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Structural Brain Imaging in Individuals at High Familial Risk of Schizophrenia

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Doctor of Philosophy University of Edinburgh

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Declaration

My primary role in the Edinburgh High Risk Study has been to learn and apply Freesurfer to the structural scans collected for this dataset. I converted all scans to Freesurfer format, and then conducted the time-consuming manual edits for all scans of the dataset (only first two time points however constitute the main part of this thesis). The original idea of this study was conceived by Professor Eve Jonhstone, and the clinical and brain imaging collected by experienced clinicians. I then conducted all analyses, both in Freesurfer and R, by myself, and wrote the four first-author papers associated with this thesis. 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 of professional qualification.

Signed: _____

Catherine Bois

Dated: _____

Publications

A list of first-author publications that are based directly on the work constituting this thesis are presented below:

This work constitutes Chapter 1:

Bois C, Whalley HC, McIntosh AM, et al. (2014). Structural magnetic resonance imaging markers of susceptibility and transition to schizophrenia: a review of familial and clinical high risk population studies. *Journal of Psychopharmacology*, 29(1), 144-154.

This work constitutes Chapter 3:

Bois C, Ronan L, Levita L, et al. (2015). Cortical surface area differentiates familial high risk individuals who go on to develop schizophrenia. *Biological Psychiatry*.
[doi:10.1016/j.biopsych.2014.12.030](https://doi.org/10.1016/j.biopsych.2014.12.030)

This work constitutes Chapter 5:

Bois C, Levita L, Ripp I, et al. (2014). Longitudinal changes in hippocampal volume in the Edinburgh high risk study of schizophrenia. *Schizophrenia Research*.
[doi:10.1016/j.schres.2014.12.003](https://doi.org/10.1016/j.schres.2014.12.003)

Bois C, Levita L, Ripp I, et al. (2015). Hippocampal, amygdala and nucleus accumbens volume in first-episode schizophrenia patients and individuals at high familial risk: A cross-sectional Comparison. *Schizophrenia Research*.
[doi:10.1016/j.schres.2015.03.024](https://doi.org/10.1016/j.schres.2015.03.024)

These are included at the back of the present thesis. A list of other papers that I am also first, second and co-author on based on work I have conducted on the Edinburgh High Risk Study and other datasets are Presented in Appendix 1, along with first-author conference abstracts that I have presented at international and domestic conferences and that have been published.

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List of Abbreviations

ANCOVA Analysis of covariance

FDR False-Discovery rate

fMRI Functional magnetic resonance imaging

HR-T High Risk that transition to Schizophrenia

HR-NT High risk that do not transition to sSchizophrenia

ANCOVA Analysis of variance

HR High Risk

GWAS Genome-wide association study

MRI Magnetic Resonance Imaging

ROI Region of interest

CT Computerized Tomography

LGI Local Gyrification Index

GI Gyrification Index

Abstract

Schizophrenia is often a debilitating psychiatric disorder, characterised by both positive and negative symptoms, and cognitive and psychosocial impairments. The established disorder has been associated with a number of brain abnormalities, however it is at present unknown whether these brain changes occur prior to onset of schizophrenia, or in unaffected relatives with a familial vulnerability to develop the disorder, or only in those at high risk that go on to develop the disorder. Furthermore, most studies have been conducted cross-sectionally, which may have obscured subtle longitudinal changes in familial high risk individuals, and these studies tend to have focused on localized cortical gray matter, and thus it is unclear whether they affect different cortical parameters differentially. Prospective familial high risk studies utilizing surface based MRI programmes provide a good method to investigate this.

In the Edinburgh High Risk Study, structural magnetic resonance imaging (MRI) scans of 150 young individuals at familial high risk of schizophrenia, 34 patients with first-episode schizophrenia and 36 matched controls were obtained. Of the high risk participants with scans suitable for analysis, 17 developed schizophrenia after the scans were taken, whilst 57 experienced isolated or sub-clinical psychotic symptoms, and 70 remained well. We used Freesurfer to extract volumetric and surface-based measurements of several cortical and localized sub-cortical regions with the aim of assessing whether any alterations found were

present in all those at high risk, or selectively in the high risk cohort based on future clinical outcome, or only in those experiencing their first-episode of psychosis.

It was found that those experiencing their first episode of schizophrenia exhibited significantly more widespread brain alterations compared to those at high risk or controls, both on a more global cortical level and in more localized regions of the cortex, with cortical thickness being generally thinner than in the other groups, and cortical surface area and gyrification increased compared to the other groups. An increased global surface area was also shared with the HR[ill] group, suggesting that this could be a marker that is predictive of future transition to psychosis. Within the high risk cohort, some brain alterations seemed to present as general vulnerability markers, specifically in the temporal lobe at baseline, whilst longitudinally both localized and global cortical alterations distinguished the high risk cohort from the control group, and a different developmental trajectory of the hippocampus was also found.

These findings show that some brain alterations may be more accurately characterized as general vulnerability markers of the disorder, whilst some are specifically present in patients who have experienced their first episode of schizophrenia, whilst some also occur before disorder onset in those at high risk that go on to develop schizophrenia. The findings have some clinical implications, as they suggest that it is possible to assess who at high risk will go on to develop schizophrenia based on brain structural alterations. This may provide clinicians with an early window of opportunity for intervention, as it has been found that early intervention may improve patient's prognosis. The findings also have important implications for the understanding of the underlying etiology of schizophrenia, as they suggest that some of these alterations are present before illness onset, and not associated with medication effects, thus potentially lying on the causal path of developing schizophrenia.

Lay Summary

Schizophrenia is a complex and often debilitating psychiatric disorder. Although research into its underlying causes have been ongoing for more than a century, the disorder's underlying causes remain relatively unknown. Altered brain structure has been implicated in its underlying causes, however most studies have looked at patients that have been on antipsychotic medication for several years, which could have caused some of these alterations, rather than these changes being specifically associated with the disorder itself. In order to bypass this problem, studies of high risk individuals that have a family history of schizophrenia can examine brain structure prospectively. This thesis therefore investigates brain structure in a familial high risk cohort with the aim to see whether these individuals have altered brain structure compared to a sample of patients in their first episode of psychosis, and a sample of healthy controls. We also wanted to look at whether within the high risk cohort there were more brain alterations in those that became ill, compared to those that did not. We found that there were some widespread and localized brain alterations specific to the patients in their first episode, suggestive of illness specific mechanisms. We also found that those at high risk that became ill had more alterations compared to those that remained well. But, we also found changes distributed across the whole high risk cohort, suggesting that these may form a stable underlying biological vulnerability to schizophrenia. We conclude therefore that brain alterations are not statically associated with schizophrenia, but rather occur at different stages of the illness, and in order to find windows of opportunity for early interventions, clinicians may want to focus on brain alterations that specifically occur in those at high risk that go on to become ill.

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CHAPTER 1

Structural Brain Imaging in Patients with Schizophrenia and those at High Risk of developing Schizophrenia

1. Introduction

Schizophrenia is a severe and debilitating psychiatric disorder that primarily manifests in adolescence or early adulthood. With a lifetime prevalence of 1% it places a huge financial burden on society, as well as personal suffering on affected individuals and their families and is one of the leading causes of disability worldwide (Jablensky, 1997, Murray & Lopez, 1997, Csoboth et al. 2015). The symptoms of schizophrenia are heterogeneous and complex, including psychotic symptoms such as delusions, hallucinations and disorganization of thought and speech, as well as a loss of social functioning, emotional blunting and apathy, the latter of which are generally associated with a worse clinical prognosis (Andreasen & Olsen, 1982, Pogue-Geile & Harrow, 1985, Hunter & Barry 2012). These symptoms are often also accompanied by profound cognitive and psychosocial impairments, and may be masked by a prodromal period of anxiety, depression and/or substance misuse (Bromet & Fennig 1999, Dixon 1999, Pallanti et al. 2013).

1.1 Schizophrenia: An Overview

The clinical syndrome of schizophrenia was first described in detail by Kraepelin over a hundred years ago, who referred to the disorder as “Dementia Praecox”, who drew comparisons with dementia, with respect to the deteriorating function over time, however also the much earlier age of onset (Andreasen, 1997). The typical age of onset for schizophrenia is in adolescence or early adulthood, with males experiencing a generally earlier age of onset than females, peaking around 20-24 years. Women also experience a prominent peak at about this age, a year later on average, followed

by a second smaller peak at around 40-45 years of age (Andreasen, 1997). However, schizophrenia can also occur in childhood, or much later in life, and there is some evidence of differences in clinical course dependent on age of onset (Bromet & Fennig 1999, Cohen et al. 2013, Bartlett 2014).

Modern clinical descriptions of schizophrenia no longer refer to the disorder as Dementia Praecox, but rather focus on dividing the syndrome up into positive and negative symptoms, the former referring to symptoms characterized by phenomena such as hallucinations and delusions that are abnormal by their presence, whilst the latter refers to a loss of normal functioning (Lewine et al. 1983). Diagnosis is at present based upon the description and observation of a patient's mental state using standardized criteria as outlined in the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM 5) or the international classification of diseases (ICD – 10, World Health Organization 1993). Appendix 1.1 shows the diagnostic criteria as outlined in DSM -5.

Although the symptomatology of schizophrenia is well-described, at present there exists no preclinical marker that could aid risk stratification, resulting in there often being a period of time elapsing between the first appearance of symptoms, where these are left untreated, and the actual clinical diagnosis of schizophrenia being made (Fraguas et al. 2014. Gumley et al. 2014). However, evidence is accumulating that those that are diagnosed before the symptoms have become too severe or debilitating often experience better clinical prognoses, and not as marked a loss of social

functioning (Frangou & Byrne 2000). As available treatments focus on treating symptoms once they have fully set in, there is a considerable need to identify predictive markers of the disorder that may aid clinicians with intervening at an early stage to improve prognosis, or in aid of facilitating preventative strategies.

1.1.1 Schizophrenia as a Disorder of Neurodevelopment

Research is now converging on schizophrenia being a complex and multi-dimensional disorder, mediated by both environmental and genetic factors, as well as their interactions (McDonald & Murray 2000, Ripke et al. 2013, Stepniak et al. 2014). The stress-diathesis model is often applied to schizophrenia, with research suggesting that schizophrenia may be best characterized as an initial neurodevelopmental disruption, which may lie dormant until environmental stressors precipitate it (Zubin & Spring 1977). Another commonly applied model is that of a neurodevelopmental lesion which is released by typical developmental processes, such as synaptic pruning, which takes place during late childhood/early adolescence, when the symptoms of schizophrenia often begin to manifest (Weinberger 1987). This process is that of synapse elimination that occurs between early childhood and the onset of puberty in many mammals, this process is what is referred and it has been suggested that this may occur excessively by an early lesion may contribute to the symptoms of psychosis (Weinberger 1987). A lack of gliosis found in post-mortem studies of patients (Roberts et al. 1987) has also strengthened support for schizophrenia having a neurodevelopmental component, as has findings of pre-morbid abnormalities in cognition, brain functioning and structure (Walder et al. 2014, Bois et al. 2014). As

schizophrenia is also hypothesized to have a large genetic component, and at least 30% of human genes are expressed exclusively in the brain (Sutcliffe et al. 1984), it is possible that an early genetic defect contributes to the alterations in brain processes described above.

1.1.2 Schizophrenia and the Brain

Although the notion of schizophrenia being a disorder characterized by brain alterations dates back to the time of Kraepelin, it is only in the past fifty years or so that we have been able to investigate this *in vivo*, specifically with the advent of Computerized Tomography (CT), which found that enlarged ventricles distinguished patients with schizophrenia from healthy controls (Johnstone et al., 1976). With the advent of more modern imaging techniques, such as magnetic resonance imaging (MRI) (For a more detailed explanation of the principles underlying MRI, please see chapter 2), more sophisticated analysis of the brain's structure and function has been able to take place, leading researchers to now suggest that schizophrenia is in large part a disorder of widespread disruption of brain structure and function (Lewis & Levitt., 2002, White & Hilgetag, 2011). There is now overwhelming evidence that patients with established schizophrenia exhibit widespread structural brain abnormalities, with the most consistent findings being enlarged lateral ventricles, reduced whole-brain, temporal and frontal lobe volumes (Gur et al., 2007, Haijma et al., 2013). However, evidence also exists for the involvement of other brain regions, such as the parietal and occipital cortices (Olabi et al., 2011, Kubicki et al., 2007), as well as volumetric reductions of the insula and anterior cingulate (Smieskova et al.,

2013). Increasingly, methods looking at investigating brain structure and function in schizophrenia are moving away from assessing circumscribed alterations in specific regions, and rather to assess the brain as a whole, specifically connections between brain regions (Friston & Frith, 1995). The hypothesis that brain alterations in schizophrenia are primarily characterized by problems in connectivity has emerged as a central theory of the underlying pathophysiology of schizophrenia (Friston 1998, Pettersson-Yeo et al., 2011, Friston & Frith, 1995, Stephan et al., 2009, Frith 1997).

Evidence from functional connectivity studies of patients have indicated that disruptions occur in multiple brain systems concurrently, demonstrating that the brain alterations in schizophrenia may be primarily characterized as dysfunctional connections across distributed brain systems (Lawrie et al., 2002, Guo et al., 2014, Ma et al., 2014, Fornito et al., 2012, Collin et al. 2013) Multivariate machine-based learning methods have also been implemented (Koutsouleris et al. 2009), and there is some evidence that this technique has the capacity to predict disorder status at a single-patient level, thus having a strong clinical potential in elucidating markers of the disorder (Please see Zarogianni et al. 2013 for detailed review). Methods aiming to measure graph and complex network properties are being increasingly utilized to investigate structural brain alterations in schizophrenia as well as other psychiatric disorders (Bullmore and Sporns 2009), and diffusion tensor imaging studies have showed an abnormal organization of water flow through neurones as constrained by several white matter tracts of the brain in patients compared to controls (Sprooten et al., 2011).

Structural MRI (sMRI), aims to look at specific structural parameters of the brain, such as gray or white matter, in order to assess whether these differ in patients or high risk cohorts compared to controls. Anatomical networks derived from inter-regional covariance estimates of gray matter of cortical networks have also shown differences between patients and controls, suggesting that disturbed structure may arguably underlie disturbed function in schizophrenia (Boch et al., 2013).

Converging evidence suggests that structural abnormalities evident in schizophrenia precede disorder onset, and may be attributed to connectivity issues outlined above, which may be more macroscopically displayed as structural alterations in brain parameters such as cortical thickness, surface area and gyrification, or in more circumscribed regions such as the hippocampus (Fusar-Poli et al., 2011). It has also been suggested that, if schizophrenia is partially caused by an early brain insult it may, when coupled with ongoing pathological processes such as alterations in synaptic pruning, lead to longitudinal and progressively displayed structural abnormalities (Olabi et al. 2011).

However, many of the studies mentioned above did not use first-episode patient samples. As length of illness increases, several confounding influences become more prominent. Elevated substance abuse associated with the disorder, as well as high doses of anti-psychotic medication and length of illness itself have all been shown to have significant effects on structural brain alterations in patients (Fusar-Poli et al., 2011, Van Haren, 2011). Hence, some of the ambiguity pertaining to the relationship

between the timing of the development of these structural abnormalities in various regions and the onset of schizophrenia may be avoided by exploring whether these alterations can be observed in patients that are in/or have just experienced their first episode of schizophrenia. It is important to explore brain structure early in the disorder as it may be that these regions reflect the core regions that are abnormal in schizophrenia, and therefore provide a key for earlier diagnosis (Chakos et al. 2005). By focusing on the extent to which brain alterations form predictive markers of the disorder, researchers aim to facilitate pre-onset/first-episode interventions (Pantelis et al. 2005) as early diagnosis has been associated with an improved clinical outcome.

1.1.3 Clinical and Familial High Risk Paradigms

High risk/first episode paradigms may serve to disentangle the extent to which alterations of brain structure are associated with an early neurodevelopmental vulnerability to develop schizophrenia, or whether they only occur with the manifestation of frank psychosis. One reason why high risk studies can elucidate this is because they are more robustly able to determine the extent to which structural abnormalities in schizophrenia form part of the underlying risk and/or pathophysiology. As one of the primary aims of investigating both functional and structural brain abnormalities in schizophrenia is to understand whether they are associated with the illness itself, predisposing genetic factors, and/or secondary factors relating to the illness such as substance abuse, paradigms that allow as clear as possible investigation of this remain paramount. sMRI studies of patients may

inadvertently incorporate confounding factors that obfuscate our understanding of the causality of any abnormalities.

In order to more directly infer whether brain abnormalities in schizophrenia are of neurodevelopmental origin, prospective studies of relatives, or individuals exhibiting clinical symptoms without yet having made the transition to psychosis (high-risk studies, HR), have been employed. If indeed structural alterations are biological precursors of the disorder, reflective of its neurodevelopmental origin, these should also be present in individuals at high risk of schizophrenia, albeit perhaps to a lesser extent. As individuals at high risk, for either clinical or familial reasons, have an increased vulnerability of transitioning to the disorder (Pantelis et al. 2003), this paradigm has the capacity to distinguish between those high risk individuals that transition to the disorder (HR-T) compared to those that do not (HR-NT), in order to evaluate whether structural alterations underlie a general vulnerability to the disorder or are specifically associated with mechanisms that go above and beyond a general disposition, selectively present in HR-T. These studies may therefore also elucidate risk factors exaggerating brain abnormalities, as well as those conferring resilience to the disorder.

Clinical high risk individuals are selected on the basis that they exhibit prodromal signs of schizophrenia, however have not yet made the transition to full psychosis (Cornblatt et al., 2002). In general, these individuals most often present mild and/or transient positive and negative (Riecher-Rossler et al. 2009) psychotic symptoms,

without reaching the level required for clinical diagnosis, and may also present with mild cognitive and social deficits (Brewer et al. 2006). The rates of conversion for these individuals are significantly higher than for the general population, yet not inevitable, with a large proportion of individuals never developing full-blown psychosis. Rates of conversion vary across studies, with some reporting as many as 35% of cohorts transitioning (Pantelis et al. 2003, Yung et al. 2004). However, the definitions of these high risk individuals often differ based on the centre of investigation and criteria used for inclusion (for a detailed summary of the different symptomatology used to define these individuals see Smieskova et al., 2010, & Fusar-Poli et al. 2013 for a detailed review of clinical high risk individuals in general).

In familial high risk paradigms, individuals are selected on the basis that they have at least one affected family member, usually including first/second-degree relatives (Johnstone et al., 2005). Twin studies of monozygotic and dizygotic twins discordant for schizophrenia have also been conducted (Cannon et al., 2002). Schizophrenia is known to have a significant heritable component (Lawrie et al., 2001, Harrison and Weinberger, 2005) and by assessing relatives that share a high proportion of genes with affected family members during the critical ages of illness development, familial high risk paradigms have the capacity to more directly infer the extent to which variations in brain structure form part of the genetic risk of the disorder or are associated with aspects of symptomatology and/or environmental factors. Although familial high risk investigations tend to report lower conversion rates than clinical high risk studies, with estimates of approximate 13% transition rate (Lawrie et al.

2001), they enable researchers to more thoroughly assess whether brain abnormalities form part of a general vulnerability to schizophrenia, as this paradigm allows investigation of individuals that are deemed at familial high-risk but may not be presenting with any symptoms. Thus, if any brain alterations are present, these can be more directly inferred to be associated with an underlying vulnerability to develop the disorder.

Most studies that investigate familial and clinical high risk cohorts have been cross-sectional, however some have shown that dynamic changes occur before disorder onset in those at high risk for familial or clinical reasons (Pantelis et al. 2003, Job et al. 2005, Carletti et al. 2012, McIntosh et al. 2011). This suggests that a vulnerability to schizophrenia entails both initial structural abnormalities coupled with aberrant development. Clearly, cross-sectional studies cannot distinguish whether incipient changes alone characterize susceptibility for psychosis, or whether such vulnerability is also associated with additional abnormal developmental trajectories. Longitudinal analyses of those at familial high risk for schizophrenia are thus a critical complement to cross-sectional observations.

Most clinical high risk patients experience symptoms, although some 10% or so are defined as having a family history and functional decline, and accordingly often have higher rates of transition than familial high risk studies (Pantelis et al. 2003). Thus, the transition may be easier to assess than in familial studies, whilst some familial high risk studies may allow fuller study of trait factors and longer study of the

prodrome without the confound of antipsychotic medication. Both groups can also be investigated at ages when the risk of onset has diminished, to allow the extent to which abnormalities represent trait markers of the disorder to be evaluated more thoroughly. Overall, both clinical and familial high risk paradigms involve individuals with genetic and environmental risk factors and likely index some common pathophysiology of schizophrenia.

Several aspects of familial and clinical high risk paradigms overlap, as many people at clinical risk also have a family history of the disorder, and individuals at familial high risk exhibit more symptoms than the general population (Pantelis et al., 2005, Fusar-Poli et al. 2011). Indeed, a recent systematic review found overlapping brain alterations in familial and clinical high risk samples in the frontal, temporal, and cingulate regions (Smieskova et al., 2013), suggesting that common markers of schizophrenia can be identified based on these two high risk paradigms, and thus to increase the evidence base for this chapter, both paradigms will be discussed, although the following data-containing Chapters will focus only on familial high risk individuals.

1.1.4 Scope of Present Chapter

The aim of this chapter is to review the sMRI studies conducted on familial and clinical high risk individuals and as such assess the extent to which evidence for structural markers indicating a general vulnerability to the disorder exist, as well as those indicating that transition to psychosis is likely. First, hand-traced and/or semi-automated structural ROI studies of high risk individuals will be discussed, and I will

subsequently move on to other approaches that have been widely applied in high risk research, such as Voxel-Based Morphometry (VBM) and Diffusion Tensor Imaging (DTI), as well as more recently applied surface-based methods.

1.2 sMRI studies of Familial and Clinical High Risk Cohorts

Most early sMRI studies of high risk cohorts focused on specific regions of the brain thought to be implicated in schizophrenia; however more recently whole-brain analyses as well as approaches measuring putative indices of structural connectivity have emerged. Below we present findings from studies to date, and go on to discuss the aims and objectives of the present thesis in the framework of the studies evaluated in this Chapter.

1.2.1 Hand-Tracing and Semi-Automated Investigations of Gray Matter Volume in High Risk Individuals.

The majority of structural studies of high risk subjects have been conducted on the volume of temporal and frontal regions as these were thought to be critically involved in the pathogenesis of schizophrenia, as these are thought to mediate functions that are impaired in the disorder, such as executive functioning and episodic memory (Smieskova et al., 2010). Familial high risk studies have found significant volume reductions bilaterally in the amygdala and hippocampus, as well as the thalamus, compared to healthy controls (Lawrie et al., 1999, Seidman et al., 1999, Keshavan et al., 2002). This has also been confirmed in a meta-analysis which

also found evidence of a reduction in total cerebral cortex volume (Boos et al., 2007). However some other studies have failed to report this direction of difference in clinical or familial high risk subjects in these regions (Table 1.1).

One of the reasons why regions such as the hippocampus, amygdala and nucleus accumbens have been so extensively studied is because several post-mortem studies have implicated the limbic system as a possible substrate for the cognitive and emotional abnormalities observed in patients suffering from schizophrenia (Bogerts et al. 1990, Lawrie et al. 2001, Lauer et al. 2001). Some of the evidence for this derives from the observation of schizophrenia-like symptoms observed in cases of known limbic-system neuropathology, such as temporal-lobe epilepsy, encephalitis and brain tumours (Torrey & Peterson, 1974), as well as signs of limbic-system dysfunction in some patients diagnosed with schizophrenia. Abnormal neurological signs in patients, including autonomic dysfunction, proprioception, vestibular dysfunction, and eye-tracking, also point towards disturbances in the subcortical area of the brain which includes the limbic system (Torrey & Peterson, 1974). The nucleus accumbens has been reported to be smaller in patients with schizophrenia compared to controls (Pakkenberg 1990) and is an integral and important part of limbic and prefrontal cortico-striato-pallidal-thalamic circuitry and is involved in several cognitive, emotional and psychomotor functions altered in schizophrenia (Lauer et al. 2001). In animal models, developmental disturbances within the hippocampus and amygdala induce a dysregulation of inputs to the nucleus accumbens, resulting in schizophrenia-like symptoms (Lauer et al. 2001).

Nonetheless, due to the complex neuroanatomy of the human ventral striatum hardly any morphometric data on the nucleus accumbens are available, and up until recently methods used to delineate the hippocampus and amygdala separately have been scarce and time-consuming. Therefore, research on these structures not only in high risk samples but also in patients, is lacking.

However, with the advent of techniques such as Freesurfer, which enable less time-consuming estimates of these brain structures and place less importance on inter-rater reliability, it is becoming increasingly possible to study these three important subcortical structures in more depth in relation to schizophrenia. To our knowledge, no current study has used Freesurfer to investigate these three structures in both a patient and a familial high risk sample concurrently, and thus doing so would provide highly important and novel information about the importance of these limbic system structures in schizophrenia. Especially in a large dataset such as the present, when detailed manual tracing techniques are not feasible within specific allocated time-frames. However, although Freesurfer has many strengths, it is also important to recognize some of its limitations in relation to delineating these three structures, which will be outlined in greater detail in section 1.3 below.

Authors	Subjects	Method	Results	Further Comments
Phillips et al. (2002)	32 FEP 20 HR-T 40 HR-NT 139 HC	Manual Tracing /ANALYZE	HR ↓ bilateral hippocampal volume than HC HR-T ↑ left hippocampal volume compared to HR-NT	Volumetric increases in the hippocampus associated with subsequent transition to psychosis
Wood et al. (2005)	35 familial HR 44 clinical HR 49 HC	Manual Tracing	No significant differences between HR and HC in hippocampal volume Left hippocampus ↓ clinical HR compared to familial HR No differences based on outcome (eg HR-T or HR-NT)	Clinical HR showed hippocampal abnormalities not present in familial HR individuals, authors suggest that hippocampal abnormalities may be more environmental than genetically driven.
Velakoulis et al. (2006)	89 chronic SZ 162 FEP 96 clinical HR-NT 39 HR-T	Manual Tracing	Both HR-T and HR-NT had normal hippocampal and amygdala volume whilst only FEP and chronic SZ displayed hippocampal reductions	Authors conclude that medial temporal changes do not occur before illness onset
Buehlmann et al. (2010)	16 clinical HR-T 21 clinical HR-NT 23 FEP 22 HC	Manual Tracing	Left hippocampus ↓ in FEP compared to HR and HC. Left hippocampus ↑ in HR-T compared to FEP	Moderate evidence for hippocampal abnormalities reflecting a vulnerability marker of psychosis

Table 1.1 Region of interest based approaches to brain structure in clinical and familial high risk studies.

HC: healthy control; **HR:** high-risk; **HR-NT:** HR-no transition; **HR-T:** HR-transition; **FEP:** first episode patient.

Familial high risk studies have also used automated ROI extraction of pre-frontal, as well as temporal lobe volume, to investigate baseline and longitudinal change in HR-T compared to HR-NT, before HR-T transitioned to the disorder. The Edinburgh High Risk Study (EHRS) found no differences in temporal lobe volumes amongst those at high risk. However, at baseline they found that all high risk individuals had significantly smaller left and right pre-frontal lobes compared to controls, but reported no differences between HR-T and HR-NT. Longitudinally, the authors report significantly greater reductions in left and right frontal lobe volume in HR-T compared to HR-NT (McIntosh et al., 2011). Reduced superior temporal gyrus (STG) volume has also been found in ROI studies, both familial (Rajarethinam, 2004) and clinical (Takahashi et al., 2009a). The latter study also found longitudinal gray matter reductions in the left superior temporal gyrus in HR-T compared to HR-NT.

Several other brain regions that have been associated with schizophrenia have also been investigated in those at high risk. A study of the insular cortex of clinical high risk individuals found that HR-T had significantly smaller bilateral volumes of the insular cortex compared to HR-NT as well as controls in the right hemisphere. Longitudinally, HR-T subjects showed greater reductions bilaterally in the insular cortex compared to controls and HR-NT (Takahashi et al., 2009b). Another longitudinal study of familial high risk individuals measured several ROIs, and found that relatives declined but controls increased or remained stable in bilateral lateral orbitofrontal, left rostral anterior cingulate, left medial prefrontal, right inferior

frontal gyrus and left temporal pole volumes at follow-up relative to baseline. This study also found that smaller volumes predicted more severe prodromal symptoms at follow-up and that greater longitudinal decreases in volumes predicted worsening of these symptoms over time (Bhojraj et al., 2011).

Some of the inconsistency regarding disparate findings on the same brain regions in ROI studies may have arisen from the considerable variation associated with the labelling and selecting of neuroanatomical regions for ROI analysis. Studies have found that specifically for smaller subcortical structures there exists less reliability in tracing (Whalley & Wardlaw 2001). These studies may have been limited in their ability to capture the true extent of brain alterations in those at clinical or familial high risk of the disorder, as they only focus on very circumscribed regional variation using disparate methodologies, whereas studies are converging to suggest that several brain regions are implicated in both those at clinical and familial high risk (Fusar-Poli et al., 2011). Therefore, there remains a gap in knowledge regarding a) the extent of which brain alterations do occur in schizophrenia and b) whether the localized subcortical alterations occur in part as a function of the disparate methodologies described above, that rely a lot on reliability across tracers.

Methods such as Freesurfer, a semi-automated segmentation software, aim to reduce the variance associated with manual tracing that can sometimes occur in ROI studies that rely on hand tracing, as they rely on normalizing the image to a template based on probabilistic information, thus reducing inter-rater variability (Fischl et al. 2002). Furthermore, these methods significantly reduce the time required to process large

and complex datasets, allowing use of more scans and larger sample sizes. If the underlying pathophysiology of schizophrenia indeed affects mechanisms of synaptic efficacy/plasticity, regional abnormalities as well as structural and functional disconnections may be expected in regions showing high plastic potential (Friston et al. 1995). It is interesting that ROIS implicated in studies of those at high risk do indeed involve regions that have the molecular machinery for synaptic remodelling and neuromodulatory control, such as the hippocampus, and thus it is important to not bypass key subcortical regions when investigating the brain in relation to schizophrenia, even when also assessing more global structures.

1.2.2 Voxel-Based Morphometry Approaches of Gray Matter Volume in in High Risk Individuals

Voxel-based morphometry (VBM) is an automated method whereby local differences in the composition of brain tissue, such as gray matter density, can be identified between groups on a voxel-wise basis (Ashburner and Friston, 2000). VBM studies are increasingly used in high risk research as they do not define anatomical borders *a priori*, producing an unbiased view of gray or white matter change across the whole brain.

1.2.2.1 Cross-sectional Findings

Several cross-sectional studies and meta-analyses of VBM studies have been conducted in familial and clinical high risk cohorts. Cannon et al. (2002) used three-

dimensional cortical surface mapping of monozygotic and dizygotic twins discordant for chronic schizophrenia, as well as matched control twins. This study found that decreasing gray matter volume was associated with increased genetic risk for schizophrenia in dorsolateral and polar prefrontal cortex, as well as several other areas, including Broca's and Wernickes area. Job et al. (2003) also used the same familial high risk cohort as that described in the present thesis and found volumetric reductions in the anterior cingulate as well as a trend for a reduction in the left parahippocampal gyrus in those at high risk compared to healthy controls, whilst no differences were reported between HR-T and HR-NT at baseline. Meisenzahl et al. (2008) found gray matter reductions in clinical high risk subjects, compared to a healthy control group, in frontal, lateral temporal and medial temporal regions, but did not assess whether subjects transitioned or not. Borgwardt et al. (2007) found that HR-T had smaller gray matter volumes of the posterior cingulate gyrus, precuneus and paracentral lobule bilaterally and in the superior parietal lobule, coupled with volumetric increases in a left parietal/posterior temporal region.

A recent voxel-based meta-analysis concluded that individuals at an enhanced risk of psychosis, for both clinical and familial reasons, had reduced gray matter volume in the right superior temporal gyrus, left precuneus, left medial frontal gyrus, right middle frontal gyrus, bilateral hippocampal/parahippocampal regions and bilateral anterior cingulate (Fusar-Poli et al. 2011). This meta-analysis also found that further gray matter reductions were present selectively in HR-T in the right inferior frontal gyrus and in the right superior temporal gyrus. Another meta-analysis found volumetric reductions in temporal, cingulate, insular, prefrontal cortex and

cerebellum of HR-T compared to HR-NT, occurring before transition to psychosis (Smierskova et al. 2010). Cooper et al. (2014) investigated familial high risk subjects and found that they exhibited volumetric increases in the left medial frontal gyrus, compared to controls, but also decreased gray matter in the left thalamus and putamen, as well as right superior frontal gyrus and left insula.

1.2.2.2 Longitudinal Findings

Whether abnormal developmental trajectories are implicated in the volumetric changes seen in high risk subjects remains to a large extent unknown. The main reason for this is that there are relatively few longitudinal studies of normal brain development, let alone of high risk subjects as they transition to the disorder. However, Pantelis et al. (2003) found that HR-T exhibited longitudinal gray matter reductions in the left parahippocampal, fusiform, orbitofrontal and cerebellar cortices, as well as the cingulate gyri as they transitioned, whilst HR-NT had changes restricted to cerebellum. Borgwardt et al. (2008) also investigated clinical high risk subjects and found that HR-T had cortical gray matter volume reductions in the orbitofrontal cortex that included the right orbital and left rectal gyrus and in the right inferior temporal, superior frontal, superior parietal and the left precuneus and the right cerebellum. This, along with Pantelis et al (2003) study suggests that some of the structural alterations in high risk subjects occur during transition to psychosis, thus reflecting mechanisms that go beyond a general vulnerability to the disorder. A study by Job et al. (2005) used VBM to map changes in gray matter over time in familial high risk subjects and also found progressive alterations in HR-T occurring

in the right frontal and temporal lobes, that were not present in HR-NT, and some of these changes were also able to differentiate those with psychotic symptoms who did not make the transition from those who had symptoms and did develop schizophrenia.

1.2.2.3 Summary Gray Matter Volumetric Approaches

Neuroimaging studies are gradually moving away from ROI approaches, as it is increasingly clear that multiple brain regions are implicated in an elevated risk for developing schizophrenia. There is now also converging evidence from meta-analyses that whole-brain parameters are altered in high risk individuals. Smieskova et al. (2010) revealed small to medium effect sizes for larger global volumes in HR-T relative to HR-NT, but also compared to first episode patients. These global measures included whole-brain volume, intracranial volume and total gray matter volume. White and Hilgetag (2011) argue that a symptomatology as complex as that of schizophrenia is likely to be associated with a global deficit and therefore it remains debatable to what extent the high risk state is associated with only small-scale alterations, as opposed to widespread alterations. Longitudinal studies suggest that markers to transition may involve disruption of the developmental trajectory of the brain, as opposed to static abnormalities. However, it is also possible that these longitudinal findings are related to risk factors such as substance abuse, and/or medication status changes as symptom severity increases. More longitudinal studies of both healthy and high risk individuals are required to elucidate possibly deviant developmental trajectories, which may be obscured cross-sectionally, potentially hindering the search for reliable markers.

However despite some advantages over standard ROI studies, VBM is problematic to an extent in that it is designed to be sensitive to small-scale structural differences, while discounting both important structural positional information, as well as large-scale volumetric changes (Ashburner & Friston, 2000). Although global measures, such as brain volume or whole brain grey matter, may have the potential to have more predictive validity for classification and prognosis schemes, they are still at present unable to inform about the underlying psychophysiology of schizophrenia. Future studies that are able to more specifically investigate connectivity may be better able to inform our studies of what drives brain alterations in those at high risk, and whether they are specific to those that transition to schizophrenia, or form general vulnerability markers.

1.2.3 White matter Investigations

The white matter (WM) of the human brain consists of glial cells and myelinated axons, which connect regions of the cerebral cortex (Kubicki et al., 2007).

Myelination is thought to be a critical process affecting neuronal connectivity, and abnormalities have been observed in post-mortem studies of patients (Uranova et al., 2001, 2004). There are two main approaches to examining WM, either using volumetric measures derived from T1 images or by the study of white matter integrity from DTI. Several studies have indicated both global and regional changes in white matter volume in patients with schizophrenia (see Kubicki et al., 2007 for a detailed review). DTI maps the diffusion anisotropy of white matter tracts and

outputs measures such as mean diffusivity (MD) and fractional anisotropy (FA), which indicate how coherent and organized the water is as it flows through the molecules, forming a putative index of white matter integrity potentially associated with myelination (Alexander et al., 2007). It has therefore been suggested that white matter integrity can form an indirect marker of disrupted structural connectivity. An overview of cross-sectional DTI studies of both clinical and familial high risk subjects is provided in Table 1.2.

Table 1.2. Overview of cross-sectional diffusion tensor imaging (DTI) studies of both clinical and familial high-risk (HR) subjects

Authors	Participants	Method	Results	Further Comments
Maniega et al. (2008)	22 familial HR 31 SC 51 HC	VBA-ROI	HR ↓ in anterior limb of internal capsule compared to controls	SZ ↓ FA bilateral uncinate, left arcuate, left and right ALIC
Hoptman et al. (2008)	22 familial HR 37 HC 23 SC	VBA-WB	HR ↓ FA left inferior frontal gyrus, bilateral cingulate, angular gyrus compared to HC. HR ↑ FA middle frontal gyri subgenual anterior Cingulum bundle L, pontine tegmental Bilateral compared to HC No group differences	Most SC family members of HR
Peters et al. (2008)	10 Clinical HR 10 SZ, 10 SA 10 HC	ROI - Fiber-tracking	No group differences	
Peters et al. (2009)	Same subjects as Peters et al. (2008)	VBA	UHR: FA ↓ superior and middle frontal BL	SZ,SA: FA ↓ temporal & parietal BL, frontal L
Hao et al. (2009)	34 familial HC 34 SC 32 HC	VBA-WB	SC and HR shared ↓ FA in left prefrontal cortex and the hippocampus compared to HC.	Familial HR siblings of the SC patients of the study. SC ↓ FA in anterior cingulate.
Karlsgodt et al. (2009)	36 clinical HR 25 HCs	TBBS-ROI	This difference not significantly different between SC and HR. HR ↓ FA in superior LF	↓ FA in MTL and LF predicted deterioration in social and role functioning at 15-month follow-up.
Camchong et al. (2009)	Study 1: 18 miz twin pairs Study 2: 22 relatives of SC	VBA-TBBS & ROI	Study 1: FA values more strongly correlated between MZ twin pairs than randomly paired in genu of corpus callosum, anterior cingulum, and forceps minor. Study 2: HR ↓ reduced FA in medial frontal white matter compared to HC. HR-NT ↓ FA in bilateral medial frontal compared to controls. HR-T ↓ FA temporal compared to HR-NT ↑ FA in L MTL	Anatomical connectivity highly heritable and may serve as endophenotype for SC Positive PANSS scores correlated negatively with FA in temporal regions
Bloemen et al. (2010)	27 HR-NT 10 HR-T 10 HC	VBA	No group differences	
Peters et al. (2010)	7 HR-T 10 HC	ROI Fiber-tracking	No group differences	
Von Hohenberg et al. (2013)	28 clinical HR 34 HC	TBBS-WB	HR ↑ MD in R superior longitudinal fasciculus, posterior corona radiata and corpus callosum compared to HC HR ↑ RD restricted area posterior parietal lobe compared to HC	Rate of conversion low White matter alterations may form markers

FA: fractional anisotropy; HC: healthy control; HR: high-risk; L; left; MD: mean diffusivity; R: right; HR-NT: HR-no transition; HR-T: HR-transition; ALIC: anterior limb of the internal capsules; LF: longitudinal fasciculus; MTL: medial temporal lobe; MZ: monozygotic; PANSS: the positive and negative syndrome scale for schizophrenia; RD: radial diffusivity; ROI: region of interest; SA: schizoaffective; SZ: schizophrenia; VBA: voxel-based analysis; TBBS: tract-based analysis; VBA: voxel-based analysis; TBBS: tract-based spatial statistics; HR: high risk; WB: whole brain ,temporal lobe; MZ: monozygotic; PANSS: the positive and negative syndrome scale for schizophrenia; RD: radial diffusivity; ROI: region of interest; SA: schizoaffective; SZ: schizophrenia; VBA: voxel-based analysis; TBBS: tract-based

The majority of these studies indicate that WM is widely affected in individuals at an elevated risk; however the majority of these studies have not assessed differences between HR-T and HR-NT, potentially producing a pool of heterogeneous participants. Furthermore, at present there exist very few longitudinal DTI studies of high risk individuals. Carletti et al (2012) conducted a longitudinal clinical high risk study and found group differences over time, driven by progressive reductions in FA in HR-T of left frontal white matter, compared to HR-NT. Additionally, it remains unclear to what extent DTI really outputs an indirect measure of myelination (Davis et al. 2003) and this needs to be understood further.

1.2.4 Cortical Surface-Based Approaches

Theories are converging to posit cortical dysconnection as central to the pathophysiology of schizophrenia (For a review see White and Hilgetag, 2011). With the advent of sMRI tools specifically designed to investigate cortical morphology, researchers have been able to more precisely delineate separable components of the cortex. This may have significant implications for understanding the biological underpinnings of schizophrenia, as cortical gray matter is a product of cortical thickness and surface area, which have been shown to have distinct neurodevelopmental and genetic antecedents (Panizzon et al., 2009). However, at

present, most studies of high risk individuals have used volumetric, as opposed to surface based approaches, to investigate the cortex.

Palaniyappan and Liddle (2012) used surface based morphometry (SBM) and VBM in a sample of schizophrenia patients, and found that SBM parameters, such as thickness, surface area and gyrification made distinct but regionally variable contributions to group differences, suggesting that differences in cortical gray matter volume are partially mediated by contrasting cortical parameters. Therefore, SBM methods may be able to specify markers of psychosis that lie closer to the action of specific genetic processes than volumetric methods, which is especially important when looking at familial high risk cohorts.

1.2.4.1 Cortical Thickness and Surface Area

1.2.4.2 Cross-sectional Findings

Cortical thinning has been found in patients with schizophrenia (Narr et al., 2005; van Haren, 2011). Sprooten et al. (2013) found that those at familial high risk displayed cortical thinning in a restricted portion of the left middle temporal lobe but found no differences between HR-T and HR-NT on a single scan on average of 2.5 years before transition. A clinical high risk paradigm found that compared with controls, those at increased risk showed reduced cortical thickness in the right parahippocampal gyrus. Consistent with Sprooten et al. (2013), this study reported no significant differences in cortical thickness between the HR-T and HR-NT some 6

months before transition (Tognin et al., 2013). Another familial study, conducted by Goghari et al (2007), evaluated sulcal thickness, surface area and gray matter volume in frontal and temporal regions and found that high risk individuals had reversed hemispheric asymmetry in their cingulate sulcal thickness and bilaterally thinner cortices in the superior temporal sulcus compared to controls. Interestingly, no significant differences in surface area or gray matter volume were found. Haller et al. (2009) investigated cortical thickness in a clinical high risk sample and found no thickness differences between groups, yet found that cortical thickness asymmetry distinguished first episode patients from healthy controls, with a trend for this relationship between high risk individuals and controls. Benetti et al. (2013) investigated cortical thickness and gray matter and found evidence of cortical thinning in the right superior temporal gyrus in individuals at clinical high risk, with no concurrent gray matter changes. Goldman et al. (2009) investigated heritability estimates and cortical thickness on a node-by-node basis in healthy controls, patients with schizophrenia and their unaffected siblings to investigate the extent to which cortical thickness reductions were mediated by genetic risk. This study identified widespread cortical thickness reductions in patients, primarily in the prefrontal cortex, however found only a trend level for similar reductions of thickness in siblings. Thus, the authors conclude that thickness may not be a strong intermediate phenotype for schizophrenia.

Goghari et al. (2006) also used a familial high risk paradigm to investigate surface area and cortical thickness, as well as gray matter volume. The authors included both global measures, such as total gray matter volume, surface area and cortical thickness,

as well as regional parcellations derived from an automated methodology. Compared to controls, relatives had decreased volume and surface area in the right cingulate gyrus, bilateral decrease in cingulate thickness and decreased surface area in the superior temporal lobe. In addition, relatives had a subtle increase in gray matter volume and surface area in the left hemisphere, bilaterally in the parahippocampal gyri, left middle temporal lobe. Gogtay et al. (2007) investigated cortical development in non-psychotic siblings of patients diagnosed with child onset schizophrenia and found that siblings showed significant deficits in left prefrontal and bilateral temporal cortices, however also that these deficits seemed to disappear by age 20.

1.2.4.3 Longitudinal Findings

Very few longitudinal studies of cortical thickness and/or surface area have been conducted in familial high risk studies. However, a familial high risk study conducted by Prasad et al. (2010) found that at baseline those at high risk showed reduced gyral surface area in the fronto-parietal lobes, along with increased sulcal curvature and thinner parietal gyral cortices, compared to controls. Longitudinally, there was a decrease of surface area, particularly in bilateral frontal and occipital regions, unaccompanied by changes in thickness. In contrast, healthy individuals showed preserved or increased surface area and cortical thinning. However, Prasad et al. (2010) did not distinguish between HR-T and HR-NT. In a study of individuals at clinical high risk for psychosis, Sun et al. (2009) found contraction of the right dorsolateral prefrontal cortical surface area over one year among HR-T, compared to

HR-NT. Fornito et al. (2008) investigated the anterior cingulate cortex longitudinally, including gray matter volume, surface area and cortical thickness and found that relative to controls, HR-T displayed bilateral thinning of the rostral paralimbic anterior cingulate cortex, whereas HR-NTs showed a relative thickening of dorsal and rostral regions that was correlated with anxiety ratings. Ziermans et al. (2012) also investigated rate of change over time of cortical thickness in clinical high risk subjects, finding that HR-T exhibited widespread cortical thinning compared to controls in the left anterior cinuglate, precuneus and temporo-parietal-occipital area.

1.2.4.4 Gyrification

Gyrification is the term used to describe the way in which the cortex forms its characteristic convoluted shape, and refers specifically to the extent of folding of the cortex (White & Hilgetag, 2011). This is often measured as a gyrification index (GI), which provides an estimate of both the mean value of gyrification across the whole brain as well as specific analysis of particular brain regions (Zilles et al. 1988). The pattern and extent of gyri and sulci in the human brain started receiving attention as far back as 1907 with the work of Spitzka, who spent a considerable amount of research effort investigating the gross morphology of the brain (Zilles et al. 1988). However, it is only with the advent of more modern imaging techniques that gyrification was measured in more depth, by investigating the GI as defined by the ratio of inner and outer contours of the convolutions of the cortex (Zilles et al. 1988). Hence, a cortex with a large amount of folding will have a greater GI, compared to cortices with less folding, which will have a smaller GI.

The first studies to use this measure often used classical two-dimensional GI, which is the ratio of the total pial cortical surface over the perimeter of the brain but on two-dimensional coronal sections (2D-GI). Because of its easy interpretation and its simplicity in implementation, many studies have been conducted using the Gyrification Index to identify abnormal cortical complexity in neurodevelopmental and psychiatric disorders (Schauer et al. 2008). However, some issues with these original 2D-GI were that the two dimensional coronal slices did not accurately take into account the inherent three-dimensional nature of the cortical surface, and thus these estimates could easily become biased by slice orientation, or by the presence of buried sulci. Hence, more recent studies using Freesurfer have been able to bypass these problems by incorporating sophisticated three dimensional GI (3D-GI) techniques (For a more in depth explanation of gyrification and the Freesurfer technique, Please see Chapter 4).

Empirical studies have shown that gyrification does not occur due to the constraints of an inflexible cranium, as folding of the cortex has been demonstrated in cultured brains of rodents in the absence of skull (Kingsbury et al., 2003). Van Essen's (1997) prominent theory posits gyrification as an intrinsic property of the growing cortex, suggesting that the neuronal connectivity established during the second trimester of development produces fibre tension of axons, drawing together densely connected regions, forming gyri, whereas less heavily connected regions drift, and create sulci. Thus the characteristic shape of the cortex is linked to an effective way to transit neural signals, ie neuronal connectivity (White and Hilgetag, 2011).

There are inconsistent findings regarding abnormal gyrification both in patients (Schultz et al., 2013, Nesvåg et al., 2014) as well as high risk individuals. Harris et al. (2007) used hand-traced gyrification indices in a familial high risk sample and found increases in right prefrontal lobe GI values in HR-T individuals compared to HR-NT. Interestingly; these indices were from individuals that did not transition to schizophrenia until an average of 2.5 years later. Falkai et al. (2007) investigated frontal as well as parieto-occipital GI in families affected with schizophrenia and found that frontal, but not parieto-occipital GI was significantly higher in schizophrenic patients as well as unaffected relatives compared to controls. However, Jou et al. (2005) found reduced left frontal cortical folding in unaffected relatives. Schaer et al. (2006) investigated individuals with the 22q11.2 deletion syndrome, a genetic condition associated with increased risk for developing schizophrenia. Those with the deletion, compared to controls, showed a significant decrease of GI in the frontal and parietal lobes compared with controls. Disparate sample sizes and methodologies across studies have been cited as reasons for inconsistent findings in gyrification across studies.

1.2.5 Summary Surface Based Approaches

Cross-sectional SBM studies suggest that some minor reductions in cortical thickness is associated with the high risk state, but studies remain too few to date to clearly delineate markers of psychosis as opposed to a general vulnerability, or even to determine whether these exist. These techniques remain relatively few and new in

high risk research; several of them implement ROI strategies, as opposed to whole-brain methods. Future studies of cortical thickness across the cortex will be required to determine in an unbiased fashion the extent of any abnormalities.

Longitudinal surface-based investigations to date, although few, indicate that cortical surface area and thickness are differentially affected in schizophrenia and also in those at high risk, which is in keeping with empirical studies that have found evidence that these parameters develop differentially over time, even in typically developing individuals (Wierenga et al., 2014). Thus, surface-based investigations may be more sensitive than volumetric studies, as the latter may obscure important biological information pertaining to disrupted genetic/neurodevelopmental processes. However, studies of these parameters, as well as gyrification, of which there exist no longitudinal studies in high risk individuals, are not consistent in the direction of this divergence and remain too few at present time to provide definitive answers as to the underlying causes of it.

Gyrification remains the cortical parameter that is perhaps most linked to underlying neuronal connectivity (Van Essen 1997). Yet, this method is arguably the one associated with the most methodological limitations of previous studies. Many studies of gyrification in high risk individuals have focused their analysis on a very small numbers of slices, probably due to the time-consuming nature of manually hand-tracing these indices. This greatly reduces the reliability of interpretations of gyrification studies to date. Neuroimaging packages such as Freesurfer now provide less time-consuming ways of measuring gyrification (Schaer et al. 2008), and do so on a whole-brain basis.

1.3 The Freesurfer Technique: Strengths and Limitations in Relation to the Present Thesis

The present thesis uses Freesurfer, a relatively new structural neuroimaging package, to investigate both cortical and subcortical alterations in the Edinburgh High Risk Study of Schizophrenia. Although its strengths and limitations are mentioned in more depth in the following Chapters, it is worth mentioning here some of its most important strengths and weaknesses in order to justify why this technique was chosen for this thesis, and in relation to what brain structures/brain parameters.

The FreeSurfer Software Suite is an open source software suite for processing and analysing human brain MRI images. Skull stripping, image registration, subcortical segmentation, cortical surface reconstruction, cortical segmentation, cortical thickness estimation, longitudinal processing, fMRI analysis, tractography, FreeView visualization are some of the mentionable features of FreeSurfer (Fischl et al 2002). This software tool was developed at the Martinos Center for Biomedical Imaging by the Laboratory for Computational Neuroimaging. FreeSurfer executables are available for download from, <http://surfer.nmr.mgh.harvard.edu/fswiki/Download> and is free to download.

The EHRS is a relatively old dataset, relying on older scans with lower voxel resolution and field strength than is commonly used in present studies. Although all data were acquired with 1 Tesla scanner, which is a low field strength, the ultimate quality of the data is dependent on the quality of the reconstructions obtained with

Freesurfer. Each surface reconstruction for every individual was carefully quality-checked and manually edited when errors were found. Although low field strength can reduce signal to noise, higher fields can be more liable to different problems, such as susceptibility artefacts, and so there is no hard-and fast rule as to field strength affecting the results of Freesurfer analyses. Indeed, this is one of the reasons why multi-centre collaborations such as ENIGMA (Enhancing NeuroImaging Genetics through Meta-Analysis) stress data pre-processing consistency and reliability and do not rule out low field strength data (For a more detailed overview, please see Thompson et al. 2014). All scans were therefore manually checked for inaccuracies by a trained rater (CB) blinded to group or subsequent diagnostic status. A more detailed explanation of the quality checking procedures used for the present study are outlined in Chapter 2.

Several advantages exist for using Freesurfer compared to manual segmentation approaches. In relation to the subcortical structures investigated in the present study, one key advantage is that it is less labour intensive and thus less time-consuming than hand-tracing methods and thus arguably more accurate in larger datasets such as the present, especially for the smaller subcortical structures analysed in the present study, which could be very influenced by interrater reliability and rater fatigue (Whalley & Wardlaw, 2001). Furthermore, Freesurfer assigns one of 37 labels to each voxel, such as the hippocampus, amygdala and nucleus accumbens, and by doing this it employs a registration procedure that is robust to anatomical variability, including the ventricular enlargement typically associated with neurological diseases and aging (Fischl et al. 2002). Freesurfer has even been shown to be of sufficient

sensitivity to detect the very subtle changes in subcortical structures preceding Alzheimers disease (Fischl et al. 2002). FreeSurfer has been shown to have good reliability when compared to manual segmentation (Kuperberg et al. 2003, Morey et al. 2009, Salat et al. 2004) and has been shown to have good test-retest reliability (Clarkson et al. 2011) with the subcortical structures.

In relation to the cortical parameters investigated in the present study, the surface-based stream enables a detailed investigation of cortical thickness, area and gyrification, as opposed to merely investigating cortical gray matter volume. As described in the section above, this may provide more sensitive information that may be crucial when looking at the brain in relation to schizophrenia, as these parameters lie closer to the neurobiological action of neurodevelopment and genes than gray matter volume (Rakic, 1971).

However, it is also important to recognize some of the limitations associated with using Freesurfer. It has been shown that the choice of algorithms for intensity correction and skull-stripping impacts the quality of resulting segmentations (Acousta-Cabronera et al. 2008) and it is important to note here that although automated, Freesurfer requires visual inspection and manual intervention at certain processing steps, thus still requiring some time consuming edits. Furthermore, research has also shown that the version of Freesurfer as well as type of workstation and operating system used can influence the final results (Fischl et al. 2012), as well as scanner-specific parameters, such as type of scanner, field strength and pulse

sequence, which have been shown to impact on regional brain volume, cortical thickness and area (Schnack et al. 2010, Jovicich et al. 2009). However, some studies have suggested that although Freesurfer is advantageous when using larger datasets as they significantly reduce the time required to process each scan, that for some structures, accuracy may be slightly better when using manual tracing methods (Whalley & Wardlaw 2001). Hence, when using Freesurfer it is important to not update or change scanner or workstation or operating system used for data processing, as well as being important not to forget some of its limitations.

1.4 General Summary and Aims/Objectives of Present Thesis

To summarise, there is a large sMRI evidence base suggesting that structural changes occur in schizophrenia and that these precede disorder onset. However, as is clear, the presentation of these in different samples are heterogeneous, which could in part be attributed to some studies often using samples of patients that have had schizophrenia for a number of years, and thus remain confounded by secondary factors such as medication and/or substance use, which high risk studies can bypass. This Chapter presents findings that suggest that these alterations occur in several distributed regions of the brain before disorder onset, and there is evidence suggesting that brain abnormalities in additional regions distinguish HR-T from HR-NT, although the direction, nature and extent of these alterations remains inconsistent. There is a lack of prospective longitudinal studies of familial high risk studies that

examine the nature of brain changes over time using a surface based as well as a volumetric approach.

Although at present sMRI techniques are only able to provide indirect measures of disrupted neurobiology, structural imaging has reached a level whereby some improvement in assessing structure in relation to underlying neurobiology can be made. In order to thoroughly assess susceptibility to psychosis, it is important that some understanding of the underlying disrupted processes, however indirect, can be inferred. Standard gray matter volumetric measures of the cortex may yield little further understanding of these processes as research is increasingly demonstrating that thickness and surface area are mediated by separable genetic and neurodevelopmental influences (Panizzon et al., 2009, Winkler et al., 2010). In the search for reliable markers of psychosis, surface-based approaches may therefore be more sensitive to the neurobiological changes leading up to the clinical manifestation of schizophrenia. Another reason why surface-based approaches may provide useful clinical tools is because evidence exists that the morphology of the cortex is intrinsically linked to underlying neuronal connectivity (Van Essen 1997). Gyrification is, according to some theories, a marker of underlying neuronal connectivity, and thus surface based methods may be able to link brain structure to the hypothesized deficit underlying schizophrenia.

Clarifying whether potential abnormalities in structure are progressive or fixed across time is also central to elucidating markers of psychosis, as aberrant

development itself may be a marker of schizophrenia. Generally the evidence from longitudinal comparisons of HR-T and HR-NT suggest that a pre-existing vulnerability interacts with abnormal development as well as disease-specific processes, causing both fixed and progressive abnormalities that develop as high risk individuals' transition to the disorder. Despite some differences between familial and clinical high risk approaches, this Chapter has evaluated both in order to critically assess the information pertaining to whether there exists markers specific to HR-T and HR-NT.

The evidence reviewed in the present Chapter shows a wealth of subtle brain abnormalities in divergent and multiple brain regions in HR-NT, with more pronounced deficits present in HR-T. However, studies have yielded inconsistent results and thus it remains largely unknown to what extent widespread/localized alterations are present before/after illness onset in HR-T, and in unaffected relatives of individuals at high risk, as very few studies have investigated both global and more localized alterations using both a surface-based method in conjunction with an automated segmentation method. Furthermore, very few studies have specifically looked at those individuals that do not develop schizophrenia, but do develop symptoms, and whether these individuals are different from those at high risk that have no symptoms and HR-T. Thus, it remains controversial whether alterations are associated with transient symptomology, predate the onset of illness, only emerge as a function of illness onset or if they are related to medication effects, duration of illness or severity of symptoms.

In order to address the gaps in present knowledge identified above, I therefore present findings from a prospective longitudinal study of sMRI measures in individuals at high familial risk of developing schizophrenia who either remained well during a long follow-up or developed transient symptoms that were never severe enough to warrant a diagnosis of schizophrenia, or developed schizophrenia on average 2.5 years after the scanning took place. This thesis therefore aims to investigate a sample of individuals that had experienced a first episode of psychosis, but had no family history of psychosis, and a sample of healthy controls. This thesis thus investigates cross-sectional and longitudinal alterations in those at high risk, controls and patients in cortical parameters using a surface-based method (Chapters 3 and 4) and specific subcortical (Chapter 5) regions using a volume-based method.

CHAPTER 2

General Methodology

2. Introduction

As is clear from Chapter 1, schizophrenia has been associated with several structural brain abnormalities, and these have been shown to be present to a lesser extent in populations deemed at high risk of developing schizophrenia, for either clinical or familial reasons, with more alterations in HR-T than HR-NT. However, schizophrenia remains a complex and heterogeneous disorder, with a relatively unknown aetiology, and the nature, direction and extent of brain alterations remains unclear (Bois et al. 2014, Smieskova et al. 2013, Cooper et al. 2014). Understanding whether brain alterations in schizophrenia reflect a genetic disposition to schizophrenia, and may thus be specified as trait markers of schizophrenia, or whether they are better characterized as state markers, associated with some aspect of the clinical illness itself, has crucial implications in our understanding of the disorder's underlying aetiology. Looking at the brain in a biologically informed manner may aid our quest in this matter. Furthermore, this understanding may also aid investigators and clinicians in their search for effective intervention strategies, as markers enabling early diagnosis may facilitate a better prognosis/outcome for patients, as an increased duration of untreated psychosis has been associated with

worse clinical prognosis (Gumley et al. 2014).

Familial and prospective high risk studies also have the added benefit of allowing researchers to more firmly establish causality regarding any brain alterations, as these paradigms are, unlike patient studies, unconfounded by the effects of length of illness and/or medication effects on brain structure. However, most investigations of familial cohorts to date have employed cross-sectional designs. Yet, as outlined in Chapter 1, evidence suggests that a familial risk for developing schizophrenia may be specifically associated with abnormal developmental trajectories, and alterations may therefore display themselves subtly over time (Gutierrez-Galve et al. 2015, Cannon et al. 2015). Hence, to further increase the sensitivity of familial high risk studies, the importance of employing longitudinal designs is clear.

Other issues that may have confounded findings to date is that most studies on those at familial high risk have to date have only utilized volumetric approaches when assessing brain structure (McIntosh et al. 2011, Job et al. 2005). However, increasingly, surface-based techniques, such as Freesurfer, are being utilized, as they are thought to confer greater sensitivity when assessing cortical brain alterations, which are thought by some to be the locus of underlying connectivity issues thought to be central in schizophrenia. As we discuss in greater depth in Chapter 1, surface-based approaches may be better able to distinguish subtle cortical alterations occurring due to potentially disrupted genetic/neurodevelopmental processes, as they can divide the cortex into surface area, cortical thickness and gyrification, rather than

merely focusing on cortical gray matter volume. Please see Figure 2.1 for a visual demonstration of the difference between cortical gray matter volume analyses versus looking at cortical area and thickness separately.

Figure 2.1. Figure adapted from Winkler et al. (2010). Figure illustrates the relationship between cortical thickness, surface area and gray matter volume, and shows the difference between utilizing a volumetric technique compared to a surface based technique.

Using a surface-based approach when investigating the cortex may thus further improve sensitivity when assessing cross-sectional and longitudinal brain alterations in those at high risk of developing schizophrenia for familial reasons, as using volumetric approaches when investigating the cortex may have obscured subtle and differential effects.

2.1 Structural Neuroimaging; A brief overview

Kraepelin hypothesized over 100 years ago that schizophrenia had an organic basis characterized in the form of brain alterations, and 40 years ago it was shown using modern non-invasive imaging techniques that the brain was altered in some way in schizophrenia compared to healthy controls – with the seminal finding of enlarged ventricles in patients (Johnstone et al. 1976). With the advent of more sophisticated sMRI techniques using MRI scanners with better resolution, researchers have been able to more thoroughly investigate brain structure in relation to schizophrenia. MRI scanners rely on a set of physical principles governing the changes in behaviour of protons in the presence of a magnetic field. When a subject is placed in the scanner, the protons of the tissue align longitudinally with the static magnetic field, either in a lower energy state (parallel) or higher energy state (anti-parallel). Magnetic effects of protons in parallel and anti-parallel alignment cancel each other out, but there are more protons aligned in the lower energy state than the higher which results in longitudinal magnetization (Huettel et al. 2009). A radio frequency pulse is then introduced, producing a synchronous precession of the protons, which in turn results in a process called transverse magnetization. The radio-frequency pulse also provides sufficient energy for some of the protons to move to the higher (anti-parallel) state, which has the effect of cancelling out some of the longitudinal magnetization (Huettel et al. 2009). When the pulse is turned off, the protons return back to their

original energy state, which has the effect of returning energy to the surrounding structural lattice, which increases the longitudinal magnetization referred to as T1 relaxation time. The transverse magnetization decreases as the precession of the protons become asynchronous once more, being termed the T2 relaxation time (Huettel et al. 2009). Different tissues have different relaxation times, depending on the amount of hydrogen they contain, and this process can be manipulated to form different types of scans that give different information. T1 weighted MRI scans are often used to maximise the contrast between gray and white matter, whilst other methods such as DTI that looks at white matter integrity of the brain relies on a different type of scan. These scans can then be used in combination with different sMRI software packages, such as SPM or Freesurfer, to produce the parameters of interest, such as estimations of gray matter, cortical thickness or surface area.

2.2 The Edinburgh High Risk Study of Schizophrenia (EHRS)

2.2.1 Recruitment Procedure for the EHRS

The Edinburgh High Risk Study (EHRS) is a large prospective study of individuals at high familial risk of schizophrenia. Recruitment details have been described previously in more thorough depth (Hodges et al. 1999, Byrne 2003, Johnstone et al. 2000, Johnstone et al. 2005). Recruitment originally centred in Edinburgh and South-east Scotland, but later also extended to also include Argyll and Clyde, Borders, Galloway, Forth Valley, Highlands and Islands, and the towns of Dumfries and Perth. Potential high risk participants were identified from families with at least two family members with a confirmed diagnosis of schizophrenia using the OPCRIT

(Operational Criteria Checklist) computer program (McGuffin et al. 1991).

Individuals were identified by examining case-notes of patients with schizophrenia known to individual hospitals. Where there appeared to be two related cases, consent was sought to speak to a healthy family member to obtain a detailed family history to determine if there was any possibility of there being a close relative between the age of 16-25. As described, 229 individuals were in this way identified and 162 of these provided some data in the first phase of the study, 146 that had brain scans. Thus, high risk individuals aged 16 to 25 years with no personal history of psychiatric disorder at the first point of contact were found throughout Scotland. Importantly, these participants were not recruited based on emerging symptomatology, and therefore this study differs from the clinical high risk paradigm explained in more detail in Chapter 1. 37 healthy controls without personal or family history of major psychiatric disorder were recruited from the same social and geographical networks as those at high risk, in order to minimize potential confounding environmental influences. At baseline scanning, the vast majority of both high risk and controls were either in full-time employment or in higher education. Structural MRI scans used in the present study were conducted for both high risk and controls at baseline and repeated after a mean scan interval of 1.87 years. Furthermore, 36 first episode patients were recruited at first admission to psychiatric hospitals in Edinburgh and the Lothians, Scotland (For exact numbers of each group with MRI scans suitable for analysis, please see the Demographics Tables in section 2.12 of this Chapter). Case notes were reviewed using the Operational Criteria (OPCRIT) check-list (McGuffin et al., 1991) and diagnosis was confirmed using the Present State Examination (PSE) (Wing et al., 1974). All first-episode patients met DSM-IV (American Psychiatric

Association, 2000) criteria for schizophrenia or schizophreniform disorder but did not have a familial history of schizophrenia. The smaller numbers were collected with the underlying rationale of being matched to the number of individuals within the high risk cohort that were expected to develop schizophrenia, at which point follow-up studies of the high risk subjects ceased. The first episode patient group only had baseline scans as this was a specific part of the study design. Ten to fifteen percent of the high risk group were predicted to develop schizophrenia by the age of 30 years based on the known frequency of schizophrenia in individuals with this degree of heredity. The collection for this study began in 1994 and was funded by the Medical Research Council and the original idea for this study was conceived by Professor Johnstone.

The high risk individuals were recruited within the age range of 16-25, as this reflects the range within which the risk of developing schizophrenia is the greatest, and this, combined with the longitudinal design, was designed to capture the highest numbers possible of high risk individuals that went on to develop schizophrenia, thus hopefully minimizing any following changes to clinical status that may have occurred after the study period which could have altered results obtained. However, many of the high risk individuals were informally followed up after the study period's finish, by senior clinicians involved in the study, and as far as we are aware none of the high risk diagnoses have changed nor have other high risk individuals become unwell after the first ten years of observations.

2.2.2 Data used for present Study

This first phase of the Edinburgh High Risk study (1994-1999) employed repeated assessments every 18-24 months, including clinical and neuropsychological assessments as well as structural neuroimaging. The present thesis uses data from the first two time-points of the study. On completion of recruitment, at the end of 1998, 229 high risk individuals had been identified. At baseline assessment, 162 high risk participants provided some data: clinical, neuropsychological and/or a structural MRI scan. Due to rolling recruitment, smaller numbers reached the 18-24 month follow-up assessments within that five year time-frame. Finally, 29 had a third assessment, but due to this significant decrease of numbers and the associated drop in power, the present thesis focuses only on the first two time-points of the first phase. For the present study, after the 14 scans at baseline had been excluded (See section on Freesurfer reconstructions in this Chapter for further details) there were thus 201 scans that were included: 140 high risk individuals, 27 patients with first episode and 34 controls that were included. At follow-up, there were 82 scans of which 18 were controls and 64 were high risk individuals.

2.2.3 Ethical Consent

Prior to use in this analysis, all participants volunteered to be a part of the EHRS study, and had the right to withdraw at any time. Informed consent was obtained from all participants, as approved by the Psychiatry and Clinical Psychology subcommittee of the Multi-Centre Research Ethics Committee for Scotland. All permissions were obtained from participants for the usage of their brain scans and patient information for the duration of this study. All applications for continuation

and amendment to this study have been filed appropriately with the Scotland Research Ethics Committee, and accordance with the Helsinki Declaration.

2.2.4 Structured Psychiatric Interviews

Around the time of the scan all individuals underwent a structured psychiatric interview to identify any psychotic symptoms (the Present State Examination, PSE, Wing et al. 1974). A scoring system (Johnstone et al. 2002) was administered by three experienced clinicians (Professor E Johnstone, Professor D Owens, and Professor S Lawrie). A score of 4 was assigned for definite schizophrenia based on the PSE and a clinical diagnosis of schizophrenia in terms of the ICD-10 (World Health Organization, 1993). A score of 3 was assigned for any fully rated psychotic feature from PSE items 55-92 including thought reading, echo, broadcast, auditory, visual or other hallucinations, delusions of control or other, and from PSE items 128-129 and 135-7 (blunted affect, incongruous affect, neologisms or idiosyncratic use of words, flight of ideas or incoherence of speech. A score of 2 was assigned if any of the features of 3 were partially held or present to a mild degree, plus items 49-54 (perceptual disorders other than hallucination and behavioural items 108-9, 118, and 125-6 (self-neglect, bizarre appearance, behaves as if hallucinated, suspicious, perplexed), fully rated, and items 133 (muteness), partially or fully rated. Thus the outcome of this scoring was:

4 = Schizophrenia

3 = fully rated psychotic symptoms

2 = partially rated psychotic symptoms

1 = fully or partially related non-psychotic symptoms

0 = no symptoms

For the purposes of examining clinical state effects on brain structure, the high risk individuals were thus grouped into three groups: fully or partially held scores (2/3) HR[symp], absence of psychotic symptoms, (0/1) HR[well] or diagnosis of schizophrenia (score 4), HR[ill]. The high risk individuals were also grouped together, eg HR[all], to examine trait effects of a genetic predisposition on brain structure.

During the course of the study, 21 high risk individuals developed schizophrenia, 19 of whom had full clinical assessments and 17 who had at least one structural MRI scan. Those in the HR[ill] group were formally diagnosed after an average of 929 days (SD = 138) and were not offered rescanning once this diagnosis has been made. Once a diagnosis of schizophrenia had been made these individuals were not formally followed-up, nor were those participants that dropped out for other reasons. None of those at high risk that were scanned were on any form of anti-psychotic medication at baseline, nor at follow-up scanning.

2.3 Main Findings from First Phase Related to Present Thesis

At baseline there were no significant differences between those at high risk and controls in age, gender, or social class at birth (paternal) (Johnstone et al. 2002). There were also no significant differences in terms of alcohol, cannabis or other drug use (Johnstone et al. 2002). However, the high risk group did present poorer educational and employment attainment, and had more social work contact in childhood than the group of healthy controls (Johnstone et al. 2002). In terms of psychopathology, high risk individuals also presented more symptomatology than controls, including partial and definite psychotic and non-psychotic symptoms. Isolated psychotic symptoms occurred in 2-3 times as many individuals than were expected to develop schizophrenia. In those subjects that went on to develop schizophrenia, the florid condition had been preceded by isolated psychotic symptoms in approximately 70% of cases (Johnstone et al. 2002)

2.4 Overall aims of Thesis

This study examines cross-sectional and longitudinal brain changes in the Edinburgh High Risk Study of Schizophrenia (EHRS) using Freesurfer. Although previous sMRI studies on this cohort have been published, the present thesis used Freesurfer, a sMRI package not available when the previous studies were conducted, to investigate in depth cortical morphology and sub-cortical structure in this cohort. As outlined in Chapter 1, evidence suggests that different cortical parameters have different genetic and neurodevelopmental underpinnings (Panzzion et al. 2009, Rakic et al. 1988,

1995), and thus the rationale for investigating this in a familial high risk study is clear. As mentioned above, the dataset consists of a sample of young individuals deemed at high genetic risk of developing schizophrenia for familial reasons, a sample of healthy controls and a sample of patients experiencing their first-episode of schizophrenia. Due to the nature of this prospectively acquired dataset, the results are not confounded by the effects of chronicity of illness nor any putative effect of anti-psychotic medication effects on brain structure (for more details on the effects of anti-psychotic medication and length of illness on brain structure, see Chapter 1). For the group of familial high risk individuals that developed schizophrenia, all the scans were taken before disorder onset and thus any changes occurring selectively in this group can truly be attributed to the clinical state itself, and not confounding issues related to the disorder.

As mentioned in Chapter 1, increasingly, evidence is pointing towards schizophrenia being associated with widespread cortical changes along with some key subcortical regions, and therefore we aimed to assess global and lobar cortical measures, along with some key subcortical structures, that have been previously implicated in the disorder, in order to assess both global and localized cortical and subcortical brain structure.

Thus, the main aims of this thesis are as follows:

- 1. To investigate whether cross-sectional/baseline structural brain abnormalities exist in those at high risk of developing schizophrenia compared to healthy**

controls and first-episode patients, and whether they are stratified based on subsequent clinical outcome within the high risk cohort.

The purpose of these investigations was to assess whether any brain alterations were present in the whole high cohort compared to a sample of first episode patients and healthy controls. The rationale behind this approach was to assess whether there were any general high risk brain abnormalities that distinguished this high risk cohort from healthy controls, thus potentially forming part of a general predisposition for the disorder. Furthermore, this approach would also help assess whether any alterations were actually only present in the first-episode cohort and could therefore be more accurately specified as “state” markers, associated with the manifestation of symptoms itself. I then went on to assess whether any cross-sectional alterations were differentially present in HR[well], HR[ill] and HR[symp] at baseline, and thus whether brain alterations were tied more specifically to clinical familial risk, as opposed to a general disposition to develop schizophrenia. These group comparisons form part of the cross-sectional analyses presented in Chapter 3, 4 and 5. Specific Hypotheses relating to this aim are thus presented in these Chapters.

2. To assess whether longitudinal structural brain abnormalities exist in those at high risk of developing schizophrenia compared to healthy controls and whether they are stratified based on subsequent clinical outcome within the high risk cohort.

As evidence suggests progressive brain abnormalities form part of the aetiology of schizophrenia, indicative of disturbed development over time, another primary aim

of this thesis was to investigate whether any structural brain abnormalities were present longitudinally. As no longitudinal data is available for the first-episode patients for the present study, this was investigated by first comparing HR[all] to controls. It was then investigated whether they were differentially present in HR[well], HR[ill] and HR[symp], thus assessing whether brain alterations were tied more specifically to clinical risk, as opposed to a general disposition to develop schizophrenia. These group comparisons form part of the longitudinal analyses presented in Chapter 3, 4 and 5. Specific hypotheses relating to this aim are thus presented in these Chapters.

3. To assess whether surface area, cortical thickness and gyrification are differentially affected, both cross-sectionally and longitudinally, in high risk individuals compared to healthy controls and first-episode patients, and/or whether they are stratified based on subsequent clinical outcome within the high risk cohort.

As was outlined in more depth in Chapter 1, evidence suggests that analysing thickness, surface area and cortical gyrification separately may improve the sensitivity of sMRI studies compared to investigations of gray matter volume, as these parameters develop differentially and are thought to be mediated by different developmental processes. Therefore, it was investigated, in Chapter 3 and 4, group differences in the same manner as that described above, in global and lobar cortical thickness, surface area and gyrification, as opposed to measuring cortical gray matter volume. Specific Hypotheses relating to this aim are thus presented in these Chapters.

2.5 MRI Acquisition

Concurrently with baseline and follow-up clinical assessment, participants underwent structural MRI. The present analysis focuses on those individuals with either one or two scans. The scans were taken between 1994 and 1999 and on the same scanner, a 42 SPE Siemens (Erlangen, Germany) Magnetom operating at 1.0 T. The scanning sequence was the same for both scans and the scanner was not upgraded between the two scans. The sequence was a three-dimensional magnetization prepared rapid acquisition gradient echo sequence consisting of a 180° inversion pulse followed by a fast low angle-shot collection (flip angle 12°, repetition-time 10 msec, echo-time 4 msec, inversion time 200 msec, relaxation-delay-time 500 msec, field of view 250 mm § 250 mm), giving 128 contiguous slices with a thickness of 1.88 mm. The sequence was selected in order to obtain optimal gray/white matter contrast.

2.5.1 Freesurfer Pre-Processing

Freesurfer is a set of automated tools used for both cortical and subcortical segmentation and reconstruction of the brain's surface based on sMRI data. Cortical reconstructions were generated using the surface-based stream of the software FreeSurfer, version 5.3, and subcortical segmentations using the volume-based stream. (<http://surfer.nmr.mgh.harvard.edu/fswiki/recon-all/>). It is freely available for numerous platforms, and was developed by the laboratory for computational

neuroimaging at the Martinos Center for biomedical imaging in Boston, USA.

Broadly, there are two different processing streams that are used for structural MRI data, however it can also be used for fMRI and DTI purposes. The volume-based stream facilitates the segmentation of visible subcortical brain structures and subsequent calculation of their volumes. As this stream was used only for Chapter 5, it will be described in more detail there and mentioned only briefly here. In this Chapter I focus on the surface based stream as this is used for both Chapter 3 and 4. Firstly, all scans were required to be converted from original dicoms to mgz files, and subsequently to nifti files in order to be able to be further processed by the Freesurfer cortical (surface-based) and subcortical (volume-based) stream.

As mentioned in Chapter 1, there are several advantages of using methods such as Freesurfer compared to manual segmentation approaches. It is less labour intensive and thus less time-consuming than hand-tracing methods and thus preferable in larger datasets such as the present. However it has been shown that the choice of algorithms for intensity correction and skull-stripping impacts the quality of resulting segmentations (Acousta-Cabronera et al. 2008) and it is important to note here that although automated, Freesurfer requires visual inspection and manual intervention at certain processing steps. Furthermore, research has also shown that the version of Freesurfer as well as type of workstation and operating system used can affect the segmentation algorithms and influence the final results (Fischl et al. 2012). And scanner-specific parameters such as type of scanner, field strength and pulse sequence have been shown to impact on regional brain volume, cortical thickness and area (Schnack et al. 2010, Jovicich et al. 2009). Hence, when using Freesurfer it is important to not update or change scanner or workstation or operating system used

for data processing. Furthermore, as mentioned previously, the surface-based stream enables a detailed investigation of cortical thickness, area and gyrification, as opposed to merely investigating cortical gray matter volume, thus providing more sensitive information that may be crucial when looking at the brain in relation to schizophrenia. FreeSurfer has been shown to have good reliability when compared to manual segmentation (Kuperberg et al. 2003, Morey et al. 2009, Salat et al. 2004) and has been shown to have good test-retest reliability (Clarkson et al. 2011).

2.5.2 Cortical Reconstructions using Freesurfer

The surface-based stream facilitates the reconstruction of the cortical surfaces and is able to provide several measures, including but not restricted to curvature, gyrification, thickness and surface area. Freesurfer defines cortical as any region that is considered to be part of the cerebral cortex. So, for example, although the hippocampus is strictly speaking part of the cerebral cortex, it is located inferior to the cortices, and is thus processed in the volume-based stream.

For the surface-based stream, Freesurfer aligns the MRI scans to the Desikan-Killiany atlas template (Desikan et al. 2006). This is created by averaging MRI scans of 40 subjects who were between 19 and 86 years old (26 females, 14 males) of which 10 had a diagnosis of Alzheimers disease. The rationale for this has been to capture brain atrophy typically found in studies that use Freesurfer (Desikan et al. 2006).

Firstly, a subject's directory is created, and each person's scan organized in a separate folder. Then, the command `recon-all -subject -all` is run for each subject. This command performs all steps required to generate a complete Freesurfered brainmask and the relevant statistics used for analysis. This processing includes motion correction and averaging (Fischl et al. 2001) of T1 weighted images, removal of non-brain tissue using a hybrid watershed/surface deformation procedure , automated Talairach transformation, intensity normalization (Fischl et al. 1999), tessellation of the gray matter / white matter boundary, automated topology correction (Fischl & Dale 2000, Rosas et al, 2002), and surface deformation to place the gray/white and gray/cerebrospinal fluid borders optimally (Fischl et al. 2001). Once the cortical models are complete, a number of deformable procedures can be performed in further data processing and analysis including surface inflation (Rosas et al, 2002), and creation of a variety of surface based data. This method uses both intensity and continuity information from the entire three dimensional MR volume in segmentation and deformation procedures to produce representations of cortical thickness, calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface (Fischl & Dale, 2000). The maps are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity.

2.5.3 Manual Editing of Scans.

Although the procedures for the measurement of cortical thickness have been validated against histological analysis and manual measurements (Rosas et al. 2002),

the process is prone to error and required manual editing. Although all data were acquired with 1T scanner, low field strength, the ultimate quality of the data is dependent on the quality of the reconstructions obtained with Freesurfer. Each surface reconstruction for every individual was carefully quality-checked and manually edited when errors were found. We note that while low field strength can reduce signal to noise, higher fields can be more liable to different problems, such as susceptibility artefacts, and so there is no hard-and fast rule as to field strength affecting the results of Freesurfer analyses. Indeed, this is one of the reasons why multi-centre collaborations such as ENIGMA (Enhancing NeuroImaging Genetics through Meta-Analysis) stress data pre-processing consistency and reliability and do not rule out low field strength data (For a more detailed overview, please see Thompson et al. 2014). All scans were therefore manually checked for inaccuracies by a trained rater (CB) blinded to group or subsequent diagnostic status. This was done for all stages of the longitudinal processing; edits were first made to the cross-sectional templates which are carried through to the base and then if required also the long runs to ensure accuracy of the borders (Please see section 2.10 in this Chapter for a detailed explanation of the longitudinal pipeline). After these steps, 14 of the baseline scans and 3 of the follow-up scans had to be excluded due to defective surface generation that was not fixed by manual intervention procedures. An 'edit' in this context is a manual intervention by required when the automated stream (recon-all) does not produce output that is correct or suitable as determined by the user, or if the automated stream fails outright. Manual interventions occur via the volume editing tool tkmedit and then implementation of these through the recon-all command-line options. 'Persistent' in this context means that an edit, once made, is

treated as a permanent change: it is saved to a file either a voxel change in a volume, or a text file listing points or commands and it is automatically re-used by recon-all if recon-all is run again. These edits for both the cross-sectional and longitudinal pipeline took approximately 12 months to complete for the whole dataset.

2.5.3.1 White matter edits

In order for the cortical surface to be reconstructed accurately, edits to the white matter of the Free-surfer reconstruction are required. Sometimes the white matter is not segmented correctly: sometimes voxels that should be white matter are excluded, and other times voxels that should not be white matter are included in error. This causes problems to the cortical and subcortical reconstruction by also affecting the surface borders of the cortex. Figure 2.2, 2.3 and 2.4 show examples of white matter edits that were required for the EHR dataset

Figure 2.2. **A)** Example of an area on the right hemisphere where the white surface (yellow line) does not follow the surface of the brain, but in fact cuts into it. This geometric inaccuracy is caused by a lesion where white matter has been marked as non-white matter. **B)** With the `wm.mgz` volume loaded into `tkmedit` you can see that this area has been left out of the `wm` volume

Figure 2.3. Example of **A)** Non-white matter being inaccurately included in the brainmask, **B)** The same inaccuracy in the white matter mask and **C)** An edit made to remove this inaccuracy.

Figure 2.4. Example of **A)** White matter being missed out inaccurately, and control points added to fix this error and **B)** An edit made to remove this inaccuracy.

2.5.3.2 *Pial Edits*

The pial surface is created by expanding the white matter surface so that it closely follows the gray-CSF intensity gradient as found in the brainmask.mgz volume. Once an accurate white surface is created then you can work on correcting the pial surface

if needed. The pial surface boundary and white matter surface boundary should not cross. After the pial surface has been generated, it's a good idea to visually check it for defects that may have been created during automatic topology fixing.

Figure 2.5 Example of **A)** Non gray-matter included in the brainmask and **B)** Edit made to fix this inaccuracy

2.5.3.3 Skull Strips

Sometimes the skull strip process malfunctions with freesurfer. Figure 2.6 shows examples of this.

Figure 2.6 Example of **A)** Too aggressive skull-strip and **B)** Edit made to fix this inaccuracy

2.6 Brain Regions Investigated in Present thesis.

For Chapter's 3 both global and lobar surface area and cortical thickness were used, and for Chapter 4 lobar and global gyrification. For Chapter 5, the hippocampus, amygdala and nucleus accumbens were investigated.

2.7 Gyrification Procedure

This is detailed in Chapter 4.

2.8 Subcortical Segmentations

This is detailed in Chapter 5.

2.9 Freesurfer Longitudinal Pipeline Processing

Importantly the present study used the longitudinal pipeline available with Freesurfer. As research has shown that inter-scan variability due to limited accuracy of Freesurfer may be in the same magnitude as the changes in brain morphology over time, the use of this pipeline is thought to increase reliability and validity significantly (Klauschen et al. 2009). To extract reliable structural estimates for the longitudinal analyses, images were automatically processed with the longitudinal stream, implemented in Freesurfer v 5.3 (Reuter et al., 2012). Specifically an unbiased within-subject template space and image (Reuter and Fischl, 2011) is created using robust, inverse consistent registration (Reuter et al., 2010). Several processing steps, such as skull stripping, Talairach transforms, atlas registration, as well as spherical surface maps and parcellations, are then initialized with common information from the within-subject template, significantly increasing reliability and statistical power (Reuter et al., 2012). From this template, each subject's individual time-point is rerun. This pipeline allows analysis of individuals with only even one time-point in Matlab and Freesurfer, using specific tools designed for this. Due to the longitudinal nature of the data, this editing was also required for the bases that were created, and sometimes also the long runs.

2.10 Batch Scripting

As this was a large study of many subjects and two time-points, the image reconstruction, pre-processing commands and commands used for specific sub-

sections of this thesis were performed using batch scripts devised by the author and based on commands available with Freesurfer.

2.11 Statistical Analysis

All statistical analyses for the present thesis were conducted in R (version 3.2.0). As the statistical methodology used is somewhat different for the different chapters, these will be outlined separately in each data containing chapter. Cross-sectional analyses of cortical and subcortical brain measures were conducted using separate analyses of covariance (ANCOVA) for each brain region and parameter of interest to compare group differences (more specific information will be outlined in each upcoming Chapter). The longitudinal analyses used linear mixed-effects models. The linear mixed-effects model is less often used than the commonly applied repeated-measures ANOVA, however is thought to be more robust for a number of reasons. It permits the inclusion of incomplete datasets, so that deletion of missing data is not required, which is important for this dataset where the follow-up numbers are smaller than at baseline (Cnaan et al. 1997). Furthermore, the intercept term is treated as a random effect that varies by individual so that intrindividual correlations among the structural brain measures per person are taken into account.

2.12 Demographic characteristics of sample

As mentioned above, some scans had to be excluded due to abnormalities/Freesurfer segmentation errors, and thus the following tables are for those individuals that had

at least one (or two) scans suitable for the MRI analyses. Relevant demographics for both baseline and longitudinal analyses for those subjects that were included are presented in Table 2.1, 2.2 and 2.3. Sample errors of the brains that were excluded are included in Appendix 2.1

Table 2.1 shows demographic characteristics of the individuals that had brain scans suitable for analysis

	First Episode	Controls	High Risk	Statistic(p)
Number of Participants	27	34	140	
Age in years (SD)	21.59(3.63)	21.17(2.37)	21.19(3.01)	F=.033(0.56)
Male	16	16	71	X ² =2.75(0.25)
Right-Handed	31	31	127	
Duration of Illness	6(2.5- 18.75)			
<i>Antipsychotic Medication</i>				
Typical	17			
Atypical	7			
Unmedicated	2			

HC: Healthy Controls; HR: individuals at high familial risk for schizophrenia; FES; first-episode schizophrenia patients

Table 2.2 Demographics of high risk participants with MRI scans by clinical outcome

	HR[well]	HR[symp]	HR[ill]	Statistic(p)
Number of Participants at Scan	68	55	17	
Age in Years (SD) at Scan	21.14(3.11)	20.98(2.83)	20.55(3.02)	F=0.46(0.50)
Male:Female	35:33	23:32	11:6	X2 = 3.12(0.21)
Handedness (right/left/mixed)	63/4/1	48/2/5	16/0/1	NA

Table 2.3 Mean Ages and WAIS IQ's and associated standard errors and statistics for HR[symp], HR[well], HR[ill] and controls, along with numbers and sex distributions of each group and scan intervals.

		Controls	HR[well]	HR[symp]	HR[ill]	Statistic
WAIS-IQ	Range	79-140	77-139	77 – 128	75-129	
	Mean(se)	105(2.17)	99(1.58)	96(1.67)	100(3.07)	F(3,169)=3.48, $p<.05$
Age	Range	16-26	16-27	16-26	16-23	
	Mean(se)	21(.28)	22(.25)	21(.29)	20(.40)	F(3,175)=1.6, ns
Numbers at Baseline Scan	Male:Female	17:19	36:32	24:33	11:6	
Numbers at Follow-up	Male:Female	11:7	18:12	11:15	5:3	

Scan				
Mean Scan	2.21(.031)	1.90(.108)	1.65(.05)	1.64(.19)
Interval				

HR[well]: individuals at high familial risk of schizophrenia who remained well during follow-up period; HR[symp]: individuals at high familial risk of schizophrenia who had psychotic symptoms during follow-up period; HR[ill]: individuals at high familial risk of Schizophrenia who developed schizophrenia during follow-up period.

CHAPTER 3

A cross-sectional and longitudinal investigation of cortical thickness and surface area

3. Introduction

As described in Chapter 1, schizophrenia is a highly heritable disorder, that generally becomes manifest in adolescence or early adulthood. The established condition has been associated with a host of functional and structural brain alterations (Olabi et al. 2011, Kubicki et al. 2007, Sasamoto et al. 2013), however to date it remains unclear whether these alterations occur due to mechanisms associated with the disorder itself, or with confounding secondary factors of the disorder, such as medication, length of illness, as well as other conditions that are commonly comorbid with the disorder itself, such as depression and anxiety (Van Haren et al. 2011, Fusar-Poli et al. 2011).

Evidence is increasingly accumulating that some of the brain alterations present in patients actually precede disorder onset, and thus may form part of a neurodevelopmental component of the disorder (For a more detailed review, please see Chapter 1). Furthermore, some of these alterations are also present in individuals at familial or clinical high risk of developing schizophrenia but that do not transition to the disorder, and therefore it has been suggested that some brain alterations may be more accurately specified as “trait” markers, as opposed to predictive markers of transition to psychosis (Pantelis et al. 2003). In order for our understanding of the aetiology of schizophrenia to progress, it is imperative that researchers understand how brain structural alterations relate to the disorder itself, and whether they occur before illness onset, or after. Understanding both the timing and nature of brain alterations that occur in schizophrenia may provide a useful means to help guide risk

stratification and the potential for early intervention efforts.

The cerebral cortex of the brain has been shown to be both structurally and functionally abnormal in the established disorder of schizophrenia (Xiao et al. 2015, Tseng et al. 2015) as well as in those considered at high risk for familial reasons (Callicott et al. 2003, Li et al. 2012, Nenadic et al. 2015). A lot of research has focused on assessing the frontal and temporal lobes in a ROI approach (McIntosh et al. 2011, Pantelis et al. 2003), however evidence is increasingly showing that both localized and widespread structural perturbations of the cortex occur in schizophrenia (White & Hilgetag 2011). This has lead researchers to propose that a symptomatology as complex and heterogeneous as that of schizophrenia is unlikely to be wholly explained by only localized brain deficits, and as the cortex itself is the largest continuous structure of the brain, the importance of not bypassing global structural parameters when investigating the cortex is clear.

However, in order to accurately specify markers of psychosis, the choice of adequate structural parameters is crucial. To date, the majority of structural analysis of the cortex has focused on cortical gray matter volume, although findings are currently heterogeneous (Cooper et al. 2014, Honea et al. 2008, Smieskova et al. 2013). The migration of neurons formed through mitosis during foetal development gives rise to the cortex (Rakic 1988, Rakic 1995, Rakic & Zecevic 2003). Cortical thickness is formed by the asymmetrical division and proliferation of radial glia in the ventricular and sub-ventricular zones (Rakic 1988, Rakic 1995), whilst surface area is

determined by symmetrical division of progenitor cells in these cortical layers, forming cortical columns. These processes occur at distinct periods of development, and are thought to be mediated by different genes (Panzzoni et al. 2009). This is referred to as the radial unit hypothesis of cortical formation (Rakic 1977). Interestingly, these processes occur earlier in development than cortical folding (This process will be explained in more depth in Chapter 4).

For this reason investigating thickness and area separately in individuals at familial high risk of schizophrenia may help improve the sensitivity of structural imaging studies to different developmental disruptions (Winkler et al. 2010). Cross-sectional studies have found divergent effects of these parameters, in that larger surface areas are often linked to thinner cortices (Ronan et al. 2012, Kingsbury et al. 2003) and longitudinal studies have shown a negative relationship between area and thickness over time such that as area increases, thickness decreases (Hogstrom et al. 2012, Storsve et al. 2014, Gur et al. 2007). As evidence suggests that cortical thickness and surface area have different longitudinal trajectories, the importance of employing longitudinal designs within the high risk paradigm becomes even more apparent, as cross-sectional studies may be inherently limited by bypassing important developmental information. To our knowledge, no familial high risk paradigm has looked at both global and lobar cortical thickness and area estimates longitudinally.

The aim of the present study was to assess cross-sectional and longitudinal change in both global and lobar cortical thickness and surface area in the EHRS (For a more

detailed description of the cohort, please see Chapter 2). It was investigated whether any alterations were evident at baseline when comparing those at high risk to healthy controls and a sample of patients that had experienced their first episode of psychosis. Another aim was to assess whether any changes occurred over time. As a secondary aim it was also investigated whether these alterations could be more accurately specified as markers of clinical risk, and thus only present in those at high risk that developed schizophrenia after the two scans, HR[ill], compared to high risk individuals that remained well, HR[well,] and those that presented only isolated psychotic symptoms, HR[symp].

Based on existing evidence that surface area and thickness reflect distinct developmental processes, the hypotheses for this chapter are that cortical thickness and surface area would be differentially affected in those at high risk compared to controls at baseline, with the patients exhibiting the largest cortical alterations. Longitudinally, it was predicted that there would be a different developmental trajectory in both thickness and area in those at high risk compared to controls. It was also predicted that within the high risk cohort, those that subsequently became ill would show the greatest alterations, as it has been suggested that the brain alterations found in schizophrenia may be present before disorder onset to a greater extent in those at high risk that go on to transition to the disorder, compared to those that do not.

3.1 Methods

3.1.1 Participants

There are identical to those presented in Chapter 2, which also noted the number of individuals excluded due to gross anatomical abnormalities in their brain scans, and individuals that were excluded due to reconstruction errors in Freesurfer.

3.1.2 Imaging parameters

These are identical to those presented in Chapter 2.

3.1.3 Freesurfer Reconstruction

This is identical to that presented in Chapter 2. For this Chapter, the parameters of interest were average global and lobar (parietal, cingulate, occipital, temporal, frontal and insular) cortical thickness and surface area per hemisphere, which were extracted from individual images and compared across groups.

3.1.4 Statistical Analysis

All statistical analyses were performed using R version 3.0.2 (<http://www.r-project.org>). Any variables that were non-normally distributed were transformed

using the box-cox transform, implemented in package geoR. Additionally, for the patient group, chlorpromazine equivalents of current medication dose were calculated according to Woods et al. (2003) and Atkins et al. (1997) and Spearman's rank correlation coefficients between thickness and area, both lobar and global, and chlorpromazine equivalents and duration of illness (in months) were also calculated.

3.1.4.1 Cross-sectional Analysis

For the cross-sectional analyses, Analyses of Covariance (ANCOVAs) were implemented using packages lsmeans and car. Comparisons were first conducted between healthy controls and those at high risk, and the first-episode patients. These analyses were then repeated between high risk participants based on their clinical outcome: HR[well], HR[symp], HR[ill]. These comparisons were conducted for average left and right cortical thickness and surface area, as well as for the lobar measures: parietal, occipital, frontal, temporal, insular and cingulate. All models controlled for age and sex, whilst the lobar analyses additionally controlled for whole-brain measures (eg average surface area/thickness per hemisphere). Lobar comparisons were corrected for multiple comparisons using false-discovery rate (FDR) deriving from the method of Benjamini & Hochberg 1995, and calculated using package fdr-tool implemented in R.

3.1.4.2 Longitudinal Analysis

For the longitudinal analysis, linear mixed models were conducted using package

nlme (version 3.1-109). These models were used for the longitudinal analyses, as opposed to repeated-measures Analysis of Variance (ANOVAs), as they have been shown to be more robust against missing time-points and variable scan interval (Cnaan et al. 1997). Lobar comparisons were corrected for multiple comparisons in the same manner as the cross-sectional lobar analyses. Covariates were entered as in the cross-sectional analyses, however also including the group-by-time interaction and scan-interval. A quadratic term for age was entered and removed if it did not improve the model's fit.

3.2 Results

Relevant Demographics are the same as those presented in Table 2.1, 2.2 and 2.3 in Chapter 2. No significant correlations occurred between any measure of thickness or area (eg both left and right global and lobar) and chlorpromazine equivalents and duration of illness and are presented in Table 3.1.

Table 3.1 Table of Spearman's rank correlation coefficients between investigated thickness and area regions and chlorpromazine equivalents and duration of illness (in months).

Hemisphere	Region	Thickness				Surface Area			
		Duration of Illness		Chlorpromazine Equivalents		Duration of Illness		Chlorpromazine Equivalents	
		R	p	R	p	R	p	R	p
Left	Global	-0.064	0.670	-0.204	0.183	0.016	0.915	-0.049	0.749
	Frontal	-0.178	0.242	-0.135	0.379	0.008	0.957	-0.065	0.670
	Parietal	0.146	0.339	-0.155	0.311	0.097	0.523	-0.049	0.749
	Occipital	0.0324	0.831	-0.229	0.136	-0.081	0.595	-0.114	0.456
	Cingulate	0.0284	0.852	0.0205	0.894	0.097	0.523	0.106	0.488
	Insula	-0.324	0.0335	-0.171	0.263	0	1	-0.073	0.631
	Temporal	-0.064	0.670	-0.270	0.078	0.008	0.957	0.008	0.957
Right	Global	-0.134	0.380	-0.151	0.324	0.024	0.873	-0.040	0.790
	Frontal	-0.134	0.380	-0.066	0.669	-0.073	0.632	-0.090	0.558
	Parietal	-0.243	0.111	-0.171	0.263	0.097	0.523	-0.081	0.594
	Occipital	0.097	0.523	-0.163	0.286	-0.089	0.559	-0.057	0.709
	Cingulate	-0.008	0.957	0.106	0.488	0.073	0.632	0.040	0.790
	Insula	0.0284	0.852	0.036	0.810	-0.044	0.292	-0.094	0.540
	Temporal	-0.0691	0.651	-0.282	0.066	0.016	0.915	0.016	0.915

3.2.1 Cross-sectional Findings

3.2.1.1 Global Comparisons in Cortical Thickness and Surface Area between First Episode Patients, High Risk Individuals and Controls.

There was a significant main effect of group on left ($F = 13.6$ $df = 2, 197$, $p = 0.001$) and right ($F = 9.22$ $df = 2, 197$, $p = 0.001$) global cortical thickness. Subsequent post-hoc testing showed that this was due to patients having significantly thinner left global cortex compared to controls, ($t = 5.001$, $df = 197$, $p < .0001$), and compared to the high risk cohort ($t = -4.52$, $df = 197$, $p < .0001$). The same pattern of results

occurred for right global thickness; patients had significantly thinner right global cortex compared to controls ($t = 3.83$, $df = 197$, $p = 0.0005$) and those at high risk ($t = -4.058$, $df = 197$, $p = 0.002$), whilst no significant differences occurred between controls and those at high risk in either left or right global cortical thickness. These results are illustrated in Figure 3.1.

Figure 3.1 Figure showing mean differences in global a) left cortical thickness and b) right cortical thickness. * shows significantly thinner left and right cortical thickness in first-episode patients compared to controls and those at high risk

There was also a significant main effect of group on left ($F = 5.28$, $df = 2,197$, $p < .001$) and right ($F = 4.76$, $df = 2,197$, $p < .001$) global surface area, which subsequent post-hocs revealed were due to patients having significantly larger surface areas compared to controls ($t = -2.38$, $df = 198$, $p = 0.048$) and compared to those at high risk ($t = 3.062$, $df = 198$, $p = 0.0070$). This effect also occurred for right global surface area, with patients having significantly larger right global surface areas compared to both controls ($t = -2.64$, $df = 198$, $p = 0.0242$) and those at high risk ($t = 3.19$, $df = 198$, $p = 0.0047$). This is illustrated in Figure 3.2.

Figure 3.2 Figure showing mean differences in global a) left cortical surface area and b) right cortical surface area. * shows significantly larger left and right area in first-episode patients compared to controls and those at high risk

Cortical thickness and surface area results are summarized along means and associated standard errors in Table 3.2 and 3.3

Table 3.2 Table of main results for global cortical thickness and surface area group comparisons between those at high risk, controls and first-episode patients

Hemisphere	Region	Cortical Thickness			Surface Area		
		<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>p</i>
Left	Global	13.6	2,197	<0.001	5.28	2,197	0.005
Right	Global	9.22	2,197	0.0001	4.76	2,197	0.009

Table 3.3 Table of means and associated standard errors of the means for global cortical thickness and surface area group comparisons between those at high risk, controls and first-episode patients

Hemisphere	Region	Cortical Thickness			Surface Area		
		First Episode	Controls	High Risk	First Episode	Controls	High Risk
Left	Global	2.19(0.016)	2.30(0.014)	2.27(0.007)	109760(1716)	103723(1508)	103759(768)
Right	Global	2.20(0.017)	2.29(0.014)	2.28(0.007)	109685(1727)	104209(1517)	103892(772)

3.2.1.2 Lobar Comparisons in Cortical Thickness and Surface Area between First Episode Patients, High Risk Individuals and Controls.

After controlling for multiple comparisons, all left lobes remained significant, whilst all bar the parietal lobe remained significant for the right hemisphere. Subsequent post-hocs showed that this was because patients had significantly thinner left frontal thickness compared to controls ($t = 4.42$ $df = 197$, $p = 0.001$) and those at high risk ($t = -3.96$ $df = 197$, $p = 0.0003$), and right frontal thickness compared to controls ($t = 3.66$ $df = 197$, $p = 0.0009$) and those at high risk ($t = -4.26$ $df = 197$, $p = 0.0001$), thinner left ($t = 3.63$ $df = 197$, $p = 0.011$) and right ($t = 3.32$, $df = 197$, $p = 0.0030$) cingulate compared to controls and thinner left ($t = -4.92$ $df = 197$, $p < 0.001$) and right ($t = -3.92$, $df = 197$, $p = 0.0004$) cingulate compared to those at high risk.

Patients also had significantly thinner left ($t = 3.67$ $df = 197$, $p = 0.0009$) and right ($t = -4.40$ $df = 197$, $p = 0.001$) insula compared to controls and thinner left ($t = -3.71$ $df = 197$, $p = 0.0008$) and right ($t = -4.40$ $df = 197$, $p = 0.0001$) insula compared to those at high risk. Patients also had significantly thinner left ($t = -5.48$ $df = 197$, $p < 0.0001$) and right ($t = 5.38$ $df = 197$, $p < 0.0001$) occipital cortex compared to

controls and thinner left ($t = -4.9$ $df = 197$, $p < 0.0001$) and right ($t = -5.37$ $df = 197$, $p < 0.0001$) occipital compared to those at high risk. For the left parietal lobe, the only significant contrast was between controls and patients in that patients had significantly thinner cortex in the left ($t = 3.11$ $df = 197$, $p = 0.0059$) hemisphere. For left temporal thickness those at high risk had significantly thinner cortex than controls ($t = 2.42$ $df = 197$, $p = 0.043$), with patients having also significantly thinner temporal cortex than controls ($t = 4.01$ $df = 197$, $p = 0.0003$) and also those at high risk ($t = -2.72$ $df = 197$, $p = 0.020$), whilst for the right temporal lobe patients had significantly thinner cortex compared to controls ($t = 2.90$ $df = 197$, $p = 0.012$) and those at high risk ($t = -2.59$ $df = 197$, $p = 0.028$). These results are illustrated in Figure 3.3 and 3.4

Figure 3.3 Figure showing mean differences in left a) frontal b) parietal c) cingulate d) occipital e) insula f) temporal cortical thickness. * indicates significantly thinner cortex for patients with first-episode schizophrenia in all 6 lobes, compared to those at high risk, except for temporal cortex, where there was also a difference between those at high risk and first-episode patients.

Figure 3.4 Figure showing mean differences in right a) frontal b) occipital c) cingulate d) insula e) temporal cortical thickness. * indicates significantly thinner cortex for patients with first-episode schizophrenia in all 5 lobes

When adjusting for multiple comparisons, significant main effects for group remained for left surface area of the occipital lobe which subsequent post-hocs showed were because patients had significantly larger surface areas compared to those at high risk ($t = 2.71$, $df = 197$, $p = 0.0197$). For the left temporal lobe, those at high risk had significantly larger surface areas ($t = -2.81$, $df = 197$, $p = 0.015$) compared to the patient group. For the left cingulate lobe patients had significantly larger ($t = 2.75$, $df = 197$, $p = 0.018$) surface areas compared to those at high risk. For the right parietal surface area, patients had smaller parietal area compared to both controls ($t = 2.76$ $df = 197$, $p = 0.017$) and high risk ($t = -3.38$, $df = 197$, $p = 0.0025$). These results are shown in Figure 3.5

Figure 3.5 Figure showing mean differences in left a) occipital, with* showing significantly increased surface area in first-episode patients compared to high risk and controls b) temporal, with * showing significantly increased area in those at high risk compared to patients and c) cingulate surface area * showing significantly increased surface area in first-episode patients compared to high risk and controls, d) right parietal with patients showing * significantly decreased parietal surface area

The main effects for cortical thickness and surface area lobar results are summarized in Table 3.4 and means and associated standard errors in Table 3.5.

Table 3.4 Table of main results for lobar cortical thickness and surface area group comparisons between those at high risk, controls and first-episode patients

Hemisphere	Region	Cortical Thickness			Surface Area		
		<i>F</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>df</i>	<i>p</i>
Left	Frontal	10.6	2,197	0.000	0.045	2,197	0.956
	Parietal	4.87	2,197	0.008	0.045	2,197	0.638
	Occipital	15.2	2,197	0.000	4.58	2,197	0.011
	Cingulate	12.1	2,197	0.000	3.78	2,197	0.024
	Insula	8.01	2,197	0.004	1.9	2,197	0.151
	Temporal	8.05	2,197	0.000	3.95	2,197	0.020
Right	Frontal	9.53	2,197	0.001	0.025	2,197	0.974
	Parietal	1.26	2,197	0.284	0.88	2,197	0.332
	Occipital	17	2,197	0.000	2.1	2,197	0.124
	Cingulate	8.02	2,197	0.004	2.57	2,197	0.078
	Insula	10.9	2,197	0.001	3.68	2,197	0.026
	Temporal	4.52	2,197	0.0120	1.36	2,197	0.25879

Table 3.5 Table of means and associated standard errors of the means for lobar cortical thickness and surface area group comparisons between those at high risk, controls and first-episode patients

Hemisphere	Region	Cortical Thickness			Surface Area		
		First Episode	Controls	High Risk	First Episode	Controls	High Risk
Left	Frontal	2.32(0.022)	2.45(0.019)	2.41(0.001)	31783(178)	31787(153)	31827(78)
	Parietal	2.021(0.018)	2.10(0.016)	2.064(0.008)	21248(159)	21197(137)	21333(70)
	Occipital	1.77(0.016)	1.87(0.014)	1.86(0.007)	12144(170)	11932(147)	11636(75)
	Cingulate	2.28(0.026)	2.40(0.022)	2.42(0.011)	4487(69)	4306(60)	4278(30)
	Insula	2.73(0.028)	2.86(0.024)	2.84(0.012)	2274(34)	2212(29)	2201(15)
	Temporal	2.51(0.021)	2.62(0.018)	2.57(0.009)	22188(153)	22570(132)	22662(67)
Right	Frontal	2.31(0.023)	2.42(0.020)	2.41(0.010)	32019(197)	32075(170)	32041(87)
	Parietal	2.048(0.020)	2.090(0.017)	2.075(0.009)	21757(161)	22347(139)	22357(71)
	Occipital	1.79(0.017)	1.91(0.0145)	1.89(0.007)	12130(160)	11898(138)	11777(70)
	Cingulate	2.28(0.025)	2.39(0.028)	2.39(0.011)	4359(81)	4235(70)	4162(36)
	Insula	2.67(0.030)	2.84(0.026)	2.81(0.013)	2379(41)	2253(36)	2258(18)
	Temporal	2.55(0.022)	2.63(0.019)	2.61(0.009)	21799(145)	21683(125)	21907(64)

3.2.1.3 Global Comparisons in Cortical Thickness and Surface Area between High Risk Individuals based on subsequent Clinical Outcome

There were no significant differences in either left or right global thickness when the high risk individuals were considered based on subsequent clinical outcome.

However, a significant main effect occurred for left ($F = 4.51$, $df = 2, 137$, $p = 0.0127$) and right global surface area ($F = 4.50$, $df = 2, 136$, $p = 0.0128$). Subsequent post-hocs revealed that this was due to HR[ill] having significantly larger left ($t = -2.63$, $df = 137$, $p = 0.0254$) and right ($t = -2.52$, $df = 137$, $p = 0.034$) global surface areas compared to HR[well]. There was a trend for HR[symp] to have significantly larger left ($t = -2.17$, $df = 137$, $p = 0.079$) and right ($t = -2.32$, $df = 137$, $p = 0.057$) surface areas compared to HR[well]. No significant differences emerged between HR[symp] and HR[ill]. These findings are illustrated in Figure 3.6, and the results and group means for these investigated structures are presented in Table 3.6 and 3.7.

Table 3.6 Table of main results for global cortical thickness and surface area group comparisons between those HR[ill], HR[symp] and HR[well].

Hemisphere	Region	Cortical Thickness			Surface Area		
		<i>F</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>df</i>	<i>p</i>
Left	Global	0.155	2,136	0.856	4.51	2,137	0.0127
Right	Global	0.152	2,136	0.859	4.50	2,137	0.0128

Table 3.7 Table of means and associated standard errors of the means for global cortical thickness and surface area group comparisons between HR[ill], HR[symp] and HR[well].

Hemisphere	Region	Cortical Thickness			Surface Area		
		HR[well]	HR[symp]	HR[ill]	HR[well]	HR[symp]	HR[ill]
Left	Global	2.27(0.018)	2.28(0.012)	2.27(0.022)	101427(955)	104732(1061)	107505(1955)
Right	Global	2.27(0.010)	2.27(0.011)	2.26(0.021)	101517(959)	105020(1064)	107355(1960)

Figure 3.6. Figure showing * significantly increased global surface area in a) left and b) right hemisphere in HR[ill] compared to HR[well]

3.2.1.4 Lobar Comparisons in Cortical Thickness and Surface Area between High Risk Individuals based on subsequent Clinical Outcome

No significant differences emerged for any of the six lobes investigated when the high risk cohort was considered based on subsequent clinical outcome, and this is shown in Table 3.8 and 3.9.

Table 3.8 Table of main results for lobar cortical thickness and surface area group comparisons between HR[ill], HR[symp] and HR[well].

Hemisphere	Region	Cortical Thickness			Surface Area		
		<i>F</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>df</i>	<i>p</i>
Left	Frontal	0.842	2,136	0.433	0.089	2,137	0.914
	Parietal	0.891	2,136	0.413	1.02	2,137	0.362
	Occipital	1.58	2,136	0.209	2.31	2,137	0.103
	Cingulate	0.754	2,136	0.472	0.264	2,137	0.768
	Insula	0.608	2,136	0.545	0.059	2,137	0.307
	Temporal	0.137	2,136	0.872	1.05	2,137	0.351
Right	Frontal	0.079	2,136	0.924	2.26	2,137	0.108
	Parietal	0.001	2,136	0.864	0.051	2,137	0.950
	Occipital	0.011	2,136	0.505	0.862	2,137	0.424
	Cingulate	0.005	2,136	0.879	1.21	2,137	0.302
	Insula	0.001	2,136	0.991	1.01	2,137	0.345
	Temporal	0.406	2,136	0.669	0.38	2,137	0.684

Table 3.9 Table of means and associated standard errors of the means for global cortical thickness and surface area group comparisons between HR[ill], HR[symp] and HR[well].

Hemisphere	Region	Cortical Thickness			Surface Area		
		HR[well]	HR[symp]	HR[ill]	HR[well]	HR[symp]	HR[ill]
Left	Frontal	2.42(0.014)	2.41(0.015)	2.38(0.029)	31322(114)	31352(123)	31436(212)
	Parietal	2.08(0.012)	2.08(0.013)	2.05(0.025)	20742(225)	21242(250)	21954(461)
	Occipital	1.85(0.010)	1.87(0.016)	1.89(0.021)	11484(132)	11476(147)	11811(270)
	Cingulate	2.42(0.017)	2.42(0.019)	2.37(0.035)	4094(67)	4301(75)	4388(138)
	Insula	2.85(0.025)	2.82(0.026)	2.84(0.041)	2070(27)	2249(56)	2249(56)
	Temporal	2.58(0.014)	2.56(0.015)	2.57(0.028)	21995(272)	22581(301)	23505(555)
Right	Frontal	2.42(0.015)	2.41(0.016)	2.42(0.030)	31542(124)	31626(134)	31083(238)
	Parietal	2.06(0.011)	2.07(0.012)	2.056(0.023)	20742(225)	21242(250)	21954(461)
	Occipital	1.88(0.011)	1.90(0.012)	1.89(0.023)	11484(132)	11476(147)	11811(270)
	Cingulate	2.39(0.016)	2.39(0.018)	2.41(0.033)	4068(65)	4119(72)	4187(133)
	Insula	2.80(0.018)	2.81(0.020)	2.82(0.037)	2070(27)	2269(30)	2249(56)
	Temporal	2.61(0.014)	2.60(0.016)	2.58(0.029)	21167(208)	21953(232)	22451(427)

3.2.2 Longitudinal Findings

3.2.2.1 Global Comparisons in Cortical Thickness and Surface Area between High Risk Individuals and Controls

There was a significant group by time interaction for left ($F = 5.46$, $df = 1, 78$, $p = 0.022$) global cortical thickness, which occurred due to those at high risk not showing the increase in cortical thickness that occurred in controls ($t = -3.98$, $df = 78$, $p = 0.0001$), who in contrast underwent a relative thinning. The time by group interaction was also significant for right cortical thickness ($F = 3.99$, $df = 78$, $p = 0.0001$), in the same direction as left thickness ($t = -3.011$, $df = 78$, $p = 0.0034$). This is illustrated in Figure 3.7.

Figure 3.7. Baseline scan values of cortical thickness were normalized to 100%, and the percentage value of the follow-up scan in relation to this was obtained. Y axis represents the % difference between the two scans in a) left and b) right cortical thickness between controls and those at high-risk. *Indicates significant difference in cortical thickness change over time.

For left surface area, a significant group effect emerged ($F = 5.46$, $df = 1,78$, $p = 0.022$), which subsequent post hoc tests revealed were due to those at high risk showing a lesser decrease in surface area compared controls ($t = 2.32$, $df = 78$, $p = 0.023$). A trend for the same effect occurred also occurred for right surface area. This is shown in Figure 3.8. Table 3.10 shows the results of these comparisons, with Table 3.11 showing the means at baseline and follow-up.

Figure 3.8. Baseline scan values of surface area were normalized to 100%, and the percentage value of the follow-up scan in relation to this was obtained. Y axis represents the % difference between the two scans in a) left and b) right surface area between controls and all those at high-risk .
*Indicates significant difference in surface area change over time.

Table 3.10 Table of main results for global time by group interactions for global cortical thickness and surface area group comparisons between those at high risk and controls

Hemisphere	Region	Cortical Thickness			Surface Area		
		<i>F</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>df</i>	<i>p</i>
Left	Global	15,37	1,78	0.002	5.46	1,78	0.022
Right	Global	7.77	1,78	0.006	3	1,78	0.090

Table 3.11 Table of means and associated standard errors of the means for global cortical thickness and surface area group comparisons at baseline and follow-up scan between those at high risk and controls

		Cortical Thickness				Surface Area			
		High Risk		Controls		High Risk		Controls	
Hemisphere	Region	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Left	Global	2.27(0.007)	2.26(0.009)	2.29(0.014)	2.34(0.015)	103961(719)	101453(780)	104079(1418)	100538(1492)
Right	Global	2.27(0.007)	2.26(0.001)	2.29(0.015)	2.32(0.018)	103979(721)	101896(774)	104425(1427)	101645(1490)

3.2.2.2 Lobar Comparisons in Cortical Thickness and Surface Area between High Risk Individuals and Controls

When controlling for multiple comparisons, none of the surface area lobar comparisons survived multiple comparisons. For left and right thickness, after multiple comparisons control, a significant effect remained for frontal, temporal, occipital and cingulate cortex. Subsequent post-hocs showed that for the frontal lobe this was because of controls increasing significantly more over time in both left ($t = -3.65$, $df = 78$, $p = 0.0004$) and right ($t = -1.93$, $df = 78$, $p = 0.0068$) compared to high risk individuals, who in contrast underwent a relative thinning. This also occurred for left ($t = -2.57$, $df = 78$, $p = 0.0116$) and right ($t = 2.84$, $df = 57$, $p = 0.0053$) cingulate, left ($t = -3.22$, $df = 78$, $p = 0.0017$) and right ($t = -2.045$, $df = 57$, $p = 0.0432$) temporal and left ($t = -2.81$, $df = 78$, $p = 0.0057$) and right occipital ($t = -2.18$, $df = 78$, $p = 0.032$). This is illustrated in Figure 3.9, which combines the results for both lobes as these results showed a similar pattern bilaterally. Table 3.12 shows the results of these comparisons, unadjusted for multiple comparisons, and along with the global comparisons, with Table 3.13 showing the means of all investigated structures for each group.

Figure 3.9. Baseline scan values of thickness were normalized to 100%, and the percentage value of the follow-up scan in relation to this was obtained. Y axis represents the % difference between the two scans in a) temporal b) frontal c) occipital d) cingulate between controls and all those at high-risk . *Indicates significant difference in surface area change over time.

Table 3.12 Table of main results for lobar time by group interactions for cortical thickness and surface area group comparisons between those at high risk and controls

Hemisphere	Region	Cortical Thickness			Surface Area		
		<i>F</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>df</i>	<i>p</i>
Left	Frontal	15.83	1,78	0.001	0.194	1,78	0.66
	Parietal	3.94	1,78	0.047	0.656	1,78	0.418
	Occipital	8.4	7.49	0.006	0.026	1,78	0.873
	Cingulate	6.07	1,78	0.014	0.295	1,78	0.587
	Insula	1.7	1,78	0.197	0.292	1,78	0.589
	Temporal	12.36	1,78	0.007	0.969	1,78	0.325
Right	Frontal	7.64	1,78	0.005	2.9	1,78	0.09
	Parietal	2.69	1,78	0.101	4.3	1,78	0.038
	Occipital	4.87	1,78	0.027	1.44	1,78	0.229
	Cingulate	7.15	1,78	0.007	0.489	1,78	0.485
	Insula	4.48	1,78	0.038	0.059	1,78	0.81
	Temporal	5.6	1,78	0.018	6.57	1,78	0.01

Table 3.13 Table of means and associated standard errors of the means for lobar cortical thickness and surface area group comparisons at baseline and follow-up scan between those at high risk and controls

Hemisphere	Region	Cortical Thickness				Surface Area			
		High Risk		Controls		High Risk		Controls	
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Left	Frontal	2.41(0.016)	2.40(0.012)	2.44(0.019)	2.50(0.022)	31398(78)	31340(95)	31356(149)	31264(171)
	Parietal	2.07(0.009)	2.06(0.012)	2.09(0.017)	2.11(0.021)	21074(70)	21052(91)	20930(132)	20839(160)
	Occipital	1.86(0.008)	1.86(0.009)	1.87(0.015)	1.90(0.017)	31586(85)	31732(102)	31615(164)	31626(185)
	Cingulate	2.41(0.011)	2.41(0.015)	2.40(0.023)	2.45(0.027)	4207(32)	4206(43)	4239(59)	4216(73)
	Insula	2.84(0.012)	2.83(0.018)	2.86(0.024)	2.90(0.031)	2195(19)	2116(31)	2211(32)	2111(50)
	Temporal	2.57(0.009)	2.56(0.012)	2.63(0.019)	2.67(0.024)	22376(67)	22380(86)	22276(127)	22355(151)
Right	Frontal	2.41(0.001)	2.39(0.013)	2.42(0.020)	2.45(0.024)	31586(85)	31732(102)	31615(164)	31626(184)
	Parietal	2.06(0.008)	2.06(0.010)	2.09(0.016)	2.12(0.019)	22111(71)	21987(101)	22109(131)	21771(169)
	Occipital	1.89(0.008)	1.88(0.009)	1.91(0.016)	1.92(0.018)	11641(73)	11751(92)	11743(139)	11956(163)
	Cingulate	2.39(0.011)	2.43(0.016)	2.43(0.028)	2.39(0.022)	4083(35)	4157(42)	4153(67)	4249(76)
	Insula	2.81(0.013)	2.81(0.019)	2.83(0.026)	2.90(0.033)	2251(22)	2200(35)	2251(38)	2210(56)
	Temporal	2.61(0.001)	2.59(0.0135)	2.63(0.019)	2.67(0.024)	21674(65)	21536(86)	21454(121)	21527(149)

3.2.2.3 Global Comparisons in Cortical Thickness and Surface Area between High Risk Individuals based on subsequent Clinical Outcome

No significant longitudinal findings occurred for either left or right cortical thickness or surface area when the high risk individuals were considered based on subsequent clinical outcome. Results and means are presented in Table 3.14 and 3.15.

Table 3.14 Table of main results for global time by group interactions for cortical thickness and surface area group comparisons between HR[well], HR[symp] and HR[ill]

		Cortical Thickness			Surface Area		
		<i>F</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>df</i>	<i>p</i>
Left	Global	0.97	2,57	0.387	1.05	2,57	0.357
Right	Global	0.11	2, 57	0.896	0.951	2,57	0.392

Table 3.15 Table of means and associated standard errors of the means for global cortical thickness and surface area group comparisons at baseline and follow-up scan between HR[well], HR[symp] and HR[ill]

		Cortical Thickness						Surface Area					
		HR[well]		HR[symp]		HR[ill]		HR[well]		HR[symp]		HR[ill]	
Hemisphere	Region	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Left	Global	2.27(0.018)	2.26(0.014)	2.28(0.012)	2.27(0.147)	2.27(0.022)	2.24(0.026)	101427(955)	100715(1063)	104732(1061)	103385(1105)	106277(1977)	
	Right	Global	2.27(0.010)	2.26(0.013)	2.27(0.011)	2.28(0.013)	2.26(0.021)	2.24(0.023)	101517(959)	100788(1055)	105020(1064)	103800(1100)	10670(1972)

3.2.2.4 Lobar Comparisons in Cortical Thickness and Surface Area between High Risk Individuals based on subsequent Clinical Outcome

No significant longitudinal findings survived correction for multiple comparisons in any of the six investigated lobes for either left or right cortical thickness or surface area when the high risk individuals were considered based on subsequent clinical outcome. Results and means are presented in Table 3.16 and 3.17.

Table 3.16 Table of main results for lobar time by group interactions for cortical thickness and surface area group comparisons between HR[well], HR[symp] and HR[ill]

		Cortical Thickness			Surface Area		
		<i>F</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>df</i>	<i>p</i>
Left	Frontal	2.16	2, 57	0.125	0.98	2,57	0.382
	Parietal	0.3	2, 57	0.745	0.201	2,57	0.819
	Occipital	0.47	2, 57	0.628	0.599	2,57	0.553
	Cingulate	0.38	2, 57	0.685	3.2	2,57	0.048
	Insula	1.65	2, 57	0.202	5.28	2,57	0.007
Right	Temporal	0.83	2, 57	0.442	1.74	2,57	0.184
	Frontal	0.08	2, 57	0.922	0.87	2,57	0.426
	Parietal	0.83	2, 57	0.442	0.616	2,57	0.543
	Occipital	0.47	2, 57	0.628	0.614	2,57	0.545
	Cingulate	0.11	2, 57	0.893	0.052	2,57	0.949
	Insula	1.06	2, 57	0.352	3.25	2,57	0.046
	Temporal	0.26	2, 57	0.773	3.25	2,57	0.046

Table 3.17 Table of means and associated standard errors of the means for lobar cortical thickness and surface area group comparisons at baseline and follow-up scan between between HR[well], HR[symp] and HR[ill]

Hemisphere	Region	Cortical Thickness						Surface Area					
		HR[well]			HR[symp]			HR[ill]			HR[symp]		
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Left	Frontal	2.42(0.014)	2.41(0.018)	2.41(0.015)	2.42(0.018)	2.38(0.029)	2.33(0.032)	31322(114)	31185(154)	31352(123)	31313(148)	31436(212)	31409(240)
	Parietal	2.08(0.012)	2.05(0.016)	2.08(0.013)	2.07(0.017)	2.05(0.025)	2.05(0.030)	20742(225)	20567(251)	21242(250)	21005(261)	21954(461)	21704(466)
	Occipital	1.85(0.010)	1.84(0.013)	1.87(0.016)	1.87(0.014)	1.89(0.021)	1.20(0.024)	11484(132)	11411(150)	11476(147)	11483(155)	11811(270)	11736(276)
	Cingulate	2.42(0.017)	2.41(0.023)	2.42(0.019)	2.43(0.023)	2.37(0.035)	2.38(0.041)	4094(67)	4221(80)	4301(75)	4221(80)	4388(138)	4263(142)
	Insula	2.85(0.025)	2.85(0.051)	2.82(0.026)	2.85(0.045)	2.84(0.041)	2.78(0.059)	2070(27)	2102(36)	2249(56)	2181(38)	2249(56)	2302(66)
	Temporal	2.58(0.014)	2.56(0.018)	2.56(0.015)	2.57(0.018)	2.57(0.028)	2.56(0.032)	21995(272)	21898(303)	22581(301)	22287(314)	23505(555)	23125(561)
Right	Frontal	2.42(0.015)	2.41(0.020)	2.41(0.016)	2.39(0.020)	2.42(0.030)	2.37(0.036)	31542(124)	31595(167)	31626(134)	31774(161)	31083(238)	31259(260)
	Parietal	2.06(0.011)	2.056(0.015)	2.07(0.012)	2.07(0.015)	2.056(0.023)	2.03(0.027)	20742(225)	20567(251)	21242(250)	21005(261)	21954(461)	21704(466)
	Occipital	1.88(0.011)	1.88(0.014)	1.90(0.012)	1.89(0.014)	1.89(0.023)	1.88(0.025)	11484(132)	11411(150)	11476(147)	11483(155)	11811(270)	11736(276)
	Cingulate	2.39(0.016)	2.35(0.022)	2.39(0.018)	2.35(0.023)	2.41(0.033)	2.35(0.040)	4068(65)	4034(74)	4119(72)	4091(77)	4187(133)	4171(137)
	Insula	2.80(0.018)	2.78(0.027)	2.81(0.020)	2.83(0.028)	2.82(0.037)	2.86(0.049)	2070(27)	2102(36)	2269(30)	2181(38)	2249(56)	2302(66)
	Temporal	2.61(0.014)	2.60(0.019)	2.60(0.016)	2.60(0.020)	2.58(0.029)	2.56(0.035)	21167(208)	21114(235)	21953(232)	21654(243)	22451(427)	22079(434)

3.3 Discussion

The aim of this Chapter was to examine cross-sectional and longitudinal brain alterations to global and lobar measures of cortical thickness and surface area. Cross-sectionally, it was first assessed whether any differences were present between the high risk familial cohort, a sample of controls, and a sample of first-episode patients, in order to establish whether any cross-sectional alterations could be characterized as “trait” markers of schizophrenia, and thus present in the familial high risk cohort compared to controls, or whether they were more specifically associated with the disorder itself, and therefore selectively present in the sample of patients with first episode schizophrenia. Furthermore, it was also investigated whether baseline abnormalities in surface area and cortical thickness were present in the high risk sub-groupings based on subsequent clinical outcome, in order to investigate whether brain structural alterations in fact precede illness onset. Changes in these measures were also assessed (without the first episode patients as there was no longitudinal data for these individuals), to determine whether the longitudinal trajectory differed between these groups, rather than or in addition to static alterations present at one time-point.

Based on existing evidence that surface area and thickness reflect distinct developmental processes, we hypothesized that cortical thickness and surface area would be differentially affected in those at high risk compared to controls at baseline. Specifically, it was predicted that cortical thickness would show decreases, with the

first episode patients exhibiting the largest cortical thickness decreases, and that the alterations to area would be different to those in thickness, so thus either remain unaltered or show decreases. Longitudinally, it was predicted that there would be different developmental trajectories in both thickness and area in those at high risk compared to controls, and based on previous research that cortical thickness would decrease over time, whilst the surface area trajectory would be different. It was also predicted that within the high risk cohort, those that subsequently became ill would show the greatest alterations, as it has been suggested that the brain alterations found in schizophrenia may be present before disorder onset to a greater extent in those at high risk that go on to transition to the disorder, compared to those that do not.

The findings presented in this chapter are consistent with surface area and thickness being controlled by different developmental processes. This is consistent with the literature suggesting that early brain development is regulated by several different processes, that may interact and that form the complex product of the cortex. Cortical migration (Rakic, 1971) is a crucial developmental process that forms the columnar architecture of the cortex, as described in the radial unit hypothesis of cortical formation (Rakic 1977), and the findings presented in this chapter, are indicative of this process being disturbed early in neurodevelopment in people that go on to develop schizophrenia, and thus that this is an illness-specific developmental abnormality. In contrast, our altered thickness findings could be indicative of synaptic pruning alterations, eg excessive synaptic elimination, in people at high risk of developing schizophrenia compared to typically developing controls.

3.3.1 Cross-sectional Findings

In accordance with our hypothesis, it was found that the extremes of abnormalities were to be found in the patient group, as there was evidence for widespread cortical alterations distinguishing patients from controls and from those at high risk. Firstly, globally, patients had significantly thinner cortex than high risk individuals and healthy controls, as well as having significantly larger surface areas compared to both these groups. The findings are thus in accordance with the hypothesis that surface area and thickness would not be affected in the same manner, as they are thought to reflect distinct developmental processes.

The findings also generally support the hypothesis that those at high risk would show alterations compared to controls, as there were alterations in the temporal lobe at baseline and thickness alterations longitudinally in both global and more local regions of the cortex. Cortical thickness was notably thinner in patients compared to both those at high risk and controls. However, patients and high risk individuals had a shared thinning in the temporal lobe, compared to controls. This may be reflective of synaptic pruning mechanisms, for example excessive synaptic elimination occurring in those at high risk, compared to controls, occurring close to/right before disorder onset, and it is especially interesting that there is a shared alteration in the temporal lobe, which has previously shown to be abnormal in both those at high risk and patients (Harris et al. 2007). This shared localized alteration may therefore reflect a “trait” marker, or general vulnerability to developing schizophrenia.

The results of relatively unaltered thickness between high risk individuals and controls at baseline suggest that processes involved in the proliferation and numbers of cells within cortical columns are unaltered at baseline but may interact with developmental processes, such as synaptic pruning, to produce longitudinal alterations with both global and localized consequences in those at high risk of developing schizophrenia for familial reasons (Rakic 1988, Rakic 1995).

In contrast, surface area was most notably increased in patients. Specifically, it was found that patients had significantly increased surface area of both the left cingulate and occipital lobe, compared to those at high risk, whilst the opposite was found for the temporal lobe; with those at high risk having significantly increased surface area compared to patients. The right parietal lobe in contrast was smaller in patients. Interestingly, impaired structural connectivity between occipital, cingulate, and temporal and parietal lobes have previously been found in patients (Zalesky et al. 2011). As the “dysconnectivity” hypothesis suggests either increased or decreased connectivity amongst and between brain regions as being part of the central brain deficit of schizophrenia, it remains possible that the increases and decreases in surface area in these regions in the patient group are reflective of altered connectivity across these regions, which may be a function of altered cortical column formation early in development.

As the increases in surface area in both occipital and cingulate surface area were

only significant compared to those at high risk, but not controls, it remains possible that a relatively smaller surface area of these regions in those in the high risk group may reflect a resilience factor that acts to compensate for other alterations, such as the increased temporal surface area found in this cohort. It is interesting to note that although the cortical thickness and global alterations appear bilateral, the localized surface area in patients findings suggest lateralization to the left hemisphere, which is in keeping with other studies of the cortex suggesting the left hemisphere to be more affected in schizophrenia, compared to the right (Harris et al. 2007).

When the high risk individuals based on their clinical outcomes, there were no thickness alterations in either global or lobar thickness, however we found that at baseline, HR[ill] had a significantly larger cortical surface area compared to HR[well]. Intriguingly, this effect did not seem to have a more regional locus, as there was no evidence for group differences in baseline lobar surface area, but only a significantly larger mean surface area. Thus the results suggest a more global alteration in surface area may serve as a putative marker for subsequent transition to psychosis. No cortical thickness differences between the groups were evident at baseline, in either the global or lobar analyses.

A recent meta-analysis found a moderate effect size for increased whole-brain volume and gray matter volume in high risk individuals, compared to both controls and first-episode patients (Cooper et al. 2014). It is possible that these changes were driven by an increase in surface area, as previous studies have suggested that volume

is more driven by area than thickness (Hogstrom et al. 2012). As these alterations are present in HR[ill] before disorder onset, the findings presented in this thesis suggest a neurodevelopmental disruption to genes/processes involved in surface area expansion. Previous studies have also found that larger surface areas are associated with more gyrified cortices (Hogstrom et al. 2012, Storsve et al. 2014), and therefore it is important to also investigate gyrification when assessing the cortex.

3.3.2 Longitudinal Findings

The longitudinal findings suggest that different trajectories in thickness and area may differentiate those at high risk from controls. In controls, area decreased over time whilst thickness increased. However, for those at high risk, surface area was relatively preserved over time whilst thickness decreased. Previous studies have found that larger surface areas are linked to thinner cortices, while reduced surface area is linked to increased thickness (Hogstrom et al. 2012, Storsve et al 2014). This inverse relationship may reflect the differential effect of genes which control the developmentally distinct periods of symmetric and asymmetric division. As this inverse relationship seems altered in those at high risk compared to controls, in that they showed a relatively preserved area coupled with increased thinning, it may be that genes involved in regulating the pattern of change in these two parameters may be disrupted in those at high risk, and may reflect a general vulnerability marker to schizophrenia.

Longitudinally, it was found that all high risk participants had a relatively preserved developmental trajectory of area compared to controls, who in contrast underwent a significantly greater decrease in area. These longitudinal abnormalities may thus be accurately specified as a general vulnerability marker of psychosis. These results are consistent with the literature suggesting that those at high risk share certain alterations, with further brain abnormalities differentiating those that transition to psychosis (Job et al 2003, Job et al. 2005) Dazzan et al. 2011, Pantelis et al. 2003).

Longitudinal analysis indicated that controls showed a significantly greater increase in global cortical thickness compared to all high risk individuals, who in contrast showed a relative thinning. Furthermore, significant regional thickness abnormalities were found in the frontal, cingulate and occipital and temporal lobes in that those at high risk who showed thinning over time, whilst controls underwent a significant relative thickening over time. These abnormalities were not linked to subsequent clinical outcome, suggesting that these alterations may also be specified as markers of a general vulnerability to psychosis. Areas of these lobes have previously been shown to be abnormal both in high risk research (Borgwardt et al. 2007, Asami et al. 2014, Rapp et al. 2013), as well as established schizophrenia (Salgado-Pineda et al. 2014, Schultz et al. 2013) suggesting that these regions may form part of localized brain networks disrupted in psychosis.

3.3.3 Implications of Cross-sectional and Longitudinal Findings

Identifying markers of schizophrenia that are present in those that go on to develop schizophrenia, before disorder onset, could enhance the power of early detection of psychosis. To our knowledge, this study is the first study to investigate baseline and longitudinal cortical thickness and area in a familial high risk cohort using both global and more localized measures, whilst also distinguishing between HR-T and HR-NT, as well as those at high risk that developed transient symptoms.

This methodology allows researchers to delineate which structural changes are more likely to be specific markers of the disorder, facilitating the predictive value of brain-imaging in clinical settings. As many individuals deemed at high risk for psychosis due to familial or clinical reasons never go on to develop psychosis, this approach may also help researchers examine whether aspects of brain structure confer resilience to the disorder in HR[well]. It is possible that a relatively smaller surface area in HR[well] compared to HR[ill] serves as a resilience factor against schizophrenia, and warrants further investigation. It is interesting to note that the abnormalities in surface area found in the present study are on a global scale, whilst the cortical thinning was also evident in localized regions of the cortex. To our knowledge, this is the first longitudinal study of global surface area in individuals at familial high risk of developing psychosis. Localized cortical thinning may serve as a general vulnerability indicator in high risk individuals, whilst global surface area abnormalities are associated to a larger degree with subsequent psychosis and illness.

However, it also remains possible that a larger sample size or longer follow-up period would have enabled us to detect more regionally localized alterations to surface area as well as lobar differences between HR[ill], HR[symp] and HR[well].

Nonetheless, this study remains the largest longitudinal study of individuals at familial high risk of psychosis. Given that the scans were acquired prior to disorder onset, our results have allowed us to quite robustly distinguish general and more specific markers of the disorder in antipsychotic-naïve high risk individuals. Future work is required to determine whether these abnormal developmental trajectories are further augmented with progression of the disease.

3.3.4 Conclusions

The findings presented in this chapter suggest that widespread cortical alterations, at baseline, occur in patients that have experienced their first episode of schizophrenia, compared to those at high risk and healthy controls. However, shared alterations exist between patients and HR[ill]; specifically an increased global surface area, suggesting that this may be a marker of clinical risk, whilst the shared alteration between all those at high risk and patients in the temporal lobe may be more indicative of a general vulnerability factor. Abnormal cortical development, as indexed by cortical thickness occurs over time both globally and in specific cortical regions, more specifically in the frontal, occipital and cingulate cortices compared to controls and may be tied to a general familial risk of the disorder. It was found that

baseline measures of surface area distinguished HR[ill] from HR[well], and that this was not specifically localized to any lobe, potentially reflecting a disrupted developmental process with global consequences. This alteration may be associated with mechanisms related to the transition to psychosis, as opposed to a general familial disposition to the disorder. Progressive changes in both area and thickness were not related to subsequent clinical outcomes of the high risk cohort, but rather seemed to reflect differential developmental trajectories present in all high risk individuals, and may therefore serve as a marker of a general vulnerability to develop schizophrenia.

CHAPTER 4

A Cross-sectional and Longitudinal Investigation of Gyrification

4. Introduction

The previous Chapter focused on both cross-sectional and longitudinal group differences in global and lobar indices of cortical thickness and surface area. It was also mentioned that the relationship between thickness and area may also be influenced by a third cortical parameter, namely gyrification. As the cortex develops, it folds and fissures, resulting in the characteristically complex morphology of the human brain. Its surface consists of many sulci, which are depressions or small grooves of the cortex that are surrounded by gyri, the ridges of the cortex, which forms the characteristic convoluted or “folded” appearance of the brain (Armstrong et al. 1995). Figure 4.1 illustrates this appearance.

Figure 4.1. Diagrammatic representation of the characteristic morphology of the cortex

The process by which the cortex develops a convoluted appearance is referred to as gyrification. It is thought that gyrification occurs in part to accommodate the expanding surface area of the cortex (White & Hilgetag 2011). The human brain has acquired a disproportionately large increase in surface area during evolutionary development which has not been observed other mammals, and which is not accompanied by a proportionate increase in thickness (Sol et al. 2008, Barton 2007). Researchers have suggested that this evolutionary trend of increasing surface area and gyrification, as opposed to thickness, ie “cortical stretching” maximises brain connectivity and functional development (Hogstrom et al. 2012). This has been empirically confirmed in computational modelling studies that have shown that increased gyrification and areal expansion provide an efficient means by which to facilitate brain connectivity and functional development (Murre & Sturdy 1995, Ruppin et al. 1993)

Several theories exist regarding the mechanisms by which the brain develops from its smooth, lissencephalic form to its final gyrified one. Empirical studies have shown that gyrification does not occur due to the constraints of an inflexible cranium, as folding of the cortex has been demonstrated in cultured brains of rodents in the absence of a skull (Kingsbury et al., 2003), and therefore many theories posit gyrification to be an intrinsic property of the growing cortex, although contrasting theories do exist (Ronan et al. 2014). The first theory emerged over a century ago and postulated that higher and lower growth rates of different brain regions separate gyral regions as a result of tension and local tissue deformation (His, 1874). One of the most prominent contemporary theories of gyrification is that of Van Essen (1997),

which posits that the neuronal connectivity established during the second trimester of development produces fibre tension of axons, drawing together densely connected regions, forming gyri, whereas less heavily connected regions drift, and create sulci. Thus the characteristic shape of the cortex is linked to an effective means by which to transit neural signals. If tension produced by these neuronal connections is indeed involved in the mechanisms of gyrification, then brain surface morphology could be an indirect marker of regional neuronal connectivity (White and Hilgetag, 2011). Some empirical animal evidence has supported this theory, as it has been found that changes in gyrification occur after induced white-matter lesions in the primate brain (Goldman-Rakic 1980, Goldman-Rakic & Rakic 1984). The idea that gyrification is a marker of connectivity has crucial implications in the search of sMRI markers of schizophrenia, as the disorder is increasingly being referred to as a disorder primarily of “dys-connectivity”, ie decreased/increased connectivity within/between brain regions (For a more detailed overview of the connectivity topic, please see Chapter 1)

Gyrification is established early in fetal life, with dramatic changes occurring in the third trimester of foetal development (Chi et al. 1977), when the brain develops its final gyrified form. It is thought that this developmental period is particularly sensitive to small developmental perturbations, as it involves several processes of neuronal development (see Chapter 1 for a more detailed overview of symmetric and asymmetric division of neuronal cells), which could dramatically influence the gyrification of the brain. As folding of the cortex occurs after the processes giving rise to cortical thickness and surface area (Rakic 1988), which can also be influenced by later developmental and degenerative processes, investigation of this third cortical

parameter may provide a more detailed time-scale of any postulated neurodevelopmental disruption present in schizophrenia, than investigations of thickness and area alone.

Gyrification has also been shown to be altered in several psychiatric disorders, such as autism and depression (Palaniyappan & Liddle 2012, Kates et al. 2009, Zhang et al. 2009, 2010). However, research on gyrification in schizophrenia has so far provided disparate findings, with some studies indicating that patients are characterized by increased gyrification (hypergyria), whereas others suggest decreased gyrification (Harris et al. 2004, 2007, Nandas et al. 2014). Investigations of individuals at high risk of developing schizophrenia for either familial or clinical reasons have also produced disparate findings. Harris et al. (2004) used hand-traced gyrification indices in a familial high risk sample and found increases in right prefrontal lobe GI values in those that transitioned to schizophrenia compared to those that did not. Interestingly, these differences were apparent in individuals that did not go on to transition until an average of 2.5 years later. Vogeley et al. (2001) found right hypergyria in affected and unaffected siblings in families multiply affected with schizophrenia. Falkai et al. (2007) investigated frontal as well as parieto-occipital GI in families affected with schizophrenia and found that frontal, but not parieto-occipital GI was significantly higher in patients with schizophrenia as well as unaffected relatives, compared to a healthy control group. However, Jou et al. (2005) found reduced left frontal cortical folding in unaffected relatives. Whilst Schaer et al. (2006) investigated individuals with the 22q11.2 deletion syndrome, a genetic condition associated with increased risk for developing schizophrenia and

found that those with the deletion showed a significant decrease of GI in the frontal and parietal lobes.

As the findings on gyrification, both in those at high risk of developing schizophrenia, as well as those with the established disorder currently remain so diverse and relatively few, it is unclear whether these alterations reflect part of an underlying neurodevelopmental disruption, or are perhaps more specifically associated with illness related factors. Furthermore, very few longitudinal studies of gyrification have been conducted in schizophrenia, and as far as we know, none have been conducted in a prospective familial high risk cohort. Adolescence is characterized by several maturational brain processes that cause drastic changes in gray matter and thickness (Gotgay et al. 2004), and it remains possible that baseline changes in GI can be confidently attributed to early neurodevelopmental events whilst longitudinal change could perhaps be secondary to changes in thickness or area.

The gyrification index is a metric that quantifies the amount of cortex buried within the sulcal folds, as compared with the amount of cortex on the outer visible cortex. Thus, a cortex with extensive folding has a large gyrification index, whereas a cortex with limited folding has a small gyrification index. Previously, classical two-dimensional Gyrification index (2D-GI, Zilles et al. 1988) has measured the ratio of the total pial cortical surface over the perimeter of the brain but on two-dimensional coronal sections. This original 2-D GI index delineates surfaces on coronal sections, which does not take into account the inherent 3-D nature of the cortical surface. Hence, this measurement may be biased by slice orientation, and buried sulci cannot always be included. These studies have often also relied on manual delineation of the

contours, which raises the question of reliability and reproducibility in the context of large-scale studies such as ours. However, Freesurfer now implements a vertex-wise extension of this method which is thought to bypass several of the limitations associated with the 2-D coronal approach by implementing the gyrification index three-dimensionally, across the cortex.

The dataset used for the present study is exactly the same as outlined in Chapter 2. The aim of this study was to look at baseline measures of global and lobar estimates of gyrification in individuals at high risk of developing schizophrenia for familial reasons, healthy controls and a sample of first episode patients, and to then look at differences between high risk individuals based on their subsequent clinical outcomes. Based on previous studies conducted on the same cohort, we predicted that HR[ill] would have increases in gyrification, along with the patients in frontal regions. As no previous studies have measured gyrification in a high risk cohort longitudinally, we aimed to explore this research question in a hypothesis free manner.

4.1 Methods

4.1.1 Participants

These are the same as those reported in Chapter 2.

4.1.2 Imaging Parameters

These are the same as those reported in Chapter 2.

4.1.3 Freesurfer Reconstruction

This is the same as that reported in Chapter 2.

4.1.4 Longitudinal Pipeline

This is the same as that reported in Chapter 2.

4.1.5 Gyrification Procedure

The gyrification index (GI) is a measure that quantifies the amount of cortex buried within the sulcal folds, as compared with the amount of cortex on the outer visible cortex. Thus, a cortex with extensive folding will be reflected in a large GI, whilst a less folded cortex will have a smaller GI. Unlike surface area and thickness, gyrification is not an automated processing step in the surface-based stream, and requires some additional steps. The flag (-localGI) is used and runs in combination with scripts in Matlab, and is based on a method which computes measurements of gyrification across the whole cortical surface (Schaer et al. 2008). This method is thought to be advantageous compared to the classical two-dimensional Gyrification index mentioned in more depth in Chapter 1 (Zilles et al. 1988), which is the ratio of the total pial cortical surface over the perimeter of the brain but on two-dimensional coronal sections.

Figure 4.2. Figure adapted from Freesurfer showing creation of 3-D Gyrification Indices

With the gray–white interface constructed via surface registration and cortical inflation from the Freesurfer workflow, a pial surface is first obtained by constructing a set of lines perpendicular to the gray–white interface. Smoothing is then conducted to ensure that the local curvature at all points on the smoothed pial surface is less than the curvature of a 15-mm radius sphere. The chosen radius of 15 mm for the smoothing is designed to ensure that the hull surface remains external to the sulcal dips (Schaer et al. 2008). This surface then acts as the outer perimeter, whereas the original pial surface provides the inner perimeter (Schaer et al. 2008). Both inner and outer surfaces are tessellated with numerous vertices that are formed by the meeting points of triangles. For each vertex on the outer surface, a spherical region of interest is created with the vertex as the centre point and a standard 25-mm

radius. This sphere yields two area measures for each vertex. The outer measure is area of that part of the hull defined by the intersection of this sphere with the hull surface. To measure the corresponding pial surface area, the respective pial region of interest for the given vertex on outer hull surface is determined as follows. Initially all vertices within Area jO (on the hull surface) are identified. After this, the nearest pial vertex to each of these hull vertices is identified. These pial vertices define the outline of pial mesh, whose area is then calculated with sum of areas of all included triangular tessellations (Schaer et al (2008)). The ratio of the pial surface area to the outer surface area gives the LGI for each vertex on the outer surface. These outer surface values are redistributed to the pial surface with a weighted sum of all outer surface LGIs to which each pial vertex contributed during the prior computation. The mean LGI calculated with all vertices present within a predefined sulco-gyral region of the atlas is used as the LGI of that anatomical sub-region (Schaer et al. 2008). In this analysis, LGI values for global, and lobar (frontal, temporal, occipital, parietal, cingulate and insula) were used.

4.1.6 Statistical Analysis

All statistical analyses were computed with R version 3.0.2 (<http://www.r-project.org>). Where variables were found to be non-normally distributed, data was transformed using a Box Cox transform. Post-hoc testing of significance values was conducted using the package *lsmeans* (version 2.00-1). Additionally, in the patient group, chlorpromazine equivalents of current medication dose were calculated according to Woods et al. (2003) and Atkins et al. (1997) and Spearman's rank

correlation coefficients between gyrification, both lobar and global, and chlorpromazine equivalents and duration of illness (in months) were also calculated.

4.1.6.1 Cross-sectional Analysis

ANCOVAs were used for all cross-sectional analyses. Firstly, we conducted cross-sectional analyses comparing mean gyrification per hemisphere in the first-episode patients, high risk cohort and healthy controls. These analyses were then repeated for the high risk cohort based on their subsequent clinical outcome. We then performed the same analyses for the frontal, temporal, cingulate, insular, parietal and occipital lobes. Results for lobar comparisons were corrected for multiple comparisons using false-discovery rate. For the baseline analysis, differences between groups were modelled including the fixed effects of age and sex. Furthermore, as the degree of gyrification has been shown to be positively associated with surface area, we controlled for global surface area in all analyses (Ronan et al. 2013).

4.1.6.2 Longitudinal Analysis

Linear mixed models implemented in package nlme (version 3.1-109) for the longitudinal analyses. For longitudinal comparisons, covariates were entered as in the cross-sectional analyses, with the addition of the group-by-time interaction and co-varying for scan-interval. A quadratic term for age was entered and removed if it did not improve the model's fit, as were other variables.

4.2 Results

Relevant Demographics are the same as those presented in Table 2.1, 2.2 and 2.3 in Chapter 2. No significant correlations occurred between any measure of gyrification (eg both left and right global and lobar) and chlorpromazine equivalents and duration of illness (Table 4.1)

Table 4.1 Table of Spearman's rank correlation coefficients between investigated gyrification regions and chlorpromazine equivalents and duration of illness (in months).

Hemisphere	Region	Duration of Illness		Chlorpromazine Equivalents	
		R	<i>p</i>	R	<i>p</i>
Left	Global	-0.03	0.91	-0.02	0.92
	Parietal	-0.24	0.3	0.05	0.84
	Frontal	-0.04	0.88	0.04	0.86
	Cingulate	0.13	0.58	-0.02	0.94
	Temporal	0.02	0.94	0.1	0.68
	Occipital	0.15	0.52	0.11	0.64
	Insula	-0.16	0.5	0.13	0.59
Right	Global	-0.06	0.77	0.08	0.72
	Parietal	-0.1	0.68	0.16	0.49
	Frontal	-0.18	0.44	0.18	0.45
	Cingulate	0.11	0.66	0.05	0.82
	Temporal	-0.07	0.77	0.19	0.42
	Occipital	0.09	0.7	0.03	0.92
	Insula	-0.19	0.41	0.14	0.55

4.2.1 Cross-sectional Findings

4.2.1.1 Global Comparisons in gyrification between First-episode patients, High Risk Individuals and Controls

For mean gyrification of the left hemisphere, there was a significant group difference ($F = 16.3$ $df = 2,197$ $p < .0001$). Subsequent post-hocs revealed that this was due to patients showing significantly greater gyrification indices compared to controls ($t = -5.035$, $df = 2,197$, $p < .0001$) as well as compared to those at high risk ($t = 5.36$, $df = 2,197$, $p < .0001$) (Figure 4.3). There were however no significant differences between controls and those at high risk. For the right hemisphere, a significant main effect of group occurred in mean gyrification, ($F=10.14$, $df, = 2,197$ $p < .0001$). Post-hoc testing showed this was due to patients showing increased gyrification compared to controls ($t= -3.469$, $df = 2,197$, $p < .05$), and those at high risk ($t = 4.456$, $df =2,197$, $p < .0001$). No significant differences emerged between controls and those at high risk. A summary of these analyses is presented in Table 4.2, and means and standard errors in Table 4.3.

Figure 4.3 Mean gyrification index of a) the left hemisphere and b) the right hemisphere for controls, those at high risk and first episode Patients. * indicates a significantly larger gyrification for patients compared to controls and those at high risk.

Table 4.2 Table of main results for global gyrification group comparisons between those at high risk, controls and first-episode patients

		Gyrification		
Hemisphere	Region	<i>F</i>	<i>df</i>	<i>p</i>
Left	Global	16.3	2,197	0.0001
Right	Global	10.1	2,197	0.001

Table 4.3 Table of means and associated standard errors of the means for global gyrification group comparisons between those at high risk, controls and first-episode patients

		Gyrification		
Hemisphere	Region	First Episode	Controls	High risk
Left	Global	3.17(0.024)	3.03(0.025)	3.05(0.017)
Right	Global	3.15(0.026)	3.04(.026)	3.03(0.018)

4.2.1.2 Lobar Comparisons in Gyrification between First Episode Patients, High Risk Individuals and Controls

After applying the FDR correction to all results, gyrification for the left parietal, frontal, cingulate, temporal, occipital showed significant hypergyria compared to those at high risk and controls. However, for the temporal lobe, post-hoc tests revealed both those at high risk and patients showed significant hypergyria compared to controls, with no significant differences emerging between those at high risk and patients. For the right hemisphere, only the right cingulate lobe remained significant after controlling for multiple comparisons and post-hoc tests revealed this was due to patients showing significant hypergyria compared to controls and those at high risk. Figure 4.4 shows significant lobar results for the left and right hemisphere. These results are summarized in Table 4.4 and Table 4.5 summarizing means for each group for each investigated region.

Figure 4.4. Figure of all significant lobar analyses for a = left frontal b = left cingulate c = left temporal, d = left occipital , e = left parietal and e = right cingulate. Gyrification is measured in mm. * Indicates significantly increased gyrification in patients compared to controls and high risk, except for c which shows increased gyrification in high risk and patients compared to controls

Table 4.4 Table of main results for lobar gyrification group comparisons between those at high risk, controls and first-episode patients

Hemisphere	Region	Gyrification		
		<i>F</i>	<i>df</i>	<i>p</i>
Left	Frontal	4.99	2,197	0.008
	Parietal	5.90	2,197	0.003
	Occipital	9.89	2,197	0.0001
	Cingulate	14.5	2,197	0.004
	Insula	1.27	2,197	0.280
	Temporal	5.94	2,197	0.003
Right	Frontal	1.68	2,197	0.189
	Parietal	1.23	2,197	0.295
	Occipital	3.58	2,197	0.029
	Cingulate	5.56	2,197	0.004
	Insula	0.007	2,197	0.994
	Temporal	3.92	2,197	0.022

Table 4.5 Table of means and associated standard errors of the means for lobar gyrification group comparisons between those at high risk, controls and first-episode patients

Gyrification				
Hemisphere	Region	First Episode	Controls	High risk
Left	Frontal	2.76(0.020)	2.69(0.018)	2.71(0.012)
	Parietal	3.15(0.020)	3.23(0.021)	3.16(0.014)
	Occipital	2.60(0.020)	2.70(0.022)	2.61(0.013)
	Cingulate	2.29(0.020)	2.36(0.02)	2.28(0.010)
	Insula	4.40(0.160)	4.51(0.21)	4.40(0.100)
	Temporal	3.25(0.023)	3.34(0.025)	3.30(0.015)
Right	Frontal	2.72(0.040)	2.86(0.520)	2.72(0.260)
	Parietal	3.29(0.050)	3.40(0.670)	3.17(0.033)
	Occipital	2.68(0.062)	2.78(0.080)	2.67(0.040)
	Cingulate	2.29(0.021)	2.37(0.024)	2.28(0.011)
	Insula	4.57(0.163)	4.87(0.210)	4.31(0.100)
	Temporal	3.28(0.710)	3.46(0.098)	3.23(0.046)

4.2.1.3 *Global comparisons in Gyrification between High Risk Individuals based on subsequent Clinical Outcome*

No significant group differences in mean gyrification occurred for the left or right hemisphere when the high risk group was considered based on subsequent clinical outcome. All results are presented in Table 4.6 and Table 4.7.

Table 4.6 Table of main results for global gyrification group comparisons between HR[ill], HR[symp] and HR[well].

Hemisphere	Region	Gyrification		
		<i>F</i>	<i>df</i>	<i>p</i>
Left	Global	2.02	2,137	0.135
Right	Global	0.432	2,137	0.651

Table 4.7 Table of means and associated standard errors of the means for global gyrification group comparisons between HR[well], HR[symp] and HR[ill]

Hemisphere	Region	Gyrification		
		HR[well]	HR[well]	HR[ill]
Left	Global	2.99(0.009)	2.97(0.009)	2.98(0.018)
Right	Global	2.98(0.009)	2.96(0.940)	2.96(0.018)

4.2.1.4 Lobar Comparisons in Gyrification between High Risk Individuals based on subsequent Clinical Outcome

No significant differences occurred in mean gyrification per lobe in any of the high risk sub-groupings occurred for either the left or right hemisphere. These results are presented in Table 4.8, and 4.9.

Table 4.8 Table of main results for lobar gyrification group comparisons between HR[ill], HR[symp] and HR[well].

		Gyrification		
		<i>F</i>	<i>df</i>	<i>p</i>
Left	Frontal	0.15	2,137	0.89
	Parietal	1.67	2,137	0.27
	Occipital	1.26	2,137	0.22
	Cingulate	0.26	2,137	0.77
	Insula	3.15	2,137	0.04
	Temporal	1.7	2,137	1.87
Right	Frontal	0.16	2,137	0.85
	Parietal	1.73	2,137	1.8
	Occipital	0.32	2,137	0.73
	Cingulate	1.32	2,137	0.3
	Insula	0.6	2,137	0.55
	Temporal	0.15	2,137	0.86

Table 4.9 Table of means and associated standard errors of the means for lobar gyrification group comparisons between HR[ill], HR[symp] and HR[well].

Hemisphere	Region	Gyrification		
		HR[well]	HR[symp]	HR[ill]
Left	Frontal	2.68(0.014)	2.69(0.016)	2.68(0.03)
	Parietal	3.13(0.015)	3.10(0.017)	3.11(0.030)
	Occipital	2.56(0.014)	2.55(0.016)	2.59(0.029)
	Cingulate	2.20(0.014)	2.22(0.016)	2.22(0.029)
	Insula	4.51(0.035)	4.48(0.039)	4.52(0.071)
	Temporal	3.25(0.017)	3.23(0.019)	3.27(0.034)
Right	Frontal	2.68(0.014)	2.67(0.016)	2.65(0.030)
	Parietal	3.15(0.014)	3.14(0.015)	3.12(0.028)
	Occipital	2.62(0.016)	2.61(0.017)	2.63(0.032)
	Cingulate	2.24(0.016)	2.24(0.017)	2.26(0.032)
	Insula	4.41(0.031)	4.41(0.041)	4.29(0.075)
	Temporal	3.22(0.016)	3.19(0.018)	3.17(0.033)

4.2.2 *Longitudinal Findings*

4.2.2.1 *Global comparisons in Gyrfication between High Risk individuals and Controls*

When all those at high risk were compared against controls, a trend emerged for a significant time by group interaction in left gyrfication, ($F = 3.36$, $df = 1, 78$, $p = 0.0706$). Analysis of means indicated that this trend was due to those at high risk having significantly less decrease over time in left gyrfication compared to controls, ($t = 1.5$, $df = 78$, $p = 0.06$). For the right hemisphere, no significant group by time nor trend for such an interaction. These results are presented in Table 4.10 and 4.11

Table 4.10 Table of main results for global time by group interactions for gyrification group comparisons between those at high risk and controls

		Gyrification		
		<i>F</i>	<i>df</i>	<i>p</i>
Left	Global	3.36	1,78	0.071
Right	Global	1.89	1,78	0.173

Table 4.11 Table of means and associated standard errors of the means for global gyrification group comparisons at baseline and follow-up scan between those at high risk and controls

		Gyrification			
		High Risk		Controls	
Hemisphere	Region	Baseline	Follow-up	Baseline	Follow-up
Left	Global	2.99(0.006)	2.96(0.007)	2.96(0.012)	2.92(0.014)
Right	Global	2.97(0.06)	2.95(0.010)	2.97(0.012)	2.94(0.014)

4.2.2.2 *Lobar comparisons in Gyrification between High Risk Individuals and Controls*

No significant time by group interaction between those at high risk and controls occurred in lobar gyrification. These results are presented in Table 4.12 and 4.13

Table 4.12 Table of main results for lobar time by group interactions for gyrification group comparisons between those at high risk and controls

		Gyrification		
		<i>F</i>	<i>df</i>	<i>p</i>
Left	Frontal	0.36	1,78	0.548
	Parietal	3.74	1,78	0.068
	Occipital	1.1	1,78	0.298
	Cingulate	0.08	1,78	0.776
	Insula	2.1	1,78	0.152
	Temporal	2.69	1,78	0.105
Right	Frontal	2.05	1,78	0.156
	Parietal	0.34	1,78	0.561
	Occipital	1.06	1,78	0.307
	Cingulate	0.09	1,78	0.769
	Insula	2.05	1,78	0.155
	Temporal	2.62	1,78	0.109

Table 4.13 Table of means and associated standard errors of the means for global gyrification group comparisons at baseline and follow-up scan between those at high risk and controls

Gyrification					
Hemisphere	Region	High Risk		Controls	
		Baseline	Follow-up	Baseline	Follow-up
Left	Frontal	2.67(0.007)	2.65(0.008)	2.65(0.014)	2.61(0.016)
	Parietal	3.11(0.008)	3.08(0.009)	3.09(0.016)	3.034(0.018)
	Occipital	2.57(0.008)	2.56(0.008)	2.57(0.015)	2.54(0.017)
	Cingulate	2.57(0.008)	2.56(0.008)	2.57(0.015)	2.54(0.017)
	Insula	4.51(0.018)	4.47(0.021)	4.47(0.035)	3.39(0.040)
Right	Temporal	3.24(0.009)	3.22(0.010)	3.19(0.018)	3.15(0.020)
	Frontal	2.67(0.007)	2.65(0.008)	2.64(0.014)	2.61(0.016)
	Parietal	2.67(0.007)	2.65(0.008)	2.64(0.014)	2.61(0.016)
	Occipital	2.57(0.008)	2.56(0.009)	2.56(0.015)	2.54(0.017)
	Cingulate	2.21(0.007)	2.19(0.009)	2.22(0.015)	2.21(0.017)
	Insula	4.51(0.018)	4.47(0.021)	4.47(0.035)	3.39(0.040)
	Temporal	3.24(0.009)	3.22(0.011)	3.19(0.018)	3.14(0.020)

4.2.2.3 *Global comparisons in gyrification between High Risk individuals based on subsequent Clinical Outcome*

No significant time by group interaction emerged for left gyrification when the high risk group were considered based on subsequent clinical outcome, nor for right gyrification. This is presented in Table 4.14 and 4.15.

Table 4.14 Table of main results for global time by group interactions for gyrification group comparisons between HR[well], HR[symp] and HR[ill]

Hemisphere	Region	Gyrification		
		<i>F</i>	<i>df</i>	<i>p</i>
Left	Global	0.87	2, 57	0.397
Right	Global	0.78	2, 57	0.709

Table 4.15 Table of main results for global time by group interactions for gyrification group comparisons between HR[well], HR[symp] and HR[ill]

Hemisphere	Region	Gyrification					
		HR[well]		HR[symp]		HR[ill]	
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Left	Global	2.99(0.009)	2.97(0.011)	2.97(0.009)	2.94(0.011)	2.98(0.018)	2.96(0.021)
Right	Global	2.98(0.009)	2.96(0.011)	2.96(0.94)	2.94(0.12)	2.96(0.018)	2.95(0.020)

4.3.2.4 *Lobar comparisons in Gyrification between High Risk Individuals based on subsequent Clinical Outcome*

No significant time by group interaction occurred for any of the clinical sub-groups in lobar gyrification. These results are presented in Table 4.16 and 4.17.

Table 4.16 Table of main results for lobar time by group interactions for gyrification group comparisons between HR[well], HR[symp] and HR[ill]

Hemisphere	Region	Gyrification		
		<i>F</i>	<i>df</i>	<i>P</i>
Left	Frontal	0.97	2,57	0.38
	Parietal	0.87	2,57	0.42
	Occipital	1.76	2,57	0.18
	Cingulate	2.36	2, 57	0.09
	Insula	0.06	2, 57	0.94
	Temporal	0.51	2, 57	0.6
Right	Frontal	0.61	2, 57	0.54
	Parietal	0.54	2,57	0.58
	Occipital	1.09	2, 57	0.34
	Cingulate	1.06	2, 57	0.35
	Insula	0.06	2, 57	0.94
	Temporal	0.8	2, 57	0.45

Table 4.17 Table of means and associated standard errors of the means for lobar gyrification group comparisons at baseline and follow-up scan between between HR[well], HR[symp] and HR[ill]

Hemisphere	Region	Gyrification					
		HR[well]		HR[symp]		HR[ill]	
		Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Left	Global	2.99(0.009)	2.97(0.011)	2.97(0.009)	2.94(0.011)	2.98(0.018)	2.96(0.021)
Right	Global	2.98(0.009)	2.96(0.011)	2.96(0.94)	2.94(0.12)	2.96(0.018)	2.95(0.020)

4.3 Discussion

The aim of this study was to investigate cross-sectional differences in global and lobar values of gyrification in a sample consisting of first episode patients, high risk individuals and healthy controls. Another aim was to assess the extent to which any further alterations in both global and lobar gyrification existed in high risk individuals based on their subsequent clinical outcome. For the longitudinal comparisons, changes between scans in both global and lobar gyrification firstly in high risk individuals compared to controls, and then stratified based on the subsequent clinical outcomes of the high risk cohort, were assessed. Based on previous studies conducted on the same cohort, it was predicted that HR[ill] would have increases in gyrification, along with the patients in frontal regions. There were no pre-existing hypotheses about how gyrification would develop longitudinally, as no previous studies have measured gyrification in a high risk cohort longitudinally, and therefore this was examined in an exploratory fashion.

4.3.1 Cross-sectional Findings

Cross-sectionally, patients demonstrated increased gyrification both on a bilateral global and lateralized lobar scale, compared to both high risk and controls. For the localized lobar values, hypergyria that survived the FDR threshold were primarily lateralized to the left hemisphere, specifically to the parietal, frontal, cingulate,

temporal, and occipital lobe in patients, compared to both high risk and healthy controls. For the temporal lobe, increased gyrification in those at high risk compared to controls was found, with no significant differences emerging between patients and those at high risk. For the right hemisphere, only the cingulate lobe remained significant after FDR correction, in that patients had significantly higher gyrification indices than both those at high risk and controls.

4.3.2 Longitudinal Findings

Longitudinally, very few differences between those at high risk and controls was found, or between the high risk groupings based on their subsequent clinical outcome, apart from a trend for a significantly smaller decrease in gyrification in the left global gyrification for high risk individuals, compared to controls. No right hemisphere group effects were found, nor were any lobar effects found that survived correction for multiple comparisons.

4.3.3 Implications of Findings

4.3.3.1 Cross-sectional

Our cross-sectional findings of increased gyrification in patients are consistent with studies indicating that hypergyria occurs in patients with schizophrenia (Nesvag et al. 2004, Ronan et al. 2013, Harris et al. 2004). Although researchers have indicated the importance of early neurodevelopmental processes in gyrification, increasingly it has been shown that variance within-species in this parameter appears to also be

mediated by non-genetic influences, highlighting the importance of also investigating non-heritable and possibly much later maturational events in gyrification and its relationship to schizophrenia (Rogers et al. 2010 ; Zilles et al. 2013). In a study of monozygotic and dizygotic twins (Bartley et al. 1997) it was found that cortical patterns are determined primarily by random environmental factors. Even though the development of the sulcal and gyral patterns in the brain is strongly influenced by genetic processes (Piao et al. 2004) studies of monozygotic twins who share the same genetic complement, show considerable differences in their surface morphology (Thompson et al, 2001, White et al, 2002), indicating the importance of investigating gyrification changes in adolescence/early adulthood.

Overall, the results suggest that the increases in cortical gyrification in the patients reflect illness-related mechanisms, as opposed to being trait markers of schizophrenia. As most of these widespread alterations were not present in those at high risk nor specifically in HR[ill], it remains possible that aspects of brain development influencing gyrification may only be altered around the time of or after disorder onset. Thus, widespread alterations in gyrification may not be related to a general vulnerability of developing the disorder. However, this is in contrast to previous studies on this same cohort that have found gyrification differences. These studies however did not use a stringent method of multiple comparisons, nor did they control for surface area like the present study, which may have limited generalizability across studies.

It is interesting to note however that the patients and those at high risk both exhibited increased gyrification in the temporal lobe, especially as the previous study found alterations in the high risk cohort in this lobe. As evidence has suggested that high risk individuals exhibit similar, but lesser brain structural abnormalities to those with the established disorder, it remains possible that this shared temporal alteration reflects a marker of altered neurodevelopment ie a trait, as opposed to state marker of schizophrenia. Temporal areas have been shown to be being among the last to develop mature gyri (Battin et al., 1998), and thus future studies may wish to assess whether alterations to the temporal lobe reflect disturbed adolescent neuro-development, with the aim of pinpointing this to specific developmental processes.

Disturbed gyrification is in line with a model of illness in which neural connectivity develops and/or matures abnormally. Several reasons have been suggested as to why increased gyrification, as opposed to decreased gyrification, would characterize those that have schizophrenia. Animal experiments have demonstrated wide-ranging misconnections in regions of hypergyrification (Goldman-Rakic, (1980). Van Essen's (1997) theory predicts that if distant connections were malformed, regional gyrification would be increased. It has been proposed that the mechanism by which this would occur would be through decreased gyral width and increased sulcal depth, which may consequently restrict contact with underlying connectivity, eg in the form of white matter (Vogely et al 2001). Thus, increased gyrification does not necessarily equate to increased connectivity, and our results indicate that increased gyrification on patients, as well as the increased temporal lobe gyrification in those at high risk suggests that there may exist an upper threshold of gyrification required for

optimal function (Kesler et al. 2006, Janssen et al. 2009)

4.3.3.2 Longitudinal

To my knowledge, this is the first study looking at longitudinal changes in gyrification in a sample at high risk of developing schizophrenia for familial reasons. The lack of longitudinal alterations in both global and lobar gyrification in the high risk cohort when considered as a whole and compared to the healthy controls, as well as when considered based on subsequent clinical outcome, are to a certain extent consistent with developmental studies that have suggested that gyrification remains relatively stable after birth, with few changes. However, this hypothesis has been put under scrutiny, as evidence is increasingly showing the dynamic nature of gyrification across the life-span and in particular adolescence, a period of development characterized by several large changes in brain anatomy and function (Chi et al. 1997, Vogeley 2001). It remains plausible therefore that although gyrification may vary across the life-span, that this trajectory of change does not interact with group status for our high risk cohort, ie that longitudinal development in gyrification does not occur as a function of group status. In this light, as in keeping with previous findings suggestive of a lack of synaptic pruning in later adolescence in (pre)-schizophrenia (Feinberg 1990)

Although the findings presented in this chapter suggest that gyrification alterations only occur early in disorder onset, there are some limitations in the present study that limit the generalizability of the findings. It is important to note here that due to the

way that the data was collected and the aims of the study, the patients do not have a

family history of schizophrenia, and there is some debate regarding the heterogeneity of the aetiology for those with a family history of schizophrenia versus those without. It may be that this group did indeed have gyrification alterations before disorder onset; however the design of this study makes it impossible for us to know this. Future studies may wish to examine thoroughly the differences in brain structure between familial and non-familial cohorts and whether there are some alterations specific to each group. Several researchers have suggested that the discrepancies across studies may stem from large methodological differences in the manner by which gyrification indices are acquired. As is clear from above, the findings to date on gyrification in relation to schizophrenia are divergent and sometimes contradictory. Other limitations to this study are similar to the other Chapters and will be summarized in Chapter 6.

Thus, the cross-sectional findings are indicative of widespread alterations in gyrification mainly around the time of disorder onset in the patient group. As large cross-sectional differences in the patients compared to high risk and controls, the findings suggest that gyrification alterations are primarily tied to the manifestation of the disorder itself, as opposed to an underlying risk or in those that go on to develop the disorder. In Chapter 3 evidence was found for significant changes over time in both cortical area and thickness in the high risk cohort as a whole, and as no longitudinal gyrification changes were found, this could suggest that the

neurodevelopmental lesion that disrupts thickness and area occurs in foetal

development before folding, which may remain unaltered up until very close to or after disorder onset. Up until the third trimester, the cortex is mainly lissencephalic, and investigating these three parameters separately may help researchers to elucidate a temporal time-frame by which specific disruptions occur and thus aid researchers in developing more specific hypotheses regarding the underlying aetiology of schizophrenia. Indeed, Janssen et al (2004) found normal gyrification in regions showing decreased thickness, again highlighting the importance of looking at these cortical parameters separately.

The findings in this chapter suggest that gyrification should not be classified as a trait marker for schizophrenia, except for potentially alterations to the temporal cortex, but rather that most alterations in this parameter seems to be associated with the disorder itself. Previous studies on the same cohort (Harris et al. 2004, Harris et al. 2007) did find increased hypergyria in the HR cohort, specifically the HR [ill] subgroup. This could plausibly be attributed to the large differences in methodology used; previous studies on this cohort have used two-dimensional gyrification indices requiring manual tracing, whilst the present study relied on an automated, whole-brain technique. As was mentioned in the methods section, gyrification has been shown to vary drastically with technique used (For a review, see Schaer et al. 2006), and thus this study also shows the importance of using generalizable techniques across studies.

4.3.4 Conclusions

In summary, the present study finds evidence for increased gyrification in patients

compared to both those at high risk and controls. These alterations are seemingly not based on subsequent clinical outcome in the high risk sub-groups, and thus the findings suggest that altered cortical complexity occurs early in the disorder and may reflect an illness-specific marker of disturbed brain development. In contrast, there also seems to be a shared abnormality of the left temporal lobe present in the high risk cohort as a whole, potentially reflecting a neurodevelopmental disruption that may be accurately specified as a trait marker of schizophrenia. Intriguingly, the results of the present study found very little evidence for changes over time in gyrification, in contrast to the group differences found in area and thickness in Chapter 3. This would suggest that the mechanisms controlling the development of these three parameters are differentially affected in the context of schizophrenia, and thus that future work may be able to elucidate important temporal information regarding the time-frame of the development of these parameters, which may aid our understanding of the underlying pathology of the disorder, and in the future provide potential windows for interventions.

CHAPTER 5

A Cross-sectional and Longitudinal Investigation of the Hippocampus, Amygdala and Nucleus Accumbens

5. Introduction

Temporal lobe structures and their projections have been credited with a central role in the pathophysiology of schizophrenia (Goghari et al., 2011; Cullen et al., 2012; Shan et al., 2013). Both functional and structural abnormalities of the hippocampus, amygdala as well as other temporal lobe structures have been extensively documented in schizophrenia (Bogerts et al., 1993; Qui et al., 2013; Lappin et al. 2014). The hippocampus plays an integral part in mediating encoding and retrieval of multi-modal sensory information (Heckers & Konradi, 2002) whilst the amygdala is crucially implicated in emotional learning (Exner et al., 2004; Lawrie et al., 2006). Abnormalities in these structures have therefore been attributed key roles in the deficits of episodic memory and emotional learning seen in schizophrenia (Dereet al., 2010). There is also increasing evidence relating changes in the nucleus accumbens and psychiatric disorders (Belujon et al. 2014, Greer et al. 2014, Womer et al. 2014), as it is known to be involved in several relevant behaviours, such as anticipation and responses to rewards and aversive events which are known to be abnormal in schizophrenia (Chau et al. 2004, Greer et al. 2014). As these three structures share several efferent and afferent anatomical connections, it has been suggested that brain circuits involving these regions are impaired in schizophrenia (Moore et al. 1999), which has been confirmed in a number of studies finding functional (Hall et al. 2010) as well as volumetric alterations in these structures in schizophrenia (Lauer et al.

Increasingly, it is being hypothesized that the brain deficit in schizophrenia is one of “dysconnectivity”, i.e, either increased or decreased connectivity between regions (For a more detailed review, Please see chapter 1). As the hippocampus, amygdala and nucleus accumbens share several efferent and afferent anatomical connections, it has been suggested that brain circuits involving these regions are impaired in schizophrenia (For a review, see Grace, 2003). Indeed, both functional and structural studies of patients have found connectivity alterations, including these regions (Hall et al. 2010, Whalley et al. 2011). However, reported data on the extent of changes to these structures in patients are inconsistent, and no clear patterns of alterations have yet been established (Tanskanen et al. 2005, Stefanis et al. 1999). Methodological differences such as sample size, chronicity of illness and medication use, are often reported as factors influencing the generalizability across studies to date (Smieskova et al. 2013, Fusar-Poli et al. 2011, Cooper et al. 2014).

Empirical evidence derived from family and twin studies reports that some of the alterations to these three regions appear to a lesser extent in unaffected relatives of patients with schizophrenia (Cannon et al., 2002, Seidman et al. 1999, Lawrie et al. 1999, Staal et al. 2000, Job et al. 2003, Cannon et al. 2000) with more severe abnormalities distinguishing HR-T compared to HR-NT (Cooper et al., 2014). Clinical high risk studies have found a similar pattern of results, with HR-NT showing some alterations compared to controls, and HR-T showing further

alterations that distinguish them from HR-NT (Pantelis et al., 2005 and Wood et al., 2008). However, not all clinical and high risk studies have found more changes in HR-T compared to HR-NT, and the direction of these abnormalities is somewhat inconsistent, with some studies showing increases in the hippocampus, and others decreases (Phillips et al., 2002, Sun et al., 2009 and McIntosh et al., 2011). Studies have also reported no differences in the hippocampus and amygdala in healthy relatives of patients (Arnold et al. 2014) or in individuals at high clinical risk of the disorder (Velakoulis et al. 2006).

To date, most studies of subcortical regions in high risk cohorts are cross-sectional. However, as abnormal developmental trajectories have been implicated in schizophrenia, cross-sectional investigations remain inherently limited as they cannot elucidate the extent to which abnormalities are statically evident at a given time-point, or whether they instead reveal themselves dynamically over time. Evidence from longitudinal imaging studies of individuals with established schizophrenia have suggested that progressive alterations occur during the early or even prodromal phase of the disorder in these regions (Gur et al., 1999 and Thompson et al., 2001) with further anatomical deterioration as the disorder worsens and becomes chronic (Pantelis et al., 2005 and Olabi et al., 2011). Although longitudinal studies in patient populations provide further information regarding the dynamic nature of abnormalities they are still confounded by medication and secondary factors associated with long-standing illness and are therefore unable to discern if abnormalities are present prior to illness onset. Prospective longitudinal studies of individuals at high risk can more thoroughly assess whether abnormal developmental

trajectories characterise those at high risk of the disorder, eliminating the confounds associated with the established disorder (For a more detailed review, please see Chapter 1). Both clinical and familial high risk studies have indicated that progressive changes occur in subcortical regions before disorder onset, with some evidence of greater longitudinal change in those that go on to transition to the disorder (Lawrie et al., 2002, Pantelis et al., 2003, McIntosh et al., 2011, Haijma et al., 2013 and Bois et al., 2014b). However, very few studies if any have looked at the nucleus accumbens.

Some studies have found evidence for longitudinal change in the hippocampus and amygdala in all those at high risk compared to controls, suggesting that alterations to this structure may form part of a general vulnerability to schizophrenia, whilst others have found no evidence for progressive structural changes (Lawrie et al. 2002, Job et al. 2003, Pantelis et al. 2003, Walter et al. 2012). Identifying whether longitudinal changes in the hippocampus, amygdala and nucleus accumbens reflect a general predisposition to developing the disorder or more specifically with frank psychosis would help understand their role in the aetiology of schizophrenia.

Longitudinal studies previously published on this cohort have found alterations in the hippocampus-amygdala complex (Job et al. 2005, Lawrie et al. 2002). In contrast to these previous studies conducted on this cohort, the present study utilizes an automated ROI technique that enables us to examine the hippocampus and amygdala independently, rather than examining the hippocampal- amygdala complex as a

whole (Lawrie et al. 2002). Furthermore, we also examine the nucleus accumbens, which previous studies published on this cohort, have not. Furthermore, the present paper used a linear mixed modelling approach, which, unlike Lawrie et al (2002) and Job et al. (2005) provides a statistical analysis approach that is able to model a group by time comparison and include individuals with missing time-points, thus utilising a larger sample size than these two previous papers.

Thus, at present it remains unclear to what extent abnormalities of the hippocampus, amygdala and nucleus accumbens form part of an underlying vulnerability to schizophrenia, or whether they are more specifically associated with the illness itself. To address this question the present study compared cross-sectional and longitudinal gray matter volumes of the amygdala, hippocampus and nucleus accumbens between the first-episode patient group, individuals at familial high risk for the disorder, and an age-matched control group without a family history of psychiatric illness. Secondly, we tested whether these structures were differentially affected in individuals at high risk who subsequently developed schizophrenia compared to those who presented only with isolated psychotic symptoms, and those at high risk who remained well.

Based on other studies conducted in the same cohort, it was predicted that the patient group and to a lesser extent high risk individuals would show sub-cortical brain alterations in all three structures, with the high risk sub-group that subsequently became ill showing the largest volume alterations compared to those at high risk that

remained well or developed only transient symptoms. Longitudinal changes in hippocampal, amygdala and nucleus accumbens volumes were also investigated in the high risk and control groups. Secondly, it was investigated whether longitudinal trajectories were differentially altered in those at high risk who subsequently developed schizophrenia versus those who presented with isolated psychotic symptoms, as well as those at high risk that remained well. It was hypothesised that those at high risk will show altered developmental trajectories compared to controls in both the hippocampus, amygdala, and nucleus accumbens and that those at high risk that went on to develop schizophrenia would show the greatest alterations compared to the other high risk sub-groups.

5.1 Methods

5.1.1 Participants

These are identical to those presented in Chapter 2, as is the time between scans and the scan interval presented in Chapter 2.

5.1.2 Imaging Parameters

These are identical to those presented in Chapter 2.

5.1.3 Freesurfer Reconstruction

Automated subcortical segmentations were conducted using Freesurfer 5.30 (<http://surfer.nmr.mgh.harvard.edu>) with the volume-based stream. This method has been shown to have good validity when compared to manual segmentation (Dewey

et al. 2010) and generally good test-retest reliability, except for some smaller subcortical structures (Morey et al. 2009). Parcellation of the subcortical anatomy and calculations of the total intracranial volume was performed by delineating anatomical divisions via automatic parcellation methods, in which the statistical knowledge base derives from a training set incorporating the anatomical landmarks and conventions described by Duvernoy (1991). First, non-parametric non-uniform intensity normalization is carried out to correct for intensity non-uniformity in the MRI data. Then, the registration to the MNI-305 atlas occurs, designed to be insensitive to individual variations and thus maximise the accuracy of the segmentations. The MNI-305 atlas is a template of the average of 305 volumetric MRI scans of healthy control subjects which were each registered to the Talairach atlas brain (Talairach and Tournoux 1988, Evans et al. 2003). Several other processes also use Talairach coordinates as seed points. Next, an intensity normalization is carried out to correct for fluctuations in intensity due to field inhomogeneity and all voxels are scaled so that the mean intensity of the white matter is 110. Subsequently, non-brain tissue is removed using a hybrid watershed deformation procedure (Segonne et al. 2004). The resulting skull-stripped brain images were then carefully inspected.

This procedure assigns a neuroanatomical label to each voxel in an MRI volume and segments the white matter and deep grey volumetric brain measures (Fischl et al. 2002, Fischl et al. 2004) based on probabilistic information estimated from a manually labelled training set. The classification technique employs a non-linear registration procedure that is robust to anatomical variability (Fischl et al. 2002). The

segmentation uses three pieces of information to disambiguate labels: (1) the prior probability of a

given tissue class occurring at a specific atlas location, (2) the likelihood of the image given what tissue class and (3) the probability of the local spatial configuration of labels given the tissue class. The gray matter volumes of the hippocampus, amygdala and nucleus accumbens were then extracted from Freesurfer. An example of Freesurfer extractions of these volumes are illustrated in Figure 5.1

Figure 5.1. Example Figure of a) = A = Amygdala, H = Hippocampus, and b) = NA = Nucleus Accumbens extractions using Freesurfer.

5.1.3.1 Longitudinal Pipeline

This is identical to that presented in Chapter 2.

5.1.4 Statistical Analysis

Where gray matter volume was found to be non-normally distributed, a boxCox transform implemented in package geoR (Version 1.74) was conducted. Additionally, in the patients with first episode schizophrenia, chlorpromazine equivalents of current medication dose were calculated according to Woods (2003) and Atkins et al.

(1997). Spearman rank correlation coefficients between the three subcortical regions and chlorpromazine equivalents and duration of illness (in months) were also calculated.

5.1.4.1 Cross-sectional Analysis

The gray matter volumes of the left and right hippocampus, amygdala and nucleus accumbens were extracted from Freesurfer. Two general analyses were performed on the extracted subcortical parcellations: one compares the patients with first-episode schizophrenia, those at high risk and the healthy controls, and a second comparing the high risk sub-groups to test for brain differences relating to clinical outcome. These comparisons were performed using separate Analyses of Covariance (ANCOVAs; adjusting for intracranial volume, age and sex. Where the ANCOVAs indicated significant between-group differences, pairwise comparisons were performed using package lsmeans (Version 2.10) implemented in R.

5.1.4.2 Longitudinal Analysis

For the longitudinal analysis, linear mixed models were conducted using package nlme (version 3.1-109). These models were used for the longitudinal analyses, as opposed to repeated-measures Analysis of Variance (ANOVAs), as they have been shown to be more robust against missing time-points and variable scan interval (Cnaan et al. 1997). Lobar comparisons were corrected for multiple comparisons the

same manner as the cross-sectional lobar analyses. Covariates were entered as in the cross-sectional analyses, however also including the group-by-time interaction and scan-interval. A quadratic term for age was entered and removed if it did not improve the model's fit, as where the other covariates listed above.

5.2 Results

5.2.1 Cross-sectional Findings

Medication had non-significant effects for all sub-cortical regions investigated in the present study, as did length of illness and are presented in Table 5.1

Table 5.1 Table of Spearman's rank correlation coefficients between investigated subcortical regions and chlorpromazine equivalents and duration of illness (in months).

Hemisphere	Region	Duration of Illness		Chlorpromazine Equivalents	
		R	<i>p</i>	R	<i>p</i>
Left	Hippocampus	0.235	0.123	-0.032	0.831
	Amygdala	-0.016	0.915	-0.180	0.241
	Nucleus				
	accumbens	0.081	0.595	-0.049	0.749
Right	Hippocampus	0.081	0.595	-0.212	0.166
	Amygdala	0.097	0.523	-0.049	0.749
	Nucleus				
	accumbens	0.162	0.288	-0.049	0.749

5.2.1.1 Hippocampal Volume in First Episode Patients, High Risk Participants and Controls.

For the left hippocampus, there was a significant main effect of group, ($F = 5.7$, $df = 2, 197$, $p = .004$). Subsequent contrasts revealed that this was due to patients having significantly smaller left hippocampi than controls ($t = 3.036$, $df = 197$, $p = .008$), as well as high risk individuals, ($t = -3.167$, $df = 197$, $p = .005$). No significant differences were found between controls and those at high risk, ($t = 0.586$, $df = 197$, $p = 0.877$). For the right hippocampus, when all those at high risk were considered together and compared against controls and patients, there was a significant main effect of group on volume ($F = 5.55$ $df = 2, 197$ $p = .004$). Subsequent contrasts revealed that this was due to patients having significantly smaller right hippocampi than controls ($t = 2.64$, $df = 197$, $p = 0.0239$), as well as patients having significantly smaller right hippocampi compared to high risk individuals ($t = -3.29$, $df = 197$, $p = .003$). These results are illustrated in Figure 5.2. No significant differences emerged between high risk individuals and controls, ($t = -0.090$, $df = 197$, $p = .996$).

Figure 5.2. Mean Gray Matter Volume of a) the left hippocampus and b) the right hippocampus for Controls, those at High Risk and First Episode Patients. * indicates significantly smaller mean volumes for First Episode patients in both these structures compared to the other groups.

5.2.1.2 Amygdala Volume in First Episode Patients, High Risk Participants and Controls.

For the left amygdala, there was a significant main effect of group on volume, ($F=5.2$, $df = 2,197$, $p = .006$). Subsequent contrasts revealed that this was due to patients having significantly smaller left amygdala compared to controls ($t = 3.026$ $df = 197$ $p = .007$), as well as compared to those at high risk ($t = -2.93$ $df = 197$ $p = 0.011$). No significant differences emerged between high risk individuals and controls ($t = 0.840$, $df = 197$, $p = 0.678$). There was no significant main effect of group on the volume of the right amygdala. These results are illustrated in Figure 5.3

Figure 5.3. Mean Gray Matter Volume of a) the left amygdala, * indicates significantly smaller mean volumes for First Episode patients in a) compared to the other groups. and b) the right amygdala for controls, those at High Risk and First Episode Patients.

5.2.1.3 Nucleus Accumbens Volume in First Episode Patients, High Risk

Participants and Controls.

There was a significant main effect of group on the right nucleus accumbens which subsequent post-hoc testing revealed was due to patients having significantly smaller right nucleus accumbens volumes than controls ($t = -2.766$, $df = 197$, $p = 0.017$). No significant differences emerged between high risk individuals and controls, or high risk individuals and patients. There was no significant main effect of group on the volume of the left nucleus accumbens. These results are illustrated in Figure 5.4

Figure 5.4 Mean Gray Matter Volume of a) the left nucleus accumbens and b) the right nucleus accumbens for controls, those at high risk and first episode Patients. * indicates a significantly smaller right mean volume between First Episode patients compared to controls.

All group comparisons for the hippocampus, amygdala and nucleus accumbens between controls, those at high risk and the patient group are presented in Table 5.2, along with the means and associated standard errors for these structures in Table 5.3

Table 5.2 Table of main results for hippocampus, amygdala and nucleus accumbens group comparisons between those at high risk, controls and first-episode patients

Hemisphere	Region	<i>F</i>	<i>df</i>	<i>p</i>
Left	Hippocampus	5.70	2.197	0.004
	Amygdala	5.25	2.197	0.006
	Nucleus			
	accumbens	0.65	2.197	0.524
Right	Hippocampus	5.55	2.197	0.005
	Amygdala	1.87	2.197	0.155
	Nucleus			
	accumbens	4.15	2.197	0.017

Table 5.3 Table of means and associated standard errors of the means for hippocampus, amygdala and nucleus accumbens group comparisons between those at high risk, controls and first-episode patients

Hemisphere	Region	Group		
		First Episode	Controls	High risk
Left	Hippocampus	3739(87)	4033(81)	3991(53)
	Amygdala	1181(44)	1329(41)	1299(27)
	Nucleus			
	accumbens	608(24)	633(22)	611(15)
Right	Hippocampus	3698(86)	3952(80)	3958(53)
	Amygdala	1291(49)	1387(46)	1373(30)
	Nucleus			
	accumbens	573(19)	640(17)	631(8)

***5.2.1.4 Hippocampus, Amygdala and Nucleus Accumbens Volume Compared
between High Risk Participants based on subsequent Clinical Outcome***

No significant Group differences emerged for any of these structures when those at high

risk were considered based on subsequent illness transition or not. These results are summarized in Table 5.4 and 5.5

Table 5.4 Table of main results for hippocampus, amygdala and nucleus accumbens group comparisons between HR[well], HR[symp], HR[ill]

Hemisphere	Region	<i>F</i>	<i>df</i>	<i>p</i>
Left	Hippocampus	1.90	2,137	0.156
	Amygdala	0.915	2,137	0.405
	Nucleus accumbens	0.911	2,137	0.406
Right	Hippocampus	0.814	2,137	0.369
	Amygdala	1.21	2,137	0.302
	Nucleus accumbens	0.167	2,137	0.123

Table 5.5 Table of means and associated standard errors of the means for hippocampus, amygdala and nucleus accumbens group comparisons between HR[well], HR[symp], HR[ill]

Hemisphere	Region	HR[well]	HR[symp]	HR[ill]
		Baseline	Baseline	Baseline
Left	Hippocampus	3955(50)	4108(54)	4046(97)
	Amygdala	1228(25)	1298(27)	1328(48)
	Nucleus			
	accumbens	606(14)	641(15)	605(25)
Right	Hippocampus	3955(50)	4108(54)	4046(97)
	Amygdala	1378(29)	1427(31)	1410(54)
	Nucleus			
	accumbens	680(15)	680(64)	619(27)

5.2.1.5 Correction for Multiple Comparisons

The results reported above are uncorrected for multiple comparisons. However, we also conducted a false discovery rate (FDR) correction for multiple comparisons, and all results for all significantly reported structures remained significant, except for the right nucleus accumbens comparison between high risk, patients and controls. This is presented in Table 5.6

Table 5.6 Multiple comparisons testing for baseline comparisons between those at high risk, controls and patients with first episode schizophrenia

Hemisphere	Region	p value	q value
Left	hippocampus	0.003	0.008
	amygdala	0.005	0.008
	nucleus accumbens	0.524	0.524
Right	hippocampus	0.004	0.013
	amygdala	0.155	0.025
	nucleus accumbens	0.017	0.155

5.2.2 Longitudinal Findings

5.2.2.1 Hippocampal, Amygdala and Nucleus Accumbens Volume in High Risk Participants and Healthy Controls.

There was no significant group by time interaction for the volume of the left hippocampus. However for the right hippocampus, a significant group by time interaction emerged, ($F = 15.5$, $df = 1, 78$, $= 0.0002$). Subsequent post-hocs revealed that this was controls increased significantly more in right hippocampal volume compared to those at high risk, who in contrast underwent a relative reduction in right hippocampal volume. This is illustrated in Figure 5.5.

Figure 5.5 Graph showing change over time in Healthy controls and the High Risk Cohort between Baseline and Follow-up scan in Right Hippocampal volume (mm³).

No significant group by time interactions emerged for either the left or right amygdala or nucleus accumbens, and all results for this section are summarized in Table 5.7 and 5.8.

Table 5.7 Table of main results for subcortical time by group interactions for group comparisons between those at high risk and controls

Hemisphere	Region	<i>F</i>	<i>df</i>	<i>p</i>
Left	Hippocampus	2.35	1,78	0.125
	Amygdala	0.412	1,78	0.523
	Nucleus accumbens	0.23	1,78	0.633
Right	Hippocampus	15.5	1,78	0.001
	Amygdala	0.001	1,78	0.987
	Nucleus accumbens	0.212	1,78	0.646

Table 5.8 Baseline and follow-up mean values and associated standard errors for each hemisphere and region between controls and those at high risk

		High Risk		Controls	
		Baseline Scan	Follow-up	Baseline	Follow-up
Left	Hippocampus	4004(34)	3997(37)	4068(69)	4117(73)
	Amygdala	1271(17)	1256(20)	1301(34)	1266(39)
	Nucleus				
	accumbens	622(10)	601(15)	644(19)	614(23)
Right	Hippocampus	4055(35)	4006(39)	4064(70)	4207(75)
	Amygdala	1390(19)	1388(24)	1405(38)	1402(45)
	Nucleus				
	accumbens	658(19)	629(13.4)	673(18)	650(22)

5.2.2.2 Hippocampal, Amygdala and Nucleus Accumbens volume in High Risk

Participants based on subsequent clinical outcome

No significant group by time interaction emerged when the high risk individuals were considered based on subsequent clinical outcome for any of the investigated subcortical structures (Table 5.9). Baseline and follow-up means are presented in Table 5.10.

Table 5.9 Table of main results for subcortical time by group interactions for group comparisons between HR[well], HR[symp] and HR[ill]

Hemisphere	Region	<i>F</i>	<i>df</i>	<i>p</i>
Left	Hippocampus	0.950	2,58	0.393
	Amygdala	0.114	2,58	0.892
	Nucleus accumbens	.398	2,58	0.674
Right	Hippocampus	1.46	2,58	0.240
	Amygdala	.094	2,58	0.910
	Nucleus accumbens	1.53	2,58	0.225

Table 5.10 Baseline and follow-up mean values and associated standard errors for each hemisphere and region between HR[well], HR[symp] and HR[ill]

Hemisphere	Region	HR[well]	HR[symp]	HR[ill]			
		Baseline	Follow-up	Baseline	Follow-up		
Left	Hippocampus	3955(50)	3844(62)	4108(54)	4036(62)	4046(97)	4017(105)
	Amygdala	1228(25)	1216(37)	1298(27)	1296(35)	1328(48)	1304(56)
	Nucleus accumbens	606(14)	587(20)	641(15)	630(19)	605(25)	573(30)
Right	Hippocampus	3955(50)	3844(62)	4108(54)	4036(62)	4046(97)	4017(105)
	Amygdala	1378(29)	1326(48)	1427(31)	1392(45)	1410(54)	1350(69)
	Nucleus accumbens	680(15)	643(19)	680(64)	643(19)	619(27)	619(31)

5.2.2.3 Correction for Multiple Comparisons

The results reported above are uncorrected for multiple comparisons. However, we also conducted a false discovery rate (FDR) correction for multiple comparisons, and all results for all significantly reported structures remained significant (Table 5.11)

Table 5.11 Multiple comparison testing for longitudinal high risk versus control contrasts

Hemisphere	Region	p value	q value
Left	hippocampus	0.125	0.375
	amygdala	0.523	0.775
	nucleus accumbens	0.633	0.775
Right	hippocampus	0.000	0.001
	amygdala	0.987	0.987
	nucleus accumbens	0.646	0.772

5.3 Discussion

This study assessed cross-sectional and longitudinal estimates of the gray matter volumes of the hippocampus, amygdala and nucleus accumbens. Cross-sectionally, it was done in a sample of individuals at high familial risk of developing schizophrenia, along with a group of patients experiencing their first episode of psychosis, and healthy controls. The main aim was to investigate whether there were any changes in these structures based on whether an individual was at high risk, or after their first episode of psychosis, compared to controls. As a secondary aim it was examined whether volume changes were selectively present in those at high risk that went on to develop schizophrenia on average 2.5 years after the scans took place, compared to those at high risk that remained well or developed only transient psychotic symptoms. The rationale behind this approach was to assess whether any structural alterations were present before illness onset in those that went on to develop the disorder, or whether any alterations were specifically found in those that were experiencing their first episode of psychosis. Longitudinally, it was assessed whether those at high risk would show altered developmental trajectories compared to controls, and whether those at high risk that subsequently developed schizophrenia would show the greatest alterations to both hippocampal, amygdala and nucleus accumbens structures over time, compared to those at high risk that remained well, or developed only transient symptoms.

Based on other studies conducted in the same cohort, it was predicted that the patient

group and to a lesser extent high risk individuals would show sub-cortical brain alterations in all three structures, with the high risk sub-group that subsequently became ill showing the largest volume alterations compared to those at high risk that remained well or developed only transient symptoms. It was hypothesised that those at high risk would show altered developmental trajectories compared to controls in both the hippocampus, amygdala, and nucleus accumbens and that those at high risk that went on to develop schizophrenia would show the greatest alterations compared to the other high risk sub-groups.

5.3.1 Cross-sectional Findings

Significant bilateral volumetric reductions of the hippocampus in patients, compared to controls and high-risk participants, were found which was in accordance with our hypotheses. For the amygdala, this effect was lateralized to the left hemisphere, whilst the opposite effect was observed for the nucleus accumbens. Unexpectedly, no statistically significant abnormalities in high-risk patients when compared to controls, nor when the high-risk individuals were separately considered based on subsequent clinical outcome, which did not support our hypotheses.

5.3.2 Longitudinal Findings

A significant group-by-time interaction was found for the right hippocampus, which subsequent tests revealed was due to controls undergoing a significant volumetric increase between the two scans, with those at high risk undergoing a relative reduction. Unexpectedly, no longitudinal alteration in the volume of the amygdala or nucleus accumbens over time was found. There were no significant group-by-time interactions when the high risk groups were split based on subsequent clinical outcome. Hence, some of the longitudinal findings supported the hypothesis that those at high risk will show altered developmental trajectories compared to controls, whilst the lack of HR[ill] findings did not.

5.3.3 Implications of Findings

The findings of reduced volumes in the three subcortical structures in those in their first episode of schizophrenia were not significantly associated with medication intake, or duration of illness. This suggests that these alterations may be more associated with pathological mechanisms occurring early in the disease process (Moore et al. 1999, Grace 2003), rather than the effects of medication.

Most of the patients used in the present study were scanned six months or less after

diagnosis, thus also suggesting that the brain alterations demonstrated in the present study were not merely a by-product of duration of psychosis. It is interesting that these structural alterations occur in the hippocampus, amygdala and nucleus accumbens, as these structures are thought to be critically involved in reward and aversive event behaviour, that is typically altered in schizophrenia (Grace, 2003), and future studies may wish to examine how structural alterations in these regions relate to functional alterations.

The findings support the more general hypothesis that the developmental trajectory of the hippocampus would be different in those at high risk compared controls. As evidence is increasingly suggesting that a genetic predisposition to schizophrenia may involve an altered neurodevelopmental trajectory (Lawrie et al., 2002), our findings support the literature suggesting that altered development of processes affecting brain growth and maturation may play a critical role in a predisposition to schizophrenia. As none of the high risk sample used in the present study was on anti-psychotic medication during either of the scans, the developmental alteration in hippocampal volume cannot be ascribed to effects of medication. Our finding of progressive changes in high risk individuals, and indeed seemingly distributed similarly in the high risk cohort as a whole, as opposed to being stratified based on subsequent clinical outcome, potentially has important implications for the investigation of the underlying pathophysiology of the disorder. If longitudinal abnormalities of hippocampal volume are present in high risk individuals in general,

this suggests that these alterations may reflect a general, possibly genetic predisposition to develop the disorder, as opposed to more specific illness-related mechanisms. It remains possible that further alterations to the volume of the hippocampus, or even the amygdala and nucleus accumbens, occur nearer to the time of disorder onset and/or shortly after in those at high risk that go on to develop the disorder. However, we cannot investigate this in this sample, as scanning was not repeated once these individuals had developed schizophrenia.

Previous research has indicated a robust maturational process resulting in increased volumes of the hippocampus occurring during development (Suzuki et al., 2005, Gogtay et al 2006). The hippocampus and amygdala have previously been shown to have an age-related increase in healthy subjects aged 8–38 years (Sowell and Jernigan 1998), and another study also demonstrated an age-related increase in volume in the hippocampus in female subjects aged 4–18 years (Giedd et al. 1996A and Giedd et al 1996b). These findings are consistent with our findings of volumetric increases in controls, suggesting that this is reflective of a typical development over time in this structure. The human hippocampus is capable of undergoing neurogenesis (Komack and Rakic 1999), and our results are suggestive of some form of delay/failure of volumetric increases occurring in those at high risk of schizophrenia. Mapping typical development and how it differs from those at high risk is imperative in understanding the significance of brain alterations found in schizophrenia, and the present findings could be suggestive of a delayed maturational process, linked in some way to a general genetic predisposition to schizophrenia, and should be further examined in future studies. However, as the role of environmental

factors in the aetiology of schizophrenia is becoming increasingly clear, it remains possible that the effects of stress/trauma, as opposed to a familial disposition to develop the disorder, may be associated with the disorder, and future studies may thus wish to investigate whether stress/trauma also has a role to play in longitudinal alterations of the hippocampus. Indeed, unpublished data from my research suggests that those at high risk of developing schizophrenia may be more vulnerable to the effects of childhood maltreatment by this directly affecting the volumes of the hippocampus (Bois et al, *in prep*)

5.3.4 Limitations and Methodological Considerations

There are some important limitations to the present study. Examining the hippocampus and amygdala separately may have reduced power to find volume reductions at baseline in high risk subjects across both structures as we previously reported in this cohort (Lawrie 1999). Although as we described earlier, Freesurfer has some advantages over hand-traced studies, it is important here to note that there has also been research that has suggested that Freesurfer methods may be less able to pick up very subtle anatomical changes compared to other methodological approaches, specifically in small structures such as the amygdala, which is a difficult structure to define anatomically, as it shares many arbitrary borders with the hippocampus, and consists of many sub-nuclei. Certainly, some VBM studies have found changes over time in the hippocampal and bordering regions in those at HR that become ill (Job et al. 2003 and Pantelis et al 2003) and thus some caution must

be inferred when interpreting the present results. Furthermore, the registration of the scans to the MNI-305 template may have acted to reduce some subtle important intraindividual variation across the brain scans, and as this template is based on a healthy sample, it may not be the best fit for our patient group.

The present study found no significant subcortical differences between high risk individuals and controls. This is consistent with some previous studies using Freesurfer (Arnold et al. 2015, Sprooten et al. 2013) but not our preceding studies using hand-traced (Lawrie et al. 1999) or voxel-based (Job et al 2003) methods. A previous study conducted on the same sample as that of the present study measured the hippocampal-amygdalar complex using manual tracing methods and found significant reductions in the high risk cohort compared to controls (Lawrie et al. 1999). However, the present study used Freesurfer, semi-automated parcellation software that is able to separately delineate the hippocampus and amygdala, finding no significant differences. It remains possible therefore that by investigating these structures separately and using a different sMRI technique, subtle structural changes occurring in the high risk individuals may be lost, as evidence suggests that any alterations found in high risk individuals will be present to a lesser extent than those with established schizophrenia (Lawrie et al. 2008). It is noteworthy that all subcortical structures investigated in the present study were slightly smaller in those at high risk compared to controls. It remains possible that use of an automated brain segmentation method such as Freesurfer, compared to manual tracing methodologies, typically considered the “gold standard” of ROI studies, are less able to pick up the subtle structural differences hypothesized to exist between those at high risk and

controls. Indeed, a recent study conducted by Arnold et al. (2015) found evidence for Freesurfer to produce significantly larger volumes than manual tracing methods, suggesting the need to consider methodological discrepancies when interpreting the results of the study. Furthermore, when investigating structures such as the nucleus accumbens, specific limitations to the delineation protocol may inherently arise to produce inconsistencies across studies, as this structure has previously shown low accuracy in automated segmentation studies (Babalola et al. 2009).

A third possibility is that the results reported here are correct, and that any differences in familial high risk subjects are primarily in entorhinal cortex bordering the hippocampal-amygdala complex; indeed a previous study conducted with Freesurfer found a trend for a significant difference between controls and those at high risk located in the entorhinal cortex, as did a voxel-based study (Job 2003; Spooten 2013). This is an important point to consider in future high risk sMRI studies. However, another important point to consider is the low transition rate in this study, of approximately 13% (Whalley et al. 2007), and hence the possibility that this sample reflects a specific group of familial high risk participants with less vulnerability to develop schizophrenia than those presenting to clinics with psychotic symptoms. The present results do highlight the need for future sMRI studies to incorporate standardized methods across studies, in order to increase the generalizability and interpretability of findings. However, the current findings also reiterate findings that the alterations found in schizophrenia in sub cortical structure; namely the hippocampus, amygdala and nucleus accumbens occur early in the disorder and do so independently of medication and length of illness.

5.3.5 Conclusions

The present findings suggest that structural alterations of the hippocampus, amygdala and nucleus accumbens occur early in the first episode of schizophrenia, and are not associated with medication effects. These alterations were not shared with people at high familial risk of developing the disorder, nor selectively in those at high risk that developed schizophrenia on average 2.5 years after scanning. However, this study also presents the need to consider the importance of methodological discrepancies across studies when investigating brain alterations in relation to a risk for schizophrenia. This study demonstrated a significant group by time interaction in the volume of the hippocampus in individuals at familial high risk of developing schizophrenia compared to a sample of controls. This effect was not present when the sample was stratified by clinical outcome, suggesting that longitudinal hippocampal alterations may reflect a general vulnerability indicator of schizophrenia, however power and segmentation issues should also be considered. In addition to investigating high risk individuals before transition to the disorder, future studies should also assess whether a so called “second-hit” of further brain changes occurs in high risk individuals that develop schizophrenia during the critical period

of transition.

CHAPTER 6.

General Discussion

6. Introduction

The work presented in this thesis has used Freesurfer, a structural neuroimaging software, to examine both global and more localized cortical brain alterations, along with some key sub-cortical structures that have been implicated in the disorder previously, in a cohort at familial high risk of schizophrenia, a sample of controls and patients in their first episode of schizophrenia. This work is the first ever longitudinal study to employ the Freesurfer technique in a high risk sample such as this, to investigate both cortical and subcortical brain alterations. Since Freesurfer provides highly sensitive and novel information about the cortex by investigating thickness, surface area and gyrification separately, and allows a less subjective analysis of subcortical regions than manual tracing methods, the work presented in this thesis is novel in the field of finding objective and early biomarkers for schizophrenia. As schizophrenia becomes more chronic and severe as the disorder progresses, it also becomes much harder to treat and chances of a good prognosis may decrease (Lawrie et al. 2001). Hence, finding objective biomarkers of schizophrenia has crucial implications for its treatment, as it may provide methods for early intervention and preventative strategies.

Baseline data was available to examine differences in these three groups, as well as within the high risk cohort based on their subsequent clinical outcomes. However, there was no longitudinal data for the patients in their first episode of psychosis, and

thus the longitudinal analyses for this thesis conducted two general analyses; first we looked to compare all of those at high risk compared to the controls, and then the high risk cohort based on their subsequent clinical outcomes.

As was outlined in Chapter 1, both functional and structural studies of the brain have found widespread alterations in schizophrenia. However, it has not been clear whether these brain alterations are associated with a vulnerability to develop the disorder, illness-specific mechanisms occurring before/after disorder onset, and/or secondary confounding effects, such as length of illness and/or medication effects. It has also not been clear whether these alterations present themselves statically, or whether they are associated with altered longitudinal trajectories. One of the key reasons for wanting to elucidate these issues is to understand whether alterations found in sMRI studies could help distinguish between high risk individuals that later make the transition to schizophrenia. This would have critical implications for our understanding of the disorder's underlying aetiology in terms of how and when it affects the brain.

One reason for the uncertainty pertaining to the issues outlined above may stem from methodological differences across studies. Many studies of sub-cortical regions have relied on essentially manual delineations, which risk unreliability across studies if the manual delineations differ across studies, potentially producing a large margin of error for small structures such as the hippocampus and amygdala. In contrast, Freesurfer can produce semi-automated and/or fully automated estimates of sub-

cortical regions relying on probabilistic information, reducing time drastically, which is key when working with large, complex datasets as that presented in this thesis. Another reason for discrepancies across studies may be that many studies to date have used voxel-based volumetric approaches, as opposed to surface-based ones, which may have obscured findings, by examining gray matter volume as opposed to the cortical parameters of thickness, area and gyrification separately. As was outlined in Chapter 1, these three cortical parameters are thought to be mediated by the action of different genes and developmental processes, and thus studies that have focused on gray matter volume may be obscuring subtle differential effects of these parameters, which may have crucial implications when assessing a familial high risk cohort. This thesis therefore aimed:

1. To investigate cross-sectional brain structure in a sample at high risk of developing schizophrenia, patients with first episode schizophrenia and controls to determine whether brain alterations are best characterized as part of an underlying vulnerability to develop schizophrenia, or are more associated with illness specific mechanisms; eg “trait” and “state”.

2. To determine whether any alterations reveal themselves longitudinally in those at high risk compared to controls, and in those at high risk based on subsequent clinical outcome.

3. To assess whether surface area, cortical thickness and gyrification are differentially affected, both cross-sectionally and longitudinally, in high risk individuals compared to healthy controls and first-episode patients, and/or whether they are stratified based on subsequent clinical outcome within the high risk cohort.

The cross-sectional analyses were conducted to assess whether alteration to both the cortex and/or key subcortical regions existed in HR[ill] before disorder onset, or whether any alterations were selectively present in the patient group, or more accurately specified as trait markers of schizophrenia. The longitudinal analysis explored whether differential developmental trajectories occurred as a function of familial risk for schizophrenia.

As the aims of this thesis was not to assess function of the cortical regions, I did not have specific hypotheses regarding which cortical regions would be more affected than others, but rather our hypotheses focused on whether brain alterations could more accurately be specified as markers of clinical risk present before or after disorder onset, or whether they were better characterized as markers of general vulnerability to develop schizophrenia. As most studies to date have focused on very localized regions of the cortex, but evidence is increasingly showing widespread perturbations in brain structure, it was also assessed whether alterations were more widespread, as opposed to localized, and the nature of these alterations over time. At baseline, it was predicted that those in their first-episode of schizophrenia would show more widespread alterations compared to the high risk group and controls,

however we also predicted that those in the HR[ill] group would show larger brain structural alterations compared to the other high risk individuals. It was predicted that there would be longitudinal alterations both in HR[all], specifically to the hippocampus as this has been shown in previous studies, with more alterations in HR[ill]. Based on existing evidence that cortical thickness, surface area and gyrification reflect different neurodevelopmental and genetic processes, it was predicted that these would have differential presentations both at baseline and cross-sectionally, but I had no specific hypotheses regarding the direction of these alterations in these three parameters, as so few studies in this type of cohort have previously been conducted.

The aim of the present Chapter is merely to broadly integrate the findings from the previous experimental chapters and discuss to what extent our hypotheses were met, as each of these contains their separate discussion section. I also aim to discuss general strengths and limitations of the work described in the three chapters, and possible future directions and implications stemming from the highly novel work presented in this thesis.

6.1 Summary of Main Results

Rather than repeat the main findings of each chapter textually, Figure 6.1 summarizes the main findings of this thesis. Overall, the present thesis found that

when investigating both cortical and sub-cortical brain structure in familial high risk participants, first-episode patients, and healthy controls, widespread alterations to the cortex in thickness, area and gyrification occur in patients that have experienced their first episode of schizophrenia. Furthermore, decreased volume of the hippocampus, amygdala and nucleus accumbens were also found, supporting the general hypotheses that schizophrenia affects both global and localized regions of the brain. These alterations do not occur as a function of medication intake and length of illness, and it is therefore plausible that they are related to an illness-specific mechanism that takes place early in the disorder. Hence, these findings support the hypotheses that patients would exhibit more brain alterations than those at high risk and healthy controls. Several studies are now converging to show that the brain is abnormal in patients early in the disorder onset, and thus are not caused by secondary factors associated with the disorder. This has important implications for the study of schizophrenia, as it suggests that brain alterations in schizophrenia may be mediated at least in part by an illness specific mechanism that either interacts with normal developmental processes or occurs as a function of an early neurodevelopmental lesion. Furthermore, this mechanism affects both subcortical gray matter volume, surface area, thickness and gyrification, suggesting that it is a mechanism with large consequences in the brain, and thus the importance of finding an early objective biomarker is clear.

There also appear to be cortical alterations that represent a general vulnerability to develop schizophrenia which, at baseline, seem localized to the temporal lobe, and longitudinally appear more global in that thickness, both globally and in the frontal,

temporal, occipital and cingulate lobe are affected. The hippocampus showed altered developmental trajectories compared to controls. This supports the general hypotheses that those at high risk would show different developmental trajectories compared to controls. Hence, our longitudinal findings suggest that some maturational process is altered in all those at high risk compared to controls, potentially forming a trait marker to develop schizophrenia. However, there were no alterations to the subcortical structures in those at high risk at baseline, which did not support our hypotheses, which we had based on previous studies conducted on this cohort. However, as is discussed in section 6.2 of this Chapter, this could be due to a number of methodological differences, as was the lack of HR[ill] specific subcortical alterations that was predicted.

However, at baseline, the findings were that HR[ill] had a globally increased surface area, which could thus be interpreted as a marker of later transition to schizophrenia, and was also shared with the patient group. Cortical column formation may thus be a specific neurodevelopmental process that is altered in schizophrenia, whilst cortical thickness, determined by cortical cell proliferation, may occur in interaction with synaptic pruning to produce progressively displayed alterations that occur longitudinally and represent a marker of risk, but also show drastic changes close to disorder onset. However, as those in the HR[ill] were not offered rescanning after diagnosis, we were unable to investigate this further.

By investigating a familial rather than a clinical high risk cohort, we are able to look at brain alterations in individuals at high risk, yet without even the confounding effects of symptoms on brain structure in the HR[well] sub-group. As not everyone at high risk goes on to develop schizophrenia, this thesis directly compared brain structure between high risk individuals that remained well, and those that developed transient symptoms or a diagnosis of schizophrenia. As diagnosis of schizophrenia is currently based solely on symptomatology, there is a huge need for an objective biomarker capable of distinguishing different levels of risk, before disorder onset. Thus, this type of familial high risk paradigm has the capacity to stratify individuals, and thus also potentially investigate mechanisms of resilience, as well as risk. Taken together, the results of the present thesis suggests that that the pathophysiology underlying schizophrenia is in part mediated by early structural brain alterations that may be neurodevelopmental in nature, with specific alterations to global surface area potentially acting to enhance risk for developing schizophrenia.

Figure 6.1 Summary figure of main results of Chapter 3,4 and 5 of this thesis.

6.2 Overall Implications of Present Study

This thesis presents novel findings regarding premorbid cortical brain alterations in a sample at high familial risk of developing schizophrenia. These findings have crucial implications towards both understanding and treating schizophrenia, as currently, symptoms of schizophrenia are treated with anti-psychotic medications that have variable efficacy and severe side effects. There has been much interest in the understanding both the clinical and familial high risk state and the possibility of preventing schizophrenia by interfering with the aberrant postnatal brain maturation associated with this disorder. As our findings suggest pre-morbid cortical and sub-cortical alterations in those at high risk for familial reasons, along with some further changes in surface area specific to those that transition, our findings enable some further understanding into the mechanisms that underlie the long-term progression to full disease manifestation, which may in the future identify the best targets and approaches towards the important goal of finding early intervention and preventative strategies.

The present study has investigated both cortical and subcortical alterations cross-sectionally and longitudinally in a sample at high familial risk of schizophrenia, healthy controls and patients with first episode schizophrenia. As mentioned previously, the cortical parameters of thickness, area and gyrification are important to investigate separately, as they are determined early in neurodevelopment by distinct genetic and neurodevelopmental processes (Panzzion et al. 2009), such as cortical migration and cellular division, and are thus potentially able to provide more sensitive clues into the disturbed processes in schizophrenia than studies of gray matter volume alone. Hence, the clinical implications stemming from this study are huge, as this longitudinal study presents the largest sample size to investigate these cortical parameters separately in a prospective familial high risk study.

Young cortical neurones migrate across an inter mediate zone to produce the cortical plate from which the mature cerebral cortex develops. The cells forming the cortical plate are arranged in such a way that the first to be generated remain nearest to the proliferative zone and subsequent generations are to be found progressively further away (Hickey & Hitchcock, 1984), and this process results in cortical column formation, and thus the cortex. This developmental process is often referred to as the radial unit hypothesis of cortical formation (Rakic 1977). It is interesting to note that we found an increased surface area in HR[ill], which could be linked to early disturbances in the process described above. If neuronal migration is disrupted, an

abnormality in cell position results (Kovelman & Scheibel, 1984), which could affect surface area, cortical thickness and gyrification differentially, which could support our different group results in these three parameters. As evidence for schizophrenia to be a disorder of neurodevelopment is continuing to increase, the results presented in this thesis have highly important implications in understanding this disrupted development.

Although schizophrenia is primarily a psychiatric disorder that occurs on early adulthood, the evidence here provides more support for the idea of schizophrenia being a disorder of altered neurodevelopment, in which disturbances in susceptibility genes during early neurodevelopment initiate neurophysiological changes over a long time course, both in those at high familial risk that do not transition to psychosis and those that do, in which these disturbances culminate in the onset of full-blown disease later on. It is especially interesting that cortical thickness alterations reveal themselves progressively in those at high risk, as this may very plausibly be linked to excessive synaptic pruning, caused by an early genetic defect in these individuals. Thus, our results provide further evidence for aberrant prenatal brain maturation as an essential mechanism underlying the disease and the familial high risk state. Thus, our findings of altered cortical thickness and surface area in those at high risk compared to the healthy control group is highly important, as these two parameters are regulated in prenatal development by the processes of progenitor cell proliferation, neural migration and cortical column formation. Other evidence for altered neurodevelopment also comes from the minor physical anomalies, in particular in the craniofacial region and limbs observed in patients and are thought to be effected by events of the first and second trimester, which is also when progenitor

cell proliferation and neural migration take place. It has been suggested that these reflect outcomes in response to disconnectivity arising from earlier neurodevelopmental insults, such as defects of neuroprogenitor cell proliferation and migration. Taken together, subtle disturbances in the prenatal/perinatal period in progenitor cell control, neuronal migration, dendritic growth and arborization might contribute to the later anatomical changes observed in schizophrenia by neuropathology and brain imaging.

Increasingly, schizophrenia is thought to have a polygenic component that acts to either predispose or increase risk of schizophrenia (Thompson et al. 2014). Since we do not expect to find that this genetic component codes directly for the symptoms of schizophrenia itself, we need to find an intermediate phenotype that lies closer to the action of the genes themselves. Thus, we may expect to find that this genetic component of schizophrenia causes some defect in the control of neurodevelopment which cause a set of structural brain changes, which predisposes to cause later schizophrenia. As cortical thickness, surface area and gyrification are known to be mediated by different neurodevelopmental processes, this study has the capacity to sensitively assess which of these, if any, is more disrupted in schizophrenia. With the advent of GWAS and the search for genetic factors implicated in the aetiology of schizophrenia, by looking at brain parameters closer to the action of genes themselves it is more conceivable to in the future pinpoint specific genes and processes disrupted in schizophrenia, as neuronal migration etc are affected by these. Additional environmental factors might be crucial for full manifestation of the genetic effects.

6.2 Strengths and Limitations of Present Study

One of the main strengths of the present study is the prospective longitudinal design. Thus, our results within the high risk cohort are entirely unconfounded by length of illness and medication effects, and we were able to examine HR[ill] individuals in great detail before disorder onset. This has very important clinical implications, as the duration of untreated psychosis is correlated with worse outcome of the disorder (Gumley et al. 2014). Thus, predictive markers of schizophrenia that occur without confounding influences may offer clinicians an early window of opportunity whereby to intervene, with the aim of alleviating distress of symptoms and/or even preventing the disorder. The design of this study provides investigators with an excellent opportunity to look at brain alterations within this framework.

By using Freesurfer, rather than only volumetric approaches, we may be one step closer to finding this biomarker, as we are investigating the brain closer to the biological action of genes compared to investigations of gray matter. Although the present study is purely means based, and may not be able to offer much information on an individualized level, the importance of investigating the brain in a biologically informed manner to provide us with a greater understanding of the underlying brain deficit(s) in schizophrenia is clear. Recent advances within theoretical neurobiology have however investigated connections within superficial versus deep cortical layers, which may be implicated in schizophrenia (Reveley et al. 2014). With the advent of high field MRI and laminar imaging, it may prove possible to take structural

phenotyping beyond cortical thickness to address the structural sequelae of aberrant synaptic message passing at the level of individual cortical layers.

Another important strength of this work is that we looked at both global as well as more localized brain alterations. As it is becoming clear how complex and heterogeneous schizophrenia is, it is also becoming increasingly clear that it is unlikely to be mediated only by very circumscribed, localized brain alterations, but rather by widespread alterations in structure and function, potentially caused by altered connectivity between and across brain regions. Hence, it is important to not bypass global information when investigating the brain in relation to schizophrenia, and the present study did indeed find several more global brain alterations, that occurred both cross-sectionally and longitudinally (See Figure 6.1 above for more details), which ROI studies may have missed.

However, there are a number of pertinent limitations of the present study that may limit the generalizability of our findings. As mentioned in Hafner et al. (2013), the age of onset is earlier for males than for females, and although we found that more males than females became ill in the HR[ill] sub-group, this may reflect the earlier age of onset typical for males, as opposed to increased incidence, and thus a potential limitation of the present study is that we were perhaps unable to capture the true extent to which women were affected by schizophrenia in our cohort, if they developed schizophrenia at a more advanced age. One potential limitation of the present study is that more males than females became ill in the HR[ill] group. This

may, however, reflect sex differences in the incidence of schizophrenia (Iacono et al 1992) and moreover, since we controlled for sex in all analyses performed, it is unlikely to have confounded our main findings. As the results are based on clinical data available from the last assessment of these individuals, and that although a number of these individuals were followed up for a number of years by senior clinicians, some of these statuses may have changed, which could potentially alter some findings. Furthermore, we were unable to control for potentially important environmental factors, such as social functioning and socio-economic status, which could have impacted on the results found.

Another limitation of this study is that it uses a between-subject design to look at pre and post disorder differences in brain structure, as the patient group experiencing their first episode, and HR[ill] groups consist of two different sets of individuals. It is also important to note that the first episode patient group was selected with the rationale of them being sporadic cases that did not have a positive family history of schizophrenia, in contrast to the HR[ill] group. Although this was an important part of the original study design, one potential limitation to the present findings is that there may be differences in aetiology between these groups, which could have produced some margin of error on our present findings (McDonald et al. 2002, Scherr et al. 2012). It has been reported that up to 60% of patients with schizophrenia report neither having a first nor second degree relative with the disease (Gottesman & Erlenmeyer-Kimling 2001). Hence, some of the brain structural changes seen in the patient group may reflect a specific mechanism of non-familial schizophrenia, which potentially limits the generalizability of our findings. However, as mentioned

in Chapter 1, several aspects of clinical and familial high risk paradigms overlap, and the same goes for familial versus non-familial schizophrenia. Another limitation is the small number of HR[ill] individuals, which may have reduced our ability to find subtle pre-illness changes, by not having enough numbers to produce sufficient power. This may be especially important when looking at very small regions such as the hippocampus, nucleus accumbens and amygdala, where any alterations are thought to be subtle in nature and thus perhaps a larger sample size is required to elucidate these. It remains possible that our lack of finding further changes in the HR[ill] sub-group could reflect a lack of power deriving from the relatively small sample size of this group, and that future studies incorporating larger numbers of individuals that go on to transition or a longer follow-up between scans would be able to find subtle anatomical differences. Furthermore, as individuals were not offered rescanning after diagnosis, the present research is unable to directly assess whether the hippocampal volume reductions described occurred closer to disorder onset or after disorder onset, as the patient versus HR[ill] means it is a between subject analysis.

Another methodological limitation that may have contributed to the lack of high risk sub-group findings that we had hypothesized is the use of 1.0 T scanner and a relatively thick slice thickness, which may have made very subtle pre-illness effects harder to detect. Another potential reason for the present study not finding any significant high risk versus control differences in the investigated structures is because it remains possible that as the HR[ill] group did not develop schizophrenia until on average 2.5 years after the scans were acquired, that further progressive

changes may have occurred closer to the time of disorder onset. Despite these limitations, it is important to bear in mind that this study remains one of the largest, prospective and longitudinal studies of familial high risk studies of its kind, and can still provide us with invaluable information regarding familial high risk individuals.

6.3 Suggestions for further research

One of the aims of this thesis was to look at whether brain alterations were better characterized as “trait”, or “state” markers of schizophrenia. However, research in high risk paradigms is increasingly also using more sophisticated and advanced methods that can also look into what *drives* these alterations. Our findings are indicative of neurodevelopmental, general processes and illness-specific mechanisms affecting the brain in schizophrenia and in those at high risk, but increasingly it is also being shown that there are several environmental factors that are also implicated in driving these alterations. Recent work from our lab suggests that hippocampal alterations in high risk individuals may be driven by past childhood maltreatment (Bois et al. 2015, Barker et al. 2015). Other environmental factors such as life events, drug use or adverse social settings, or even secondary factors of the disorder, such as effects of medication or the symptomatology itself, as these have all been correlated with altered brain function and structure (Ellman et al. 2010, Welch et al. 2011, Harald & Falkai 2006). Some evidence suggests that a familial disposition to develop schizophrenia may interact with environmental factors to produce an elevated sensitivity to adverse environmental factors. Thus in order to further understand the underlying pathophysiology of schizophrenia, it is imperative to identify both genetic

and environmental factors which may be associated with brain alterations in high risk individuals.

Additionally, evidence is pointing to a substantial part of the genetic variation associated with the disorder being polygenic, consisting of many common single-nucleotide polymorphisms (SNPs), each of small effect size (Schizophrenia working group of the psychiatric genomics consortium, 2014). Indeed, work currently being prepared in our centre suggests that a polygenic load for schizophrenia may be associated with cortical brain structure (Bois et al. 2015). This type of methodology, as opposed to only looking at groups when investigating familial risk may provide a more subtle and dimensional aspect to genetic risk for schizophrenia, which may be particularly important when trying to relate the actions of genes to brain structure. Furthermore, by identifying genetic pathways as a mechanism that produces the brain alterations in schizophrenia, to get a better grasp of the neurobiological processes driving them.

In order to facilitate the translation of brain alterations into diagnostic markers, some standardization of methodology will be required. Recent initiatives, such as ENIGMA (Enhancing Neuro-Imaging Genetics through Meta-Analysis), have pooled data across sites to increase the power of each individual study by combining them through meta-analytical and genetic techniques (Thompson et al. 2014), signalling important progress in attempts to integrate data and provide a framework for future investigations. Identifying genetic risk variants in high risk individuals and

combining this with appropriate sMRI methods may further our understanding of risk to schizophrenia than familial HR approaches alone, as it will provide critical information regarding the direct influence of genetic risk and heritability on brain structure. The steady identification of risk genes for schizophrenia in summed polygenic risk scores is increasingly explaining a large proportion of brain structure in patients with schizophrenia (Terwisscha van Scheltinga et al. 2013).

Combining measures of gyrification with DTI may yield further progress in understanding ways in which connectivity of the brain is affected and thus in this way facilitate the search for reliable markers. Additionally, techniques that allow testing of the hypothesis that abnormal integration across several brain regions produces the complex symptomatology of schizophrenia have the potential to provide diagnostic markers of schizophrenia, by incorporating important theoretical notions regarding the underlying psychopathology of the disorder. Novel techniques drawing from complex properties of large-scale networks as well as techniques looking at inter-regional connectivity, for example thickness correlations across the cortex, may be of paramount importance in future sMRI study to find new quantitative measures that can serve as diagnostic markers between patient groups and controls (Boch et al., 2013)

More longitudinal studies mapping normal development are also required, as we need to have a better understanding of normal structural variation before we can fully understand what can be characterized as pathological. It also remains possible that

dynamic interactions across time and regions make some regions more involved at any given time-point and that this changes as individuals go from mild cognitive impairment to full-blown psychosis.

One possibility for subsequent work would be to create a multivariate model that combines these environmental and genetic risk factors for schizophrenia with our findings of cortical and sub-cortical brain alterations, in order to design a training set and see if we can predict who develops schizophrenia based on this model. As we mentioned in the limitations sections, there are several difficulties associated with taking statistical group differences to form the basis for clinical inferences. However by combining the findings from this thesis with that approach, a more sensitive and viable clinical methodology may be possible.

Overall, our findings suggest that sMRI techniques have the capacity to distinguish between abnormalities specific to a predisposing vulnerability to the disorder, as well as changes signalling that transition to psychosis is likely. The predictive value of sMRI studies may be improved by also assessing global changes in brain structure associated with the high risk state, as evidence suggests widespread alterations in brain structure, as well as looking at thickness, area and gyrification separately, as opposed to gray matter volume only. By integrating findings into a coherent framework capable of guiding future investigations, we may be better able to identify markers of the disorder and translate these into useful diagnostic tools, allowing early intervention strategies to take place.

6.4 Conclusions

The results from the series of analyses presented in this thesis have indicated that there exist both cortical and sub-cortical structural brain alterations in a cohort at familial high risk of developing schizophrenia. These include both “general” vulnerability markers, potentially reflecting a trait marker of schizophrenia, as well as alterations specific to those that transition to schizophrenia, potentially more reflective of mechanisms associated with the illness, over and above a familial predisposition. Hence, some brain alterations seem to be associated with inherited risk, as opposed to medication/disease effects. However, more specifically localized alterations at baseline, both cortical and sub-cortical, are not present in the high risk cohort, and may therefore occur very close to/right after disorder onset, as we found evidence of widespread alterations in both cortical and subcortical structure in patients experiencing their first episode of schizophrenia. I have also sought to highlight that in order for a susceptibility of psychosis to be accurately and reliably mapped out, morphometric variables closer to the biological action of genes are required, and that it is important to assess thickness, area and gyrification separately. By choosing adequate structural parameters and methodologies to elucidate neuroanatomical risk of schizophrenia, we will be better able to map mechanisms of susceptibility and potentially identify markers of schizophrenia in high risk

individuals that could at some stage be useful in the clinic. The application of advances in medical imaging research investigated in the present thesis is increasingly enabling scientists to conduct quantitative clinical imaging studies using data collected across multiple sites, to test new hypotheses on larger cohorts, increasing the power to detect subtle effects, and thus increasing our chances of finding objective biomarkers of schizophrenia that will increase our chances of treating this disorder.

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Appendices

Appendices

Appendix 1. List of Conference First-Author Abstracts and other publications derived from my work on the EHRS dataset

Papers:

Bois C., et al (2015) Effects of Childhood Maltreatment on Subcortical structure in the Edinburgh High Risk Study of Schizophrenia, *British Journal of Psychiatry*. *Under Review*.

Bois C., et al. (2015) Polygenic risk for schizophrenia is associated with transition to

disease in a familial high risk sample. *In Prep.*

Bois C., et al. (2015) The effects of cannabis on cortical thickness and surface area in individuals at high familial risk of developing the disorder. *In Prep.*

Barker V., **Bois C.**, et al. (2014) Childhood maltreatment in individuals at familial risk of schizophrenia. *Psychological Medicine. Under Review.*

Published First-Author Conference Abstracts:

Bois C., et al. (2015). Polygenic risk for schizophrenia is associated with transition to disease in a familial high risk cohort. *Journal of Neuropsychopharmacology Supplement.*

Bois C., et al. (2015). Cortical thickness and surface area in the EHRS. *Biological Psychiatry Supplement.*

Presented First-Author Conference Abstracts:

Bois C., et al. Longitudinal Changes in Hippocampal Volume in the Edinburgh High Risk Study of Schizophrenia

Bois C., et al. A Longitudinal Study of Global Cortical Morphology in the Edinburgh High Risk Study of Schizophrenia

Bois C., et al. Increased Polygene Scores for Schizophrenia are associated with Altered Surface Area and Gyrification in a cohort at Familial High Risk of Developing Schizophrenia

Chapter 1:

Appendix 1.1 Diagnostic criteria for Schizophrenia according to DSM-5.

Schizophrenia

Diagnostic Criteria

295.90 (F20.9)

- A. Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated). At least one of these must be (1), (2), or (3):
1. Delusions.
 2. Hallucinations.
 3. Disorganized speech (e.g., frequent derailment or incoherence).
 4. Grossly disorganized or catatonic behavior.
 5. Negative symptoms (i.e., diminished emotional expression or avolition).
- B. For a significant portion of the time since the onset of the disturbance, level of functioning in one or more major areas, such as work, interpersonal relations, or self-care, is markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, there is failure to achieve expected level of interpersonal, academic, or occupational functioning).
- C. Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or by two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).
- D. Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either 1) no major depressive or manic episodes have occurred concurrently with the active-phase symptoms, or 2) if mood episodes have occurred during active-phase symptoms, they have been present for a minority of the total duration of the active and residual periods of the illness.
- E. The disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.
- F. If there is a history of autism spectrum disorder or a communication disorder of childhood onset, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations, in addition to the other required symptoms of schizophrenia, are also present for at least 1 month (or less if successfully treated).

Specify if:

The following course specifiers are only to be used after a 1-year duration of the disorder and if they are not in contradiction to the diagnostic course criteria.

First episode, currently in acute episode: First manifestation of the disorder meeting the defining diagnostic symptom and time criteria. An *acute episode* is a time period in which the symptom criteria are fulfilled.

First episode, currently in partial remission: Partial remission is a period of time during which an improvement after a previous episode is maintained and in which the defining criteria of the disorder are only partially fulfilled.

First episode, currently in full remission: Full remission is a period of time after a previous episode during which no disorder-specific symptoms are present.

Multiple episodes, currently in acute episode: Multiple episodes may be determined after a minimum of two episodes (i.e., after a first episode, a remission and a minimum of one relapse).

Multiple episodes, currently in partial remission

Multiple episodes, currently in full remission

Continuous: Symptoms fulfilling the diagnostic symptom criteria of the disorder are remaining for the majority of the illness course, with subthreshold symptom periods being very brief relative to the overall course.

Unspecified

Specify if:

With catatonia (refer to the criteria for catatonia associated with another mental disorder, pp. 119–120, for definition).

Coding note: Use additional code 293.89 (F06.1) catatonia associated with schizophrenia to indicate the presence of the comorbid catatonia.

Specify current severity:

Severity is rated by a quantitative assessment of the primary symptoms of psychosis, including delusions, hallucinations, disorganized speech, abnormal psychomotor behavior, and negative symptoms. Each of these symptoms may be rated for its current severity (most severe in the last 7 days) on a 5-point scale ranging from 0 (not present) to 4 (present and severe). (See Clinician-Rated Dimensions of Psychosis Symptom Severity in the chapter "Assessment Measures.")

Note: Diagnosis of schizophrenia can be made without using this severity specifier.

Chapter 2.

Appendix 2.1

Examples of scans that had to be excluded: