controls (11).

With regards to the potential involvement of BD risk genes in limbic system dysregulation, there are reports of an association between *CACNA1C* SNP rs1006737 and amygdala activity. In a study of 64 healthy individuals, carriers of the risk allele (AA/AG) had increased activity in the right amygdala in a monetary reward paradigm (12). Another group reported enhanced activity in AA/AG individuals compared to GG individuals in the right amygdala during a fearful faces paradigm (N = 41 BD patients, 25 relatives and 50 healthy controls) (13). Thus, there is some evidence supporting the hypothesis that *CACNA1C* SNP rs1006737 affect amygdala activity during different paradigms related to limbic system functioning.

Interestingly, two recent mega-analyses reported that *CACNA1C* was one out of three common genes for BD and SZ (14,1). These findings on the molecular genetic level are consistent with similarities in clinical and cognitive characteristics (15), and the continuum hypothesis for severe psychiatric disorders (16). However, to the best of our knowledge *CACNA1C* has not been investigated with fMRI in SZ. Thus, it remains unclear whether the effect of this gene on amygdala activity is general or confined to one or more diagnostic categories.

The primary aim of the current study was to test for altered amygdala activity in carriers of the *CACNA1C* SNP rs1006737 risk allele. Secondarily, we aimed to determine the specificity of such associations, by testing for potential differences between healthy controls and patients with BD or SZ. Therefore, we measured fMRI amygdala blood-oxygen-level

dependence (BOLD) responses during a faces matching paradigm (17) in 250 genotyped individuals of Norwegian origin, including 66 BD cases, 61 SZ cases and 123 healthy control subjects.

MATERIALS AND METHODS

Sample characteristics

The total number of individuals in this study was 250, including 66 BD cases, 61 SZ cases and 123 healthy control subjects. All participants were of Norwegian origin, part of the ongoing TOP (Thematic Organized Psychosis) Study, and included from 2003 to 2009.

To be included in the study, patients had to be between 18 and 65 years, have a DSM-IV diagnosis of a bipolar spectrum or schizophrenia spectrum disorder, and be willing and able to provide written informed consent. Exclusion criteria were an IQ score below 70 and reporting a history of head injury or neurological disorder. In the healthy control group, we also excluded subjects if they or their close relatives had a lifetime history of a severe psychiatric disorder. Subjects with a history of a medical condition potentially interfering with brain function (hypothyroidism, uncontrolled hypertension and diabetes), and an illicit drug abuse/addiction diagnosis were also excluded.

Patients were recruited from psychiatric in- and out-patient hospital units in the Oslo area, and had been diagnosed with bipolar I disorder (N = 30), bipolar II disorder (N = 32), bipolar disorder not otherwise specified (N = 4)), schizophrenia (N =48), schizoaffective disorder (N = 9) or schizophreniform disorder (N = 4), according to DSM-IV using the Structural Clinical Interview for DSM-IV (SCID) (18). Diagnostic evaluation was performed by trained psychologists and psychiatrists, of whom all participated regularly in diagnostic meetings supervised by professors in psychiatry. Reliability measures of the diagnostic assessment in the TOP study were performed, and the overall agreement for the DSM-IV diagnostic categories tested was 82 % and the overall Kappa 0.77 (95 % CI: 0.60-0.94). Information on education, age of onset, number of relapses, medication status, alcohol and illegal substance abuse were obtained during an initial clinical interview. Neurocognitive and psychosocial functioning was also assessed with clinical interviews. On the day of scanning,

patients underwent an abbreviated re-interview including Young Mania Rating Scale (YMRS) (19), Inventory of Depressive Symptoms (IDS) (20) and Positive and Negative Syndrome Scale (PANSS) (21). For patients lacking data for this re-interview, we used data from the clinical interview.

The healthy control subjects were randomly recruited from the same catchment area as the patients, and underwent an initial interview where demographic and clinical information was obtained. Clinical assessment of the patients and healthy controls participating in the TOP study is described in details in a previous report (22). Demographic and clinical characteristics are presented in Table 1.

The Norwegian Scientific-Ethical Committees and the Norwegian Data Protection Agency approved the study. All subjects have given written informed consent prior to inclusion into the project.

Genotyping

Genomic DNA was extracted from whole blood. *CACNA1C* SNP rs1006737 was genotyped in the 250 subjects participating in this study using Affymetrix Gene Chip Genome-Wide SNP 6.0 array (AffymetrixInc, Santa Clara, CA, USA), as described in details elsewhere (23,24).

Experimental paradigm

A widely used and validated paradigm was employed to elicit amygdala reactivity (17). In this task participants select which of two stimuli (displayed at the bottom of the screen) matches a target stimulus (displayed at the top). The images displayed were either human faces expressing anger or fear (faces matching task) or geometrical shapes (the sensorimotor control task). Participants completed 4 blocks of the faces matching task, where each block consisted

of 6 emotion-specific face trios derived from a standard set of facial affect pictures (25). Interleaved between these blocks, participants completed 5 blocks of the sensorimotor control task. Each trial (faces or shapes) was presented for 5.4 seconds with no inter-stimulus interval, for a total block length of 32.6 seconds. The total paradigm lasted 310 seconds. E-prime software (version 1.0 Psychology Software Tools, Inc, Pittsburgh, PA, USA) controlled the presentations of the stimuli using VisualSystem (NordicNeuroLab, Bergen, Norway). Response times and accuracy were recorded through MR-compatible ResponseGrips (NordicNeuroLab, Bergen, Norway). Behavioural data was missing for 13 individuals.

Image acquisition

MRI scans were acquired on a 1.5 T Siemens Magnetom Sonata scanner (Siemens Medical Solutions, Erlangen, Germany) supplied with a standard head coil. Volumes (n = 152, 24 axial slices, 4 mm thick with 1 mm gap) covering the whole brain were acquired in the axial plane, using a BOLD EPI sequence (TR=2040 ms, TE=50ms, flip angle=90º, matrix 64 x 64, FOV 192 x 192 mm). The first seven volumes were discarded. Prior to BOLD fMRI scanning, a sagittal T1-weighted 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) scan (TR= 2000 ms, TE=3.9 ms, flip angle =7º, matrix 128 x 128, FOV 256 x 256 mm) was collected for better localization of functional data.

fMRI data analyses

All fMRI volumes were preprocessed and analysed with Statistical Parametric Mapping (SPM2) (http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB7.1 (The Mathworks Inc, Natick, Massachusetts). All of the functional images were realigned to the first image in the time series to correct for head motion (26). All subjects moved less than 2.5 mm in any direction during the scan. Subsequently, the mean functional image and the anatomical image

were coregistered to ensure that they were aligned. The images were spatially normalized to the stereotactical MNI template (26), and resampled at 2x2x2 mm voxels. The images were smoothed using a 6 mm full width-half maximum (FWHM) isotropic kernel. Data were high-pass filtered using a cutoff value of 128 s. The fMRI data for all subjects were first analysed using a single-subject fixed-effect model. The model was built by convolving boxcar functions for the onsets of the two different conditions (faces and figures) with a canonical hemodynamic response function (HRF). Individual contrast images were created by subtracting "figures" from "faces". The contrast images for faces versus figures for each subject were entered into a random effects statistical model. These data were analysed with a region of interest (ROI) approach and a pre-defined anatomical mask (bilateral amygdala) derived from the Wake Forest University PickAtlas for SPM2 (27).

Statistical analysis

For the overall sample (N = 250), we used an ANCOVA model comparing amygdala activity in risk-allele carriers (AA/AG) with the corresponding activity in carriers of the protective allele (GG), using sex, age, diagnostic category (BD, SZ, healthy control) and medication status as covariates. Medication status was dichotomised for the categories Antipsychotics, Lithium, Antidepressants, Anticonvulsives and Hypnotics. We also tested for potential specific effects in each diagnostic subcategory, for genotype x diagnosis interactions, and for effect of diagnostic category on amygdala activity. Findings were corrected for multiple testing with Family-Wise Error (FWE) within the respective ROI.

RESULTS

Behavioural results

Genotype group and diagnosis did not influence the accuracy rate significantly. The mean response time (RT) was significantly longer for individuals in the BD group (RT = 1255 milliseconds (ms)) and SZ group (RT = 1231 ms) than those in the healthy control group (RT = 1065 ms) ($P < 0.001$), but did not differ significantly with respect to genotype group. For details, see Table 1.

Sociodemographic and clinical results

Carriers of the risk allele did not differ significantly from the GG homozygotes with respect to demographical variables within the total sample or any of the diagnostic groups (Table 1). Further, the clinical characteristics did not differ between genotype groups, except that risk allele carriers in the total patient sample had significantly lower Global Assessment of Functioning-symptom score (GAF-S) than those with the GG genotype ($P = 0.01$). However, this was not seen in the subgroups, and was probably due to a higher frequency of the risk allele in the SZ group (43/61 (70.5 %)) compared to the BD group (34/66 (51.5 %)). For details regarding results across diagnostic categories, see Table 1.

fMRI results

The fMRI results are shown in Table 2 and Figure 1. In the total sample (N = 250), carriers of the *CACNA1C* SNP rs1006737 risk allele (AA/AG) showed significantly increased activation in the left amygdala (x = -24, y = -2, z = -14; Z = 3.47; cluster-size = 72), with an FWE-corrected P-value of 0.026. The risk allele was also significantly associated with enhanced activity in the left amygdala (x = -24, y = 0, z = -14; Z = 3.35; cluster-size = 91) in the BD subgroup (FWE-corrected $P = 0.041$). There were also AA/AG associated activations in the

right amygdala in the total sample (x = 26, y = 0, z = -16, Z = 2.65, cluster-size = 32) and in the BD subgroup (x = 22, y = 0, z = -20, Z = 2.54, cluster-size = 61), but these did not reach significance level. There were no significant findings in the SZ subgroup or the healthy control group, but the activation patterns had the same direction in all subgroups, of which reason there was no significant genotype x diagnosis interaction effect between BD and the other subgroups. Furthermore, there was no significant effect of diagnosis on amygdala activity.

DISCUSSION

The main finding in this study was an enhanced activation in the left amygdala in carriers of the *CACNA1C* SNP rs1006737 risk allele. This effect was also significant in the BD subgroup, but did not reach significance level in the SZ or healthy control subgroup.

To the best of our knowledge, no studies of amygdala activity and *CACNA1C* polymorphisms have investigated the specificity in BD. We found a significant association in the BD subgroup, with an even larger cluster size (k = 91) than in the total sample (k = 72. The fact that the most significant association was found in the BD sample, might indicate a stronger effect for this *CACNA1C* polymorphism in amygdala in BD than in SZ and healthy controls, although there was no significant diagnosis x genotype interaction. Interestingly, recent GWA studies found the presently investigated SNP to be significantly associated with BD ($P = 1.7 \times 10^{-5}$; OR = 1.11) as well as SZ ($P = 1.2 \times 10^{-6}$; OR = 1.11) (14,1). This suggests susceptibility of similar effect sizes on the clinical phenotype level, while the present results indicate different specificity with regards to amygdala activity. This hypothesis is underpinned by the fact that other genes involved in calcium channel regulation have been found to be associated with neurological disorders such as migraine, ataxia and epilepsy (28). Thus, genetically conditioned calcium channel dysregulation might be a common mechanism increasing the risk for developing several neuropsychiatric disorders, by affecting various brain structures including amygdala.

The current finding that *CACNA1C* SNP rs1006737 risk allele is associated with increased amygdala activity is in line with two previous studies (13,12). One of the studies found that individuals with the risk allele have increased activity in the right amygdala during a reward paradigm (12), and the other reported increased activity in the right amygdala during a fear-face paradigm (13). In the current study, we found significantly enhanced activation in the left amygdala during a negative faces paradigm. But there was also nominally

significantly increased activity in the right amygdala in our sample (Table 2). Taken together, previous and present results suggest that carriers of the *CACNA1C* SNP rs1006737 A allele might have increased activity in amygdala bilaterally during processing of emotional and reward stimuli.

As this increased activity has been reported for different paradigms, it is uncertain whether these responses reflect different neurobiological pathways, like positive and negative conditioning, or whether this enhanced response to emotional stimuli with different valences indicates that amygdala is occupied with relevance detection in general, rather than with only fear-related or reward-related information (29,30). Furthermore, it remains unclear if amygdala over-activation is a cause or secondary effect of disease-related processes, and how this over-activation is causally related to prefrontal functioning, as there is evidence of reduction of volume (31) as well as activity (11) in the prefrontal cortex in BD. It is possible that enhanced amygdala activation is a primary biological feature of BD, but it may also be a consequence of decreased prefrontal inhibition, which in turn may result from neurodevelopmentally conditioned prefrontal volume reduction. A combination of these two neurobiological processes should also be taken into consideration, and the relative contribution could be different in SZ and BD. Additionally, we lack knowledge on how environmental factors may modulate the neurobiological aspects of severe psychiatric disorders like BD and SZ. Pharmacological treatment could also affect this relationship, but we found no effect of ongoing treatment in the current sample.

Genetic variations in *CACNA1C* have been shown to imply additional psychiatric manifestations to those observed in BD and SZ. Patients with the above-mentioned Timothy syndrome are characterized by symptoms like autism and cognitive disability. In a proposed model of a spectrum of psychiatric disorders with autism in the neurodevelopmental end and MDD in the affective end (16), it is possible that polymorphisms in or around the *CACNA1C*

gene could increase the risk of developing less severe conditions than those observed in Timothy syndrome, like BD and SZ. This is further supported by the fact that rs1006737 is situated in one of the introns of *CACNA1C,* thus probably affecting pathophysiological pathways related to these disorders by regulating the expression of the protein, and not by altering the structure, as is the case with the de novo mutations in Timothy syndrome, which are located in one of the exons (32).

As for the potential pharmacological consequences of the association between *CACNA1C* and BD and SZ, calcium channel blockers have been studied in affective disorders and substance abuse/dependence (33). Some promising preliminary results have been reported for dihydropyridine-based blockers that bind specifically to $Ca_v1.2$ channels in the treatment of BD, but more studies are needed (34). Future pharmacological studies could investigate the potentially modulating effect of calcium channel blockers on the endophenotype level, like for example amygdala activity in BD.

Taken together, the current findings provide evidence that the *CACNA1C* SNP rs1006737 is involved in neurobiological processes underpinning severe psychiatric disorders, most pronounced in BD. The present study further strengthens the hypothesis that these mechanisms may include increased amygdala activity.

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Table 1

Demographic data and clinical characterization for individuals genotyped for rs1006737 and participating in a negative faces functional MRI study

	Clinical group				Genotype			Clinical group x genotype								
	BD	SZ	CTR		AA + AG	GG		BD			SZ			CTR		
				P			P	AA + AG	GG	P	AA + AG	GG	P	AA + AG	GG	P
N (Females, %)	66 (57.6)	61 (36.1)	123 (43.9)	0.05	148 (43.2)	102 (49.0)	0.37	34 (52.9)	32 (62.5)	0.43	43 (34.9)	18 (38.9)	0.77	71 (43.7)	52 (44.2)	0.95
Mean age (SD)[a]	34.8 (10.6)	33.7 (8.6)	34.6 (9.0)	0.77	33.9 (8.5)	35.2 (10.4)	0.30	34.2 (10.0)	35.5 (11.4)	0.62	33.5 (8.5)	34.1 (9.0)	0.81	34.0 (7.8)	35.3 (10.4)	0.43
Education (years), mean (SD)	14.1 (3.0)	13.7 (2.5)	14.3 (2.3)	0.32	14.2 (2.5)	13.9 (2.7)	0.32	13.9 (3.0)	14.4 (3.0)	0.52	14.0 (2.4)	13.1 (2.4)	0.21	14.6 (2.1)	13.9 (2.5)	0.12
WASI, mean (SD)	109.4 (11.5)	108.2 (13.7)	115.9 (10.0)	0.00	110.9 (12.6)	113.4 (10.3)	0.10	107.4 (12.7)	111.7 (9.7)	0.14	107.7 (14.1)	109.5 (13.2)	0.64	114.5 (10.6)	115.8 (9.2)	0.46
IDS, mean (SD)	15.4 (13.1)	11.7 (11.5)		0.10	13.2 (12.4)	14.2 (12.6)	0.64	13.8 (12.4)	17.0 (13.9)	0.33	12.7 (12.6)	9.4 (8.4)	0.32			
YMRS, mean (SD)	1.9 (3.5)	1.4 (4.0)		0.45	1.6 (3.7)	1.8 (3.9)	0.72	1.4 (1.9)	2.4 (4.6)	0.27	1.7 (4.6)	0.8 (1.6)	0.43			
PANSS total positive score, mean (SD)	9.5 (3.3)	13.0 (6.1)		0.00	11.8 (5.7)	10.2 (4.1)	0.10	9.5 (3.6)	9.5 (3.0)	0.96	13.6 (6.4)	11.4 (5.4)	0.22			
GAF-S, mean (SD)	58.2 (9.5)	42.6 (10.6)		0.00	48.5 (13.0)	54.2 (11.2)	0.01	57.4 (10.1)	59.0 (8.9)	0.49	41.5 (10.7)	45.7 (9.5)	0.16			
GAF-F, mean (SD)	55.3 (12.2)	44.4 (9.9)		0.00	49.0 (13.0)	51.7 (11.2)	0.23	55.3 (13.3)	55.4 (11.2)	1.00	44.0 (10.5)	45.2 (7.9)	0.67			
Age of onset, mean (SD)	21.2 (8.6)	24.8 (7.8)		0.02	23.3 (8.2)	22.4 (8.6)	0.57	20.9 (8.5)	21.7 (8.7)	0.71	25.2 (7.5)	23.8 (8.5)	0.53			
Duration of illness, mean (SD)	18.1 (10.3)	13.1 (6.2)		0.00	14.9 (8.4)	17.1 (9.5)	0.17	17.8 (9.9)	18.4 (10.9)	0.79	12.6 (6.3)	14.7 (5.8)	0.24			
No. of depressive episodes, mean (SD)	7.3 (12.9)	2.3 (6.0)		0.01	5.4 (12.7)	4.1 (5.3)	0.53	8.9 (17.2)	5.7 (5.8)	0.33	2.7 (6.9)	0.9 (0.9)	0.32			
No. of manic episodes, mean (SD)	1.5 (2.8)	0.1 (0.3)		0.00	0.6 (1.5)	1.2 (2.9)	0.15	1.2 (2.0)	1.8 (3.5)	0.86	0.1 (0.4)	0.1 (0.2)	0.68			
No. of hypomanic episodes, mean (SD)	14.0 (38.0)	0.0 (0.1)		0.01	5.8 (19.3)	9.6 (38.3)	0.47	13.2 (27.6)	14.8 (47.1)	0.40	0.0 (0.2)	0.0 (0.0)	0.53			
Alcohol abuse[b], n (%)	8 (12.0)	5 (8.2)		0.47	10 (13.2)	3 (6.0)	0.20	6 (17.6)	2 (6.3)	0.16	4 (9.3)	1 (5.6)	0.63			
Use of illegal substances[b], n (%)	4 (6.1)	7 (11.5)		0.28	7 (9.2)	4 (8.0)	0.82	3 (8.8)	1 (3.1)	0.33	4 (9.3)	3 (16.7)	0.41			
Accuracy rate, faces (%)	98.1 (10.6)	97.9 (8.4)	99.2 (29.3)	0.25	98.7 (7.3)	98.5 (6.7)	0.86	96.8 (14.5)	99.6 (12.9)	0.32	98.8 (31.6)	95.2 (15.5)	0.17	99.4 (19.0)	98.8 (39.3)	0.24
Response time (ms)	1255 (355)	1231 (46)	1065 (224)	0.00	1153 (313)	1145 (293)	0.85	1252 (463)	1258 (279)	0.95	1217 (258)	1245 (433)	0.78	1072 (227)	1054 (221)	0.67

Abbreviations: BD, bipolar disorder; SZ, schizophrenia; CTR, controls; SD, standard deviation; WASI, Wechsler Abbreviated Scale of Intelligence; IDS, Inventory of Depressive Symptoms; YMRS, Young Mania Rating Scale; PANSS, Positive and Negative Syndrome Scale; GAF-S, Global Assessment of Functioning–symptom score; GAF-F, Global Assessment of Functioning–function score; ms, milliseconds.

[a]Mean age at fMRI scanning

[b]Last six months

Table 2

Results for *CACNA1C* SNP rs1006737 (AA+AG>GG) effect on amygdala activation in a sample of bipolar disorder and schizophrenia cases and healthy controls

Hemisphere	Group	Participants (n)	x	y	z	Cluster size	Z	FWE
Left	Total sample	250	-24	-2	-14	72	3.47	0.026
	BD	66	-24	0	-14	91	3.35	0.041
	SZ	61	-	-	-	-	-	-
	CTR	123	-24	-2	-12	16	2.51	n.s.
Right	Total sample	250	26	0	-16	32	2.65	n.s.
	BD	66	22	0	-20	61	2.54	n.s.
	SZ	61	24	-4	-16	26	2.24	n.s.
	CTR	123	28	4	-16	2	1.96	n.s.

Abbreviations: BD, bipolar disorder; SZ, schizophrenia; CTR, healthy controls; FWE, Family-wise error rate. Only nominally significant results (P = <0.05) are shown.

Figure 1

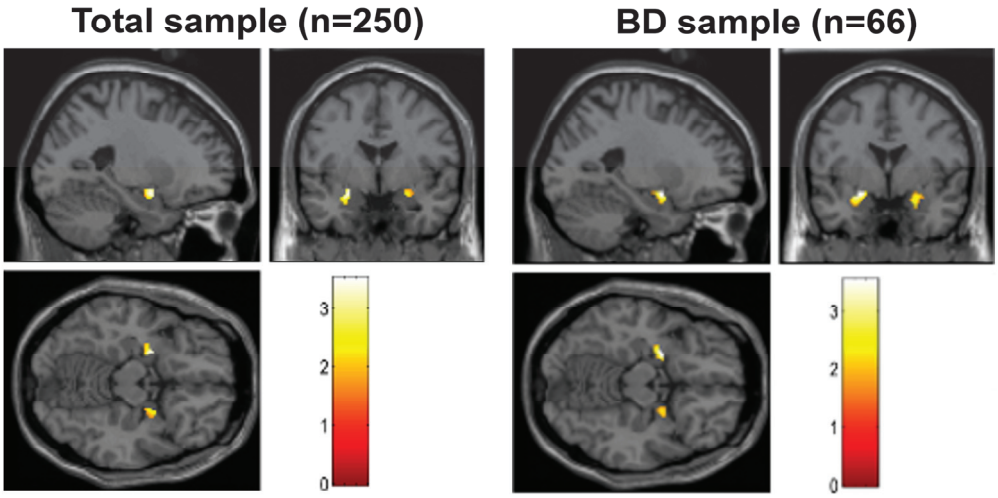

Fig. 1. Carriers of the *CACNA1C* SNP rs1006737 risk allele A have significantly increased activity in the left amygdala in the total sample (x = -24, y = -2, z = -14; FWE P = 0.026) and BD subgroup (x = -24, y = 0, z = -14; FWE P = 0.041), and non-significantly increased activity in the right amygdala in the total sample (x = 26, y = 0, z = -16) and BD subgroup (x = 22, y = 0, z = -20) compared with GG homozygotes during a negative faces paradigm.
Abbreviations: BD, bipolar disorder; FWE, family-wise error.