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Genetic determinants of white matter integrity in bipolar disorder

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Declaration

I declare that this thesis has been written by me and that the work presented herein is my own. This work has not been submitted for any other degree or professional qualification.

The Bipolar Family Study was designed by Andrew McIntosh and participants were recruited by Andrew McIntosh, Jess Sussmann and associated clinicians. Clinical assessments and psychiatric interviews were completed by Andrew McIntosh, Jess Sussmann, Jeremy Hall and April Clugston. Neuropsychology data were collected by our research group at the Division of Psychiatry (James McKirdy, Tiffany Stewart, Holly Redpath, Anna Peel, and myself). The protocol and sequences for the diffusion MRI scans were designed by Mark Bastin at the Division of Clinical Neurosciences in the Western General Hospital. Genotyping of whole blood samples was performed at the Wellcome Trust Clinical Research Facility at the Western General Hospital. My main contribution to this study, as presented in this thesis, is in the analysis and interpretation of the DTI data.

Publications

The following articles are based on the work presented in Chapters 3, 5 and 6.

Sprooten, E., Sussmann, J. E., Clugston, A., Peel, A., McKirdy, J., Moorhead, T. W. J., Anderson, S., et al. (2011). White matter integrity in individuals at high genetic risk of bipolar disorder. *Biological Psychiatry*, *70*(4), 350-356.

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Sprooten, E., McIntosh, A. M., Lawrie S. M., Hall J., Sussmann J. E., Dahmen N., Konrad A., Bastin M. E., Winterer G. A genome-wide supported psychosis variant in *ZNF804A* is not associated with white matter integrity in the human brain. *Magnetic Resonance Imaging* (In Review).

Abstract

Bipolar disorder is a heritable psychiatric disorder, and several of the genes associated with bipolar disorder and related psychotic disorders are involved in the development and maintenance of white matter in the brain. Patients with bipolar disorder have an increased incidence of white matter hyper-intensities, and quantitative brain imaging studies collectively indicate subtle decreases in white matter density and integrity in bipolar patients. This suggests that genetic vulnerability to psychosis may manifest itself as reduced white matter integrity, and that white matter integrity is an endophenotype of bipolar disorder. This thesis comprises a series of studies designed to test the role of white matter in genetic risk to bipolar disorder by analysis of diffusion tensor imaging (DTI) data in the Bipolar Family Study. Various established analysis methods for DTI, including whole-brain voxel-based statistics, tract-based spatial statistics (TBSS) and probabilistic neighbourhood tractography, were applied with fractional anisotropy (FA) as the outcome measure. Widespread but subtle white matter integrity reductions were found in unaffected relatives of patients with bipolar disorder, whilst more localised reductions were associated with cyclothymic temperament. Next, the relation of white matter to four of the most prominent psychosis candidate genes, *NRG1*, *ErbB4*, *DISC1* and *ZNF804A*, was investigated. A core haplotype in *NRG1*, and three of the four key single nucleotide polymorphisms (SNPs) within it, showed an association with FA in the anterior thalamic radiations and the uncinate fasciculi. For the three SNPs considered in *ErbB4*, results were inconclusive, but this was consistent with the background literature. Most notable however, was a clear association of a non-synonymous *DISC1* SNP, Ser704Cys, with FA extending over most of the white matter in the TBSS and voxel-based analyses. Finally, FA was not associated with a genome-wide supported risk SNP in *ZNF804A*, a finding which could not be attributed to a lack of statistical power, and which contradicts a strong, but previously untested hypothesis. Whilst the above results need corroboration from independent studies, other studies are needed to address the cellular and molecular basis of these findings. Overall, this work provides strong support for the role of white matter integrity in genetic vulnerability to bipolar disorder and the wider psychosis spectrum and encourages its future use as an endophenotype.

Abbreviations

BD: bipolar disorder

HR: high risk group (in figures and tables)

HC: control group (in figures and tables)

ALIC: anterior limb of internal capsule

AF: arcuate fasciculus

UF: uncinate fasciculus

CING: cingulum bundle

ATR: anterior thalamic radiations

CST: cortico-spinal tract

MRI: magnetic resonance imaging

DTI: diffusion tensor imaging

FA: fractional anisotropy

SVC: small-volume correction

ROI: region of interest

FWE: family-wise error

FDR: false discovery rate

IQ: intelligence quotient

FWHM: Full-width at half-maximum

SNP: single nucleotide polymorphism

GWAS: genome-wide association study

NRG1: neuregulin-1

ErbB4: v-erb-a erythroblastic leukemia viral oncogene homolog 4

DISC1: disrupted-in-schizophrenia-1

ZNF804A: zinc finger protein 804A

CACNA1C: voltage-dependent calcium channel L type alpha 1C subunit

ANK3: ankyrin 3

BDNF: brain-derived neurotrophic factor

SLC6A4: solute carrier family 6 (serotonin transporter)

DAOA: D-amino acid oxidase activator

DGKH: diacylglycerol kinase eta

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Chapter 1

Background

1.1 Introduction

Bipolar disorder (BD) is an affective disorder characterised by alternating periods of depression and mania. These periods of extreme mood typically last for weeks to months, and periods of mixed mood are also commonly experienced. During manic episodes, patients tend to be disinhibited with poor self-judgement resulting in risk taking and impulsivity, and domineering and discourteous social behaviour. Depressive episodes encompass feelings of worthlessness and hopelessness, lack of pleasure and motivation and social withdrawal. In BD, depressive episodes are associated with a suicide risk as high as 15%, which is higher than for unipolar depression (Müller-Oerlinghausen, 2002). The severity, range and frequency of BD symptoms can vary widely between patients, and the boundaries between healthy mood fluctuations and mild mood disorder is determined by the degree of functional impairment in addition to affective symptoms *per se*. Furthermore, despite a presumed predominance of affective symptoms, BD is not always clearly distinguishable from schizophrenia or other psychotic disorders and patients with BD commonly present with delusions and sometimes hallucinations. The current diagnostic criteria for manic and depressive episodes according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) are summed up in Table 1.1. BD is categorised in bipolar I and bipolar II disorder. A diagnosis of bipolar I disorder requires at least one manic or mixed episode, whilst in bipolar II disorder patients have had at least one major depressive episode and at least one hypomanic episode, a milder form of mania (Table 1.1).

Clearly, the symptoms of BD have a substantial impact on the patient's quality of life, his/her family and relationships, and professional ambitions. In between episodes, patients may be able to function well, and when treated successfully these euthymic periods can last for many years. Subclinical mood swings, cognitive disabilities and personality characteristics associated with the disorder or its medication, are likely to persist in spite of effective treatment. Although mood-stabilising medications, often combined with anti-psychotics and/or psychotherapy, are successful in eliminating full-blown mood and psychotic episodes in up to 60% of patients (Geddes *et al*., 2004), the course of BD tends to be life-long. The underlying causes of BD are poorly understood, and this is the main obstacle for developing more effective treatments.

Table 1.1: DSM-IV diagnostic criteria for episodes of mood disorder.

Criteria for Major Depressive Episode

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.

1) depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others.
2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)
3) significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.
4) insomnia or hypersomnia nearly every day
5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
6) fatigue or loss of energy nearly every day
7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

B. The symptoms do not meet criteria for a Mixed Episode.

C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

D. The symptoms are not due to the direct physiological effects of a substance or a general medical condition.

E. The symptoms are not better accounted for by Bereavement

Criteria for Manic Episode

A) A distinct period of abnormally and persistently elevated, expansive or irritable mood, lasting at least 1 week (or any duration if hospitalization is necessary)

B) During the period of mood disturbance, three (or more) of the following symptoms have persisted (four if the mood is only irritable) and have been present to a significant degree:

1) inflated self-esteem or grandiosity
2) decreased need for sleep (e.g., feels rested after only 3 hours of sleep)
3) more talkative than usual or pressure to keep talking
4) flight of ideas or subjective experience that thoughts are racing
5) distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli)
6) increase in goal-directed activity (at work, at school, or sexually) or psychomotor agitation)
7) excessive involvement in pleasurable activities that have a high potential for painful consequences

C) The symptoms do not meet criteria for a Mixed Episode

D) The mood disturbance is sufficiently severe to cause marked impairment in occupational functioning or in usual social activities or relationships with others, or to necessitate hospitalization to prevent harm to self or others, or there are psychotic features.

E) The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication or other treatment) or a general medical condition (e.g., hyperthyroidism)

Table 1.1 (continued)

Criteria for Hypomanic Episode A) A distinct period of persistently elevated, expansive or irritable mood, lasting throughout at least 4 days, that is clearly different from the usual non-depressed mood. B) During the period of mood disturbance, three (or more) of the following symptoms have persisted (four if the mood is only irritable) and have been present to a significant degree: 1) inflated self-esteem or grandiosity 2) decreased need for sleep (e.g., feels rested after only 3 hours of sleep) 3) more talkative than usual or pressure to keep talking 4) flight of ideas or subjective experience that thoughts are racing 5) distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli) 6) increase in goal-directed activity (at work, at school, or sexually) or psychomotor agitation 7) excessive involvement in pleasurable activities that have a high potential for painful consequences C) The episode is associated with an unequivocal change in functioning that is uncharacteristic of the person when not symptomatic. D) The disturbance in mood and the change in functioning are observable by others. E) The mood disturbance not severe enough to cause marked impairment in social or occupational functioning, or to necessitate hospitalization, and there are no psychotic features. F) The symptoms are not due to the direct physiological effects of a substance or a general medical condition.
Criteria for Mixed Episode A. The criteria are met both for a Manic Episode and for a Major Depressive Episode (except for duration) nearly every day during at least a 1-week period. B. The mood disturbance is sufficiently severe to cause marked impairment in occupational functioning or in usual social activities or relationships with others, or to necessitate hospitalization to prevent harm to self or others, or there are psychotic features. C. The symptoms are not due to the direct physiological effects of a substance, or a general medical condition.

Environmental and social factors undoubtedly contribute to the development of BD, but it is evident from post-mortem and brain imaging studies that BD is associated with abnormal brain structure and function. A wealth of magnetic resonance imaging (MRI) studies have demonstrated reductions in total brain volume, enlarged ventricles and increased volumes of certain sub-cortical structures. From meta-analyses of more than 250 studies, however, it appears that regional abnormalities in grey matter volume and density are less consistent (Arnone *et al*., 2009; Kempton *et al*., 2008; Hallahan *et al*., 2011; McDonald, Zanelli *et al*. 2004). Over the last decades, MRI studies as well as histological post-mortem research across all disciplines, have focused predominantly on grey matter despite early findings of white matter hyper-intensities in BD. Recently, advances in imaging techniques such as functional connectivity and diffusion tensor imaging (DTI), along with a changing climate in neuroscience appreciating the brain as a functionally integrated system instead of a modular one, have encouraged research in functional

and structural connectivity. Psychotic and mood symptoms are increasingly understood in terms of deficient connectivity between brain regions, and evidence for compromised white matter structure in BD is compelling.

There is also a significant genetic component to the causes of BD, as shown in twin, adoption and large pedigree studies. Although this is promising as treatment advances in many genetically complex medical disorders have greatly benefited from understanding their genetic causes, genetic research in psychiatric disorders seems to require more than classical association or linkage studies to discover genetic and molecular mechanisms. Measures that reflect brain structure or function can be useful as *endophenotypes*, which are assumed to be biologically closer and more sensitive to genetic variation than the clinical phenotype and which can assist to increase our understanding of the biological mechanisms underlying genetic risk.

In this thesis I explore the relationship between genetic predisposition to BD and white matter integrity by means of DTI and the application of some of the most recent analysis techniques, including tract-based spatial statistics (TBSS) and tractography. These analyses are applied to a large sample of individuals with or without first-degree family member with BD. Genetic predisposition to BD was considered in two different ways: (1) having at least one first-degree relative with the disorder, and (2) carrying a risk-allele of a specific psychosis candidate gene.

1.2 Genetics of bipolar disorder

1.2.1 Heritability

Identical twins of BD patients have an estimated 40%-70% risk to develop BD, whereas the risk for dizygotic twins and non-twin siblings lies between 5.4% and 8% (Cardno *et al*., 2002; Craddock, O'Donovan & Owen., 2009; Gershon *et al*., 1982; Lichtenstein *et al*., 2009; McGuffin *et al*., 2003), compared to a general lifetime incidence of 0.5%-2% (Kessler, 1994). This translates to a heritability, defined as the variation in liability to BD attributable to genetic factors (h^2), of around 80% (Gershon *et al*., 1982; McGuffin *et al*., 2003). There is marked overlap in heritability between unipolar depression and BD (Gershon *et al*., 1982; McGuffin *et al*., 2003), as well as between schizophrenia, bipolar and schizoaffective disorders, which is determined by genetic factors rather than shared environmental factors, as documented by twin (Cardno *et al*., 2002) and adoption (Lichtenstein *et al*., 2009) studies. This shared genetic risk is also supported

by the observation that most of the well-established risk genes are not specific to a single psychotic disorder (Craddock, O'Donovan & Owen, 2006, 2009). Thus a large portion of genetic liability appears to be general to the "functional psychoses", while environmental factors are likely to influence which specific symptoms will or will not develop (Lichtenstein *et al.*, 2009). The distinction between environmental and genetic factors in these models is of course a complex issue since they interact and co-vary on many levels. For example substance abuse can obviously be considered "environmental", but is also heritable in its own right (Bienvenu *et al*., 2011) and is one of the most familial comorbidities of BD (Schulze *et al*., 2009).

1.2.1 Linkage and association studies

The well-documented high heritability for BD naturally prompts the search for the specific genetic loci that confer this liability. Generally, there are two ways of identifying genes related to a disorder or illness: through linkage in family-based study designs or through association in the general population. Briefly, in linkage studies one looks for co-segregation between a clinical phenotype and genetic markers spread across the genome. Linkage studies require the recruitment of one or more affected families, and can initially only detect larger chromosomal candidate regions and subsequent sequencing or association is required to map the co-segregation pattern to a specific locus. Genetic association studies simply compare the frequency of an allele or haplotype between unrelated affected and non-affected individuals. In the past, single nucleotide polymorphisms (SNPs) had to be selected *a priori*, but with the advance of genome-wide association studies (GWAS) the whole genome can be tested for association to a phenotype by tagging hundreds of thousands of polymorphisms divided over linkage disequilibrium blocks.

Over the years, many linkage studies have been performed in BD and even more in schizophrenia, and regions throughout the genome have been linked without clear consistency (Baron *et al*., 2002). Meta-analysis of linkage studies have not been able to improve this situation much, with one study reporting linkage in 6q and 8q and nominally in 9p and 20p (McQueen *et al*., 2005), another revealing significant linkage of 13q and 22q regions (Badner & Gershon, 2002), and a third finding no regions reaching genome-wide significance (Segurado *et al*., 2003). Bearing in mind that the strength of linkage studies lies in their ability to detect rare

variants of large effects and that these variants may in fact lie in different genes for different families, combining many families - let alone studies - may dilute the effect of family-specific variants. Studying a single, large family can be more sensitive to this type of effect. An example of such a design is the Scottish family that led to the discovery of the *DISC1* gene (St Clair *et al*., 1990; Blackwood *et al*., 2001), which is discussed in Chapter 5.

Association studies complement linkage studies, both in terms of methodology and resulting inconsistencies. In the absence of whole-genome methods, initial association studies relied on knowledge about gene function and results from linkage studies to provide clues to *a priori* loci. Although association for the majority of resulting candidate genes have not been replicated, as highlighted in recent reviews (Barnett & Smoller, 2009; Craddock & Sklar, 2009; Serretti & Mandelli, 2008), there are a few genes for which the cumulative functional and association-based evidence is convincing. These are *NRG1*, *ErbB4*, *DISC1*, *BDNF*, *SLC6A4* and *DAOA*. The main functions of these are in neurodevelopment (*NRG1*, *ErbB4*, *DISC1*, *BDNF*), and plasticity (*NRG1*, *ErbB4*, *DISC1*, *BDNF*, *DAOA*) or serotonin transmission (*SLC6A4*). For their clear relevance to white matter development, myelination and maintenance of axon integrity *NRG1*, *ErbB4* and *DISC1* are extensively discussed in Chapters 4 and 5.

There is a degree of randomness involved in this candidate gene approach and the scientific attention for a locus tends to fluctuate over time depending on novel findings in molecular neuroscience. As pointed out by Serretti and Mandelli (2008), a large number of potentially very interesting genes have not been studied in candidate studies, and similarly not all significantly linked regions have been followed up by candidate studies. Conversely, the current "risk genes" may appear more convincing than they are because these studies are very sensitive to publication bias. Genome-wide association studies, which enable the investigation of SNPs across the whole genome simultaneously, are more data-driven, and may therefore be a more objective way to find new causal genetic variation. However, studying the entire genome introduces a major multiple testing problem, which makes it extremely difficult to distinguish between true and false positives. Generally, stringent multiple testing corrections are applied to minimise false positives but at the same time they inevitably discard a large portion of the true signal. Fortunately, sample sizes are increasing, and even more importantly, they are being combined into massive GWAS collaborations and meta-analyses. With these, novel risk genes have been discovered, several of which were replicated in independent samples *and* have some apparent functional relevance to neuropathology, which is more than has been achieved for the majority of candidate genes over

the course of several decades. Replicated risk genes are *DGKH*, *ANK3*, *CACNA1C*, and *ZNF804A* (Baum, Akula *et al*., 2008; Baum, Hamshere, *et al.*, 2008; Ferreira *et al*., 2009; Liu *et al*., 2010; O'Donovan *et al*., 2008; Sklar *et al*., 2008; Steinberg *et al*., 2011; Weber *et al*., 2011; Williams *et al*., 2011). As far as we know, *CACNA1C* is involved in plasticity through calcium-signalling, *ANK3* encodes a cell adhesion protein and *DGKH* interacts with the phosphoinositol pathway, which is an assumed therapeutic site of action of lithium (Manji & Lenox, 1999). Little is known about the immediate molecular function of *ZNF804A* apart from its association with functional connectivity during various cognitive activities and therefore its hypothetical involvement in structural connectivity, the details of which are discussed in Chapter 6.

1.2.2 Missing genetic variation

While the emergence of GWAS has revolutionised the field by both the discovery of new risk variants and the unbiased way genetic association can be quantified, the majority of the results do not replicate across studies. Also, the correspondence between the main linkage and association results is far from perfect, with *NRG1* being the only well-supported risk gene mapping to a significant linkage region from the three meta-analyses, while *DISC1* and *ANK3* each map to regions reported in two individual linkage studies which were not supported by any of the meta-analyses (Baron *et al*., 2002). The locations of *ZNF804A*, *CACNA1C* and *ErbB4* do not map to any previously linked region. Furthermore, effect sizes of all candidate genes are small and altogether they explain only a small fraction of the heritability. What is more, an innovative method recently estimated the cumulative explained variance of the whole-genome at 40% (Lee *et al*., 2011), suggesting that increasing sample sizes in univariate GWAS performed with current methods is undoubtedly going to reveal more common or rare risk variants, but they can probably never explain more than half of the genetic component. This is disconcerting because knowledge about the genetic variants that confer risk to a disorder or illness often leads to rapid progress in the understanding of its biology and consequently improved treatment, as evident in many other, non-psychiatric yet complex genetic conditions such as type II diabetes and Crohn's disease (Manolio *et al*., 2008).

Several explanations for the missing genetic variation in psychiatry have been proposed, and tremendous efforts are under way to explore these. Part of the problem could simply be that

some of the genetic variation is of such nature that it is extremely difficult to detect for statistical and epidemiological reasons. For example the detection of rare variants (SNPs, CNVs or larger chromosomal abnormalities) of large effect require well-designed family studies to see if the transmission of a variant correlates with the inheritance of the disorder. Another property that makes genetic variation difficult to detect is that psychiatric disorders are presumably polygenic: many common variants in numerous different loci are each responsible for a small increase in susceptibility. Studies that regress clinical phenotypes onto individual polygenic scores derived from independent genome-wide association samples indeed show that including many SNPs with relatively weak associations improves the explained variance of the regression model, especially in the case for bipolar disorder and schizophrenia (Evans *et al*., 2009; Purcell *et al.*, 2009). Instead of a linear polygenic model, non-linear gene-by-gene interactions could explain the missing genetic variance. Some studies have indeed shown that selected risk variants have a larger effect in the presence of another known risk variant (e.g. Norton *et al*., 2006). But to do this in a genome-wide fashion exponentially inflates the multiple testing problem.

If it is true that univariate analysis across the whole genome can explain a maximum of 40% of the liability to BD, explaining the remaining half of h^2 may be aided by considering alternative forms of the measured phenotype. The clinical diagnostic phenotype is highly heterogeneous in both clinical and – most likely – aetiological terms, and inevitably includes some confounding effects of medication and state-related factors. The current diagnoses of BD, schizophrenia and other affective and psychotic disorders likely do not have a one-to-one relationship to the biological causes. For instance, there could be many subtypes of each disorder, each having their own biological cause. Alternatively it has been proposed that there is a psychosis-affective spectrum (e.g. Craddock, O'Donovan & Owen, 2009). In general, wherever the current psychiatric nosology bears an imperfect relation to genetic causes and other biological mechanisms, the consequences for genetic association studies are that variance of interest is lost and error variance is increased. There have been several attempts to relate genetic variation to symptom dimensions or severity, or to subgroups of psychiatric samples (reviewed in Craddock & Sklar, 2009), but these have produced mixed results. There is also no general consensus on the dimensions along which to subdivide samples, and replication will likely be more difficult with diagnostic scrutiny.

From the above it can be concluded that (1) likely many genes have the potential to confer risk to psychiatric disorders, whether it concerns rare variants of large effects or common variants of

small effect or a combination of both; and (2) improving the biological validity and decreasing the complexity of the phenotype is necessary if we want to appreciate the complete genetic component of psychiatric disorders. Given that healthy individuals can easily carry one or more risk variants without the appearance of clinical disorder, searching for genetic associations with intermediate phenotypes, or *endophenotypes*, in healthy populations can be a powerful strategy.

1.3 Endophenotypes

An endophenotype is a reliably measurable trait that biologically lies more proximal to the underlying genetics of a disorder than the original phenotype (diagnosis) and is assumed to be more sensitive to genetic variation than diagnosis itself. There are five criteria for an endophenotype as defined by Gottesman and Gould (2003): (1) it must be associated with the disorder in the general population, (2) it must be highly heritable, (3) it must be independent of clinical state, (4) it must co-segregate with the disorder within families, and (5) it must also be apparent in unaffected relatives of individuals with the original phenotype. Not only can an endophenotype, once established, be a more statistically powerful measure in association studies or quantitative linkage studies, it can also fill in missing biological links between genes and clinical symptoms. If many different risk variants additively or synergistically confer risk to develop the same symptoms, it is probably because they feed into common biological pathways and processes. Endophenotypes are likely to capture the upstream effect of these common biological pathways, and will therefore be useful research constructs to further delineate relevant processes both downstream towards molecular genetics and upstream towards clinical neuroscience.

Despite the clear potential value of endophenotypes, they have not yet been widely applied to psychiatric genetics nor have many been established as true endophenotypes according to the criteria of Gottesman and Gould. A reason may be that large family-based samples are required to firmly test the criteria that they are both present in unaffected relatives but to a lesser extent than in patients themselves, implying the effects in the relatives are subtle. Sample sizes in brain imaging studies are only recently beginning to increase towards N = 50 or more. However, proposed endophenotype candidates include behavioural, neurocognitive as well as neuroimaging phenotypes (Glahn *et al*., 2007, 2010; Hasler *et al*., 2006). Large amounts of neuropsychological data are relatively economical and reliable to acquire. As such, Glahn *et al*.

(2010) have shown robust endophenotypic qualities of verbal and facial memory and digit-symbol substitution tasks. To achieve this, a complete neuropsychological test battery was administered in over 700 individuals from multiple families. To conduct brain imaging study of similar scale would be even more challenging. Nevertheless, there are a few promising brain imaging endophenotype candidates for which high heritability has already been estimated, and which are more or less consistently abnormal in patients. These include fMRI-based resting-state connectivity (Glahn *et al*., 2010), total brain, intracranial volumes, total white matter volume (van der Schot *et al*., 2009), local grey matter density (Thompson *et al*., 2001), and white matter integrity. Below I evaluate the current evidence for white matter as an endophenotype for BD. First I consider evidence for white matter pathology in BD as required by criterion 1, after which I discuss the remaining criteria.

1.4 White matter pathology in bipolar disorder

1.4.1. Hyper-intensities, histology and structural MRI in bipolar disorder

The first indications for white matter abnormalities in the brains of patients with BD came from increases in hyper-intensities in deep white matter as visible on T_2-weighted images of the brains of bipolar patients, and are the most widely reported features in the brains of bipolar patients (reviewed in Videbech 1997; meta-analyses in Kempton *et al*., 2008 and in Beyer *et al*., 2009). Histological studies of patients' post-mortem brains have, similar to imaging studies, mostly focused on grey matter. However, density and counts of glia, and in particular oligodendrocytes which are responsible for axon myelination, within cortical regions are indicative of white matter pathology in bipolar patients (Ongur *et al*., 1998; Rajkowska *et al*., 2001; Uranova *et al*., 2004; Vostrikov *et al*., 2007). More direct evidence comes from one study showing reduced myelin staining in post-mortem brains of bipolar patients (Regenold *et al*., 2007). Furthermore, there is support from microarray studies showing altered glia- and myelin-related gene expression (Hakak *et al*., 2001; Tkachev *et al*., 2003).

Early MRI studies of total cerebral or lobar white matter volumes in BD have provided mixed results, with four studies reporting significantly reduced volumes in bipolar patients and six negative studies (reviewed by Mahon *et al*., 2009). Others focused on the corpus callosum as a region of interest easy to segment in early MRI. Six out of the nine studies demonstrated

reductions in callosal total volume or within-slice area (reviewed by Mahon *et al*., 2009), the latter of which was highly significant in a meta-analysis by Kempton *et al*. (2009).

More computationally advanced voxel-based morphometry (VBM) analyses of T_1-weighted images have also demonstrated reductions in white matter density in patients with BD, although the locations of the findings vary widely. Significant reductions have been reported in the temporal and prefrontal lobes, especially surrounding the anterior limb of the internal capsules (ALIC) (Bruno *et al*., 2004; Chen *et al*., 2004; McDonald *et al*., 2005; McIntosh *et al*., 2005, 2006). Some of these reductions have been confirmed in first-episode and medication-naïve patients (Atmaca *et al*.,2007; Rosso *et al*., 2007; Strakowski *et al*., 1993), suggesting that they are not merely a consequence of medication or the progression of the disorder. Nevertheless, several negative studies not finding any significant differences in any voxel locations also exist (Beyer *et al*., 2009; Sarnicola *et al*., 2009; Scherk *et al*., 2008).

1.4.2. Diffusion tensor imaging in bipolar disorder

Considering neuro-anatomical pathology is generally quite subtle in psychiatric patients, some of the above inconsistencies may be attributable to the sub-optimal sensitivity of the T_1-weighted signal to white matter and the application of VBM, in which there can be a confusing trade-off between grey and white matter density as such that any reductions in white matter can often also be interpreted as increases in grey matter and *vice versa*. Moreover, this method is very susceptible to partial volume effects, is dependent on subjective segmentation parameters, and although it may detect gross volumetric differences is also relatively insensitive to microstructural differences on a smaller scale. DTI is an innovative approach to measure white matter integrity that is based on the anisotropic diffusion of water molecules along (rather than across) white matter fibres. The degree of this anisotropy is commonly quantified by fractional anisotropy (FA) and is assumed to reflect the overall integrity of white matter structure (for details see Chapter 2). Not only is FA, as derived from DTI scans, sensitive to white matter microstructure, DTI also allows for more advanced analysis tailored to white matter anatomy (see Chapter 2).

DTI studies have repeatedly shown FA reductions in patients with BD, although the locations of these vary widely. Integrating DTI findings by cerebral location is difficult because of the variety

in methods ranging from voxel-based to tract-based methods, from tractography to cortical and sub-cortical ROIs. Confusingly, the latter are frequently confined to predominantly grey matter regions, without further reference to the meaning of FA in a voxel classified as grey matter. Nevertheless, roughly combining results from over twenty-five DTI studies performed in BD so far, it is clear that white matter integrity reductions have been reported in every lobe, nearly every anatomical tract as well as in or underneath most Brodmann areas (as reviewed in Heng *et al*., 2010; Mahon *et al*., 2009 and Sexton *et al*., 2009). Overall, FA reductions have been most consistent in temporal and frontal areas and their connections, including the genu of the corpus callosum, the anterior thalamic radiation (ATR) and the uncinate fasciculus (UF) (Vederine *et al*., 2011). In these regions, reductions were also confirmed in first episode patients (Adler *et al*., 2006), indicating they are not merely a result of medication or of progressive aspects of the disorder. Confusingly, there are also some indications for white matter increases in the left UF (Houenou *et al*., 2007; Versace *et al*., 2008; Wessa *et al*., 2009; Yurgulun-Todd *et al.*, 2007). It is, however, possible that these increases are attributable to the partly neuroprotective effects of lithium and some anti-psychotic medications.

Overall, histological, radiological and more advanced imaging techniques strongly suggest that subtle white matter pathology is present in BD, as required by the first criterion of endophenotypes. Despite advances in DTI, enabling the modeling of white matter microstructure *in vivo*, some difficulties in interpretation of the imaging findings remain. For example, it is not entirely clear whether the connectivity in UF is increased or decreased in BD; or whether the decreased integrity in bipolar patients is a global effect or specific to one or more restricted white matter regions. Some of these contradictions between studies can be attributed to a combination of sample heterogeneity, medication effects, illness progression and effects specific to temporary mood state (Arnone *et al*., 2009; Kempton *et al*., 2008); the same issues that should be resolved by the consideration of endophenotypes.

1.5 White matter as an endophenotype for bipolar disorder

The heritability of structural MRI-derived brain measures vary with brain structure, age and intellectual ability (Chiang, *et al*., 2009; Chiang, McMahon *et al.*, 2011, Kochunov *et al*., 2010; van der Schot *et al*., 2009, 2010). A first twin study revealed that the heritability of total white matter volume is highly significant, estimating h^2 at 74%, which is also considerably higher than

that of total grey matter volume estimated at (60%) (van der Schot *et al*., 2009). A subsequent VBM study revealed that h^2 was highest for white matter density in the frontal lobes with h^2 estimates up to 74% (van der Schot *et al.*, 2010). DTI studies are in agreement with this, with heritability of whole-brain FA estimated at (52%), with maxima in frontal and corpus callosum tracts (h^2 up to 66%). For younger people, up to mid-twenties, and those from a high socio-economic background, FA is more heritable still, and reaches h^2 estimates of 85% in frontal and callosal regions (Chiang *et al*., 2009; Chiang, McMahon *et al.*, 2011). Furthermore, bivariate modelling has shown that a significant part of genetic risk for BD is explained by variance in white matter volumes and density, whereas grey matter is associated with the (unique) environmental component of BD (van der Schot *et al*., 2009, 2010). Together these studies provide ample evidence that white matter volume and integrity are heritable, as required by endophenotype criterion (2).

For criterion (3) - the endophenotype should be independent of (temporary) state - there is no direct evidence, as this technically requires a longitudinal DTI study in patients. The stability of white matter abnormalities is, however, cross-sectionally supported by their presence in euthymic, manic and mixed patients, which also include first-episode patients (e.g. Adler *et al*., 2005; Benedetti *et al*., 2011; Wang, Jackowski *et al*., 2008; Wang, Kalmar *et al.*, 2008).

A few imaging studies, including in our own research group, have conducted structural MRI in unaffected relatives of bipolar patients. Two of these found an inverse correlation between white matter density or volume and genetic liability scores based on family history (McDonald, Bullmore *et al*., 2004; Mcintosh *et al*., 2006). In the study by our group (McIntosh *et al*. 2006), this association was located in the left inferior frontal lobe and the ALIC. The results of McDonald were more distributed and included the corpus callosum, the left deep parietal white matter (around the arcuate fasciculus; AF), and the right superior parietal white matter. In a direct comparison between unaffected relatives and a control sample, however, no reductions were found in unaffected relatives who had more than two first-degree relatives with schizophrenia or BD, but there were two small frontal regions in which there were reductions in those with at least one relative with each disorder (McIntosh *et al*., 2005).

Three DTI studies have investigated white matter in unaffected relatives of BD, but none of these can be considered conclusive. Chaddock *et al*. (2009) did not detect any significant FA difference in a direct voxel-wise contrast of unaffected relatives and patients. However, this may

be due to insufficient statistical power (19 patients, 21 relatives, 18 controls), as they did find a correlation of genetic liability scores with FA in 70 clusters distributed over the whole brain (which was the principal component). Frazier *et al*. (2007) did find localised FA reductions in unaffected children of bipolar patients aged 4 to 12 years, but they failed to apply appropriate corrections for multiple comparisons and given the age and the sample size (10 patients, 7 relatives, 8 controls) these results cannot be readily generalised. The most recent study, by Versace *et al*. (2010), concerned children between the ages of 8 and 18 years, at a time window of profound developmental changes in white matter. For children under the age of fourteen, Versace *et al*. (2010) noted increased FA in the unaffected relatives compared to controls, while the opposite was true for those older than fourteen. From the viewpoint of BD as a neurodevelopmental disorder, this is a very interesting finding (as further discussed in Chapter 3), but it is not so informative when evaluating white matter as an endophenotype. Thus, although no imaging studies provided conclusive evidence for criterion (5), they are all suggestive of reductions in individuals at high risk for BD. This is also supported by the finding mentioned above: genetic factors explain at least 38% of the covariance between white matter volume and BD (van der Schot *et al*., 2009).

To summarise, white matter integrity is a promising endophenotype candidate for BD: (1) as evident from histology, radiology and advanced imaging techniques white matter structure is compromised in patients, (2) white matter volume and integrity are highly heritable, and (3) since white matter reductions have been found in euthymic and first-episode patients, it appears to be state-independent. Although correlations with genetic vulnerability scores suggest the presence of white mater abnormalities in unaffected relatives, direct evidence for criterion (5) is lacking. On the other hand, the same studies were able to convincingly show reductions in the patients themselves, in support of criterion (4).

1.6 White matter in relation to current neuro-cognitive models of psychosis

1.6.1. Emotion regulation

In general, current models of BD focus on inadequate regulation of activation in limbic and subcortical structures by higher-order areas in the prefrontal cortex. Strakowski *et al*. (2005) proposed that emotion dysregulation arises from disrupted prefrontal modulation of the striatum

and the amygdala-hippocampal complex. This model can be extended by that of Phillips *et al*. (2003a, 2003b, 2008) which divides the emotional system into voluntary and automatic processes. The former depend on a "dorsal" system which includes hippocampus and dorsal prefrontal regions, while the latter rely on a "ventral" neural network comprised of several temporal lobe structures (the amygdala, insula), the ventral striatum and ventromedial prefrontal regions. A special role is attributed to the orbitofrontal cortex, which may serve as a "hub" to modulate the connection between the two systems. Akin to this is the model by Ochsner and Gross (2005), who also divide the top-down emotional processes into a dorsal and a ventral prefrontal network. However, in this model the main function of the dorsal network is "description-based" (the attribution of emotional value and internal representation to a stimulus), whereas the ventral network's function is "outcome-based". The precise functions and integrations of each sub-system within each model are disputable, but they clearly both emphasise the importance of functional and structural connectivity in larger brain networks in accordance with the current climate in cognitive neuroscience which has moved from "functional segregation" to "functional integration" (Friston, 1999; Friston & Frith, 1995).

A healthy functional integration of emotional networks is naturally dependent on the structural integrity of its connections. According to the above models these involve mainly fronto-temporal, fronto-thalamic and fronto-stiatal connections. In terms of white matter anatomy, the direct connections between these regions are the UF, AF, ATR and cingulum bundles (CING). As reviewed above, these relevant connections have been implicated in structural imaging studies of BD. However, the striatum, thalamus and orbitofrontal cortex are potential "hubs", meaning they can be intermediate stations between any two regions. Consequentially, changes in white matter integrity in other connections could also indirectly influence each neural network.

1.6.2. Neurodevelopment and plasticity

A striking proportion of the genes currently implicated in BD and schizophrenia are involved in neurodevelopment or plasticity, each of which have been proposed to be central to psychosis, although more often in schizophrenia (Friston, 1999; Harrison & Weinberger, 2005). These processes have most often been related to grey matter but they are equally - if not more - fitting with a role for white matter as mediator of genetic effects. Firstly, grey matter peaks around the

age of 10 to 12 years, while white matter continues to mature in, and potentially beyond, late adolescence and early twenties, especially in fronto-temporal connections (DiGiorgio *et al*., 2008; Lebel et al., 2008; Paus et al., 2005; Pfefferbaum et al., 1994) approximately matching typical age of onset of psychosis. Secondly, synaptic plasticity itself by definition occurs in grey matter, but axonal integrity and the degree of myelination control the speed of signal transduction which is crucial for the initiation of long-term potentiation. Furthermore, activity-dependent structural changes are not limited to the synapse, but also occur in white matter (Demerens et al., 1996; Fields, 2008). The mutual dependence between structural and functional connectivity, grey and white matter plasticity and their shared genes and molecular pathways, suggests a web of mechanisms in which various disruptions can cause symptoms and mental disorder. As partly inferred from the more abundant research in schizophrenia and grey matter, a role for white matter in this web is highly conceivable, however relatively under-appreciated so far.

1.7 Summary and aims

Over the last decade interest in white matter has increased due to the development of advanced imaging techniques and the view of the brain as a functionally integrated system, and evidence is accumulating that white matter integrity is compromised in BD. Family and genetic studies suggest white matter integrity can be an endophenotype of BD, although there are uncertainties about the location and nature of white matter abnormalities in bipolar patients and their relatives. Diffusion tensor imaging in a sufficiently large, unmedicated sample of unaffected relatives can clarify previous inconsistencies and explore the validity of white matter as an endophenotype for BD, and potentially provide clues to the mechanisms of specific candidate genes.

Here, I present a comprehensive DTI study of white matter integrity in individuals at high genetic risk for developing BD. First, I established whether FA is reduced in unaffected relatives, as part of testing its validity as an endophenotype (Chapter 3). Then, I directly tested associations between FA and candidate genes, chosen on the basis of their combined functional and statistical evidence in association with BD and their functional relevance to white matter (Chapters 4, 5 and 6). To investigate these questions thoroughly, a number of advanced analysis techniques were applied, which are tailored to white matter structure as detailed in the next chapter.

Chapter 2

Methods

2.1 Principles of diffusion MRI

2.1.1. Diffusion MRI

DTI is an MRI application that depends on the random motion of water molecules ("diffusion") due to thermal energy and its restriction and hindrance by body tissue. Normally, in an unrestricted and unhindered environment, molecules move randomly in all directions ("random walk"), resulting overall in *isotropic diffusion*. For a sufficiently large number of molecules, the mean squared displacement ($(r-r_0)^2$) of a molecule in a uniform substance is directly proportional to the time interval *t*, as shown in Einstein's equation [2.1]. The diffusion coefficient *D* depends on the substance in question (e.g. water) and the temperature of the sample.

$$(r-r_0)^2 = 2\,Dt \qquad [2.1]$$

In a diffusion MRI sequence, two strong pulsed magnetic field gradients are used to measure the mobility of water molecules. In its simplest form, the sequence for diffusion MRI contains an RF excitation pulse, followed by two opposite (separated by an 180^o refocusing RF pulse) pulsed magnetic field gradients separated by a time interval. Because of these gradients, the phase angle of the proton spin depends on their location. As a consequence, if the water molecules do not move during the time interval, the signal recorded after the second gradient will be equal to the signal obtained in the absence of any temporary gradients. However, as we know, the water molecules do move, and the more they move, the more the proton spins are out of phase and the lower the resulting signal will be. Thus, the amount of signal attenuation in a voxel reflects the amount of water diffusion in that location.

2.1.2. The diffusion tensor and derived measures

Importantly for DTI, the random motion of molecules in a human body is hindered and restricted by tissue properties such as membranes and organelles. The anatomical architecture of white matter, with long and thin axons organised in bundles and insulated with myelin sheaths gives rise to a distinctly *anisotropic diffusion* pattern. In an abstract sense, one can think of isotropic diffusion, as in equation [2.1], as a sphere with a radius *D*, and of anisotropic diffusion as an ellipsoid with its long axis parallel to the principal axon direction (see Figure 2.1). In mathematical terms, the three-dimensional molecule displacement is best represented by a *diffusion tensor*, D, defined by a symmetrical matrix as in equation [2.2].

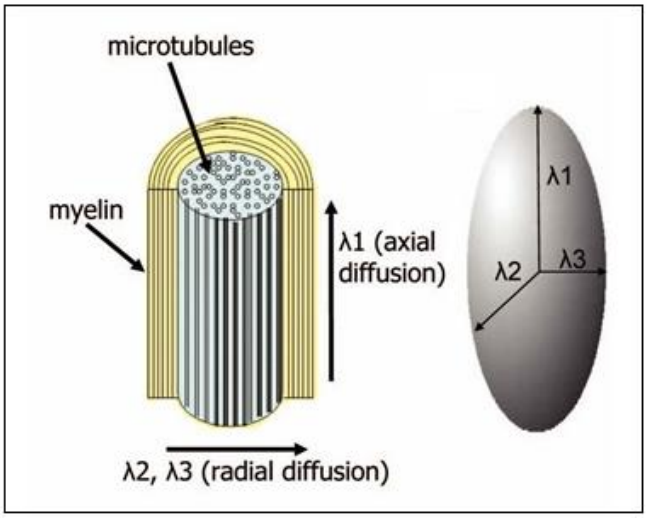
$$D = \begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yx} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix} \quad [2.2]$$

This quantity provides information on both the magnitude and directionality of water molecule displacement in three-dimensional space. To determine *D* for a given voxel, multivariate linear regression is used to estimate the six unknown variables, D_{xx}, D_{yy}, D_{zz}, D_{xy}, D_{xz} and $D_{yz,}$ from the signal intensities generated from each diffusion gradient direction and the b-value (strength of diffusion-weighting). Since there are six unknown variables, the DTI sequence has to be repeated at least six times, with diffusion gradients applied in six non-collinear directions. In practice, often more than six gradient directions are applied to increase reliability and signal-to-noise, and to allow for probabilistic tractography (see below). Acquiring more than six diffusion directions allows the probability distribution of each of the six tensor variables to be estimated, thereby incorporating an estimate of the uncertainty caused by MRI noise, local anatomical properties and imaging artefacts on the principal diffusion direction. This gives a much better representation of reality, and can be important for certain types of analysis, such as tractography (Behrens *et al*., 2003).

From the diffusion tensor, a number of useful biomarkers of white matter integrity can be calculated. This is achieved by diagonalising the diffusion tensor into three eigenvectors, which represent the orthogonal diffusion directions in each voxel (v_1, v_2, v_3), and their corresponding

eigenvalues (λ_1, λ_2, λ_3), which represent the magnitude of the diffusion along each orthogonal direction as depicted in Figure 2.1.

Figure 2.1. The diffusion tensor as a model of white matter microstructure.



(Adapted from Newcombe et al., 2007)

The first biomarker, mean diffusivity (MD), represents the overall magnitude of diffusion and is equal to the average of the eigenvalues (λ_1, λ_2, λ_3). In the brain, MD is highest in cerebrospinal fluid, since the motion is not restricted by tissue and lower in both white and grey matter. The second common biomarker provided by DTI is the fractional anisotropy (FA), which quantifies the degree of diffusion anisotropy, and indicates the weighted deviation of each of the three eigenvalues from MD. The formula to obtain FA is given by equation [2.3].

$$FA = \sqrt{\frac{3}{2}} \sqrt{\frac{(\lambda_1 - \langle D \rangle)^2 + (\lambda_2 - \langle D \rangle)^2 + (\lambda_3 - \langle D \rangle)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \qquad [2.3]$$

Since anisotropic diffusion is what distinguishes white matter best, FA is the most commonly used measure for approximation of white matter integrity, and it is the outcome measure of interest for most of the analyses presented in this thesis. Other quantifications of anisotropy also

exist, such as relative anisotropy, but these are not as commonly used because their scales are less sensitive to the variation within white matter (Hasan *et al.*, 2004). Finally, both axial and radial diffusivity are often used as measures of interest. Axial diffusivity is defined as the primary diffusion eigenvalue (λ_1) and reflects water displacement along fibres. Radial diffusivity is the perpendicular motion and is computed as the average of the second and third eigenvalues (λ_2, λ_3).

2.1.3. Validity and anatomical sources of diffusion measures

In DTI, diffusion is measured at a scale of millimetres while the anatomical restrictions to diffusion are at the scale of microns. Hence, the diffusion tensor is only a model of the anatomy we aim to measure. We can therefore not be sure of the microstructural anatomical source of variation in FA and other measures on the basis of DTI data alone, and this source can easily vary between locations and individuals. White matter has several anatomical properties that in theory can influence the degree of diffusion anisotropy: the presence of axon organelles and cell membranes hinder the intracellular diffusion across the axon, myelin restricts the diffusion, the density and parallel-ness of the fibres influence the scope of extracellular diffusion and the axon diameter has the potential to speed up the diffusion in the parallel direction. From animal models of multiple sclerosis and experimentally induced white matter pathology and post-mortem studies that combine DTI with histology, we know that FA is probably sensitive to each of the above anatomical properties (Deboy *et al.*, 2007; Gouw *et al.*, 2008; Klawiter et al, 2011; Li *et al.*, 2011; Schmierer *et al.*, 2007). Thus, FA can be seen as a non-specific measure that approximates the overall integrity of white matter.

Efforts have been made to distinguish between myelin-related pathology and axonal damage using DTI. In general, there is a belief that increases in radial diffusion (i.e. perpendicular to axon) may reflect myelin pathology, whereas decreases in axial diffusion (i.e. parallel to axon) imply axonal damage. In a series of mice experiments, Song *et al.* (2002, 2003, 2005) provided some evidence for a double dissociation between de-myelination and axonal damage and their respective effects on radial and axial diffusivity. Although axial and radial diffusivity are currently readily interpreted according to these distinct pathologies in DTI studies, claims of the distinct sensitivity of axial and radial diffusivity for myelin and axon integrity, should be interpreted with caution (Wheeler-Kingshott & Cercignani, 2009). A first problem is that axonal damage and myelination are dynamic processes, partly dependent on each other, and separating

them in *in vivo* experiments is difficult. The most important problem, however, is that both axial and radial diffusivity are absolute measures of distance, and therefore highly dependent on the overall density and permeability of local tissue, which is in turn sensitive to many processes that typically co-occur with axon damage and de-myelination *in vivo*, and with the experimental procedures used to induce them in animals. These can include inflammatory responses of all kinds such as microglia accumulation, intra- and extracellular oedema and scar formation, and there is evidence that some of these other processes are better correlated with axial and radial diffusivity over time than axonal and myelination pathology (Xie *et al.*, 2010). This view is also in line with the absence of any associations between axial and radial diffusivity with specific pathology in *ex vivo* scans of the spinal cord (DeBoy *et al.*, 2007; Klawiter *et al.*, 2011), and with the observation that the predictive value of axial and radial diffusivity on pathology type is dependent on the type of injury inflicted on the animal (Budde *et al.*, 2007).

Although in certain (clinical) circumstances it can be extremely valuable to be able to distinguish between different white matter pathologies, considering the uncertain validity of axial and radial diffusivity, these biomarkers are not considered here. For the purpose of the present study of brains with relatively unknown pathology, it was more important to have a reliable and validated outcome measure that has repeatedly been shown to be sensitive to insults in structural white matter integrity under various circumstances such as relative anisotropy (RA) and FA (Budde *et al.*, 2007; Cercignani *et al.*, 2001; DeBoy *et al.*, 2007; 2010; Gouw *et al.*, 2008; Kim, Budde *et al.*, 2006; Li *et al.*, 2011MacDonald *et al.*, 2007; Schmierer *et al.*, 2007; Song *et al.*, 2003; 2005; Underwood *et al.*, 2011).While I have applied a number of DTI analysis techniques to study white matter, FA is the outcome measure of choice for each of these - whether within voxels or averages extracted from ROIs. The main analysis procedures I have performed are later on in this chapter.

2.2 **The Bipolar Family Study**

2.2.1. Participants and clinical and neuropsychological assessments

The Bipolar Family Study is a large, prospective study of individuals at high genetic risk for BD, conducted in Edinburgh. The “high risk” participants have at least one first-degree or two second-degree relatives with a clinical diagnosis of bipolar I disorder, although most have more

than one family member with a diagnosis of an axis I psychiatric disorder. The dataset contains MRI scans, including DTI, and blood samples as well as extensive clinical and neuropsychological assessments at two time points, with an approximate 2 year interval. All analyses in this thesis are performed on the first assessments only, as the follow-up was still being conducted at the time of this project. Here follows a description of the recruitment of participants, the materials used and the sample characteristics in general. For the subsequent analyses described in the results chapters, different exclusion criteria may have been used depending on the nature of the analysis (e.g. for analysing the effects of genes, only one individual per family was used; and for the initial group comparison they were matched on IQ), so the samples differ slightly across chapters. Because of this, further details of sample demographics for each analysis are given in each result chapter.

Patients with a clinical diagnosis of bipolar I disorder were identified from the case loads of psychiatrists across Scotland. Each patient was asked to identify members of their close family aged 16-25 years and to consent to either a review of their case notes or to a structured clinical interview. The diagnosis of all affected subjects was then confirmed using the Structured Clinical Interview for DSM-IV (SCID), or the operational criteria (OPCRIT) check list for psychotic illness (McGuffin *et al.* 1991). Following informed consent, unaffected relatives of the proband with at least one first degree, or two second degree relatives with bipolar I disorder were then invited to participate in the study. Wherever possible, to optimise matching on key confounds, controls were recruited from the social networks of the high-risk subjects themselves. Written informed consent was provided by all participants and the study was approved by Committee A of the Multicentre Regional Ethics Committee for Scotland.

All participants were interviewed by a trained psychiatrist (AM McIntosh, JE Sussmann) using the SCID, the Hamilton scale for Depression (HDRS), and the Young Mania Rating Scale (YMRS). Individuals who fulfilled SCID criteria for an axis-I psychiatric disorder, including substance dependence, were excluded from further analyses. Additional exclusion criteria were major neurological disorders, history of head injury, history of learning disability and metallic implants or other contraindications to MRI examination.

Of the unaffected relatives contacted to participate in the study, eighty-one refused to participate or were ill at recruitment. A total of 259 young people took part in the study: 150 unaffected relatives and 109 healthy controls. Seven participants in the high-risk group were diagnosed with

an axis-I disorder after recruitment, and were excluded from further analysis. A further 26 unaffected relatives and 12 control participants were excluded for all analyses in this thesis because either no DTI data had been collected or because of severe imaging artefacts (movement, braces, etc.). This left 117 unaffected relatives and 97 healthy control participants for further analysis.

HDRS and YMRS scores indicated that lifetime mood and psychotic symptoms were virtually absent in our sample. Only HDRS scores differed significantly between the groups, but a lifetime absence of a depressive episode, or other axis I disorder, was confirmed in all participants included in the final sample. Substance abuse for cannabis, sedatives/anxiolytics, stimulants and hallucinogens was assessed on five-point ordinal scales based on the SCID interview: one for current (last month) and one for past substance use.Alcohol and nicotine use were assessed separately, respectively by self-report of units per week and cigarettes per day. Full-scale IQ was measured using the Wechsler Abbreviated Scale of Intelligence (WASI; Psychological corporation, 1999), which consists of four subtests of the Wechsler Adult Scale of Intelligence to measure non-verbal/performance IQ (block design and matrix reasoning) and verbal IQ (vocabulary and similarities). The main reason for the use of full scale IQ in this thesis was to exclude the possibility of confounding on other outcome measures either by ensuring subgroups of our sample were well-matched, or by including IQ as a covariate in statistical models.

2.2.2. Scan Acquisition and Pre-processing

All MRI data were collected on a GE 1.5 T Signa Horizon HDX (General Electric Milwaukee, WI, USA) clinical scanner equipped with a self-shielding gradient set (22mT/m maximum gradient strength) and manufacturer-supplied 'birdcage' quadrature head coil. Whole brain DTI data were acquired for each subject using a single-shot pulsed gradient spin-echo echo-planar imaging (EPI) sequence.. Diffusion gradients ($b = 1000$ s/mm^2) were applied in 64 non-collinear directions to increase the signal-to-noise ratio and to allow for probabilistic tractography. Fifty-three 2.5 mm contiguous axial slices were acquired with a field-of-view of 240 × 240 mm, acquisition matrix of 96 × 96 (zero-filled to 128 × 128), given an isotropic acquisition voxel dimension of 2.5 mm. In addition, 7 T_2-weighted EPI baseline scans were collected as part of the DTI sequence. Finally, a separate T_1-weighted volume was acquired for each participant with the

following acquisition parameters: acquisition matrix 192 × 192, voxel dimension 1.25 × 1.25 × 1.20 mm, 180 slices, time of inversion 500 ms, echo time 4 ms and flip angle 8°.

The diffusion MRI data were converted to 4D NIfTI-1 (http://nifti.nimh.nih.gov) volumes and pre-processed using tools available from FSL (http://www.fmrib.ox.ac.uk/fsl). First, the data were corrected for eddy current induced distortions produced by the strong diffusion-encoding gradients in the EPI sequence and bulk subject motion by registering the diffusion weighted volumes to the first T_2-weighted EPI volume within each subject. This was followed by extraction of brain tissue, as performed by "BET" in FSL, using a fractional intensity threshold of 0.3. Then diffusion tensor characteristics were calculated, including principal eigenvectors and FA values, using DTIFIT. The quality of all resulting FA maps was visually checked, and images showing severe artefacts were excluded from further analyses. Whenever there was doubt about the quality of an image, an experienced MR physicist (ME Bastin) was consulted.

2.2.3. Genotyping

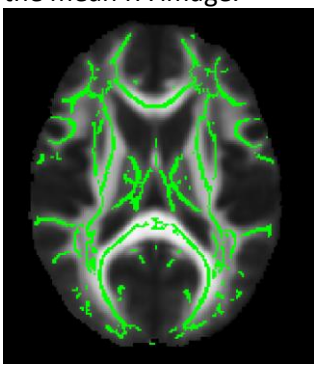
DNA was extracted from venous whole-blood samples. Genotypes at selected *DISC1*, *NRG1*, *ErbB4* and *ZNF804A* SNPs were determined at the Wellcome Trust Clinical Research Facility in Edinburgh (http://www.wtcrf.ed.ac.uk) using TaqMan polymerase chain reaction (PCR, TaqMan, AssayByDesign, Applied Biosystems, Foster City, USA) using validated assays. In brief, two types of probes, each designed to contain one of the possible alleles at the SNP of interest, anneal to one of the DNA strands. Each type of probe has its own fluorophore on one end and a quencher on the other. A primer is designed to anneal to the DNA downstream from the SNP, and the attachment of a polymerase enzyme to the primer induces PCR (meaning it synthesises a new strand of DNA, complimentary to the existing strand, starting from the primer). When the PCR gets to the probe, it breaks down the probe and the fluophore is released. The resulting amount of fluorescence shows the amount of DNA containing the probe target allele so that heterozygotes will show an approximate equal amount of each type of dye, while homozygotes will have about twice as much of the relevant dye.

2.3 Whole-brain statistics

2.3.1. Tract-based Spatial Statistics

Although FA maps are in principle suitable for voxel-wise statistics, for example in SPM (http://www.fil.ion.ucl.ac.uk/spm), after co-registration and spatial smoothing, there is considerable individual variability in white matter structure, which in places is very thin or characteristically shaped, and therefore this method is sensitive to mis-registration and partial volume effects. Tract-based spatial statistics (TBSS; Smith *et al.*, 2006) is a method especially designed for voxel-wise analysis of FA data. The unique feature of TBSS is that for each individual a white matter "skeleton" is created, which is assumed to represent the centres of the white matter structure for each person. This skeleton projection step alleviates the effect of mis-registration, smoothing thresholds and the non-normal distribution of FA values (Zalesky, 2011).

Figure 2.2. The mean FA skeleton mask template (green) used as template for TBSS, overlaid on the mean FA image.



TBSS was carried out using standard tools in FSL (http://www.fmrib.ox.ac.uk/fsl/tbss/) and according to the recommended protocol (Smith *et al.*, 2007). First, all subject's FA volumes resulting from the processes described in section *2.2.2* were non-linearly registered to a standard template using FNIRT. Second, a mean of all registered FA volumes was calculated and a white matter skeleton template was created. Upon visual inspection, a threshold of FA > 0.2 was applied to the FA skeleton template to exclude predominantly non-white matter voxels (Figure 2.2). Third, for each subject's FA volume, at each point on the skeleton the maximum voxel perpendicular to the local skeleton direction was projected onto the skeleton. This skeleton projection step is performed according to two rules: (1) when a "maximum" voxel is near two

skeleton points, it can only be projected onto the one closest to it, and (2) a Gaussian kernel weights each voxel's FA according to its distance from the skeleton so that far away voxels are less likely to be "chosen". This results in one FA skeleton map per subject, which is assumed to contain anatomically corresponding centres of white matter structure. These skeletons, overlaid on each subject's FA map, were checked by eye to ensure they were located in each subject's white matter. Voxel-wise statistics were then be performed on the skeletons, as described in detail below. In this work, I also frequently used FA averaged over the whole skeleton which proved very useful as a single outcome estimate for general white matter integrity.

2.3.2. Threshold-free cluster enhancement

In the present work, voxel-wise statistics were mostly performed using non-parametric T-tests or regressions in FSL "randomise". As with any voxel-wise analyses, whole skeleton analysis causes a massive multiple testing problem, and without corrections will inevitably lead to many false positive findings. In these datasets, statistics were performed on each of the 137832 voxels in each skeleton. Simply dividing the critical p-value by this number would be overly conservative since FA is correlated between neighbouring voxels and would unnecessarily inflate false negative findings. There are now many possible solutions for multiple testing problems in whole-brain analysis that take this spatial correlation into account, and many even take advantage of it.

In general there are two ways statistics can be considered in whole-brain analysis: at a single voxel-level and at a cluster level. The former tests the significance of each separate voxel, and is therefore sensitive to a large effect in a single location. In this case, the p-value of a voxel can be corrected for multiple testing by estimating the smoothness of the image, which gives the number of independent signals ("resels") which can then be corrected for using family-wise error (Bonferroni) or false discovery rate (Bullmore *et al.*, 1999) methods. On the other hand, cluster-based methods (Friston *et al.*, 1993; Bullmore *et al.*, 1999) are based on the logic that if many consecutive voxels show a similar effect, the effect is less likely to be a mere coincidence than if they are in random locations all over the brain. An advantage of the cluster-based methods is that they are sensitive to more subtle effects as long as they are spatially consistent, a type of effect that is probably more realistic in most situations than a large effect at a single voxel. In practice, cluster-wise statistics are typically computed by thresholding a T-map with an initial, arbitrary

threshold, and testing the significance of the cluster-size of supra-threshold voxels against a null distribution. This null-distribution can be estimated using random-field theory (Bullmore *et al.*, 1999; Friston *et al.*, 1993). However, such theoretical null-distributions for cluster sizes are not always accurate, especially in data with non-uniform smoothness (non-stationarity), small resels and small sample sizes, and when low initial thresholds are applied (Hayasaka & Nichols, 2003; Hayasaka *et al.*, 2004; Salimi-Khorshidi *et al.*, 2011; Silver *et al.*, 2011). Moreover, the latter also reduces sensitivity to subtle, widespread signals which was the main reason for using cluster-based methods in the first place.

Threshold-free cluster enhancement (TFCE; Smith & Nichols, 2009), retains the sensitivity to subtle widespread signals of cluster-based thresholds, but does not require the arbitrary choice of an initial threshold. If followed by permutation-based estimation of a null-distribution, the above problems of cluster-wise testing are mostly resolved. In practice, each T statistic is converted into a TFCE value that represents both the local effect size (or "height") and the extent of adjacent voxels "supporting" this signal ("extent"), according to equation [2.4]

$$TFCE = \int_{h=h_0}^{h} e(h)^E h^H dh \qquad [2.4]$$

Where h is the "height", e(h) is the extent given this height, and E and H are parameters to weigh these factors, typically set at 0.5 and 2 respectively. In practice, the "heights" of all supporting voxels are added to obtain the TFCE score of a particular voxel. In this process the height is increased from h_0 (usually 0) to h of the voxel in question, which is also the maximum h that can be contributed by any supporting voxel. As such, the local specificity of the voxel is retained while incorporating the spatial consistency of the signal.

Similar TFCE maps are calculated for each permuted dataset, and the maximum TFCE values make up the empirical null-distribution. At each voxel, the p-value is then the proportion of permuted TFCE values exceeding the unpermuted TFCE value, which is equal to the probability that one would obtain the observed TFCE statistic by chance alone. In this work, I applied TFCE to the TBSS skeletons, as available in the "randomise" tool from FSL, with 5000 permutations per contrast. For all whole-brain analysis, results are considered significant only at levels of $p < 0.05$, corrected at the whole-brain level. Results are reported by "cluster" extent (number of

voxels) and localised to anatomical structures using DTI-based white matter atlases, built in FSL (Mori et al., 2005; Wakana et al., 2007; http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html).

2.4 Tractography

2.4.1. Streamlines

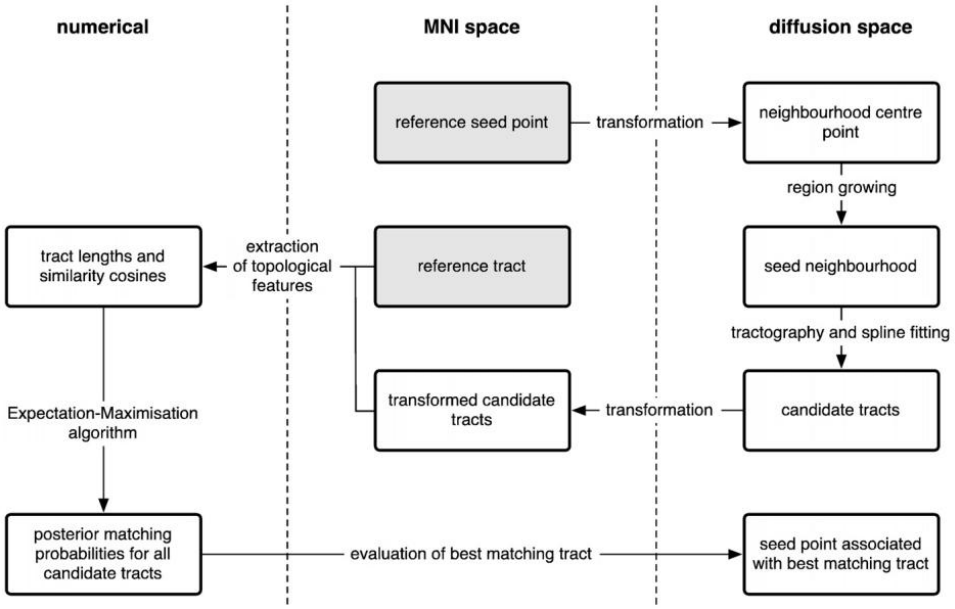
Whole-brain (or whole-skeleton) voxel-wise comparisons of FA are useful methods to determine differences in white matter integrity between a clinical group and a control group, especially in the absence of knowledge about the locations of potential abnormalities. However, even with the application of TBSS, registration errors are still present (Zalesky *et al.*, 2011). The confidence that a voxel in one subject corresponds to the same anatomical structure in another subject can be increased by segmenting specific tracts in native space. Although one can segment particular regions of interest by hand, this is very demanding and subjective work. Tractography algorithms are automatic or semi-automatic methods to segment specific tracts of interest based on DTI data. Although many different algorithms have been developed to date, they all rely on the directional information available from the diffusion tensor, and they require one or more seed points and termination criteria to stop tracing when approaching grey matter or cerebrospinal fluid. In its most basic form, a simple deterministic streamline approach, an algorithm starts from a given seed voxel in the centre of the tract of interest from where it follows the primary diffusion direction (v1) to the appropriate adjacent voxel. This procedure continues until a termination criterion is reached. Obviously, the same procedure is performed in the other direction from the seed point, and the algorithm is usually repeated for multiple seed points in the same tract. Information is retained about which voxels have been visited by the streamline(s), and average FA or other summary metrics of interest can be extracted from them.

2.4.2. Probabilistic Neighbourhood Tractography

More sophisticated tractography algorithms, such as BedpostX/ProbTrackX are not deterministic, but incorporate probabilistic information on the diffusion direction by acquiring the diffusion MRI data with more than 6 diffusion gradient directions and modelling the signal as a

combination of a purely isotropic and several anisotropic (fibres) compartments (Behrens *et al.*, 2003). From a single seed point they can then sample thousands of streamlines with different endpoints. A resulting connectivity map provides information about the likelihood that the voxels are "connected" to the seed voxel. Importantly, one has to keep in mind that the whole method is still based on the relatively large scale at which DTI data is acquired, and that "structural connectivity" remains no more than a model of the underlying axonal shapes and connections. But although tractography is no replacement for histology, methods such as BedpostX/ProbTrackX have produced convincing connectivity maps that correspond very closely to known anatomy from histological examinations (Behrens *et al.*, 2003; Johanssen-Berg *et al.*, 2005). A further advantage of probabilistic tractography algorithms is that they can model more than one fibre direction in a voxel and are potentially able to track through regions of low anisotropy, features which are not available to the diffusion tensor model (Behrens *et al.*, 2007).

Figure 2.3. Processing pipeline for probabilistic neighbourhood tractography.



From Clayden et al. (2009)

Thus, applying the BedpostX/ProbTrackX algorithm improves the quality of tractography results and is more faithful to reality by incorporating the uncertainty inherent in diffusion MRI data. But on in isolation, this method is still influenced by the (subjective) placements of the seed points. A complementary algorithm, neighbourhood tractography (Clayden *et al.*, 2006; 2009) alleviates this problem by performing ProbTrackX seeded from multiple "neighbourhood" seeds, and automatically selecting the "best" seed point based on the similarity of the resulting tract to a pre-determined atlas-based reference tract (Muñoz Maniega, Bastin *et al.*, 2008). The procedure of this method is graphically shown in Figure 2.3. In brief, the seed point from the reference tract in standard space is transformed to the subject's native space, using the inverted (standard space to native space) transformation matrix obtained from FSL's FLIRT algorithm (Jenkinson & Smith, 2001). ProbTrackX is applied to each of a neighbourhood of surrounding voxels (commonly: $7 \times 7 \times 7$) to create a set of "candidate tracts". The spatial median of each of these candidate tracts is compared using a tract-shape modelling algorithm, which calculates a posterior probability of the candidate tract matching the reference tract for shape and length. This posterior probability is determined by the angles between each of a number of b-splines on both reference and median candidate tract; and on the similarity in tract length. (Clayden *et al.*, 2006). The best seed point is then chosen and the respective streamlines (typically 5000) from this seed point are then pruned to only retain the ones for which inclusion improves the "statistical fit" to the reference tract. This *pruning* effectively removes streamlines that deviate too much from the original median line (Clayden *et al.*, 2009).

The voxels that are visited by one or more streamline after all these processes are combined into a binary mask, and the average FA is extracted from them and weighted according the proportion of streamlines going through the voxel (reflecting the probability a voxel belongs to the tract). This final FA value is now assumed to be a good representation of the integrity of the tract of interest and can subsequently be compared between groups of people or correlated with a measure of interest. The scan-rescan reproducibility of FA values resulting from probabilistic neighbourhood tractography is reasonably good (coefficient of variations between 3.9 and 7.2), although somewhat depending on the tract of interest (Clayden *et al.*, 2006). Probabilistic neighbourhood tractography has been integrated with BedpostX/ProbTrackX by Jonathan Clayden, and can be downloaded in a software package called TractoR (https://github.com/jonclayden/tractor).

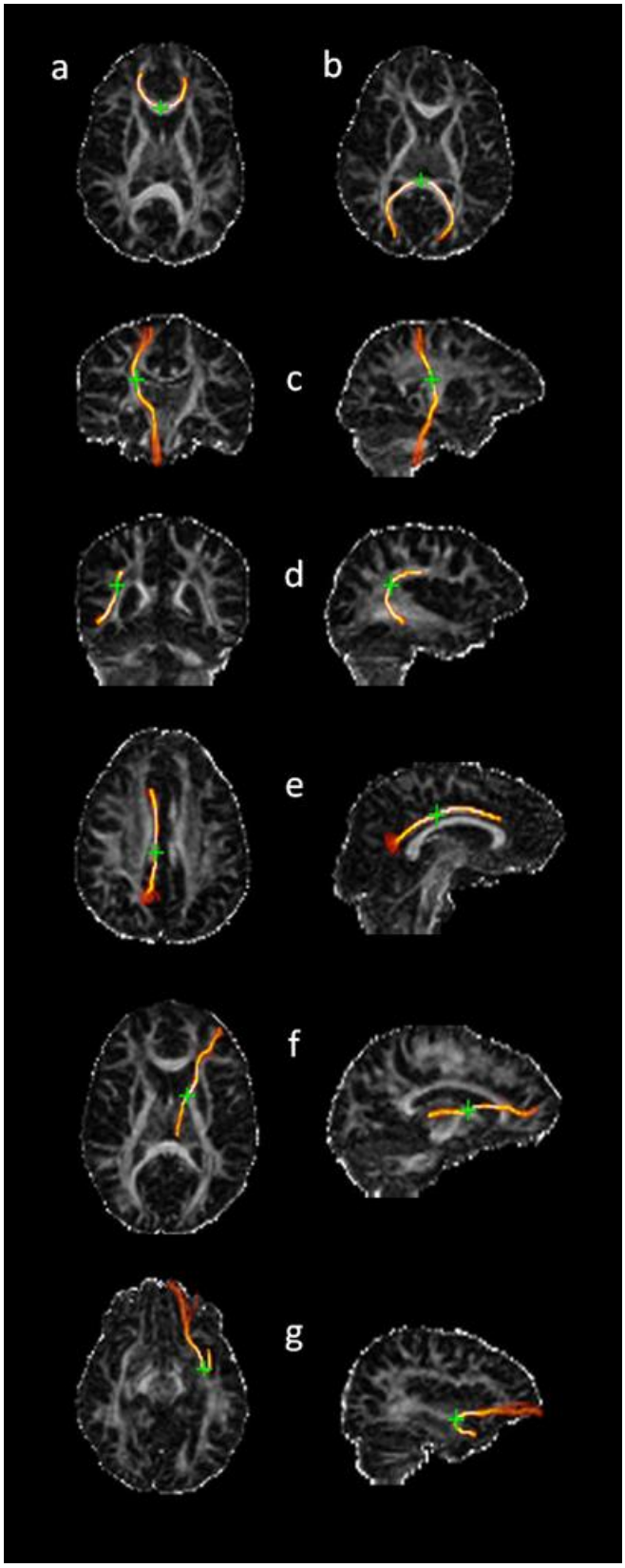


Figure 2.4 Examples of tracts included in the analysis. Typical examples shown for all tracts of interest: genu (a), splenium (b), corticospinal tract (c), arcuate fasciculus (d), cingulum bundle (e), anterior thalamic radiation (f), and uncinate fasciculus (g).

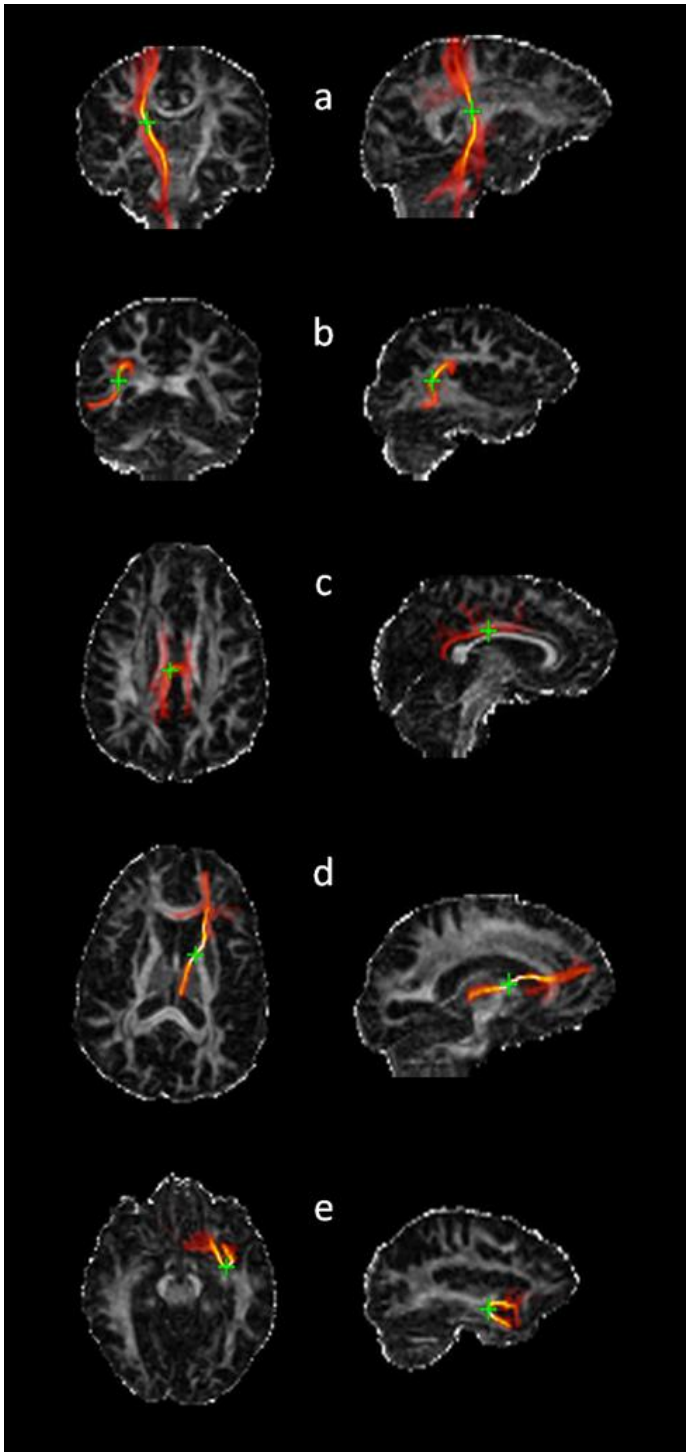


Figure 2.5 Examples of tracts excluded from the analysis. Examples are shown for the corticospinal tract (a), arcuate fasciculus (b), cingulum bundle (c), anterior thalamic radiation (d), and uncinate fasciculus (e). No tracts were excluded for genu and splenium.

2.4.3 Tractography applied to the Bipolar Family Study

Using TractoR, I applied BedpostX/ProbTrackX in combination with probabilistic neighbourhood tractography to the bipolar high-risk data for the following tracts of interest: the genu and splenium of the corpus callosum, the bilateral anterior thalamic radiations (ATR), arcuate fasciculi (AF), cingulum bundles (CING), uncinate fasciculi (UF) and corticospinal tracts (CST). I used 5000 streamlines per tract per seed, and a $7 \times 7 \times 7$ voxel neighbourhood leading to 343 candidate tracts per tract of interest.

The resulting tracts were visually inspected, and tracts that obviously deviated from known anatomy or were severely truncated were excluded. The most common reason for exclusion was for tracts to branch out into the territory of other tracts, and for this a threshold of 5% was employed (i.e. > 5% of streamlines, after pruning, determined using the tractography atlas; Mori *et al.* 2005). The numbers of subjects excluded for each tract are detailed in Table 2.1. The genu and splenium in the corpus callosum were successfully traced for all subjects, but it was necessary to exclude up to 19% and 13% of the participants for the left UF and left ATR respectively. The other tracts encountered few problems, with fewer than 9 exclusions per tract. The number of exclusions for left ATR and UF were statistically independent of group (Fisher's $p = 0.86$; $p = 0.16$, respectively), suggesting it has probably not caused significant bias to the analysis in Chapter 3. Examples of included and excluded tracts are depicted in Figures 2.4 and 2.5.

Table 2.1 Numbers and proportions of successfully traced tracts.

	HC	% HC	HR	% HR	ALL	% ALL
Genu	97	100	117	100	214	100
Splenium	97	100	117	100	214	100
AF left	93	95.8	113	96.6	206	96.3
AF right	96	99.0	110	94.0	206	96.3
ATR left	86	88.7	101	86.3	187	87.4
ATR right	96	99.0	115	98.3	211	98.6
CING left	95	97.9	111	94.9	206	96.3
CING right	96	99.0	112	95.7	208	97.2
CST left	95	97.9	116	99.1	211	98.6
CST right	96	99.0	115	98.3	211	98.6
UF left	83	85.6	91	77.8	174	81.3
UF right	96	99.0	116	99.1	212	99.1

The most common reason for exclusion of the ATR was branching into the genu of the corpus callosum. The ATR is a large tract with fibres "radiating" out into different directions from the thalamus, which likely introduces a lot if individual variation in tract shape. Also, ATR anatomy has a lot of potential to cross other fibres especially those of the genu. For the left UF, again branching into the corpus callosum was a common problem, which happened in 22 subjects. But for this tract additional problems were also encountered including branching in the ATR or other tracts, and severe truncation. The UF has relatively low FA, and has relatively few fibres, and thus streamlines are more likely to move into nearby voxels with higher FA values that are part of other tracts. Like the ATR, the UF crosses many other fibres including those of the genu, the inferior longitudinal fasciculus and the fronto-occipital fasciculus. It is unclear why tracings of the right ATR and UF were more successful than the left, but other colleagues have verified this to be the case with TractoR.

Chapter 3

White matter integrity in individuals at high genetic risk of bipolar disorder

3.1 Introduction

As discussed in Chapter 1, BD is associated with white matter abnormalities in the brain, some of which have also been found in unaffected relatives of patients. DTI is currently the most widely used and regarded as the most accurate method of studying white matter integrity *in vivo*. However, to date it is unclear if white matter integrity reductions, as measured by DTI, are present in unaffected relatives of patients with BD. Here, I set out to test this important criterion for white matter as an endophenotype. In addition, I investigate whether white matter integrity is related to cyclothymic temperament, which is thought to be genetically related to BD and which might underlie the bipolar spectrum (Akiskal & Akiskal, 2005). Three previous studies have measured FA in unaffected relatives (Chaddock *et al*., 2009; Frazier *et al.*, 2007; Versace *et al.*, 2010), all of which were suggestive of white matter integrity reductions in relation to genetic risk for BD. However, as reviewed in Chapter 1, due to methodological and demographic issues the results of these cannot be readily generalised.

Cyclothymia, as a dimension of temperament in the healthy population, reflects the affective stability of a person. It has been suggested that cyclothymic temperament is a fundamental trait underlying the bipolar spectrum, and that it plays a role in the predisposition to affective disorders (Akiskal & Akiskal, 2005). Since cyclothymia scores of individuals at high genetic risk are intermediate between patients and controls (Mendlowicz *et al.*, 2005; Chiaroni *et al.*, 2005), cyclothymic temperament could also be a potential endophenotype for BD.

I used TBSS, tractography and conventional voxel-wise analyses to compare FA in a large sample of unaffected relatives of patients with BD with a large number of individuals without a family history of psychiatric disorder. Furthermore I investigated the association of FA with cyclothymic temperament.

3.2 Materials and Methods

3.2.1 Study sample and data acquisition

Participants were recruited, clinically and neuropsychologically assessed and scanned as described in Chapter 2. To match the unaffected relatives and the healthy controls on IQ, 19 individuals in the control group with the highest IQ scores were excluded from the present analyses. For this chapter, the total sample consisted of 197 subjects, including 117 unaffected relatives and 79 controls. The groups were matched on all other demographic variables of interest, including age, sex and history of substance abuse (Table 3.1).

3.2.2 Cyclothymic temperament

Scores for cyclothymic temperament were obtained by administration of the abbreviated version of the Temperament Evaluation of Memphis, Pisa and San Diego auto-questionnaire (TEMPS-A; Akiskal, Mendlowicz *et al.*, 2005). The abbreviated TEMPS-A is a self-report questionnaire consisting of 39 items, 12 of which exclusively load on a cyclothymia factor. The cyclothymia factor has excellent internal consistency (Crohnbach's α=0.91), is the best represented factor of the abbreviated questionnaire (Akiskal, Mendlowicz *et al.*, 2005), and has high test-retest reliability (Spearman's coefficient = 0.84) in the original TEMPS-A (Matsumoto *et al.*, 2005). Although in addition to the cyclothymia factor, the anxiety factor of the TEMPS-A scale was also increased in unaffected relatives, I did not analyse the anxiety factor because it is only represented by 3 items in the abbreviated questionnaire and has a lower reliability (Akiskal, Mendlowicz *et al.*, 2005).

3.2.3 TBSS

The TBSS skeletons were obtained as described in Chapter 2. Voxel-wise comparisons between high-risk and the control groups were performed using T-tests in 'randomise' in FSL. All reported TBSS results are corrected for multiple testing at $p < 0.05$ using TFCE in FSL. Associations with cyclothymic temperament were examined by adding log-transformed cyclothymia scores to the model in 'randomise'. In addition to this main analysis, a few post-hoc

examinations were conducted to validate the results further. Firstly, in some cases more than one high-risk participant was recruited per affected family, therefore not all observations were statistically independent. In order to test for robustness against this non-independence both group and cyclothymia analyses were repeated leaving in only unrelated individuals, randomly selected for each family. In addition, because TFCE results indicated a widespread effect of FA, FA values were averaged over the whole skeleton for each subject for further analysis in SPSS. Finally, to investigate whether results could have been driven by differences in white matter volume; each subject's total white matter volume was calculated on the basis of the T_1-weighted scan, using a standard segmentation script in SPM5 and compared between groups using a T-test in SPSS.

3.2.4 Voxel-based analysis

The TBSS group comparison was cross-validated using more conventional voxel-based statistics on the whole brain FA-maps. This analysis compares FA values throughout the brain and does not utilize a common white matter skeleton. For this analysis, FA volumes created by the DTIFIT were non-linearly co-registered using the FNIRT toolbox. Subsequently, images were smoothed with a smoothing kernel with FWHM = 12 mm, as recommended by Jones *et al.* (2005). A mask was created by averaging all FA volumes and thresholding at FA > 0.15. Voxel-wise comparisons were performed on all voxels within the mask using 'randomise' with the TFCE option. Results are reported corrected on the whole-brain level according to TFCE, using permutation testing (5000 permutations, $p < 0.05$). White matter regions were localised to specific regions and/or tracts using the JHU DTI-based white matter atlas and the JHU tractography atlas.

Table 3.1: Demographics, clinical characteristics and history of substance abuse.

Variable	HC	HR	Statistic	P value
N_S / N_F	79 / 79	117 / 93		
Age (SD)[1]	20.78 (2.27)	21.03 (2.75)	T = -0.65	0.52
IQ (SD)[1]	108.88 (10.42)	106.50 (13.66)	T = 1.36	0.18
Sex[3]				
Male	37 (46.8 %)	55 (47.0 %)	$\chi^2 < 0.01$	0.98
Female	42 (53.2 %)	62 (53.0 %)		
Handedness[3]				
Left	7 (8.9 %)	8 (6.8 %)	$\chi^2 = 0.30$	0.59
Right	70 (88.6 %)	107 (91.5 %)		
Unknown	2 (2.5 %)	2 (2.0 %)		
Occupation parent[3]				
Manual	32 (40.5 %)	52 (44.4 %)	$\chi^2 = 0.55$	0.46
Non-manual	38 (48.1 %)	49 (41.9 %)		
Unclassified	9 (11.4 %)	16 (13.7 %)		
Cyclothymia[2] *	1 (0 – 5)	2 (0 – 6)	U = 3033	0.04
HDRS[2] *	0 (0-1)	0 (0-3)	U = 3653	0.02
YMRS[2]	0 (0-0)	0 (0-0)	U = 4038	0.07
Substance use				
Alcohol (SD, units/wk)[2]	15 (0 – 33)	10 (0 – 27)	U = 4023	0.16
Cigarettes[3]	24 (31.2 %)	42 (35.9 %)	$\chi^2 = 0.46$	0.50
if yes: cig/day (SD)[2]	10 (1 – 19)	10 (0 – 21)	U = 498	0.94
Cannabis[3]	53 (67.9 %)	81 (69.2 %)	$\chi^2 = 0.04$	0.85
Stimulants[3]	25 (32.1 %)	37 (31.6 %)	$\chi^2 < 0.01$	0.95
Hallucinogenic[3]	15 (19.2 %)	21 (17.9 %)	$\chi^2 = 0.05$	0.82
Opiates[3]	2 (2.6 %)	6 (5.1 %)	NA	0.31
Sedatives[3]	4 (5.1 %)	12 (10.3 %)	NA	0.16

[1]group means and standard deviations for variables normally distributed; [2]medians and interquartile ranges for variables not normally distributed; [3]frequency and percentages for categorical variables.

HC = Healthy control group, HR = High risk group, N_S: number of subjects; N_F: number of families; NA: statistics could not be validly calculated due to insufficient number of observations per cell or floor effects.

3.2.5 Tractography

Probabilistic neighbourhood tractography was performed according to the methods explained in Chapter 2. In SPSS, two types of mixed-model regressions were applied: (1) a large mixed model regressing the between-subject variable 'group' and within-subject variables 'tract' and 'hemisphere' on the dependent variable FA; and (2) for each tract of interest with hemisphere as the within-subject factor, group as the between-subject factor, and average FA within the segmented tract as the dependent variable. As detailed in Chapter 2, some subjects were excluded for specific tracts due to truncation, excessive branching, and severe deviation from known anatomy. In addition, a few extreme outliers (> 3 standard deviations) were also removed. This led to 2 missing values for the genu, 1 for the splenium, 7 for the left AF, 7 for the right AF, 22 for the left ATR, 3 for the right ATR, 7 for the left cingulum, 5 for the right cingulum, 2 for the left CST, 2 for the right CST, 28 for the left UF and 1 for the right UF. The advantage of using mixed-model regressions in this respect is that they allow for the unilateral exclusion of a value whilst retaining the data for the other hemisphere. Associations with cyclothymic temperament were tested for by adding log-transformed cyclothymia scores to each of the mixed regression models above.

Comparing FA for these tracts in seven regressions, naturally comes with the risk of inflated type I error due to multiple testing. A simple Bonferroni-based correction can be calculated by dividing 0.05 by 7 resulting in a corrected significance threshold of 0.007. However, in this specific situation there are a few factors that alleviate the probability of type I error and make the Bonferroni adjustment overly conservative: (1) FA is highly correlated between tracts (Penke *et al.*, 2011); (2) if there are any significant effects in the first large regression model it indicates a true effect is present, whether it is be a subtle effect over all tracts together or caused by one or more tracts in particular; and (3) correspondence of tractography results with the location of the voxel-wise results would increase confidence in a significant effect being a "true" effect. For these reasons, initially uncorrected p-values are reported, followed by a discussion of their validity with respect to Bonferroni-corrected significance levels and correspondence with voxel-wise results is also considered.

3.3 Results

3.3.1 Group Comparison: voxel-wise analysis

TBSS followed by TFCE resulted in one large, diffuse cluster (K = 46 101 voxels, $p_{FWE} < 0.05$) extending over most of the white matter skeleton, including the corpus callosum, internal and external capsules (including ATR), inferior and superior longitudinal fasciculi, inferior fronto-occipital fasciculi, uncinate fasciculi, parts of the CST and subcortical white matter around the central sulci (Figure 3.1). Around two thirds of these voxels (K = 29 292) were still significant when the corrected significance level was made more stringent at $p_{FWE} < 0.01$. There were no clusters in which FA was increased in the high-risk group compared to the controls for any voxel-wise analysis.

Similar results were obtained after random removal of related siblings in the unaffected relatives group, again with a cluster covering most of the skeleton (K = 35 820 voxels) significant at $p_{FWE} < 0.01$. Although the groups were well-matched on all demographic variables, additional analyses confirmed that these results were not confounded by age, IQ or sex: a widespread cluster of reduced FA remained after including these variables in the design matrix (K = 41 921; $p_{FWE} < 0.05$).

Furthermore, the post-hoc analysis of average FA extracted across the whole skeleton revealed that FA was significantly reduced in the unaffected relatives compared to controls (T = 2.87, p = 0.005). The groups did not differ significantly in total white matter volume (T = 0.55, p = 0.58). And again in line with the TBSS results, the voxel-based analysis conducted without a white matter skeleton also indicated reduced FA in the unaffected relatives (K = 387 442 voxels, $p_{FWE} < 0.05$) compared to controls (Figure 3.2).

3.3.2 Group comparison: Tractography

Results of the different mixed-model regressions of group on FA are summarised in Table 3.2. Figure 3.3 and Table 3.3 summarise the average FA for each tract, comparing the high-risk group to the control group. The first large regression model for all tracts together revealed a significant effect of group on FA (F = 8.135, p = 0.005). This is a reflection of FA being lower in the high risk group compared to the control group, and consistently so across tracts, which is in line with

the voxel-wise results (Figures 3.1 & 3.2). As can be expected, main effects of tract and hemisphere were also observed, but more importantly there were no group-by-tract or group-by-hemisphere interactions. However, separate regressions for each tract revealed that the effect was certainly stronger in some tracts and nearly absent in others.

To summarise Figure 3.3 and Table 3.2, there was a highly significant effect of group on FA in the AF ($F = 13.595$, $p < 0.001$) across both hemispheres, which comfortably survives a Bonferroni correction at $p < 0.007$ (0.05 / 7 tracts). There was also a nominally significant effect of group on FA in the genu ($F = 4139$, $p = 0.043$). For the other tracts, none of the differences were significant. The only tract giving some indication for a group-by-hemisphere interaction was the ATR ($F = 2.229$, $p = 0.137$), with a trend for the left ATR ($T = 1.748$, $p = 0.065$) but not for the right. All of these tractography results remained similar when co-varying for age, sex and IQ, after exclusion of left-handed people and after random exclusion of related individuals.

Figure 3.1. TBSS results of the group comparison. TBSS revealed reduced FA in the high-risk group compared to the control group in one large cluster (K= 46 101 voxels; $P_{FWE} < 0.05$). For better visibility the results are thickened using the 'tbss_fill' command. The images are in radiological convention.

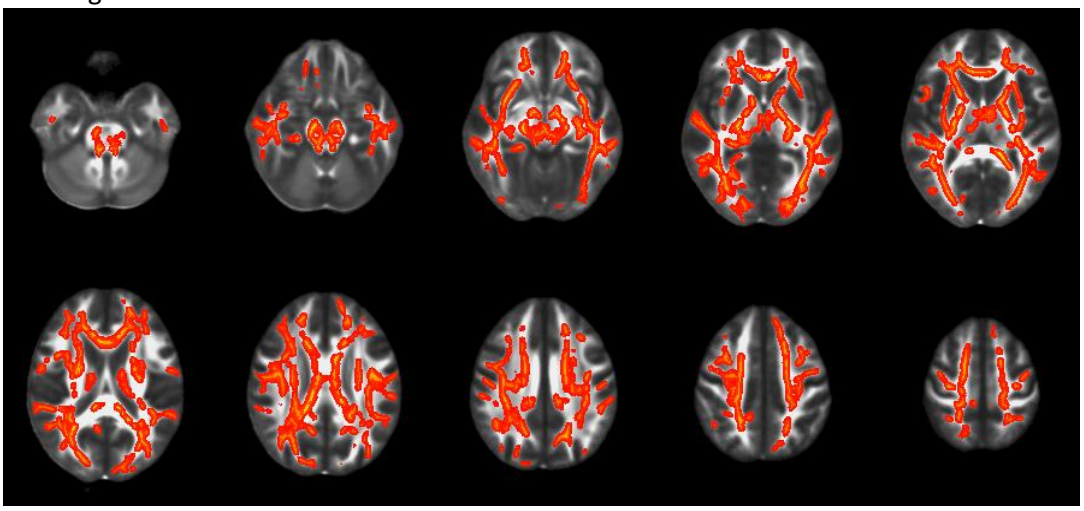


Figure 3.2. Voxel-based result of the group comparison. Voxel-based analysis revealed reduced FA in unaffected relatives of patients with bipolar disorder compared to the control group in one large cluster extending over most parts of the white matter (K = 387 442 voxels, $p_{FWE} < 0.05$). The images are in radiological convention.

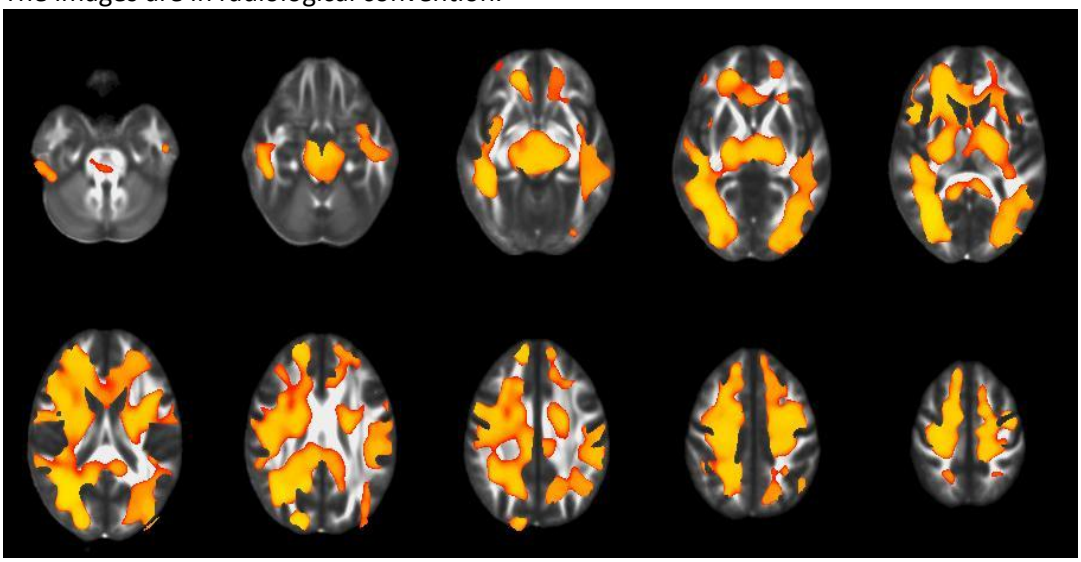


Figure 3.3. Tractography results of the group comparison. Tractography revealed significant FA reductions in the AF and the genu of the corpus callosum of the unaffected relatives of patients with bipolar disorder. Overall, there were no tracts in which FA was increased in the unaffected relatives and a large mixed model also revealed an overall effect of group in the absence of group-by-tract or group-by-hemisphere interactions on FA.

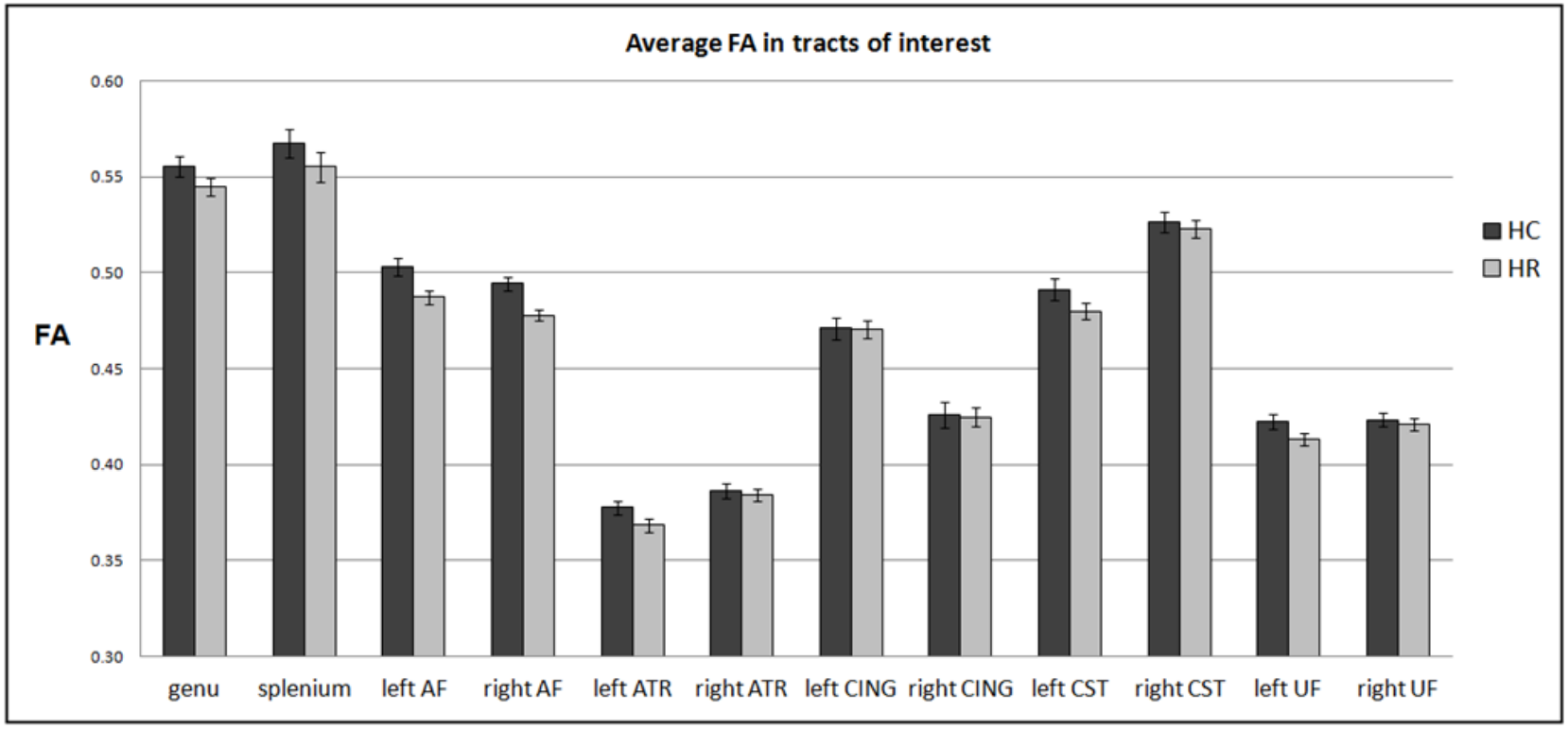


Table 3.2. Results of mixed regressions

LARGE MODEL:	**Group**	**Tract**	**Hem**
	F=8.135 (p=0.005)*	F=391.687 (p<0.001)	F=0.147 (p=0.701)
groupXtract	**Group X Hem**	**Hem X Tract**	**Group X Tract X Hem**
1.110 (p=0.353)	F=0.815 (p=0.367)	F=47.001 (p<0.001)	F=0.215 (p=0.930)
BY TRACT:	**Group**	**Hemisphere**	**Group X Hem**
AF	***F=13.595 (p < 0.001)****	F=8.927 (p=0.003)	F=0.039 (p=0.843)
ATR	F=1.759 (p=0.186)	F=20.170 (p<0.001)	F=2.229 (p=0.137)
CING	F=0.016 (p=0.899)	F=114.899 (p<0.001)	F=0.001 (p=0.974)
CST	F=1.989 (p=0.160)	F=96.340 (p<0.001)	F=0.820 (p=0.366)
UF	F=1.594 (p=0.208)	F=2.978 (p=0.086)	F=1.611 (p=0.206)
GENU	*F=4.139 (p=0.043)**	-	-
SPLENIUM	F=2.330 (p=0.128)	-	-

Statistics in bold are significant under a Bonferroni-type correction (p-value multiplied by number of unilateral tracts; See text) Hem=Hemisphere; AF=arcuate fasciculus; ATR=anterior thalamic radiation; CING=cingulum bundle; CST=cortico-spinal tract; UF=uncinate fasciculus;

Table 3.3. Simple effects per unilateral tract (corresponding to Figure 3.3)

Means per group, statistics and standardised effect sizes (Cohen's *d*)

TRACT	**HC**		**HR**		**Statistics**			
	Mean	**SE**	**Mean**	**SE**	**T**	**df**	**p**	***d***
genu	0.55531	0.00503	0.54503	0.00456	1.490	192	0.138	0.215
splenium	0.56755	0.00744	0.55502	0.00747	1.188	193	0.236	0.171
left AF	0.50295	0.00446	0.48717	0.00374	2.694	187	*0.008**	0.394
right AF	0.49445	0.00357	0.47785	0.00314	3.460	187	*0.001**	0.506
left ATR	0.37758	0.00338	0.36839	0.00362	1.855	172	0.065	0.283
right ATR	0.38604	0.00384	0.38401	0.00327	0.401	191	0.689	0.058
left CING	0.47091	0.00581	0.47058	0.00451	0.045	187	0.964	0.007
right CING	0.42595	0.00696	0.42476	0.00491	0.140	189	0.889	0.020
left CST	0.49118	0.00549	0.47978	0.00421	1.665	192	0.098	0.240
right CST	0.52640	0.00515	0.52258	0.00450	0.566	192	0.872	0.082
left UF	0.42212	0.00399	0.41326	0.00319	1.748	156	0.083	0.280
right UF	0.42336	0.00362	0.42115	0.00320	0.451	193	0.652	0.065

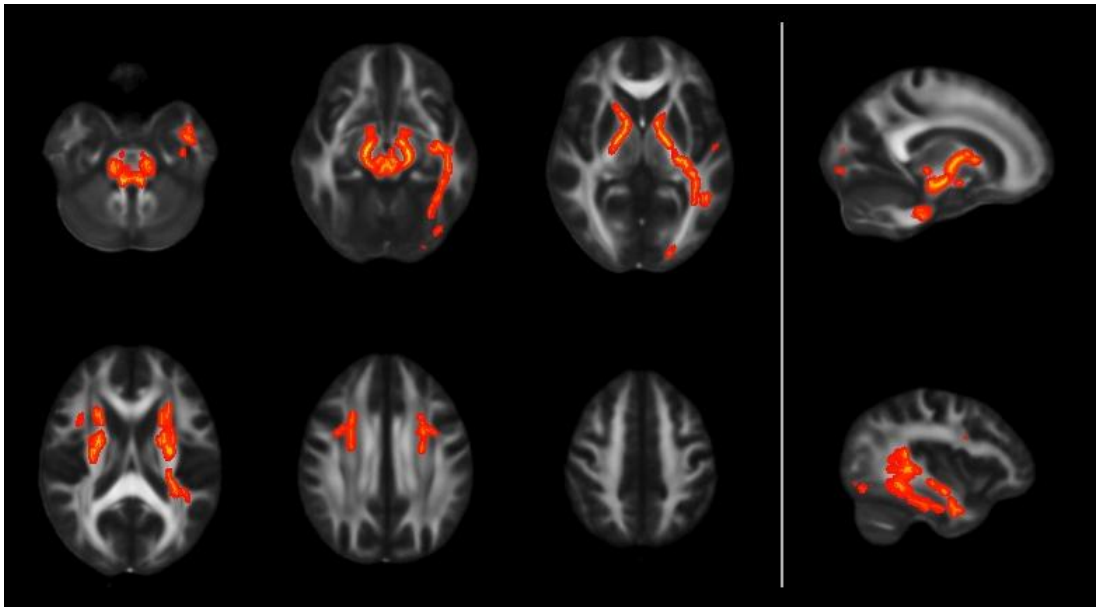
Note that the main analyses were not performed on simple effects per unilateral tract, but as mixed regressions, across hemispheres (and tracts). This table is included to provide meaningful effect sizes that allow for comparisons across studies, not for assessing the statistical significance of the present findings per se. The values correspond to those illustrated in Figure 3.3.

3.3.3 Cyclothymic Temperament: voxel-wise analysis

Unaffected relatives had significantly higher cyclothymia scores than the controls (U = 3033, p =0.04; log-transformed: T= 2.06, p = 0.04). Voxel-wise association of cyclothymia scores with FA within the TBSS skeleton revealed three clusters in which FA was negatively associated with cyclothymia (Figure 3.4). The largest one (K = 10 128 voxels, $p_{FWE} < 0.05$) consisted of the internal capsules bilaterally connected via deep sub-thalamic white matter; and two smaller clusters (K = 236 voxels, K = 2 voxels, both at $p_{FWE} < 0.05$) in the left hemisphere containing several fronto-temporal and fronto-thalamic connections including the external capsule, inferior longitudinal fasciculus, anterior parts of the left AF, dorsal left UF as well as left occipital white matter. After random removal of related siblings, a trend for the effect of cyclothymia remained, with similar results to the full sample when the significance level was relaxed to $p_{FWE}< 0.08$.

The between group difference in FA was also significant in this analysis in three clusters (K= 9 349, K=250 and K=198 contiguous voxels, $p_{FWE}< 0.05$) mostly in the right hemisphere and the corpus callosum, indicating that the group effect is at least partly independent of the cyclothymia effect. There were no voxels in which there was a significant interaction between group and cyclothymia scores (all $p_{FWE}> 0.40$).

Figure 3.4. Negative association of FA with cyclothymic temperament. TBSS results for the effect of cyclothymia on FA, corrected for the effects of group, showing effects in the bilateral internal capsules and in the left temporal white matter. For better visibility the results are thickened using the 'tbss_fill' command. The images are in radiological convention.



3.3.4 Cyclothymic temperament: Tractography

The large mixed regression model including cyclothymia scores revealed no significant effect of cyclothymia scores on overall FA, and no interactions with tract, hemisphere or group. Effects of group, tract and hemisphere all remained significant, as in the model without cyclothymic temperament. Separate regressions per tract resulted in significant negative associations of cyclothymic temperament on FA in the AF bilaterally ($F = 9.847$, $p = 0.002$, Figure 3.5) and more nominally in the UF ($F = 4.414$, $p = 0.037$), in the absence of any interactions with hemisphere or group. Again, the result in the AF comfortably survives Bonferroni correction at $p < 0.007$, but the one in the UF does not. And yet again the only tract giving any indication of laterality was the ATR with a significant cyclothymia-by-hemisphere interaction effect on FA ($F = 4.211$, $p = 0.042$), which was caused by a negative association of cyclothymia with FA in the left ATR ($T = -2.025$, $p = 0.045$) but not in the right.

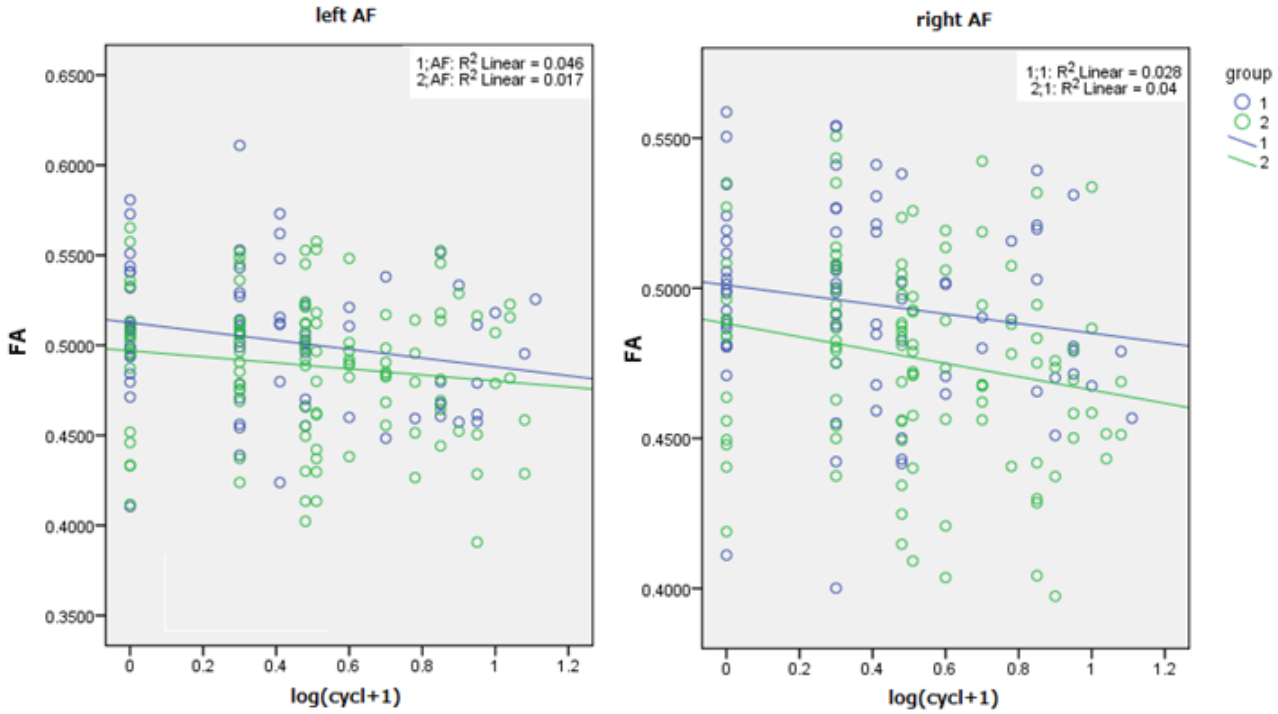
3.4 Discussion

Here, I report widespread reductions in white matter integrity in our sample of unaffected relatives of patients with BD. These findings support the notion of white matter integrity as an endophenotype of BD, and suggest that impaired white matter integrity may be one of the underlying mechanisms of genetic predisposition to BD. In addition, I demonstrate an association between cyclothymic temperament and white matter integrity in specific temporal and fronto-thalamic regions, further supporting the view that white matter abnormalities have behavioural associations that are related to the symptomatology of the clinical disorder.

3.4.1 White matter and genetic risk for BD

As reviewed previously, others have found white matter abnormalities in unaffected relatives of patients with BD, most of them using white matter density or volume derived from T_1-weighted MRI scans. Kiesappä *et al.* (2003) reported reduced left hemisphere white matter volumes in the unaffected twins of BD patients, McIntosh *et al.* (2005) found that unaffected relatives of patients had reduced white matter density in prefrontal areas, and McDonald *et al.* detected an inverse correlation between genetic liability to BD and white matter volume in several frontal,

Figure 5.3. Negative association of cyclothymic temperament with FA in the left and right arcuate fasciculus (AF).



AF = Arcuate Fasciculus; cycl = cyclothymic score; group 1 = control group; group 2 = high risk group.

and temporo-parietal regions (McDonald, Bullmore *et al.*, 2004). Two previous DTI studies of relatives of bipolar patients applied voxel-based analysis of whole brain FA values. Frazier *et al.* (2007) compared seven unaffected relatives with eight controls and found reduced FA in two clusters in the superior longitudinal fasciculi. However, the extremely small sample size, the lack of adequate correction for multiple comparisons, the very young age of the participants (4-12 years) and the presence of axis I disorders and other comorbidities in the majority of the children greatly limited the interpretation of that first study. In another DTI study, Chaddock *et al.* (2009) did not detect any differences contrasting twenty-one relatives to eighteen controls, but found that genetic liability was associated with widespread FA reductions. Finally, Versace *et al.* (2010) applied TBSS to a group of children of patients with BD . Although in these unaffected children, they found an overall increase of FA in the corpus callosum and the right temporal lobe,

their main finding was an interaction with age where FA in the high risk group decreased with age whilst in the control group it increased leading to an overall reduction of FA in those at high risk over the age of fourteen. Overall, our results of widespread FA reductions are most in line with the negative correlation of FA with a genetic risk score reported by Chaddock *et al.* (2009) although their sample may have been too small to detect this in a direct group contrast. The results of Frazier *et al.* (2007) and Versace *et al.* (2010) were much more confined to specific regions, albeit they did not agree, and this may be explained by statistics. First, our sample was much larger leading to an increase in statistical power which may be why we found more extended reductions than the studies by Frazier *et al.* (2007) and Versace *et al.* (2010). Furthermore, the other studies did not apply TFCE, and therefore would have required larger effect sizes within any given voxel to reach significance. Given the late development of some white matter tracts, another important difference with both of these previous studies is age: Frazier *et al.* (2007) studied FA in children as young as 4 years old and Versace *et al.* (2010) adolescents were between 8 and 17, whilst the unaffected relative in Chaddock's study were on average about twenty years older than our participants. These age differences may not only affect results in the case of age-by-group interactions, but also in terms of clinical characteristics of the samples because the younger the children are, the less confident one can be they will be unaffected as the typical age of onset has not been reached.

Another advantage of our study compared to previous studies is in the application of three different analyses methods, and the ability to corroborate voxel-wise results with tractography. The latter enables localisation to specific tracts and does not require co-registration, thereby better accounting for anatomical variability between individuals. Overall, the results of the three methods corresponded well and all indicated subtle but widespread FA reductions in the high risk group. However, tractography clearly indicated a large effect in the AF and modest effects in the genu, and could not confirm effects in other regions. On the other hand, the absence of any interactions between tract and group effects in the large mixed model seems to imply the effect, however small, is consistent across tracts. This is also visible in Figure 3.3, where most tracts display a distinct group difference in FA albeit non-significant. Whilst TFCE is a strict method for correction of multiple testing, it allows for quite substantial differences in effect sizes across voxels, and may include voxels with very small effect sizes as long as there are many

consecutive voxels with similar tendencies. Altogether, the three methods suggest widespread, but in places subtle, FA reductions in the unaffected relatives, and any discrepancies between tractography and the voxel-wise methods in "significant" and "non significant" locations can be explained by the underlying statistics (TFCE vs. univariate statistics). In line with the idea of globally reduced FA, unaffected relatives also had lower FA averaged over the whole skeleton. This measure of whole brain white matter integrity is also estimated to have high heritability (> 50%), and has been associated with mood-related genetic variation using quantitative trait linkage analysis (Kochunov *et al.*, 2010). For these reasons this summary measure could be particularly useful in delineating multivariate genetic effects on white matter.

3.4.2 White matter and cyclothymic temperament

Here, the term temperament is used to describe a relatively stable, non-pathological trait to describe individual differences both in the healthy population and in clinical populations. In this context, cyclothymia has been described as defining "a subaffective disorder as well as a temperament" (Chiaroni *et al.*, 2005). According to the theory by Akiskal and others cyclothymic temperament is one five affective temperament dimensions that together define a continuous space of affective stability and reactivity onto which the general population as well as the entire spectrum of affective disorders could be mapped (e.g. Akiskal & Akiskal, 2005). To what extent this overall continuum theory is true, and moreover, to what extent cyclothymic temperament is phenomenologically and biologically similar to bipolar disorder (as its presumed extreme), is a matter of debate. However, several studies at least provide support for the internal consistency, test-retest reliability (stability), power to predict recurrent depression, power to disciminate between unipolar and bipolar disorders, and correlations with quality of life measures in both healthy and patient populations (Matsumoto *et al.*, 2005; Mazzarini *et al.*, 2009; Nilsson *et al.*, 2012; Vasquez *et al.*, 2008). Furthermore, the finding that unaffected relatives of bipolar patients have increased cyclothymic temperament suggests it may share a genetic component with BD (Vasquez *et al.*, 2008). In this chapter I do not make assumptions about the phenomenological and theoretical relationship of cyclothymic temperament to clinical BD, but the following discussion interprets the results under the more narrow assumption that cyclothymic temperament and BD have - to some extent - overlapping biological components.

Using TBSS I found a negative association between cyclothymic temperament and white matter integrity in the internal capsules bilaterally and in the left temporal lobe, across both high-risk subjects and controls. Following tractography, the association with FA in the temporal regions appeared in the UF and the AF, bilaterally. In contrast, the effect in the ATR, which runs through the internal capsules, was only found in the left hemisphere. Thus, although the overall locations of the cyclothymia associations were compatible with the TBSS results, being in fronto-temporal and fronto-thalamic connections, there were differences in laterality. Whilst the ATR connects the frontal lobes to the thalamus and the striatum, the UF and AF are the two main fronto-temporal fibre tracts. As reviewed in Chapter 1, these connections are assumed to play key roles in emotion perception and regulation according to various neuro-cognitive and neurobiological models, and they have been widely associated with BD in brain imaging studies. Our findings can be integrated into the models of emotion regulation of Phillips *et al.* (2003a, 2003b, 2008), Strakowski *et al.* (2005) and Ochsner and Gross (2005): genetic predisposition to BD may become manifest through subtle reductions in overall white matter integrity, making unaffected relatives more vulnerable to more localised disruptions in emotional brain networks. The latter localized disruptions can result from genetic factors or environmental stressors, and may correspond to the associations of cyclothymia with FA in parts of the prefrontal-thalamic-limbic network I present here. However, the literature does not imply any differences in laterality, although a few studies have found that reductions in white matter (Bruno *et al.*, 2008; McIntosh, Muñoz Maniega, Lymer *et al.*, 2008), and temporal lobe volumes (Hallahan *et al.*, 2010) of affected individuals are stronger in the left hemisphere. The present laterality inconsistency between TBSS and tractography may also have a methodological source. Both the left ATR and the left UF were decidedly more difficult to trace than their right counterparts and the resulting unilateral exclusions of some individuals may have caused a bias towards either hemisphere.

3.4.3 Limitations

Given the inconsistencies in previous reports, and the fact that both groups in our study consisted of healthy individuals only, we anticipated that any between-group differences would be subtle. We therefore maximized the sensitivity of our analyses using high resolution DTI data in 64 gradient directions in a large well-matched sample of unaffected relatives and controls. Furthermore, we analysed data across the whole brain, avoiding the need for anatomically-

specific hypotheses, and used state-of-the art methods including TBSS and probabilistic tractography. But of course there are a several limitations to this investigation. Firstly, since there was no affected patient group in our study, I could not directly assess whether unaffected relatives were intermediate between patients and controls with respect to FA, although the existing literature indicates that this is the case (Chaddock *et al.*, 2009). Secondly, it is not possible to distinguish between genetic effects and the environmental consequences of growing up in proximity to someone with major mental illness. However, since white matter integrity is under strong genetic control (Konrad *et al.*, 2009; Winterer *et al.*, 2008; Sprooten *et al.*, 2009; Chiang *et al.*, 2009; Kochunov *et al.*, 2010), it is likely that genetic factors are the strongest determinant of the current findings. Thirdly, tractography of all tracts was not successful for all individuals and after visual evaluation of the segmentations, a substantial number of individuals were excluded for some of the tracts, particularly the left UF and ATR. We cannot tell from this analysis whether these exclusions were random of whether this may have introduced a selection bias, as we do not know the true FA values of the discarded tracts, however it is reassuring that the numbers of exclusions per tract did not differ between the groups. Finally, in the absence of any clear pre-hypothesised regions and following from the widespread results of the voxel-wise analysis, it was necessary to exploring the effects of genetic risk across 12 tracts of interest using tractography. But this comes with a potential type I error inflation, for which there are no standard ways of correcting. Both genetic and cyclothymia effects on the bilateral AF comfortably survive a conservative Bonferroni correction, but the more modest effects and trends in the genu, ATR and UF do not and need to be interpreted with caution.

3.4.4. Conclusions

By taking advantage of the high sensitivity of DTI to white matter integrity in combination with state of the art methodology, we were able to demonstrate white matter integrity reductions in a large sample of unaffected relatives of patients with BD for the first time. Whilst the effects of genetic risk were widespread, the associations with cyclothymia were more localized to fronto-temporal and prefrontal-thalamic connections. This suggests that reduced fronto-temporal and fronto-thalamic white matter integrity is a structural substrate of mood instability in both healthy controls and unaffected relatives at high genetic risk for BD. Given that white matter integrity as measured by FA is highly heritable, our findings of widespread but subtle reductions in FA in the

unaffected relatives support the consideration of white matter integrity as an endophenotype for BD, and encourage future studies assessing its relationship to risk-associated genetic variation.

Chapter 4

White matter integrity in relation to *NRG1* and *ErbB4*

4.1. Introduction

The protein NRG1 is a trophic factor which directly activates ErbB4 receptor kinases. Both NRG1 and ErbB4 have many isoforms, which in combination can serve a multitude of distinct functions. Their genes are abundantly expressed in the brain, and have spatially and temporally controlled expression profiles. The gene *NRG1* is located on chromosome 8 (at 8p12), a genomic region historically linked to psychiatric disorders (Tabarés-Seisdedos *et al.*, 2009). The gene for ErbB4 is located at 2q34 and regions nearby in 2q have also been linked to psychiatric disorders (e.g. Paunio *et al*., 2001). Evidence suggestive of the involvement of *NRG1* and *ErbB4* in genetic risk for psychiatric phenotypes comes from several angles of research including statistical evidence from association studies, but perhaps even more compelling, evidence of NRG1 / ErbB4 function. In this introduction I will summarise evidence from association studies, followed by a brief description of what is known about NRG1 and ErbB4 functions in the brain and their relationships to white matter. I will then present results of my research relating *NRG1* and *ErbB4* genotypes to FA as part of the Bipolar Family Study.

4.1.1 Association studies of NRG1 and ErbB4

When Stefansson *et al.* (2002; 2003) identified and replicated the association of schizophrenia with a haplotype at the 5'end of the gene, *NRG1* became one of the most widely accepted candidate genes for psychotic disorders. The core Icelandic at-risk haplotype ("Hap_{ICE}") consists of 5 SNPs (SNP8NRG221132, rs35753505, SNP8NRG241930, rs6994992, SNP8NRG433E1006) and two microsatellites. Hap_{ICE}, its individual SNPs and overlapping haplotypes have since been associated with schizophrenia and BD in various populations (Alearts *et al.*, 2009; Corvin *et al.*, 2004; Georghieva *et al.*, 2008; Goes *et al.*, 2009; Green et al, 2003; Kim, Lee *et al.*, 2006; Thomson *et al.*, Petryshen *et al.*, Yang *et al.*,

2003; Tang *et al.*, 2004; Li *et al.*, 2004; Prata *et al.*, 2009; Walker *et al.*, 2010; Williams *et al.*, 2003). But, as with every candidate gene negative findings also exist (Cassidy *et al.*, 2006; Lachman *et al.*, 2006; Ikeda *et al.*, 2008; Iwata *et al.*, 2004). Confusingly, several studies have associated psychotic disorder with opposite alleles, or different haplotypes (Alearts *et al.*,2009; Bakker et al, 2004; Corvin *et al.*, 2004; Georgieva *et al.*, 2008; Kim, Lee, *et al.*, 2006; Lachman *et al.*, 2006; Prata *et al.*, 2009; Thomson *et al.*, 2007). The four meta-analyses on *NRG1* case-control associations available to date also vary in their conclusions and they all stress significant heterogeneity across studies and ethnicities, even within the European population and especially for Hap_{ICE} and for rs35753505 (Gong *et al.*, 2009; Li, Collier & He, 2006; Munafa *et al.*, 2006, 2008). A further complicating factor is that most of the commonly associated SNPs are functionaly ill-defined and are assumed to exert their effect via linkage disequilibrium and/or interactions with one or more other SNPs and genes. Hence, the discrepancies between studies could be explained by population differences in linkage disequilibrium structure between tagging and functional SNPs, as well as more general interactions with genetic and environmental background. It is therefore not surprising that the most convincing association results have arisen from confined populations such as the Icelandic sample, and that much larger effect sizes have been reported in small studies of individuals at high clinical or genetic risk in which all individuals homozygous for the risk alleles at rs6994992 presented with psychotic symptoms compared to only 14%-46% of the non-risk carriers (Hall *et al.*, 2006; Keri, Kiss & Kelemen, 2009).

Association studies of *ErbB4* with psychiatric disorders are less abundant. Silberberg et al (2006), found an association with schizophrenia and 3-SNP haplotype comprised of rs7598440, rs83523 and rs707284 (A-G-G). Nicodemus *et al.* (2006) found two haplotypes in *ErbB4* to be significantly more common in patients, one of which was replicated in two independent samples and overlapped with Silberberg's haplotype albeit with opposite risk alleles. Association with the other significant haplotype of that study was later replicated in a Han Chinese sample in addition to an association with one of the SNPs within it (Lu *et al.*, 2010). Yet others have found no (Shiota *et al.*, 2008) or only marginal evidence for association with schizophrenia (Norton *et al.*, 2006) but instead they found significant interactions with *NRG1* genotypes.

None of the SNPs in Hap_{ICE} or the Silberberg *ErbB4* haplotypes are exonic. Nevertheless, at least some of them seem to have a functional consequence in regulating expression of the respective

genes. In post-mortem studies, Law *et al.* (2006a, 2006b) found that the *NRG1* SNP rs6994992 and each of the SNPs in Silberberg *ErbB4* haplotype are associated with mRNA expression levels of their respective genes. Furthermore, *NRG1* expression is increased in the hippocampus of bipolar and schizophrenia patients who have different concentrations of the distinct *ErbB4* mRNA isoforms (Law *et al.*, 2006b; Silberberg *et al.* 2006).

4.1.2 Functions of NRG1-ErbB4 signalling

Both *NRG1* and *ErbB4* produce a large multitude of isoforms by alternative RNA splicing and cleavage, the combinations of which define their distinct functions. There is a wealth of in vitro, post-mortem and animal research describing the functions of NRG1-ErbB4 signalling. It is beyond the scope of this chapter to describe these studies but for reviews see Harrison & Law (2006), Mei & Xiong (2008) and Corfas *et al.* (2004). In general, NRG1 and ErbB4 play important roles in neurodevelopmental processes such as neuronal and glial proliferation, differentiation and migration as well as axon guidance (Anton *et al.*, 1997; Rio *et al.*, 1997; Lopez-Bendito *et al.*, 2006). NRG1-ErbB4 signalling is also vital for many plasticity-related processes continuing throughout adulthood such as regulation of NMDA and GABA receptor function (Hahn *et al.*, 2006; Gu *et al.*, 2005) and neuron-glia signalling to promote myelination (Chen *et al.*, 2006; Taveggia *et al.*, 2008). Many of these processes are akin to key hypotheses of schizophrenia: the glutamate hypothesis (Konradi & Heckers, 2003), dysconnectivity hypothesis (Friston 1995, 2002; Stephan, Friston & Frith, 2009), GABA-ergic dysfunction (Blum & Mann, 2002), thereby further supporting *NRG1* and *ErbB4* as candidate genes. Interestingly for the present research, disruption of most of the above processes would have a direct impact on white matter structure.

A few imaging genetics studies indeed found evidence for associations of *NRG1* and *ErbB4* with white matter integrity. Our group have found that healthy individuals homozygous for the risk-allele ('T') at rs6994992 had reduced white matter density (derived from T_1-weighted images) and FA in the ALIC (McIntosh, Moorhead *et al.*, 2008), which was later confirmed to be in the ATR using tractography (Sprooten *et al.*, 2009). In line with this, Winterer *et al.* (2008) reported reduced medial prefrontal FA in carriers of the C-allele at rs35753505. Similarly, with respect to *ErbB4* both our groups have found reduced FA in association with risk alleles in the *ErbB4*

region. In particular, Konrad *et al.* (2008) found reduced FA in the left temporal white matter, including the external capsule in healthy individuals homozygous for the risk-allele ('G') at rs839523; whilst Zuliani *et al.* (2011) found an association of FA in the ALIC, in particular the left, in with rs4673628, the locus previously interacting with Hap_{ICE} on risk for schizophrenia (Norton *et al.*, 2008).

In summary, numerous association studies support the involvement of *NRG1* and *ErbB4* in psychiatric disorders, but there is notorious disagreement about the genomic locations and the directions of the risk variants, especially concerning Hap_{ICE} in *NRG1*. This suggests a complicated picture in which genetic background and environmental factors modulate or mediate the effects of genetic variation at the *NRG1* and *ErbB4* regions. Using intermediate phenotypes is one way to reduce the number of environmental factors that may affect clinical phenotypes, and considering the functions of NRG1-ErbB4 signalling white matter integrity seems a logical mediator between gene and symptomatology. In this chapter I present an investigation of the relationship of FA with four *NRG1* SNPs in Hap_{ICE} and with the three *ErbB4* SNPs that make up the haplotype identified by Silberberg *et al.* (2006).

4.2 Methods

4.2.1 Study sample

Participants were recruited as part of the Bipolar Family Study. All scanning, genotyping and screening were performed as described in Chapter 2. DTI and *NRG1* / *ErbB4* genotype data were available for 196 participants, of which 26 were excluded because they had a first or second degree relative who also participated in the study. These excluded participants were selected at random for each family. After this, 170 individuals were left, including 87 unaffected relatives of patients with BD.

4.2.2 Genotyping and haplotype estimation

Seven SNPs were genotyped: four SNPs in the *NRG1* region (NRG221132, rs35753505, NRG241930, and rs6994992) and three SNPs in the *ErbB4* region (rs7598440, rs839523 and

rs707284). For all SNPs, allele frequency distributions were in accordance with the Hardy-Weinberg equilibrium although one SNP, rs6994992, had a p-value of 0.09. As expected, SNPs within haplotypes were in high linkage disequilibrium with each other. See Table 4.1 for genotype distributions and Table 4.2 for allele frequencies and for statistics of Hardy-Weinberg equilibrium tests and linkage disequilibrium. Haplotypes were estimated using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/), which applies an expectation–maximization algorithm. The 4 SNP *NRG1* core Icelandic risk haplotype was identified as 'GGCT' at NRG221132, rs35753505, NRG241930, and rs6994992 respectively. The 3 SNP haplotype as identified by Silberberg *et al.* (2006) and Law *et al.* (2007) is 'TCC' at rs7598440, rs839523 and rs707284, respectively. Both risk haplotypes were common in our sample with frequencies of 0.36 for Hap_{ICE} and 0.58 for the *ErbB4* haplotype. Haplotype estimation was successful for all participants with most posterior probabilities equal to 1, and all exceeding 0.78.

In our sample, there was an association of the rs6994992 *NRG1* SNP with at-risk status (χ^2 = 4.091, p = 0.043), where the minor allele ("C") was less common in the high risk group than in the control group. Other allele frequencies had similar frequencies in both groups (all p > 0.14). Neither haplotype frequency was significantly higher in the unaffected relatives (Hap_{ICE}: p = 0.13; Hap_{ErbB4}: p = 0.87). For NRG221132 only 3 individuals were homozygous for the A-allele, so they were combined with the heterozygotes into an A-carrier group. IQ differed significantly between the *NRG1* haplotype and SNP groups in the healthy control group (all p < 0.05). There was also some statistical dependence of sex with the Hap_{ICE} in the controls (χ^2 = 5.918, p = 0.052); and with rs35753505, rs6994992 and Hap_{ICE} in the unaffected relatives (χ^2 = 8.669, p = 0.034; χ^2 = 6.597, p = 0.06; χ^2 = 4.968, p = 0.088; respectively). Potential confounding effects of these demographic measures were examined in post-hoc analyses. Age did not differ between the *NRG1* genotype groups (p > 0.42). All *ErbB4* genotypes were well-matched for age, IQ and sex (all p > 0.18).

4.2.3 TBSS and tractography

TBSS was performed according to standard procedures available from FSL and as described in detail in Chapter 2. Average FA over the whole skeleton was also extracted, and ANOVA's were performed in SPSS to test any effects of genotypes on this summary measure. For its increased power as a summary measure, whole-skeleton FA was also used to test for interactions between

NRG1 and *ErbB4* haplotypes, and for interactions with age. Age is of special concern in the case of NRG1-ErbB4 signalling because of their temporally controlled expression profile and its

Table 4.1. Number of participants per genotype

SNP / Haplotype	**HC** (N = 83)*	**HR** (N = 87)*	**HWE (p)**
HapICE (0 – 1 - 2)	30 – 38 - 15	39 – 33 – 15	-
rs35753505 (AA-AG-GG)	29 – 37 - 14	37 – 33 - 14	0.24
rs6994992 (CC-CT-TT)	27 – 36 - 20	35 – 35 - 15	0.09
NRG221132 (AA-AG-GG)	1 – 17 - 65	2 – 16 – 69	1
NRG241930 (AA-AC-CC)	11 – 37 - 35	13 – 37 - 37	0.24
HapErbB4 (0 – 1 - 2)	15 – 40 - 28	13 – 49 - 25	-
rs707284 (CC-CT-TT)	40 – 35 - 8	45 – 35 – 7	0.74
rs7598440 (CC-CT-TT)	14 – 40 - 28	12 – 50 – 25	0.58
rs839523 (CC-CT-TT)	44 – 32 - 7	48 – 33 – 6	0.72

*There are a few missing values, due to undetermined genotypes

Table 4.2: SNP-by-SNP linkage disequilibria

	Linkage Disequilibrium (D')			
NRG1	**NRG221132**	**rs35753505**	**NRG241930**	**rs6994992**
NRG221132	1			
rs35753505	1.000	1		
NRG241930	1.000	0.976	1	
rs6994992	1.000	0. 943	1.000	1
ErbB4	**rs7598440**	**rs839523**	**rs707284**	
rs7598440	1			
rs839523	0.980	1		
rs707284	0.982	1.000	1	

involvement in brain development. Indeed, different patterns of grey matter development have been found in children carrying *NRG1* risk-alleles (Addington *et al.*, 2007).

Probabilistic neighbourhood tractography was performed as described in Chapter 2. Visual inspection of the tract segmentations led to the exclusion of a significant number of people for deviations of known anatomy of some of the tracts. These exclusions were done on a tract-by-tract basis, and the application of mixed models allows for appropriate within-subject analyses with missing data for one hemisphere but not for the other. As discussed in Chapter 2, the left UF and ATR in particular proved difficult to trace. For the left UF, 26 unaffected relatives and 14 controls were excluded, whilst for the left ATR 15 unaffected relatives and 11 healthy controls were excluded. Seven individuals were excluded for the right AF. Exclusions for the other tracts were minimal ranging from 1 to 3 individuals.

4.2.4 Multiple testing and corrections

Inflation of type I error due to multiple testing should be considered for several factors, namely tests across many voxels in TBSS, tests across multiple tracts and hemispheres and tests across multiple SNPs and haplotypes. TFCE of course corrects for multiple testing across voxels. All reported p-values resulting from the TBSS analysis were corrected in this fashion. For each SNP, two genetic models were tested, a dominant model (risk-carriers vs. non-risk homozygotes), and a recessive model (risk-homozygotes vs. non-risk carriers). Because both homozygotes remain in the same group, these models are also statistically dependent. No correction was performed for this, but results were examined to see if they were in agreement with one another. To the extent one considers these tests independent, a higher agreement between the two results implies a lower probability of a false positive.

For the 2 haplotypes and 7 SNPs, traditional Bonferroni corrections would be overly conservative since the SNPs and haplotypes within each gene are in extremely high linkage disequilibrium (Table 4.1). Dividing the p-value threshold by a factor of two (for the separate genes) would be more appropriate, albeit arguably somewhat anti-conservative. I will consider this for any significant initial contrast in the results section.

FA values are also highly correlated across tracts (Penke *et al.*, 2010), again making a Bonferroni correction overly conservative. Hence, for initial detection of significant effects the reported p-values are uncorrected. Especially for interaction terms which have less statistical power it would be important to keep type II error low. Similar to Chapter 3, after analyses, any significant p-values are compared to Bonferroni corrected significance thresholds which were computed for each result separately depending on the number of tests performed to obtain it. Thus, the initial alpha 0.05 is first divided by the number of mixed models performed, one for each tract, i.e. 5 unilateral + 2 corpus callosum = 7 tracts. If any interaction was observed and two subsequent tests were performed to examine effects within group or hemisphere, the resulting significant threshold was further divided by 2 (either hemispheres or group) or 4 (if it concerns a unilateral effect within one group). However, it should be borne in mind that this remains a conservative way of performing a multiple testing correction given the intercorrelations between tract FA.

4.3 Results

4.3.1. NRG1

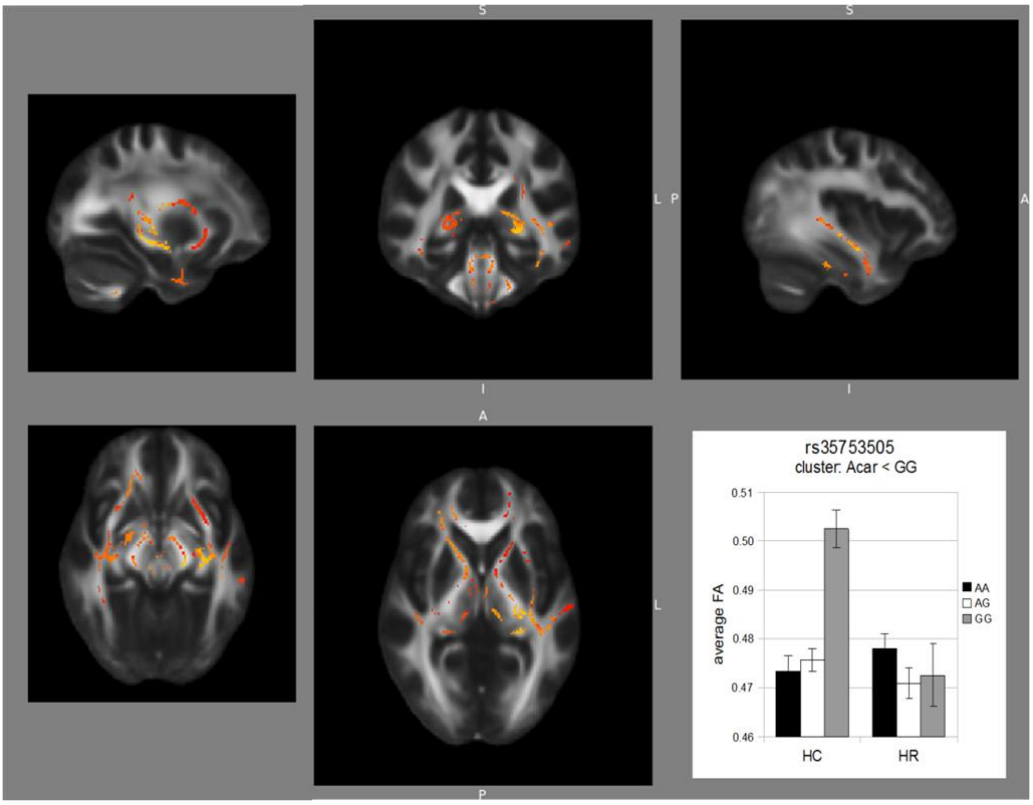
4.3.1.1. TBSS

In the healthy control group, individuals homozygous for the risk-alleles at NRG241930, rs35753505, and at rs6994992 had significantly *increased* FA in several frontal white matter areas, most prominently in the ATR ($p_{FWE} < 0.05$). Other regions less consistently associated with genotype across *NRG1* SNPs were the fornix / stria terminalis, external capsules, brain stem, left cerebellar peduncle, temporal lobes bilaterally, and left temporo-parietal white matter ($p_{FWE} < 0.05$). Figure 4.1 shows the significant effect of rs35753505 on FA in all of these regions. Corrected p-maps for NRG241930 were very similar, while for rs6994992 significant results were confined to the bilateral ATRs, the brainstem and the left temporal lobe. The same areas showed a trend for increased FA in individuals carrying two risk-haplotypes compared to heterozygotes and non-risk carriers combined, with family p_{FWE} between 0.06 and 0.10.

In the high risk group there were no significant effects for any SNP, nor for the *NRG1* core haplotype. There was a trend for *decreased* FA in the splenium of the corpus callosum in association with the risk alleles at rs6994992 and at rs35753505, at p_{FWE} between 0.07 and 0.10.

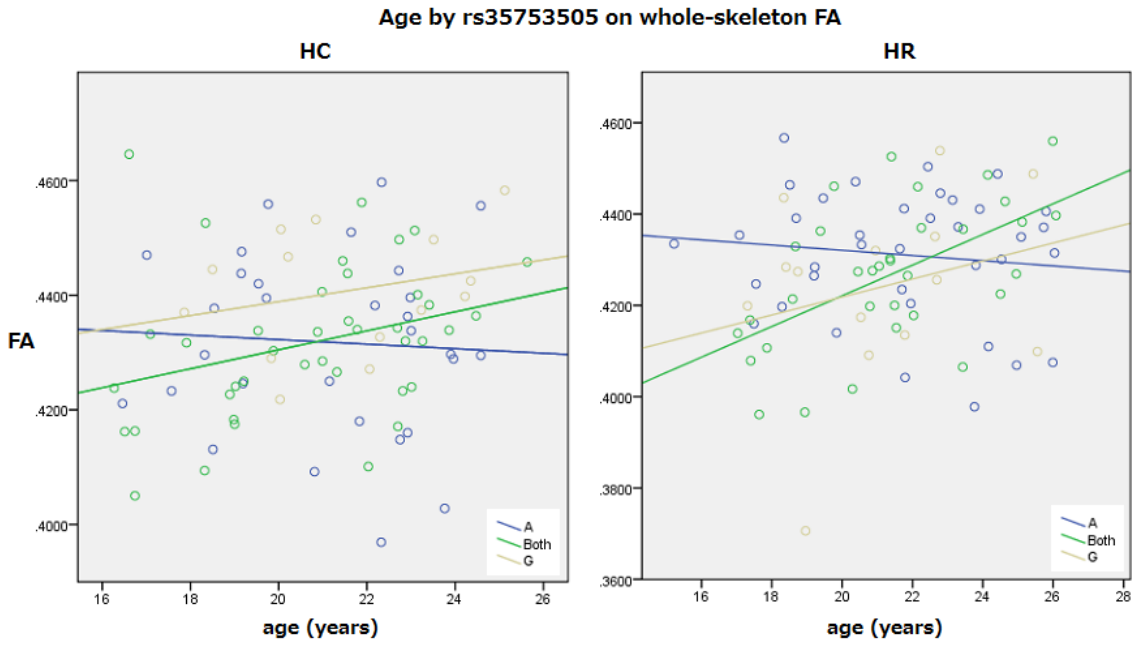
The above findings, as well as Figure 4.1, suggest *NRG1* genotypes have a different effect in the controls than in the high risk group. However, voxel-wise F-tests were not significant for genotype-by-group interactions (all $p > 0.99$). TFCE-based F-tests in FSL have appeared to be very conservative in other instances[1], where they produced corrected p-values $p_{FWE} > 0.99$ whilst T-tests of the same 'randomise' contrasts produced corrected p-values at trend-levels ($p_{FWE} < 0.08$). Unsurprisingly, group-by-genotype interactions were highly significant on the extracted FA averages within the significant clusters (all F between 6.06 and 12.47, all $p < 0.003$).

Figure 4.1. The effects of NRG1 SNP rs35743505 on FA in the control group, obtained using TBSS ($p_{FWE} < 0.05$). Note that the bar graph is added to illustrate the directions of effects of the 3-by-2 design, but extracted values were not used for statistical inference.



[1]See forum post on this topic:
https://www.jiscmail.ac.uk/cgi-bin/webadmin?A2=ind1101&L=FSL&D=0&P=302882

Figure 4.2. Effects of age on the association between NRG1 SNP rs35743505 and FA. The "risk-allele" originally reported in Stefansson et al. (2002) was "G".
Genotype key: "A"=AA; "Both"=AG; "G"=GG



None of the SNPs had a significant effect on the extracted whole-skeleton FA, although there was a trend for rs35743505 in the control group ($F = 2.493$, $p = 0.089$). For Hap_{ICE}, however, there was a Hap_{ICE}-by-group interaction on whole-skeleton FA ($F = 5.793$, $p = 0.017$), which was driven by significantly increased FA in the controls homozygous for the risk-haplotype ($F = 4.669$, $p = 0.034$) whilst unaffected relatives homozygous for the risk-haplotype had on average lower whole-skeleton FA than the non-risk carriers, albeit non-significantly so ($F = 1.724$, $p = 0.193$).

Post-hoc analyses of the cluster averages including sex and IQ in the model (since they were differently distributed across *NRG1* genotype groups) all resulted in genotype effects of $p < 0.001$, indicating that the above results were not caused by differences in sex or IQ. The genotype groups were well-matched for age, but there was a significant age-by-genotype interaction for rs35753505 on average FA from the skeleton ($F = 5.374$, $p = 0.006$, Figure 4.2). A similar effect was found for rs6994992-by-age interactions ($F = 2.861$, $p = 0.015$). For both groups, it appears that in subjects homozygous for the A-allelle, which has most frequently (yet

inconsistently) reported to be the non-risk or protective allele, FA is negatively associated with age, while in others FA increases with age as would be expected in young people. Note, however, that these data are cross-sectional, as is further discussed in the next section.

Considering the multiple testing corrected p-value for testing two genes, only the contrast comparing A carriers to G homozygotes at rs35743505 contained voxels significant at $p_{FWE} < 0.025$, with over 3000 voxels located in the left temporal lobe and posterior part of the ATR and brainstem.

4.3.1.2. Tractography

In keeping with the TBSS analysis, tractography revealed several significant effects of *NRG1* genotypes on FA. Individuals carrying two copies of the *NRG1* core risk haplotype had significantly higher FA in the bilateral ATRs ($F = 4.525$; $p = 0.012$) compared to the other two groups. This was also the case for the bilateral UFs in the healthy control group ($F = 4.311$, $p = 0.017$), but not in the high risk group ($F = 0.324$, $p = 0.724$; group-by-haplotype interaction: $F = 3.157$, $p = 0.045$). A similar pattern was seen for the SNP rs35753505: in the bilateral ATRs the risk allele was associated with increased FA across both groups ($F = 4.590$, $p = 0.012$) and in the bilateral UFs this was only the case in the healthy control group ($F = 4.089$, $p = 0.021$; group-by-genotype interaction: $F = 3.338$, $p = 0.038$). Again, for rs6994992 the same pattern occurred in the ATR, bilaterally, across both groups ($F = 4.657$, $p = 0.011$). Although there were no group-by-genotype interactions on FA in the ATR (all $p > 0.20$), the association was much stronger in the control group (Hap_{ICE}: $p = 0.002$; rs35753505: $p = 0.001$; rs6994992: $p = 0.008$). These results in the ATR and UF are illustrated by Figure 4.3 and Table 4.3, and Figure 4.4 and Table 4.4, respectively.

Post-hoc analyses including IQ and sex in the mixed models indicated that none of these significant effects were caused by confounding of IQ or sex. In fact, the effects of *NRG1* haplotypes and rs35753505 genotypes on the UF in the control group became dramatically more significant at $p = 0.002$ and $p = 0.001$, whilst all the above genotype effects on FA in the ATR across both groups remained significant at $p = 0.030$. Whilst the main effect of rs35743505 in the ATR would not survive the most stringent multiple comparison corrections at $p < 0.0071$ (0.05 / 7), the effect in the control group alone would for Hap_{ICE} and rs35753505 at $p < 0.0036$ (0.05 / [7

* 2]). The IQ-corrected effects in the UF in the controls are also below this corrected threshold at $p < 0.0036$. And more restrictively, only the effect of rs35743505 in the ATR in the control group ($p = 0.001$) would survive yet another correction of testing two genes (0.05 / [7 * 2 * 2]). There were no significant results for the genu, splenium, AF, CST or cingulum. There were also no significant effects of NRG241930 and NRG221132 on FA in any of the segmented tracts.

4.3.2. ErbB4

4.3.2.1 TBSS

Using TBSS, we did not detect any effects of *ErbB4* SNPs that were significant on the whole-brain level, neither in the control sample (all $p_{FWE} > 0.11$) nor the high risk sample (all $p > 0.30$). Similarly, there were no effects of the *ErbB4* haplotype on the whole-brain level in the control group (all $p > 0.17$), or in the high risk group (all $p_{FWE} > 0.35$). The same was true for the combined sample, in which T-tests and F-tests in 'randomise', whilst co-varying for group, were all non-significant on the whole-brain level (all $p_{FWE} > 0.31$). There were also no significant group-by-genotype interactions detected by the F-test in the large model of combined groups ($p_{FWE} > 0.64$).

There were also no significant effects of *ErbB4* on whole-skeleton FA, and importantly, no significant interactions between *NRG1* and *ErbB4* on whole-skeleton FA.

4.3.2.2. Tractography

Compared to *NRG1*, the effects of *ErbB4* on FA, if any, were less clear. Overall, significant effects were confined to the CST and the cingulum. In the cingulum, there was a significant interaction between rs707284 and group ($F = 3.298$, $p = 0.039$), driven by a significant effect of rs707284 on the bilateral cingulum in the control group ($F = 3.192$, $p = 0.046$), but not in the high risk group ($F = 0.712$, $p = 0.494$). In this tract, similar trends were observed for the *ErbB4* core haplotype (interaction: $F = 2.310$, $p = 0.103$; within control group: $F = 3.408$, $p = 0.038$) and for rs7598440 (interaction: $F = 2.986$, $p = 0053$; within control group: $F = 2.931$, $p = 0.056$). These results are illustrated by Figure 4.5.

Figure 4.3. Effects of NRG1 on FA in the ATR, separated for group and hemisphere. Effects are shown for Hap_{ICE}, and two SNPs within Hap_{ICE}: rs35753505 (risk-allele "G") and rs6994992 (Risk allele "T"). Significant effects of genotype on FA were found for all three genetic markers, across hemispheres. "A"=AA; "G"=GG; "C"=CC; "T"=TT; "Both"= heterozygous.

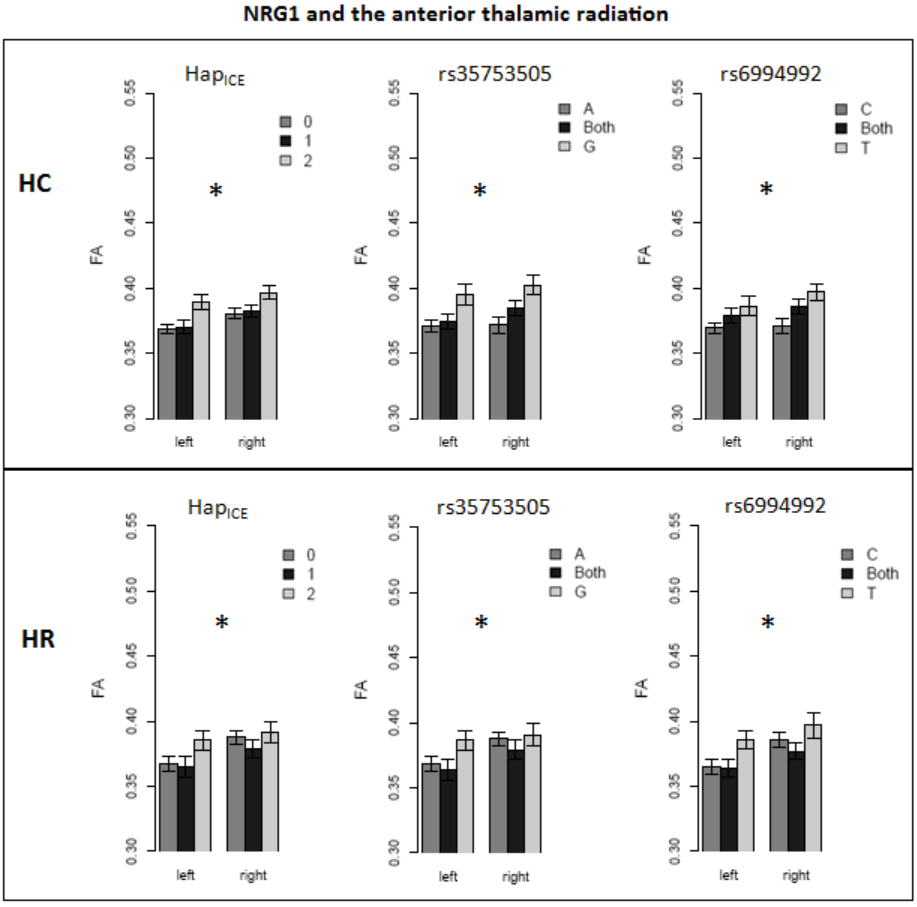


Table 4.3. Summary of simple effects of HAP_{ICE} on FA in the ATR, separated per group

HAP_{ICE}	Mean (SD)			Statistics			
	0	1	2	F	p	df	η^2
HC							
left	0.3700 (0.0217)	0.3758 (0.0309)	0.3929 (0.0302)	3.239	0.043	70	0.085
right	0.3721 (0.0312)	0.3856 (0.0332)	0.4017 (0.0278)	4.525	0.014	80	0.101
HR							
left	0.3672 (0.0312)	0.3647 (0.0438)	0.3853 (0.0248)	1.475	0.236	73	0.040
right	0.3874 (0.0325)	0.3788 (0.0419)	0.3916 (0.0326)	0.810	0.448	82	0.018

Note that the main analyses were not performed on simple effects per unilateral tract, but as mixed regressions, across hemispheres (and tracts). This table is included to provide meaningful effect sizes that allow for comparisons across studies, not for assessing the statistical significance of the present findings per se. The values correspond to those illustrated in Figure 4.3. df = degrees of freedom; η^2 (eta squared) = effect size as proportion of variance explained by Hap_{ICE} genotype.

Figure 4.4. Effects of NRG1 on FA in the UF, separated for group and hemisphere. Effects are shown for Hap_{ICE}, and two SNPs within Hap_{ICE}: rs35753505 (risk-allele "G")and rs6994992 (Risk allele "T"). Significant effects of genotype on FA were found for Hap_{ICE} and rs35753505 across hemispheres, in the control group only. "A"=AA; "G"=GG; "C"=CC; "T"=TT; "Both"= heterozygous.

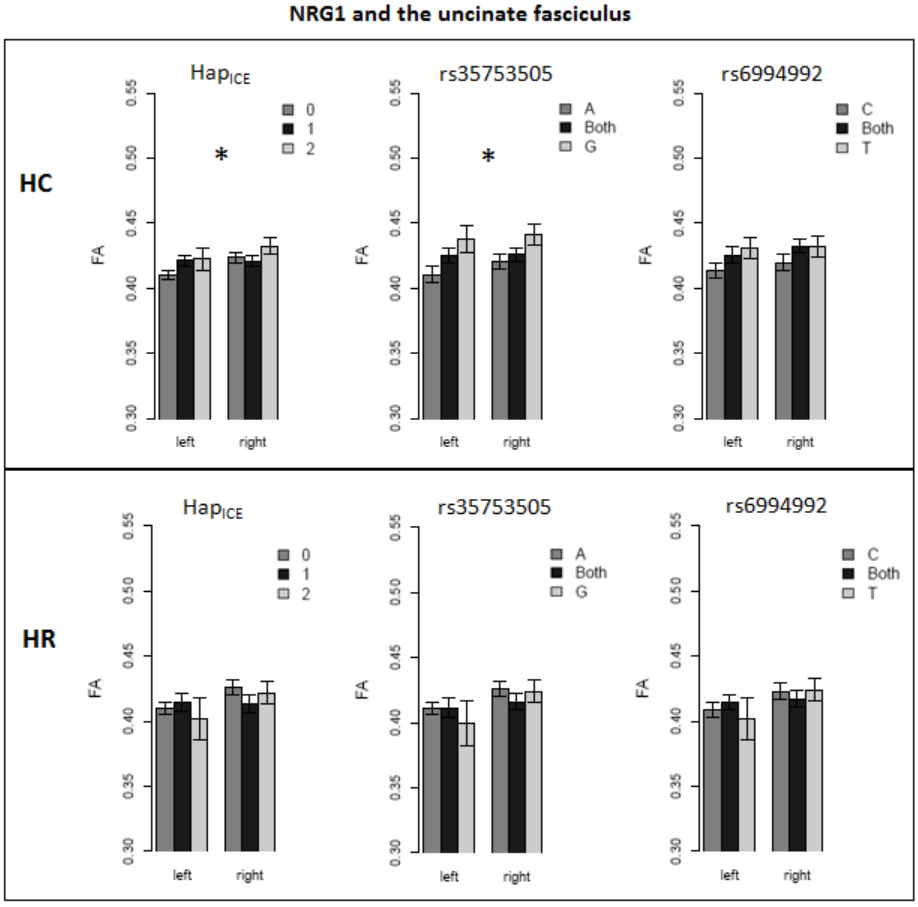


Table 4.4. Summary of simple effects of HAP_{ICE} on FA in the UF, separated per group

HAP_{ICE}	Mean (SD)			Statistics			
	0	1	2	F	p	df	η^2
HC							
left	0.4111 (0.0297)	0.4261 (0.0317)	0.4365 (0.0341)	3.215	0.046	68	0.082
right	0.4209 (0.0304)	0.4270 (0.0335)	0.4431 (0.0311)	2.435	0.094	80	0.061
HR							
left	0.4096 (0.0282)	0.4146 (0.0320)	0.4017 (0.0472)	0.522	0.569	62	0.015
right	0.4259 (0.0359)	0.4135 (0.0374)	0.4219 (0.0323)	1.06	0.351	83	0.027

Note that the main analyses were not performed on simple effects per unilateral tract, but as mixed regressions, across hemispheres (and tracts). This table is included to provide meaningful effect sizes that allow for comparisons across studies, not for assessing the statistical significance of the present findings per se. The values correspond to those illustrated in Figure 4.4. df = degrees of freedom; η^2 (eta squared) = effect size as proportion of variance explained by HAP_{ICE} genotype.

Figure 4.5 Effects of ErbB4 genotypes on FA in the cingulum, separated for group and hemisphere. Effects are shown for the ErbB4 haplotype and two SNPs within the haplotype: rs707284 (risk-allele "C) and rs7598440 (risk-allele "T"). Significant effects were found for the haplotype and genotype at rs707284, and a trend for rs7598440, in the control group only. "C"=CC; "T"=TT; "Both"= heterozygous.

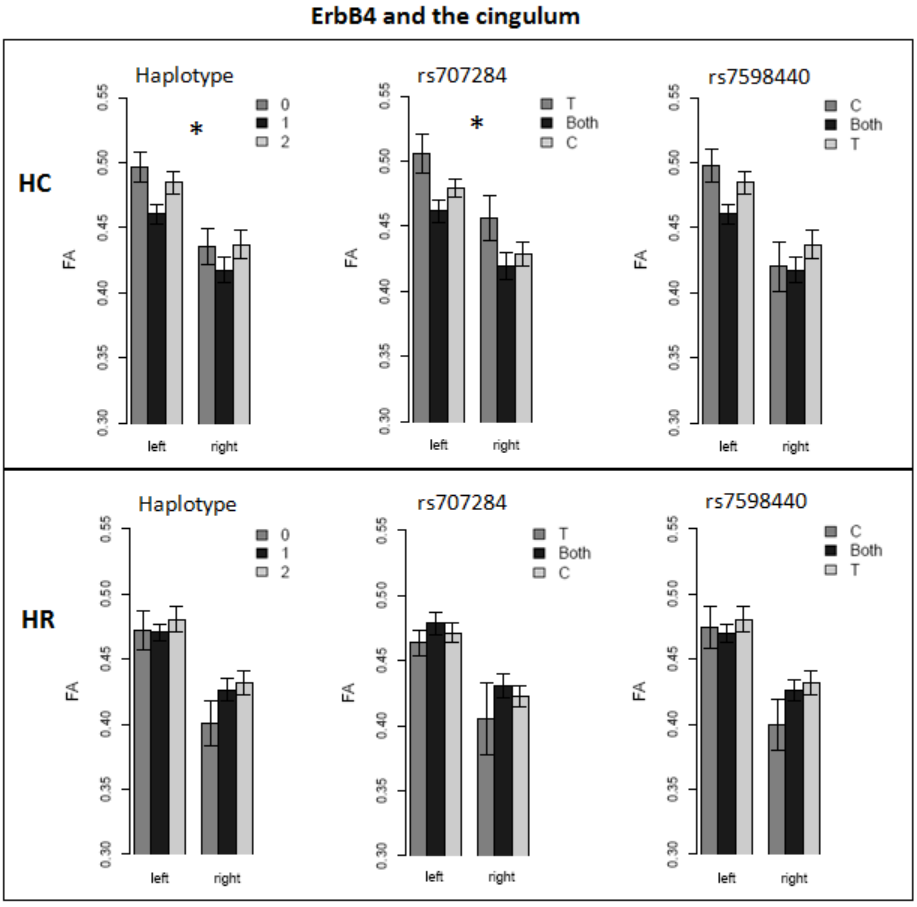
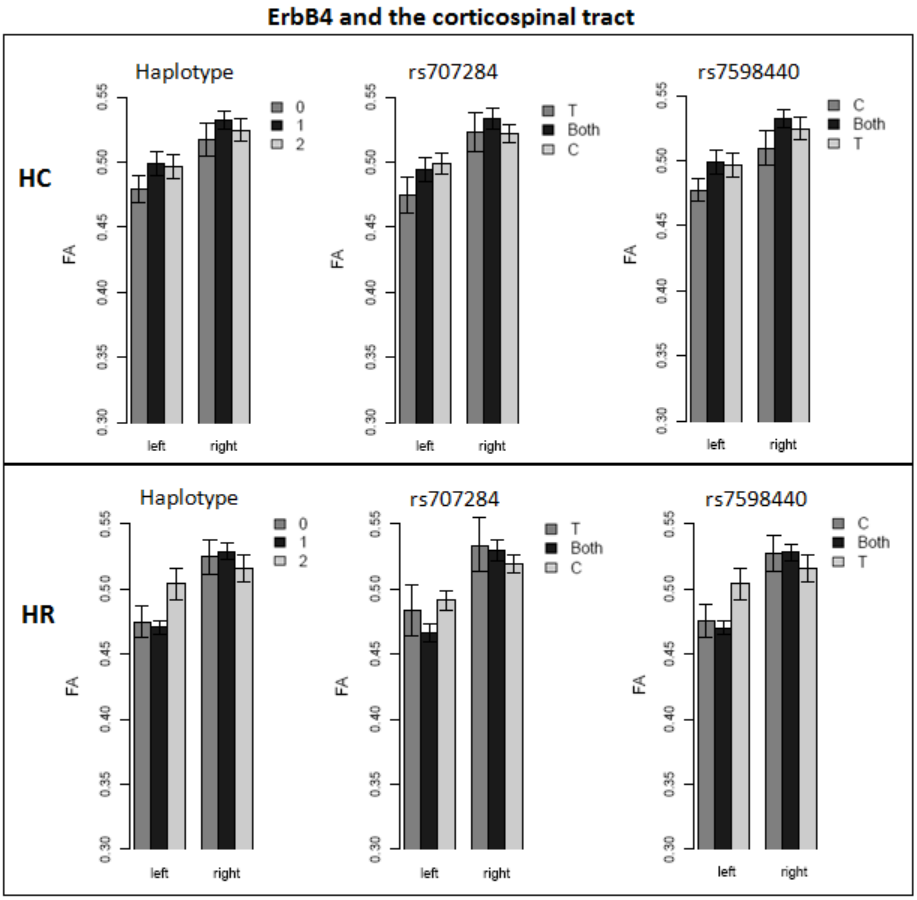


Figure 4.6. Effects of ErbB4 genotypes on FA in the CST, separated for group and hemisphere. Significant hemisphere-by-genotype interactions were found for the Haplotype and genotypes at rs707284 and rs7598440, but simple effects were not significant. “C”=CC; “T”=TT; “Both”= heterozygous.



In the CST, on the other hand, there were hemisphere-by-genotype interactions for the *ErbB4* haplotype ($F = 3.653$, $p = 0.028$), rs707284 ($F = 4.453$, $p = 0.013$) and rs7598440 ($F = 3.516$, $p = 0.032$). However, what may have driven this interaction is not entirely clear. For both SNPs and the *ErbB4* haplotype, there were merely trends for genotype effects in the left CST ($p < 0.10$) with no trends in the right hemisphere. Looking at Figure 4.6, it may also be caused by essentially a 3-way group*genotype*hemisphere interaction, but breaking down the analyses for groups, hemispheres and genotype groups may have left too little power to detect subtle 3-way interactions. Also, it should be noted that the distribution of FA in the CST deviates from normality. Although mixed-effects models, as regressions, are fairly robust against deviations of normality, strange observations may occur because of extreme violations of the normality assumption. None of the effects of *ErbB4* genotypes survives our Bonferroni corrected p-values. No significant main effects or interactions on FA were detected for rs839523 in any of the tracts of interest.

4.4 Discussion

Here, I present an investigation of white matter integrity in relation to *NRG1* and *ErbB4*. Genetic variation in *NRG1* was associated with FA in the bilateral ATR and UF. These are the same two regions as found previously in similar analyses of both *NRG1* and *ErbB4* genotype effects in healthy individuals (Konrad *et al.*, 2009; McIntosh, Moorhead *et al.*, 2008; Sprooten *et al.*, 2009; Winterer *et al.*, 2008; Zuliani *et al.*, 2011). The clearest effects were observed in the control group, with in the unaffected relatives weaker effects of *NRG1* variants on the ATR and no effects on the UF. From the present study, the effects of *ErbB4* on FA are inconclusive, with some weak evidence for interactions with genetic background in the CST and the cingulum, regions not highlighted in previous studies.

4.4.1 Risk variants in NRG1 and ErbB4 are associated with increased FA in the ATR and UF

As noted in Chapter 1, the ATR and the UF, connecting the prefrontal cortex to the thalamus and the medial temporal lobe respectively, are assumed to be central to emotion regulation and BD pathology. White matter integrity is these regions is also highly heritable (Chiang *et al.*, 2009;

Chiang, McMahon *et al.*, 2011; Kochunov *et al.*, 2010) and is also associated with genetic liability to psychosis (McIntosh *et al.*, 2006; Sprooten *et al.*, 2011; also see Chapter 3). Whilst the locations of the effect of *NRG1* genotypes were entirely in line with what would be expected, the direction of the effect was not: the risk-alleles and risk-haplotype were consistently associated with increased FA, thus contradicting all previous studies of white matter and *NRG1* / *ErbB4* (Konrad *et al.*, 2009; McIntosh, Moorhead *et al.*, 2008; Sprooten *et al.*, 2009; Winterer *et al.*, 2008; Zuliani *et al.*, 2011). Although contradictory, it is not completely out of line with the background literature and there are several possible explanations for this finding. First, as mentioned in the introduction, there is disagreement on the definition of the "risk-alleles" at *NRG1* loci, with high heterogeneity between studies and populations, especially for rs35753505 and Hap_{ICE}. Although the Icelandic risk-allele finding at rs35753505 was initially replicated in a Scottish sample (Stefansson *et al.*, 2003), a subsequent Scottish association study found the opposite allele to be associated with increased risk (Thomson *et al.*, 2007). A series of studies in other populations also associated these "opposite" alleles at rs35753505 and/or rs6994992 with increased risk (Alearts et al., 2009; Georgieva *et al.*, 2008; Kim, Lee *et al.*, 2006; Prata *et al.*, 2009). Interestingly, I found a marginally significant association of risk-status with the C-allele at rs6994992 in our own sample, which is again the opposite allele initially indentified as the risk-allele in the Icelandic study. Since affected individuals were excluded from our at-risk sample, this could imply a protective genetic effect of this allele, but considering most people in our sample are younger than the typical age of onset for psychosis it more likely reflects population heterogeneity. Different linkage disequilibrium patterns surrounding the studied SNPs with the more functional SNPs and different genotypes at interacting gene's locations could be responsible for what appears to be a paradoxical finding both in the case-control association studies cited above and in our own association with white matter integrity.

Secondly, environmental or demographic factors can also interact with *NRG1* genotypes to increase or decrease FA, especially since NRG1 is involved in plasticity (Hahn *et al.*, 2006; Gu *et al.*, 2005; Mei & Xiong, 2008). Obviously in our young sample, one demographic variable of special interest is age. In contrast to our previous sample (McIntosh, Moorhead *et al.* 2008, Sprooten *et al.* 2009), the present participants were at an age at which white matter, especially in limbic and prefrontal areas, continues to develop quite rapidly (Barnea-Goraly *et al.*, 2005; Bava *et al.*, 2010; DiGiorgio *et al.*, 2008; Kochunov *et al.*, 2010; Lebel *et al.*, 2008). Furthermore, heritability estimates of FA vary with age in prefrontal white matter (Chiang, McMahon *et al.*,

2011) and *NRG1* and *ErbB4* expression, and the concentrations of their distinct isoforms, also changes during development (Mei & Xiong, 2008). *NRG1* and *ErbB4* genotypes are associated with different patterns of grey matter development in children (Addington *et al.* 2007), and I also found some evidence for an age-by-genotype interaction on FA (with rs35743505). Together, these pieces of information imply complex interactions between *NRG1*, white matter development and age, and further longitudinal studies incorporating a wider age range, will be necessary to disentangle these.

Since NRG1 directly binds to *ErbB4* via the epidermal growth factor domain, epistasis between *NRG1* and *ErbB4* genotypes on FA are plausible. *NRG1*-by-*ErbB4* interactions would perhaps be the most compelling explanation for our inconclusive findings for *ErbB4* combined with the counterintuitive direction of effect for *NRG1*. And indeed, two studies report such epistasis on risk of schizophrenia (Norton *et al.,* 2006; Shiota *et al.*, 2008), although Addington *et al.* (2007) failed to find support for epistasis on grey matter development. Similarly, in the present analyses we could not find any interactions between *NRG1* and *ErbB4* on whole-skeleton FA. However, technically our numbers within *ErbB4*/*NRG1* genotype cells are too small to interpret a negative finding, especially, since the most interesting contrast in our results requires splitting up the homozygous *NRG1* risk group, which is the smallest (N = 15), along *ErbB4* genotypes. I also did not test *ErbB4 / NRG1* interactions in the voxel-wise analyses because that would require even larger samples.

4.4.2. Effects of NRG1 and ErbB4 are more pronounced in the control group

The present results are more pronounced in the control group, and each effect is more or less absent in the unaffected relatives. This is contrary to the idea that candidate genes might have more pronounced effects against a vulnerable genetic background, but there are several possible explanations for why we failed to observe such increased sensitivity in the high risk group. Individuals at high genetic risk already have widespread FA reductions (Chapter 3; Sprooten *et al.*, 2011), and so we may be looking at a "floor effect", in the sense that when white matter integrity is already reduced it becomes more resistant to any further reductions. Sampling bias may also have contributed to this, because further reductions may have behavioural or clinical consequences in which case high-risk participants at the extreme end of the spectrum would have

been excluded from our study. In addition, the high risk group may be more heterogeneous as a sample. During brain development a great multitude of interacting genetic and environmental variables impact upon brain structure and these may have a broader range in individuals of affected families. The complexity of these interactions and the wide variety of environmental circumstances can obscure subtle effects of single genes on brain structure, let alone single SNPs or haplotypes. A third explanation comes from looking at the data in the high risk subjects, where a pattern emerges of heterozygous individuals having the lowest FA. This implies a possible interaction of the biological mechanisms of *NRG1* on the molecular level with genetic background. The mechanisms of such interactions remain open to speculation, but may involve a compensatory mechanism for extremely high or extremely low *NRG1* expression in one of the homozygous groups.

4.4.3. Limitations

A first limitation, as is the case in other chapters, is that tractography was not always successful leading to the exclusion of a number of participants for some tracts, especially for the left ATR and the left UF. It is unclear whether these exclusions are random since the real FA within these tracts cannot be known. But it is encouraging that the TBSS and tractography results are compatible with one another. Perhaps the most important limitation is the multiple tests that were performed. As mentioned in the methods section, the probability of false positives is not simply the number of tests times the p-values since neither FA across tracts, nor SNPs are independent measures. In the TBSS analyses, only one contrast of one SNP would survive a liberally corrected p-value (i.e. only divided by the number of genes). In the tractography analysis, the effects of *NRG1* on the ATR and the UF in the control group were significant at a post-hoc Bonferroni corrected p-value, but none of the *ErbB4* effects were. It should also be borne in mind that the ATR would have been the top-ranked *a priori* region given our previous studies (McIntosh, Moorhead *et al.*, 2008; Sprooten *et al.*, 2009; Zuliani *et al.*, 2011), and the consistency between TBSS and tractography methods further reduces the possibility of false positives. Nevertheless, in isolation the associations we found between *NRG1* and FA should be treated with caution, and it is only in integrating them with previous findings and by observing their consistency, that we can be more confident of the association of *NRG1* with white matter integrity in the ATR and possibly the UF. A final limitation worth discussing here derives from

the observation that for both genes the haplotypes appeared less sensitive to FA differences than the SNPs. Given the heterogeneity at the *NRG1* locus, as observed by all four meta-analyses (Gong *et al.*, 2009; Li, Collier & He, 2006; Munafa *et al.*, 2006, 2008), it may mean that the particular *NRG1* and *ErbB4* haplotypes are not appropriate in our population for either white matter or clinical phenotypes. Ideally, sample-specific determination of haplotypes by sequencing would enlighten this problem. However, this was beyond the scope of the current project.

4.4.3. Conclusions

In our sample of young healthy individuals I found modest associations of *NRG1* with white matter integrity in frontal and temporal fibres. Whether *ErbB4* genotypes also influence white matter integrity remains unclear. In unaffected relatives of patients with BD we could also not detect any clear associations. Integrating these findings with the available literature, it seems that NRG1-ErbB4 signalling has an impact on many neurodevelopmental processes including white matter development, but its mechanisms likely vary with age and environmental factors.

Chapter 5

White matter integrity and *DISC1*

5.1 Introduction

5.1.1 Evidence for the involvement of DISC1 in psychiatric disorders

More than two decades ago St Clair *et al.* (1990) discovered a remarkable co-segregation of a genetic mutation with schizophrenia and affective disorders in a large Scottish family. In this family 29 of the 37 people who inherited a translocation between chromosomes 1 and 11, developed schizophrenia, BD or major depression, whilst none of the 38 relatives with normal karyotypes were diagnosed with a major psychiatric disorder (Blackwood *et al.*, 2001). With log odds ratios of 3.6 with schizophrenia and 7.1 with the broader psychiatric phenotype, this co-segregation was highly significant in this family, and inspired a wealth of research during the following decades.

Muir *et al.* (1995) identified the breakpoints of the translocation at 1q42 on chromosome 1 and 11q14 on chromosome 11. Sequencing of the breakpoint region on chromosome 1 revealed that two genes were disrupted by the 1:11 translocation: "disrupted in schizophrenia 1" (*DISC1*) on the anti-sense strand and "disrupted in schizophrenia 2" (*DISC2*) on the sense strand (Millar *et al*., 2000). Since *DISC1* is on the anti-sense strand, it is the gene responsible for encoding the protein *DISC1*, whereas *DISC2* may be involved in the regulation of *DISC1* expression but not in protein translation itself. Recently a gene disrupted by the breakpoint on chromosome 11, *DISC1* Fusion Partner 1 (*DISC1FP1*) has also been identified, but due to its recent discovery very little research has been conducted on its function so far (Zhou *et al*., 2008). *DISC1* is the main focus of this chapter, as it remains the most likely causal gene of the psychiatric phenotype in the 1:11 family to this date.

Family studies are useful for the identification of rare genetic variants and mutations that may convey a substantial increase in risk for a clinical phenotype. On the other hand, a finding in one family may be specific to the genetic background or a specific subtype of the disorder within that

family, and not provide insight into the causes of the disorder in the general population. Hence, association and linkage studies in other samples were conducted to confirm the link between the *DISC1* gene and psychiatric phenotypes. Although no study has reported an effect (i.e. odds ratio) of genetic variation in *DISC1* as strong as in the original 1:11 family, linkage and association studies of *DISC1* SNPs and haplotypes outside the 1:11 family have provided independent evidence for the involvement of common *DISC1* variants in psychiatric disorders in a wide variety of populations including Finnish, North American, Chinese and various Asian populations (reviewed in Chubb *et al*., 2008). A recent study combined data from the four European association studies, and identified three SNPs as most significantly associated with BD (Hennah *et al*., 2008).

5.1.2 Functions of DISC1

Despite a decade of intensive research into *DISC1* function, the precise mechanisms by which genetic variation in the *DISC1* region impacts upon mental health remain to be elucidated. The protein DISC1 interacts with a multitude of other proteins and molecular pathways, but so far these have converged on several candidate causal mechanisms rather than a single disrupted function. *DISC1* is abundantly expressed in the brain, most prominently in the hippocampus and other limbic regions, and its expression levels vary at different stages of human development (Chubb *et al*., 2008). Evidence from animal studies and cell cultures indicate that *DISC1* regulates several neuro-developmental processes relevant to the aetiology of psychiatric disorders including, but not limited to, neuronal proliferation, differentiation and migration (Ishizuka *et al*., 2011; Kamiya *et al*., 2005; Mao *et al*., 2009), axon growth (Brandon *et al*., 2009; Hur & Zhou, 2010; Mao *et al*., 2009) and axon myelination (Wood *et al*., 2008). Although complete *DISC1* knockout is impossible in rodents, (partially) mimicking the 1:11 translocation or reducing *DISC1* expression in transgenic mice can result in several depression- and schizophrenia-like deficits in working memory, pre-pulse inhibition, as well as reductions in total brain volume, dendritic arborisation and possibly enlarged ventricles (Clapcote *et al*. 2007; Pletnikov *et al*. 2008). Notably, *DISC1* knockdown in zebrafish leads to severe disruption of axonal development (Wood *et al*., 2008). Thus, from the available literature it appears that variation in *DISC1* increases susceptibility to psychiatric disorders by (directly or indirectly) influencing the development and plasticity of the brain. In contrast to grey matter, white matter

continues to develop during late adolescence and early adulthood (Lebel *et al*., 2008) compatible with the average age of onset of psychotic and affective disorders, making white matter a plausible site for the effects of *DISC1* and other (genetic and environmental) risk factors to converge and impact upon mental wellbeing.

A complementary approach to animal research and cell culture to gain understanding in gene function is to investigate intermediate phenotypes relevant to psychiatric disorders in the human brain *in vivo*. In the original 1:11 family study it was already established that *DISC1* has an effect on auditory processing in the brain; the P300 ERP peaked later and with lower amplitude in carriers of the translocation compared to non-carriers, similar to what is observed in schizophrenic patients (Blackwood *et al*., 2001). Common *DISC1* risk-haplotypes and SNPs have also been associated with working memory impairments, long-term memory impairments and grey matter reductions in the prefrontal and temporal cortices (Burdick *et al*., 2005; Cannon *et al*., 2005; Carless *et al*., 2011; Hennah *et al*, 2005). A recent analysis by our group has shown interesting interactions between the three SNPs associated with BD in the combined European samples (Hennah *et al*., 2008) and diagnoses of BD and schizophrenia on BOLD signal in various brain areas during a language task (Chakirova *et al*., 2011).

One of the few non-synonymous, exonic *DISC1* SNPs, rs821616, resulting in a serine to cysteine substitution (Ser704Cys), has particularly received a lot of attention in the context of intermediate phenotypes. Apart from three studies reporting an association of Ser704Cys with schizophrenia, BD and major depression (Callicott *et al*., 2005; Hashimoto *et al*., 2006; Qu *et al*., 2007), this SNP has been associated with several cognitive performance measures including short and long-term memory, logical and abstract reasoning and verbal reasoning skills in the elderly (Callicott *et al*., 2005; Kamiya *et al*., 2006; Thomson *et al*. 2005). The latter finding is complicated by a distinct sex by genotype interaction, suggesting it is important to examine any interactions and between group differences with respect to sex. Using MRI this SNP has been associated with hippocampal structure and function, hippocampus–prefrontal functional connectivity, and grey matter density in a number of cortical regions including the bilateral fusiform gyri, bilateral middle temporal gyri, medial frontal regions, right inferior parietal cortex and left precuneus (Callicott *et al*., 2005; DiGiorgio *et al*., 2008, Hashimoto *et al*., 2006). So far, evidence for an effect of *DISC1* on human white matter integrity is very limited, with only one study reporting slight FA reductions in prefrontal areas in relation to the Ser704Cys SNP, but

here uncorrected statistical thresholds were used in only a small sample of healthy individuals (Hashimoto *et al*., 2006). Notably, the above intermediate phenotype measures are among the most consistently replicated neural and cognitive abnormalities in schizophrenic patients, and to a lesser extent in affective disorders (Arnone *et al*., 2009; Forbes *et al*., 2009; Hallahan *et al*., 2011; Lewandowski *et al*., 2011; Mcintosh *et al*., 2005; Stephanopoulou *et al*., 2009).

In summary, for its unique translocation and hereditary patterns of psychiatric phenotypes, the 1:11 family has been tremendously valuable in the discovery of the *DISC1* locus. Since then it has become clear that genetic variation in the 1q32-42 region is associated with psychiatric disorders in the general population. To understand the mechanisms by which genetic variation in *DISC1* can potentially cause clinical symptoms, *DISC1* function needs to be examined on all scales, from the sub-molecular to the system level. The prevailing notion at the moment is that DISC1 is involved in several neural developmental processes, including neuronal proliferation and migration and axon myelination, and that this has various consequences for cognitive functioning and the structure and function of the mature human brain. MRI studies have previously demonstrated associations between *DISC1* genotypes and grey matter density and activation in healthy people, however to this point no convincing evidence exist for an association with white matter integrity. Using DTI followed by various state-of-the-art analysis methods, I investigated in detail whether genetic variation in the common, non-synonymous Ser704Cys SNP is associated with white matter integrity in the healthy human brain.

5.2 Methods

5.2.1 Study sample

Participants were recruited as part of the Bipolar Family Study, and they were interviewed, neuro-psychologically assessed, scanned and genotyped according to the methods described in Chapter 2. In cases where participants were genetically related to one another, one person of each family was randomly selected to be included in the analysis sample. Demographic information of the study sample and *DISC1* rs821616 genotype frequencies are summarised in Table 5.1. Note that rs821616 is a symmetric SNP, and that in our case the T-allele is the minor allele which results in the translation of Cysteine.

Table 5.1: Demographic and genotype information of the study sample.

Control Group	**AA** (N = 39)	**AT** (N = 38)	**TT** (N = 10)	**statistic (p-value)**
age in years (SD**)**	20.7 (2.33)	21.1 (2.38)	21.6 (2.82)	F = 0.55 (p = 0.58)
male (%)	17 (44%)	16 (40%)	6 (60%)	χ^2 = 1.07 (p = 0.59)
handedness (N left handed)	3	2	0	NA
IQ (SD)	113 (2.11)	111 (2.26)	119 (3.00)	F = 1.45 (p = 0.24)
occupation parent (% manual)	15 (38%)	14 (37%)	3 (33%)	χ^2 = 0.37 (p = 0.83)
History of substance abuse[1]				
nicotine	11 (29%)	10 (26%)	1 (10%)	χ^2 = 1.51 (p = 0.27)
alcohol (units / week)[2]	14	11	7.5	p = 0.851
cannabis	26 (67%)	24 (63%)	7 (70%)	χ^2 = 0.21 (p = 0.90)
hallucinogens	5 (13%)	8 (21%)	0 (0%)	χ^2 = 3.01 (p = 0.22)
stimulants	9 (23%)	10 (26%)	3 (30%)	χ^2 = 0.24 (p = 0.89)
sedatives	1	0	0	NA
High Risk Group	**AA** (N = 41)	**AT** (N = 37)	**TT** (N = 12)	**statistic (p-value)**
age in years (SD**)**	21.8 (2.78)	20.8 (2.68)	22.0 (3.05)	F = 1.40 (p = 0.25)
male (%)	19 (46%)	17 (46%)	5 (42%)	χ^2 = 0.09 (p = 0.96)
handedness (N left handed)	1	4	1	NA
IQ (SD)	108 (12.94)	103 (15.48)	106 (12.70)	F = 0.99 (p = 0.38)
occupation parent: manual (%)	18 (44%)	18 (49%)	7 (58%)	χ^2 = 1.85 (p = 0.76)
History of substance abuse[1]				
nicotine	14 (34%)	13 (35%)	7 (58%)	X^2 = 2.50 (p = 0.29)
alcohol (units / week)[2]	15	15	6	F = 2.21 (p = 0.12)
cannabis	28 (68%)	25 (68%)	9 (75%)	X^2 = 0.246 (p = 0.88)
hallucinogenics	10 (26%)	5 (14%)	1 (8%)	X^2 = 0.242 (p = 0.30)
stimulants	14 (34%)	9 (24%)	4 (33%)	X^2 = 0.82 (p = 0.66)
sedatives	4 (10%)	3 (11%)	2 (17%)	X^2 = 0.74 (p = 0.69)

[1] History of substance abuse refers to having ever used any of the substances, and differences were tested using Chi-squared tests. [2]Alcohol abuse was measured in units per week, and differences were tested using analyses of variance. NA: valid statistics could not be computed due to small numbers per cell or floor effects.

5.2.2 TBSS

TBSS was performed according to standard methods in FSL, as described in Chapter 2. In the following statistical analyses, I first investigated whether there was any evidence for group-by-genotype interactions using a general linear model in 'randomise' in FSL. In addition to the results of the F-test, I looked at the results of the separate T-tests for any differential genotype effects between the two groups, since from previous analyses it appeared that F-tests are surprisingly less sensitive than T-tests in FSL (also see my post regarding this problem on the FSL forum[2]). Secondly, three voxel-wise comparisons were performed on the TBSS skeletons in high risk and control groups separately: A-allele homozygotes vs. T-allele carriers, A-allele carriers vs. T-allele homozygotes and A-allele homozygotes vs. T-allele homozygotes. As mentioned in the introduction, there is evidence for sex-specific effects of *DISC1*. For this reason, I tested for sex-by-genotype interactions using an F-test as well as multiple T-tests. In an additional model I co-varied for sex to rule out any effects of rs821616 genotype could have been confounded by sex-differences. Finally, because unilateral brain differences may exist between left- and right-handed people, especially around the AF, additional analyses were performed on right-handed people only. Because genotype groups were well-matched on demographic variables known to impact on white matter integrity (Table 5.1), no further covariates were added to the statistical models. All reported results are significant at $p < 0.05$, corrected on the whole brain-level via TFCE (Smith & Nichols, 2009).

5.2.3 Voxel-based analysis

Any significant results in the TBSS analysis, were further explored using the more conventional voxel-based analysis of FA. Here, all individual FA maps were normalised to a standard MNI template using the FNIRT tool in FSL and smoothed with a FWHM kernel of 12mm, as it has the best trade-off between false-positives and sensitivity in diffusion data using parametric non-stationary cluster inference (Hayasaka *et al*., 2004; Jones *et al*., 2005). To create an explicit white matter mask all FA maps were averaged, thresholded at FA > 0.15 and binarised. Voxel-wise comparisons between *DISC1* genotype groups were performed in SPM5 for each significant

[2] https://www.jiscmail.ac.uk/cgi-bin/webadmin?A2=ind1101&L=FSL&P=R58244&1=FSL&9=A&J=on&d=No+Match%3BMatch%3BMatches&z=4

contrast in the TBSS analysis. To perform cluster-wise statistics, an initial threshold of $p < 0.01$ (uncorrected) was applied to the statistical image. Subsequently, the whole-brain corrected significance of subthreshold clusters was assessed using the non-stationary cluster inference toolbox. This toolbox accounts for local variation in smoothness by using observed resel sizes in the calculation of cluster-wise statistics (Hayasaka *et al*., 2007*).* This way, an important source of false positive findings for cluster-wise testing is eliminated (Smith & Nichols, 2004; Moorhead *et al*. 2005).

5.2.4 Tractography

Probabilistic neighbourhood tractography was performed using TractoR , as described in Chapter 2. Average FA, weighted according to the probability of the tract going through each voxel, was extracted from each tract of interest, namely the ATR, UF, CST and cingulum bundles bilaterally, and the genu and splenium of the corpus callosum. Average FA within each tract was analysed using mixed model regressions in SPSS 14, with hemisphere as within-subject variable and (risk) group and rs821616 genotype as between subject variables. For the genu and the splenium of the corpus callosum, I performed univariate ANOVAs with group and rs821616 genotype as independent variables. Although genotype groups were well-matched on demographic variables known to impact on white matter integrity, in a post-hoc analysis age, sex and IQ were added to the models to ensure any significant results could not be attributed to these key variables.

5.3 Results

5.3.1 TBSS

In contrast to what happened in the *NRG1* analysis in the previous chapter, the F-test was significant for the group-by-genotype interaction in a total of 18078 voxels ($p_{FWE} < 0.05$), indicating the effect of genotype on FA is substantially different between the two groups. The T-tests of the interaction effects were in agreement with this, with 2 significant ($p_{FWE} < 0.05$) contrasts out of 6 and one trend ($p_{FWE} < 0.06$).

Significant group-by-genotype interactions led to analyses separate for the control and the high-risk groups. In the control group, A-allele carriers at rs821616 had reduced FA compared to T-homozygotes in three clusters ($p_{FWE} < 0.05$; K = 33 929; K = 170; K = 46 voxels), together covering large parts of the TBSS skeleton (Figure 5.1, Table 5.2). Similarly, the contrast of A-homozygotes versus T-homozygotes resulted in ten clusters ($p_{FWE} < 0.05$; K = 21 259; K = 1061; K = 509; K= 381; K = 322; K = 272; K = 118; K = 14; K = 7; K = 6 voxels) widely distributed over the TBSS skeleton (Table 5.2). Part of these clusters, namely around the right AF, remained significant under a very stringent threshold of $p_{FWE} < 0.005$ (Figure 5.2, Table 5.2). Contrasting A-homozygotes to the T-allele carriers resulted in four relatively smaller clusters (K = 285; K = 77; K = 75; K = 50 voxels), mainly confined to the right AF and similar to the $p < 0.005$ results in the other two contrasts (Table 5.2). In the high risk group, no significant differences between genotype groups were observed.

T-tests and F-tests revealed no significant genotype-by-sex interactions in the healthy control group on the whole-brain level (all $p_{FWE} > 0.33$). This is also illustrated by Figure 5.3, showing that the effects of rs821616 on FA were similar in men and women in all significant clusters. Furthermore, results in the control group were very similar when left-handed individuals were excluded from the analysis.

5.2.2 Voxel-based Analysis

In the healthy control group, an ANOVA comparing all three rs821616 genotype groups resulted in one large cluster in the right hemisphere, strikingly following the curve of the AF (K = 14804, $p_{FWE} < 0.004$; Table 2). An additional, smaller cluster was visible in the left temporo-occipital white matter, around the inferior longitudinal fasciculus (K = 4983, $p_{FWE} = 0.049$; Table 5.2). In line with the TBSS results, post-hoc T-contrasts revealed that A-carriers had reduced FA compared to T-homozygotes in three clusters distributed over most of the cerebral white matter (Figure 5.4, Table 5.2), and AA homozygotes had reduced FA compared to TT homozygotes in 4 similarly widespread clusters (Table 2). A striking overlap of the most significant voxels ($p_{FWE} < 0.005$) in this analysis with those from the TBSS analysis, which are mostly located around the right AF, is illustrated in Figure 5.2. In the high-risk group there were no significant effects of rs821616 and no supra-threshold trends (all corrected p-values > 0.4).

Figure 5.1. Reduced FA in carriers of the A allele at rs821616 in *DISC1* in the control group, as demonstrate by TBSS ($p_{FWE} < 0.05$). For better visibility, the significant results are thickened using the "tbss_fill" command. The images are in radiological convention.

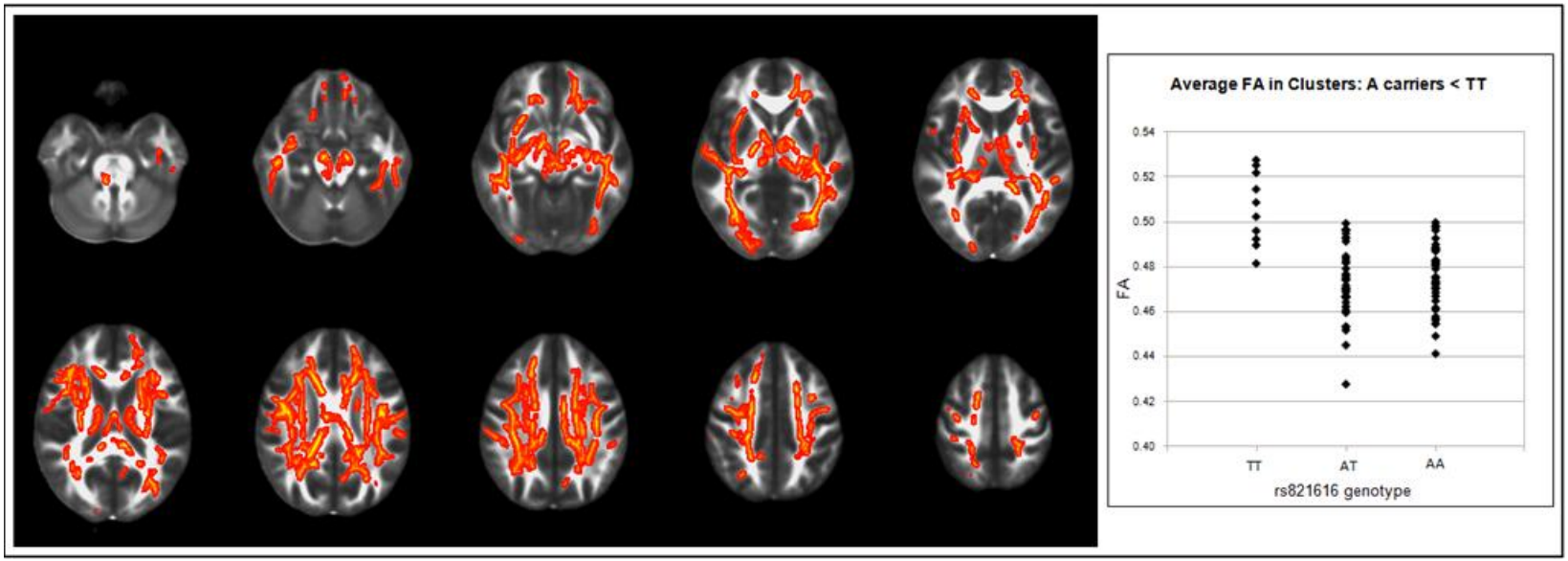


Figure 5.2. Comparison of most significant ($p_{FWE} < 0.005$) results between TBSS and voxel-based analysis in the control group.

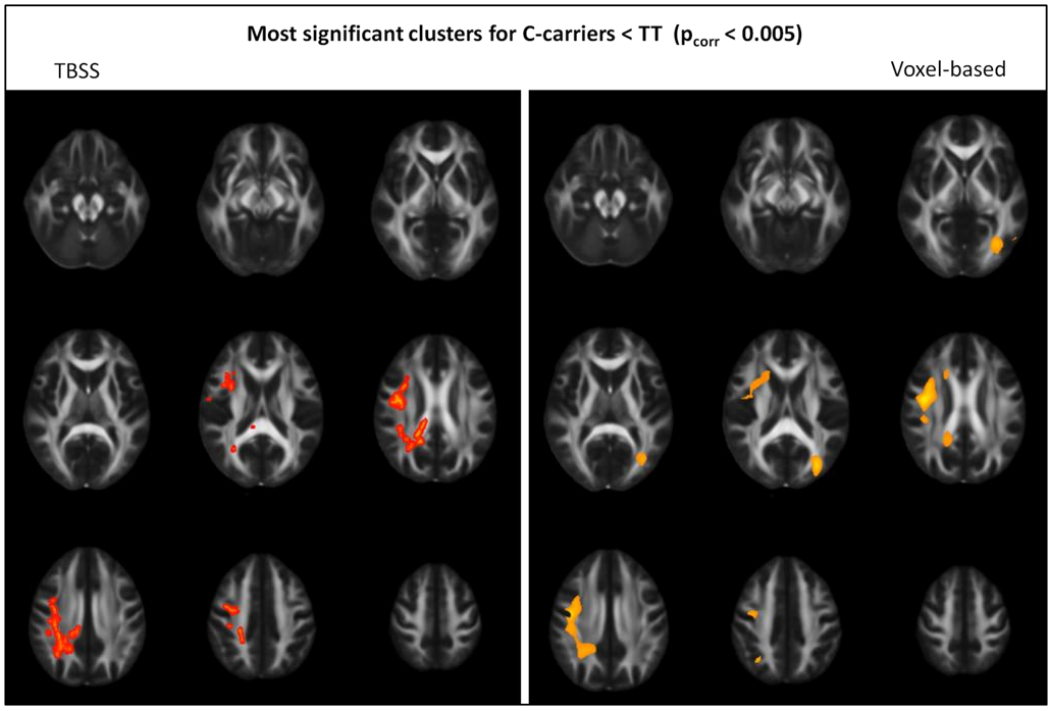


Table 5.2. Summary of voxel-based and TBSS results in the control group.

Method	Contrast	p_{FWE} (RFT / TFCE)	Location	Size (voxels)
Voxel-based	F-test	0.004	Right AF	14804
	F-test	0.049	Left TL and OL	4983
	Acar < TT	< 0.001	Distributed over RH and posterior LH	121059
	Acar < TT	0.009	Left TL and OL, cerebellum	13415
	Acar < TT	0.004	Left FL and PL	20577
	AA < TT	< 0.001	Distributed over RH	67846
	AA < TT	0.015	Left cerebellum	11265
	AA < TT	0.001	Left TL and OL	24007
	AA < TT	0.008	Left FL and PL	16301
TBSS – TFCE	AA < Tcar	0.05	Right AF	258, 77, 75, 50
	Acar < TT	0.05	Widely distributed	33929, 170, 46
	Acar < TT	0.005	Right AF	3040
	AA < TT	0.05	Widely distributed	> 23000
	AA < TT	0.005	Right AF	2082, 23, 4

RFT = Random-field theory; TFCE = Threshold-free cluster enhancement; AF = arcuate fasciculus, TL = Temporal lobe; OL = Occipital lobe; PL = Parietal lobe; FL = Frontal lobe; RH = Right hemisphere; LH = Left Hemisphere; Acar = A-carriers

Figure 5.3. Average FA extracted from the significant clusters from each contrast in the TBSS analysis in the control group, separated for men and women, to confirm the absence of sex-by-genotype interactions.

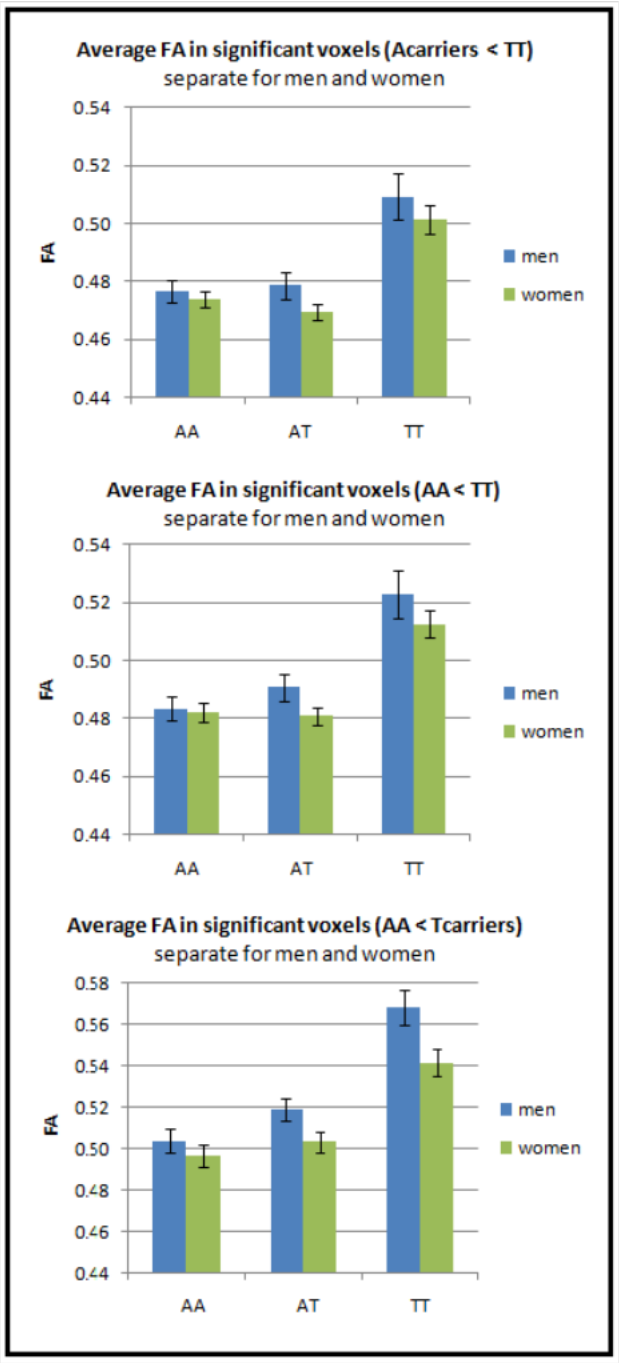
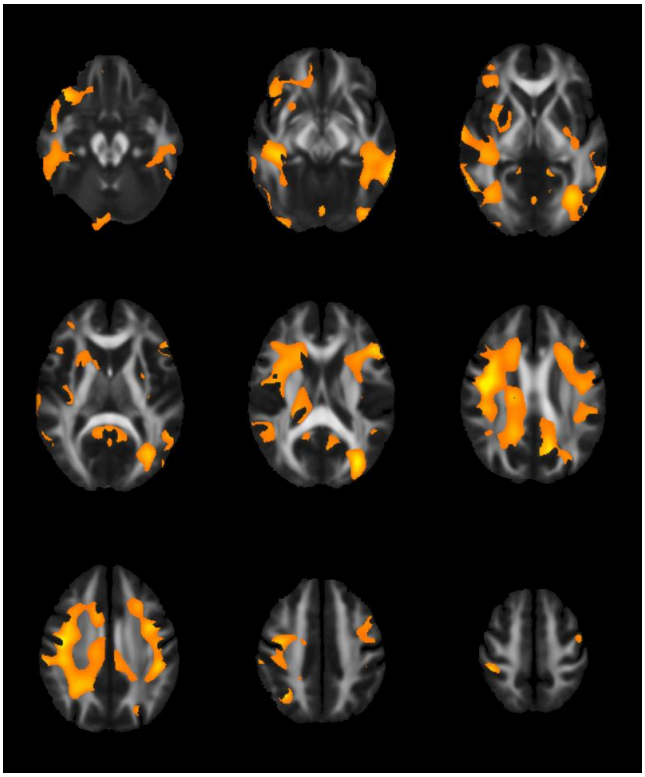


Figure 5.4. Voxel-based analysis showing significant ($p_{FWE} < 0.05$) FA reductions in carriers of the DISC1 rs821616 risk variant.



5.2.3 Tractography

Figure 5.5 gives an overview of the effects of *DISC1* genotype on FA averages within each tract, separated for group. For the AF, there was a group-by-genotype interaction (F = 3.829, p = 0.023). Mixed model regressions separate for the control group and the high risk group revealed that rs821616 had a highly significant effect on the AF in the control group (F = 6.167, p = 0.003), but not at all in those at high familial risk for BD (F = 0.097, p = 0.907). Individuals with the TT genotype had higher FA in both the left and the right AF (Figure 5.5). For the ATR, the effects of rs821616 on FA were more complex, with a three-way interaction between group, hemisphere and genotype (F = 4.795, p = 0.010). This is explained by a significant SNP-by-hemisphere interaction in the high risk group (F = 7.309, p = 0.001), but not in the healthy control group (F = 0.417, p = 0.661). Specifically, in individuals at high genetic risk for BD, a strong effect of rs821616 genotype on FA in the right ATR was observed (F = 6.515, p = 0.002),

but not on FA in the left ATR ($F = 0.395$, $p = 0.675$). Here, the AT genotype at rs821616 was associated with higher FA compared to both homozygous groups. For the UF, a trend for an interaction between rs821616 genotype and hemisphere was found ($F = 2.558$, $p = 0.081$). After splitting the analysis between the left and the right tracts, a moderately significant effect was found for the right UF across both groups ($F = 3.147$, $p = 0.045$), but not for the left ($F = 0.345$, $p = 0.709$). Post-hoc tests revealed that, again, the AT genotype was associated with the highest FA compared to both homozygous groups although looking at Figure 5.5 this seems to be mainly driven by the high risk group.

Using a two-way ANOVA for the genu of the corpus callosum indicated that there was a trend for an effect of rs821616 genotype ($F = 2.717$, $p = 0.069$) across both control and high risk groups. This was found in the absence of a group-by-genotype interaction ($F = 0.908$, $p = 0.405$), although from the FA averages within genotype groups (Figure 5.5) it appears this effect is mostly driven by TT genotype in the control group and by the AT genotype in the high risk group. In this case, interaction effects may be difficult to detect because of the low T-allele frequency. Concerning the cingulum, there was a significant effect of rs821616 genotype on FA in the control group ($F = 3.517$, $p = 0.034$), but not in the high risk group ($F = 0.142$, $p = 0.868$), although the group-by-genotype interaction was not significant ($F = 1.396$, $p = 0.251$). Again, this paradoxical finding is probably due to the small TT-genotype group, which clearly plays a major role in the genotype effect, but at the same time may have too little influence to drive a group-by-genotype interaction. For the splenium and CST there were no indications for any effects of rs821616.

All tractography results were very similar, and most effects were more pronounced, when accounting for effects of sex, age and IQ in the statistical models. This was also the case when left-handed people were excluded from the analyses, apart from in the UF, where only a trend for an effect of genotype remained ($F = 2.583$, $p = 0.079$). The finding in the AF in the healthy controls comfortably survives a stringent - and overly conservative - Bonferroni correction of $0.05 / [8(\text{tracts}) * 2(\text{groups})] = 0.0031$. The result in the right ATR in the high risk group also marginally passes its Bonferroni corrected p-value of $0.05 / [12(\text{unilateral tracts}) / 2(\text{groups})] = 0.00208$.

Figure 5.5. Effects of DISC1 SNP rs821616 (Ser704Cys) on FA across all tracts of interest

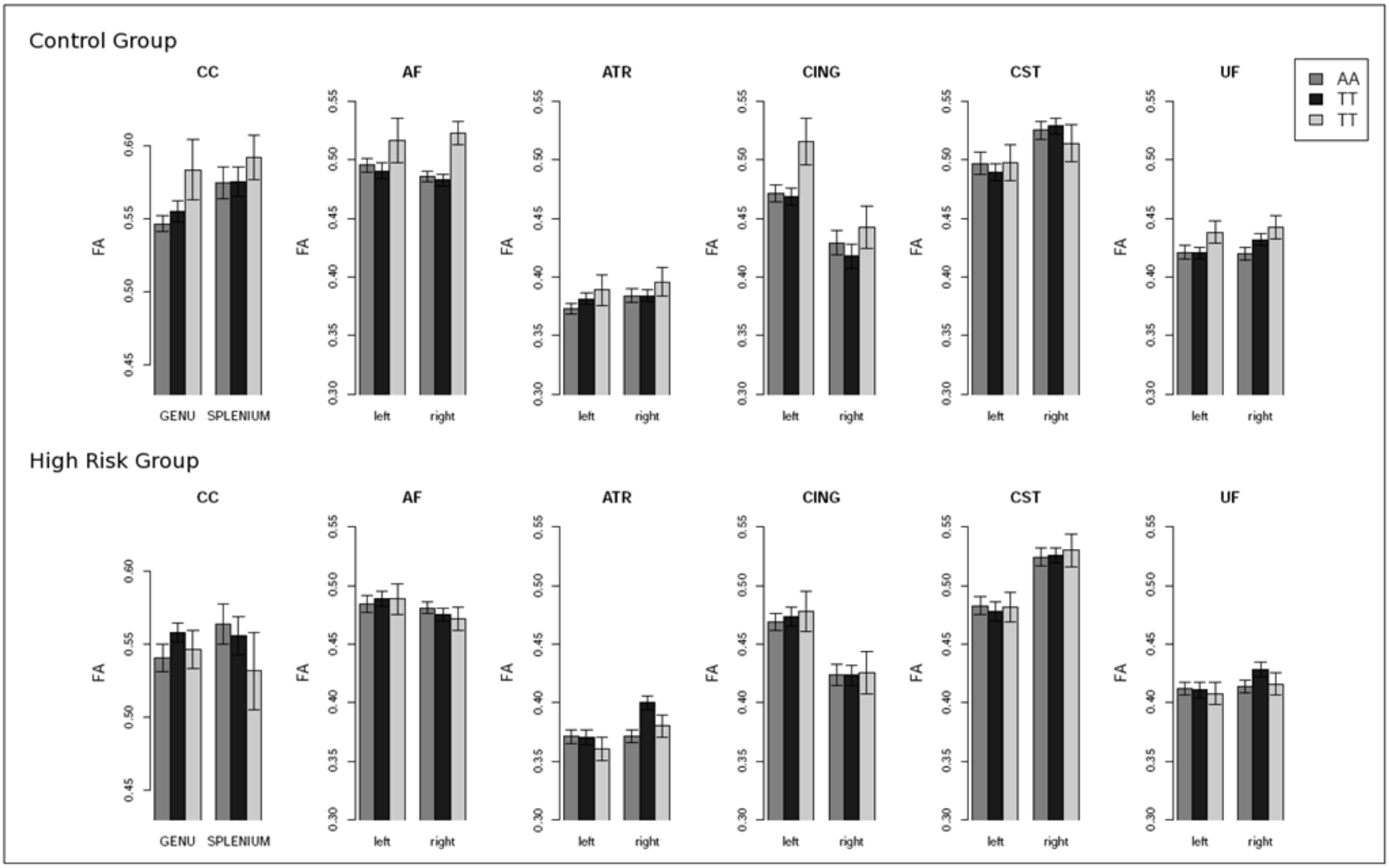


Table 5.3. Summary of effects of rs821616 genotype on FA, separated per tract.

TRACT	HC				HR			
	F	p	df	η^2	F	p	df	η^2
genu	2.645	0.077	83	0.060	1.731	0.183	87	0.038
splenium	0.301	0.741	83	0.008	0.677	0.511	87	0.015
left AF	1.492	0.231	80	0.036	0.110	0.896	85	0.003
right AF	6.634	*0.002**	82	0.139	0.506	0.605	81	0.012
left ATR	1.361	0.263	73	0.036	0.395	0.675	76	0.010
right ATR	0.495	0.612	83	0.012	6.515	*0.002**	85	0.133
left CING	4.031	*0.021**	81	0.091	0.205	0.815	84	0.005
right CING	0.707	0.496	82	0.017	0.008	0.992	83	0.001
left CST	0.238	0.789	81	0.006	0.093	0.912	87	0.002
right CST	0.375	0.689	82	0.009	0.072	0.931	85	0.002
left UF	1.097	0.334	71	0.030	0.057	0.944	65	0.002
right UF	2.510	0.087	83	0.057	1.640	0.200	86	0.037

Note that the main analyses were not performed on simple effects per unilateral tract, but as mixed regressions, across heispheres (and tracts). This table is included to provide meaningful effect sizes that allow for comparisons across studies, not for assessing the statistical significance of the present findings per se. The values correspond to those illustrated in Figure 5.6.df = degrees of freedom; η^2 (eta squared) = effect size (proportion of variance explained by rs821616 genotype.

5.4 Discussion

In this chapter I present the first thorough investigation of the effects of genetic variation in *DISC1* on white matter structure. I performed whole-brain analyses using TBSS as well as conventional voxel-based comparisons and I examined FA in specific connections of interest using probabilistic neighbourhood tractography. Overall, the results suggest that healthy people carrying the major allele (Serine) at the common, exonic SNP rs821616 have reduced white matter integrity compared to those homozygous for the minor (Cysteine) allele in many regions distributed over the cerebral white matter, and most robustly so in the right AF in which tractography, TBSS and voxel-based analyses all showed a highly significant genotype effect surviving the most rigorous corrections for multiple testing.

5.4.1. DISC1 and possible interactions with genetic background

Interactions between familial risk-status and rs821616 genotype effects were common for many regions. In most cases they were driven by a larger genotype effect in the control group than in the high risk group. In agreement with this, two other studies also found pronounced effects of the Ser704Cys SNP on prefrontal activation (Prata *et al*., 2008) and grey matter density (Takahashi *et al*., 2009) in healthy individuals, but not in patients. This was also the case for the effects of *NRG1* genotypes (Chapter 4), and in the case of *DISC1* these may be similarly caused by floor effects and possibly sampling bias in the high risk group, or increased heterogeneity of the high risk sample.

More speculatively, the present data also allow for an explanation on the biological level. The functional consequences of the Serine/Cysteine substitution are largely unknown, but it is possible that these are different against a vulnerable genetic background from functional consequences in the general population. A general pattern emerging from tractography analyses is that in the high-risk group, heterozygous individuals have the highest FA, whereas in the control group a more linear relationship exists between the number of T alleles and FA. It is possible that genetic variants within *DISC1* or elsewhere interact with the functional consequences of the Serine/Cysteine substitution, making those processes that are beneficial to white matter integrity in the general population detrimental against a vulnerable genetic background. For example, Hashimoto *et al*. (2006) found that DISC1 resulting from the Serine variant (sDISC1) interacted more with the ERK pathway than DISC1 transcribed from the Cysteine variant. The ERK pathway is involved in neuronal development, plasticity and neuron and oligodendrocyte survival (Domercq *et al*., 2011), its activity is reduced in schizophrenic and bipolar patients (Yan *et al*., 2010), and it is a therapeutic target of mood stabilisers and antipsychotics (Einat *et al*., 2003; Engel *et al*., 2009; Xia *et al*., 2008). An increase in ERK-related neuroprotective and growth stimulating processes should have a beneficial effect on white matter integrity in normal circumstances, but an atypical linkage disequilibrium pattern within the *DISC1* region or the presence of risk-variants elsewhere in the genome could prevent an interaction with ERK or instigate the opposite effect. Of note, although the ERK pathway is to this date the only known functional consequence of the Serine/Cysteine substitution, it constitutes only one of the many DISC1 interactomes, and undoubtedly other pathways will be identified in due time that could provide similar explanations for the differential effect of

Serine/Cysteine in our two studied groups. For instance, the Serine/Cysteine SNP is located in the *DISC1* region of which the protein products interact with various other key proteins in neuronal development and migration, and axon outgrowth (FEZ1, NUDEL1, LIS1). What is more, rs821616 genotype affects mRNA expression levels of each of these proteins in the *post mortem* brain of schizophrenia patients but not controls, while the expression of DISC1 itself was not affected in this study (Lipska et al., 2006). A third example of known DISC1 protein interactions is through DISC1's function in the Wnt pathway via GSK3β (Brandon *et al*., 2009; Chubb *et al*., 2007; Mao *et al*., 2009), where the phosphorylation of DISC1 changes its role from stimulating progenitor cell proliferation to inhibiting it to initiate cell migration (Ishizuka *et al*., 2011). These opposite roles of unphosphorylated versus phosphorylated DISC1 give way to many more possible explanations of how changes in DISC1 protein structure could have opposite effects on white matter development, depending on genetic and environmental backgrounds, as I presented here. Generally speaking, our data and the available literature can be integrated by imagining the *DISC1* Serine/Cysteine SNP as one small player in a system of many genetic and environmental risk factors, which together disrupt the balance and functioning of protein cascades necessary for healthy brain structure.

5.4.2. The risk-allele at DISC1 rs821616

Despite the abundance of studies associating Ser704Cys with psychiatric illness and intermediate phenotypes, the literature disagrees substantially about which allele at rs821616 is the risk variant. Briefly, three studies found the major allele (Serine) was associated with an increased incidence of schizophrenia (Callicott *et al*., 2005; Chen *et al*., 2007; Zhang *et al*., 2005), whereas three studies found the minor the minor allele (Cysteine) to be over-transmitted in patients with schizophrenia (Song *et al*., 2007; Qu *et al*., 2007) or major depression (Lepagnol-Bestel *et al*., 2010). Negative findings, mostly in smaller samples, also exist (Devon *et al*., 2001; Kim *et al*., 2008; Rastogi *et al*., 2009; Saetre *et al*., 2008; Song *et al*., 2010; Zhang *et al*., 2006). With respect to intermediate phenotypes, most studies found the Serine variant related to decreased cognitive function and grey matter volumes, but opposite and negative findings also exist. Functional imaging findings are more difficult to categorise in terms of "risk" and "protective" allele effects, as discussed below. See Table 5.3 for a summary of intermediate phenotype studies in association to rs821616.

Reasons for inconsistencies between studies could, as always, be sample heterogeneity and variations of methodology, but in this case an additional source could be the ambiguity inherent to A/T and C/G SNPs because of their strand symmetry ("A" on the positive strand is equivalent to "T" on the negative strand). More fundamentally, as others have also pointed out (e.g. Palo *et al*., 2007; Cannon *et al*., 2005; Thomson *et al*., 2005b), complex clinical phenotypes such as psychotic disorders are likely to be associated with multi-SNP haplotypes and even more distal multi-gene interactions making the effects of single polymorphisms, no matter how functionally relevant, difficult to detect. Intermediate phenotypes, on the other hand, are likely to be more sensitive to variation in specific, functionally relevant SNPs (Glahn, Thomson & Blangero, 2007).

Table 5.4. Summary of studies associating rs821616 (Ser704Cys) with intermediate phenotypes.

1st author	"risk" allele	"risk" Amino Acid	intermediate phenotype	(sub)population
Callicott	major	Serine	hippocampal grey matter and activation (task-dependent opposing effects)	Caucasian
Hashimoto	minor	Cysteine	prefrontal FA, cing gyrus grey matter	Asian
Thomson	major minor	Serine Cysteine	cognitive impairment with ageing cognitive impairment with ageing	Male Caucasian Female Caucasian
DiGiorgio	minor	Cysteine	hipp activation and functional connectivity, hipp grey matter (trend)	Caucasian
Raznahan	major	Serine	cortical development (thickness) in temp & front lobes	Caucasian
Takahashi	major ns	Serine ns	frontal and temporal grey matter no clear effects on grey matter	Healthy Asian SCZ & SCZAFF Asian
Palo	major	Serine	visuospatial task, verbal fluency	Caucasian
Prata	unsure ns	unsure ns	increased prefrontal activation during verbal fluency in Ser/Ser trend decreased prefrontal activation during verbal fluency in Ser/Ser	Healthy Caucasian SCZ & BD Caucasian

But here, other problems can cause heterogeneity between studies. Firstly, reductions in volumetric or density measures of brain tissue do not always represent pathology. Dependent on the region or the measure, increases may also be indicative of pathology. This is especially true for functional MRI data in which increases in activation are commonly interpreted in terms of compensatory mechanisms or inefficiency. Grey matter measures derived from voxel-based morphometry methods can also be ambiguous for several reasons. Firstly, whilst gross measures derived from MRI on the voxel-level can give information about the tissue type, and estimate the amount of tissue both regionally and globally, they do not directly inform about the integrity of the microstructure or about the specific the cell-type or sub-cellular compartments within a voxel. Secondly, in tissue classification a trade-off can exist between white and grey matter, which can cause white matter pathology (or "reductions") to manifest as "increases" in the adjacent grey matter. Thus, if *DISC1* affects white matter integrity, as the above results suggest, different classification methods and grey matter measures could produce contradictory results. Finally, both association and intermediate phenotype studies commonly combine the heterozygous (Ser/Cys) individuals with individuals homozygous for the minor allele (Cys/Cys) into one group to increase statistical power. Whether or not this is justified depends on whether the data agrees with a dominant, recessive or linear model of genetic effects. For example, from the present study, it was the small homozygous (Cys/Cys) group that deviated most from the two other groups, at least amongst the healthy controls, meaning that in this case combining the minor allele carriers into one group would not give robust results.

5.4.3. Limitations

A few limitations to the present analysis also need mentioning. Firsly, as with most studies, individuals homozygous for the minor allele were few: 10 in the control group and 12 in the high risk group. This made it difficult to detect genotype-group interactions without combining genotype groups, and impossible to investigate gene-gene interactions or multi-SNP haplotypes. Also, it was this smallest group in the control participants who deviated most from the two larger groups, and independent replications will need to exclude the possibility this was an incidental finding. On the other hand, that probability is by definition under 5% in each analysis, regionally below 0.5%, and even smaller for all results to agree across the different methods the way they did here.

As in previous chapters, other limitations are the lack of a patient group, the number of tracts excluded after tractography because of visible deviations from known anatomy, and the multiple testing associated with tractography. Previously the latter was alleviated by the initial significance of one large regression model, in which FA was predicted by tract, group and hemisphere. However, when it comes to dividing the groups according to genotypes, the addiction of a fourth variable incrementally reduces statistical power especially for detecting interaction effects (which are very likely in this context). The effect of rs821616 on FA remained significant for two tracts after conservative Bonferroni correction, namely the AF and the right ATR. The effect in the UF, on the other hand, did not survive such corrections.

Finally, on a genetic level a few reservations on interpreting the findings should be kept in mind. Ser704Cys (rs821616) is one of the few non-synonymous, exonic SNPs in the *DISC1* region (Devon *et al*., 2001) and to this date the only one for which a direct functional consequence has been identified, supporting the possibility that this SNP itself is responsible for the above findings rather than another SNP in close linkage disequilibrium. However, more research on molecular consequences of the serine/cysteine substitution is required to unequivocally confirm (or falsify) such a direct effect. Also, a genetic variant does not need to be non-synonymous and not even protein coding, for it to be directly or indirectly functional. Increasing evidence is emerging for the importance of non-coding SNPs in regulating expression or post-translational processes, including those transcribing micro-RNA and reversely transcribed, supporting a possible role for DISC2. Future research in molecular genetics will hopefully reveal more about the mechanisms by which Ser704Cys can have an effect on brain structure and function, including white matter integrity.

Whether or not the Ser704Cys SNP is directly responsible for reductions in white matter integrity does not impede the main implications of the present study. From the above findings it can be concluded that white matter integrity is associated with genetic variation in a non-synonymous *DISC1* SNP. In individuals without a family history of psychiatric disorder this effect was evident in a large number of voxels widespread over the cerebral white matter, in which FA was increased in individuals homozygous for the minor cysteine allele. In unaffected relatives of bipolar patients, a different and somewhat less clear pattern emerged, with only tractography indicating increased FA in the genu of the corpus callosum, right ATR and right UF in heterozygous individuals. Considering *DISC1* is one of the main risk loci for psychiatric

disorders and white matter integrity may be an endophenotype of psychotic disorders (Chapter 3), the present results provide a plausible mechanistic link between enhanced genetic risk through inheritance of *DISC1* genotypes and the development of psychiatric disorders.

Chapter 6

No association of the genome-wide supported ZNF804A SNP with white matter integrity

6.1 Introduction

In the first large genome-wide association study of schizophrenia the common SNP rs1344706 of the Zinc Finger Protein 804A gene (*ZNF804A*) was identified as the most significant genetic marker ($p < 1.61 \times 10^{-7}$)(O'Donovan *et al*., 2008). In the same study, combining schizophrenia and bipolar phenotypes showed an even higher association ($p < 9.96 \times 10^{-9}$), surpassing genome-wide significance at $p < 7.2 \times 10^{-8}$ and suggesting the variant conveys susceptibility to the wider psychosis spectrum. Four independent replications have since confirmed its association to schizophrenia and BD (Riley *et al*., 2010; Steinberg *et al*., 2011; Zhang *et al*., 2011), including one independent replication of genome-wide significance, and a recent meta-analysis resulting in p-values up to 4.1×10^{-13} for the combined phenotype (Williams *et al*., 2011).

6.1.1. ZNF804A is associated with task-independent functional connectivity

Despite this abundance of statistical evidence for the association of *ZNF804A* with psychosis, only modest effect sizes have been reported with odds ratios of around 1.10 (Donohoe *et al*., 2010). Intermediate phenotypes such as functional connectivity and white matter integrity in the brain are therefore especially valuable because they are more proximal to the initial genetic effect than the clinical phenotype, and effect sizes are expected to be larger, thereby requiring smaller sample sizes to obtain a significant association (Glahn, Thompson & Blangero, 2007). An additional reason for studying intermediate phenotypes is that they enable one to examine directly which particular neuropathological processes or brain structures may be affected by a gene of interest. On the molecular level, all that is known about the *ZNF804A* function is that it is highly expressed in the brain and the presence of A-allele at rs1344704 creates a myelin transcription factor binding site (Riley *et al*., 2010; Voineskos *et al*., 2011). The most

comprehensive data on *ZNF804A* function comes from neuroimaging and neuropsychological data, collectively indicating that rs1344706 is associated with brain function. Esslinger *et al.* (2009) reported reduced functional connectivity between the left and right dorsolateral prefrontal cortices and increased fronto-temporal functional connectivity in carriers of the risk allele (A) during a working memory task. Importantly, in a subsequent study Esslinger *et al.* (2011) found that the reduced interhemispheric prefrontal connectivity was also apparent during a facial emotion processing and during rest, whereas the increased fronto-temporal connectivity appeared specific to working memory processes. This task-independent effect of *ZNF804A* genotype on interhemispheric prefrontal functional connectivity prompted the hypothesis that these effects may be mediated by effects on white matter integrity, especially in anterior interhemispheric connections. In line with this hypothesis, Lencz *et al.* (2010) found that individuals homozygous for the *ZNF804A* risk allele have reduced total white matter volumes (corrected for total intracranial volume) compared to carriers of the non-risk allele. However, total volumetric measures lack spatial specificity and are particularly susceptible to partial volume effects and segmentation difficulties, including ambiguity concerning a trade-off between white and grey matter volume. DTI is more suited to the study of white matter and FA is currently widely accepted to be the best measure for estimating white matter integrity *in vivo*. Surprisingly, using DTI tractography Voineskos *et al.* (2011) did not detect any effects of *ZNF804A* on FA in the UF, AF, CING or in the corpus callusum of 64 healthy individuals. Although they discuss the possibility of having missed any *ZNF804A* effects outside their tracts of interest, their chosen connections were the most relevant in the light of the functional connectivity differences found previously.

Taken together, the myelin transcription factor binding site determined by the A-allele at rs1344706, the task-independent effects of *ZNF804A* on functional connectivity, the high heritability of FA and its association with genetic risk all converge in the hypothesis that structural connectivity is a mediator of rs1344706 effects on functional connectivity. This hypothesis is logically and scientifically appealing, and readily testable with FA as an objectively measurable approximation of white matter integrity. It is therefore not only surprising that Voineskos *et al.* (2011) failed to detect an association between *ZNF804A* and FA, but also that it is the only available report investigating the hypothesis since Esslinger *et al*. (2009) first suggested it, while positive associations of *ZNF804A* with cognitive performance and other brain imaging measures continue to emerge (Esslinger *et al*., 2011; Lencz *et al*., 2010; Walters *et al*.;

2010; Walter *et al*., 2011; Hashimoto *et al*., 2010). This may be due to a lack of availability of combined genotype and DTI data, or to a publication bias disfavouring negative findings. In this chapter I therefore present a second, more detailed investigation of the possible effect of *ZNF804A* on FA using tract-based spatial statistics (TBSS) and tractography of the genu in the bipolar family sample.

6.2 Methods

All image acquisition, DTI pre-processing, TBSS and tractography procedures, and genotyping were performed as described in Chapter 2.

6.2.1. Study Sample

All participants were recruited as part of the Bipolar Family Study, as described in chapter 2. Both DTI data and blood samples were available from a total of 110 high-risk subjects and 83 controls. Because some high-risk subjects were genetically related to each other, I randomly excluded one of each sibling pair to avoid statistical dependence in the sample, leaving 84 high-risk and 83 controls in our final sample. Demographics and genotype results are summarised in Table 6.1. Allele frequencies at rs1344706 were similar to those previously reported (O'Donovan *et al*., 2008). For our principal analysis, subjects carrying the C-allele were combined into a C-carrier group (CC + CA), as was done in previous studies of rs1344706 (Voineskos *et al*., 2011). Assuming the genetic effect is either linear or recessive, this increases statistical power when there is only a small group of C-homozygotes. Additional 3-group analyses were preformed to rule out any dominant or otherwise non-linear genotype effects. In the healthy control group age differed significantly between genotype groups. Therefore additional analyses were performed including age as a covariate for each statistical comparison below. There were no other demographical differences between genotype groups (Table 6.1).

Table 6.1 Demographic characteristics and genotype frequencies of the study samples.

	Control Sample			High Risk Sample		
rs1344706	**AA**	**AC + CC**	***P***	**AA**	**AC + CC**	***P***
N	31	39 + 13		37	36 + 11	
Age (years)	21.8 ± 2.3	20.7 ± 2.4	0.03*	21.4 ± 2.8	21.4 ± 2.8	0.94
Sex (m / f)	14 / 17	23 / 29	0.93	17 / 20	22 / 25	0.94
IQ	111.7 ± 11.9	112.2 ± 13.1	0.84	108.0 ± 13.9	104.6 ± 14.4	0.28

6.2.2. Voxel-wise analysis

FA values within the skeletons were compared between C-carriers and individuals homozygous for the A-allele within the control and high-risk groups separately, using voxel-wise non-parametric T-tests calculated by FSL's "randomise" tool. Statistics were corrected for multiple comparisons according to family-wise error ($p < 0.05$) using TFCE (Smith *et al*., 2009). For the control group, an additional analysis was performed with age included as a covariate.

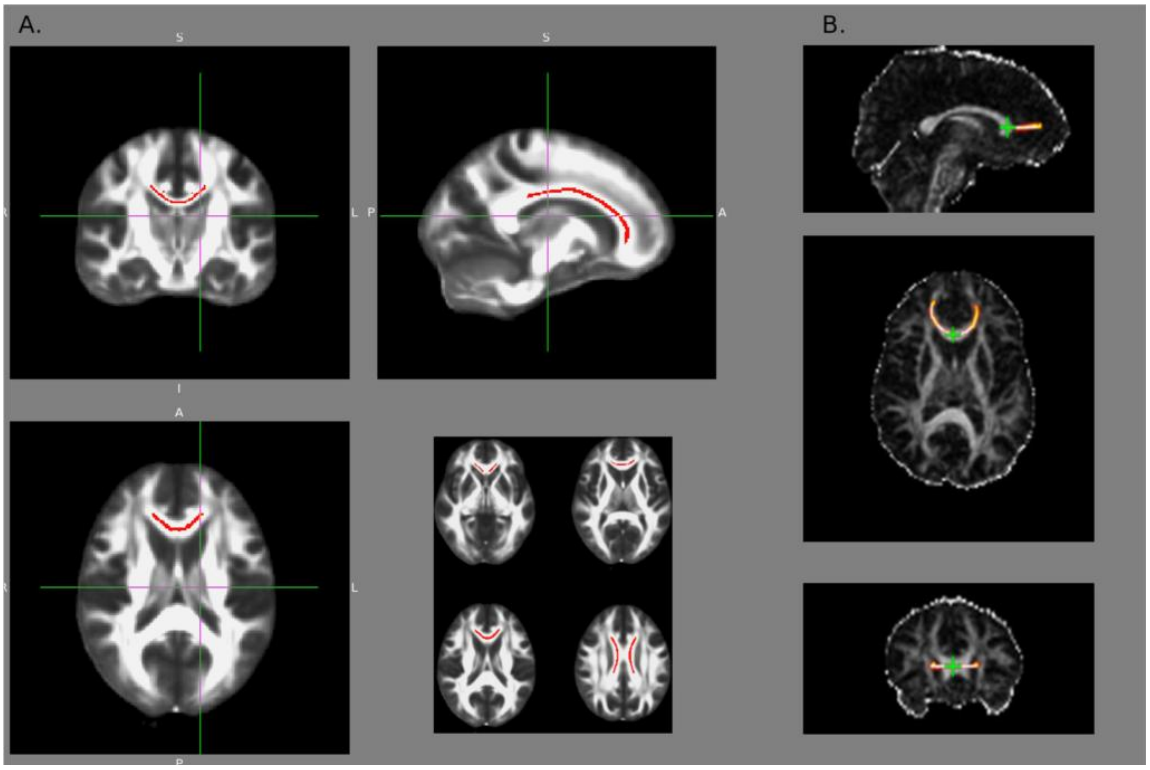
6.2.3 Small volume correction

Because the two previous studies by Esslinger *et al.* (2009, 2011) showed *ZNF804A* was related to task-independent inter-hemispheric functional connectivity between the dorsolateral prefrontal cortices, a small volume correction (SVC) was applied to include only voxels within the skeleton and the body and genu of the corpus callusum. With an SVC only voxels in an *a priori* location are included in the analysis, thereby reducing the number of voxel-wise comparisons the statistics have to be corrected for and increasing the power to detect a significant effect. The SVC was created using the John Hopkins University white matter labels atlas in MNI space (Mori & van Zijl, 2011), thresholded to include only the genu and body of the corpus callosum, smoothed (FWHM 1.1 mm), binarised and multiplied with the skeleton mask to include only voxels that were in both the skeleton and the corpus callosum SVC (Figure 6.1A). Voxel-wise analyses were re-run for the control and the high-risk group with this SVC applied as a mask.

6.2.4 Region of interest analysis

In addition to using the SVC as a region of interest (ROI), the average FA was extracted from this corpus callosum region and compared between genotype groups using independent sample T-tests. Because one extreme outlier (> 3 interquartile ranges from the median) was observed in the high-risk group, an additional analysis excluding this individual was performed. Additional ANOVAs with all three genotype groups were also performed to verify there were no dominant or otherwise non-linear genotype effects.

Figure 6.1. Segmentation of corpus callosum regions of interest using TBSS and a white matter atlas (Mori & van Zijl , 2011) (A), and of the genu using tractography (B).



6.2.5 Tractography

As described in chapter 2, probabilistic neighbourhood tractography was applied to trace the genu of the corpus callusum (Figure 6.1B). The weighted average FA values were compared between genotype groups using independent samples T-tests for the control group and high-risk group separately. Again, there was one extreme outlier in the high-risk group who was removed in an additional T-test. In addition, to confirm that any dominant or otherwise non-linear effects of *ZNF804A* on FA were not obscured by combining the CC and AC genotype groups, I performed analyses of variance (ANOVAs) of all three genotype groups on average FA within the genu.

6.2.6 Power considerations

Post-hoc power calculations are a controversial endeavour because they are often based on the observed effect size involving circular reasoning and a "power paradox" where higher (less significant) p-values correspond to both lower observed power and more evidence for the null-hypothesis (Hoenig & Heisey, 2011). Therefore I took into account several power considerations, each no doubt with its own controversy or opponents, but altogether informative to interpret negative results. Power analysis of whole brain voxel-wise statistics are especially complicated, even without the consideration of TFCE. Sometimes a post-hoc power analyses is performed on the voxel that displayed the maximum effect. But this method assumes that if the alternative hypothesis is true, it concerns the effect in this particular voxel, ignoring the tens of thousands of other voxels, with smaller effect sizes, that could be considered for this role. If the alternative hypothesis were true in the population, it is extremely unlikely that it concerns the maximum voxel out of all possible voxels. Thus, such method ignores a multiple testing problem and by using the maximum effect size would result in a severe over-estimation of the likelihood of any true effect in the population. Methods have been developed for overcoming this problem in the more conventional MRI analysis techniques, but these are not widely accepted and none have been customised to TBSS and/or TFCE[3]. Considering this important limitation I have not performed a quantitative post-hoc power analysis for the voxel-wise TBSS analysis. However,

[3] Link for power tool: http://www.fmripower.org/,

histograms of T-distributions across the TBSS skeleton were examined, which can give an indication of any supra-threshold trends. For example, in the case of a widely distributed signal (between-group difference) of small effect size, the whole histogram is shifted to the left or the right and in the case of focal signals of large effect size a small proportion of voxels stands out on one or both sides of the histogram.

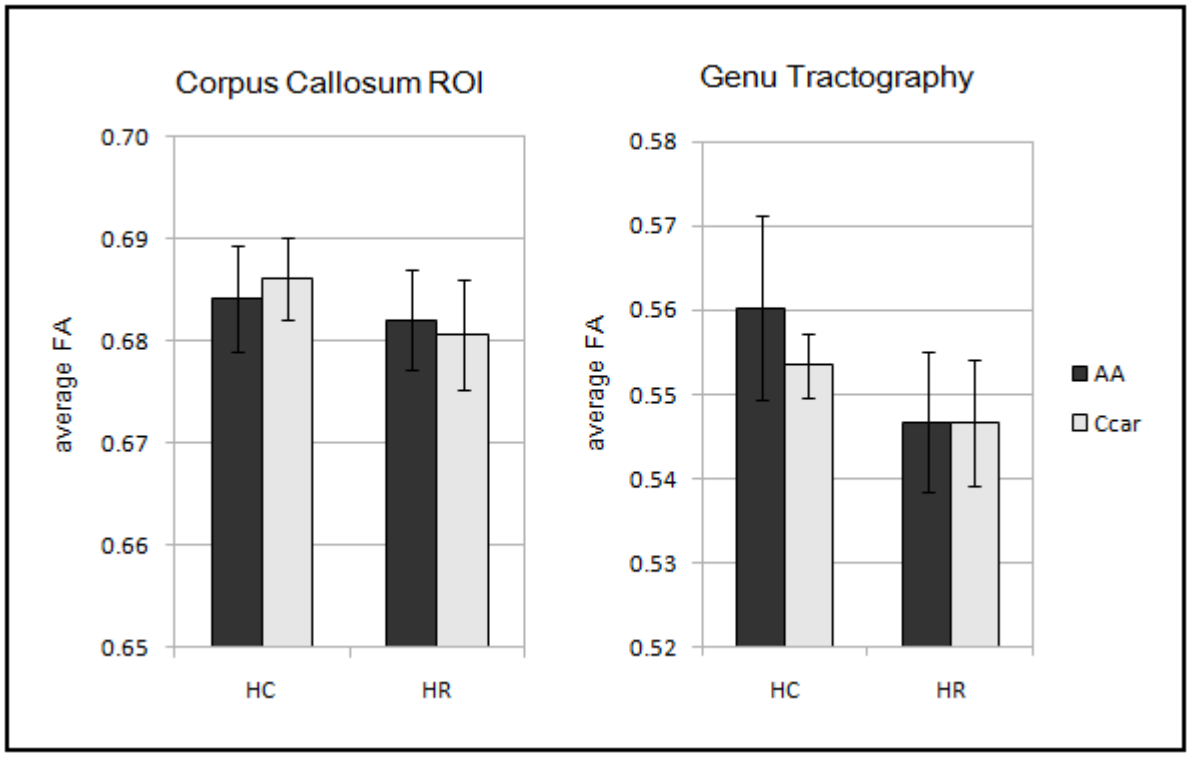
For the tractography and ROI analysis I examined statistical power in three ways: (1) confidence intervals around the observed group differences in relation to effects typically observed in similar studies and (2) the power of our study to detect such a typical effect size, and (3) the sample size that would be required to obtain a significant result given our own observed effect size. In each of these calculations I avoided using both our observed effect size and observed standard errors at the same time. I did not correct for multiple testing in these ROI analyses, because here it is more important to reduce type-II error than type-I error. However, it should be borne in mind that if our results were positive, a correction for multiple testing would be required and would increase p-values substantially. Therefore all calculations are under-estimations of the true requirements to obtain significance under multiple testing corrected p-values.

6.3 Results

Allele frequencies of rs1344706 in our samples were similar to those reported previously (Table 6.1) (O'Donovan *et al*., 2008). There were no significant deviations from Hardy-Weinberg equilibrium. No statistically significant differences (all $P > 0.84$) between rs1344706 genotype groups were found in age, sex, education, IQ for any of the samples, apart from an age difference in the Scottish control sample (Table 6.1).

For TBSS, no significant differences in FA were found between the genotype groups in either the control or high-risk samples (all $p_{FWE} > 0.38$). No significant differences were found in the control sample after the model was adjusted for the effect of age ($p_{FWE} > 0.37$). Histograms of raw T-statistics were normally distributed around zero, indicating that there were no trends for an effect of rs1344706 genotype on FA in either direction.

Figure 6.2. No effect of *ZNF804A* genotype on average FA extracted from the corpus callosum ROI and genu. HC=healthy control group; HR=High risk group; "Ccar"=C-carriers.

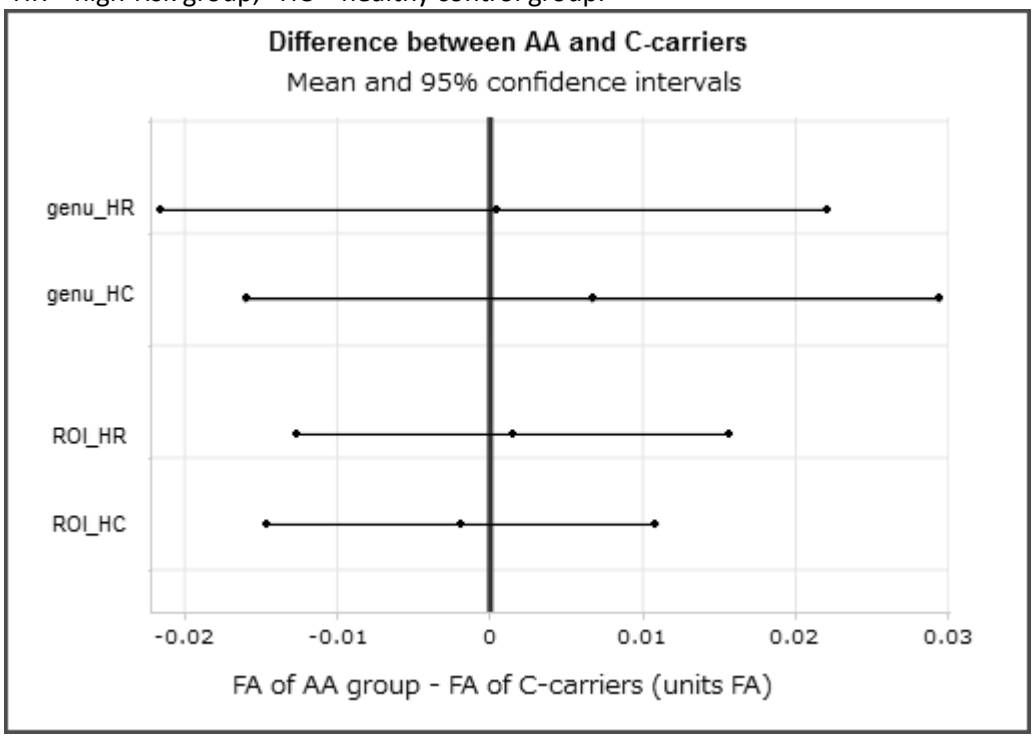


The application of a SVC over the body and genu of corpus callosum did not detect any FA differences between *ZNF804A* genotype groups using voxel-wise statistics with TFCE ($p > 0.37$). Average FA within the corpus callusum ROI did also not differ between genotype groups for the control group ($T = -0.29$, $p = 0.78$), nor for the high-risk group ($T = -0.23$, $P = 0.82$). Correspondingly, no significant genotype effects were found with tractography of the genu (controls: $T = 0.58$, $P = 0.57$; high-risks: $T = 0.55$, $P = 0.58$). Removal of the extreme outlier in the high-risk group did not change this negative result for either genu tractography ($T = 0.02$, $p = 0.99$) or the corpus callosum ROI ($T = 0.20$, $P = 0.84$). As shown in Figure 6.3, there were no obvious trends in either direction for any of the comparisons. Finally, analyses of variance comparing all three genotype groups with respect to average FA within the genu and the corpus callosum ROI were all non-significant, with or without outlier (all $F < 0.75$, all $P > 0.49$), indicating that there were no non-linear or dominant effects of the risk-allele that may have been obscured by combining the CC and AC groups.

Out of the four corpus callosum analyses (tractography and ROI for each sample), only for the corpus callosum ROI in the healthy controls was the effect of *ZNF804A* genotype in the expected direction. For tractography of the genu, individuals homozygous for the A-allele had on average higher FA than C-carriers in both high risk and control groups, as was the case for the ROI analysis in the high risk group (Figure 6.2, Figure 6.3). Figure 6.3 shows that the maximum effect under the 95% confidence interval in either direction is just below 0.03 FA units. The maximum difference in the expected direction under the 95% confidence interval is 0.0216 FA units, for genu in the high risk group. In comparison, we previously reported a significant effect of *NRG1* on FA the left ATR with an effect size of around 0.07 FA units (Sprooten *et al*., 2009) and voxel based FA studies have reported effects of BDNF (Chiang, Barysheva *et al.*, 2011), *ErbB4* (Konrad *et al*., 2009) and *NRG1* (Winterer *et al*., 2008) genotypes of 0.05 to 0.11 FA units. In our analysis of *DISC1* genotypes (Chapter 5) the mean FA difference within the cluster was 0.04 FA units. These effects sizes are far outside the confidence intervals in the present study and we can therefore be fairly confident that *ZNF804A* has no such effect on FA in the corpus callusum. Conversely, Liu *et al*. (2010) reported effects of COMT haplotypes around 0.02 FA units, marginally within the scope of our widest confidence interval (genu in high-risk subjects). Correspondingly, with our own sample size and standard deviation, we had 83% power to detect an effect of 0.02 FA units in a two-tailed T-test ($P < 0.05$). Of note, the chosen effect size here, 0.02 FA units, is the smaller of mean differences reported in similar studies, and corresponds to Cohen's d ~ 0.5.

Finally, using Statistical Analysis System (SAS Institute Inc.; http://www.sas.com) we calculated the required sample size to obtain significant results given the observed effect size. Considering the genu ROI in the healthy control group, individuals homozygous for the risk allele (AA) had on average 0.0019 lower FA than carriers of the C-allele, with standard deviations of SD_{AA} = 0.0255 and $SD_{Ccarriers}$= 0.0288. For 0.0019 to be significant with 80% power in a two-tailed T-test, given the observed variance and equal genotype group sizes, a sample size of N = 7324 would be required. I refrained from calculating required sample sizes for the other three analyses, because there the AA group had on average higher FA than the C-carriers, opposite of what was hypothesised.

Figure 6.3. Mean differences in FA between genotype groups, and 95% confidence intervals. "HR"=high-risk group; "HC"=healthy control group.



6.4 Discussion

To date, rs1344706 locus of the *ZNF804A* gene is statistically the best supported SNP in association with schizophrenia and the wider psychosis phenotype (O'Donovan *et al*., 2008; Riley *et al*., 2010; Steinberg *et al*., 2011; Williams *et al*., 2011; Zhang *et al*., 2011), but the mechanisms by which it may affect susceptibility to psychosis are poorly understood. Associations of *ZNF804A* with cognitive (Esslinger *et al*., 2011; Walters *et al*., 2010; Hashimoto *et al*., 2010) and imaging (Voineskos *et al*., 2011; Esslinger *et al*., 2009, 2011; Lencz *et al*., 2010) phenotypes indicate that the gene modulates brain function and is involved in higher cognitive processes. So far most emphasis has been on the association of *ZNF804A* with functional connectivity, and more specifically with its effect on interhemispheric prefrontal connectivity which is apparent during various cognitive tasks as well as during rest (Esslinger *et al*., 2009, 2011).

Here, I present a thorough investigation of the relationship between genotype at rs1344706 of the *ZNF804A* gene and white matter integrity of the brain. Our study was motivated by a strong *a priori* hypothesis based on previous associations of this SNP with task-independent functional connectivity (Esslinger *et al*., 2009, 2011), the recent knowledge that the risk genotype at this SNP is responsible for creating a myelin transcription factor binding site (Riley *et al*., 2010; Voineskos *et al*., 2011), and FA as an established intermediate phenotype. Despite the use of various analyses methods and efforts to increase statistical power in two adequately sized samples, results were remarkably and consistently negative.

Because proving equivalence statistically entails more than the absence of significant difference, we corroborate our negative findings with extensive examination of statistical power and any on-significant trends. On closer examination of genotype group averages across methods and samples no trends were observed, with genotype group means differing seemingly at random in either direction, and histograms of T-statistics normally distributed around zero. More quantitative power calculations all seem to suggest the same: if there were any real effects at all they must be far smaller than what is typical for imaging genetics studies to have remained undetected in the present study.

Although our DTI study should not be seen as an attempt to replicate the functional connectivity results of Esslinger and colleagues (2009, 2011), the question arises as to what can cause the discrepancy between the observed effects of *ZNF804A* on functional connectivity and its lack of effect on structural connectivity. With regard to methodology, possible explanations are population heterogeneity, lack of statistical power in the current study, and limitations of both DTI and fMRI-based functional connectivity. It is also possible that the reported effects of *ZNF804A* were sample specific, since most previous observations of *ZNF804A* effects on cognitive and imaging phenotypes were derived from the same or largely overlapping samples (Esslinger *et al*., 2009, 2011; Walter *et al*., 2011). In general, the interpretation of functional connectivity derived from fMRI is complicated by factors such as task-dependent effects and the possibility of activation in a third area modulating the correlation between two areas of interest, as well as by limitations of BOLD fMRI in the first place including regional differences in BOLD response and limited temporal and spatial resolution. Hence, the relationship of functional connectivity to underlying brain physiology or structural connectivity is not entirely clear. On the other hand, DTI is also limited by spatial resolution and tensor modelling, and

voxel-wise FA analysis is obscured by co-registration difficulties and partial volume effects. Although we alleviated some of these limitations by applying several techniques such as TBSS and tractography in native space, DTI would fail to detect small-scale differences in structural integrity, especially when close at the synapse or near grey matter and away from large fibre bundles. But perhaps the most likely explanation is that *ZNF804A* has an effect on functional connectivity but not on white matter structure, for example by interacting with neurotransmitter synthesis or release, with receptor affinity or density, or because of common thalamic input. Grey matter integrity is also a possible mediator, for example through local dendrite density or growth or, as suggested by Voineskos *et al.* (2011), oligodendrocytes within the cortical neuropil. The latter is compatible with the A-allele in rs1344706 creating a myelin transcription factor binding site (Riley *et al*., 2010; Voineskos *et al*., 2011), and with the association with regional variation in cortical thickness (Voineskos *et al*., 2011). *In vitro* and animal research into the molecular and cellular functions of *ZNF804A* should investigate the plausibility of such mechanisms.

In conclusion, we were unable to detect any effects of *ZNF804A* genotype on white matter integrity, in any of our three samples, using four different DTI analysis methods. Following this study the biological mechanisms of possible *ZNF804A* effects on functional connectivity remain unclear. After the paper by Voineskos and colleagues (2011) this is the second thorough investigation, using state-of-the-art imaging methods and larger sample sizes than most imaging studies, that has reported no association of *ZNF804A* with FA in the brains of healthy individuals. Therefore, any effects of variation in rs1344706 genotype on functional connectivity appear unlikely to be mediated by structural integrity differences in large, long-range connections.

Chapter 7

General conclusions and implications for the future

I applied DTI to a unique sample of 150 young, unaffected relatives of patients with BD and 109 well-matched healthy control participants to explore associations between genetic risk for BD and white matter structure in the human brain. First, the potential for white matter integrity as an endophenotype was considered by testing whether FA is reduced in unaffected relatives of patients with BD. After establishing this, I further investigated associations between FA and four of the best supported risk genes of BD - *NRG1*, *ErbB4*, *DISC1* and *ZNF804A* - which were selected because of known functions that could impact on white matter development or plasticity. The use of white matter as an endophenotype in this context can provide clues to the mechanisms by which these genes confer risk to BD, and can aid in generating new, testable hypotheses for molecular, cellular and imaging research in psychiatry. In this last chapter I will summarise the key findings of this thesis along with its limitations, and suggest some ideas for future research using white matter integrity as an endophenotype.

7.1. Summary of main findings

In our sample, unaffected relatives of patients with BD had subtle but widespread FA reductions, as consistently demonstrated using traditional voxel-based methods, and the more innovative techniques of TBSS and probabilistic tractography. This was the only criterion of endophenotypes (Gottesman and Gould, 2003) that remained thus far unconfirmed for white matter integrity. In line with previous suggestive findings of either white matter volume (Kieseppä, *et al*., 2003), or density (McIntosh *et al*., 2005) reductions in unaffected relatives or, less directly, associations with scalar measures of genetic liability (Chaddock *et al*., 2009; McDonald, Bullmore *et al*., 2004;McIntosh *et al*., 2006), the present results support white matter integrity as an endophenotype for BD. Although we could not confirm more pronounced reductions in patients themselves, as would be required to exclude the possibility of confounding genetic or environmental factors unrelated to BD *per se*, there is ample evidence from post-mortem and imaging research demonstrating white matter abnormalities in BD (as reviewed in

Chapter 1). This is also supported by an inverse correlation of cyclothymic temperament with FA in our own sample.

Having confirmed the association of white matter to genetic risk for BD in general, it is natural to wonder whether part of this association could be attributed to one or more specific risk variants. I investigated potential contributions of known risk-variants in *DISC1*, *ZNF804A*, *NRG1* and *ErbB4*, for their known roles in white matter development and plasticity and their previous associations with structural and/or functional connectivity in other samples. Modest associations were found between FA and genetic variation in *NRG1* and to a lesser extent the gene encoding its receptor, *ErbB4*. Although the effects of *NRG1* were most convincingly located in pre-hypothesised fronto-thalamic and fronto-limbic regions, confusingly, the variants most commonly associated with risk for psychiatric illness were associated with increased rather than reduced FA. Longitudinal studies in even larger samples are required to determine whether this could be a result of complex interactions with age or environmental factors, or is attributable variation elsewhere on the genome specific to our sub-population. A more striking result was a strong and robust association of FA with of variation in a non-synonymous *DISC1* SNP, rs821616. This effect was highly significant in both voxel-based and TBSS analyses and was widespread over the cerebral white matter. The tractography results were overall less pronounced, and confined to selected tracts including the AF, genu and cingulum bundles for the control group and the ATR and UF in the high risk group. This slight discrepancy between methods may be caused by differences in statistical sensitivity, although we cannot exclude possible misregistration effects in the voxel-wise analyses. Overall, however, these findings are in line with known neurodevelopmental functions of *DISC1* and encourage further research into its role in development and maintenance of white matter structure. Perhaps equally remarkable was the lack of association between *ZNF804A* and FA in any of the analysis conducted despite employing particularly lenient statistical thresholds and extra sensitive region-of-interest analysis. This lack of association was not attributable to insufficient statistical power and it strongly disagrees with a previously convincing, but untested, hypothesis which was based on a series of functional connectivity experiments (Esslinger *et al*., 2009; 2011). This negative finding has also been replicated in an independent German sample of healthy individuals (unpublished data).

A few remarks are required on phenomena observed more or less consistently across several results chapters. Firstly, both overall risk-status and *DISC1* appeared to affect white matter

integrity diffusely over the brain. Although much research has focused on *DISC1* expression in the hippocampus, *DISC1* is abundantly expressed across the brain (Schurov *et al*., 2004) and has numerous interactomes through which it can impact on a wide range of brain functions. Among these, axon guidance and myelination are the most likely mediators of the effects observed here, and these are not regionally specific. Hence, disruption in *DISC1* function may well have a similar effect on white matter across the cerebrum. A similar global effect is even more understandable for overall genetic risk, which reflects an increased likelihood of possessing *any* BD risk variants. The polygenic nature of BD means that the genomic location of this risk-associated variation should vary widely and randomly between unrelated individuals, which can logically lead to an average diffuse effect extending over the whole brain. Secondly, the effects of *DISC1*, *NRG1* and *ErbB4* were more pronounced in the control group than in the high risk group. Although this may be counter-intuitive, as discussed in the respective chapters, it could reasonably be a result of a floor effect in the high risk group, increased heterogeneity in the high risk group, or an increased impact of environmental factors that may obscure the subtle effects of single genetic variants. It should, however, be mentioned that imaging artefacts, such as those related to motion, could also be more common in the high risk group, and that this may account for reduced sensitivity to detect gene-effects.

All of the results presented here require replications in independent samples. Some because they are very novel (i.e. the effects of *DISC1*), yet robust against all kinds of post-hoc validations, and others because the results bring about more questions than answers (e.g. *NRG1/ErbB4*). Nevertheless, all results are consistent with the idea that white matter integrity plays an important role in genetic risk for BD, as is not only demonstrated by a direct contrast between unaffected relatives and control participants, but also supported by the cumulatively convincing evidence for associations with *DISC1*, *NRG1* and *ErbB4*.

7.2. Methodological remarks and limitations

With regard to methodology, a first noteworthy observation was the good correspondence between the voxel-based, TBSS and tractography results. Overall, the voxel-based results were somewhat less significant than the TBSS results, presumably reflecting the superior registration in the latter, and the less severe family-wise error-correction required when testing only the

voxels within the skeleton. It is slightly surprising that tractography results were also less pronounced than TBSS in most cases, despite the strict multiple testing corrections required for voxel-wise comparisons, and the assumed registration-related error. However, as noted elsewhere, TFCE could also increase sensitivity if the signal is of small effect but of large extent (i.e. spatially consistent). Although the correspondence between the methods greatly increases confidence in the current findings, it should be kept in mind that all analyses were based on the same dependent variable: FA. As reviewed in Chapter 2, DTI and derived measures such as FA are only an approximation of white matter structure and the scale at which they are measured limits biological interpretations of their variation. FA can be regarded a sensitive and reliable, albeit non-specific, measure of white matter integrity, but post-mortem animal studies are necessary to confirm the relatively crude imaging results and to distinguish properly between the various sources of variation in FA (i.e. myelination, axonal damage) and their causes (i.e. developmental or plasticity-related, and the role of oligodendrocytes).

The narrow age range in our sample is a strength since it means the sample is relatively uniform, as well as a limitation, as discussed in previous chapters. The age of our subjects could have influenced the results for two clinical reasons. On the one hand, a small proportion of the unaffected relatives is likely to develop BD after the assessment, although they are unlikely to have driven the present results given the absence of outliers and their small proportion. On the other hand, a few individuals were excluded because they were diagnosed with major depression or BD, but given their small number this is unlikely have skewed our sample to the extent that the remaining subjects have significantly more protective genetic variants. In addition, considering their known roles in brain development, the investigated genes are likely to interact with age, something that, because of the narrow age range, could not be properly addressed in the current sample. Follow-up assessments of our participants have recently been completed and further analyses of this data should clarify some of these issues. In addition, other studies have investigated particularly young children or adolescents (Versace *et al*., 2010; Frazier *et al*. 2007) or older participants (Chaddock *et al*., 2009), and integrating our results with those, it seems that genetic risk (as in familial risk) clearly interacts with white matter development, but mostly only up to the age of 17 years (Versace *et al*., 2010).

7.3. Implications for the future

In the early 20th century Carl Wernicke first suggested that disrupted connectivity may lie at the core of psychotic symptoms. More recently, the most comprehensive theories of psychosis, and in particular hallucinations, as well as affective disorders are based on similar ideas (Friston, 1999; Frsiton & Frith, 1995; Phillips *et al*., 2008; Strakowski *et al*., 2005). Despite this, in scientific research white matter has always been only of secondary interest to grey matter, as can be clearly deduced from systematic reviews and from a simple PubMed search. The recent availability of DTI has caused a surge in imaging studies focusing on white matter, and during this short period evidence for white matter involvement in psychosis has mounted to compelling heights. However, as mentioned, DTI on its own is never enough to confirm and fully understand subtle white matter pathology, and there remains a shortage of histological, animal, cellular and molecular studies focussing on white matter. For example, as mentioned in Chapter 1, all post-mortem studies of BD have only looked at cortical regions, even those that addressed myelin or glia pathology did not stain deep white matter. This lack of research in white matter is not only apparent in pathology, but also in basic neuroscience. For example, the mechanisms of synaptic plasticity in grey matter have been known since the 1960s (Lømo, 2003), but it has only recently emerged that similar activity-dependent structural changes can occur in white matter (Fields *et al*. 2008; Demerens *et al*. 1996). Thus, while it is slowly becoming clear that white matter is far more complex than just a set of static "wires" that passively conduct nerve impulses, a lot remains to be discovered before we can make better informed hypotheses about the mechanisms that cause its pathology.

Following from the present findings, white matter is arguably the best supported biological endophenotype for BD. Although it is presumably rather unspecific to BD *per se*, it could be an extremely helpful phenotype in animal studies, which do not have many superior alternatives when it comes to psychiatric research. Furthermore, as an endophenotype white matter integrity can be assumed to be somewhere midway between genetics and clinical phenotype, and can therefore be useful as a basis for initial hypotheses to test the biological function of novel genetic risk-variants.

As mentioned above, neurodevelopmental processes are assumed to be involved in BD, as compatible with the functions of many risk-genes. Longitudinal studies, incorporating wider age ranges, are required to explore gene-age interactions on brain structure, as recently demonstrated

by Versace *et al*. (2010). In particular, the expression patterns and functions of *DISC1* vary across developmental stages and for example it remains to be elucidated whether there are any critical time-windows for *DISC1* disruption to affect white matter structure, or whether *DISC1* influences myelination and/or axonal integrity in the mature brain, in addition to its known role in the developing brain.

Having established the potential of white matter integrity as an endophenotype, probably the most obvious next step is to use this quality in the discovery of new genetic variation and in the confirmation and functional understanding of known variants. In addition to the present efforts to increase understanding of *DISC1*, *NRG1*, *ErbB4* and *ZNF804A* function, we recently started using average FA extracted from the TBSS skeleton as a phenotype in a GWAS in our own dataset. However preliminary given our small dataset, the results sensibly confirm the over-representation of relevant gene-ontology categories and nominal effects of known psychosis-risk genes (unpublished data), which is more than what has been achieved in many case-control GWAS in much larger samples. Nevertheless, no SNP or gene reached genome-wide significance and larger datasets are required to conduct an adequately powered white matter GWAS.

7.4. General conclusion

In our sample, subtle but widespread white matter integrity reductions were present in 150 young, unaffected relatives of patients with BD compared to 109 well-matched healthy control participants, in support of white matter integrity as an endophenotype of BD. This is also supported and further extended by a striking association of white matter integrity with a non-synonymous *DISC1* SNP and more modest associations with genetic variation in *NRG1* and *ErbB4*. Together, the results provide compelling evidence for white matter structure as an endophenotype for BD, and encourage the use of white matter integrity as an endophenotype in genetic research and animal studies.

References

Addington, A. M., Gornick, M. C., Shaw, P., Seal, J., Gogtay, N., Greenstein, D., Clasen, L., et al. (2007). Neuregulin 1 (8p12) and childhood-onset schizophrenia: susceptibility haplotypes for diagnosis and brain developmental trajectories. *Molecular Psychiatry*, *12*(2), 195-205.

Adler, C. M., Adams, J., DelBello, M. P., Holland, S. K., Schmithorst, V., Levine, A., Jarvis, K., & Strakowski, S. M. (2006a). Evidence of white matter pathology in bipolar disorder adolescents experiencing their first episode of mania: a diffusion tensor imaging study. *The American Journal of Psychiatry*, *163*(2), 322-324.

Adler, C. M., Holland, S. K., Schmithorst, V., Wilke, M., Weiss, K. L., Pan, H., & Strakowski, S. M. (2004). Abnormal frontal white matter tracts in bipolar disorder: a diffusion tensor imaging study. *Bipolar Disorders*, *6*(3), 197-203.

Akiskal, H. S., Djenderedjian, A. M., Rosenthal, R. H., & Khani, M. K. (1977). Cyclothymic disorder: validating criteria for inclusion in the bipolar affective group. *The American Journal of Psychiatry*, *134*(11), 1227-1233.

Akiskal, H. S., Mendlowicz, M. V., Jean-Louis, G., Rapaport, M. H., Kelsoe, J. R., Gillin, J. C., & Smith, T. L. (2005). TEMPS-A: validation of a short version of a self-rated instrument designed to measure variations in temperament. *Journal of Affective Disorders*, *85*(1-2), 45-52.

Akiskal, H. S., Rosenthal, R. H., Rosenthal, T. L., Kashgarian, M., Khani, M. K., & Puzantian, V. R. (1979). Differentiation of primary affective illness from situational, symptomatic, and secondary depressions. *Archives of General Psychiatry*, *36*(6), 635-643.

Akiskal, K. K., & Akiskal, H. S. (2005). The theoretical underpinnings of affective temperaments: implications for evolutionary foundations of bipolar disorder and human nature. *Journal of Affective Disorders*, *85*(1-2), 231-239.

Alaerts, M., Ceulemans, S., Forero, D., Moens, L. N., De Zutter, S., Heyrman, L., Lenaerts, A.-S., et al. (2009). Support for NRG1 as a susceptibility factor for schizophrenia in a northern Swedish isolated population. *Archives of General Psychiatry*, *66*(8), 828-837.

Anand, A., Li, Y., Wang, Y., Lowe, M. J., & Dzemidzic, M. (2009). Resting state corticolimbic connectivity abnormalities in unmedicated bipolar disorder and unipolar depression. *Psychiatry Research*, *171*(3), 189-198.

Anton, E. S., Marchionni, M. A., Lee, K. F., & Rakic, P. (1997). Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. *Development*, *124*(18), 3501-3510.

Arnone, D., Cavanagh, J., Gerber, D., Lawrie, S. M., Ebmeier, K. P., & McIntosh, A. M. (2009). Magnetic resonance imaging studies in bipolar disorder and schizophrenia: meta-analysis. *The British Journal of Psychiatry*, *195*(3), 194-201.

Badner, J. A., & Gershon, E. S. (2002). Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Molecular Psychiatry*, *7*(4), 405-411.

Bakker, S. C., Hoogendoorn, M. L. C., Selten, J.-P., Verduijn, W., Pearson, P. L., Sinke, R. J., & Kahn, R. S. (2004). Neuregulin 1: genetic support for schizophrenia subtypes. *Molecular Psychiatry*, *9*(12), 1061-1063.

Barnea-Goraly, N., Chang, K. D., Karchemskiy, A., Howe, M. E., & Reiss, A. L. (2009). Limbic and corpus callosum aberrations in adolescents with bipolar disorder: a tract-based spatial statistics analysis. *Biological Psychiatry*, *66*(3), 238-244.

Barnett, J. H., & Smoller, J. W. (2009). The genetics of bipolar disorder. *Neuroscience*, *164*(1), 331-343

Baron, M. (2002). Manic-depression genes and the new millennium: poised for discovery. *Molecular Psychiatry*, *7*(4), 342-358.

Baum, A. E., Akula, N., Cabanero, M., Cardona, I., Corona, W., Klemens, B., Schulze, T. G., et al. (2008). A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. *Molecular Psychiatry*, *13*(2), 197-207.

Baum, A. E., Hamshere, M., Green, E., Cichon, S., Rietschel, M., Noethen, M. M., Craddock, N., et al. (2008). Meta-analysis of two genome-wide association studies of bipolar disorder reveals important points of agreement. *Molecular Psychiatry*, *13*(5), 466-467.

Bava, S., Thayer, R., Jacobus, J., Ward, M., Jernigan, T. L., & Tapert, S. F. (2010). Longitudinal characterization of white matter maturation during adolescence. *Brain Research*, *1327*, 38-46.

Behrens, T. E. J., Berg, H. J., Jbabdi, S., Rushworth, M. F. S., & Woolrich, M. W. (2007). Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? *NeuroImage*, *34*(1), 144-155.

Behrens, T. E. J., Woolrich, M. W., Jenkinson, M., Johansen-Berg, H., Nunes, R. G., Clare, S., Matthews, P. M., et al. (2003). Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magnetic Resonance in Medicine*, *50*(5), 1077-1088.

Benedetti, F., Yeh, P.-H., Bellani, M., Radaelli, D., Nicoletti, M. A., Poletti, S., Falini, A., et al. (2010). Disruption of White Matter Integrity in Bipolar Depression as a Possible Structural Marker of Illness. *Biological Psychiatry*.

Beyer, J. L., Young, R., Kuchibhatla, M., & Krishnan, K. R. R. (2009). Hyperintense MRI lesions in bipolar disorder: A meta-analysis and review. *International Review of Psychiatry (Abingdon, England)*, *21*(4), 394-409.

Bienvenu, O. J., Davydow, D. S., & Kendler, K. S. (2011). Psychiatric “diseases” versus behavioral disorders and degree of genetic influence. *Psychological Medicine*, *41*(1), 33-40.

Blackwood, D. H., Fordyce, A., Walker, M. T., St Clair, D. M., Porteous, D. J., & Muir, W. J. (2001). Schizophrenia and affective disorders--cosegregation with a translocation at chromosome 1q42

that directly disrupts brain-expressed genes: clinical and P300 findings in a family. *American Journal of Human Genetics*, *69*(2), 428-433.

Blum, B. P., & Mann, J. J. (2002). The GABAergic system in schizophrenia. *The International Journal of Neuropsychopharmacology*, *5*(2), 159-179.

Brandon, N. J., Millar, J. K., Korth, C., Sive, H., Singh, K. K., & Sawa, A. (2009). Understanding the role of DISC1 in psychiatric disease and during normal development. *The Journal of Neuroscience*, *29*(41), 12768-12775.

Bruno, S. D., Barker, G. J., Cercignani, M., Symms, M., & Ron, M. A. (2004). A study of bipolar disorder using magnetization transfer imaging and voxel-based morphometry. *Brain: A Journal of Neurology*, *127*(Pt 11), 2433-2440.

Bruno, S., Cercignani, M., & Ron, M. A. (2008). White matter abnormalities in bipolar disorder: a voxel-based diffusion tensor imaging study. *Bipolar Disorders*, *10*(4), 460-468.

Budde, M. D., Kim, J. H., Liang, H.-F., Schmidt, R. E., Russell, J. H., Cross, A. H., & Song, S.-K. (2007). Toward accurate diagnosis of white matter pathology using diffusion tensor imaging. *Magnetic Resonance in Medicine*, *57*(4), 688-695.

Bullmore, E. T., Suckling, J., Overmeyer, S., Rabe-Hesketh, S., Taylor, E., & Brammer, M. J. (1999). Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Transactions on Medical Imaging*, *18*(1), 32-42.

Burns, J., Job, D., Bastin, M. E., Whalley, H., Macgillivray, T., Johnstone, E. C., & Lawrie, S. M. (2003). Structural disconnectivity in schizophrenia: a diffusion tensor magnetic resonance imaging study. *The British Journal of Psychiatry*, *182*, 439-443.

Callicott, J. H., Straub, R. E., Pezawas, L., Egan, M. F., Mattay, V. S., Hariri, A. R., Verchinski, B. A., et al. (2005). Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(24), 8627-8632.

Cardno, A. G., Rijsdijk, F. V., Sham, P. C., Murray, R. M., & McGuffin, P. (2002). A twin study of genetic relationships between psychotic symptoms. *The American Journal of Psychiatry*, *159*(4), 539-545.

Cassidy, F., Roche, S., Claffey, E., & McKeon, P. (2006). First family-based test for association of neuregulin with bipolar affective disorder. *Molecular Psychiatry*, *11*(8), 706-707.

Cercignani, M., Inglese, M., Pagani, E., Comi, G., & Filippi, M. (2001). Mean diffusivity and fractional anisotropy histograms of patients with multiple sclerosis. *AJNR. American Journal of Neuroradiology*, *22*(5), 952-958.

Chaddock, C. A., Barker, G. J., Marshall, N., Schulze, K., Hall, M. H., Fern, A., Walshe, M., et al. (2009). White matter microstructural impairments and genetic liability to familial bipolar I disorder. *The British Journal of Psychiatry*, *194*(6), 527-534.

Chakirova, G., Whalley, H. C., Thomson, P. A., Hennah, W., Moorhead, T. W. J., Welch, K. A., Giles, S., et al. (2011). The effects of DISC1 risk variants on brain activation in controls, patients with bipolar disorder and patients with schizophrenia. *Psychiatry Research*, *192*(1), 20-28.

Chen, H. H., Nicoletti, M. A., Hatch, J. P., Sassi, R. B., Axelson, D., Brambilla, P., Monkul, E. S., et al. (2004). Abnormal left superior temporal gyrus volumes in children and adolescents with bipolar disorder: a magnetic resonance imaging study. *Neuroscience Letters*, *363*(1), 65-68.

Chen, Q.-Y., Chen, Q., Feng, G.-Y., Lindpaintner, K., Wang, L.-J., Chen, Z.-X., Gao, Z.-S., et al. (2007). Case-control association study of Disrupted-in-Schizophrenia-1 (DISC1) gene and schizophrenia in the Chinese population. *Journal of Psychiatric Research*, *41*(5), 428-434.

Chen, S., Velardez, M. O., Warot, X., Yu, Z.-X., Miller, S. J., Cros, D., & Corfas, G. (2006). Neuregulin 1-erbB signaling is necessary for normal myelination and sensory function. *The Journal of Neuroscience*, *26*(12), 3079-3086.

Chiang, M.-C., Barysheva, M., Shattuck, D. W., Lee, A. D., Madsen, S. K., Avedissian, C., Klunder, A. D., et al. (2009). Genetics of brain fiber architecture and intellectual performance. *The Journal of Neuroscience*, *29*(7), 2212-2224.

Chiang, M.-C., Barysheva, M., Toga, A. W., Medland, S. E., Hansell, N. K., James, M. R., McMahon, K. L., et al. (2011). BDNF gene effects on brain circuitry replicated in 455 twins. *NeuroImage*, *55*(2), 448-454.

Chiang, M.-C., McMahon, K. L., de Zubicaray, G. I., Martin, N. G., Hickie, I., Toga, A. W., Wright, M. J., et al. (2011). Genetics of white matter development: a DTI study of 705 twins and their siblings aged 12 to 29. *NeuroImage*, *54*(3), 2308-2317.

Chiaroni, P., Hantouche, E.-G., Gouvernet, J., Azorin, J.-M., & Akiskal, H. S. (2005). The cyclothymic temperament in healthy controls and familially at risk individuals for mood disorder: endophenotype for genetic studies? *Journal of Affective Disorders*, *85*(1-2), 135-145.

Chubb, J. E., Bradshaw, N. J., Soares, D. C., Porteous, D. J., & Millar, J. K. (2007). The DISC locus in psychiatric illness. *Molecular Psychiatry*, *13*(1), 36-64.

Clapcote, S. J., Lipina, T. V., Millar, J. K., Mackie, S., Christie, S., Ogawa, F., Lerch, J. P., et al. (2007). Behavioral phenotypes of Disc1 missense mutations in mice. *Neuron*, *54*(3), 387-402.

Clayden, J. D., Bastin, M. E., & Storkey, A. J. (2006). Improved segmentation reproducibility in group tractography using a quantitative tract similarity measure. *NeuroImage*, *33*(2), 482-492.

Clayden, J. D., Storkey, A. J., Muñoz Maniega, S., & Bastin, M. E. (2009). Reproducibility of tract segmentation between sessions using an unsupervised modelling-based approach. *NeuroImage*, *45*(2), 377-385.

Corfas, G., Roy, K., & Buxbaum, J. D. (2004). Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. *Nature Neuroscience*, *7*(6), 575-580.

Corvin, A. P., Morris, D. W., McGhee, K., Schwaiger, S., Scully, P., Quinn, J., Meagher, D., et al. (2004). Confirmation and refinement of an "at-risk" haplotype for schizophrenia suggests the EST cluster, Hs.97362, as a potential susceptibility gene at the Neuregulin-1 locus. *Molecular Psychiatry*, *9*(2), 208-213.

Craddock, N., & Sklar, P. (2009). Genetics of bipolar disorder: successful start to a long journey. *Trends in Genetics: TIG*, *25*(2), 99-105.

Craddock, N., O'Donovan, M. C., & Owen, M. J. (2006). Genes for schizophrenia and bipolar disorder? Implications for psychiatric nosology. *Schizophrenia Bulletin*, *32*(1), 9-16.

Craddock, N., O'Donovan, M. C., & Owen, M. J. (2009). Psychosis genetics: modeling the relationship between schizophrenia, bipolar disorder, and mixed (or "schizoaffective") psychoses. *Schizophrenia Bulletin*, *35*(3), 482-490.

DeBoy, C. A., Zhang, J., Dike, S., Shats, I., Jones, M., Reich, D. S., Mori, S., et al. (2007). High resolution diffusion tensor imaging of axonal damage in focal inflammatory and demyelinating lesions in rat spinal cord. *Brain*, *130*(Pt 8), 2199-2210.

Demerens, C., Stankoff, B., Logak, M., Anglade, P., Allinquant, B., Couraud, F., Zalc, B., et al. (1996). Induction of myelination in the central nervous system by electrical activity. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(18), 9887-9892.

Devon, R. S., Anderson, S., Teague, P. W., Burgess, P., Kipari, T. M., Semple, C. A., Millar, J. K., et al. (2001). Identification of polymorphisms within Disrupted in Schizophrenia 1 and Disrupted in Schizophrenia 2, and an investigation of their association with schizophrenia and bipolar affective disorder. *Psychiatric Genetics*, *11*(2), 71-78.

Domercq, M., Alberdi, E., Sánchez-Gómez, M. V., Ariz, U., Pérez-Samartín, A., & Matute, C. (2011). Dual-specific phosphatase-6 (Dusp6) and ERK mediate AMPA receptor-induced oligodendrocyte death. *The Journal of Biological Chemistry*, *286*(13), 11825-11836.

Donohoe, G., Morris, D. W., & Corvin, A. (2010). The psychosis susceptibility gene ZNF804A: associations, functions, and phenotypes. *Schizophrenia Bulletin*, *36*(5), 904-909.

Einat, H., Yuan, P., Gould, T. D., Li, J., Du, J., Zhang, L., Manji, H. K., et al. (2003). The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. *The Journal of Neuroscience*, *23*(19), 7311-7316.

Engel, S. R., Creson, T. K., Hao, Y., Shen, Y., Maeng, S., Nekrasova, T., Landreth, G. E., et al. (2009). The extracellular signal-regulated kinase pathway contributes to the control of behavioral excitement. *Molecular Psychiatry*, *14*(4), 448-461.

Esslinger, C., Kirsch, P., Haddad, L., Mier, D., Sauer, C., Erk, S., Schnell, K., et al. (2011). Cognitive state and connectivity effects of the genome-wide significant psychosis variant in ZNF804A. *NeuroImage*, *54*(3), 2514-2523.

Esslinger, C., Walter, H., Kirsch, P., Erk, S., Schnell, K., Arnold, C., Haddad, L., et al. (2009). Neural mechanisms of a genome-wide supported psychosis variant. *Science*, *324*(5927), 605.

Evans, D. M., Visscher, P. M., & Wray, N. R. (2009). Harnessing the information contained within genome-wide association studies to improve individual prediction of complex disease risk. *Human Molecular Genetics*, *18*(18), 3525-3531.

Ferreira, M. A. R., O'Donovan, M. C., Meng, Y. A., Jones, I. R., Ruderfer, D. M., Jones, L., Fan, J., et al. (2008). Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nature Genetics*, *40*(9), 1056-1058.

Fields, R. D. (2008). White matter in learning, cognition and psychiatric disorders. *Trends in Neurosciences*, *31*(7), 361-370.

Forbes, N. F., Carrick, L. A., McIntosh, A. M., & Lawrie, S. M. (2009). Working memory in schizophrenia: a meta-analysis. *Psychological Medicine*, *39*(6), 889-905.

Frazier, J. A., Breeze, J. L., Papadimitriou, G., Kennedy, D. N., Hodge, S. M., Moore, C. M., Howard, J. D., et al. (2007). White matter abnormalities in children with and at risk for bipolar disorder. *Bipolar Disorders*, *9*(8), 799-809.

Friston, K. J. (1999). Schizophrenia and the disconnection hypothesis. *Acta Psychiatrica Scandinavica. Supplementum*, *395*, 68-79.

Friston, K. J., & Frith, C. D. (1995). Schizophrenia: a disconnection syndrome? *Clinical Neuroscience*, *3*(2), 89-97.

Friston, K. J., Worsley, K. J., Frackowiak, R. S. J., Mazziotta, J. C., & Evans, A. C. (1993). Assessing the significance of focal activations using their spatial extent. *Human Brain Mapping*, *1*(3), 210-220.

Geddes, J. R., Burgess, S., Hawton, K., Jamison, K., & Goodwin, G. M. (2004). Long-term lithium therapy for bipolar disorder: systematic review and meta-analysis of randomized controlled trials. *The American Journal of Psychiatry*, *161*(2), 217-222.

Georgieva, L., Dimitrova, A., Ivanov, D., Nikolov, I., Williams, N. M., Grozeva, D., Zaharieva, I., et al. (2008). Support for neuregulin 1 as a susceptibility gene for bipolar disorder and schizophrenia. *Biological Psychiatry*, *64*(5), 419-427.

Gershon, E. S., Hamovit, J., Guroff, J. J., Dibble, E., Leckman, J. F., Sceery, W., Targum, S. D., et al. (1982). A Family Study of Schizoaffective, Bipolar I, Bipolar II, Unipolar, and Normal Control Probands. *Archives of General Psychiatry*, *39*(10), 1157-1167.

Di Giorgio, A., Blasi, G., Sambataro, F., Rampino, A., Papazacharias, A., Gambi, F., Romano, R., et al. (2008). Association of the SerCys DISC1 polymorphism with human hippocampal formation gray matter and function during memory encoding. *The European Journal of Neuroscience*, *28*(10), 2129-2136.

Di Giorgio, A., Watkins, K. E., Chadwick, M., James, S., Winmill, L., Douaud, G., De Stefano, N., et al. (2010). Longitudinal changes in grey and white matter during adolescence. *NeuroImage*, *49*(1), 94-103.

Glahn, D. C., Thompson, P. M., & Blangero, J. (2007). Neuroimaging endophenotypes: strategies for finding genes influencing brain structure and function. *Human Brain Mapping*, *28*(6), 488-501.

Glahn, D. C., Winkler, A. M., Kochunov, P., Almasy, L., Duggirala, R., Carless, M. A., Curran, J. C., et al. (2010). Genetic control over the resting brain. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(3), 1223-1228.

Goes, F. S., Willour, V. L., Zandi, P. P., Belmonte, P. L., MacKinnon, D. F., Mondimore, F. M., Schweizer, B., et al. (2009). Family-based association study of Neuregulin 1 with psychotic bipolar disorder. *American Journal of Medical Genetics*, *150B*(5), 693-702.

Gong, Y. G., Wu, C. N., Xing, Q. H., Zhao, X. Z., Zhu, J., & He, L. (2009). A two-method meta-analysis of Neuregulin 1(NRG1) association and heterogeneity in schizophrenia. *Schizophrenia Research*, *111*(1-3), 109-114.

Gottesman, I. I., & Gould, T. D. (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *The American Journal of Psychiatry*, *160*(4), 636-645.

Gouw, A. A., Seewann, A., Vrenken, H., van der Flier, W. M., Rozemuller, J. M., Barkhof, F., Scheltens, P., et al. (2008). Heterogeneity of white matter hyperintensities in Alzheimer's disease: post-mortem quantitative MRI and neuropathology. *Brain: A Journal of Neurology*, *131*(Pt 12), 3286-3298.

Green, E. K., Raybould, R., Macgregor, S., Gordon-Smith, K., Heron, J., Hyde, S., Grozeva, D., et al. (2005). Operation of the schizophrenia susceptibility gene, neuregulin 1, across traditional diagnostic boundaries to increase risk for bipolar disorder. *Archives of General Psychiatry*, *62*(6), 642-648.

Gruber, O., Falkai, P., Schneider-Axmann, T., Schwab, S. G., Wagner, M., & Maier, W. (2008). Neuregulin-1 haplotype HAP(ICE) is associated with lower hippocampal volumes in schizophrenic patients and in non-affected family members. *Journal of Psychiatric Research*, *43*(1), 1-6.

Gu, Z., Jiang, Q., Fu, A. K. Y., Ip, N. Y., & Yan, Z. (2005). Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. *The Journal of Neuroscience*, *25*(20), 4974-4984.

Hahn, C.-G., Wang, H.-Y., Cho, D.-S., Talbot, K., Gur, R. E., Berrettini, W. H., Bakshi, K., et al. (2006). Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. *Nature Medicine*, *12*(7), 824-828.

Hakak, Y., Walker, J. R., Li, C., Wong, W. H., Davis, K. L., Buxbaum, J. D., Haroutunian, V., et al. (2001). Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(8), 4746-4751.

Hall, J., Whalley, H. C., Job, D. E., Baig, B. J., McIntosh, A. M., Evans, K. L., Thomson, P. A., et al. (2006). A neuregulin 1 variant associated with abnormal cortical function and psychotic symptoms. *Nature Neuroscience*, *9*(12), 1477-1478.

Hallahan, B., Newell, J., Soares, J. C., Brambilla, P., Strakowski, S. M., Fleck, D. E., Kieseppä, T., et al. (2010). Structural Magnetic Resonance Imaging in Bipolar Disorder: An International Collaborative Mega-Analysis of Individual Adult Patient Data. *Biological Psychiatry*.

Harrison, P. J., & Law, A. J. (2006). Neuregulin 1 and schizophrenia: genetics, gene expression, and neurobiology. *Biological Psychiatry*, *60*(2), 132-140.

Harrison, P. J., & Weinberger, D. R. (2005). Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Molecular Psychiatry*, *10*(1), 40-68; image 5.

Hasan, K. M., Alexander, A. L., & Narayana, P. A. (2004). Does fractional anisotropy have better noise immunity characteristics than relative anisotropy in diffusion tensor MRI? An analytical approach. *Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, *51*(2), 413-417.

Hashimoto, R., Numakawa, T., Ohnishi, T., Kumamaru, E., Yagasaki, Y., Ishimoto, T., Mori, T., et al. (2006). Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. *Human Molecular Genetics*, *15*(20), 3024-3033.

Hashimoto, R., Ohi, K., Yasuda, Y., Fukumoto, M., Iwase, M., Iike, N., Azechi, M., et al. (2010). The impact of a genome-wide supported psychosis variant in the ZNF804A gene on memory function in schizophrenia. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, *153B*(8), 1459-1464.

Hasler, G. (2006). Evaluating endophenotypes for psychiatric disorders. *Revista Brasileira De Psiquiatria (São Paulo, Brazil: 1999)*, *28*(2), 91-92.

Hayasaka, S., & Nichols, T. E. (2003). Validating cluster size inference: random field and permutation methods. *NeuroImage*, *20*(4), 2343-2356.

Hayasaka, S., Peiffer, A. M., Hugenschmidt, C. E., & Laurienti, P. J. (2007). Power and sample size calculation for neuroimaging studies by non-central random field theory. *NeuroImage*, *37*(3), 721-730.

Hayasaka, S., Phan, K. L., Liberzon, I., Worsley, K. J., & Nichols, T. E. (2004). Nonstationary cluster-size inference with random field and permutation methods. *NeuroImage*, *22*(2), 676-687.

Heng, S., Song, A. W., & Sim, K. (2010). White matter abnormalities in bipolar disorder: insights from diffusion tensor imaging studies. *Journal of Neural Transmission (Vienna, Austria: 1996)*, *117*(5), 639-654.

Hennah, W., Thomson, P., McQuillin, A., Bass, N., Loukola, A., Anjorin, A., Blackwood, D., et al. (2009). DISC1 association, heterogeneity and interplay in schizophrenia and bipolar disorder. *Molecular Psychiatry*, *14*(9), 865-873.

Hoenig, J. M., & Heisey, D. M. (2001). The Abuse of Power. *The American Statistician*, *55*(1), 19-24.

Houenou, J., Wessa, M., Douaud, G., Leboyer, M., Chanraud, S., Perrin, M., Poupon, C., et al. (2007). Increased white matter connectivity in euthymic bipolar patients: diffusion tensor tractography between the subgenual cingulate and the amygdalo-hippocampal complex. *Molecular Psychiatry*, *12*(11), 1001-1010.

Hur, E.-M., & Zhou, F.-Q. (2010). GSK3 signalling in neural development. *Nature Reviews. Neuroscience*, *11*(8), 539-551.

Ikeda, M., Takahashi, N., Saito, S., Aleksic, B., Watanabe, Y., Nunokawa, A., Yamanouchi, Y., et al. (2008). Failure to replicate the association between NRG1 and schizophrenia using Japanese large sample. *Schizophrenia Research*, *101*(1-3), 1-8.

Ishizuka, K., Kamiya, A., Oh, E. C., Kanki, H., Seshadri, S., Robinson, J. F., Murdoch, H., et al. (2011). DISC1-dependent switch from progenitor proliferation to migration in the developing cortex. *Nature*, *473*(7345), 92-96.

Iwata, N., Suzuki, T., Ikeda, M., Kitajima, T., Yamanouchi, Y., Inada, T., & Ozaki, N. (2004). No association with the neuregulin 1 haplotype to Japanese schizophrenia. *Molecular Psychiatry*, *9*(2), 126-127.

Jenkinson, M., & Smith, S. (2001). A global optimisation method for robust affine registration of brain images. *Medical Image Analysis*, *5*(2), 143-156.

Johansen-Berg, H., Behrens, T. E. J., Sillery, E., Ciccarelli, O., Thompson, A. J., Smith, S. M., & Matthews, P. M. (2005). Functional-anatomical validation and individual variation of diffusion tractography-based segmentation of the human thalamus. *Cerebral Cortex (New York, N.Y.: 1991)*, *15*(1), 31-39.

Jones, D. K., Symms, M. R., Cercignani, M., & Howard, R. J. (2005). The effect of filter size on VBM analyses of DT-MRI data. *NeuroImage*, *26*(2), 546-554.

Kamiya, A., Kubo, K.-ichiro, Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., Sawamura, N., et al. (2005). A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nature Cell Biology*, *7*(12), 1167-1178.

Kawashima, T., Nakamura, M., Bouix, S., Kubicki, M., Salisbury, D. F., Westin, C.-F., McCarley, R. W., et al. (2009). Uncinate fasciculus abnormalities in recent onset schizophrenia and affective psychosis: a diffusion tensor imaging study. *Schizophrenia Research*, *110*(1-3), 119-126.

Kempton, M. J., Geddes, J. R., Ettinger, U., Williams, S. C. R., & Grasby, P. M. (2008). Meta-analysis, database, and meta-regression of 98 structural imaging studies in bipolar disorder. *Archives of General Psychiatry*, *65*(9), 1017-1032.

Kéri, S., Kiss, I., & Kelemen, O. (2009). Effects of a neuregulin 1 variant on conversion to schizophrenia and schizophreniform disorder in people at high risk for psychosis. *Molecular Psychiatry*, *14*(2), 118-119.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, *62*(6), 593-602.

Kieseppä, T., van Erp, T. G. M., Haukka, J., Partonen, T., Cannon, T. D., Poutanen, V.-P., Kaprio, J., et al. (2003). Reduced left hemispheric white matter volume in twins with bipolar I disorder. *Biological Psychiatry*, *54*(9), 896-905.

Kim, H.-J., Park, H. J., Jung, K. H., Ban, J. Y., Ra, J., Kim, J. W., Park, J. K., et al. (2008). Association study of polymorphisms between DISC1 and schizophrenia in a Korean population. *Neuroscience Letters*, *430*(1), 60-63

Kim, J. H., Budde, M. D., Liang, H.-F., Klein, R. S., Russell, J. H., Cross, A. H., & Song, S.-K. (2006). Detecting axon damage in spinal cord from a mouse model of multiple sclerosis. *Neurobiology of Disease*, *21*(3), 626-632.

Kim, J. W., Lee, Y.-S., Cho, E.-Y., Jang, Y. L., Park, D. Y., Choi, K.-S., Jeun, H. O., et al. (2006). Linkage and association of schizophrenia with genetic variations in the locus of neuregulin 1 in Korean population. *American Journal of Medical Genetics*, *141B*(3), 281-286.

Klawiter, E. C., Schmidt, R. E., Trinkaus, K., Liang, H.-F., Budde, M. D., Naismith, R. T., Song, S.-K., et al. (2011). Radial diffusivity predicts demyelination in ex vivo multiple sclerosis spinal cords. *NeuroImage*, *55*(4), 1454-1460.

Kochunov, P., Glahn, D. C., Lancaster, J. L., Winkler, A. M., Smith, S., Thompson, P. M., Almasy, L., Duggirala, R., Fox, P. T., & Blangero, J. (2010). Genetics of microstructure of cerebral white matter using diffusion tensor imaging. *NeuroImage*, *53*(3), 1109-1116.

Konrad, A., Vucurevic, G., Musso, F., Stoeter, P., Dahmen, N., & Winterer, G. (2009a). ErbB4 genotype predicts left frontotemporal structural connectivity in human brain. *Neuropsychopharmacology, 34*(3), 641-650.

Lachman, H. M., Pedrosa, E., Nolan, K. A., Glass, M., Ye, K., & Saito, T. (2006). Analysis of polymorphisms in AT-rich domains of neuregulin 1 gene in schizophrenia. *American Journal of Medical Genetics*, *141B*(1), 102-109.

Law, A. J., Kleinman, J. E., Weinberger, D. R., & Weickert, C. S. (2007). Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. *Human Molecular Genetics*, *16*(2), 129-141.

Law, A. J., Lipska, B. K., Weickert, C. S., Hyde, T. M., Straub, R. E., Hashimoto, R., Harrison, P. J., et al. (2006). Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5' SNPs associated with the disease. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(17), 6747-6752.

Lebel, C., Walker, L., Leemans, A., Phillips, L., & Beaulieu, C. (2008). Microstructural maturation of the human brain from childhood to adulthood. *NeuroImage*, *40*(3), 1044-1055.

Lee, S. H., Wray, N. R., Goddard, M. E., & Visscher, P. M. (2011). Estimating missing heritability for disease from genome-wide association studies. *American Journal of Human Genetics*, *88*(3), 294-305.

Lencz, T., Szeszko, P. R., DeRosse, P., Burdick, K. E., Bromet, E. J., Bilder, R. M., & Malhotra, A. K. (2010). A schizophrenia risk gene, ZNF804A, influences neuroanatomical and neurocognitive phenotypes. *Neuropsychopharmacology*, *35*(11), 2284-2291.

Lepagnol-Bestel, A.-M., Dubertret, C., Benmessaoud, D., Simonneau, M., Adès, J., Kacha, F., Hamdani, N., et al. (2010). Association of DISC1 gene with schizophrenia in families from two distinct French and Algerian populations. *Psychiatric Genetics*, *20*(6), 298-303.

Lewandowski, K. E., Cohen, B. M., & Ongur, D. (2011). Evolution of neuropsychological dysfunction during the course of schizophrenia and bipolar disorder. *Psychological Medicine*, *41*(2), 225-241.

Li, D., Collier, D. A., & He, L. (2006). Meta-analysis shows strong positive association of the neuregulin 1 (NRG1) gene with schizophrenia. *Human Molecular Genetics*, *15*(12), 1995-2002.

Li, J., Li, X.-Y., Feng, D.-F., & Gu, L. (2011). Quantitative evaluation of microscopic injury with diffusion tensor imaging in a rat model of diffuse axonal injury. *The European Journal of Neuroscience*, *33*(5), 933-945.

Lichtenstein, P., Yip, B. H., Björk, C., Pawitan, Y., Cannon, T. D., Sullivan, P. F., & Hultman, C. M. (2009). Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. *Lancet*, *373*(9659), 234-239.

Lin, F., Weng, S., Xie, B., Wu, G., & Lei, H. (2011). Abnormal frontal cortex white matter connections in bipolar disorder: a DTI tractography study. *Journal of Affective Disorders*, *131*(1-3), 299-306.

Liu, B, Li, J, Yu, C, Li, Y, Liu, Y, Song, M, Fan, M, et al. (2010). Haplotypes of catechol-O-methyltransferase modulate intelligence-related brain white matter integrity. *NeuroImage*, *50*(1), 243-249.

Liu, Y., Blackwood, D. H., Caesar, S., de Geus, E. J. C., Farmer, A., Ferreira, M. A. R., Ferrier, I. N., et al. (2011). Meta-analysis of genome-wide association data of bipolar disorder and major depressive disorder. *Molecular Psychiatry*, *16*(1), 2-4.

Lømo, T. (2003). The discovery of long-term potentiation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *358*(1432), 617-620.

López-Bendito, G., Cautinat, A., Sánchez, J. A., Bielle, F., Flames, N., Garratt, A. N., Talmage, D. A., et al. (2006). Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. *Cell*, *125*(1), 127-142.

Lu, C.-L., Wang, Y.-C., Chen, J.-Y., Lai, I.-C., & Liou, Y.-J. (2010). Support for the involvement of the ERBB4 gene in schizophrenia: a genetic association analysis. *Neuroscience Letters*, *481*(2), 120-125.

Mac Donald, C. L., Dikranian, K., Bayly, P., Holtzman, D., & Brody, D. (2007). Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. *The Journal of Neuroscience*, *27*(44), 11869-11876.

Mahon, K., Burdick, K. E., & Szeszko, P. R. (2010). A role for white matter abnormalities in the pathophysiology of bipolar disorder. *Neuroscience and Biobehavioral Reviews*, *34*(4), 533-554.

Manji, H. K., & Lenox, R. H. (1999). Ziskind-Somerfeld Research Award. Protein kinase C signaling in the brain: molecular transduction of mood stabilization in the treatment of manic-depressive illness. *Biological Psychiatry*, *46*(10), 1328-1351.

Mao, Y., Ge, X., Frank, C. L., Madison, J. M., Koehler, A. N., Doud, M. K., Tassa, C., et al. (2009). Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. *Cell*, *136*(6), 1017-1031.

Matsumoto, S., Akiyama, T., Tsuda, H., Miyake, Y., Kawamura, Y., Noda, T., Akiskal, K. K., et al. (2005). Reliability and validity of TEMPS-A in a Japanese non-clinical population: application to unipolar and bipolar depressives. *Journal of Affective Disorders*, *85*(1-2), 85-92.

Mazzarini, L., Pacchiarotti, I., Colom, F., Sani, G., Kotzalidis, G. D., Rosa, A. R., Sanna, L., et al. (2009). Predominant polarity and temperament in bipolar and unipolar affective disorders. *Journal of Affective Disorders*, *119*(1-3), 28–33.

McDonald, C., Bullmore, E. T., Sham, P. C., Chitnis, X., Wickham, H., Bramon, E., & Murray, R. M. (2004a). Association of genetic risks for schizophrenia and bipolar disorder with specific and generic brain structural endophenotypes. *Archives of General Psychiatry*, *61*(10), 974-984.

McDonald, C., Bullmore, E., Sham, P., Chitnis, X., Suckling, J., MacCabe, J., Walshe, M., et al. (2005). Regional volume deviations of brain structure in schizophrenia and psychotic bipolar disorder: computational morphometry study. *The British Journal of Psychiatry*, *186*, 369-377.

McDonald, C., Zanelli, J., Rabe-Hesketh, S., Ellison-Wright, I., Sham, P., Kalidindi, S., Murray, R. M., et al. (2004). Meta-analysis of magnetic resonance imaging brain morphometry studies in bipolar disorder. *Biological Psychiatry*, *56*(6), 411-417.

McGuffin, P., Farmer, A., & Harvey, I. (1991). A polydiagnostic application of operational criteria in studies of psychotic illness. Development and reliability of the OPCRIT system. *Archives of General Psychiatry*, *48*(8), 764-770.

McGuffin, P., Rijsdijk, F., Andrew, M., Sham, P., Katz, R., & Cardno, A. (2003). The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. *Archives of General Psychiatry*, *60*(5), 497-502.

McIntosh, A. M., Job, D. E., Moorhead, T. W. J., Harrison, L. K., Forrester, K., Lawrie, S. M., & Johnstone, E. C. (2004). Voxel-based morphometry of patients with schizophrenia or bipolar disorder and their unaffected relatives. *Biological Psychiatry*, *56*(8), 544-552.

McIntosh, A. M., Job, D. E., Moorhead, T. W. J., Harrison, L. K., Lawrie, S. M., & Johnstone, E. C. (2005). White matter density in patients with schizophrenia, bipolar disorder and their unaffected relatives. *Biological Psychiatry*, *58*(3), 254-257.

McIntosh, A. M., Job, D. E., Moorhead, W. J., Harrison, L. K., Whalley, H. C., Johnstone, E. C., & Lawrie, S. M. (2006). Genetic liability to schizophrenia or bipolar disorder and its relationship to brain structure. *American Journal of Medical Genetics*, *141B*(1), 76-83.

McIntosh, A. M., Moorhead, T. W. J., Job, D., Lymer, G. K. S., Muñoz Maniega, S., McKirdy, J., Sussmann, J. E. D., et al. (2008). The effects of a neuregulin 1 variant on white matter density and integrity. *Molecular Psychiatry*, *13*(11), 1054-1059.

McIntosh, A. M., Muñoz Maniega, S., Lymer, G. K. S., McKirdy, J., Hall, J., Sussmann, J. E. D., Bastin, M. E., et al. (2008). White matter tractography in bipolar disorder and schizophrenia. *Biological Psychiatry*, *64*(12), 1088-1092.

McQueen, M. B., Devlin, B., Faraone, S. V., Nimgaonkar, V. L., Sklar, P., Smoller, J. W., Abou Jamra, R., et al. (2005). Combined analysis from eleven linkage studies of bipolar disorder provides strong evidence of susceptibility loci on chromosomes 6q and 8q. *American Journal of Human Genetics*, *77*(4), 582-595.

Mei, L., & Xiong, W.-C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nature Reviews. Neuroscience*, *9*(6), 437-452.

Mendlowicz, M. V., Jean-Louis, G., Kelsoe, J. R., & Akiskal, H. S. (2005). A comparison of recovered bipolar patients, healthy relatives of bipolar probands, and normal controls using the short TEMPS-A. *Journal of Affective Disorders*, *85*(1-2), 147-151. doi:10.1016/j.jad.2004.01.012

Millar, J. K., Wilson-Annan, J. C., Anderson, S., Christie, S., Taylor, M. S., Semple, C. A., Devon, R. S., et al. (2000). Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Human Molecular Genetics*, *9*(9), 1415-1423.

Nilsson, K. K., Straarup, K. N., Jørgensen, C. R., & Licht, R. W. (2012). Affective temperaments' relation to functional impairment and affective recurrences in bipolar disorder patients. *Journal of Affective Disorders*, *138*(3), 332–336.

Moorhead, T. W. J., Job, D. E., Spencer, M. D., Whalley, H. C., Johnstone, E. C., & Lawrie, S. M. (2005). Empirical comparison of maximal voxel and non-isotropic adjusted cluster extent results in a voxel-based morphometry study of comorbid learning disability with schizophrenia. *NeuroImage*, *28*(3), 544-552.

Mori S, Faria AV, van Zijl PCM, & Oishi K. (2011). *MRI atlas of human white matter* (2nd ed.). Amsterdam: Elsevier/Academic Press.

Müller-Oerlinghausen, B., Berghöfer, A., & Bauer, M. (2002). Bipolar disorder. *Lancet*, *359*(9302), 241-247.

Muir, W. J., Gosden, C. M., Brookes, A. J., Fantes, J., Evans, K. L., Maguire, S. M., Stevenson, B., et al. (1995). Direct microdissection and microcloning of a translocation breakpoint region,

t(1;11)(q42.2;q21), associated with schizophrenia. *Cytogenetics and Cell Genetics*, *70*(1-2), 35-40.

Munafò, M. R., Attwood, A. S., & Flint, J. (2008). Neuregulin 1 genotype and schizophrenia. *Schizophrenia Bulletin*, *34*(1), 9-12.

Munafò, M. R., Thiselton, D. L., Clark, T. G., & Flint, J. (2006). Association of the NRG1 gene and schizophrenia: a meta-analysis. *Molecular Psychiatry*, *11*(6), 539-546.

Muñoz Maniega, S., Bastin, ME, McIntosh, AM, Lawrie, SM, & Clayden, JD. (2008). Atlas-based reference tracts improve automatic white matter segmentation with neighbourhood tractography. *Proceedings of the ISMRM 16th Scientific Meeting and Exhibition* (3318). Berkeley, CA: ISMRM.

Muñoz Maniega, S., Lymer, G. K. S., Bastin, M. E., Marjoram, D., Job, D. E., Moorhead, T. W. J., Owens, D. G., et al. (2008). A diffusion tensor MRI study of white matter integrity in subjects at high genetic risk of schizophrenia. *Schizophrenia Research*, *106*(2-3), 132-139.

Nicodemus, K. K., Luna, A., Vakkalanka, R., Goldberg, T., Egan, M., Straub, R. E., & Weinberger, D. R. (2006). Further evidence for association between ErbB4 and schizophrenia and influence on cognitive intermediate phenotypes in healthy controls. *Molecular Psychiatry*, *11*(12), 1062-1065.

Norton, N., Moskvina, V., Morris, D. W., Bray, N. J., Zammit, S., Williams, N. M., Williams, H. J., et al. (2006). Evidence that interaction between neuregulin 1 and its receptor erbB4 increases susceptibility to schizophrenia. *American Journal of Medical Genetics*, *141B*(1), 96-101.

O'Donovan, M. C., Craddock, N., Norton, N., Williams, H., Peirce, T., Moskvina, V., Nikolov, I., et al. (2008). Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nature Genetics*, *40*(9), 1053-1055.

Ochsner, K. N., & Gross, J. J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences*, *9*(5), 242-249.

Ongür, D., Drevets, W. C., & Price, J. L. (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(22), 13290-13295.

Paunio, T., Ekelund, J., Varilo, T., Parker, A., Hovatta, I., Turunen, J. A., Rinard, K., et al. (2001). Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. *Human Molecular Genetics*, *10*(26), 3037-3048.

Paus, T. (2005). Mapping brain maturation and cognitive development during adolescence. *Trends in Cognitive Sciences*, *9*(2), 60-68.

Penke, L., Muñoz Maniega, S., Murray, C., Gow, A. J., Hernández, M. C. V., Clayden, J. D., Starr, J. M., et al. (2010). A general factor of brain white matter integrity predicts information processing speed in healthy older people. *The Journal of Neuroscience*, *30*(22), 7569-7574.

Petryshen, T. L., Middleton, F. A., Kirby, A., Aldinger, K. A., Purcell, S., Tahl, A. R., Morley, C. P., et al. (2005). Support for involvement of neuregulin 1 in schizophrenia pathophysiology. *Molecular Psychiatry*, *10*(4), 366-374, 328.

Phillips, M. L., Drevets, W. C., Rauch, S. L., & Lane, R. (2003a). Neurobiology of emotion perception I: The neural basis of normal emotion perception. *Biological Psychiatry*, *54*(5), 504-514.

Phillips, M. L., Drevets, W. C., Rauch, S. L., & Lane, R. (2003b). Neurobiology of emotion perception II: Implications for major psychiatric disorders. *Biological Psychiatry*, *54*(5), 515-528.

Phillips, M. L., Ladouceur, C. D., & Drevets, W. C. (2008). A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. *Molecular Psychiatry*, *13*(9), 829, 833-857.

Pletnikov, M. V., Ayhan, Y., Nikolskaia, O., Xu, Y., Ovanesov, M. V., Huang, H., Mori, S., et al. (2008). Inducible expression of mutant human DISC1 in mice is associated with brain and behavioral abnormalities reminiscent of schizophrenia. *Molecular Psychiatry*, *13*(2), 173-186, 115.

Psychological Corporation. (1999). Wechsler Abbreviated Scale of Intelligence (WASI) manual. San Antonio, TX: Author.

Prata, D. P., Breen, G., Osborne, S., Munro, J., St Clair, D., & Collier, D. A. (2009). An association study of the neuregulin 1 gene, bipolar affective disorder and psychosis. *Psychiatric Genetics*, *19*(3), 113-116.

Prata, D. P., Mechelli, A., Fu, C. H. Y., Picchioni, M., Kane, F., Kalidindi, S., McDonald, C., et al. (2008). Effect of disrupted-in-schizophrenia-1 on pre-frontal cortical function. *Molecular Psychiatry*, *13*(10), 915-917, 909.

Prata, D. P., Mechelli, A., Picchioni, M., Fu, C. H. Y., Kane, F., Kalidindi, S., McDonald, C., et al. (2011). No association of Disrupted-in-Schizophrenia-1 variation with prefrontal function in patients with schizophrenia and bipolar disorder. *Genes, Brain, and Behavior*, *10*(3), 276-285.

Purcell, S. M., Wray, N. R., Stone, J. L., Visscher, P. M., O'Donovan, M. C., Sullivan, P. F., & Sklar, P. (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*, *460*(7256), 748-752.

Qu, M., Tang, F., Yue, W., Ruan, Y., Lu, T., Liu, Z., Zhang, H., et al. (2007). Positive association of the Disrupted-in-Schizophrenia-1 gene (DISC1) with schizophrenia in the Chinese Han population. *American Journal of Medical Genetics*, *144B*(3), 266-270.

Rajkowska, G., Halaris, A., & Selemon, L. D. (2001). Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biological Psychiatry*, *49*(9), 741-752.

Rastogi, A., Zai, C., Likhodi, O., Kennedy, J. L., & Wong, A. H. (2009). Genetic association and post-mortem brain mRNA analysis of DISC1 and related genes in schizophrenia. *Schizophrenia Research*, *114*(1-3), 39-49.

Regenold, W. T., Phatak, P., Marano, C. M., Gearhart, L., Viens, C. H., & Hisley, K. C. (2007). Myelin staining of deep white matter in the dorsolateral prefrontal cortex in schizophrenia, bipolar disorder, and unipolar major depression. *Psychiatry Research*, *151*(3), 179–188.

Riley, B., Thiselton, D., Maher, B. S., Bigdeli, T., Wormley, B., McMichael, G. O., Fanous, A. H., et al. (2010). Replication of association between schizophrenia and ZNF804A in the Irish Case-Control Study of Schizophrenia sample. *Molecular Psychiatry*, *15*(1), 29-37.

Rio, C., Rieff, H. I., Qi, P., Khurana, T. S., & Corfas, G. (1997). Neuregulin and erbB receptors play a critical role in neuronal migration. *Neuron*, *19*(1), 39-50.

Saetre, P., Agartz, I., De Franciscis, A., Lundmark, P., Djurovic, S., Kähler, A., Andreassen, O. A., et al. (2008). Association between a disrupted-in-schizophrenia 1 (DISC1) single nucleotide polymorphism and schizophrenia in a combined Scandinavian case-control sample. *Schizophrenia Research*, *106*(2-3), 237-241.

Salimi-Khorshidi, G., Smith, S. M., & Nichols, T. E. (2011). Adjusting the effect of nonstationarity in cluster-based and TFCE inference. *NeuroImage*, *54*(3), 2006-2019.

Sarnicola, A., Kempton, M., Germanà, C., Haldane, M., Hadjulis, M., Christodoulou, T., Koukopoulos, A., et al. (2009). No differential effect of age on brain matter volume and cognition in bipolar patients and healthy individuals. *Bipolar Disorders*, *11*(3), 316–322.

Schmierer, K., Wheeler-Kingshott, C. A. M., Boulby, P. A., Scaravilli, F., Altmann, D. R., Barker, G. J., Tofts, P. S., et al. (2007). Diffusion tensor imaging of post mortem multiple sclerosis brain. *NeuroImage*, *35*(2), 467-477.

van der Schot, A. C., Vonk, R., Brans, R. G. H., van Haren, N. E. M., Koolschijn, P. C. M. P., Nuboer, V., Schnack, H. G., et al. (2009). Influence of genes and environment on brain volumes in twin pairs concordant and discordant for bipolar disorder. *Archives of General Psychiatry*, *66*(2), 142-151.

van der Schot, A. C., Vonk, R., Brouwer, R. M., van Baal, G. C. M., Brans, R. G. H., van Haren, N. E. M., Schnack, H. G., et al. (2010). Genetic and environmental influences on focal brain density in bipolar disorder. *Brain*, *133*(10), 3080-3092.

Schulze, T. G., Hedeker, D., Zandi, P., Rietschel, M., & McMahon, F. J. (2006). What is familial about familial bipolar disorder? Resemblance among relatives across a broad spectrum of phenotypic characteristics. *Archives of General Psychiatry*, *63*(12), 1368-1376.

Segurado, R., Detera-Wadleigh, S. D., Levinson, D. F., Lewis, C. M., Gill, M., Nurnberger, J. I., Jr, Craddock, N., et al. (2003). Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: Bipolar disorder. *American Journal of Human Genetics*, *73*(1), 49-62.

Serretti, A., & Mandelli, L. (2008). The genetics of bipolar disorder: genome “hot regions,” genes, new potential candidates and future directions. *Molecular Psychiatry*, *13*(8), 742-771.

Sexton, C. E., Mackay, C. E., & Ebmeier, K. P. (2009). A Systematic Review of Diffusion Tensor Imaging Studies in Affective Disorders. *Biological Psychiatry*, *66*(9), 814-823.

Shiota, S., Tochigi, M., Shimada, H., Ohashi, J., Kasai, K., Kato, N., Tokunaga, K., et al. (2008). Association and interaction analyses of NRG1 and ERBB4 genes with schizophrenia in a Japanese population. *Journal of Human Genetics*, *53*(10), 929-935.

Silberberg, G., Darvasi, A., Pinkas-Kramarski, R., & Navon, R. (2006). The involvement of ErbB4 with schizophrenia: association and expression studies. *American Journal of Medical Genetics*, *141B*(2), 142-148.

Silver, M., Montana, G., & Nichols, T. E. (2011). False positives in neuroimaging genetics using voxel-based morphometry data. *NeuroImage*, *54*(2), 992-1000.

Sklar, P., Smoller, J. W., Fan, J., Ferreira, M. A. R., Perlis, R. H., Chambert, K., Nimgaonkar, V. L., et al. (2008). Whole-genome association study of bipolar disorder. *Molecular Psychiatry*, *13*(6), 558-569.

Smith, S. M., & Nichols, T. E. (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *NeuroImage*, *44*(1), 83-98.

Smith, S. M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T. E., Mackay, C. E., Watkins, K. E., et al. (2006). Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *NeuroImage*, *31*(4), 1487-1505.

Smith, S. M., Johansen-Berg, H., Jenkinson, M., Rueckert, D., Nichols, T. E., Miller, K. L., Robson, M. D., et al. (2007). Acquisition and voxelwise analysis of multi-subject diffusion data with tract-based spatial statistics. *Nature Protocols*, *2*(3), 499-503.

Song, S.-K., Sun, S.-W., Ju, W.-K., Lin, S.-J., Cross, A. H., & Neufeld, A. H. (2003). Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *NeuroImage*, *20*(3), 1714-1722.

Song, S.-K., Sun, S.-W., Ramsbottom, M. J., Chang, C., Russell, J., & Cross, A. H. (2002). Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *NeuroImage*, *17*(3), 1429-1436.

Song, S.-K., Yoshino, J., Le, T. Q., Lin, S.-J., Sun, S.-W., Cross, A. H., & Armstrong, R. C. (2005). Demyelination increases radial diffusivity in corpus callosum of mouse brain. *NeuroImage*, *26*(1), 132-140.

Song, W., Li, W., Feng, J., Heston, L. L., Scaringe, W. A., & Sommer, S. S. (2008). Identification of high risk DISC1 structural variants with a 2% attributable risk for schizophrenia. *Biochemical and Biophysical Research Communications*, *367*(3), 700-706.

Song, W., Li, W., Noltner, K., Yan, J., Green, E., Grozeva, D., Jones, I. R., et al. (2010). Identification of high risk DISC1 protein structural variants in patients with bipolar spectrum disorder. *Neuroscience Letters*, *486*(3), 136-140.

Sprooten, E., Lymer, G. K. S., Muñoz Maniega, S., McKirdy, J., Clayden, J. D., Bastin, M. E., Porteous, D., et al. (2009). The relationship of anterior thalamic radiation integrity to psychosis risk associated neuregulin-1 variants. *Molecular Psychiatry*, *14*(3), 237-238, 233.

Sprooten, E., Sussmann, J. E., Clugston, A., Peel, A., McKirdy, J., Moorhead, T. W. J., Anderson, S., et al. (2011). White matter integrity in individuals at high genetic risk of bipolar disorder. *Biological Psychiatry*, *70*(4), 350-356.

St Clair, D., Blackwood, D., Muir, W., Carothers, A., Walker, M., Spowart, G., Gosden, C., et al. (1990). Association within a family of a balanced autosomal translocation with major mental illness. *Lancet*, *336*(8706), 13-16.

Steinberg, S., Mors, O., Børglum, A. D., Gustafsson, O., Werge, T., Mortensen, P. B., Andreassen, O. A., et al. (2011). Expanding the range of ZNF804A variants conferring risk of psychosis. *Molecular Psychiatry*, *16*(1), 59-66.

Stephan, K. E., Friston, K. J., & Frith, C. D. (2009). Dysconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. *Schizophrenia Bulletin*, *35*(3), 509-527.

Strakowski, S. M., Delbello, M. P., & Adler, C. M. (2005). The functional neuroanatomy of bipolar disorder: a review of neuroimaging findings. *Molecular Psychiatry*, *10*(1), 105-116.

Sussmann, J. E., Lymer, G. K. S., McKirdy, J., Moorhead, T. W. J., Muñoz Maniega, S., Job, D., Hall, J., et al. (2009). White matter abnormalities in bipolar disorder and schizophrenia detected using diffusion tensor magnetic resonance imaging. *Bipolar Disorders*, *11*(1), 11-18.

Tabarés-Seisdedos, R., & Rubenstein, J. L. R. (2009). Chromosome 8p as a potential hub for developmental neuropsychiatric disorders: implications for schizophrenia, autism and cancer. *Molecular Psychiatry*, *14*(6), 563-589.

Tang, J. X., Chen, W. Y., He, G., Zhou, J., Gu, N. F., Feng, G. Y., & He, L. (2004). Polymorphisms within 5' end of the Neuregulin 1 gene are genetically associated with schizophrenia in the Chinese population. *Molecular Psychiatry*, *9*(1), 11-12.

Taveggia, C., Thaker, P., Petrylak, A., Caporaso, G. L., Toews, A., Falls, D. L., Einheber, S., et al. (2008). Type III neuregulin-1 promotes oligodendrocyte myelination. *Glia*, *56*(3), 284-293.

Thomson, P. A., Christoforou, A., Morris, S. W., Adie, E., Pickard, B. S., Porteous, D. J., Muir, W. J., et al. (2007). Association of Neuregulin 1 with schizophrenia and bipolar disorder in a second cohort from the Scottish population. *Molecular Psychiatry*, *12*(1), 94-104.

Thomson, P. A., Harris, S. E., Starr, J. M., Whalley, L. J., Porteous, D. J., & Deary, I. J. (2005). Association between genotype at an exonic SNP in DISC1 and normal cognitive aging. *Neuroscience Letters*, *389*(1), 41-45.

Tkachev, D., Mimmack, M. L., Ryan, M. M., Wayland, M., Freeman, T., Jones, P. B., Starkey, M., et al. (2003). Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet*, *362*(9386), 798-805.

Underwood, C. K., Kurniawan, N. D., Butler, T. J., Cowin, G. J., & Wallace, R. H. (2011). Non-invasive diffusion tensor imaging detects white matter degeneration in the spinal cord of a mouse model of amyotrophic lateral sclerosis. *NeuroImage*, *55*(2), 455-461.

Uranova, N. A., Vostrikov, V. M., Orlovskaya, D. D., & Rachmanova, V. I. (2004). Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium. *Schizophrenia Research*, *67*(2-3), 269-275.

Vázquez, G. H., Kahn, C., Schiavo, C. E., Goldchluk, A., Herbst, L., Piccione, M., Saidman, N., et al. (2008). Bipolar disorders and affective temperaments: a national family study testing the 'endophenotype' and 'subaffective' theses using the TEMPS-A Buenos Aires. *Journal of Affective Disorders*, *108*(1-2), 25–32.

Vederine, F.-E., Wessa, M., Leboyer, M., & Houenou, J. (2011). A meta-analysis of whole-brain diffusion tensor imaging studies in bipolar disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *35*(8), 1820-1826.

Versace, A., Almeida, J. R. C., Hassel, S., Walsh, N. D., Novelli, M., Klein, C. R., Kupfer, D. J., et al. (2008). Elevated left and reduced right orbitomedial prefrontal fractional anisotropy in adults with bipolar disorder revealed by tract-based spatial statistics. *Archives of General Psychiatry*, *65*(9), 1041-1052.

Versace, A., Ladouceur, C. D., Romero, S., Birmaher, B., Axelson, D. A., Kupfer, D. J., & Phillips, M. L. (2010). Altered development of white matter in youth at high familial risk for bipolar disorder: a diffusion tensor imaging study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*(12), 1249-1259, 1259.e1.

Videbech, P. (1997). MRI findings in patients with affective disorder: a meta-analysis. *Acta Psychiatrica Scandinavica*, *96*(3), 157-168.

Voineskos, A. N., Lerch, J. P., Felsky, D., Tiwari, A., Rajji, T. K., Miranda, D., Lobaugh, N. J., et al. (2011). The ZNF804A Gene: Characterization of a Novel Neural Risk Mechanism for the Major Psychoses. *Neuropsychopharmacology.*

Vostrikov, V. M., Uranova, N. A., & Orlovskaya, D. D. (2007). Deficit of perineuronal oligodendrocytes in the prefrontal cortex in schizophrenia and mood disorders. *Schizophrenia Research*, *94*(1-3), 273-280.

Walker, R. M., Christoforou, A., Thomson, P. A., McGhee, K. A., Maclean, A., Mühleisen, T. W., Strohmaier, J., et al. (2010). Association analysis of Neuregulin 1 candidate regions in schizophrenia and bipolar disorder. *Neuroscience Letters*, *478*(1), 9-13.

Walter, H., Schnell, K., Erk, S., Arnold, C., Kirsch, P., Esslinger, C., Mier, D., et al. (2011). Effects of a genome-wide supported psychosis risk variant on neural activation during a theory-of-mind task. *Molecular Psychiatry*, *16*(4), 462-470.

Walters, JT, Corvin, A, Owen, MJ, Williams, H, Dragovic, M, Quinn, EM, Judge, R, et al. (2010). Psychosis susceptibility gene ZNF804A and cognitive performance in schizophrenia, *67*(7), 692-700.

Wang, F., Jackowski, M., Kalmar, J. H., Chepenik, L. G., Tie, K., Qiu, M., Gong, G., et al. (2008). Abnormal anterior cingulum integrity in bipolar disorder determined through diffusion tensor imaging. *The British Journal of Psychiatry*, *193*(2), 126-129.

Wang, F., Kalmar, J. H., Edmiston, E., Chepenik, L. G., Bhagwagar, Z., Spencer, L., Pittman, B., et al. (2008). Abnormal corpus callosum integrity in bipolar disorder: a diffusion tensor imaging study. *Biological Psychiatry*, *64*(8), 730-733.

Wang, F., Kalmar, J. H., He, Y., Jackowski, M., Chepenik, L. G., Edmiston, E. E., Tie, K., et al. (2009). Functional and structural connectivity between the perigenual anterior cingulate and amygdala in bipolar disorder. *Biological Psychiatry*, *66*(5), 516-521.

Weber, H., Kittel-Schneider, S., Gessner, A., Domschke, K., Neuner, M., Jacob, C. P., Buttenschon, H. N., et al. (2011). Cross-disorder analysis of bipolar risk genes: further evidence of DGKH as a risk gene for bipolar disorder, but also unipolar depression and adult ADHD. *Neuropsychopharmacology*, *36*(10), 2076-2085.

Wessa, M., Houenou, J., Leboyer, M., Chanraud, S., Poupon, C., Martinot, J.-L., & Paillère-Martinot, M.-L. (2009). Microstructural white matter changes in euthymic bipolar patients: a whole-brain diffusion tensor imaging study. *Bipolar Disorders*, *11*(5), 504-514.

Williams, H. J., Norton, N., Dwyer, S., Moskvina, V., Nikolov, I., Carroll, L., Georgieva, L., et al. (2011). Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. *Molecular Psychiatry*, *16*(4), 429-441.

Williams, N. M., Preece, A., Spurlock, G., Norton, N., Williams, H. J., Zammit, S., O'Donovan, M. C., et al. (2003). Support for genetic variation in neuregulin 1 and susceptibility to schizophrenia. *Molecular Psychiatry*, *8*(5), 485-487.

Winterer, G., Konrad, A., Vucurevic, G., Musso, F., Stoeter, P., & Dahmen, N. (2008). Association of 5' end neuregulin-1 (NRG1) gene variation with subcortical medial frontal microstructure in humans. *NeuroImage*, *40*(2), 712-718.

Wheeler-Kingshott, C.A., Cercignani, M. (2009). About "Axial" and "Radial" Diffusivities. *Magnetic Resonance in Medicine* 61(5), 1255–1260.

Wood, J. D., Bonath, F., Kumar, S., Ross, C. A., & Cunliffe, V. T. (2009). Disrupted-in-schizophrenia 1 and neuregulin 1 are required for the specification of oligodendrocytes and neurones in the zebrafish brain. *Human Molecular Genetics*, *18*(3), 391 -404.

Xie, M., Tobin, J. E., Budde, M. D., Chen, C.-I., Trinkaus, K., Cross, A. H., McDaniel, D. P., et al. (2010). Rostrocaudal analysis of corpus callosum demyelination and axon damage across disease stages refines diffusion tensor imaging correlations with pathological features. *Journal of Neuropathology and Experimental Neurology*, *69*(7), 704-716.

Yang, J. Z., Si, T. M., Ruan, Y., Ling, Y. S., Han, Y. H., Wang, X. L., Zhou, M., et al. (2003). Association study of neuregulin 1 gene with schizophrenia. *Molecular Psychiatry*, *8*(7), 706-709.

Yuan, P., Zhou, R., Wang, Y., Li, X., Li, J., Chen, G., Guitart, X., et al. (2010). Altered levels of extracellular signal-regulated kinase signaling proteins in postmortem frontal cortex of individuals with mood disorders and schizophrenia. *Journal of Affective Disorders*, *124*(1-2), 164-169.

Yurgelun-Todd, D. A., Silveri, M. M., Gruber, S. A., Rohan, M. L., & Pimentel, P. J. (2007). White matter abnormalities observed in bipolar disorder: a diffusion tensor imaging study. *Bipolar Disorders*, *9*(5), 504-512.

Zalesky, A. (2011). Moderating registration misalignment in voxelwise comparisons of DTI data: a performance evaluation of skeleton projection. *Magnetic Resonance Imaging*, *29*(1), 111-125.

Zhang, F., Sarginson, J., Crombie, C., Walker, N., St Clair, D., & Shaw, D. (2006). Genetic association between schizophrenia and the DISC1 gene in the Scottish population. *American Journal of Medical Genetics*, *141B*(2), 155-159.

Zhang, R., Lu, S. M., Qiu, C., Liu, X. G., Gao, C. G., Guo, T. W., Valenzuela, R. K., et al. (2011). Population-based and family-based association studies of ZNF804A locus and schizophrenia. *Molecular Psychiatry*, *16*(4), 360-361.

Zhang, X., Tochigi, M., Ohashi, J., Maeda, K., Kato, T., Okazaki, Y., Kato, N., et al. (2005). Association study of the DISC1/TRAX locus with schizophrenia in a Japanese population. *Schizophrenia Research*, *79*(2-3), 175-180.

Zhou, S.-Y., Suzuki, M., Hagino, H., Takahashi, T., Kawasaki, Y., Nohara, S., Yamashita, I., et al. (2003). Decreased volume and increased asymmetry of the anterior limb of the internal capsule in patients with schizophrenia. *Biological Psychiatry*, *54*(4), 427-436.

Zhou, X., Geyer, M. A., & Kelsoe, J. R. (2008). Does disrupted-in-schizophrenia (DISC1) generate fusion transcripts? *Molecular Psychiatry*, *13*(4), 361-363.

van Zijl, Mori. (2005). *MRI atlas of human white matter*. Amsterdam: Elsevier.

Zuliani, R., Moorhead, T. W. J., Bastin, M. E., Johnstone, E. C., Lawrie, S. M., Brambilla, P., O'Donovan, M. C., et al. (2011). Genetic variants in the ErbB4 gene are associated with white matter integrity. *Psychiatry Research*, *191*(2), 133-137.