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Running in the family?

Structural brain abnormalities in first-degree relatives of patients with schizophrenia

Komt het voor in de familie?

Structurele hersenafwijkingen in eerstegraads familieleden van patiënten met schizofrenie

(met een samenvatting in het Nederlands)

Proefschrift

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door

Heleen Brechtje Marije Boos

geboren op 23 juli 1978 te Utrecht

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	Dr. N.E.M. van Haren

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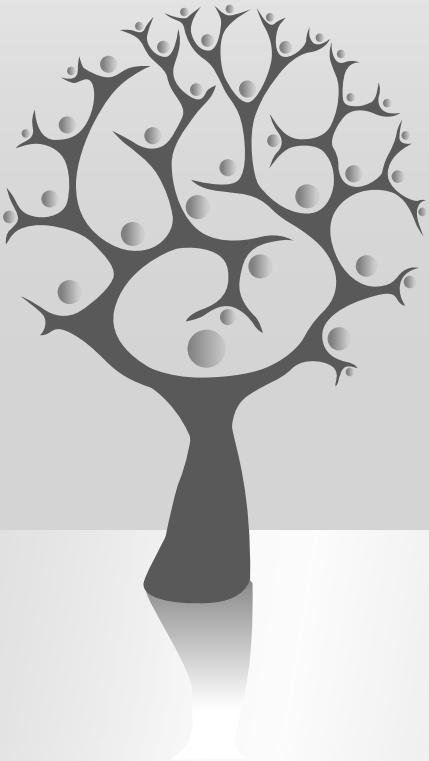
Chapter 1

Introduction



1 | Introduction

The main title of this thesis refers to a hit song ('Running in the family') from the eighties by the British band called Level 42 (1987). The song is about similarities (good or bad) that run in families. The studies conducted in this thesis explore whether first-degree non-psychotic relatives of patients with schizophrenia show structural brain abnormalities, similar (but to a lesser extent) to those consistently found in patients with schizophrenia.

1.2 | Schizophrenia

Schizophrenia is a severe and complex psychiatric brain disorder, characterized by abnormalities in the perception of reality and a disruption of thought processes and feelings. It results in great suffering for patients, but it also has a large impact on the patient's relatives and friends. Schizophrenia is a heterogeneous syndrome. Clinical presentations differ from one patient to another, due to variation in combinations of symptoms, severity, course and outcome of the illness.

1.2.1 | Etiology and Risk factors

The lifetime risk to develop schizophrenia is about 1%. It seems to occur almost one-and-a-half times as often in men as in women (Aleman et al., 2003) and generally manifests during early adulthood; in male on average earlier (around age 20-25 years) than in female (around age 25-30 years). In addition, there is a second smaller peak in female after age 45 (see review Goldstein and Lewine from Castle et al., 2000).

Emil Kraepelin (1856-1926) was the first to differentiate the illness, which he referred to as "dementia praecox" (early dementia) from manic depression (kraepelin, 1913). The term 'schizophrenia' was introduced at the beginning of the twentieth century by Eugen Bleuler (Bleuler, 1923) and is derived from two Greek words: "schizo", which means split, and "phren", meaning mind. Bleuler classified the symptoms of schizophrenia into fundamental and accessory symptoms (Bleuler, 1923). According to Bleuler, the fundamental symptoms are ambivalence, disturbance of association, disturbance of affect, and a preference for fantasy over reality. He claimed that these symptoms are present in all patients, at all stages of the illness, and are diagnostic of schizophrenia. Bleuler's accessory symptoms of schizophrenia included delusions, hallucinations, movement disturbances, somatic symptoms, and manic and melancholic states. He believed that these symptoms often occurred in other illnesses and were not present in all patients with schizophrenia. In addition, it is noteworthy that Bleuler's reconceptualization of dementia praecox as "the group of schizophrenias" is reflected in the current view that

schizophrenia is a heterogeneous group of disorders with varied aetiologies, but similar clinical presentations.

Since then, numerous attempts have been made at formalizing the definition of schizophrenia and to distinguish it from other disorders. In addition, different subtypes have been defined (i.e. acute versus chronic, and poor outcome versus good outcome subtypes). The most widely used standardized criteria to diagnose mental disorders come from the American Psychiatric Association's Diagnostic and Statistical Manual of Mental disorders; the DSM-IV (APA, 1994) (criteria for schizophrenia: see **Table 1**), and the World Health Organization's International Statistical Classification of Diseases and Related Health Problems; the ICD-10. In research, criteria according to the DSM-IV are mostly used. The DSM-IV provides several diagnostic subtypes to characterize patients with schizophrenia. For instance, paranoid schizophrenia is considered to be different from disorganized schizophrenia, with respect to psychopathology, age of onset and premorbid social functioning (Kaplan and Sadock, 1998). However, the extent to which such categorical distinctions have concurrent validity is a matter of debate. For example, differences between the diagnostic subtypes of schizophrenia (paranoid, undifferentiated, residual, catatonic and disorganized) have not been well reported (Carpenter et al., 2009). Also, the validity of diagnostic categories of schizophrenia and other non-affective psychotic disorders remains controversial.

With the forthcoming edition DSM-V, the definition of schizophrenia will most likely be revised. The DSM-V task force proposes to disregard subtypes.

Symptoms of schizophrenia are well defined by making a distinction between positive and negative symptoms. Positive symptoms, such as hallucinations, delusions and disorganized thinking, reflect an excess of normal functioning. Negative symptoms refer to a reduction or loss of normal functioning, like affective flattening, apathy, lack of energy or emotional withdrawal. Moreover, cognitive deficits are also consistently observed in patients with schizophrenia, including problems with executive functioning (planning and organization of behavior), attention, memory and concentration.

The etiology of schizophrenia remains largely unknown. Nevertheless, family, twin and adoption studies indicate that genetic factors play an important role. The risk for schizophrenia is elevated in individuals who have an affected family member; the closer the level of genetic relatedness, the greater the likelihood that a relative will also suffer from this illness (McGuffin et al., 1995; Gottesman, 1991). As shown in **Figure 1**, a healthy twin with an affected monozygotic co-twin

has the highest risk to develop schizophrenia (50%), followed by offspring of two schizophrenic parents (45%) (Cardno and Gottesman, 2000; McGuffin et al., 1995; Gottesman, 1991).

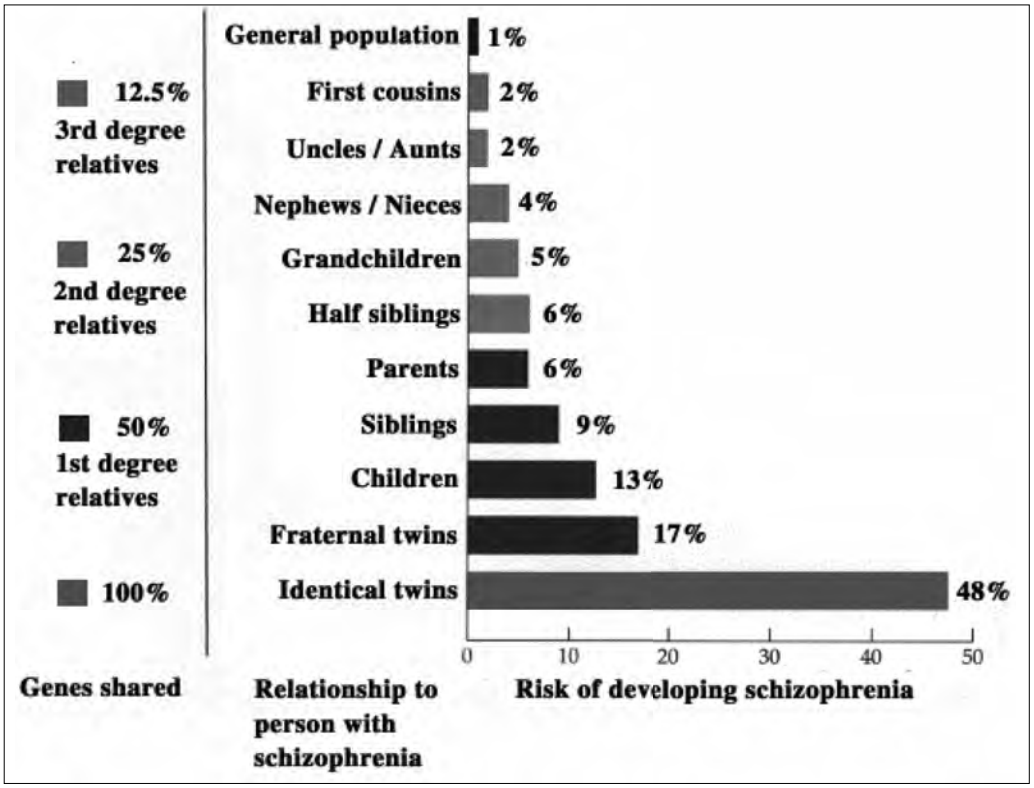


Figure 1 | Lifetime risk of developing schizophrenia (in percentage). Source: Gottesman, 1991

However, schizophrenia is not like Huntington's disease, in which a single gene is responsible for its occurrence (i.e. a genetic disorder in the Mendelian way). This is evident from the fact that monozygotic (MZ) twins have less than 100% concordance rates of schizophrenia. Findings from studies of discordant MZ twins indicate that the rate of schizophrenia is elevated in the offspring of non-affected co-twins (Gottesman and Bertelsen, 1989; Kringlen and Cramer, 1989), which suggests that some individuals possess a genetic vulnerability for schizophrenia that they pass on to their offspring despite the fact that they are never diagnosed with the illness, i.e. obligate carriers.

Table 1 | Diagnostic Criteria for Schizophrenia, according to DSM-IV

A. Characteristic symptoms: Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated):

1. delusions
2. hallucinations
3. disorganized speech (e.g., frequent derailment or incoherence)
4. grossly disorganized or catatonic behavior
5. negative symptoms, i.e., affective flattening, alogia, or avolition

B. Social/occupational dysfunction: For a significant portion of time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).

C. Duration: 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 two or more symptoms listed in criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

D. Schizoaffective and mood disorder exclusion: schizoaffective disorder and mood disorder with psychotic features have been ruled out because either (1) no major depressive episode, manic episode, or mixed episode have occurred concurrently with the active-phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.

E. Substance/general medical condition exclusion: The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

F. Relationship to a pervasive developmental disorder: If there is a history of autistic disorder or another pervasive developmental disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).

(Adapted from Diagnostic and Statistical Manual of Mental Disorders, 4th ed.)

The neurodevelopmental hypothesis of schizophrenia suggests that a disruption of brain development underlies the later emergence of psychosis during adulthood. Events that adversely affect fetal development are now considered to be potential environmental triggers of genetic vulnerability. It is also plausible that they are sufficient on their own to result in a vulnerability to schizophrenia.

There is extensive evidence that obstetric complications have an adverse impact on the development of the fetal brain. Numerous studies have shown that patients with schizophrenia are more likely to have a history of obstetric complications (for a review, see Cannon et al., 2002). Among these obstetric complications are pregnancy problems, such as preeclampsia and delivery complications.

A number of other environmental factors such as viral infections and cannabis use have also been associated with schizophrenia (Semple et al., 2005; Torrey, 1988). Cannabis (ab)use can induce psychosis and increases the risk for psychosis in adulthood (Semple et al., 2005). Nutrition (Hulshoff Pol et al., 2000), (pre- and perinatal) stress factors (Khashan et al., 2008; Norman and Malla, 1993) and social factors, such as (parental) socioeconomic status (Cantor-Graae, 2007), are other suggested environmental factors involved in the development of schizophrenia, but their specific contribution remain to be elucidated. Although the abovementioned environmental (risk) factors are associated with the illness, it is important to note that most individuals, who experience these adversities, never develop schizophrenia.

Most likely, in schizophrenia the environmental factors act in concert with genetic factors. Mathematical models show us that more than 80% of the etiological factors are of genetic origin (see meta-analysis of Sullivan et al., 2003), but it seems reasonable to believe that there is indeed a gene-environment interaction involved.

1.3 | Brain imaging

1.3.1 | History

In the early nineteen-hundreds, imaging techniques became available to investigate the brain in vivo, with techniques called ventriculography and pneumo-encephalography. In ventriculography, X-ray images of the ventricular system, were obtained by injection of infiltered air directly through holes drilled in the skull. In pneumo-encephalography, air was injected by lumbar spinal punction to enter the cerebral ventricles and cerebrospinal fluid compartments. These techniques were painful and resulted in unpleasant side effects. For this reason, healthy control subjects were often not included in the earliest studies.

Computer assisted tomography, initially called computerized axial tomography (CAT), was introduced in the early 1970s. Using this technique, it became possible to visualize the brain safely and non-invasively. Moreover, it also became possible to obtain more detailed anatomic images.

The introduction of modern imaging techniques, such as Computed Tomography (CT) enabled to investigate the hypothesized brain abnormalities in schizophrenia in a way that was less

disturbing for patients. CT uses a computer to analyze the data from a series of X-ray images. Using this technique, Johnstone and colleagues (1976) were the first who reported enlarged ventricles in patients with schizophrenia as compared to healthy control subjects. Earlier, Haug (1962) had already concluded that atrophic cerebral changes occurred in some patients with schizophrenia as he found frequently abnormal encephalogram in these subjects. In addition, he described an increase in the encephalographic abnormalities in the course of illness of patients whose clinical picture tends to be progressive.

1.3.2 | Magnetic Resonance Imaging (MRI) and schizophrenia

Schizophrenia is a disorder characterized by brain abnormalities. Magnetic resonance imaging (MRI) has been proved to be useful in revealing such structural brain abnormalities in patients with schizophrenia compared to healthy control subjects.

This imaging technique was developed in the early nineteen eighties. It was originally referred to as nuclear magnetic resonance (NMR), since the mechanism is based on the fact that certain atomic nuclei are sensitive to magnetic fields. Approximately 60-70% of the human brain consists of water, which means plentiful hydrogen nuclei (protons) that can be aligned in a strong magnetic field (longitudinal magnetization). The MRI scanner consists of a horizontal tube running through a strong magnet. The part of the body that needs to be scanned is placed in the center of the magnetic field and a radio transmitter generates electromagnetic pulses, making the protons start to spin and acts as tiny radio transmitters themselves. This radio signal generates an electric current in a receiver coil, which is measured as the MR signal. Different tissue types in the brain lead to differences in MR signal. The MRI scanner is able to determine from which location in the patient's body it receives certain signals. Finally, it integrates all this information to create a three-dimensional (3D) image.

The MR signal will be in part dependent on magnetic field strength, expressed in Tesla (T). In this thesis, subjects were scanned on a 1.5 T scanner. 3 T scanner machines are also widely available and since 2007 a 7 T scanner operates at the University Medical Center Utrecht, of which there are only a few in the world.

One of the main advantages of MRI is that brain scans are acquired in vivo without exposure to radiation. It is therefore a safe procedure. Furthermore, with MRI it is possible to quantify the gray and white matter of the brain. MR images are composed of 3D voxels, which is analogous to 2D pixels. With specific software, different brain areas can be segmented i.e., partitioned into meaningful structures (such as intracranium, total brain, gray and white matter, lateral and third ventricle) (Brouwer et al., 2010; Schnack et al., 2001a+b).

Most studies have employed a region of interest (ROI) measurement of brain structures. Although the anatomical validity of such studies is high, this type of analysis is time consuming. In addition, this method does not easily allow for comparison of many brain regions or large subject groups.

To overcome this issue, researchers employed the voxel-based morphometry (VBM; Ashburner and Friston, 2000) method, a fully automated whole-brain measurement technique, to examine structural MR images of the brain. By examining the whole brain, VBM provides a non-biased measure of highly localized regions that may not be investigated in hypothesis-based studies that use more labor-intensive ROI measurement techniques.

Another relatively new MRI technique is the measurement of cortical thickness, i.e. the thickness of the gray matter of the human cerebral cortex (Kabani et al., 2001; Fischl and Dale, 2000; Davatzikos and Bryan, 1996; Thompson and Toga, 1996). Cortical thickness is determined by the size, density and arrangement of neurons, neuroglia, and nerve fibers. Cortical thinning is frequently regionally specific and can therefore provide important additional information for characterizing disease-specific neuro-anatomical changes. Although cellular characteristics cannot be quantified directly in neuroimaging data, cortical thickness may more closely reflect cytoarchitectural abnormalities than cortical volume does (Kabani et al., 2001; Fischl and Dale, 2000).

While structural MRI has been proven to be useful in examining and detecting gray matter (GM) abnormalities in schizophrenia, particularly in the frontal and temporal regions (see meta-analysis of Wright et al., 2000); nowadays diffusion tensor imaging (DTI) has also shown to be useful for the examination of white matter (WM) fiber bundles connecting these regions. DTI imaging is a MRI method that allows the investigation of different aspects of white matter fiber bundles that cannot be measured using standard T1- or T2-weighted MRI. Growing evidence suggests that the integrity of WM fibers connecting GM regions is compromised in schizophrenia and that impaired functioning of WM is part of its pathophysiology (Davis et al., 2003).
DTI measures the degree to which water diffusion is constrained by barriers such as myelin sheaths, membranes or neuronal fiber tracts. Diffusion in white matter is anisotropic, which means that in a given white matter voxel, axial diffusion is much greater than radial diffusion. Fractional anisotropy (FA) is the most frequently used measure of the degree of diffusion and is a measure of white matter microstructure that may reflect fiber organization, fiber directional coherence, and/or fiber integrity (Beaulieu et al., 2002).

1.3.3 | MRI findings in schizophrenia

Many of the structural MRI studies in schizophrenia suffer from small sample sizes, and many (but not all) have measured different brain regions, not all in the same type of subjects. The interpretation of available data is therefore complicated. However, in a meta-analysis of 58 MRI studies (all published before September 1998), Wright et al. (2000) convincingly showed brain volume abnormalities in patients with schizophrenia; lateral ventricle volume being increased with 16%, while the cerebral volume was reduced with 2%. The latter was principally attributed to a decrease in gray matter volume (2%). However, a small but significant reduction in white matter volume (1%) was also reported. Evidence for regional pathology indicates larger reductions in overall temporal lobe volume (Harrison et al., 1999) and more specifically in medial temporal lobe structures (hippocampus and amygdala) (Nelson et al., 1998). The findings of the meta-analysis of Wright et al. (2000) are replicated in many studies (see meta-analyses Steen et al., 2006 and Vita et al., 2006). They are not detectable in all patients compared to control subjects, nor do they cluster in individual patients. They represent subtle significant deviations from population mean distributions of structural size.

A meta-analysis of voxel-based morphometry studies (Honea et al., 2005) convincingly showed that patients with schizophrenia have reduced gray matter density as compared to healthy control subjects, most pronounced in the left superior temporal gyrus and the left medial temporal lobe, but also in other areas across the brain. The findings of this meta-analysis have been replicated (Tanskanen et al., 2010; Koutsouleris et al., 2008). The diversity of regions reported in voxel-based morphometry studies is in part related to the choice of variables in the automated process, such as smoothing kernel size and linear versus affine transformation, as well as differences in patient groups. Voxel-based morphometry can be used as an exploratory whole-brain approach to identify abnormal brain regions in schizophrenia, which should then be validated by using region-of-interest analysis.

The first study to apply cortical surface reconstruction methods to investigate cortical thinning in schizophrenia, reported cortical thinning between patients and healthy control subjects, primarily in the frontal and temporal regions (Kuperberg et al., 2003). Recently, these findings have been replicated in larger samples of patients with schizophrenia (van Haren et al., submitted; Goldman et al., 2009; Nesvag et al., 2008).

Earlier DTI studies in schizophrenia reported reduced white matter integrity in patients with schizophrenia, particularly in the frontal lobe and its connections with the uncinate fasciculus,

but results have been inconsistent (see meta-analysis Ellison-Wright and Bullmore et al., 2009; reviews Kubicki et al., 2007 and Kanaan et al., 2005). In addition, reduced white matter integrity has been associated with symptomatology (see reviews Kubicki et al., 2007 and Kanaan et al., 2005). However, the use of such voxel-based morphometry methods, underpowered samples and limited reporting of effect size have limited interpretability and created challenges for replicating findings from DTI studies of schizophrenia (Konrad and Winterer, 2008).

More recently, fiber tracking techniques are used to infer fiber integrity along complete tracts, and average values of groups, such as FA can be measured along fiber tracts and can be compared between groups. Varying results have been found using such tract-based analysis.

Thus, DTI imaging has been shown to be a useful method to measure WM integrity in schizophrenia, but findings are inconclusive. The diversity of findings has been attributed to factors such as clinical heterogeneity and differences in study methodologies (Kanaan et al., 2005).

Accumulating evidence from longitudinal studies further indicates that multiple brain regions experience progressive tissue loss after the first psychotic onset of schizophrenia (Hulshoff Pol and Kahn, 2008; Pantelis et al., 2005). However, the timing and regional pattern of these brain changes remain unclear, and it is still unknown whether these changes are caused by the effect of long-term illness or the influence of illness-related factors, such as anti-psychotic medication or outcome. Nevertheless, it is clear that patients with schizophrenia show aberrant trajectories of brain volume changes during adolescence (Rapoport et al., 1999) and adulthood (van Haren et al., 2008).

1.3.4 | Family studies in schizophrenia

While it is clear that schizophrenia is associated with structural abnormalities of the brain, the extent to which these are related to a vulnerability to schizophrenia, opposed to the presence of the illness itself, is less certain. This issue can be addressed through the comparison of subjects at increased risk of schizophrenia (who are not psychotic themselves) and patients with the illness.

One group at increased risk comprises relatives of patients with schizophrenia, particularly the first-degree relatives. Valuable information has come from imaging studies of monozygotic (MZ) twins, discordant for schizophrenia. Environmental factors have been found to influence the often replicated lateral ventricle enlargement in schizophrenia by earlier twin studies (Rijsdijk

et al., 2005; Ohara et al., 1998; Suddath et al., 1990; Reveley et al., 1982), although influence of genetic factors are probably also involved (McDonald et al., 2002; Baare et al., 2001). Genetic factors have been found to play a substantial role in the whole brain abnormalities that are found in patients with schizophrenia (Rijsdijk et al., 2005).

In the past years, non-twin first-degree relatives have been examined in relation to the brain abnormalities that are consistently found in schizophrenia, but the results of these studies seem less clear than those of the recent twin studies. Most of these studies (Ho et al., 2007; Wood et al., 2005; Gogtay et al., 2003; van Erp et al., 2002; Cannon et al., 2002) showed smaller total brain volumes in first-degree relatives compared to healthy control subjects, but others (Goldman et al., 2008; Marcelis et al., 2003; Seidman et al., 2002; Lawrie et al., 2001) did not. Similarly, larger ventricular volumes have been reported in several studies (McDonald et al., 2002; Seidman et al., 1997) but three other studies did not find this (Ho et al., 2007; Gogtay et al., 2003; Cannon et al., 1998). Thus, although brain abnormalities have been found in the first-degree relatives of patients with schizophrenia compared to healthy control subjects, findings are inconsistent.

1.4 | The GROUP-project

The GROUP (Genetic Risk and Outcome of Psychosis) project has been designed to study genetic and non-genetic vulnerability and resilience factors for variation in the expression of non-affective psychotic disorders and variation in the course of these disorders in a naturalistic cohort study. This longitudinal observational study investigates the interaction between several vulnerability and resilience factors (genetic, somatic, psychological, and social) and genetic variation.

The consortium of this GROUP-project consists of four academic psychiatric centers (in Amsterdam, Groningen, Maastricht and Utrecht) and their affiliated mental health care institutions. It covers an area of more than 10 million inhabitants and establishes a population-based cohort of patients with a recent developed psychotic disorder (N=1000), a cohort of subjects at risk (brothers/sisters, N=1000), parents (N=350 pairs) and healthy volunteers (N=350). Assessments include a psychiatric interview, questionnaires, neuropsychological tests, and blood and urine samples. Patients, siblings, and healthy volunteers are assessed at baseline and again after three and six years. Parents have only been tested at baseline.

For an overview of questionnaires and tests at first measurement of the GROUP-project: see **Table 2**.

Table 2 | Overview of questionnaires and tests at first measureent of the GROUP-project

Tests/questionnaires rated by researcher	patients	siblings	parents	controls
SCAN/CASH	X	X	X	X
AIMS	X	X	X	X
UPDRS	X	X	X	X
BARS + Dystonia	X	X	X	X
PANSS	X	(X)	(X)	(X)
Samengestelde Lijst	X			
Samengestelde Lijst – short version		X		X
CIDI	X	X	X	X
NPO	X	X	X	X
SIS-R		X	X	X
FIGS		(X)	X	X
Questionnaires rated by someone other than subject:				
CAN	X			
SDS	X			
PAS	X	X		X
Follow-up checklist Farmacogenetics	X			
Rated by subjects self:				
Medische vragenlijst	X	X	X	X
WHO QoL	X	X	X	X
OC-DUS version cannabis	X	X	X	X
Physical analysis:				
blood drawing	X	X	X	X
urine screening	X	X		X

Presence or absence of psychopathology was established using the Comprehensive Assessment of Symptoms and History (CASH; Andreasen et al., 1992). In addition, the Schedule for the Deficit Syndrome (SDS; Kirkpatrick et al., 1989), Composite International Diagnostic Interview-Substance Abuse Module (CIDI; Andrews and Peters, 1998), Family Interview for Genetic Studies (FIGS; Maxwell, 1992), Assessment of severity of psychopathology or traits (Positive and Negative Syndrome Scale: PANSS; Kay et al., 1987), Community Assessment of Psychic

Experiences (CAPE, see http://www.cape42.homestead.com), Structured Interview for Schizotypy-Revised (SIS-R; Vollema and Ormel, 2000), Yale-Brown Obsessive-Compulsive Scale (YBOCS; Goodman et al., 1989), Extrapiramidal symptoms (EPS), Obsessive Compulsive Drug Use Scale (OCDUS; Franken et al., 2002), Premorbid Adjustment Scale (PAS; Cannon-Spoor et al., 1989), Camberwell Assessment scale of Need Short Appraisal Schedule (CANSAS; Slade et al., 1999) were administered.
Neuropsychological assessment consisted of the Visual Verbal Learning Task (VVLT; Brand and Jolles, 1985), Sustained attention: Continuous Performance Test (CPT-HQ; Wohlberg and Kornetsky, 1973), Working memory (WAIS III subtest Arithmetic; Wechsler, 1997), Speed of processing (WAIS III subtest Digit Symbol-Coding; Wechsler, 1997), Reasoning and problem solving (Block Design from the WAIS III; Wechsler, 1997), Verbal comprehension (WAIS-III Information subtest; Wechsler, 1997), Social cognition (Facial affect recognition: degraded facial affect recognition task (van 't Wout, 2004), and Theory of mind (Hinting Task; Versmissen, 2008; Corcoran, 1995). Finally, Urine screening, and blood samples for DNA were collected. For detailed description of the procedures and instruments we refer to the first and companion paper of the GROUP-project (G.R.O.U.P. et al., submitted).

From this multisite longitudinal cohort study patients with schizophrenia, their siblings, and healthy control subjects were recruited for the purpose of the structural MRI studies described in this thesis at the University Medical Center in Utrecht.

1.5 | Aim and outline of this thesis

In this thesis we described brain imaging studies examining first-degree relatives of patients with schizophrenia.

The overall aim of this work was to study focal and global brain structures and white matter integrity in non-psychotic first-degree relatives of patients with schizophrenia as compared to healthy control subjects. In **Chapter 2** we performed a meta-analysis of twenty-five studies to determine the magnitude and extent of brain volume differences in studies of first-degree relatives of patients with schizophrenia compared to healthy control subjects. The key to a meta-analysis is defining an effect-size statistic capable of representing the quantitative findings of a set of research studies in a standardized form that permits meaningful comparison and analyses across the studies (Lipsey and Wilson, 2001). In the analysis, we also examined the possibility of publication bias. **Chapter 3** describes the family study in which we examined

cortical and subcortical brain structures in a large sample of patients with schizophrenia, their related non-psychotic siblings and healthy control subjects, applying volumetric measurements, cortical thickness and voxel-based morphometry. In **Chapter 4** we compared mean fractional anisotropy (FA) along averaged white matter tracts, computed for the genu, splenium, left and right uncinate fasciculus, cingulum, inferior fronto-occipital fasciculus, fornix, arcuate fasciculus, and inferior longitudinal fasciculus. We computed mean FA for patients with schizophrenia, their related non-psychotic siblings and healthy control subjects. In addition, we examined the effect of age on mean FA between patients, their siblings and healthy control subjects.

Chapter 5 describes the study that was designed to examine brain structures in healthy parents of patients with schizophrenia as compared to control couples and its relationship with cognitive function. Finally, **Chapter 6** provides a brief summary of the abovementioned studies. Implications for future research are also discussed.

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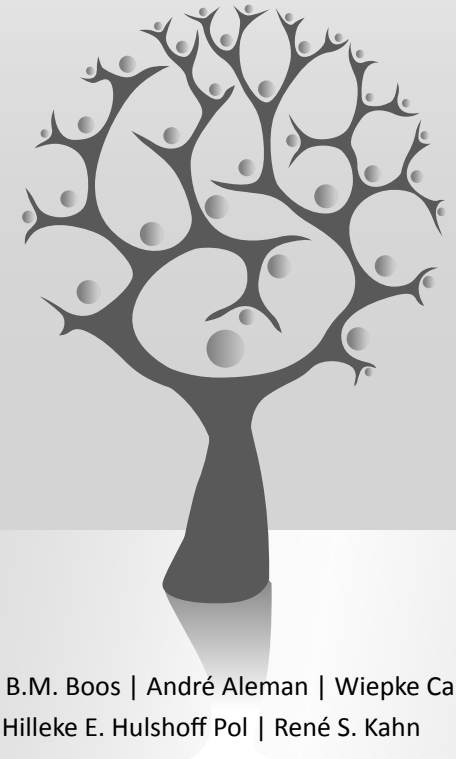
Chapter 2

Brain volumes in relatives of patients with schizophrenia: a meta-analysis



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Abstract

Context: Smaller brain volumes have consistently been found in patients with schizophrenia, particularly in gray matter and medial temporal lobe structures. Although several studies have investigated brain volumes in non-psychotic relatives of patients with schizophrenia, results have been inconsistent.

Objective: To determine the magnitude and extent of brain volume differences in first-degree relatives of schizophrenic patients.

Data Sources: A systematic search was conducted to identify relevant studies. Computer searches of the MEDLINE database were performed for English- language articles published before July 2005. Relevant abstracts published in 2005 were also selected.

Study Selection: Magnetic resonance imaging studies that examined differences in brain volumes between first-degree relatives of patients with schizophrenia and healthy control subjects were obtained through computerized databases, including MEDLINE. Studies had to report sufficient data for computation of effect sizes.

D**ata Extraction:** For each study, the Cohen d was calculated. Data extraction and calculation of the effect size was performed by 2 authors (H.B.M.B. and A.A.) who reached consensus in cases of uncertainty and discrepancies. All analyses were performed using the random-effects model.

Data Synthesis: Twenty-five studies were identified as suitable for analysis and included 1065 independent first-degree relatives of patients, 679 patients with schizophrenia, and 1100 healthy control subjects. The largest difference between relatives and healthy control subjects was found in hippocampal volume, with relatives having smaller volumes than controls (d=0.31, 95% confidence interval [CI], 0.13-0.49; 9 effect sizes). Gray matter was smaller (d=0.18, 95% CI, 0.02-0.33; 7 effect sizes) and third ventricle volume was larger (d=0.21, 95% CI=0.03-0.40; 7 effect sizes) in relatives compared with healthy control subjects.

Conclusion: Brain abnormalities are present in non-psychotic first-degree relatives of patients with schizophrenia and are most pronounced in the hippocampus.

Introduction

Structural brain abnormalities are well-established in schizophrenia. Several meta-analyses (Wright et al., 2000; Lawrie et al., 1998) have reported smaller brain volumes in schizophrenia, with more pronounced reductions in the hippocampus and amygdala. However, the nature of these brain changes is still unresolved. For instance, whether these changes are a result of the use of antipsychotic medication is a matter of debate (Cahn et al., 2002; Lieberman et al., 2001; Gur et al., 1998; Keshavan et al., 1994). Similarly, it is unclear to what extent these abnormalities are related to the vulnerability for developing the illness. Both issues can be (partially) addressed by studying brain structures in relatives of patients with schizophrenia. Clearly, the vulnerability for developing schizophrenia is highly genetic: studies (Cardno et al., 1999) in families of patients with schizophrenia have shown that the origin of the disorder has an estimated heritability of 80%, including interaction between the genes and environment. Thus, the presence of brain changes in relatives of patients would suggest these to be related to the shared genetic risk of developing schizophrenia. Moreover, brain volume differences in relatives cannot be the result of antipsychotic medication. Therefore, examining brain volumes in non-psychotic first-degree relatives of schizophrenic patients can clarify some of the causes of the brain abnormalities observed in probands.

In recent years, several studies have measured brain volumes in non-psychotic relatives of schizophrenic patients compared with those of healthy subjects. Most of these studies (Boos et al., 2005; Wood et al., 2005; Hulshoff Pol et al., 2004; van Haren et al., 2004; Gogtay et al., 2003; Cannon et al., 2002; van Erp et al., 2002; Baare et al., 2001; Noga et al., 1996) showed smaller total brain volumes in relatives, but others (Marcelis et al., 2003; Seidman et al., 2002; Lawrie et al., 2001) did not. Similarly, larger ventricular volume has been reported in several studies (van Haren et al., 2004; McDonald et al., 2002; Baare et al., 2001; Lawrie, et al., 2001; Staal et al., 1998; Seidman et al., 1997), but 2 other studies did not find this (Gogtay et al., 2003; Cannon et al., 1998). Furthermore, medial temporal lobe structures were reportedly smaller in several studies (van Erp et al., 2004; 2002; Keshavan et al., 2002; Narr et al., 2002; Seidman et al., 2002; Lawrie et al., 2001; O'Driscoll et al., 2001), but this finding has not been universally replicated (Wood et al., 2005; Hulshoff Pol et al., 2004; van Haren et al., 2004; Schulze et al., 2003; Staal et al., 1998). Thus, although brain abnormalities have been found in first-degree relatives of schizophrenic patients, the findings are inconsistent. Moreover, effect sizes in the individual studies have not been quantitatively reviewed and integrated.

The aim of the present meta-analysis was to determine the magnitude and extent of brain volume differences in first-degree relatives of schizophrenic patients. We attempted to integrate the findings from magnetic resonance imaging (MRI) studies in relatives of patients

with schizophrenia. To this end, we examined volumes of global brain structures and smaller structures in non-psychotic first-degree relatives of patients with schizophrenia compared with those of healthy control subjects. In an additional analysis, we compared brain volumes of patients with those of the unaffected relatives.

Methods

Data sources

The MRI studies that examined differences in brain volumes in first-degree relatives of patients with schizophrenia compared with healthy control subjects were obtained through computerized databases, including MEDLINE. The keywords used in the computerized search were brainabnormalit(s), relative(s) and schizophre(s). The terms relative(s), sib(s), parent(s) and schizophre(s) were also combined with brain volume(s), grey matter, white matter, ventricle(s) and hippocampus. Titles and abstracts of the articles were examined to see whether or not they could be included. Additional studies were obtained by a hand search of journals published in 2005 that most frequently publish articles on structural brain imaging in schizophrenia to find articles that had not yet been included in computerized databases. The journals included the following: Archives of General Psychiatry, The American Journal of Psychiatry, Biological Psychiatry, Schizophrenia Research, Psychiatry Research: Neuroimaging, American Journal of Medical Genetics , and Neurobiology of Disease. Bibliographies of included articles were used for a further search. Finally, abstracts from conferences on schizophrenia presented in 2005 were taken into account.

Study selection

Forty-three studies were identified as potential candidates for the meta-analysis. Studies were included if (1) they were MRI studies of brain structures published before July 2005 or they were not yet published but were presented as an abstract at the International Congress on Schizophrenia Research in 2005, (2) they compared first-degree relatives of patients with schizophrenia with a healthy control group (having no history and family history of psychosis), (3) they were published in the English language, and (4) they reported sufficient data to obtain the effect size: means, standard deviations, exact *P* values, or exact F values for a 2-group comparison. Studies in which some of the relatives had an ill family member diagnosed as having schizoaffective disorder (instead of schizophrenia) were also included in this analysis.

Fifteen studies were excluded from the meta-analysis because they did not show relevant data to enable us to compute the Cohen d values (Chapple et al., 2004; Toulopoulou et al., 2004; Job et al., 2003; Posthuma et al., 2003; Turetsky et al., 2003; Bridle et al., 2002; Cannon et al., 2002; 1994; Harris et al., 2002; Keshavan et al., 2002; Wright et al., 2002; McNeil et al., 2000; Waldo et al., 2000; Silverman et al., 1998). Five studies were excluded because they did not report brain volumes of relatives of schizophrenic patients compared with healthy control subjects (Falkai et al., 2002; Phillips et al., 2002; Thompson et al., 2001; Stefanis et al., 1999; Suddath et al., 1990). Twenty-five studies were identified as suitable for our meta-analysis and included 1065 independent first-degree relatives of patients, 679 patients with schizophrenia, and 1100 control subjects. The 25 studies that were identified as suitable reported brain volumes of different types of first-degree relatives; namely, siblings (van Haren et al., 2004; Gogtay et al., 2003; Marcelis et al., 2003; Cannon et al., 2002; 1998; Narr et al., 2002; Seidman et al., 2002; 1997; van Erp et al., 2002; Staal et al., 1998), monozygotic twins (Hulshoff Pol et al., 2004; van Erp, et al., 2004; van Haren et al., 2004; Narr et al., 2002; Baare et al., 2001; Sharma et al., 1999; Noga et al., 1996), dizygotic twins (Hulshoff Pol et al., 2004; van Erp et al., 2004; Narr et al., 2002; Baare et al., 2001; Sharma et al., 1999), parents (Boos et al., 2005; Falkai et al., 2004; Marcelis et al., 2003; Schulze et al., 2003; McDonald et al., 2002; Narr et al., 2002; Seidman et al., 2002; 1999), and offspring (Keshavan et al., 2002). Four studies did not specify first-degree relatives (Wood et al., 2005; McDonald et al., 2002; Lawrie et al., 2001; O'Driscoll et al., 2001). Together, the 25 studies reported volumes of 56 brain structures. Some of these structures were not evaluated by more than 3 studies, and in this case, these structures were not examined in the analysis. **Table 1** lists the included articles and the brain structures that were analyzed.

Data Extraction

This meta-analysis was performed to examine measurements of volumes in global brain structures and smaller structures in the medial temporal lobe in non-psychotic first-degree relatives of schizophrenic patients and healthy control subjects. The structures that were suitable for analysis included total brain, intracranial, lateral ventricle, third ventricle, gray matter, white matter, amygdala-hippocampal, hippocampal, and cerebrospinal fluid volume. If sufficient data were present, an analysis was performed to examine the effect of the side of the brain and differences in volumes between patients and relatives.

The key to meta-analysis is defining an effect size statistic capable of representing the quantitative findings of a set of research studies in a standardized form that permits meaningful comparison and analyses across the studies (Lipsey and Wilson, 2001). Therefore, for each

study in this meta-analysis, the effect size statistic Cohen d was calculated. Cohen d is the difference between the mean of the experimental group and the mean of the comparison group, divided by the pooled standard deviation. In this analysis, the mean volume of a specific brain structure for relatives of patients with schizophrenia was subtracted from the mean volume for comparison subjects and divided by the pooled standard deviation of both. When means and standard deviations were not available, d values were calculated from exact *P* values, *t* values, or F values. Data extraction and computation of the effect sizes were performed independently by 2 of the authors (H.B.M.B., and A.A.). In cases of discrepancies, a consensus was reached by means of discussion. After computing individual effect sizes for each study, meta-analytic methods were applied to obtain a combined effect size, which indicated the magnitude of the association across all studies (Hedges and Olkin, 1985). Individual effect sizes were inverse variance weighted in order to correct for upwardly biased estimation of the effect in small sample sizes (Rosenthal, 1991; Hedges and Olkin, 1985). Additionally, a homogeneity statistic, *Q*, was calculated to test whether the studies could be assumed to share a common population effect size. A significant *Q* statistic indicates heterogeneity of the individual study effect size, which poses a limitation to a reliable interpretation of the results. If significant heterogeneity is found, a moderator analysis can be performed to investigate the potential moderating factors (Rosenthal, 1991). A *t* test was subsequently performed on the null hypothesis that the d value is 0.00, which we report together with the associated *P* value. According to Cohen (Cohen, 1988), d values of 0.2 show small effects. Values between 0.4 and 0.6 are moderate effects and d values of 0.8 or higher are large effects. All analyses were performed in a random-effects model using comprehensive meta-analysis (Borenstein and Rothstein, 1999). A random-effects model assumes that the true effect size estimated by different studies varies between studies because of differences in samples or paradigms and that these true effect sizes have a normal distribution (ie, that heterogeneity exists) (DerSimonian and Laird, 1986).

To examine the possibility of publication bias, we computed a fail-safe number of studies (Rosenthal, 1991; Orwin, 1983). Publication bias implies that studies with no effect may not be published, posing a threat to the stability of the obtained effect size. The fail-safe number of studies indicates the number of unpublished studies with null effects that must reside in file drawers to reduce the observed effect size to a negligible level. The statistic can be calculated with the use of the formula given by Orwin (Orwin, 1983) and Lipsey and Wilson (Lipsey and Wilson, 2001):

$k*[(ES_k:Es_c)-1]$.

In this formula, k is the number studies, ES_k the mean weighted effect size; and Es_c the criterion effect size (which we set at a d value of 0.10).

Table 1 | Summary of 25 studies in Meta-analysis and included brain volumes.

Study	Year	No. of relatives	No. of controls	No. of patients	Included brain volumes
Baaré et al.	2001	29	58	29	IC, TB, LV, 3V
Boos et al.	2005	66	52	NA	IC, TB, LV, GM, WM, 3V
Cannon et al.	1998	60	56	75	IC, GM, WM
Cannon et al.	2002	51	54	64	TB, GM, WM
Falkai et al.	2004	51	41	45	NA
Gogtay et al.	2003	15	32	NA	GM, WM, LV
Hulshoff Pol et al.	2004	22	44	22	IC, TB, GM, WM
Keshavan et al.	2002	17	22	NA	NA
Lawrie et al.	2001	147	36	34	TB, LV, 3V, AHC
Marcelis et al.	2003	32	27	31	TB, GM, WM
McDonald et al.	2002	96	68	66	TB, LV, 3V
Narr et al.	2002	20	40	20	HC
Narr et al.	2002	20	20	NA	NA
Noga et al.	1996	12	12	12	TB
O'Driscoll et al.	2001	20	14	NA	HC
Schulze et al.	2003	96	68	66	HC
Seidman et al.	1997	6	11	NA	NA
Seidman et al.	1999	28	26	NA	TB, LV, 3V, WM, AHC
Seidman et al	2002	45	48	18	HC
Sharma et al.	1999	55	39	29	TB
Staal et al.	1998	16	32	16	GM, WM, LV, 3V, HC
van Erp et al.	2002	58	53	72	TB, HC
van Erp et al.	2004	46	109	48	IC, GM, HC
van Haren et al.	2004	32	146	32	TB, LV, 3V, HC
Wood et al.	2005	79	49	NA	TB, HC, NA

Abbreviations: AHC, amygdala-hippocampal complex; GM, gray matter; HC, hippocampus; IC, intracranial; LV, lateral ventricle; NA, not applicable/not available; TB, total brain; WM, white matter; 3V, third ventricle.

Data Synthesis

The structures that were analyzed, the number of studies included, and the number of subjects in which the structures were measured are reported in **Table 1**. The composite effect sizes (Cohen d, associated confidence intervals, *Q* statistic, and *P* values) of all studies for the different structures are reported in **Table 2**.

Only those structures for which the volumes were explored in more than 3 individual studies were analyzed. In applicable studies, brain volumes of patients were also compared with those of relatives.

Table 2 | Brain structures included in meta-analysis and results

Brain Structure	No. of Studies	No. of Relatives	No. of Controls	Mean Weighted Effect Size: Cohen d (95% CI)	Within-Category Homogeneity Statistic (*Q*)	*P* Value for *Q*
Total Brain	13	605	633	0.28 (-0.02 to 0.57)	63.99	<.001
Intracranial	8	335	369	0.12 (-0.04 to 0.27)	4.04	.77
Lateral Ventricle	7	367	412	0.11 (-0.05 to 0.27)	5.85	.44
Third Ventricle	7	414	418	0.21* (0.03 to 0.40)	8.31	.22
Gray Matter	7	249	285	0.18* (0.02 to 0.33)	4.68	.70
White Matter	7	245	284	0.40 (-0.04 to 0.83)	33.25	<.001
Amygdala-Hippocampus	12	605	675	0.52* (0.16 to 0.89)	94.17	<.001
Hippocampus total	9	421	603	0.31* (0.13 to 0.49)	13.79	.09
Hippocampus left	9	499	444	0.47* (0.34 to 0.61)	6.56	.58
Hippocampus right	9	499	444	0.23* (0.01 to 0.46)	19.43	.01
Cerebrospinal Fluid	4	96	121	0.61 (0.08 to 1.14)	9.81	.02

Abbreviations: CI, confidence interval. * *P*<.05.

Results

As presented in Table 2, the results of our meta-analysis indicate brain volume differences between first-degree relatives of patients with schizophrenia and healthy control subjects. The largest effect was found for hippocampal volume, with smaller volumes in relatives compared with healthy subjects (Figure 1). In this analysis, 9 studies were included, with a group size of 421 relatives of patients with schizophrenia and 603 healthy control subjects. One of the studies that measured hippocampal volumes controlled for intracranial volume and 8 studies for whole

brain volume. The combined-effect Cohen d of the 9 studies was 0.31 (*P*<.001). Excluding the studies that controlled for intracranial volume did not change the results, and analyzing studies (n=12) that measured hippocampal together with amygdala volume even showed a combined-effect Cohen d of 0.52 (*P*<.005). The largest effect was found in left hippocampal volume (d=0.47; *P*=.04; right hippocampal volume: d=0.23; *P*=.04). When we measured hippocampal volume in relatives compared with control subjects, the fail-safe number was 18, large enough to lend credence to our findings.

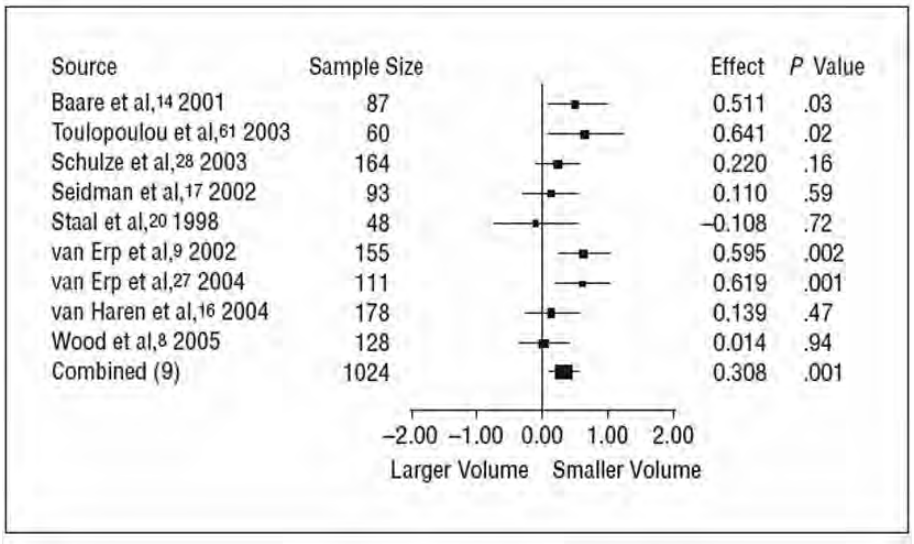

Figure 1 | Mean total hippocampal volume. Error bars indicate 95% confidence interval.

Small effects were found in cerebral gray matter (smaller in relatives vs. healthy control subjects, d=0.18; *P*=.04; fail-safe number=7) **(Figure 2)** and third ventricle volume (larger in relatives than in healthy control subjects, d=0.21; P=.02; fail-safe number=8) (**Figure 3**). The analysis of gray matter volume included 7 studies, with a group size of 249 relatives and 285 healthy control subjects. The analysis of third ventricle volume included 7 studies with 414 relatives and 418 healthy controls. Analyses of volumes of the total brain, intracranial space, lateral ventricles, and white matter did not show significant effects. However, the analysis of total brain and white matter volume did show a trend toward significance (total brain: d=0.28; P=.06, white matter: d= 0.40; *P*=.07; both smaller in relatives compared with healthy subjects).

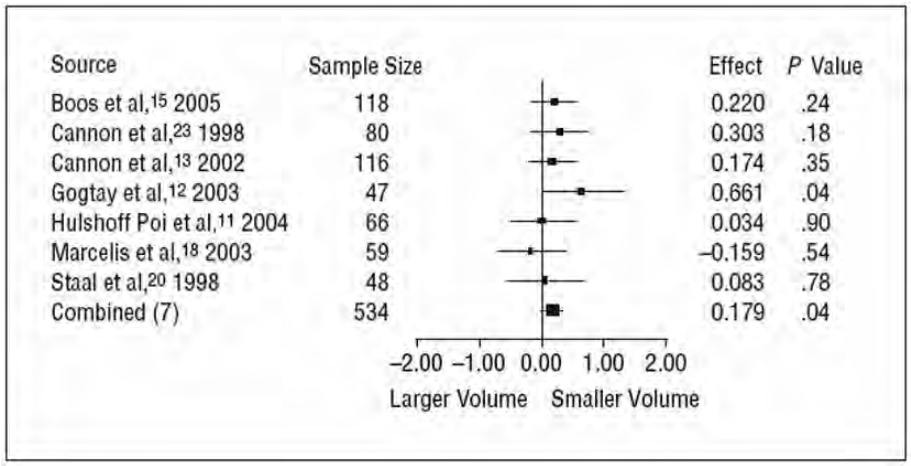

Figure 2 | Mean cerebral gray matter volume. Error bars indicate 95% confidence interval.

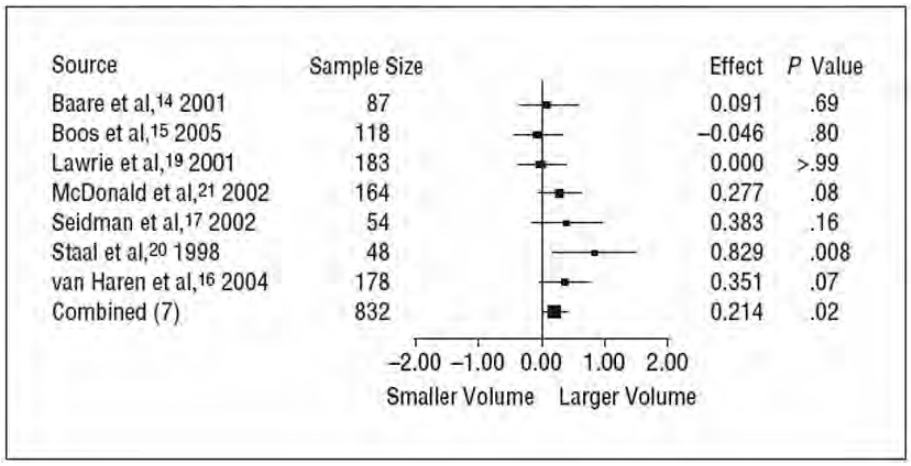

Figure 3 | Mean third-ventricle volume. Error bars indicate 95% confidence interval.

Seventeen studies also included a sample of patients (679 patients with schizophrenia and 790 non-psychotic relatives). Nine studies evaluated the hippocampus among 335 patients and 511 relatives, showing a moderate effect (d=0.43; *P*=.001; 95% confidence interval, 0.17-0.68) with patients having smaller hippocampal volumes than relatives. This result showed significant heterogeneity (*Q*=22.28; *P*=.004). However, one study was an outlier, reporting a large decrease in hippocampal volume (Suddath et al., 1990). When we excluded this study, the heterogeneity

was not significant (d=0.29; *P*<.001; *Q*=2.67; *P*=.91). The fail-safe number of studies for this analysis was 29, large enough to lend credence to our findings.
In the analysis that compared first-degree relatives with healthy control subjects, most *Q* values were non-significant (Table 2) except for those in the analyses of amygdala-hippocampal complex volume, white matter volume, total brain volume and cerebrospinal fluid volume. This significant *Q* value indicates heterogeneity of the individual study effect sizes and thus limits reliable interpretation of these results.

Comment

This meta-analysis integrated the results of 25 MRI studies that compared brain volumes of 1065 non-psychotic first-degree relatives of patients with schizophrenia with those of 1100 healthy control subjects. The results indicate that brain volumes in relatives of patients with schizophrenia differ from those of healthy control subjects, with effect sizes in the small to moderate range. The largest effect is found in hippocampal volume (d=0.31), with relatives of patients having smaller volumes than healthy control subjects. In addition, total gray matter volume (d=0.18) and third ventricle (d=0.21) volume are smaller in relatives compared with healthy control subjects. Although total brain and white matter volume did not differ significantly in relatives compared with healthy controls, both structures showed a trend toward significance (*P*=.06 and *P*=.07 respectively). The analysis that compared patients with schizophrenia with first-degree relatives showed smaller hippocampal volumes in patients (d=0.43). In addition, 3 studies that were excluded from this meta-analysis examined hippocampal volumes in first-degree relatives compared with healthy control subjects. Two studies also showed smaller volumes in relatives compared with healthy controls (Job et al., 2003; Waldo et al., 2000). However, Harris et al. (2002) did not find this.

The results of this meta-analysis suggest that brain abnormalities in schizophrenia are related (in part) to the risk of developing the disease and that these brain changes may therefore predate the clinical onset of the disorder. Moreover, they argue against the notion that the brain abnormalities in schizophrenia are solely caused by antipsychotics. These conclusions are bolstered by the finding that the brain structures affected in relatives are the same as those reported to be abnormal in patients (Wright et al., 2000). The findings are supported by two studies (Job et al., 2005; Pantelis et al., 2003) that reported reduced grey matter volumes in similar brain structures of individuals at high risk for schizophrenia. Both studies reported that those relatives who later develop psychotic symptoms have a more severe reduction before the onset of these symptoms.

The finding of hippocampal volume reduction in relatives of schizophrenic patients also dovetails with the results of recent meta-analyses regarding cognitive functioning in relatives (Sitskoorn et al., 2004; Toulopoulou et al., 2003). In these articles, lower performance in relatives of patients compared with healthy control subjects was reported on a number of cognitive domains, including verbal and declarative memory, executive functioning, and attention. Interestingly, Sitskoorn et al. (2004) found that the largest effect size was obtained for verbal memory (d=0.54), being significantly worse in relatives of patients than in healthy subjects. However, the performance of relatives on these cognitive tasks was less impaired than has been reported in patients with schizophrenia (Zakzanis et al., 2000; Aleman et al., 1999). Indeed, decreased verbal memory is one of the most robust neuropsychological findings in schizophrenia (Job et al., 2005). Deficits in verbal memory have generally been associated with smaller (left) hippocampal volume (Geuze et al., 2005) as is also the case in patients with schizophrenia (Gur et al., 1998; Goldberg et al., 1994) and their relatives (Seidman et al., 2002; O'Driscoll et al., 2001). In the present meta-analysis, the effect size was considerably larger for the left than for the right hippocampus. This finding is consistent with findings from lesion and functional MRI studies in healthy subjects, suggesting more involvement of the left hippocampus in encoding and recognition of verbal as opposed to visual or pictorial material (Powell et al., 2005). The suggestion of smaller left hippocampal volume as a vulnerability indicator for schizophrenia, put forward by Seidman et al (2002), is also consistent with these observations.

The findings of this meta-analysis suggest that a common genetic vulnerability to developing schizophrenia is reflected in brain morphologic findings. McDonald et al (2004) demonstrated that the genetic risk of schizophrenia is associated with an extensive system of gray matter deficits and white matter abnormalities. However, only a few studies have identified specific genes in relation to brain volume abnormalities in schizophrenia. Szeszko et al. (2005) studied 19 patients with schizophrenia and 25 healthy control subjects and reported a role for brain-derived neurotropic factor in determining hippocampal volume. More relevant to the finding of the current meta-analysis, Callicott et al. (2005) examined the effects of the DISC1 gene on the risk for schizophrenia and its impact on the hippocampus. They found that DISC1 increased the risk of developing the disease and was also associated with structural and functional alterations in the hippocampus.

However, smaller hippocampal volumes in relatives of patients with schizophrenia could also have been caused by environmental factors. Obstetric complications such as hypoxia are known to result in smaller brain volumes, affecting the hippocampus profoundly (McDonald et al., 2002; Kelly et al., 2000; Cannon et al., 1993). Smaller hippocampal volumes have also been associated with brain injury (Geuze et al., 2005; McAllister et al., 1998; Buckley et al., 1993) and

stress (Geuze et al., 2005; Smith et al., 2003) and have been found not only in schizophrenia but also in several other psychiatric disorders, such as major depression, posttraumatic stress disorder, obsessive compulsive disorder, and borderline personality disorder (Geuze et al., 2005). An important function of the hippocampus and amygdala is the regulation of the hypothalamic-pituitary-adrenal axis, which plays a role in stress processing. This regulation may be altered because of a genetic predisposition. In depression, the hypothalamic-pituitary-adrenal axis is strongly activated and the adrenal cortex hypersecretes glucocorticoids such as cortisol. Although less pronounced, considerable hypothalamic-pituitary-adrenal activation is also found in schizophrenia (Sapolsky et al., 1986). On the basis of earlier animal experiments, overexposure to cortisol during prolonged periods of stress is expected to damage the brain, particularly the hippocampus. Sapolsky et al. (1986) provided evidence in rats that chronic stress, with the concomitant increase in corticoid levels, causes loss of neurons in the hippocampus and subsequent deficits in memory function and cognition. In patients with depression, this glucocorticoid cascade has also been presumed to result in decreased hippocampal volume (Sapolsky, 1986), possibly explained by apoptosis (Swaab et al., 2005). Both apoptosis and neurogenesis have been shown to occur in the hippocampus (Eriksson et al., 1998). Thus, smaller hippocampal volumes in patients with schizophrenia and their first-degree relatives might also be the result of stress-related processes in the brain (McEwen, 2000).

These hypotheses regarding putative genetic and environmental factors underlying hippocampal damage in relatives of schizophrenic patients can be integrated by taking gene-environment interactions into account. Gene-environment interactions may result from genetically mediated differences in the sensitivity to environmental factors or environmentally-mediated influences on gene expression. Evidence of genetically mediated differences in environmental factor sensitivity shows that slightly elevated rates of obstetric complications are found, not only in patients with schizophrenia but also in their non-psychotic first-degree relatives (Cannon et al., 2002; Sacker et al., 1996). As reported by Cannon et al. (2000), most of these relatives exposed to obstetric complications did not develop schizophrenia, and thus these factors are incapable of causing schizophrenia on their own. Obstetric complications may act additively or interactively with genetic factors in influencing liability to schizophrenia. Van Erp et al. (2002) examined siblings of patients with schizophrenia and found that hippocampal volumes differed stepwise with each increase in genetic predisposition to schizophrenia and that hippocampal volumes of patients exposed to fetal hypoxia were smaller than those who were unexposed, whereas no such relationship was observed within the healthy control subjects. They suggested that carrying susceptibility genes for schizophrenia makes one vulnerable to perinatal damages, especially in the hippocampus.

Some limitations of this meta-analysis should be noted. First, as with all meta-analyses, the results depend on the quality of the individual studies. The adjustment of cerebral structures for whole brain or intracranial volume has been thought to facilitate differences in effects among the studies. However, the results of a moderator variable analysis failed to confirm this hypothesis. Therefore, it is unlikely that the observed differences in volume are due to differences in adjustment.

Second, structures other than those that have been evaluated in this meta-analysis may also be affected in relatives of patients with schizophrenia. The results of smaller hippocampal volumes in relatives compared with healthy control subjects might reflect broader abnormalities in the temporal lobes or even other structures, but because of the small amount of studies that measured these structures, this could not be investigated in our analysis.

Third, the results may have been influenced by publication bias. However, in the present meta-analysis, this is unlikely using a fail-safe number of studies statistic, which indicates the number of studies with null effects that must reside in file drawers before results of the obtained effect sizes are reduced to a negligible level.

Fourth, only a few studies that were included in the meta-analysis and measured brain volumes of siblings specified whether they had used independent samples or multiple siblings per family. Although this may bring in a confounding factor, because of the small number of studies included in the meta-analysis, all sibling studies that were available and met the criteria were included.

Fifth, differences in age and gender were not examined. Age and gender are known to effect brain volumes (Cannon et al., 2000); however, the studies included in this meta-analysis did not provide enough data to examine the effects of age and gender. Except for hippocampal volume, differences between left and right brain structures were not measured. The statistical test to determine the latter results requires left and right regional volumes, and these data were not generally provided by the original studies. Thus, the possibility that some of the effects found in this meta-analysis were caused by confounding factors such as sex and age, cannot be ruled out. In addition, some studies (Hulshoff Pol et al., 2004; Narr et al., 2002) suggest that white matter reduction reflects an increased risk of developing schizophrenia. Although the present meta-analysis did not find significant decreases, the analysis resulted in significant heterogeneity, which hampers a reliable interpretation and may have influenced the results. More and larger studies are needed to show whether in non-psychotic relatives total brain and white matter volume differ from healthy control subjects. Longitudinal studies on brain volumes of relatives of schizophrenic patients could also be helpful in diminishing problems of individual study characteristics and reducing heterogeneity issues. In addition, different methods have been proposed for estimating heterogeneity and publication bias. For example, other meta-analyses

have included funnel plots (plots of effect estimates against sample size) to index publication bias (Cannon et al., 2000) and the I^2 statistic to measure the proportion of inconsistency in individual studies that cannot be explained by chance. The latter approach was argued to be a better index of heterogeneity than the *Q*-statistic, especially for collections of studies with either small or large sample sizes (measuring inconsistency in meta-analyses) (Gur et al., 1991). Notably, most studies included in our analyses were of intermediate sample size. Finally, some studies included in this meta-analysis not only studied first-degree relatives but also included some second-degree relatives (Falkai et al., 2004; McDonald et al., 2002; Lawrie et al., 2001). These studies did not examine hippocampal and gray matter volume. However, in the analysis of third ventricle volume, second-degree relatives were included but the exact amount was not reported in the studies. Excluding the 3 studies that examined some second-degree relatives did not alter the results of our meta-analysis.

In summary, our results provide support for the hypothesis that non-psychotic first-degree relatives of patients with schizophrenia show structural brain abnormalities, particularly in the left hippocampus. These brain abnormalities are similar to the areas that are affected in patients with schizophrenia and parallel the findings of neuropsychological impairments (especially in verbal memory) in both patients and relatives. Although these findings reflect a vulnerability to developing schizophrenia, it is still unclear how and to what extent genes and/or environment are involved. Future studies should focus on the search for susceptibility genes in relation to brain abnormalities by using linkage and association methods.

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Chapter 3

Focal and global brain measurements in siblings of patients with schizophrenia

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Abstract

Background: It remains unclear whether structural brain abnormalities in schizophrenia are caused by genetic and/or disease related factors. Structural brain abnormalities have been found in non-psychotic first-degree relatives of patients with schizophrenia but results are inconclusive. This large MRI study examined brain structures in patients with schizophrenia, their non-psychotic siblings and healthy control subjects using global and focal brain measurements.
Methods: From 155 patients with schizophrenia, their 186 non-psychotic siblings and 122 healthy controls (including 25 sibling pairs), whole brain scans were obtained. Segmentations of total brain (TB), gray matter (GM) and white matter (WM) of the cerebrum, lateral and third ventricle and cerebellum volumes were obtained. For each subject, measures of cortical thickness and GM density maps were estimated. Group differences in volumes, cortical thickness and GM density were analysed using Structural Equation Modeling, hence controlling for familial dependency of the data.
Results: Patients with schizophrenia, but not their non-psychotic siblings, showed volumetric differences, cortical thinning and reduced GM density as compared to control subjects.
Conclusions: This study did not reveal structural brain abnormalities in non-psychotic siblings of patients with schizophrenia compared with healthy control subjects using multiple imaging methods. Therefore, the structural brain abnormalities observed in patients with schizophrenia are for the largest part explained by disease-related factors.

Introduction

Schizophrenia is characterised by gray matter (GM) reductions in cortical and subcortical regions, but the underlying mechanisms causing these abnormalities are largely unknown. Twin studies suggest that genetic influences play a role (Hulshoff Pol et al., 2006; Rijsdijk et al., 2005; Baare et al., 2001), but there is also convincing evidence that environmental influences, such as antipsychotic medication (Cahn et al., 2002; Scheepers et al., 2001; Madsen et al., 1999) (for review see: Moncrieff and Leo, 2010), obstetric complications (Falkai et al., 2003; Cannon et al., 2002; McDonald et al., 2002) and cannabis use (Bangalore et al., 2008; Rais et al., 2008; Szeszko et al., 2007) are involved. Furthermore, brain abnormalities appear to be related to clinical features such as duration of (untreated) psychosis (Cahn et al., 2008; Premkumar et al., 2008; Lappin et al., 2006) and outcome (van Haren et al., 2008; 2007).

As the heritability to develop schizophrenia is estimated to be 81% (Sullivan et al., 2003), it is thought that the brain abnormalities reported in schizophrenia may also be present in unaffected relatives of patients with this illness. Indeed, a meta-analysis, including 23 studies, reported volumetric decreases in the hippocampus and GM, as well as increases in third ventricle volume in relatives of patients with schizophrenia compared to healthy control subjects (Boos et al., 2007). This meta-analysis pooled data from neuroimaging studies (largest study: n=183) that examined various groups of relatives (i.e. twins, parents, offspring and siblings), all carrying their own specific genetic and environmental risk factors. The studies included in this meta-analysis did not provide enough data to examine the effects of age, which is relevant as off-spring and young siblings are still at risk to develop the illness, while older siblings and parents are most likely beyond the age of risk. In addition, structural brain abnormalities seem progressive, even in unaffected relatives (Brans et al., 2008).

MRI studies that were included in the meta-analysis and focussed on siblings of patients with schizophrenia reported GM reductions, most pronounced in the temporal areas (Cannon et al., 2002; 1998, but not Staal et al., 2000) and hippocampus (van Erp et al., 2002). Studies that were published after this meta-analysis came out reported GM reductions in the posterior cingulate cortex (Calabrese et al., 2008) and the inferior frontal gyrus (Harms et al., 2010). In addition, larger orbitofrontal WM was found (Fan et al., 2008), but when cortical thickness was examined, no differences were found in siblings of patients as compared to healthy control subjects (Calabrese et al., 2008). The largest sibling study to date, including 115 patients with schizophrenia, 192 non-psychotic siblings and 196 healthy control subjects, failed to find differences in global brain volumes (Goldman et al., 2008), cortical thickness (Goldman et al., 2009), and GM density (Honea et al., 2008) between siblings and healthy control subjects. Interestingly, a study including siblings of patients with childhood-onset schizophrenia found

no differences in GM volume and cortical thickness in siblings of 20 years and older, while in the younger siblings decreased (parietal) GM volume, as well as cortical thinning was reported in the prefrontal and temporal cortices (Gogtay et al., 2007).

In summary, while smaller studies report reduced volumes in siblings of patients with schizophrenia compared with healthy control subjects, the largest study to date failed to find structural brain differences between these two groups. We therefore designed this large study of 155 patients with schizophrenia, 186 of their related (relatively young) non-psychotic siblings and 122 age-matched healthy control subjects (including 25 sibling pairs). Cortical and subcortical brain structures were examined by applying volumetric measurements, cortical thickness and voxel-based morphometry. We hypothesized that non-psychotic siblings show a similar but less pronounced pattern of structural brain differences relative to patients with schizophrenia as compared to healthy control subjects. As earlier studies reported that schizotypy was found to a much higher degree in first-degree relatives compared with control subjects (Calkins et al., 2004; Kendler et al., 1995), we hypothesized that these brain differences are related to (sub-) clinical characteristics present in the siblings.

Methods and Materials

Participants

A total of 155 patients with schizophrenia, 186 related non-psychotic siblings and 122 healthy control subjects (including 25 sibling pairs) participated in this study. The recruitment was part of the baseline measurement of an ongoing longitudinal study in The Netherlands (Genetic Risk and Outcome of Psychosis; GROUP). From this study, subjects were recruited at the University Medical Center Utrecht, Utrecht, The Netherlands.

Eligible patients had to fulfil the following criteria: (1) age between 16 and 50, (2) meeting Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for a non-affective psychotic disorder (including schizophrenia, schizophreniform disorder, schizoaffective disorder), (3) fluent in Dutch, and (4) able and willing to give written informed consent. Eligible siblings (brothers and/or sisters) of participating probands had to fulfil the criteria of (1) age between 16 and 50, (2) fluent in Dutch, and (3) able and willing to give written informed consent. Eligible healthy control subjects had to fulfil the criteria of (1) age between 16 and 50, (2) no lifetime psychotic disorder and/or use of lithium medication (in the past), (3) no first- or second-degree family member with a lifetime psychotic disorder, (4) fluent in Dutch, and (5) able and willing to give written informed consent.

Patients and controls identified as potentially eligible were asked to provide consent for assessment and for contacting their siblings. Control subjects were selected through a system of random mailings to addresses in the catchment areas. Presence or absence of psychopathology was established by using Comprehensive Assessment of Symptoms and History interview (CASH; Andreasen et al., 1992), performed by at least one independent rater who was trained to assess this interview. Diagnosis was based on the DSM-IV criteria. Of all subjects, urine was screened for cocaine, amphetamines and for cannabis. Subjects with substance dependence/ abuse (based on the criteria of the Composite International Diagnostic Interview (WHO, 1994), (section B, J and L)), and a major medical or neurological illness were excluded.
Written informed consent was obtained from all subjects and the study was approved by the medical ethics committee for research in humans (METC) of the University Medical Center Utrecht.

Clinical and neuropsychological assessments

To evaluate severity of symptoms in patients with schizophrenia, the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987) was performed. In siblings and healthy control subjects, the Structured Interview for Schizotypy-Revised (SIS-R; Vollema et al., 2000; Kendler et al., 1989) was administered. The SIS-R is a semi-structured interview containing 20 schizotypal symptoms and 11 schizotypal signs, rated on a four-point scale. Scores were subdivided in positive, negative and total schizotypal features. Furthermore, the Dutch translation of the Family Interview for Genetic Studies (FIGS) was used to estimate the presence of a psychiatric illness in first- and/or second-degree family members.

Imaging and Preprocessing

Structural magnetic resonance imaging (MRI) scans of the whole brain were obtained on a 1.5 T Achieva scanner (Philips, Best, The Netherlands). A three-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head (256 x 256 matrix, TE=4.6 ms, TR=30 ms, flip angle=30˚, 160-180 contiguous slices; 1x1x1.2 mm^3 voxels, Field-Of-View (FOV) = 256 mm/70%) was acquired. Furthermore, a single-shot EPI (echo planar imaging) scan was made as part of a diffusion tensor imaging (DTI)-series (SENSE factor 2.5; flip angle=90˚;60 transverse slices of 2.5 mm; no gap; 128 x 96 acquisition matrix; FOV=240 mm; TE=78 ms) together with a magnetization transfer imaging (MTI) scan (60 transverse slices of 2.5 mm; no gap; 128 x 96 acquisition matrix; FOV = 240 mm; flip angle=8˚; TE=4.5 ms; TR=37.5 ms).
Processing was done on the computer network of the Department of Psychiatry at the University Medical Center Utrecht. All images were coded to ensure investigator blindness to subject identification and diagnosis.

Volumetric processing

The T1-weighted images were automatically put into Talairach orientation (Talairach, 1988) without scaling, by registering them to a model brain. The two other scans were registered to the T1-weigthed image by minimizing a mutual information joint entropy function (Maes et al., 1997). The co-registered scans were used for automatic segmentation of the intracranial volume, based on histogram analysis and morphology operations. The intracranial segment served as a mask for all further segmentation steps. The T1-weighted images were corrected for field inhomogeneities using the N3 algorithm (Sled et al., 1998). Our automatic processing pipeline was used for segmentation of total brain, gray (GM) and white matter (WM) of the cerebrum (Brouwer et al., 2010). In short, pure GM and WM intensities were directly estimated from the image. The amounts of pure and partial volume voxels were modelled in a non-uniform partial volume density, which is fitted to the intensity histogram. Expected tissue fractions, based on the pure intensities and the partial volume density, were subsequently computed in each voxel within the cerebrum. Total brain volume was calculated by adding the gray and white matter segments. Binary images of GM and WM were created using 0.5 as a threshold: i.e. voxels in the GM partial volume map with a fraction >0.5 were considered as GM, and similarly, voxels in the WM partial volume map with fractions >0.5 were classified as white matter.

Lateral and third ventricle and cerebellum volumes were also assessed. The software included histogram analysis, mathematical morphology operations, and anatomical knowledge-based rules to connect all voxels of interest, as was validated before (Schnack et al., 2001). The intracranial mask, ventricle, and cerebellum segments were all visually checked and edited if necessary.

Cortical thickness

To compute cortical thickness, the binarized GM and WM segments were used as input for the CLASP algorithm designed at the McConnell Brain Imaging Centre of the Montreal Neurological Institute (Kim et al., 2005; Kabani et al., 2001; MacDonald et al., 2000). A 3D surface comprising 81,920 polygons per hemisphere was fitted to the WM/GM interface, which created the inner surface of the cortex which was then expanded to fit the GM/cerebrospinal fluid interface, creating the outer cortical surface. Cortical thickness was estimated by taking the distance between the two surfaces such that each polygon vertex on the outer surface had a counterpart vertex on the inner surface. Each subject's thickness measurements were smoothed across the surface using a 20 mm (FWHM) surface-based blurring kernel (Chung et al., 2003), as was done before (Brans et al., 2010). This method of blurring improves the chances of detecting population differences, but also follows the curvature of the surface to preserve any anatomical boundaries within the cortex. The surfaces of each subject were registered to an average

surface created from 152 healthy subjects aged 18-40 years (ICBM) (Lyttelton et al., 2007), allowing comparison of cortical thickness locally between subjects.

Voxel-based morphometry (VBM)

Regional measures of GM and WM concentration (''density'') were generated using VBM in a similar manner as was done previously (Hulshoff Pol et al., 2006). VBM involved the following steps. First, a model brain was created on the total sample, similar to the method used by Grabner et al. (2006) After creation of the model brain, the partial volume GM and WM segments with voxels of 1x1x1.2 mm^3 were blurred by a 3D Gaussian kernel (FWHM=8 mm) to gain statistical power. The voxel values of these blurred partial volume GM and WM maps (between 0 and 1) reflect the local presence, or density, of GM or WM, respectively. These images are referred to as ''density maps''. To compare brain tissue at the same anatomical location in all subjects, the GM and WM segments were transformed into a standardized coordinate system (the model space). These transformations were calculated in two steps. First, the T1-weighted images were linearly transformed to the model brain. In this linear step, a mutual information metric was optimized (Maes et al., 1997). In the second step, nonlinear (elastic) transformations were calculated to register the linearly transformed images to the model brain up to a scale of 4 mm (FWHM), thus removing global shape differences between brains, but retaining local differences. For this step, the program ANIMAL (Collins et al., 1995) was used. The GM and WM density maps were now transformed to the model space by applying the concatenated linear and nonlinear transformations. Finally, the maps were resampled to voxels of size 2x2x2.4 mm^3. Voxels with an average GM density below 0.1 were excluded from the GM density voxel-based analysis. Using 'non-modulated' VBM analyses allow for direct investigation of regional differences in brain areas without being confounded by overall brain size, i.e. these individual differences in brain size and shape have been removed by linear and nonlinear transformations.

Statistical Analysis

Demographic and diagnostic data

Data were examined for outliers and normality of the distribution, using the Kolmogorov-Smirnov test for significance.

To assess whether the groups differed on demographic variables, univariate analyses of variance were conducted for non-categorical variables and χ^2 tests for categorical variables.

SPSS 15.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA) was employed for analyses of demographic data.

Group differences in brain volumes, cortical thickness and GM density maps

In the full model, total brain, GM, WM, lateral ventricle, third ventricle and cerebellum volumes were regressed on intracranial volume, gender, age, handedness and group status (patients vs. siblings vs. healthy control subjects). Cortical thickness and GM density (VBM) were regressed on gender, age, handedness, and group status. Relatedness in the patient-sibling pairs and control pairs was accounted for in the covariance structure by allowing dependencies between the residuals in the regression analyses. Group effects were tested by comparing the -2 log-likelihoods of two nested models: a model that does allow for group effects on structural brain measures (the full model), and a model that does not allow for such an effect. The difference in -2 log-likelihood between these models is χ^2 distributed. A χ^2>3.84 (1 d.f.) indicates a significant difference at α=0.05, and depicts that the discarded effect (i.e., group effect) cannot be left out of the model without seriously reducing the goodness of fit.

For group effects in volumes, cortical thickness and voxel-based morphometry, mixed model analysis was implemented using Structural Equation Modeling (SEM) with Mx software for Windows (Department of Psychiatry, Virginia Commonwealth University Richmond, Virginia). The present study aimed to examine a large group of families and variables. A distinction was made between mutual correlations between siblings and correlations between healthy control subjects. SEM is a useful design for such studies. To evaluate the differences in cortical thickness a vertex-by-vertex analysis was carried out. In each vertex, group differences in cortical thickness were calculated using regression analyses with group, age, gender and handedness as covariates. This produced χ^2-statistics at each vertex, one for the effect of group, one for the effect of age, one for the effect of gender and one for the effect of handedness. Statistical maps were created showing significant differences in cortical thickness between groups. For those cortical areas that showed significant differences, the most significant vertex was identified visually using the cortical surface viewer Brain-view developed at the Montreal Neurological Institute.

To evaluate differences in GM density, regression analysis was done through all brains for each voxel separately in the GM and WM density maps. Similar to the cortical thickness analysis, this produced χ^2-statistics at each voxel.

In all statistical analyses a correction for multiple comparisons was carried out according to the false discovery rate (FDR).

Associations with severity of illness

To address whether in patients, structural brain differences depend on severity of illness, post hoc analyses were performed. Brain measures were regressed on PANSS positive symptoms

scores, PANSS negative symptoms scores and PANSS total scores. For eight patients PANSS scores were missing. These were excluded from the analysis.

Associations with schizotypy

For the combined sample of siblings and control subjects, post-hoc analyses were performed to address whether there is an association between positive or negative schizotypal features as measured with the SIS-R and brain measures. For three siblings and one healthy control subject SIS-R scores were missing. These were excluded from the analysis.

Results

Demographic and diagnostic data

For demographic analyses, see **Table 1**. No differences between groups were found for age (siblings: 27.54 years (SD=6.75); patients with schizophrenia: age=26.91 years (SD=5.58) and healthy control subjects: 27.53 years (SD=8.24)), parental educational level (defined as the total number of years of education) and handedness. Groups differed significantly in gender distribution; male and female subjects being equally divided within the siblings (45.7% male) and control subjects (50.0%) but not in the patient group (80.6% male). Groups differed significantly in WAIS IQ (siblings: mean IQ=100.9 (SD=15.10); patients with schizophrenia: mean IQ=93.32 (SD=15.70); healthy control subjects: mean IQ=110.9 (SD=14.60)). The majority of patients (90%) were taking antipsychotic medication at time of scan, with olanzapine and risperdone being most often prescribed (N=55 and N=27 respectively). In patients, mean duration of illness was 4.02 years (SD=3.63).

Global brain volumes

After controlling for age, gender, intracranial volume and handedness, non-psychotic siblings did not differ from healthy control subjects in brain volumes. Patients with schizophrenia showed significant reductions in total brain (χ^2=23.72, p<0.01), GM (χ^2=10.82, p<0.01) and WM volumes (χ^2=6.62, p=0.01) compared with healthy control subjects (see **Table 2**). In addition, increased lateral (χ^2=14.65, p<0.01) and third ventricle (χ^2=6.94, p<0.01) volumes were found in patients relative to healthy control subjects. We have performed additional analyses in which we compared patients with their related siblings. The results of these analyses were similar to the results of the comparison of patients with control subjects. Post-hoc analyses showed no association between brain volumes and dose or type of medication at inclusion, nor did cannabis use (lifetime or past year) affect our results in patients with schizophrenia.

Table 1 | Demographic information

	N or mean (sd) [range]		
	patients (N=155)	**siblings (N=186)**	**healthy control subjects (N=122)**
age (y)	26.91 (5.6) [18.5-43.3]	27.5 (6.8) [16.6-50.5]	27.5 (8.2) [17.1-49.4]
sex (M/F)	125/30 (80.6% male)[a]	85/101 (45.7% male)	61/61 (50.0% male)
handedness (R/L))	143/12 (92.3% right)	166/20 (89.2% right)	108/14 (88.5% right)
parental education level (completed in years)	13.04 (3.6)	13.39 (3.1)	13.5 (3.1)
subject education level (completed in years)	12.04 (2.3)[a]	13.30 (2.4)[a]	14.02 (1.9)[a]
WAIS IQ	93.32 (15.7) [63-136][a]	100.9 (15.10) [68-155][a]	110.9 (14.6) [73-144][a]
Paranoid type (%)	100 (64.5)	0	0
Schizoaffective disorder (%)	20 (12.9)	0	0
Undifferentiated type (%)	17 (11.0)	0	0
Disorganized type (%)	7 (4.5)	0	0
Catatonic type (%)	1 (0.6)	0	0
Schizophreniform disorder (%)	9 (5.8)	0	0
Residual type (%)	1 (0.6)	0	0
Bipolar disorder (%)	0	7 (3.8)	0
Major depression (%)	0	36 (19.4)	0
Schizotypal Personality disorder (%)	0	1 (0.5)	0
Other disorders (%)	0	6 (3.2)	0
no psychiatric disorder (%)	0	136 (73.1)	122 (100)
PANSS positive symptoms score[b]	15.34 (5.7) [7-35]		
PANSS negative symptoms score[b]	15.41 (5.5) [6-31]		
PANSS total symptoms score[b]	62.22 (17.17) [30-113]		
SIS-R positive subscale[c]		0.19 (0.4) [0-2]	0.18 (0.24) [0-1.3]
SIS-R negative subscale[c]		0.20 (0.3) [0-1]	0.18 (0.21) [0-0.9]

[a]Significantly differed from both other groups; [b]For 8 cases information was missing; [c]For 4 cases information was missing

Table 2 | Brain volumes ml: uncorrected mean (s.d.), χ^2

Brain area	patients	siblings	controls	χ^2 (patients vs siblings)	χ^2 (siblings vs controls)	χ^2 (patients vs controls)
intracranial volume	1550.66(145.54)	1504.87(137.38)	1528.53(141.09)	2.34	1.26	1.27
whole brain	1303.20(128.90)	1286.24(123.94)	1304.69(133.59)	44.58*	0.50	23.72*
cerebral gray matter	622.79(62.08)	613.52(59.70)	622.59(66.61)	17.00*	0.00	10.82*
cerebral white matter	510.32(63.21)	507.65(60.76)	512.66(62.83)	20.87*	1.65	6.62*
lateral ventricle	16.89(9.10)	13.69(7.95)	13.16(5.83)	17.24*	0.05	14.65*
third ventricle	0.91(0.35)	0.71(0.30)	0.78(0.33)	33.07*	3.84	6.94*
cerebellum	157.42(15.56)	152.69(15.88)	156.59(15.86)	2.23	0.84	3.09

* Significant difference ($p<0.01$); Note that there were 81% male patients in this sample. In analyses means were corrected for (IC), age, gender and handedness

In urine screening, 19 patients, 18 siblings, and 6 healthy control subjects were positive for cannabis, cocaine, or amphetamines at inclusion. Excluding these subjects from the analyses did not alter the results.

After controlling for IQ or parental education level (including age, gender, intracranial volume and handedness), results were similar to those described above.

Furthermore, since there were more male than female patients, a separate analysis was performed for only male subjects (n=113 patients; n=84 siblings; n=60 healthy control subjects). The results of this analysis were similar to the results described above.

Cortical thickness

In focal cortical thickness analyses, non-psychotic siblings did not show differences in cortical thickness compared with the healthy control subjects. Patients with schizophrenia showed cortical thinning compared with healthy control subjects. **Figure 1** shows the statistical difference map of this analysis, corrected for the effect of age, gender and handedness, at a corrected threshold of χ^2>7.50 for left and χ^2>5.82 for right hemisphere (FDR; α=0.05, d.f.=1). As shown in **figure 1** and **table 3**, cortical thinning in patients was most apparent bilaterally in the frontal and temporal cortex, with patients also showing cortical thinning bilaterally in the occipital cortex, Wernicke's area, left parahippocampal and posterior cingulate gyrus, and right parietal and precentral cortex. Cortical thinning was found also in patients as compared to non-psychotic siblings (at a corrected threshold of χ^2>7.52 for left and χ^2>5.64 for right hemisphere), being most pronounced in the bilateral frontal and temporal cortex, but also in the Wernicke's area, the left parahippocampal and occipital gyrus and the right parietal cortex. Patients did not show increased cortical thickness compared with healthy control subjects or non-psychotic siblings.

Voxel-based morphometry

For GM density maps, non-psychotic siblings did not reveal differences compared with healthy control subjects. Patients with schizophrenia were significantly different from healthy control subjects as shown in **figure 2**. The critical χ^2-value of significance, corrected for multiple comparisons (FDR, α=0.05) was 9.34. After correction for age, gender and handedness, patients showed decreased GM density, most pronounced in the anterior cingulate gyrus and the insula as compared to healthy control subjects, but also in the temporal, occipital, parietal and frontal cortex, the thalamus and the head of caudate (see **Table 4**). Similar to results in the comparison of patients versus controls, patients were different from siblings. The critical χ^2-value of significance, corrected for multiple comparisons (FDR, α=0.05) was 9.13. After correction, patients showed decreased GM density as compared to siblings most pronounced in the frontal

cortex and the insula, but also in the anterior cingulate, temporal and parietal cortex, the head of caudate and the occipital cortex.

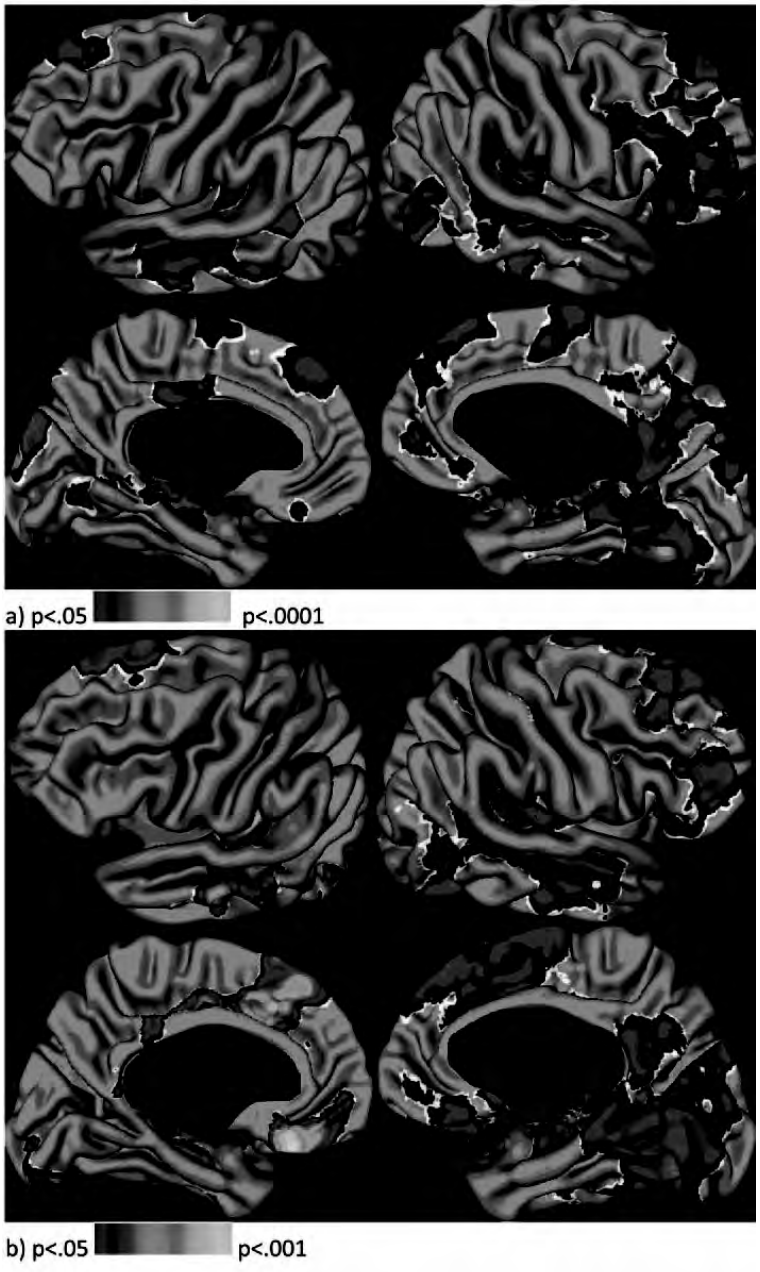

Figure 1 | Group differences in cortical thickness: Difference maps (χ^2), corrected for age, gender and handedness: a) patients versus healthy control subjects; b) patients versus siblings.

Table 3 | Significant differences in cortical thickness Areas showing cortical thinning in a) patients compared with healthy control subjects and b) patients compared with their non-psychotic siblings

a)

Brain area	Talairach coords; X, Y, Z	BA	mean patients	mean siblings	mean controls	χ^2
bilateral middle temporal	49, -32, 0		3.20(0.24)	3.27(0.25)	3.27(0.21)	19.86
bilateral inferior occipital	47, -78, -2	19	2.77(0.27)	2.81(0.22)	2.87(0.25)	19.85
bilateral superior frontal	10, 53, 42	8	3.51(0.29)	3.61(0.29)	3.63(0.29)	19.13
bilateral Wernicke's area	-44, -29, 10	41	3.03(0.20)	3.13(0.21)	3.11(0.22)	13.12
bilateral orbitofrontal	7, 48, -14	11	3.05(0.22)	3.12(0.21)	3.15(0.23)	12.19
left superior occipital	-6, -88, 23	18	2.59(0.17)	2.62(0.19)	2.71(0.20)	18.38
left parahippocampal	-37, -30, -11	36	2.84(0.19)	2.97(0.20)	3.01(0.20)	16.08
left posterior cingulate	-3, -18, 31	23	3.10(0.21)	3.16(0.21)	3.19(0.21)	12.95
right hippocampal	34, -20, -13		2.99(0.19)	3.14(0.22)	3.19(0.21)	34.72
right inferior occipital	27, -68, -7	19	2.85(0.17)	2.95(0.17)	2.97(0.17)	33.40
right middle frontal	39, 26, -8	47	3.25(0.29)	3.33(0.29)	3.37(0.29)	18.35
right posterior cingulate	4, -50, 18	30	3.23(0.29)	3.29(0.29)	3.31(0.29)	18.06
right parietal	7, -75, 44	7	2.63(0.19)	2.67(0.19)	2.71(0.19)	12.01
right superior frontal	4, 10, 49	6	3.51(0.29)	3.57(0.29)	3.58(0.29)	12.14
all significant with critical $\chi^2(\alpha=0.05)=7.50$ (left hemisphere) and $\chi^2(\alpha=0.05)=5.82$ (right hemisphere)						

b)

Brain area	Talairach coords, X, Y, Z	BA	mean patients	mean siblings	mean controls	χ^2
bilateral frontal pole	18, 25, -24	47	2.78(0.19)	2.93(0.20)	2.87(0.19)	26.05
bilateral middle temporal	48, -34, 0		3.20(0.28)	3.28(0.28)	3.27(0.28)	25.02
bilateral Wernicke's area	-43, -29, 10	41	3.03(0.21)	3.13(0.21)	3.11(0.21)	23.63
bilateral lateral superior frontal	12, 53, 40	8	3.29(0.29)	3.60(0.29)	3.61(0.29)	22.47
left parahippocampal	-9, -36, 4	27	2.21(0.25)	2.31(0.25)	2.31(0.25)	14.55
left occipital	-47, -75, 3	19	2.78(0.19)	2.85(0.22)	2.83(0.21)	11.19
right occipital	33, -80, -12	19	2.73(0.21)	2.88(0.21)	2.84(0.21)	30.73
right posterior cingulate	7, -51, 22	23	3.21(0.21)	3.28(0.21)	3.28(0.21)	18.88
right inferior frontal	44, 47, 2	10	3.02(0.21)	3.09(0.21)	3.08(0.21)	18.37
all significant with critical $\chi^2(\alpha=0.05)=7.52$ (left hemisphere) and $\chi^2(\alpha=0.05)=5.64$ (right hemisphere)						

The table shows the anatomical location (Brain area), Talairach coordinates (Talairach coords) and Brodman coordinates (BA); Mean (sd) for each group is given with the statistics (χ^2).

Table 4 | Focal decreases in gray matter density in a) patients with schizophrenia as compared to healthy control subjects and b) patients as compared to non-psychotic siblings

a)			
Brain area	**Talairach coords; X, Y, Z**	**BA**	χ^2
anterior cingulate	-2, -15, 28	23	52.94
insula	46, -12, 12	13	45.1
temporal cortex	41, 9, -24	38	28.92
occipital cortex	17, -96, 10	18	22.06
parietal cortex	-31, -80, 31	19	20.1
frontal cortex	38, 55, 4	10	19.12
thalamus	-14, 23, 3		18.63
all significant with critical $\chi2(\alpha=0.05)=9.43$			
b)			
Brain area	**Talairach coords; X, Y, Z**	**BA**	χ^2
frontal cortex	3, 40, 18	32	42.16
insula	41, 1, 5	13	42.16
anterior cingulate	6, -20, 35	31	34.8
temporal cortex	-40, 2, -14	38	32.84
parietal cortex	-28, -67, 44	7	16.67
occipital cortex	25, -91, 13	18	11.76
all significant with critical $\chi^2(\alpha=0.05)=9.13$			

The table shows the anatomical location (Brain area) with its corresponding Talairach coordinates (Talairach coords) and Brodmann area (BA).

Associations with PANSS

PANSS total symptom score was associated with total brain ($\chi2= 4.32$, $p<0.05$) and GM volume ($\chi2=7.30$, $p<0.01$), with decreased volumes related to higher scores. PANSS total positive symptom score was negatively associated with GM volume ($\chi2=8.22$, $p<0.01$) and positively with lateral ventricle volume ($\chi2=5.49$, $p<0.05$).

Associations with schizotypy

In siblings and healthy control subjects, SIS-R total, positive or negative scores were not related with brain volumes, nor with cortical thickness and GM density maps.

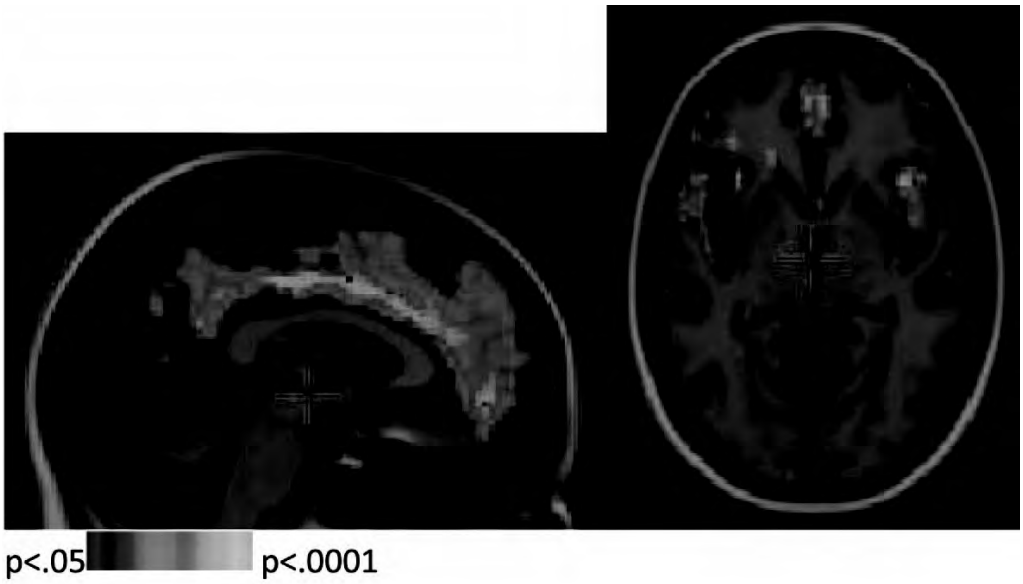

Figure 2 | Focal decreases (difference maps produced in χ^2 statistics) in GM density in patients with schizophrenia as compared to healthy control subjects from a given section from the sagittal and axial plane, respectively.

Discussion

This cross-sectional imaging study including 463 subjects examined brain structures in a relatively young sample of patients with schizophrenia (n=155), their non-psychotic siblings (n=186) and healthy control subjects (n=122, including 25 sibling pairs), using various imaging techniques. Global brain volumes of non-psychotic siblings were not different from those of healthy control subjects, nor did siblings and healthy control subjects differ in cortical thickness or GM density measured using a voxel-based morphometry approach. The paucity of cortical and subcortical brain differences in the siblings of patients is consistent with the findings from another large study in non-psychotic siblings (Goldman et al., 2009; 2008; Honea et al., 2008). The siblings in our study were about 10 years younger compared to the sample of Goldman et al. (2008) and in these analyses we were able to take into account relatedness as we included patient-sibling pairs, as well as healthy control sibling pairs. Our findings contrast with those of smaller imaging studies in non-psychotic siblings (largest study; total n=155) (Tepest et al., 2003; Staal et al., 2000; Cannon et al., 2002; 1998).

Our study did find robust structural brain differences in patients with schizophrenia as compared to healthy control subjects. Indeed, we replicate the global volumetric abnormalities in patients with schizophrenia in total brain, GM, WM, lateral ventricle and third ventricle (see meta-analyses Steen et al., 2006; Wright et al., 2000). Furthermore, the decreases in cortical thickness and GM density, particularly in the frontal and temporal cortex, as well as the anterior cingulate cortex, are consistent with earlier studies (Gutiérrez-Galve et al., 2010; Janssen et al., 2009; Kuperberg et al., 2003) and with those studies using a voxel-based morphometry approach (see meta-analysis Honea et al., 2005). These most replicated findings in the inferior frontal, middle temporal and the cingulate regions have been found to be associated with speech (see review Price et al., 2005).

Thus, our findings that brain abnormalities are expressed in patients with schizophrenia but not in non-psychotic siblings suggest that brain abnormalities in schizophrenia mainly reflect processes related to the manifestation and/or treatment of the illness.

That the illness itself causes brain changes in schizophrenia is corroborated by the findings in ultra-high risk subjects and the association between brain changes and illness-related factors in schizophrenia. Only in those subjects who later converted to psychosis cortical GM deficits were found at baseline, but deficits were not found in those subjects who did not become psychotic over time (Sun et al., 2009; Lawrie et al., 2002). Furthermore, a longitudinal study in adolescents at ultra-high risk for psychosis showed that the development of psychosis was associated with progressive abnormalities around time of onset (which was not attributed to antipsychotic medication) (Ziermans et al., 2010). In addition, studies that examined symptomatology in relation to brain imaging findings reported that reduced GM volume was related to duration of untreated psychosis (Lappin et al., 2006) and duration of psychosis (Cahn et al., 2009; Premkumar et al., 2008). In addition, various other studies (Cahn et al., 2002; Lieberman et al., 2001), but not all (DeLisi et al., 2004), reported a relationship between clinical outcome and reduced GM volume. Indeed, in the present study we found that severity of illness (total and positive symptoms) was associated with reduced GM and increased lateral ventricle volume.

There is also evidence that brain abnormalities reported in schizophrenia are related to the effects of antipsychotic treatment. While post-hoc analyses failed to show an association, in cross-sectional non-randomized studies such as ours, it is not possible to rule out medication effects on brain structure completely. A study in macaque monkeys treated long-term with olanzapine or haloperidol, reported that cortical volume was reduced by both these agents. (Dorph-Petersen et al., 2005) In contrast, in a prospective study of Lieberman et al. (2005), obtaining MRI scans at multiple intervals, brain morphology was found to be differentially affected by olazapine and haloperidol over time. In addition, other studies in patients with

schizophrenia showed that decrements in GM volume over time, particularly in prefrontal regions, were associated with the (cumulative) intake of typical, but not of atypical antipsychotic medication (van Haren et al., 2007) (for review see Moncrieff et al., 2010).

Other non-shared environmental factors, such as obstetric complications can result in brain abnormalities in patients with schizophrenia (Cannon et al., 2002; McDonald et al., 2002). Unfortunately, in our study there was not sufficient information of obstetric complications to investigate its effects on structural brain abnormalities.

To date, the neurobiological processes that underlie the brain abnormalities in patients with schizophrenia remain unclear but may reflect anomalies of synaptic plasticity and abnormal brain maturation. Early (pre- and perinatal) neurodevelopmental trauma may render the brain vulnerable to aberrant late neurodevelopmental processes, which may further interact with other causative factors associated with the onset of psychosis (e.g., substance use, stress, and dysregulation of the hypothalamic-pituitary-adrenal axis function) (Pantelis et al., 2005). Around transition to psychosis, these processes together may disrupt further brain development. Indeed, it has been suggested that the brain changes in the early state of schizophrenia are the result of the "toxic" effect of the psychotic state (Lieberman et al., 2001). Another theory was raised which guide neuroimmunology/virology studies of schizophrenia and derives from a general theoretical focus on CNS viral reactivation-induced immunological changes leading to psychosis (Waltrip et al., 1990).

That the structural brain differences are under genetic control cannot be dismissed by the negative findings of our study. MRI studies in twins do report volume decreases in whole brain, gray and white matter or hippocampus in unaffected twins who are discordant for schizophrenia (Brans et al., 2008; Hulshoff Pol et al., 2004; van Haren et al., 2004; Baare et al., 2001, but see Suddath et al., 1990). These studies included monozygotic twins, sharing 100% of the genes with their sibling, and dizygotic twins sharing 50% of the genes. Interestingly, brain volume differences in twins discordant for schizophrenia were more pronounced in the monozygotic than in the dizygotic twins, compared with healthy control twins (Hulshoff Pol et al., 2006; 2004; Rijsdijk et al., 2005; Baare et al., 2001). This suggests that the genetic contribution to brain volume reductions in schizophrenia may be subtle and is primarily detectable in subjects with high genetic loading, i.e. monozygotic discordant twins and not in the healthy siblings of patients with schizophrenia.

The presence of brain volume differences in unaffected twins but not siblings could also be explained by the contribution of environmental factors that are specific for twins, such as intra-uterine viral infections (Davis et al., 1995), prenatal environment (Brown et al., 2007) and delivery complications. (McNeil et al., 2000) 'These are common environmental factors which patients share with their (monozygotic) co-twins, while they are not shared with a

nontwin sibling (van Erp et al., 2004; Cannon et al., 2002). Stress may also be such a common environmental factor (McDonald et al., 2002). The emotional burden of the disease can be considerable in siblings of patients with schizophrenia (Schmid et al., 2006). For twins, who often have a close emotional relationship with each other, the experience of having a co-twin with a severe psychiatric disease like schizophrenia may represent a more pronounced burden. Furthermore, the heterogeneity produced by the broadly recruited sample of unaffected siblings in our study may have undermined the apparently high statistical power. Some previous computational neuroanatomical studies assessed relatively homogenous groups of unaffected relatives of patients with schizophrenia, which were chosen deliberately to maximise power through 'genetic enrichment', including high risk familial subject (Job et al., 2003) and relatives from multiply affected families (McDonald et al., 2006; 2004; McIntosh et al., 2004; 2006).

Some limitations need to be addressed. First, a selection bias may have affected our results. This is reflected in that we included only siblings of patients who were willing to participate. Those siblings whom we were not able to include in the study may be of particular interest as they might share more (sub-)clinical features with their ill proband. However, based on the Family Interview for Genetic Studies (FIGS), the included siblings were not different from those who were not included. Earlier studies reported that schizotypy was found to a much higher degree in first-degree relatives compared with healthy control subjects (Calkins et al., 2004; Kendler et al., 1995). As suggested by Diwadkar et al. (2006), relatives with high levels of schizotypy may define a hyper vulnerable sub-sample among these relatives of patients with schizophrenia. Interestingly, in the present study, siblings and healthy control subjects were similar in schizotypal scores as measured with the SIS-R, suggesting that these siblings were possibly not vulnerable to develop schizophrenia. Second, there was a preponderance of men in the sample of patients compared with siblings and healthy control subjects. The epidemiologic design of this study explains these differences. To minimize the effect of gender on brain structures, we controlled for this variable in all analyses. Male gender has been shown to be associated with larger cerebral volumes (Sowell et al., 2007; Gur et al., 1991) which disappears with head size correction (Scahill et al., 2003). Geater decline of grey matter volume with age in males has also been reported in some (Taki et al., 2004; Ge et al., 2002; Raz et al., 1997) but not other (Lemaître et al., 2005) studies. Females have also been shown to have thicker cortex across many regions of the brain (Sowell et al., 2007; Luders et al., 2006). As we know that gender but also age and handedness may influence brain structures, we have included these as covariates in our analyses. Third, it may be that cross-sectional MRI measurement might not be informative enough to find structural brain abnormalities in siblings of patients with schizophrenia. Fourth, it should be noted that the significant areas found in this study are

indicative of locations of effects; their spatial extent is influenced by the smoothing of the data (Bernall-Rusiel et al., 2010).

In conclusion, our study did not find structural brain abnormalities in non-psychotic siblings of patients with schizophrenia compared with healthy control subjects, using multiple imaging methods. This suggests that the structural brain abnormalities found in patients are most likely related to the illness itself.

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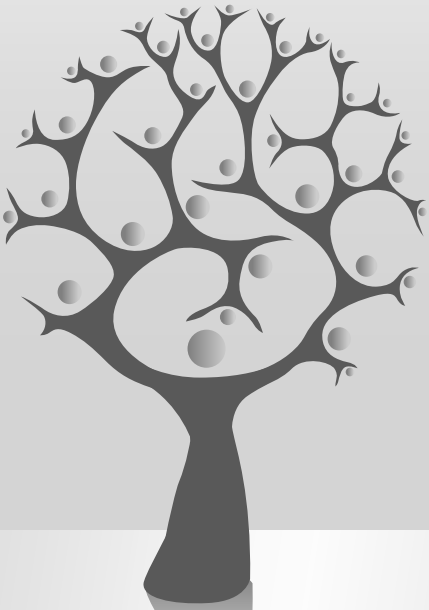
Chapter 4

Analysis of diffusion tensor imaging in patients with schizophrenia and their non-psychotic siblings



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Abstract

Background: Structural brain abnormalities have consistently been found in patients with schizophrenia. Diffusion tensor imaging (DTI) has been shown to be a useful method to measure white matter (WM) integrity in this illness, but findings of studies are inconclusive.
As having a first-degree relative with schizophrenia is the strongest known risk factor for this illness (Gottesman, 1991), differences in WM microstructure may also be present in these relatives. By examining a large group of patients with schizophrenia and their non-psychotic siblings, it is possible to investigate whether differences in WM microstructure may be related to the risk for developing schizophrenia.
Methods: From 126 patients with schizophrenia, 123 of their non-psychotic siblings and 109 healthy control subjects, DTI images were acquired with a 1.5 Tesla scanner. Mean fractional anisotropy (FA) was compared along averaged WM tracts, computed for the genu, splenium, left and right uncinate fasciculus, cingulum, inferior fronto-occipital fasciculus (IFO), fornix, arcuate fasciculus, and inferior longitudinal fasciculus (ILF).
Results: Patients with schizophrenia were not different in mean FA as compared to healthy control subjects. Siblings of patients showed higher mean FA in the left ($p=.03$) and right ($p=.02$) arcuate fasciculus as compared to patients with schizophrenia and healthy control subjects. Siblings were not different in mean FA for the other WM tracts. With increasing age, a significant stronger decline in mean FA was found in patients compared to siblings and healthy control subjects in the genu ($p=.05$), left uncinate fasciculus ($p=.05$), left inferior fronto-occipital fasciculus ($p=.01$), and left inferior longitudinal fasciculus ($p<.00$). In siblings, a significant stronger increase in mean FA with age was found in the left arcuate fasciculus ($p=.02$) as compared to patients and controls. A significant negative correlation was found between symptomatology (PANSS) and mean FA in the left and right arcuate fasciculus in patients with schizophrenia. Mean FA was not significantly different between patients using atypical versus typical medication at the time of scan.
Conclusions: This DTI study suggests that an increased familial risk for schizophrenia is associated with higher mean FA in the arcuate fasciculus bilaterally. However, overall, we found no differences in mean FA between patients with schizophrenia, their non-psychotic siblings and healthy control subjects. With increasing age, a decreased loss of WM microstructure was found in patients with schizophrenia as compared to their siblings and healthy control subjects in multiple tracts connecting the fronto-temporal cortices, particularly in the left hemisphere. This finding suggests a possible progressive loss of WM microstructure over time in schizophrenia.

Introduction

Schizophrenia is highly heritable (Gottesman et al., 1991) and structural brain abnormalities have consistently been found in patients with this illness. While standard anatomical MRI studies have revealed gray matter volume decreases in schizophrenia, particularly in the frontotemporal regions (see meta-analysis of Wright et al., 2000), nowadays diffusion tensor imaging (DTI) has shown to be useful for the examination of white matter (WM) fiber bundles connecting these regions. This MRI method measures the diffusion profile of water molecules in brain tissue. In a WM matter voxel (volume element), the diffusion profile is typically elongated, pointing in the direction of the WM fiber bundle because water diffuses more easily in the direction parallel to the fiber bundle than in the directions perpendicular to the fiber bundle. A frequently used measure that reflects the directionality (Beaulieu, 2002) of the diffusion profile is the fractional anisotropy (FA) (Basser and Pierpaoli, 1996). The FA is a scalar value ranging between 0 (no preferred direction) and 1 (one preferred direction) and is often used as an index of fiber integrity.

In schizophrenia, earlier DTI studies that mostly applied voxel-based analyses or region of interest (ROI) analyses, reported decreased WM integrity, particularly in the frontal and temporal lobe and in the fiber tracts connecting these areas (see meta-analysis: Ellison-Wright & Bullmore, 2009; review Kubicki et al., 2007 and Kanaan et al., 2005). More recently, fiber tracking techniques are used to infer fiber integrity along complete tracts, and average values of groups, such as FA can be measured along fiber tracts and can be compared between groups. A tract-based analysis approach allows detecting subtle differences in FA value that span complete tracts (Jones et al., 2008). Some studies found lower FA in brain cortical regions in patients compared to healthy control subjects (Voineskos et al., 2010; Fitzsimmon et al., 2009; Phillips et al., 2009; Kubicki et al., 2008; Nestor et al., 2008), but other studies found no differences (Chan et al., 2010; Price et al., 2008; Rosenberger et al., 2008; Szeszko et al., 2008; Mori et al., 2007; Shergill et al., 2007; Jones et al., 2006). Differences in FA in patients compared to control subjects have been associated with clinical features, such as negative and positive symptoms (Szeszko et al., 2008; Shergill et al., 2007; Hubl et al., 2004; Wolkin et al., 2003). In addition, some studies showed decreased FA with increasing age to be more pronounced in patients compared to healthy control subjects (Mandl et al. 2010; Maddah et al. 2009; Friedman et al. 2008; Rosenberger et al., 2008), but others did not (Voineskos et al, 2010).

Thus, DTI imaging has been shown to be a useful method to measure WM integrity in schizophrenia, but findings are inconclusive. The diversity of findings can be attributed to factors such as differences in sample characteristics. Differences in study methodologies have

also been suggested (Kanaan et al., 2005). In addition, the number of subjects included may be an important issue.

As having a first-degree relative with schizophrenia is the strongest known risk factor for schizophrenia (Gottesman, 1991), differences in WM microstructure may also be present in these relatives. Biological relatives of patients with schizophrenia represent a sample of individuals at risk for developing the illness, without the confounding effects of the manifestation of the illness itself. If differences in frontal WM connectivity previously found in patients with schizophrenia are present in non-psychotic relatives of patients with schizophrenia, these may be related to the (genetic) risk for developing the illness. Only few studies have been performed to examine this issue using DTI. One study measured FA in relatives of patients and found that FA was reduced in the right genu of the corpus callosum as compared to healthy control subjects (Camchong et al., 2009). Another study showed increased mean diffusivity in bilateral superior temporal regions (Narr et al., 2009). In addition, examining high-risk subjects, reduced FA in the anterior limb of internal capsules (Munoz Maniega et al., 2008) and in the bilateral cingulate and angular gyri (Hoptman et al., 2008) were found, as well as in the superior longitudinal fasciculus (Karlsgodt et al., 2008) as compared to healthy control subjects. One study has examined mean FA in a group of non-psychotic siblings measured with voxel-based analysis and found that patients with schizophrenia (N=34) and their siblings (N=34) showed reduced FA in the left prefrontal cortex and the hippocampus compared to healthy control subjects (N=32) (Hao et al., 2009).

Thus, only few studies have examined fiber integrity in first-degree relatives of patients with schizophrenia (Camchong et al., 2009; Hao et al., 2009; Narr et al., 2009; Munoz Maniega et al., 2008), but the interpretation of their findings is difficult because of the different sample characteristics (i.e. age range, duration of illness) and methods that are used.

In the present study we examined a large group of patients with schizophrenia, their non-psychotic siblings and healthy control subjects, and compared mean FA along averaged WM tracts. Average FA values were computed for the genu, splenium, left and right uncinate fasciculus, cingulum, inferior fronto-occipital fasciculus (IFO), fornix, arcuate fasciculus, and inferior longitudinal fasciculus (ILF). In addition, we examined the effect of age on mean FA between the patients, their non-psychotic siblings and healthy control subjects.

Table 1 | Demographic Information: patients with schizophrenia, non-psychotic siblings and healthy control subjects

	N or mean (sd) [range]		
	patients with schizophrenia (N=126)	**non-psychotic siblings (N=123)**	**healthy control subjects (N=109)**
age (y)	26.64 (5.58) [18-43]	26.74 (6.36) [18-49]	27.30 (8.15) [17-49]
sex (M/F)	101/25 (80.16% male)[a]	56/67 (45.53% male)	54/55 (49.54% male)
handedness (R/L))	115/11 (91.27% right)	107/16 (84.92% right)	95/14 (87.16% right)
parental education level (completed in years)	13.01 (3.53)[b]	13.33 (3.06)[b]	13.51 (3.13)[b]
subject education level (completed in years)	12.05 (2.33)[c,a]	13.19 (2.42)[c,a]	14.04 (1.97)[c,a]
WAIS IQ	92.70 (15.09)[d,a]	102.07 (15.64)[d,a]	111.69 (14.01)[d,a]
Paranoid type (%)	82 (65.1)	0	0
Schizoaffective disorder (%)	19 (15.1)	0	0
Undifferentiated type (%)	14 (11.1)	0	0
Disorganized type (%)	3 (2.4)	0	0
Schizophreniform disorder (%)	7 (5.6)	0	0
Residual type (%)	1 (0.8)	0	0
Bipolar disorder (%)	0	3 (2.44)	0
Major depression (%)	0	22 (17.89)	0
Other disorders (%)	0	5 (4.07)	0
no psychiatric disorder (%)	0	94 (76.42)	109 (100)
Duration of illness, years	4.12 (3.64) [.02-17.49]		
PANSS positive symptoms score[e]	15.46 (5.69)		
PANSS negative symptoms score[e]	15.55 (5.60)		
PANN general symptoms score[e]	32.06 (8.95)		
PANSS total symptoms score[e]	62.99 (17.55)		
SIS-R positive subscale		0.32 (0.36)[c]	0.18 (0.21)
SIS-R negative subscale		0.23 (0.22)	0.19 (0.19)
SIS-R positive subscale		4.09 (3.66)[c]	2.73 (2.50)

[a]Significantly differed from other groups, P<.05; [b]For 15 cases information was missing; [c]For 16 cases in patients, 10 in siblings and 12 in control subjects, information was missing; [d]For 11 cases in patients, 1 case in siblings and 1 in control subjects, information was missing; [e]For 5 cases information was missing.

Methods

Participants

A total of 222 families, consisting of 126 patients with schizophrenia, 123 of their related non-psychotic siblings, and 109 healthy control subjects (Ntotal=358) were included in this study (see **Table 1**). This is a subset of the sample that has already been described earlier (N=463) (Boos et al., in press). For some of the subjects participating in the first study, DTI scans were missing. In addition, for some of these subjects, we were unable to create average fiber bundles. Presence or absence of psychopathology was established by using Comprehensive Assessment of Symptoms and History interview (CASH; Andreasen et al., 1992), performed by at least one independent rater who was trained to assess this interview. Diagnosis was based on the DSM-IV criteria. Written informed consent was obtained from all subjects and the study was approved by the medical ethics committee for research in humans (METC) of the University Medical Center Utrecht. Subjects with a major medical or neurological illness were excluded.

Clinical and neuropsychological assessments

Patients met the DSM-IV criteria for schizophrenia, schizophreniform or schizoaffective disorder, as shown in **Table 1**. Patients who met DSM-IV criteria for psychotic disorder NOS, brief psychotic disorder, delusional disorder or mood disorders, anxiety disorders and substance dependence were excluded from the study. In addition, siblings were excluded if they met DSM-IV criteria for (related diagnoses of) schizophrenia or substance dependence. No healthy control subjects met the criteria for any DSM-IV axis I disorder at time of inclusion.

To evaluate severity of symptoms in patients with schizophrenia, the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987) was performed. In siblings and healthy control subjects, the Structured Interview for Schizotypy-Revised (SIS-R; Vollema and Ormel 2000; Kendler et al. 1989) was administered. The SIS-R is a semi-structured interview containing 20 schizotypal symptoms and 11 schizotypal signs, rated on a four-point scale. Scores were subdivided in positive, negative and total schizotypal features.

Image acquisition and Preprocessing

Structural magnetic resonance imaging (MRI) scans of the whole brain were obtained on a 1.5 T Achieva scanner (Philips, Best, The Netherlands). For each subject, a three-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head (256x256 matrix, TE=4.6 ms, TR=30 ms, flip angle=30 degrees, 160-180 contiguous slices; 1x1x1.2 mm^3 voxels, field-of-view=256 mm/70%) was acquired. Furthermore, a single-shot echo planar imaging (EPI)

scan was made as part of the diffusion tensor imaging (DTI)-series (SENSE factor 2.5; flip angle 90 degrees; 60 transverse slices, slice thickness 2.5 mm; no gap; 128x96 acquisition matrix; FOV 240 mm; TE=78 ms). For WM fiber tract reconstruction and computation of the FA value, 2 transverse DTI scans were acquired (32 diffusion-weighted volumes with different non-collinear diffusion directions with b-factor=1000 s/mm^2 and 8 diffusion-unweighted volumes with b-factor=0 s/mm^2; parallel imaging SENSE factor=2.5; flip angle=90 degrees; 60 slices of 2.5 mm; no slice gap; 96 x 96 acquisition matrix; reconstruction matrix 128x128; FOV=240 mm; TE=88 ms; TR=9822 ms; no cardiac gating; and total scan duration=296 s).

Image Processing

The 2 DTI scans were simultaneously realigned and corrected for possible gradient-induced distortions (Andersson and Skare, 2002). A robust estimation of the diffusion tensors was obtained using M-estimators (Chang et al., 2005) to limit the influence of possible outliers. From the diffusion tensors the FA was computed. For the T1-weighted scan, the rigid transformation was determined that spatially aligned it with the diffusion-unweighted (b=0 s/mm^2) volume of the DTI scan, using mutual information (Maes et al., 1997) as similarity metric. For each subject, a nonlinear transformation was computed using the ANIMAL software package (Collins et al., 1995) that spatially aligns the subject's T1-weighted scan with a study-specific T1-weighted model brain (Boos et al., in press). This nonlinear transformation was used at a later stage to warp the reconstructed tracts into the model space.

Reconstruction of the average fibers

Reconstruction of the average fiber tracts and the computation of the mean FA values was done in a similar way as described earlier in Mandl et al. (2010). In short, first all possible fibers in the brain were reconstructed in native space using the FACT algorithm (Fiber Assignment by Continuous Tracking; Mori and van Zijl, 2002), with 8 seed points per voxel, an FA threshold of 0.15 and maximal angle of 60 degrees. Per subject, tracts were superimposed with FA after which the tracts were warped into the study-specific model space using the concatination of the rigid "b0 to T1-weighted" transformation and the nonlinear "T1-weighted to model space" transformation. In model space a multiple region of interest (ROI) fiber bundle selection approach (Wakana et al., 2004) was used to select the fiber tracts of interest, namely the genu, the left and right uncinate fasciculus, the left and right cingulum, the left and right inferior fronto-occipital fasciculus (IFO), the left and right fornix, the left and right arcuate fasciculus, the left and right inferior longitudinal fasciculus (ILF), and the splenium. The regions of interest (ROIs) were manually defined in model space to specify the particular tracts according to (Mandl et al., 2008; Mori et al., 2002;). Because tracts were selected in model space, this was

done only once for each tract (Brouwer et al., 2010, or see http:// www.loni.ucla.edu/~narr/protocols.php). No assessment of consistency was done. Per individual we created average fiber bundles as described in Mandl et al. (2008) and Gerig et al. (2004). In short, the middle points of all fibers in the bundle were determined and the geometric center of these points served as a starting point for the average fiber. The nth coordinate of the average fiber was subsequently computed as the spatial average of the points on the fibers in the bundle at distance 2n millimeter from the starting point. This initial average fiber was smoothed and resampled to its original resolution. This procedure provided us with individual averages, which could be averaged again, providing the parts of the tract that were common to all subjects (see **figure 1**). For these parts the mean FA was computed yielding one mean FA value per tract per subject.

Statistical Analysis

Demographic and diagnostic data

Data were examined for outliers. To assess whether the groups differed on demographic variables, univariate analyses of variance were conducted for non-categorical variables and χ^2 tests for categorical variables.

SPSS 15.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA) was employed for analyses of demographic data.

Main effects of FA and interaction with age

The present study aimed to examine a large group of families and variables. For effects in FA, mixed model analysis was implemented using Structural Equation Modeling (SEM) with Mx software for Windows (Department of Psychiatry, Virginia Commonwealth University Richmond, Virginia). To test for effects between patients with schizophrenia and healthy control subjects, a full model was set up in which mean FA was regressed on sex, age, handedness, illness status (patients versus control subjects), and the interaction of ageXillness status. To test for sibling effects, mean FA was regressed on sex, age, handedness, illness status (patients versus siblings and control subjects), group status (patients and siblings versus control subjects), the interaction of ageXillness status, and ageXgroup status.

A specific effect (i.e. age) was tested by comparing the -2 log-likelihoods of two nested models: a model that does allow for that effect on WM microstructure measures (the full model), and a model that does not allow for such an effect. The difference in -2 log-likelihood between these models is χ^2 distributed. A χ^2>3.84 (1 d.f.) indicates a significant difference at α=0.05,

and depicts that the discarded effect (i.e., age effect) cannot be left out of the model without seriously reducing the goodness of fit.
Relatedness in the patient-sibling pairs and control pairs was accounted for in the covariance structure by allowing dependencies between the residuals in the regression analyses.

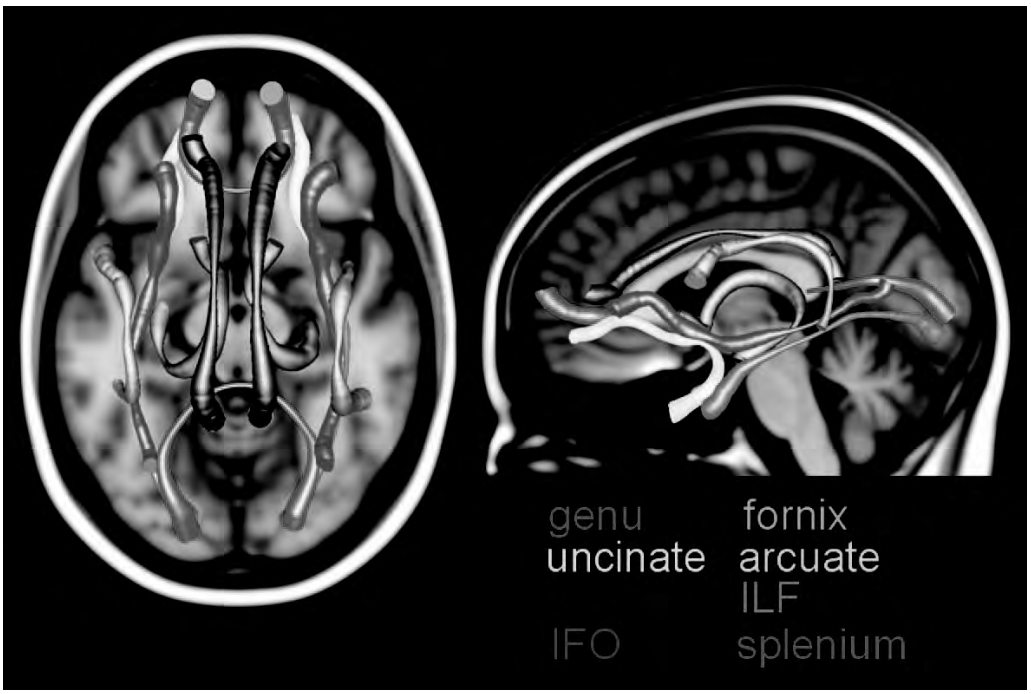


Figure 1 | By Mandl, 2010: Using tract-based analyses, mean FA was compared along averaged white matter tracts, computed for the genu (frontal tract in red), uncinate fasciculus (in yellow), cingulum (in brown), inferior fronto-occipital fasciculus (in dark blue), fornix (in green), arcuate fasciculus (in light blue), inferior longitudinal fasciculus (in pink), and splenium (posterior end of the corpus callosum: in red).

Post-hoc analyses

To address whether in patients possible differences in WM integrity depend on severity of illness, correlations with PANSS positive symptoms scores, PANSS negative symptoms scores, PANSS general scores and PANSS total scores were performed. For five patients PANSS scores were missing. These were excluded from the analysis.

Likewise, for the combined sample of siblings and control subjects, correlations were calculated to detect a possible association between positive or negative schizotypal features (as measured on the SIS-R), and WM integrity.
Correlations were calculated to analyze the effect of type of medication (atypical versus typical antipsychotic medication) and duration of illness on mean FA in patients with schizophrenia.

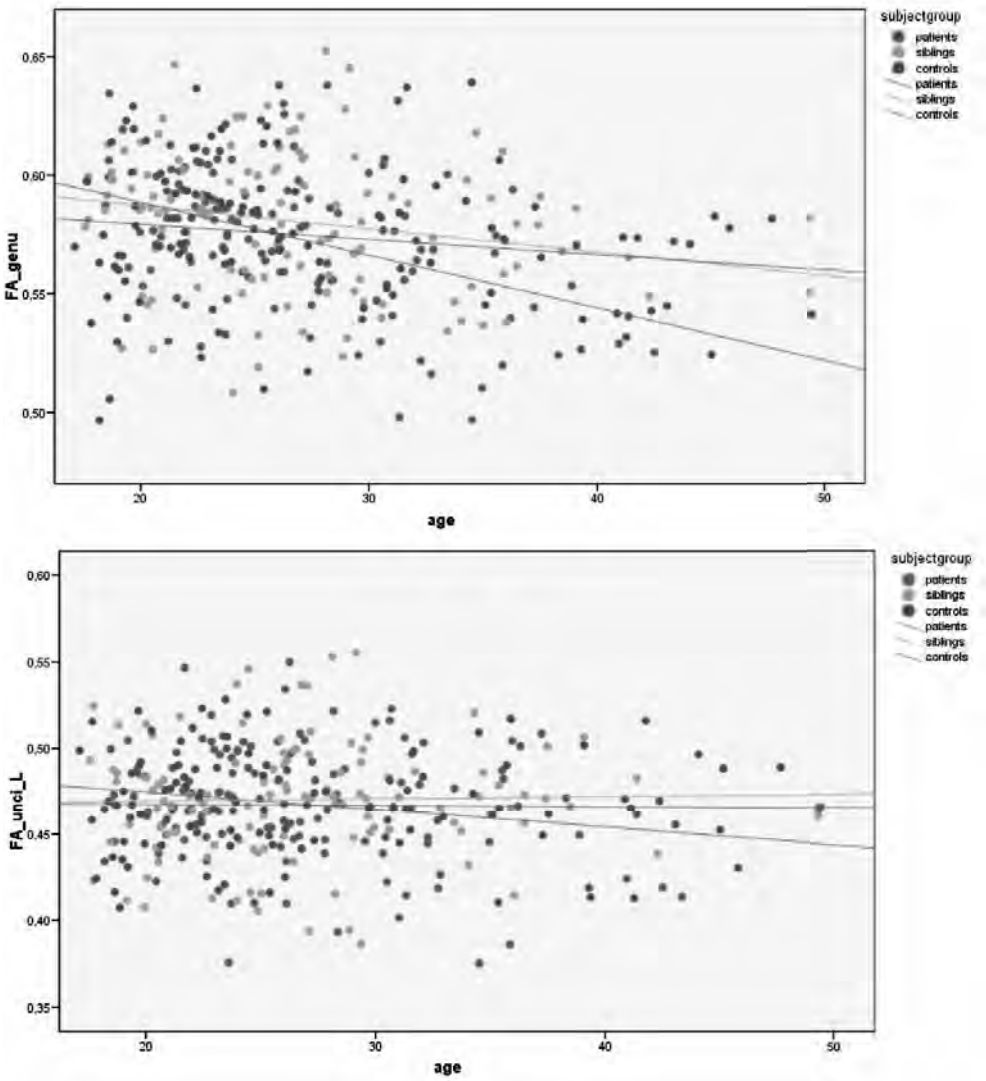
Results

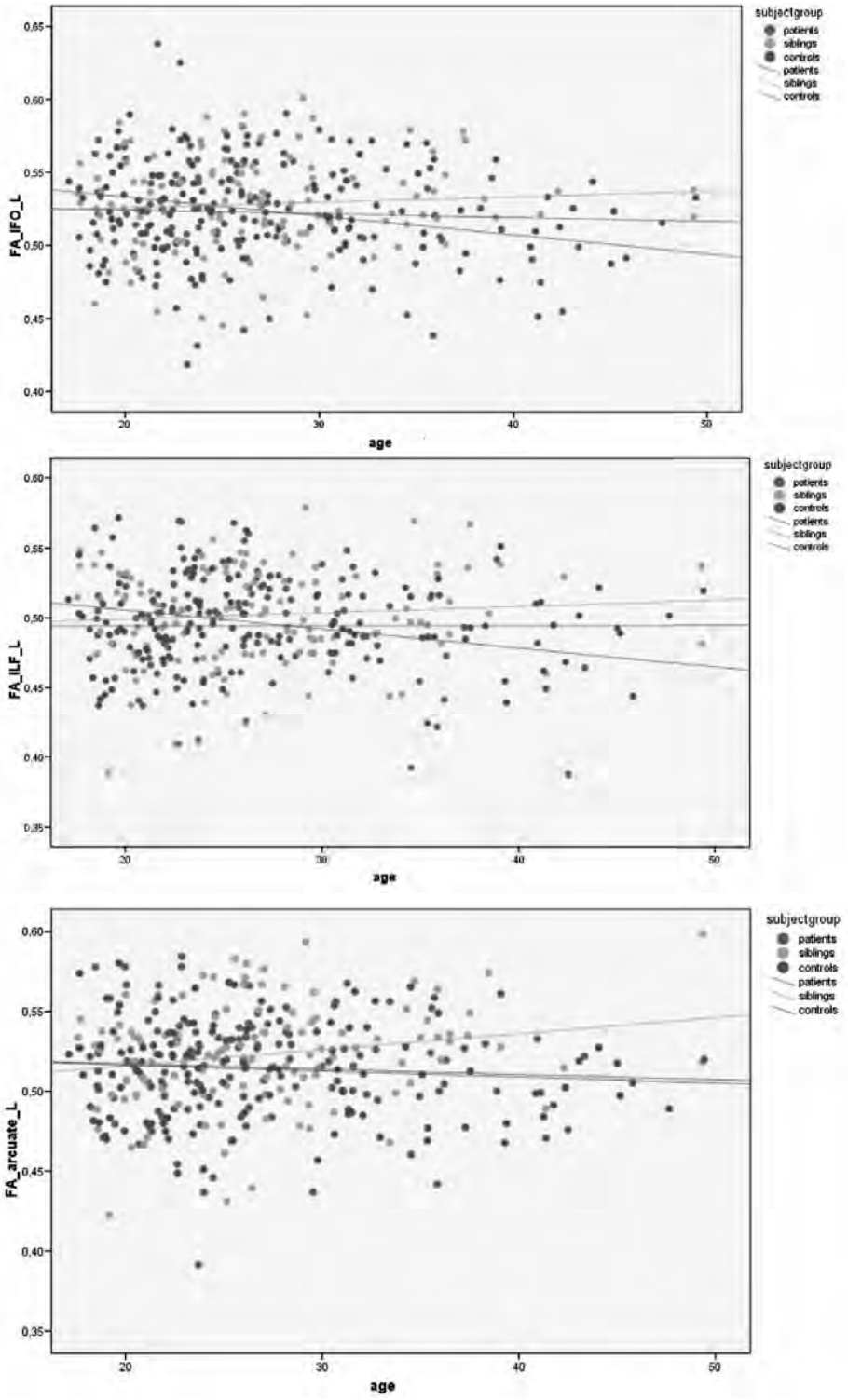
For demographic analyses, see **Table 1**. No differences between groups were found for age (patients with schizophrenia: 26.64 years (SD=5.58); siblings: mean age=26.74 years (SD=6.36) and healthy control subjects: 27.30 years (SD=8.15)), parental educational level (defined as the total number of years of education) and handedness. Groups differed significantly in sex distribution; male and female subjects being equally divided within the siblings (45.53% male) and control subjects (49.54% male) but not in the patient group (80.16% male). Groups differed significantly in WAIS IQ (patients: mean IQ=92.70 (SD=15.09); siblings: mean IQ=102.07 (SD=15.36); healthy control subjects: mean IQ=111.69 (SD=14.01)). The majority of patients (79.4%) were taking atypical antipsychotic medication at time of scan, with olanzapine and risperdone being most often prescribed (N=43 and N=21 respectively) (7.1% was taking typical antipsychotic medication and for 13.5% information on medication was missing). In patients, mean duration of illness was 4.12 years (SD=3.64).

Main effects of FA and interaction with age

Mean FA in average WM tracts for patients with schizophrenia, their non-psychotic siblings and healthy control subjects are shown in **Table 2**. After controlling for age, sex and handedness, mean FA was not significantly different between patients with schizophrenia and healthy control subjects. In siblings, mean FA was higher in the left and right arcuate fasciculus as compared to patients with schizophrenia and healthy control subjects. No differences were found for the other WM tracts between these groups. As shown in **Table 3**, there was no significant effect of age for mean FA in the average WM tracts. Comparing patients and control subjects, a significant ageXillness interaction was found for the genu (χ^2=8.486, p=.004), and the ILF (χ^2=4.693, p=.03). Including non-psychotic siblings in our analysis, a significant ageXillness interaction was found for mean FA in the genu (χ^2=3.82, p=.05), left uncinate fasciculus (χ^2=3.89, p=.05), left inferior fronto-occipital fasciculus (χ^2 =7.51, p=.01), and left inferior longitudinal fasciculus (χ^2 =8.28, p<.05), showing a more pronounced decrease in mean FA with age in these

Figure 2 | Relation between mean FA (on Y-axis) and age (on X-axis) in the genu, left uncinate fasciculus, left inferior fronto-occipital fasciculus, left inferior longitudinal fasciculus, and left arcuate fasciculus, for patients (in red), non-psychotic siblings (in orange) and healthy control subjects (in blue).

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WM tracts in patients compared to siblings and healthy control subjects (see **figures 2**). Such ageXillness interaction was not found in the other tracts. A significant ageXgroup interaction (χ^2=5.70, *p*<.02) was found in the left arcuate fasciculus, due to a stronger increase of mean FA with age in siblings as compared to patients and healthy control subjects (see also **figures 2**: last figure for left arcuate fasciculus).

Results of post-hoc analyses

A significant negative correlation was found between PANSS positive (left; right; p=.03; p=.03), PANSS negative (as shown in figure 3: p=.03; p=.01), PANSS general (p=.01; p=.01), PANSS total symptom score (p=.01; p=.01) and mean FA of left and right arcuate fasciculus in patients with schizophrenia. Patients with higher scores on the PANSS showed lower mean FA in the (left and right) arcuate fasciculus, but no such association was found for the other WM tracts.

In siblings and healthy control subjects, SIS-R total, positive or negative scores were in none of the cases correlated with mean FA.

No significant correlations were found between type of medication (atypical or typical antipsychotic medication) and mean FA. Most patients were using atypical antipsychotic medication (79.4%), but excluding those who were on typical medication or of whom we did not have information, did not alter our results.

Post-hoc analyses were performed to examine the effect of duration of illness, which showed no effect on mean FA.

Significant effects were found for handedness in the left and right fornix (χ^2=4.38, p=.04 and χ^2=5.39, p=.02), mean FA being significantly higher in right handed subjects, compared to left handed subjects. In addition, for the right ILF, a significant effect was found for sex (χ^2=6.31, p=.01), males showing higher mean FA compared to females for this particular tract.

As the sex distribution was significantly different between groups (patients versus siblings and healthy control subjects), we have performed post-hoc analyses to compare mean FA between groups only in male subjects. This analysis revealed no significant differences between groups in mean FA for all WM tracts.

Table 2 | Uncorrected mean fractional anisotropy (FA) (SD) for average WM tracts in patients with schizophrenia, their non-psychotic siblings and healthy control subjects

WM Tracts	patients	siblings	controls
genu	0.574 (0.030)	0.581 (0.029)	0.574 (0.029)
uncinate fasciculus, left	0.468 (0.033)	0.470 (0.034)	0.467 (0.029)
uncinate fasciculus, right	0.451 (0.029)	0.452 (0.031)	0.451 (0.028)
cingulum, left	0.525 (0.045)	0.528 (0.051)	0.518 (0.049)
cingulum, right	0.486 (0.052)	0.486 (0.056)	0.484 (0.055)
IFO, left	0.525 (0.037)	0.528 (0.032)	0.522 (0.029)
IFO, right	0.511 (0.032)	0.511 (0.030)	0.507 (0.034)
fornix, left	0.373 (0.035)	0.376 (0.032)	0.377 (0.030)
fornix, right	0.404 (0.053)	0.402 (0.046)	0.394 (0.048)
arcuate, left	0.515 (0.033)	0.523 (0.032)	0.514 (0.031)
arcuate, right	0.507 (0.032)	0.511 (0.031)	0.499 (0.034)
ILF, left	0.497 (0.035)	0.502 (0.033)	0.494 (0.031)
ILF, right	0.496 (0.033)	0.499 (0.033)	0.491 (0.028)
splenium	0.610 (0.037)	0.621 (0.037)	0.619 (0.329)

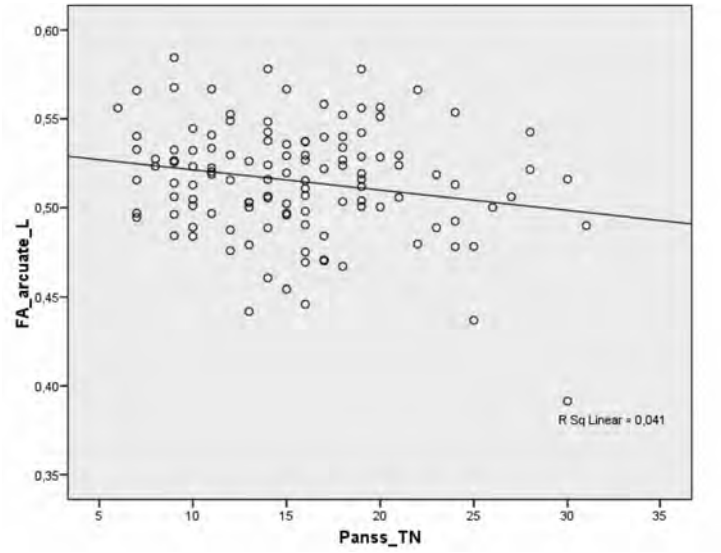

Figure 3 | Relation between mean FA (on Y-axis) of the left arcuate fasciculus and PANSS negative symptoms score (on X-axis) in patients with schizophrenia.

Table 3 | Main effect in mean FA and interaction with age (χ^2 (p))

WM fiber tract	Main effect mean FA			Interaction with age	
	patients compared to siblings and control subjects	patients and siblings compared to control subjects	Effect of age	patients compared to siblings and control subjects	patients and siblings compared to control subjects
genu	3.693 (0.055)	1.652 (0.199)	3.534 (0.060)	3.819 (0.051)*[a]↓	0.747 (0.387) ↑
uncinate fasciculus, left	0.340 (0.560)	0.125 (0.724)	0.110 (0.741)	3.886 (0.049)*[a]↓	0.211 (0.646) ↑
uncinate fasciculus, right	0.173 (0.677)	0.001 (0.971)	0.272 (0.602)	0.399 (0.527) ↓	0.284 (0.594) ↑
cingulum, left	0.308 (0.579)	2.541 (0.111)	0.048 (0.826)	1.947 (0.163) ↓	1.291 (0.256) ↑
cingulum, right	0.001 (0.982)	0.058 (0.809)	1.165 (0.281)	2.590 (0.108) ↓	0.423 (0.515) ↑
IFO, left	0.275 (0.600)	1.639 (0.200)	1.135 (0.287)	7.513 (0.006)*[a] ↓	2.147 (0.143) ↑
IFO, right	0.047 (0.828)	0.060 (0.806)	0.216 (0.642)	1.347 (0.246) ↓	0.003 (0.955) ↑
fornix, left	0.122 (0.727)	0.063 (0.802)	0.253 (0.615)	3.502 (0.061) ↓	1.524 (0.217) ↓
fornix, right	0.004 (0.948)	1.360 (0.243)	0.618 (0.432)	0.635 (0.425) ↓	0.436 (0.509) ↑
arcuate, left	3.651 (0.056)	5.112 (0.024)*[b]	0.981 (0.322)	4.364 (0.037)*[a] ↓	5.696 (0.017)*[c] ↑
arcuate, right	1.600 (0.206)	7.126 (0.008)*[b]	0.449 (0.503)	0.558 (0.455) ↓	0.020 (0.887) ↑
ILF, left	1.121 (0.290)	3.079 (0.079)	0.019 (0.892)	8.277 (0.004)*[a] ↓	1.385 (0.239) ↑
ILF, right	0.007 (0.931)	2.561 (0.110)	1.068 (0.301)	1.888 (0.169) ↓	1.148 (0.284) ↑
splenium	3.466 (0.063)	0.195 (0.659)	0.151 (0.698)	1.519 (0.218) ↓	1.018 (0.313) ↑

↓ refers to a decrease in mean FA; ↑ refers to an increase in mean FA; *A χ^2>3.84 (1 d.f.) indicates a significant difference at α=.05; [a]Decrease in FA is stronger in patients compared to siblings and control subjects; [b]Mean FA is higher in siblings compared to patients and control subjects; [c]Increase in FA in siblings compared to patients and control subjects.

Discussion

The present study examined WM integrity in a large sample of patients with schizophrenia, their related non-psychotic siblings and healthy control subjects using DTI. We found that patients (on average 27 years) did not differ from healthy control subjects in mean FA. Their non-psychotic siblings displayed a higher mean FA in the left and right arcuate fasciculus as compared to patients and healthy control subjects, but they were not different in mean FA for the other WM tracts. We suggest that an increased familial risk for schizophrenia was associated with mean FA in the arcuate fasciculus bilaterally, which seemed only to be expressed in non-psychotic siblings of patients with schizophrenia.

Using a voxel-based method, the only earlier study that examined non-psychotic siblings found reduced FA in siblings compared to control subjects in the left prefrontal cortex and hippocampus (Hao et al., 2009), but except for the anterior cingulate they had not examined other tracts such as the arcuate fasciculus. They found no differences between the non-psychotic siblings and patients with schizophrenia.

The arcuate fasciculus was previously thought to connect Wernicke's area and Broca's area, both connected to speech and language, but recent studies (Bernal and Ardila, 2009) suggest that the arcuate fasciculus instead connects Wernicke's to premotor/motor areas. As mentioned before, we found no difference between mean FA in patients with schizophrenia and healthy control subjects in the arcuate fasciculus. However, a significant negative correlation was found between symptomatology and left and right arcuate fasciculus in patients with schizophrenia. In addition, we found a significant stronger decline in mean FA with increasing age in patients as compared to siblings and healthy control subjects for multiple tracts connecting the fronto-temporal cortices, particularly in the left hemisphere. This finding suggests a progressive loss of WM microstructure over time in schizophrenia.

The significant interaction with age that we found in patients with schizophrenia in the left uncinate fasciculus supports recent findings (Mandl et al., 2010; Rosenberger et al., 2008). A study of Friedman et al. (2008) compared a group of first-episode patients with those who were chronically ill. They found less changes in WM integrity in patients at illness onset (N=40) compared to those observed in the more chronic stages of the illness (N=40), suggesting a progressive loss of WM integrity subsequent to illness onset.

That we found no differences between patients with schizophrenia and healthy control subjects using tract-based analyses is consistent with earlier DTI studies using this method (Chan et al., 2010; Price et al., 2008; Rosenberger et al., 2008; Szeszko et al., 2008; Mori et al., 2007; Shergill et al., 2007; Jones et al. 2006). However, some studies found lower FA in patients

compared with control subjects (Voineskos et al., 2010; Fitzsimmon et al., 2009; Phillips et al., 2009; Kubicki et al., 2008; Nestor et al., 2008). DTI studies using VBM methods found reductions in mean FA, particularly in the fronto-temporal tracts in patients compared to healthy control subjects (see meta-analysis Ellison-Wright and Bullmore, 2009; review Kubicki et al., 2007 and Kanaan et al., 2005).

Nevertheless, overall findings from DTI studies in patients with schizophrenia measuring mean FA are inconsistent due to differences in methodologies and sample characteristics.

The finding of a more pronounced decrease in mean FA in older patients with schizophrenia in the fronto-temporal WM tracts indicates that, although subtle and not easily detectable, even in large samples such as ours, indeed the frontal and temporal lobes seem mostly related to schizophrenia. Decreased FA over time may be due to smaller diameters of axons, fewer axons, or disruptions in myelin sheaths but the exact cause of decreased WM integrity remains unclear (Rosenberger et al., 2008). Medication effects are an important factor that should be taken into account when interpreting the results. We found no difference between use of atypical antipsychotic medication or typical medication but information of cumulative medication was not available for analysis and could therefore not be taken into account.

The present study employed a cross-sectional design. For future research it would be of great interest to examine patterns of FA across time. Longitudinal studies of large samples including first-degree relatives may be informative on whether DTI findings are indeed progressive and related to illness onset or outcome.

In conclusion, in this large tract-based diffusion tensor imaging study we found that mean FA was higher in the arcuate fasciculus for siblings compared to patients and healthy control subjects, but we found no differences in mean FA in other WM tracts between these groups. That we found a significant decrease in mean FA with increasing age in patients compared to siblings and control subjects, suggests a possible progressive loss of WM microstructure over time in schizophrenia.

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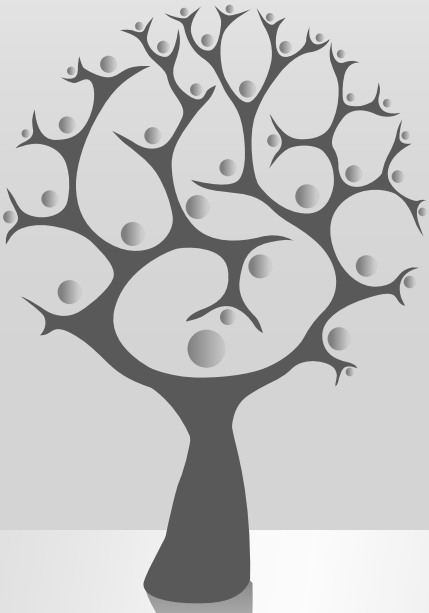
Chapter 5

Global and focal structural brain measurements in parents of patients with schizophrenia



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Submitted for publication

Abstract

Background: Neuroimaging studies have consistently shown structural brain abnormalities in patients with schizophrenia. There is evidence that these brain abnormalities are also present in the healthy relatives of patients with this illness, albeit to a lesser extent. However, only a few studies examined brain structures in the parents of patients with schizophrenia. The present study was designed to examine brain structures in healthy parents of patients with schizophrenia as compared to control couples and its relationship with cognitive function.

Methods: Whole brain Magnetic Resonance Imaging (MRI) scans were obtained from both parents of 31 patients with schizophrenia and 28 pairs of control couples. Volumes of intracranium (IC), total brain (TB), gray matter (GM), white matter (WM), lateral and third ventricles, and cerebellum were obtained. In addition, voxel-based morphometry (VBM) analyses were applied. The association between brain measures and cognitive measures of psychomotor function and verbal memory were investigated.

Results: Parents of patients with schizophrenia displayed smaller total brain volumes as compared to healthy control couples. No focal differences were found in brain volume or density between parents of patients with schizophrenia and control couples. In addition, an association between smaller total brain volume and slower psychomotor functioning was found in the total sample.

Conclusions: The finding that parents of patients with schizophrenia show a subtle decrease in total brain volume mirrors the decrease of brain volume that has consistently been reported in patients with schizophrenia. This suggests that brain tissue loss in schizophrenia is (in part) associated with the genetic risk to develop schizophrenia.

Introduction

Schizophrenia is associated with decreased global and focal gray matter (GM) volumes that appear to be most pronounced in the frontal and temporal areas (see meta-analyses Honea et al., 2005; Wright et al., 2000). There is evidence that non-psychotic first-degree relatives of patients with schizophrenia also show GM deficits, but to a lesser extent (Boos et al., 2007). These structural findings are in line with the observation that cognitive deficits, such as impairments in memory and psychomotor function that have been found in patients with schizophrenia, are also present in unaffected first-degree relatives of patients with this illness (Chen et al., 2009; Appels et al., 2004; 2003; Sitskoorn et al., 2004; Toulopoulou et al., 2003). Nevertheless, it remains unclear whether these cognitive deficits are related to the structural brain abnormalities in first-degree relatives.

The age of risk of developing schizophrenia is generally estimated to be between 15-35 years. The parents included in this study already passed this age of risk and are therefore unlikely to develop the illness themselves. Examining brain structures and their functional relevance in parents of patients with schizophrenia thus provides an advantage over the examination of other first-degree relatives (i.e. offspring or siblings).

To our knowledge, only two neuroimaging studies have examined brain structures in parents of patients with schizophrenia. A volumetric study including 9 parents of patients with schizophrenia showed lateral ventricle enlargement compared to other relatives and healthy individual control subjects (Sharma et al., 1998). The second study found reduced GM density in the thalamus, insula, and right temporal and occipital lobe (measured with voxel-based morphometry (VBM)) in 20 parents of patients with schizophrenia compared to individual control subjects (Lui et al., 2009). This study found more brain abnormalities in the parents with high familial loading. These findings suggest that there might be subtle brain abnormalities in healthy parents of patients with schizophrenia. However, these studies examined small numbers of (single) parents.

In the present MRI study, global and focal brain morphology of parent couples of patients with schizophrenia was compared to those of healthy control couples, using both a volumetric method and voxel-based morphometry. In addition, we assessed the association between cognitive functioning and brain volumes. We included both parents of patients with schizophrenia to assure that the obligate carrier (i.e. the parent who is expected to carry the schizophrenia genotype) was included in the study. Furthermore, by comparing the parents of patients with healthy control couples (i.e. both parents who do not have a psychiatric illness themselves, or a child suffering from a psychiatric illness), we were able to control for assortative

mating. Assortative mating denotes a tendency for mated pares to be more similar for some phenotypic traits than would be the case if the choice of a partner occurred at random.

Methods

Participants

Both parents of 31 patients with schizophrenia (N=62) were recruited at the University Medical Center Utrecht, as well as 28 healthy control couples (N=56). This sample has been described in previous studies (Appels et al., 2004; 2003; 2002). The Comprehensive Assessment of Symptoms and History (CASH), the Schizophrenia and Affective Disorder Schedule-Lifetime interview (SADS-L), the Structured Interview for DSM-IV personality disorders (SIDP-IV), and the Family Interview for Genetic studies (FIGS) were obtained from all participants by at least one independent psychiatrist or psychologist who was trained to assess the interviews. Diagnostic consensus was achieved in the presence of an independent psychiatrist. Psychiatric diagnosis was established according to DSM-IV criteria. At least one of the children of the parents met DSM-IV criteria for schizophrenia on the basis of the CASH. Parents of patients were excluded if they had a history of psychotic illness. For control couples, exclusion followed in case of any axis-I DSM-IV diagnosis, or diagnosis of depression, manic depression, or psychotic disorder in first-degree family, or psychotic disorder in second-degree family. In both groups all participants were physically healthy and had no history of neurological illness, or drug or alcohol abuse. As described previously in more detail (Appels et al., 2002), a comprehensive neuropsychological test battery was administered to each couple by trained psychologists. Verbal memory and psychomotor functioning were found to be impaired in parents of patients with schizophrenia. Therefore, these two cognitive domains were also examined in our study. Our sample largely overlaps with the sample of Appels et al. (2002). Verbal memory was measured using the CVLT and variables that were used for further analyses were scores on free recall short term and free recall long term. Psychomotor functioning was measured using the Finger Tapping test and Purdue Pegboard test and variables that were used for further analyses were the preferred and non-preferred hand scores. Current IQ was estimated using the Groningen Intelligence Test (GIT).

MRI acquisition and processing

Magnetic resonance imaging was performed on a 1.5 T Philips NT scanner (Philips Medical Systems, Best, The Netherlands). From all subjects T1- weighted 3D fast field echo (3D-FFE)

scans with 160-180 1.2 mm contiguous slices (TE= 4.6 ms, TR=30 ms, flip angle=30˚, FOV=256x256 mm², in-plane voxel sizes 1x1 mm²), and T2-weighted dual echo turbo spin echo (DE-TSE) scans with 120 1.6 mm contiguous coronal slices (TE1=14 ms, TE2=80 ms, TR=6350 ms, flip angle=90˚, FOV=256x256 mm², in-plane voxel sizes 1x1 mm²) of the whole head were obtained and used for quantitative measurements. The scans were coded to ensure blindness for subject identification. The T1-weighted images were put into Talairach orientation (Talairach and Tournoux, 1988) without scaling, and corrected for inhomogeneities in the magnetic field (Sled et al., 1998). Volume measures of the intracranium, total brain, GM and white matter (WM), cerebellum, third and lateral ventricles were determined. Quantitative assessment of the intracranial volume was performed with use of a full-automated computer program based on histogram analyses followed by mathematical morphology operators in the DE-TSE image. Quantitative assessment of the total brain, gray and white matter cerebrum volumes, cerebellar volume, and lateral and third ventricular volumes were performed based on histogram analyses followed by mathematical morphology operators in the 3D-FFE image, using the intracranial volume as mask (Schnack et al., 2001a; 2001b). In addition, for the cerebellum and lateral and third ventricular volumes, anatomical knowledge-based selection principles were used. For the cerebellum, this included a plane perpendicular to the sagittal plane through the aquaduct. For the third ventricle, this included the coronal slices through the anterior commissure as anterior border, the coronal slice through the posterior commissure as posterior border, and a manually outlined roof to prevent leaks into the transverse cistern, drawn in the midsagittal reconstructed slice from a point superior to the thalamus and just inferior to the plexus choroideus. For the lateral ventricles, this included automated computer-incorporated anatomical knowledge of the anatomical location of the lateral ventricles in the brain (e.g., they are surrounded by WM). All segmentations were checked after measurements and corrected manually if necessary.

Voxel based morphometry

Regional measures of GM and WM concentration ("density") were generated using VBM in a similar manner as was done previously (Hulshoff Pol et al., 2001). The binary GM masks were analyzed using voxel-based morphometry. The binary GM masks were resampled to a voxel size of 2x2x2.4 mm^3, blurred using an isotropic Gaussian kernel (full width at half maximum of 8 mm) to generate GM "density maps". The density maps represent the local concentration of GM (between 0 and 1) per voxel. Each of the scans were transformed into a standardized coordinate system in a 2-stage process using the ANIMAL algorithm (Collins et al., 1996). In the first step, a linear transformation was found by minimizing a mutual information joint entropy objective function computed on the gray level images (Maes et al., 1997). A nonlinear

transformation was computed in the second step by maximizing the correlation of the subject's image with that of a standardized brain. The nonlinear transformation is run up to a scale (full width at half maximum of 4 mm) that aligns global anatomical regions while minimally affecting local volume changes. The standardized brain was selected earlier among 200 brain scans of healthy subjects between the ages of 16 and 70 years. To select the standardized brain, all 200 brain scans were registered to the Montreal standard brain (Cosoco et al., 1997) and averaged, yielding one average brain image. The mean square error on the normalized intensity values was computed between each of the brain scans and the average brain image. The standardized brain was the brain image with the smallest mean square error. Transformations were then applied to the GM density maps to remove global differences in the size and shape of individual brains.

Statistical analyses

Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) for Windows. Data were examined for outliers, extreme values and normality of the distribution.
To assess whether parents of patients with schizophrenia differed from healthy control couples in age, IQ and structural brain volumes, analyses of variance (ANOVA) were conducted. For volumetric analyses, volume of total brain, gray (GM) or white (WM) matter of the cerebrum, lateral or third ventricles or cerebellum was entered as dependent variable. Group (parents of patients with schizophrenia versus control couples) was entered as independent variable. Intracranial volume (IC), gender and age were added as covariates.

To examine focal differences in brain structures between parents of patients with schizophrenia and healthy control couples, linear regression analysis was performed for each voxel separately in the density maps, through all brains. Predictor variables were group, gender, age, and handedness. A correction for multiple comparisons was carried out according to the false discovery rate (FDR).

In case of significant differences between the groups on brain measures, correlations were performed to assess whether cognitive performance was associated with structural brain measures. For this purpose, brain volumes were corrected for age, gender and IC using linear regression analysis and unstandardized residuals were saved. Next, the corrected brain volumes were correlated with the performance on the cognitive tasks (i.e. CVLT, Finger Tapping Test, Purdue Pegboard Test, and IQ), using Spearman Rank correlations. To compare these correlations between groups, Fisher's r-to-z transformations were used.

A post-hoc analysis was performed to investigate whether parents of patients with more than one affected first- or second-degree relative (N=27) have more structural brain abnormalities compared to parents of patients with only one affected relative, i.e. their child (N=35).

Results

Groups did not differ in age, handedness and IQ (for demographic information: see **Table 1**). Seventeen individual parents of patients received current DSM-IV diagnoses (major depression, N=14; posttraumatic stress disorder, N=1; gambling addiction, N=1; dysthymic disorder, N=1). None of the control persons had a DSM-IV axis I diagnosis.

Parents of patients with schizophrenia had significantly smaller total brain volumes (1.5%, F=4.2; df=1; p=0.04) as compared to control pairs. No volumetric differences were found between the groups in cerebral GM and WM, lateral ventricles, third ventricle and cerebellum.

Corrected for age, gender and handedness, parents of patients with schizophrenia did not differ from healthy control pairs in GM and WM density.

A positive correlation was found between psychomotor function (Finger Tapping test) and total brain volume (preferred hand score: r=.26; p=.01, non-preferred hand score: r=.29; p<.00). No difference was found between the groups in the correlations between psychomotor function and total brain volume. Moreover, no significant correlations were found between verbal memory and IQ and between verbal memory and total brain volume in the total sample, nor in the individual groups.

Finally, there were no differences in age, gender, handedness and IQ in parents of patients with more than one relative with schizophrenia (N=27) compared to parents with one ill child. Parents with more than one relative with schizophrenia did not differ on brain volumes and GM density as compared to those parents with one child with the illness.

Conclusions

This structural MRI study was designed to examine brain structures in both parents of patients with schizophrenia as compared to control couples, using a volumetric and voxel-based morphometry approach. Parents of patients with schizophrenia showed smaller total brain volumes (-1.5%) as compared to the healthy control couples, but no differences were found in volumes of cerebral GM and WM, cerebellum and ventricles or in GM density. A significant correlation was found between total brain volume and psychomotor functioning as measured with the Finger Tapping test in the total sample. A larger brain volume was associated with faster performance on the motor task.

Subtle brain abnormalities in parents of patients with schizophrenia have been found previously. Earlier MRI studies found increased lateral ventricle volume (Sharma et al. 1998) (N=9) and reduced GM density in the right insula, extending to the right temporal and parietal

lobe (Lui et al., 2009) in parents of patients (N=20) compared to healthy control subjects. This was only found in parents who had more than one relative suffering from schizophrenia compared to the other parents and controls subjects. This finding could not be confirmed in our study. A major strength of our study in contrast to the earlier studies examining parents of patients with schizophrenia is that we included both parents in comparison with healthy control couples. In this design we were able to include the obligate carrier and to control for assortative mating.

Table 1 | Demographics, brain volumes, and cognitive task performance (mean (SD)) in parents of patients with schizophrenia and healty control couples

Mean (sd)	**parents of patients (N=62)**	**control parents (N=56)**
age (y)	53.4 (4.0)	53.8 (4.6)
handedness (percentage right)	83,9%	94,6%
IQ	117.0 (14.2)	118.9 (12.2)
major depression (N)	14	0
dysthymic disorder (N)	1	0
posttraumatic stress disorder (N)	1	0
gambling addiction (N)	1	0
no psychiatric disorder (N)	45	56
intracranium volume (ml)	1459.1 (125.1)	1470.1 (133.1)
total brain volume (ml)	1216.3 (114.4)	1241.2 (100.7)
lateral ventricles volume (ml)	20.8 (12.2)	19.4 (9.5)
third ventricle volume (ml)	1.2 (0.7)	1.2 (0.5)
gray matter volume (ml)	590.2 (60.1)	602.8 (53.8)
white matter volume (ml)	474.5 (59.2)	485.6 (60.4)
cerebellum volume (ml)	137.2 (11.6)	141.0 (12.1)
CVLT, free recall short term	9.86 (2.45)	9.77 (4.40)
CVLT, free recall long term	10.71 (2.56)	10.38 (4.40)
Finger Tapping test, preferred hand	44.39 (8.42)	42.56 (15.94)
Finger Tapping test, non-preferred hand	37.68 (9.84)	37.61 (13.82)
Purdue Pegboard test, preferred hand	13.32 (3.17)	13.98 (3.49)
Purdue Pegboard test, non-preferred hand	12.79 (3.12)	13.73 (3.56)

There is consistent and overwhelming evidence of decreased total brain volume in patients with schizophrenia (Steen et al., 2006; Wright et al., 2000).

Our finding of loss of whole brain volume in the parents of patients with schizophrenia is in line with findings in unaffected twins discordant for schizophrenia (Brans et al., 2008; Hulshoff Pol et al., 2004; van Haren et al., 2004; Baare et al., 2001, but not Suddath et al., 1990). Also, in healthy siblings of 52 patients with childhood-onset schizophrenia, significant GM deficits in the left prefrontal and bilateral temporal cortices and smaller deficits in the right prefrontal and inferior parietal cortices compared to healthy control subjects were found (Gogtay et al., 2007). These cortical deficits in siblings disappeared by age 20 years and the process of deficit reduction correlated with overall functioning (GAS scores) at the last scan. In contrast, however, earlier large sibling studies showed no differences in structural brain measures between adult siblings of patients and matched control subjects (Boos et al., in press; Goldman et al., 2008).

To date, the (patho)physiological processes that are responsible for brain changes in schizophrenia are still unknown. The brain volume loss that we show in parents of patients with schizophrenia may represent altered plasticity in adulthood. There is compelling evidence that the brain continues to show plasticity during adulthood, at least in some areas. Neurogenesis is known to occur in the adult human hippocampus (Eriksson et al., 1998) and olfactory bulb (Bedard and Parent, 2004). Adult neurogenesis has also been suggested in other areas such as the amygdala (Gould, 2007). In addition, it has been proposed that the progressive volume loss that is associated with the liability to develop schizophrenia might represent aberrant plasticity of adult functional neural networks (Brans et al., 2008).

The relationship between brain morphology and cognitive function in schizophrenia has been previously investigated (for review see Antonova et al., 2004). A positive relationship has been found between total brain and GM volumes and global cognitive functioning in patients with schizophrenia (Antonova et al., 2005). Deficits, particularly in verbal memory have been related to smaller hippocampal volumes in patients with schizophrenia (Toulopoulou et al., 2004; Goldberg et al., 1994), but also in their first-degree relatives (Seidman et al., 2002; O'Driscoll et al., 2001). In an earlier paper by Appels et al. (2003), the parents of patients with schizophrenia differed from control couples on those cognitive ability domains that are generally considered to be most impaired in schizophrenia patients, i.e. verbal memory and psychomotor functioning. Interestingly, we found an association between total brain volume and psychomotor function in general. In our study groups were not different in association between psychomotor slowing and smaller volume of the brain. In addition, there was no association between total brain volume and verbal memory.

That our sample is relatively small, unfortunately not only limits the power to detect differences, it also increases the risk of type I errors.

In conclusion, the present study indicates that parents of patients with schizophrenia have decreased total brain volumes as compared to healthy control subjects. In general, total brain volume was associated with psychomotor functioning. The abnormalities found in parents mirror the loss of brain tissue found in patients, albeit to a lesser extent. Our findings suggest that at least some structural brain abnormalities are associated with the genetic risk to develop schizophrenia.

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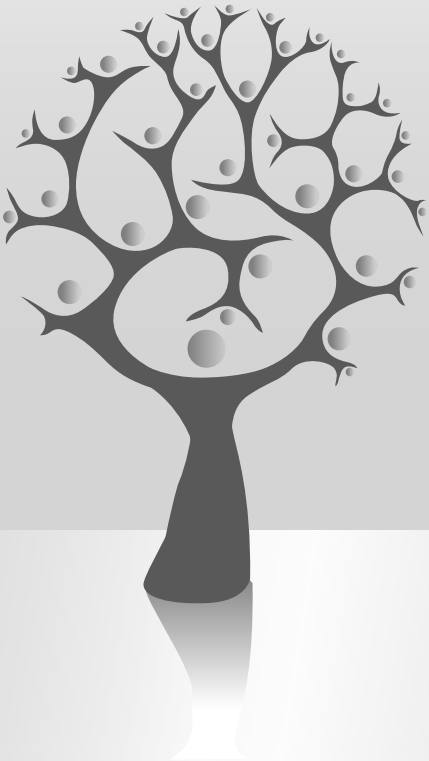
Chapter 6

Concluding words



Running in the family?

The main question in this thesis was whether the brain abnormalities consistently found in patients with schizophrenia are also present in their non-psychotic first-degree relatives. In other words: does it run in the family?

Summary and discussion

The studies conducted in this thesis explored brain structures in patients with schizophrenia, their first-degree relatives and healthy control subjects. For this purpose we set out to examine a large sample of patients with schizophrenia, their non-psychotic siblings, parents of patients, and healthy control subjects.

In this final chapter, a summary and discussion of the main findings are provided. Furthermore, possible implications and directions for future research are suggested.

In **Chapter 2** we described a meta-analysis that we performed to determine the magnitude and extent of brain volume differences in first-degree relatives of patients with schizophrenia. We therefore conducted a search in the MEDLINE database to identify relevant structural MRI studies that examined differences in brain volumes between first-degree relatives of patients with schizophrenia and healthy control subjects.

Twenty-five studies were identified as suitable for the analysis, including 1065 first-degree relatives of patients, 679 patients with schizophrenia, and 1100 healthy control subjects. The largest difference that we found between relatives and healthy control subjects was in hippocampal volume, with relatives of patients with schizophrenia having smaller volumes than control subjects. Gray matter was smaller and third ventricle volume was larger in first-degree relatives compared to healthy control subjects. These brain abnormalities are similar to the areas that are affected in patients with schizophrenia and parallel the findings of neuropsychological impairments (especially in verbal memory) in both patients and relatives (Sitskoorn et al., 2004; Aleman et al., 1999).

In the conclusions of this chapter we suggested that our findings may reflect a vulnerability to develop schizophrenia but that it is still unclear how and to what extent genetic and/or environmental influences are involved.

In **Chapter 3** we described the large cross-sectional MRI study in which we examined brain structures in patients with schizophrenia, their non-psychotic siblings and healthy control subjects using global and focal brain measurements. Group differences in volumes, cortical thickness and gray matter density were analyzed using Structural Equation Modeling. Global brain volumes of non-psychotic siblings were not different from those of healthy control subjects, nor did siblings differ in cortical thickness or gray matter density using a voxel-based morphometry approach. We replicated the global volumetric abnormalities in patients with schizophrenia in total brain, gray matter and white matter volumes, lateral and third ventricle (see meta-analyses Steen et al., 2006; Wright et al., 2000). Furthermore, the decreases in cortical thickness and gray matter density, particularly in the frontal and temporal cortex, as well as the anterior cingulate cortex, are consistent with earlier studies (Nesvag et al., 2008; Kuperberg et al., 2003) and with those studies using a voxel-based morphometry approach (see meta-analysis Honea et al., 2005).

In the conclusion of this chapter we emphasized that we did not reveal structural brain abnormalities in non-psychotic siblings of patients with schizophrenia as compared to healthy control subjects using multiple imaging methods. Our finding is consistent with the findings from another large unaffected sibling sample (Goldman et al., 2009; 2008; Honea et al., 2008), but contrast with those of several smaller imaging studies in non-psychotic siblings (Tepest et al., 2003; Cannon et al., 2002; 1998; Staal et al., 2000). We suggested that the structural brain abnormalities observed in patients may (for the largest part) be explained by disease-related factors.

The study that we performed and described in **Chapter 4** examined mean fractional anisotropy (FA) in multiple white matter tracts in patients with schizophrenia, their non-psychotic siblings and healthy control subjects, using tract-based analysis.

Only few studies have examined first-degree relatives, using DTI methods (Camchong et al., 2009; Hao et al., 2009; Narr et al., 2009; Munoz Maniega et al., 2008).

In this chapter we described that mean FA was compared along averaged WM tracts, computed for the genu, splenium, left and right uncinate fasciculus, cingulum, inferior fronto-occipital fasciculus (IFO), fornix, arcuate fasciculus, and inferior longitudinal fasciculus (ILF). We found that mean FA was higher in the left and right arcuate fasciculus for siblings compared to patients and healthy control subjects, but no differences were found in mean FA in other WM tracts between these groups.

In the conclusions of this chapter we suggested that an increased familial risk for schizophrenia may be associated with higher mean FA in the arcuate fasciculus bilaterally. That we found a significant decrease in mean FA with increasing age in patients compared to siblings and control subjects, suggests a possible progressive loss of WM microstructure over time in schizophrenia.

The study described in **Chapter 5** was designed to examine brain structures in both the healthy parents of patients with schizophrenia compared to control couples, in relation to cognitive function. The association between brain measurements (global volumes and gray matter density) and cognitive measures of psychomotor function, verbal memory and IQ were investigated. Parents of patients with schizophrenia showed smaller total brain volumes as compared to healthy control couples, but no differences were found in volumes of cerebral gray matter, white matter, cerebellum, ventricles, or gray matter density. In addition, in the total sample, total brain volume was associated with faster performance on the motor task.

We concluded this chapter by showing that our findings in parents (to a lesser extent) mirror the decrease of brain volume that has consistently been reported in patients with this illness. The finding of brain volume loss in parents of patients in this study suggests that at least to some extent brain tissue loss seems associated with the genetic risk to develop schizophrenia.

Final remarks and conclusions

In the preceding chapters of this thesis, structural magnetic imaging studies in patients with schizophrenia, their non-psychotic first-degree relatives and healthy control subjects were described.
A number of methodological issues or limitations must be taken into account interpreting the results of these neuroimaging studies.

The use of different methodological approaches may account for discrepancies between studies. In the study that we described in Chapter 3, we intended to use an unbiased approach not only by comparing whole brain gray matter volume and density, but also by investigating the thickness of the cortex without any a priori assumptions of localization of structural brain deficits between patients with schizophrenia, their non-psychotic siblings and healthy control subjects. In contrast to the cortical thickness measurements, voxel-based morphometry also provides information on gray matter density from subcortical structures. Previous findings suggest that cortical surface area and cortical thickness are independent, both globally and

regionally, and that gray matter volume/density is a function of these two indices (Panizzon et al., 2009; Pakkenberg and Gundersen, 1997).

Another important question that may arise reading this thesis is whether longitudinal, rather than cross-sectional studies, are better suited to pick up possible brain abnormalities in the non-psychotic siblings of patients with schizophrenia. In schizophrenia, different lines of evidence suggest that some structures appear abnormal before the first sign of symptoms and therefore during a first episode, while some of these same structures and others show a higher than expected volume loss over time and thus appear different only at follow-up compared to healthy control subjects (Pantelis et al., 2005). Indeed, progressive changes in the brain seem to be present during the initial years after the first episode and continue even in the more chronic phase of illness (Hulshoff Pol and Kahn, 2008). As the siblings included in the studies described in the chapters 3 and 4 of this thesis were still at risk to develop schizophrenia, it might be that they will show subtle brain abnormalities over time. However, an earlier study by Brans et al. (2008) found no such progressive changes in healthy siblings over a 5-year scan-interval. Their suggestion for this finding was that the included siblings were possibly very healthy, as they were beyond the risk to develop the illness and were selected to be healthy.

Another consideration we should take into account when interpreting the results of the described studies, is antipsychotic medication. The intake of antipsychotic medication itself may modulate regional brain volumes. It is suggested that antipsychotic medication acts regionally rather than globally on the brain, with different effects on different brain structures. In our studies, where necessary and relevant, we controlled for type of medication at time of scan on significant findings. It never appeared to influence our results. However, a difficulty in these studies was that we did not have enough information to control for cumulative medication intake.

In the meta-analysis that we described in Chapter 2, we showed that volumetric brain abnormalities are more frequent in non-psychotic first-degree relatives than in healthy control subjects. One difficulty in interpreting the results of this meta-analysis, however, is that studies in different types of relatives were included in the analysis, i.e. twins, siblings, parents and offspring of patients with schizophrenia. These different types of relatives all exhibit their own risk to develop the illness (Gottesman et al., 1991). In addition, in this meta-analysis differences in age and sex were not examined. These two factors are known to affect brain volumes (Cannon et al., 2000), but the individual studies did not provide sufficient data to examine these effects in our study.

The patients, siblings and control subjects who were included for the studies described in the chapters 3 and 4 are for largest part participating in the GROUP-project (G.R.O.U.P., submitted), which is a multisite longitudinal cohort study. As shown in the introduction (**Table 2**) of this thesis, a large battery of tests and questionnaires are being scored and the subjects are currently coming back for the second, and (starting from beginning 2011), for the third measurement. In addition, in Utrecht, these subjects are still undergoing MRI scans, and future analyses will focus on possible (subtle) changes over time in subjects at risk. In addition, the effects of (cumulative) antipsychotic medication on brain structures should be better examined in future. Another question on which future research may focus is whether there are predictors for subjects at risk (i.e. siblings in GROUP) to become ill. These predictors can be examined in GROUP and can also be investigated in relation to brain measures. Environmental factors such as life-events and obstetric complications have been more extensively examined in the second (and will be in the third) measurement of GROUP and are such factors to be of great interest to take into account in the examination of longitudinal family MRI studies. In addition, as we have also collected genetic information from the subjects included in the GROUP-study, combining imaging and genetic data will provide great value to future research.

Conclusion

Taken together, the studies presented in this thesis showed that first-degree relatives of patients with schizophrenia in general, and specifically parents of patients, display subtle brain abnormalities, similar (but to a lesser extent) to those found in patients with this illness.
Thus, the answer on the main question of this thesis is: *Yes*; indeed there are subtle structural brain abnormalities in the first-degree relatives of patients with schizophrenia. However, using multiple brain imaging measurements, we have not found brain differences in the non-psychotic siblings compared to healthy control subjects.

There is evidence that genes play an important role in the development of schizophrenia and that siblings of patients with schizophrenia are at high risk to developing this illness. However, our studies do not clearly suggest that structural brain abnormalities are indeed present in this group at risk for schizophrenia. It therefore remains to be elucidated to what extent genetic and environmental influences are involved in the exact cause of brain abnormalities in schizophrenia.

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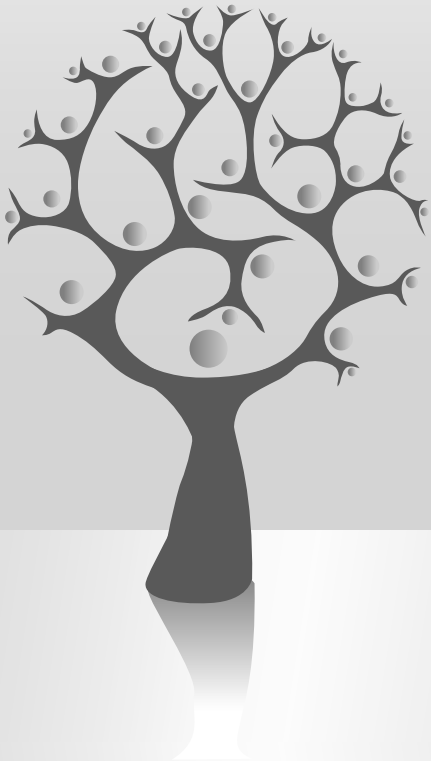
Nederlandse samenvatting



Komt het voor in de familie?

De belangrijkste vraag in dit proefschrift was of de hersenafwijkingen die consequent teruggevonden worden bij patiënten met schizofrenie, ook aanwezig zijn bij hun eerstegraads familieleden. Met andere woorden: komt het voor bij familieleden van patiënten met schizofrenie?

De studies die zijn uitgevoerd in dit proefschrift onderzochten de hersenstructuren van patiënten met schizofrenie, hun eerstegraads familieleden en gezonde controles. Voor dit doel hebben we een groot sample van patiënten, hun niet-psychotische broers en zussen, ouders van patiënten en gezonde controles geïncludeerd.

In **hoofdstuk 2** beschreven we een meta-analyse om de grootte en omvang van volume verschillen van de hersenen in eerstegraads familieleden van patiënten met schizofrenie te kunnen bepalen. Om dat te kunnen doen doorzochten we de Medline database op relevante structurele MRI studies die de verschillen in structurele hersenvolumes tussen eerstegraads familieleden van patiënten met schizofrenie en gezonde controles onderzochten.

Vijfentwintig studies werden geïdentificeerd die geschikt waren voor de analyse, met in totaal 1065 eerstegraads familieleden van patiënten, 679 patiënten met schizofrenie, en 1100 gezonde controles. Het grootste verschil tussen familieleden en gezonde controles vonden we in de hippocampus; familieleden van patiënten hadden een kleiner volume dan gezonde controles. Eerstegraads familieleden hadden minder grijze stof volume en meer volume in het derde ventrikel in vergelijking met gezonde controles. Deze gebieden zijn tevens het meest aangedaan bij patiënten met schizofrenie en de bevindingen in de hippocampus gaan gelijk op met bevindingen van neuropsychologische afwijkingen (vooral in het verbale geheugen) in zowel patiënten als familieleden (Sitskoorn et al., 2004; Aleman et al., 1999).

In the conclusies van dit hoofdstuk suggereerden we dat de bevindingen van onze meta-analyse een kwetsbaarheid tot de ontwikkeling van schizofrenie zou kunnen weerspiegelen, maar het is nog steeds onduidelijk in hoeverre genetische en/of omgevingsinvloeden hierbij precies betrokken zijn.

In **hoofdstuk 3** beschreven we de grote cross-sectionele MRI studie waarin we de hersenstructuren van patiënten met schizofrenie, hun niet-psychotische broers en zussen en gezonde controles, door middel van globale en focale meetmethodes onderzochten. Globale

hersenvolumes van broers en zussen verschilden niet van gezonde controles en de corticale dikte en grijze stof dichtheid (gemeten door middel van een voxel-based morphometrische methode; VBM) verschilden ook niet. We repliceerden globale volume afwijkingen in patiënten met schizofrenie in totaal brein, grijze en witte stof, lateraal en derde ventrikel volume (zie meta-analyses Steen et al., 2006; Wright et al., 2000). Ook vonden we afnames in corticale dikte en grijze stof dichtheid, vooral in de frontale, temporale, en anterior cingulate cortex en dit komt overeen met eerdere studies (corticale dikte: Nesvag et al., 2008; Kuperberg et al., 2003; VBM: zie meta-analyses Fornito et al., 2009; Glahn et al., 2008; Honea et al., 2005).

In de conclusie van dit hoofdstuk benadrukten we dat we geen hersenafwijkingen hebben gevonden in de niet-psychotische broers en zussen van patiënten met schizofrenie in vergelijking met gezonde controles. Onze bevindingen komen overeen met eerdere grote studies naar niet-aangedane broers en zussen van patiënten (Goldman et al., 2009; 2008; Honea et al., 2008), maar niet met kleinere neuroimaging studies bij niet-psychotische broers en zussen (Tepest et al., 2003; Cannon et al., 2002; 1998; Staal et al., 2000). Uit deze studie bleek dat de structurele hersenafwijkingen die we in patiënten vinden (voor het grootste gedeelte) verklaard kunnen worden door ziekte-gerelateerde factoren.

In de studie die we in **hoofdstuk 4** uitvoerden onderzochten we de gemiddelde fractionele anisotropie (FA) in verschillende witte stof banen met behulp van een tract-gebaseerde analyse in hetzelfde sample patiënten, broers en zussen en gezonde controles als de studie hierboven beschreven. FA wordt verondersteld de microstructurele eigenschappen binnen de witte stof banen te weerspiegelen. De diversiteit aan eerdere DTI bevindingen kan toegeschreven worden aan factoren zoals verschillen in studie methodologieën en sample karakteristieken. Eerdere studies onderzochten eerstegraads familieleden van patiënten met schizofrenie door middel van DTI methoden (Camchong et al., 2009; Hao et al., 2009; Narr et al., 2009; Munoz Maniega et al., 2008), maar er werd nog niet eerder naar siblings gekeken door middel van tract-gebaseerde technieken.

In dit hoofdstuk vergeleken we de gemiddelde FA langs de gemiddelde witte stof banen, gemeten voor de genu, het splenium, de rechter en linker uncinate fasciculus, cingulum, inferior fronto-occipital fasciculus (IFO), fornix, arcuate fasciculus, en inferior longitudinal fasciculus (ILF). We vonden dat de gemiddelde FA hoger was in de linker en rechter arcuate fasciculus bij broers en zussen in vergelijking met gezonde controles, maar er waren geen verschillen in FA in de andere witte stof banen tussen de groepen. Tevens bleek dat in patiënten met schizofrenie

voor een aantal witte stof banen FA waardes significant afnamen met toenemende leeftijd ten opzichte van de andere groepen.
Op basis van deze data veronderstellen we dat een verhoogd familiair risico op schizofrenie geassocieerd zou kunnen zijn met een hogere FA in de linker en rechter arcuate fasciculus. Een significante afname in FA met leeftijd bij patiënten met schizofrenie ten opzichte van gezonde mensen zou kunnen wijzen op een mogelijke progressieve afname van de microstructurele eigenschappen van de witte stof in schizofrenie.

De studie die we in **hoofdstuk 5** beschreven was ontworpen om de hersenstructuren van gezonde ouders van patiënten met schizofrenie te vergelijken met gezonde koppels, en de relatie tot het cognitief functioneren. De associatie tussen hersenmaten (globale volumes en grijze stof dichtheid) en cognitieve maten van psychomotorisch functioneren, verbaal geheugen en IQ werden onderzocht. Ouders van patiënten met schizofrenie hadden een kleiner totaal brein volume vergeleken met gezonde koppels, maar er werden geen verschillen gevonden in volumes van cerebraal grijze stof, witte stof, cerebellum, ventrikels, of grijze stof dichtheid. In het totale sample was totaal brein volume geassocieerd met een betere score op de motor taak.

We concludeerden in dit hoofdstuk dat onze bevindingen in ouders (in mindere mate), de afname van hersenvolume die consequent gerapporteerd wordt in patiënten met schizofrenie, weerspiegeld. De bevinding van afname van hersenvolume bij ouders van patiënten in deze studie suggereert dat dit verlies tot op zeker hoogte lijkt te zijn geassocieerd met het genetisch risico om de ziekte te ontwikkelen.

Slot opmerkingen en conclusies

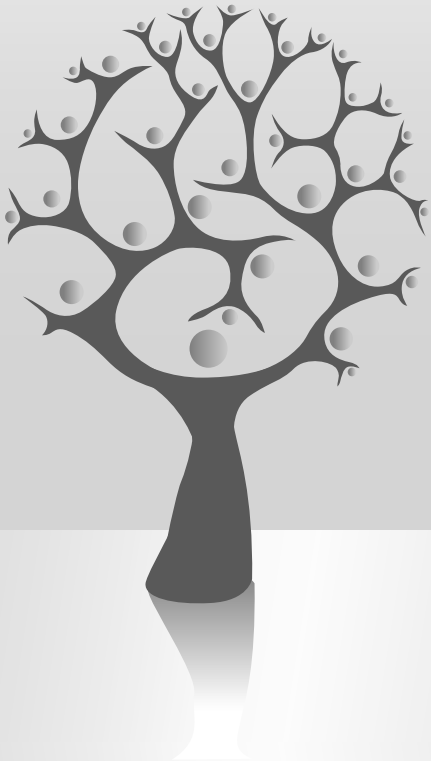
Samenvattend laten de studies in dit proefschrift zien dat eerstegraads familieleden in het algemeen, en in het bijzonder ouders van patiënten met schizofrenie subtiele hersenafwijkingen hebben. Deze verschillen in de structuren ten opzichte van gezonde controles lijken op de gebieden die aangedaan zijn bij patiënten met schizofrenie (maar in mindere mate).

Het antwoord op de belangrijkste vraag van dit proefschrift is dus: *ja*, er zijn inderdaad hersenafwijkingen gevonden bij eerstegraads familieleden van patiënten met schizofrenie. Echter, met het gebruik van verschillende neuroimaging maten hebben we geen verschillen gevonden tussen niet-psychotische broers en zussen van patiënten en gezonde controles. Het

is de vraag of de verschillen niet te subtiel zijn om met de huidige MRI methoden op te kunnen pikken.

Er is bewijs dat genen een belangrijke rol spelen in de ontwikkeling van schizofrenie en dat broers en zussen van patiënten een verhoogd risico hebben om deze ziekte ook te krijgen. Onze studies suggereren echter niet duidelijk dat structurele hersenafwijkingen aanwezig zijn in deze groep familieleden. De rol die de genetische en omgevings factoren precies spelen bij het ontstaan van de hersenafwijkingen die wij vinden bij patiënten met schizofrenie zal nog verder moeten worden onderzocht.

Dankwoord



De afgelopen jaren heb ik met plezier gewerkt aan het onderzoek dat in dit proefschrift beschreven is. Soms duurde het misschien te lang voordat ik resultaten zag; aan wetenschap komt klaarblijkelijk nooit een einde en daar heb ik tijdens dit traject wel eens moeite mee gehad. Maar geduld is een schone zaak; dat is een belangrijke les die ik geleerd heb tijdens dit proces. Het is eindelijk zover; mijn proefschrift is nu bijna een feit.

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List of publications

Boos HB, Aleman A, Cahn W, Hulshoff PH, Kahn RS (2007). Brain volumes in relatives of patients with schizophrenia: a meta-analysis. *Arch Gen Psychiatry*, 64:297-304.

Boos HBM, Cahn W, van Haren NEM, Derks EM, Brouwer RM, Schnack HG, Hulshoff Pol HE, Kahn RS. Focal and global brain measurements in siblings of patients with schizophrenia. *In press Schizophr Bull.*

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Curriculum Vitae

Heleen Boos was born on July 23, 1978, in Utrecht, the Netherlands. In 1996, she graduated from the Regionale Scholengemeenschap in Bergen op Zoom. In the same year, she started her Masters Gezondheidswetenschappen at Maastricht University. She did an internship at the department of Psychiatry at the University of Cambridge (UK) and carried out a research project on the insight of patients with schizophrenia. When she finished her Masters degree in 2001, she went back to Cambridge and started another research project in schizophrenia at the same department, resulting in an MPhil-degree. In 2003, she started a PhD-project at the Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, at the University Medical Center Utrecht. She investigated brain structures in first-degree relatives of patients with schizophrenia, under supervision of Prof. Dr. René Kahn and Prof. Dr. Hilleke Hulshoff Pol and this research resulted in the current thesis. From January 2011, Heleen will be working as a GZ-psychologist in training at the department of psychotic disorders, adult psychiatry of the University Medical Center Utrecht.

Heleen Boos werd op 23 juli 1978 geboren te Utrecht. Ze behaalde in 1996 het VWO-diploma aan het Regionale Scholengemeenschap te Bergen op Zoom. In datzelfde jaar begon zij aan haar Masters Gezondheidswetenschappen aan de Universiteit Maastricht. Ze heeft stage gelopen op de afdeling psychiatrie aan de Universiteit van Cambridge (UK) en voerde een onderzoeksproject uit over het inzicht van patiënten met schizofrenie. Nadat ze haar Masters afgerond had is ze in 2001 teruggegaan naar Cambridge en startte ze een nieuw project op naar schizofrenie. Dit project rondde Heleen in 2003 in het kader van een MPhil af en vervolgens begon zij bij het Rudolf Magnus Instituut voor Neurowetenschappen op de afdeling psychiatrie van het Universitair Medisch Centrum Utrecht, aan een promotietraject. Onder supervisie van Prof. Dr. René Kahn en Prof. Dr. Hilleke Hulshoff Pol heeft zij onderzoek gedaan naar hersenstructuren bij eerstegraads familieleden van patiënten met schizofrenie, hetgeen heeft geleid tot dit huidige proefschrift. Vanaf januari 2011 zal Heleen werkzaam zijn als GZ-psychologe in opleiding op de afdeling psychotische stoornissen, volwassenen psychiatrie van het Universitair Medisch Centrum Utrecht.