Characterization of brain morphology in patients with schizophrenia

A PhD thesis

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Abstract

Background

Schizophrenia is a chronic and severe disease which affects about 1 % of the population across the world. Most patients are severely impaired, needing long-term mental health care, and they are at higher risk of premature death due to suicide and somatic diseases. Current treatment options are not sufficient to help patients regain their everyday functioning. The risk for developing schizophrenia involves genetic and environmental factors, but the precise etiology and pathophysiological processes leading to disease are not known. Impairment in neural circuitry affecting practically all areas of the brain, in particular prefrontal and temporal cortices, striatum and thalamus, is supposed to be involved. Morphological brain abnormalities have consistently been found in patients with schizophrenia. Studies of the nature, cause, and development over time of the brain abnormalities may help elucidate on etiology and pathophysiology of the disease.

Aim

The aim of this thesis was to characterize structural brain abnormalities in schizophrenia in order to better understand the pathophysiological processes in the disease. In three separate studies the importance of age, gender, alcohol consumption, antipsychotic medication and type and severity of symptoms on the variation in brain morphology was investigated. In a fourth study cluster analysis was applied to investigate possible subgroups of patients determined by cortical thickness measurements.

Methods

Ninety-six patients in a stable phase of schizophrenia, and 107 age- and gender matched healthy control subjects underwent a comprehensive diagnostic and clinical evaluation. MRI and computer analysis software programs were used to measure brain tissue volumes and cortical thickness in all regions of the brain. General linear models and cluster analysis were applied to investigate relationships between brain morphology and clinical parameters.

Results

The patients had on average smaller volumes of grey matter in the temporal lobe, and smaller volumes of white matter in the frontal and temporal lobes compared to the healthy subjects. Patients had also thinner cortex in widespread areas of the frontal and temporal brain regions. Alcohol consumption was related to smaller white matter volumes in the total brain and in the temporal lobe, but could not explain the group differences in brain tissue volumes. Antipsychotic medication had a negligible effect on brain morphology. Group differences in cortical thickness were of equal magnitude across the age span. Positive symptoms were in general associated with smaller grey matter and cortical volumes, while negative symptoms were mainly associated with larger grey matter and cortical volumes throughout the brain. No evidence for subgroups of patients based on cortical thickness measurements was found.

Conclusion

Smaller brain tissue volumes and thinner cerebral cortex in prefrontal and temporal brain areas seem to be an intrinsic feature of schizophrenia. Morphological brain abnormalities may serve as markers of the pathophysiological processes in the disease. Positive and negative symptoms may be related to different neurobiological mechanisms. Lack of evidence for subgrouping of patients based on measurements of cortical thickness suggests that schizophrenia is a unique disease entity, in at least with regard to the cerebral cortex. Further studies are called for to establish the relationship between etiological factors and morphological brain abnormalities in schizophrenia. Once the precise etiology and pathophysiology of the disease are known, more targeted and hopefully more efficient treatments may be offered to patients with schizophrenia.

List of studies included in thesis

Study I

Ragnar Nesvåg, Arnoldo Frigessi, Erik G. Jönsson, Ingrid Agartz. Effects of alcohol consumption and antipsychotic medication on brain morphology in schizophrenia. *Schizophrenia Research 2007; 90:52-61.*

Study II

Ragnar Nesvåg, Glenn Lawyer, Katarina Varnäs, Anders M. Fjell, Kristine B. Walhovd, Arnoldo Frigessi, Erik G. Jönsson, Ingrid Agartz. Regional thinning of the cerebral cortex in schizophrenia: Effects of diagnosis, age and antipsychotic medication. *Schizophrenia Research 2008; 98:16-28.*

Study III

Ragnar Nesvåg, Erik G. Jönsson, Peter Sætre, Glenn Lawyer, Ingrid Agartz. The relationship between symptom severity and regional cortical and grey matter volumes in schizophrenia. *Under review.*

Study IV

Glenn Lawyer, Ragnar Nesvåg, Katarina Varnäs, Ingrid Agartz. Investigating possible subtypes of schizophrenia patients and controls as defined by brain cortical thickness. *Psychiatry Research - Neuroimaging in press.*

List of abbreviations

3D	Three-dimensional
ANN	Artificial neuronal network
AUDIT	Alcohol Use Disorder Identification Test
CSF	Cerebrospinal fluid
CT	Computer-assisted tomography
D2	Dopamine 2
DSM-IIIR	Diagnostic and statistical manual of mental disorders, third revised version
DSM-IV	Diagnostic and statistical manual of mental disorders, fourth version
DTI	Diffusion tensor imaging
FDR	False Discovery Rate
GLM	General linear model
HUBIN	Human Brain Informatics
ICV	Intracranial volume
MRI	Magnetic resonance imaging
OPCRIT	Operational criteria
PET	Positron emission tomography
RF	Radiofrequency
SANS	Scale for the Assessment of Negative Symptoms
SAPS	Scale for the Assessment of Positive Symptoms
SCID-I	Structured clinical interview for the DSM-IV

1. Introduction

Schizophrenia is a chronic, severe and disabling psychotic disease which affects about 0.5 % of the population (Jablensky, 2000) with a lifetime prevalence of 1 % (Mueser and McGurk, 2004). Despite more than a century of scientific investigations into the pathology and phenomenology of the disease, the precise etiology and pathophysiological mechanisms underlying schizophrenia are still unknown (Tandon et al., 2008). Current treatment options are insufficient for enabling patients to fully enter the social and vocational life. Twin, family, and adoption studies have demonstrated a high degree of heritability for the disease (Cardno et al., 1999). Abnormalities in brain structure have been found in post-mortem (Harrison, 1999a) and *in vivo* magnetic resonance imaging (MRI) studies (Honea et al., 2005; Shenton et al., 2001; Wright et al., 2000). Results from functional neuroimaging studies have shown abnormalities in neural response (Bender et al., 2007; Fallgatter, 2001) and brain activation (Ragland et al., 2007) among patients during cognitive and emotional processing. Neuropsychological tests have demonstrated both general and specific deficits as characteristic for the disease, although a number of patients have intact cognitive functioning. Disturbances in dopamine (Kapur, 2003) and glutamate (Farber, 2003) transmission seem to be involved in the pathophysiology. Some authors have proposed a circuitry based model (Andreasen, 1999; Fallon et al., 2003), in which alterations in structure and/or function of connected brain regions are involved.

A number of questions regarding the brain abnormalities in schizophrenia are still unsolved, e.g. what is the nature, why and how have they arisen, what are the functional consequences, and do they progress over time (DeLisi et al., 2006). Based on findings from neuropathological and MRI studies, schizophrenia is currently regarded to be a neurodevelopmental disorder, in which the normal brain development is altered due to genetic and/or environmental factors. A number of longitudinal MRI studies have also provided evidence for progression of brain abnormalities, indicating a neurodegenerative process. In order to acquire knowledge of the etiology and pathophysiological processes underlying schizophrenia, further preclinical and clinical investigations are needed. Establishing specific genetic and neurobiological markers of the disorder may eventually lead to more specific, and hopefully more efficient, treatment of the patients.

2. Objectives

2.1 Aim

The aim of the present thesis was to characterize structural brain abnormalities in a large group of patients with schizophrenia. To disentangle the specific effect of having a diagnosis of schizophrenia on the variation in brain morphology, the importance of age, alcohol consumption, antipsychotic medication and gender was investigated. To further explore the nature of the abnormalities, the importance of type and severity of symptoms, as well as evidence for subgroups of patients were investigated.

2.2 Hypotheses

When designing the study, we aimed to test the following hypotheses:

- Using MRI and computer image analysis it is possible to characterize morphological brain abnormalities in schizophrenia.
- Differences between patients and healthy control subjects in lobar brain tissue volumes and cortical thickness measurements are predominantly found in the frontal and temporal brain regions.
- Within the age span, differences between patients and healthy control subjects are more pronounced in higher age.
- Moderate consumption of alcohol can affect brain structure.
- Patients with schizophrenia are more susceptible to the effect of alcohol on brain structure than healthy control subjects are.
- Typical, but not atypical, antipsychotic medication is related to smaller grey matter volumes and thinner cerebral cortex.
- Gender is a significant factor for variation in cortical thickness measurements.
- Severity of negative symptoms is correlated with smaller grey matter and cortical volumes.
- Severity of positive symptoms is not correlated with grey matter or cortical volumes.
- Patients with schizophrenia may be subgrouped based on cortical thickness.

3. Background

3.1 Schizophrenia

3.1.1 History of schizophrenia

Schizophrenia as a clinical entity was first described as *Dementia Praecox* by the German physician Emil Kraepelin in the third edition of his textbook of psychiatry, published in 1896 (Kraepelin, 1919). He regarded the clinical picture of onset in early adulthood and poor outcome as a juvenile form of dementia resembling the adult form of dementia earlier described by his colleague Alois Alzheimer. He also proposed that a deteriorating clinical course without recovery was a "proof of the diagnosis". Based on post-mortem studies Kraepelin postulated that the origin of Dementia Praecox was in the brain, the nature of which would eventually be revealed by appropriate methods of investigation. A contemporary Swiss psychiatrist, Eugen Bleuler, suggested the term *Schizophrenia* for the disorder described by Kraepelin as *Dementia Praecox*. Bleuler hypothesized that the symptoms of schizophrenia could be explained as a pervasive split in patients' affect and behaviour, hence the term (gr. Schizis=division, Phrene=mind). In his textbook "Dementia Praecox or the group of schizophrenias" (Bleuler, 1950), he argued that the disease was in fact a group of clinical conditions with presumably different etiologies. Bleuler noted that some patients recovered from the disease, which was an argument against a degenerative nature of the disease. In his later works, Kraepelin revised his opinion on schizophrenia as he also found a group of patients with a better outcome.

3.1.2 Clinical picture

Symptoms and diagnosis

The definition of schizophrenia according to the latest version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994) is essentially unchanged from the descriptions of Kraepelin and Bleuler a century ago. The diagnosis is based on the presence of specific symptoms for at least six months

and a marked decline in general functioning. Symptoms are usually divided into positive, disorganized and negative symptoms. Positive symptoms include sensory hallucinations, delusions of reference, thought control, persecution, and somatic illness. Disorganized symptoms include disorganization of thought and behavior. Negative symptoms include social withdrawal, blunted affect, poverty of speech, and diminished motivation and drive. While positive symptoms usually fluctuate with time, being most severe in first-episode patients and during exacerbation of the disorder, negative symptoms are more stable and usually present at all stages of the disease (Arndt et al., 1995). Even though Bleuler pointed to the impairment in thinking and associations as fundamental in schizophrenia, cognitive deficits have only recently been called into attention as clinically important in the disease (Andreasen, 1999). Most studies on the topic report selective deficits in sustained and selective attention, executive control, working memory, and emotional processing when comparing patients with healthy control subjects. Cognitive deficits are shown to be quite stable during the course of the illness (Kurtz, 2005), further suggesting that they are fundamental to the disease process.

Longitudinal course

Onset of disease, defined as the first psychotic episode, usually occurs in the first half of the third decade of life, although a number of patients may experience their first symptom during childhood or adolescence (Perkins et al., 2006) or in their fourth or fifth decade. The longitudinal course of schizophrenia is heterogeneous (Hegarty et al., 1994). The majority of patients are characterized by a fluctuation in symptom severity and level of functioning in the course of the illness, with repeated psychotic exacerbations and periods of hospitalization. About ten percent of patients have a severe course with long-lasting symptoms and functional impairment needing long-term mental health care, and some patients need to stay in mental health hospitals for several years. A striking aspect of the disorder is the differences in outcome between genders. Women have on average onset two years later than men, and they also have a more benign form of the disease and better outcome (Leung and Chue, 2000).

3.1.3 Etiology

Genetic factors

From studies of twins concordant and discordant for schizophrenia the heritability contribution to the etiology of the disorder has been estimated to about 80 % (Cardno et al., 1999; Sullivan et al., 2003a). While some linkage studies have shown a number of genetic loci for the disease (O'Donovan et al., 2003), the evidence is not uniform (Crow, 2007). Association studies have pointed to several possible candidate genes. In a recent review the following genes were linked to elevated risk of developing schizophrenia in at least three independent studies: Catecholamin-O-methyl transferase (COMT), dysbindin (DTNBP1), neuregulin 1 (NRG1), regulator of G-protein signaling 4 (RGS4), disrupted-in-schizophrenia 1 (DISC-1), metabotrophic glutamate receptor-3 (mGluR3), and G72 (Harrison and Weinberger, 2004). The lack of specific genes and protein markers for the disease suggest that expression of a number of genes with moderate effect contributes to the disease process. Another explanation is that multiple, individually rare structural mutations may affect the expression of other genes, resulting in a cascade of alterations in the development of neuronal structure and functioning (Walsh et al., 2008).

The link between certain genetic variants and manifest neurobiology may be affected by epigenetic factors and interactions between different genes, gene products, and genes and environment, which further complicate the search for genetic markers for schizophrenia. Thus the unequivocally large contribution of genetic factors to the susceptibility for schizophrenia is far from being explained at a molecular level. In order to bridge findings from genetic studies with clinical features in schizophrenia, a number of recent studies have aimed to define “endophenotypes” of the disease. Endophenotypes are measurable and heritable components along the putative causal pathway between the genotype and the phenotype (Gottesman and Gould, 2003). Neurocognitive deficits, electrophysiological patterns, and morphological brain abnormalities have been suggested to be endophenotypes in schizophrenia (Prasad and Keshavan, 2008). If disease-specific endophenotypes are found, it will presumably lead to a better understanding of the disease process in schizophrenia.

Other risk factors

A number of demographic and environmental factors have been associated with higher risk for developing schizophrenia, e.g. winter or spring birth (Torrey et al., 1997a), living in urban areas (Torrey et al., 1997b), social and geographical migration (Harrison et al., 1988), use of cannabis (Arseneault et al., 2004), obstetric complications (Cannon et al., 2002), and high paternal age (Sipos et al., 2004). Each factor has a modest contribution to the risk for developing schizophrenia, indicating that genetic and environmental factors interact in the pathological process of schizophrenia.

3.1.4 Pathophysiology

Neurodevelopment vs neurodegeneration

At present, the neurodevelopmental model is the dominating explanatory framework for schizophrenia. According to this model normal brain development is disturbed by genetic and/or environmental factors during gestation and early life leading to abnormal pruning and maturation of the cortical neurons (Marenco and Weinberger, 2000). The higher prevalence of obstetric complications, minor physical anomalies, and neurological soft signs (Compton et al., 2007; McNeil and Cantor-Graae, 2000) among patients who later develop schizophrenia and their relatives supports this model. Some patients have been described as slow learners and with delayed development of language during childhood. These early signs, although not diagnostically specific, indicate a neurodevelopmental nature of the disease. Brain morphology studies showing smaller brain volumes even before the emergence of psychotic symptoms, and some longitudinal studies showing only subtle and regional volume reduction, support the neurodevelopmental hypothesis for schizophrenia (Rapoport et al., 2005). The general lack of pathological signs of degeneration, e.g. glial hypertrophy and neurofibrillar tangles, further argue against a classical neurodegenerative aspect of the pathological process in schizophrenia. An integrative model has been proposed based on the fluctuating course of the disorder (Pantelis et al., 2005). Neurocognitive deficits, negative symptoms, and general functional impairment may be related to the abnormal development of the brain, while the psychotic episodes and clinical deterioration in some patients may be due to limited neurodegenerative processes.

Neurotransmitters

The discovery of dopamine as a neurotransmitter in the brain by Arvid Carlsson approximately 50 years ago, and the subsequent insight provided by Paul Greengard into the cellular signalling mechanisms triggered by dopamine, were rewarded with the Nobel Prize for Medicine and Physiology in 2000. Their discovery was the foundation of the dopamine hypothesis for schizophrenia (Iversen and Iversen, 2007). The first-generation antipsychotic drugs were selective blockers of dopamine D2-receptors in the brain. Positron Emission Tomography (PET) studies have shown increased density of D2-receptors in mesolimbic regions of the brain among unmedicated patients with schizophrenia (Nordstrom et al., 1993). The selective blockade of D2 receptors by antipsychotic medication correlates with reduction in positive psychotic symptoms (Agid et al., 2006). PET-studies have provided robust evidence for dopamine as an essential neurotransmitter for the emergence of psychotic symptoms (Kapur, 2003). Other studies have included the excitatory transmitter glutamate in the pharmacology of schizophrenia (Krystal, 2002), based on the schizophrenia-like symptoms arising from the use of phencyclidine (PCP) and ketamine. While dopamine and glutamate seem to be important for the positive psychotic symptoms in schizophrenia, different mechanisms are supposed to underlie the negative symptoms and neurocognitive deficits. Antipsychotic medication has a negligible effect on these symptom domains, which have for long time been regarded as more fundamental to the disease process (Bleuler, 1950). A puzzling phenomenon is the superior efficacy of clozapine, which has a minimal effect on the dopamine system (Kapur et al., 1999). This indicates that dopamine is not the only neurotransmitter of importance in the pathophysiology of schizophrenia.

3.1.5 Treatment

Psychosurgery and physical treatment

During the first half of the 20th century patients with schizophrenia were confined to specially designed psychiatric hospitals with few effective treatment options. Cold baths and insulin coma were methods used, but none of them seemed to be advantageous for the patients. During a biologically oriented movement in psychiatry, psychosurgery was introduced as a treatment option in 1936. Neurosurgeons performed lesions in the internal

capsule, cingulum, and corpus callosum on severely ill patients, yielding a dramatic effect on reducing disorganized and agitated behavior, but also leading to changes in personality rendering the patients introvert, apathetic and affectively blunted. Psychosurgery was at the time regarded as a major advance in the treatment of schizophrenia, reflected by the nomination of the founder of the procedure, Egas Moniz, for the Nobel Prize for Medicine and Physiology in 1949.

Psychopharmacological treatment

Following the serendipitous discovery of the antipsychotic effect of chlorpromazine in 1952, a range of antipsychotic agents were developed, all of which had a selective effect on blocking postsynaptic dopamine D2 receptors in the brain. The antipsychotic medication had a distinct effect on patients' positive symptoms, but they were hampered by severe side-effects including extrapyramidal motor disturbances and hormonal changes. In the early 1990s a second generation of antipscyhotic agents was developed. In addition to modulating D2-transmission, these agents affected other monoamine systems such as serotonin and noradrenaline, resulting in fewer dopaminergic side-effects (Kapur et al., 1999). Subsequent studies showed that the second-generation antipsychotic could induce weight gain, higher prolactin levels, and elevated risk for diabetes II. This not withstanding, most patients with schizophrenia currently receive second-generation antipsychotic agents, even if no superior efficacy compared to the first-generation agents have been demonstrated (Geddes et al., 2000). Importantly, no antipsychotic medication has shown a substantial effect on alleviating negative symptoms and cognitive deficits (Buckley and Stahl, 2007). In two large governmentally sponsored comparison studies only minor differences in the efficacy of antipsychotic agents were found (Kahn et al., 2008; Lieberman et al., 2005a). Due to side effects and lack of efficacy, more than half of the patients discontinued their medication during the study period, which illustrates the shortcomings of present psychopharmacological treatment. New promising antipsychotic agents which modulate noradrenergic and glutamatergic transmission are under development, but their clinical efficacy and safety have not yet been documented. Thus there is still a need for more targeted pharmacological agents, with effect on all symptom domains.

Psychosocial treatment

Integrated psychosocially orientated treatments for patients with schizophrenia have also been developed, of which family-oriented psychoeducative therapy have shown best results (Rummel-Kluge and Kissling, 2008). Cognitive remediation programs and targeted cognitive behavioral therapy have also been introduced, showing an effect on ameliorating positive and negative symptoms (Rector and Beck, 2001), but the long-term efficacy has yet to be documented.

3.1.6 Outcome

Despite developments in detection and treatment of schizophrenia, most patients remain severely impaired in their everyday functioning. They have difficulties in holding a job in the competitive job market and need a high degree of support from relatives and public health care. The general outcome of patients is still poor, and the majority will to some degree be affected by their disease for the rest of their lives. However, some patients have a better outcome. Roughly one third of the patients show only mild functional impairment after the first episode of psychosis. Moreover, in some longitudinal studies a group of patients with full recovery of the disease has been identified (Harrow et al., 2005). Patients with acute onset in adulthood, dominated by positive symptoms, and good response to antipsychotic medication usually have a better outcome. In contrast, early onset of illness, insidious emergence of symptoms, presence of negative symptoms, and poor response to antipsychotic medication are factors linked to poor prognosis. In a multinational World Health Organization study, patients in developing countries had on average a better outcome than patients in developed countries (Jablensky et al., 1992). The finding may be explained by differences in social structures, the importance of family bonds, and beliefs about mental illness. Most pharmacologically oriented studies focus on symptomatic response, while few studies include psychosocial measurements in their definitions of treatment effect. Recently, a consensus definition of remission in schizophrenia was suggested, which include cognitive and functional measures in addition to severity of symptoms (Andreasen et al., 2005). This definition will hopefully be applied in future outcome studies to better illustrate the profound impact of the disease on the patients' lives.

3.2 Neuroimaging

3.2.1 Early neuroimaging techniques

During the 20th century, methods were developed which allowed for visualization of the central nervous system. Early radiological methods depended on infusion of contrast agents to indirectly visualize brain tissue. Pneumoencephalography was introduced in 1919 as the first method for *in vivo* investigation of the brain. The method implied drainage of cerebrospinal fluid (CSF) through a lumbar punction, and subsequent infusion of air. The air-filled ventricular system could then be visualized on radiological films. The invasive and potentially harmful nature of this method restrained recruitment of healthy volunteers, complicating the interpretation of results. The introduction of computer-assisted tomography (CT) in the 1970s enabled a non-invasive direct visualization of brain tissue and CSF. However, the distinction between grey and white matter was not clear, and the cerebellum could not be readily visualized due to its surrounding bone tissue.

3.2.2 MRI

When MRI was introduced in the 1980s, it represented a new era in brain imaging. At the time MRI represented a completely new concept of visualizing human tissue. The MR method is based on the interaction between the spin of protons and a strong static magnetic field (in which the body organ to be imaged is positioned), a gradient magnetic field, and radiofrequency (RF) pulse signals. Due to the large amount and wide distribution of water in all tissues of the human body, protons in hydrogen atoms are used for MRI in humans. Protons are magnetic dipoles that will orient themselves – align – in the direction of the static magnetic field. When they are affected by the RF pulse, they will spin out of the direction of the static magnetic field, i.e. become excited. When the pulse is terminated the protons return to their original spin – relax – and emit radiofrequency waves, which are used in the reconstruction of images of the tissue. The relaxation process can be divided into different aspects such as the T1 and T2 relaxation time constants that are measured in milliseconds. The T1, or spin-lattice relaxation time

constant, indicates how the proton is bound in the surrounding tissue, if it moves freely as in the CSF or is bound as for instance in fat. The T2, or spin-spin relaxation time constant, tells more about the intrinsic factors of the magnetic spin of the protons. The tissue contrast is dependent on T1 and T2 relaxation times, the number of affected protons, flow and temperature.

Tissue types have different magnetic properties and will therefore generate different changes in the net magnetic moments and produce different signals. MR images of the brain may therefore be reconstructed with good discrimination between bone, grey matter, white matter, CSF, and blood vessels. In a 1.5 Tesla scanner, the MR images have a spatial resolution of about 1 mm which allows for visualization of anatomical structures in great detail. However, each volume unit (voxel) in an image represents millions of synapses and neurons. Since the MR method does not imply ionizing radiation, MRI may be repeated with no risk of harmful effects for the subject.

3.2.3 Post-processing of MR images

Early quantitative studies of brain structure using CT and MRI were performed by manual tracing and measurements of regions of interest on the raw images. Manual tracing is time consuming and dependent on the operator's accuracy. During the last 15 years a number of computerized image analysis methods have been developed to automatically measure brain structures from MR-images. These programs automatically remove non-brain tissue and align the MR images from different subjects into a common coordinate system. Brain tissue may be segmented into different types based on MR signal intensity (Ashburner and Friston, 2000). Specific methods have been developed to measure global and localized brain tissue volumes (Andreasen et al., 1993) and characteristics of the cerebral cortex (Dale et al., 1999; Fischl et al., 1999a; Fischl and Dale, 2000). Such automated programs may be used to analyze large sets of MR images with high degree of accuracy. MRI and computerized programs have been introduced in a wide variety of settings, and have dramatically increased our knowledge of the healthy and pathological brain.

3.3 Brain morphology in schizophrenia

3.3.1 Neuroimaging of the brain in schizophrenia

Kraepelin suggested that the cause of dementia praecox would eventually be found in the brain (Kraepelin 1919). At that time, post-mortem examinations were the only available methods. The first study based on pneumoencephalography in patients with schizophrenia was published in 1927, showing dilatation of the lateral ventricles. Results from CT studies later confirmed this finding, as the ventricle-to-brain ratio was found to be larger in patients than controls (Johnstone et al., 1976; Weinberger et al., 1979). During the last two decades, several hundreds of studies investigating brain morphology in schizophrenia patients using MRI methods have been published. Although there is some discrepancy among different studies, the main finding is smaller grey matter volumes in prefrontal, temporal and parietal regions of the brain (Honea et al., 2005; Shenton et al., 2001; Wright et al., 2000), and larger volumes of the lateral ventricles (DeLisi et al., 2004). Smaller volumes have also been reported in specific regions of the cerebellar vermis (Okugawa et al., 2003) and in parts of thalamus, hippocampus and amygdala, while results for white matter and the basal ganglia are more mixed. Morphological brain abnormalities have even been found in patients before their first psychotic episode (Job et al., 2003), among healthy relatives (Boos et al., 2007; Goghari et al., 2006), and in childhood-onset (Rapoport et al., 1999) and first-episode patients (Keshavan et al., 2005). These findings suggest that morphological brain abnormalities are markers of the disorder, and not an epiphenomenon due to treatment, hospitalization, or having a chronic mental disease. Although some longitudinal studies have demonstrated that brain alterations progress during the course of the illness (Cahn et al., 2002; Mathalon et al., 2001; van Haren et al., 2007), a uniform pattern and rate of progression has not been established (Weinberger and McClure, 2002). The progression has been linked to antipsychotic medication (Lieberman et al., 2005b), and a deteriorating course (Ho et al., 2003; Lieberman et al., 2001).

3.3.2 Specificity of brain abnormalities in schizophrenia

There are several caveats to the interpretation of results from morphometric studies in schizophrenia. First, the variation in brain measures is large, both in patients and healthy subjects, with a considerable overlap between groups, indicating no discrete bimodal distribution. Second, several other factors may affect brain morphology, of which the natural loss of grey matter with increasing age is the most important (Salat et al., 2004). Third, there are some reports of differences in brain morphology between genders (Nopoulos et al., 1997). Fourth, cognitive measures are associated with brain morphology, reflected in the fact that patients with dementia have widely distributed atrophy of cortical grey matter (Thompson et al., 2001). Fifth, a number of possible confounders to brain morphology findings in schizophrenia have been put forward (Weinberger and McClure, 2002). Factors known to affect the brain include hydration, antipsychotic medication (Scherk and Falkai, 2006), stress (Sapolsky, 1996), nutrition (Swayze et al., 2003), alcohol consumption (Mann et al., 2001), and illicit drug use (Szeszko et al., 2007). Sixth, the heterogeneous phenotype of schizophrenia, overlapping with symptoms of bipolar disorders, obsessive-compulsive disorder, autism, Asperger's syndrome, attention deficit hyperkinetic disorder, schizotypal disorder and schizoid personality disorder, indicates that the neurobiological findings in schizophrenia may not be specific for the disease.

3.3.3 Post-mortem brain studies

Early pathological studies of the post-mortem brains from patients with schizophrenia and healthy control subjects demonstrated smaller frontal lobe grey matter volumes and ventricular enlargement in the patients. Kraepelin suggested that specific layers of the prefrontal cerebral cortex are involved in the pathological process. This has been supported by some post-mortem findings (Selemon et al., 2003), but results across studies are not consistent (Harrison, 1999a). Glial proliferation, a general marker for neuronal loss in neurodegenerative diseases, has not been found in the brains of patients with schizophrenia. Moreover, neuronal cell numbers are similar in patients and healthy control subjects (Pakkenberg, 1993), which suggests that neuronal death does not occur. The observed thinning of the cortex may be explained by reduction in neuronal size,

neuropil, or reduction in glia cells (Selemon and Goldman-Rakic, 1999). Small sample sizes of patients in a late stage of the disorder confounded by medication, hospitalization, and somatic disturbances leading to or occurring during the death process, have been limitations in post-mortem studies of schizophrenia. Results from post-mortem studies have not yet sufficed to explain the specific pathophysiological process in schizophrenia.

3.4 The HUBIN project

The Human Brain Informatics (HUBIN) project is an interdisciplinary study which was planned and performed at the Karolinska Institutet and Hospital in Stockholm. The main aim of the HUBIN project was to identify genotypes and phenotypes in schizophrenia. Based on the recruitment and characterization of a large group of representative patients with schizophrenia and healthy subjects, the results from the HUBIN project were expected to make a scientific contribution to the knowledge of etiological factors and pathophysiological mechanisms in the disease. A second aim of the project was to identify subtypes among the heterogeneous population of patients with schizophrenia. Beginning in 1995, more than 200 patients and healthy control subjects have been characterized by MRI, diagnostic and clinical evaluation, neurocognitive testing and genetic analysis. At the time, the HUBIN project represented a new concept of research, where data from several sources of information were collected from large numbers of subjects to reveal hitherto unknown relationships across multi-parametric data sets. The data were entered into a database from which subsets could be extracted for the purpose of addressing specific research questions. All studies in the present thesis are based on cross-sectional data obtained from the HUBIN project.

4. Material and methods

4.1 Ethical aspects

The HUBIN project was conducted in accordance with the Declaration of Helsinki and approved by the local and regional committees for Research Ethics. All subjects have given their written consent to participate after being informed about the project's aim and procedures.

4.2 Recruitment

4.2.1 Patients

Patients with a preliminary diagnosis of schizophrenia were recruited through their treating psychiatrist from four psychiatric clinics specialized in the treatment of psychosis in Stockholm County, Sweden, between 1998 and 2003. Most patients had received treatment for several years before inclusion in the project. Patients were first contacted by letter and then by telephone and asked to participate in the study. Permission from patients was requested to access their medical record. They were asked to participate in a clinical interview, deliver blood-samples for biochemical and genetic investigation, and to undergo an MR investigation of the brain.

4.2.2 Comparison subjects

Healthy control subjects were recruited from population registers, and among hospital staff and students at the participating centers.

4.2.3 Exclusion criteria

Patients and healthy control subjects were excluded if they had a history of head injury resulting in loss of consciousness for more than five minutes. Subjects were also excluded if they had any general medical condition which could affect the brain. A diagnosis of current or lifetime alcohol or drug use disorders was not an absolute criterion

for exclusion, but no subjects were actively treated for such disorders at the time of investigation. Healthy control subjects were excluded if they had a history of previous mental illness, or psychotic illness among first-degree relatives. An overview of the number and gender distribution of patients and healthy control subjects included in each of the studies in the present thesis are presented in the table below.

Number of patients and healthy control subjects included in each study of the thesis				
	Patients		Healthy subjects	
	Men	Women	Men	Women
Study I	50	19	63	34
Study II	70	26	73	34
Study III	38	15	n.a.	n.a.
Study IV	70	26	73	33

4.2.4 Representativity of the subject sample

The patients had to be in a stable phase of the disease to be able to give informed consent and complete the comprehensive study protocol. Although the subject sample was large, the strategy for case finding in specialized mental health care facilities puts some restrictions to the representativity of the sample. Patients not treated at psychiatric clinics or who were not able or willing to participate in the study, were not eligible for investigation.

Most of the healthy control subjects were recruited from population registers, but some were recruited among hospital staff and students at the participating centers. One would expect a bias towards people who are well adjusted to the society, have higher education, and are more interested in research than in the general population.

4.3 Clinical assessment

4.3.1 Patients

The Swedish Psychiatric Inpatient Register (SPIR) was used to detect patients with a psychotic disorder according to the register diagnosis. Eligible patients underwent the Structured Clinical Interview for the DSM-IIIR (SCID-I) (Spitzer et al., 1988) and were included if they fulfilled DSM-IIIR or DSM-IV criteria for a diagnosis of schizophrenia or schizoaffective disorder. All interviews were performed by a research psychiatrist at the Psychiatry Section at the Karolinska Hospital. In addition to the clinical interview patients' total medical records were scrutinized and summarized in an Operational Criteria (OPCRIT) protocol.

Psychiatric symptoms were assessed using the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1983) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984) as part of the clinical interview. The SANS includes 25 items from five dimensions of negative symptoms: affective flattening, alogia, avolition, anhedonia, and attentional impairment. The SAPS includes 34 items from four dimensions of positive symptoms: hallucinations, delusions, bizarre behavior, and thought disorder. Each item is scored from zero (not present) to five (present to a severe degree).

Consumption of alcohol was assessed in conjunction with the clinical interview and rated according to the Alcohol Use Disorder Identification Test (AUDIT) (Saunders et al., 1993). AUDIT is a ten-item screening questionnaire covering amount and frequency of alcohol consumption, and signs of harmful use and alcohol dependence. Each item is scored from zero to four, yielding a total score ranging from zero to 40. A score of eight for men and six for women is used as a cut-off score for detecting harmful use or alcohol dependence both in clinical and non-clinical populations (Reinert and Allen, 2007). The E-module in SCID-I was used to determine the presence or absence of a diagnosis of current or lifetime alcohol or drug use disorders.

Patients' current use of psychopharmacological medication was noted, and for 65 patients the full history of their use of psychopharmacological medication was obtained by scrutinizing their medical records. An estimate of lifetime use of antipsychotic medication was computed by multiplying current dose with duration of illness. In the studies in the present thesis, data on current and estimated lifetime use of antipsychotic medication were used.

4.3.2 Comparison subjects

The healthy control subjects were interviewed using the SCID-non patient version (Spitzer et al., 1986). Alcohol consumption was assessed using the AUDIT, and current or lifetime diagnoses of alcohol or drug use disorders were determined using the E-module in SCID.

4.4. MRI procedures

4.4.1. Acquisition of MR images

All subjects were investigated in the same 1.5 Tesla General Electronics Signa system at the MR Research Center at the Karolinska Institutet. Data from both T1- and T2-weighted images were used. T1-weighted images were acquired using a three-dimensional spoiled gradient recalled (SPGR) pulse sequence with the following parameters: 1.5 mm coronal slices, no gap, 35° flip angle, repetition time 24 ms, echo time 6.0 ms, number of excitations 2, field of view 24 cm, acquisition matrix 256 × 192. T2-weighted images were acquired using 2.0 mm coronal slices with no gap, repetition time 6000 ms, echo time 84 ms, number of excitations 2, field of view 24 ms, and acquisition matrix 256 x 192. From visual inspection, all scans were judged to be excellent without obvious motion artifacts. All scans were found to lack gross pathology when evaluated by a neuroradiologist.

4.4.2 Computer analysis of MR images

BRAINS

T1- and T2- weighted images were investigated using the software suite BRAINS, version one, developed by Professor Nancy Andreasen and coworkers at the Iowa Mental Health Clinical Research Center in Iowa, USA (Andreasen et al., 1993; Andreasen et al., 1996; Harris et al., 1999). The software performs an automatic segmentation of brain tissue into five tissue classes: grey matter, white matter, CSF, venous blood, and mixed tissue. The first step in the tissue classification process is the automatic identification of training classes that are entered into a discriminate analysis function that classifies the tissue into grey matter, white matter, CSF, and venous blood. The generated image is a continuous representation of the tissue types. This classification identifies the predominant (constituting more than 50 % of the volume) and next most likely tissue type within each voxel. The BRAINS program also provides a discrete classification, forcing the choice of only one predominant tissue type within each voxel. The continuous classification implies an intensity range, while the discrete classification implies a distinct level of grey scaling for each tissue type. The segmented brain image is oriented in standardized space according to an anatomical atlas developed by Talairach and Tournoux (Collins et al., 1994; Talairach and Tournoux, 1988). Each voxel is assigned to a specific region of the brain corresponding to the cerebral lobes, subcortical, cerebellar and brainstem regions in each hemisphere. Extra-cerebral tissue is excluded using an artificial neuronal networks (ANN) algorithm automatically tracing the intracranial volume (ICV) (Magnotta et al., 1999). ANNs are massive parallel arrays of simple processing units that can be used for computationally complex tasks such as image processing, machine and computer vision. The method is well suited for a rapid and robust determination of whether a voxel is included or not in the specific structure in question. In BRAINS, the continuous tissue classified image is used as input into the neural network structure identification module. The neural network has been trained based on human operator's definition of a structure and thereby taught to identify the brain.

FreeSurfer

To measure thickness, volume and surface area across the cerebral cortex the FreeSurfer software tools were developed by Bruce Fischl, Anders M. Dale and co-workers at the A. Martinos Center for Biomedical Imaging at Harvard Medical School in Massachusets, USA (Dale et al., 1999; Fischl et al., 1999a; Fischl et al., 1999b; Fischl and Dale, 2000; Fischl et al., 2001). The software reconstructs 3D representations of the white/grey matter border and the pial surface based on T1-weighted images. A triangular grid with approximately 1 mm spacing is applied to the grey/white matter surface allowing for the calculation of point-wise cortical thickness as the shortest distance between the white/grey matter border and the pial surface. The white/grey matter surface of each individual subject's brain is inflated and aligned according to gyral and sulcal anatomy. Data on cortical thickness, regional cortical surface area and volume is mapped onto the aligned surfaces, enabling further statistical analysis of the measurements. Applying a manually defined training set, based on anatomical landmarks, FreeSurfer performs parcellation of the cortex into 83 areas in each hemisphere (Desikan et al., 2006; Fischl et al., 2004). Data from these parcellations such as area, mean thickness, and cortical volume, may be extracted for further statistical analysis. The FreeSurfer software also includes a module for automated tracing and measurement of volumes of subcortical structures, but these measurements were not included in the studies of the present thesis. A thorough description of the processing steps in FreeSurfer is found at the website http://surfer.nmr.mgh.harvard.edu/.

4.5 Statistical analysis

4.5.1 General linear models

The statistical analyses in study I-III in the present thesis are based on general linear models (GLM) of two or more continuous or categorical variables. Group comparisons were analyzed using Student's T-test for normally distributed and Mann-Whitney U-test for non-normally distributed continuous variables. When comparing distribution of categorical variables Chi-square tests were applied. Linear regression models were used

when analyzing the separate effects of age, diagnosis, alcohol consumption and gender. In the case of different variance among the covariates, a heteroschedastic linear regression model was applied. Correlation analyses were used to investigate the relationship between two independent continuous variables. Pearson's product moment correlation analysis was applied to normally distributed variables, and Spearman's Rho was applied to non-normally distributed variables.

Measures of grey matter, white matter and CSF from the automated segmentation in BRAINS were used for statistical analyses in study I and III.

A GLM module is included with the FreeSurfer software. The module analyses specified contrasts using F-tests on age-regressed measurements at each point of the cortical mantle. In study II, results of the case-control analysis were visualized as color maps on the brain surface. To specifically investigate the effect of age on cortical measurements across groups, a model allowing for different age regression slopes between patients and healthy control subjects was applied in study II. Data from this model were used to compare age-regression slopes between patients and controls both globally across the cortical mantle, and within specified manually traced regions of the cortex. Data on mean cortical thickness and cortical volumes within cortical parcellations were extracted and analyzed in study II and III. In study II, differences in mean cortical thickness between patients and healthy control subjects were analyzed using Student's T-test. In study III, the relationship between cortical volumes and symptom severity was analyzed using partial correlation coefficients controlling for age and ICV.

4.5.2 Cluster analysis

Cluster analysis may be used to determine the number of classes, or clusters, which best explain variation in a variable across subjects. One approach is to measure how well the data is explained by different numbers of clusters. In study IV, this approach to cluster analysis was applied to measurements of cortical thickness at numerous points across the cortical mantle. The optimal clustering was determined by the k-means algorithm which minimized the within-cluster variance and maximized the between-cluster difference. The

number of clusters was limited to five. Higher number of clusters implies higher inter-subject variation, which may complicate interpretation of the results. The goodness of fit measure was derived from the Bayesian Information Criteria as this criterion is asymptotically consistent. The output was displayed as parameter maps of the cortex in which different colors represented different number of clusters at each point.

4.5.3 Controlling for multiple testing

Given the large number of measurements (83 cortical parcellations and approximately 160 000 vertices per hemisphere), a strict adjustment to prevent against type I errors is needed. A Bonferroni correction, which implies multiplying the initial p-values with the number of performed tests, is usually applied. Thus for a given significance level, say 0.05, the number of Bonferroni-adjusted rejections is much less, which protects against false positives. However, Bonferroni can be too conservative and not suitable for large numbers of tests of interdependent data. In FreeSurfer, a False Discovery Rate (FDR) adjustment (Benjamini and Hochberg, 1995) may be applied to the output of the analyses. In FDR the significant p-values are ranked, and the alpha-level is adjusted to a predefined acceptable level of false positive tests.

4.6 Validity and reliability

4.6.1 Clinical assessment

Diagnostic procedures

Determining psychiatric diagnoses based on the review of case notes using OPCRIT has previously been shown to have high validity for psychotic diseases (Craddock et al., 1996; Mihalopoulos et al., 2000). A detailed evaluation of the diagnostic procedures in the HUBIN study has previously been reported (Ekholm et al., 2005; Jonsson et al., 2006; Vares et al., 2006). Psychometric measures rely exclusively on report from study participants, their relatives, and clinical records. The reliability of the report is dependent on the patients' precise recollection and description. In this study much effort was made

to collect all relevant information from patients and medical records. All ratings were made by research psychiatrists trained in the use of OPCRIT by the British developers of the instrument. Diagnostic reliability was ascertained by calculating the unadjusted agreement and Cohen's un-weighted nominal kappa among the research psychiatrists performing clinical interviews.

Assessment of symptoms

SANS and SAPS are well established scales for the assessment of positive and negative symptoms. The scales have been used in a wide variety of settings and cross-validated against other symptom scales, e.g. the Positive and Negative Syndrome Scale (PANSS (Kay et al., 1987)) (Norman et al., 1996). Factor analytic studies have demonstrated largely consistent factors across and within studies using SANS (Blanchard and Cohen, 2006), while less consistent factor solutions have been found for SAPS (Peralta and Cuesta, 1999). In the HUBIN project, symptom assessment was performed by experienced clinical research psychiatrists, and such a procedure has been shown to yield good intra-rater and inter-rater reliability in other studies (Norman et al., 1996). The symptom assessments in the HUBIN study was not subjected to formal reliability testing.

Assessment of alcohol consumption

The AUDIT is a well established screening instrument for detecting alcohol use disorders (Reinert and Allen, 2007) which has been applied and evaluated in a variety of clinical and non-clinical settings. Assessment of consumption of alcohol and illicit drugs may also be associated with a number of uncertainties. People generally tend to under-report their use of alcohol and illicit drugs, and find it hard to remember the exact frequency and amount of drugs taken in the past. The AUDIT questionnaire was administered as a clinical interview. Since AUDIT includes questions covering the previous 12 months, the data is dependent on the patients' recollection. In previous studies, assessment of alcohol consumption based on self-report has been shown to be superior to assessment based on other sources, such as collateral report and medical and biochemical tests (Wolford et al., 1999). High validity of the AUDIT in patients with schizophrenia has previously been

reported (Dawe et al., 2000). In the HUBIN project no formal validity or reliability testing for AUDIT scores was performed.

Assessment of psychopharmacological treatment

Complete information about psychopharmacological medication depends on a number of factors. First, some patients do not know which medication they are given at certain points in time. Second, a long duration of illness usually implies in a number of changes in dose and type of medication. To obtain this information researchers have to scrutinize the patients' clinical records, which are often incomplete. Third, patients' non-compliance with pharmacological medication is a source of measurement error. Taking medication under supervision and regularly measuring serum levels of the prescribed medication may be used to ascertain compliance to the treatment. Aside from the usual procedures of the clinics, such monitoring was not an integral part of the HUBIN protocol. For the studies included in the present thesis, lifetime use of antipsychotic medication was estimated by multiplying the current dose and duration of illness. This is a rough estimate, not taking account of changes in prescription or medication-free periods during the illness.

4.6.2 Brain structure measurements

BRAINS

Measurements of brain tissue volumes using BRAINS have been validated by comparing the automated measures and the results from manual segmentation by the Andreasen group, yielding good validity (Harris et al., 1999). Validity testing was not performed for the automated segmentation of MR images using BRAINS in the HUBIN project. Results from reliability testing of the semi-automated segmentation using BRAINS in the HUBIN project have previously been reported (Agartz et al., 2001). Ten MR scans obtained within a four week period were segmented independently by two operators. The same scans were further segmented by one of the operators on two occasions within a 52-74 days interval. The general agreement was excellent, with inter-rater correlations above 0.96 and intra-rater correlations above 0.99. To determine the test-retest reliability of

measurements from MR-images, eleven subjects not included in the inter- and intra-rater reliability were rescanned within a 6-77 days interval. The segmentation procedures were performed by one operator who was blind for the identity of the scan. Test-retest correlations were found to be high (above 0.97). Thus the semi-automated segmentation procedures for MR images obtained within the HUBIN project were judged to have high degree of reliability.

FreeSurfer

Measurements of cortical thickness obtained using FreeSurfer have been validated by comparing automated and manual measurements on post-mortem (Rosas et al., 2002) and *in vivo* (Kuperberg et al., 2003) MR scans by the group at Harvard, yielding good agreement. No formal validity or reliability testing was performed for the processing of MR images using FreeSurfer for the HUBIN subjects. Since the automated procedures need only minimal manual intervention, and were performed without knowledge of the subjects' identity, the reliability was provisionally judged to be adequate. The processing was done by laboratory assistants under close supervision by senior researchers at the Institute of Psychology in Oslo. The senior researchers had been instructed in the use of FreeSurfer by the group at Harvard.

5. Results

5.1 Synopsis of the studies

5.1.1 Study I

The aim of study I was to investigate the putative effect of alcohol consumption and antipsychotic medication on brain tissue volumes in the total brain, and in the frontal and temporal lobe, among patients with schizophrenia and healthy control subjects. For the analyses, AUDIT total scores were dichotomized using a cut-off score of eight for men and six for women. Current dose of antipsychotic medication was converted to equivalent doses of haloperidol. To obtain a measure of lifetime use of antipsychotic medication, current dose was multiplied with duration of illness. The effect of alcohol consumptions and antipsychotic medication on variation of segmented grey matter, white matter and CSF volumes in total brain, and in frontal and temporal lobes (both hemispheres combined) was investigated from MR images using BRAINS. Full data sets were available for 69 patients (50 men, 19 women) and 97 healthy control subjects (63 men, 34 women). None of the subjects were receiving any treatment for alcohol use disorders at the time of investigation, indicating a non-clinical level of alcohol consumption.

There were no significant differences in measures of alcohol consumption between the patients and the healthy control subjects. Alcohol consumption was correlated with smaller volumes of white matter in the total brain and in the temporal lobes in the combined group of patients and healthy control subjects. The associations between alcohol consumption and smaller volumes of frontal white matter, and larger volumes of total and temporal CSF, reached a trend level of significance. Alcohol consumption was not significantly related to variation of grey matter volumes. Current and estimated lifetime dose of antipsychotic medication was related to larger volumes of CSF in the temporal lobes. A trend level effect of antipsychotic medication was found for smaller grey matter volumes in the frontal lobes. Patients had on average smaller volumes of total, frontal and temporal white matter, smaller volumes of total and temporal grey matter, and larger volumes of total, frontal and temporal CSF. The group differences in

brain tissue volumes remained significant when the effects of alcohol consumption and antipsychotic medication were accounted for. The results indicate that moderate levels of alcohol consumption and clinical doses of antipsychotic medication have a limited effect on brain structure, but these factors can not explain the overall group differences in brain tissue volumes. Nevertheless, the results demonstrate that use of alcohol and antipsychotic medication should be taken into account when studying brain morphology in schizophrenia.

5.1.2 Study II

The aim of study II was to investigate differences in cortical thickness between patients with schizophrenia and healthy control subjects. The importance of age, gender and antipsychotic medication for cortical thickness measurements was explored. Since no effect of alcohol consumption on grey matter volumes was found in study I, data on alcohol consumption were not included in study II. Measurements of cortical thickness at numerous points across the cortical mantle, and mean cortical thickness within predefined cortical regions, were obtained from MR images in 96 patients (70 men, 26 women) and 107 healthy control subjects (73 men, 34 women) using FreeSurfer. In separate analyses the importance of gender and antipsychotic medication was investigated. Within cortical regions where case-control differences were found, a region-of-interest analysis was performed to compare age-regression slopes between patients and control subjects.

Patients had thinner cortex in widespread areas of the prefrontal and temporal brain regions in both hemispheres. There were no significant differences in cortical thickness between men and women. Current or estimated lifetime dose or type of antipsychotic medication had no significant effect on cortical thickness measurements. Furthermore, similar age regression slopes were found in patients and control subjects, indicating that the group differences in cortical thickness were of equal size across the age span.

Thinner prefrontal and temporal cortex seems to be a specific marker of schizophrenia, not influenced by antipsychotic medication. Differences in cortical thickness were not

more pronounced across subject groups at higher age, indicating a fixed cortical reduction in patients with schizophrenia across the lifespan.

5.1.3 Study III

The aim of study III was to investigate the relationship between symptoms (type and severity) and brain volumes (lobar grey matter and regional cortical volumes). Measurements of grey matter volumes in the frontal, temporal, parietal, and occipital lobes using BRAINS, and cortical volumes within 36 parcellations across brain regions using FreeSurfer, were obtained from MR images. Symptom assessments using SANS and SAPS were available for 53 patients (38 men, 15 women), representing a subset of the patients included in study II. Based on previously published factor analysis studies, items from SANS and SAPS were grouped into five factors called negative, relational, inattention, disorganization, and reality distortion. The negative and relational factors contained items from SANS, the disorganization and reality distortion factors contained items from SAPS, and the inattention factor included one item from SAPS, but otherwise items from SANS. The negative, relational and inattention factors were therefore regarded as negative symptom factors, while the disorganization and reality distortion factors were regarded as positive symptom factors. The relationship between symptom factors and brain volumes was analyzed by partial correlation coefficients controlling for age and ICV.

Patients had on average higher scores on negative than positive symptoms, reflecting the fact that most of them were investigated in a stable phase of their illness. Severity of negative symptoms was in general related to larger frontal grey matter volumes in both hemispheres and larger cortical volumes in all regions of the brain. Severity of positive symptoms was related to smaller frontal and temporal grey matter volumes in both hemispheres and smaller cortical volumes throughout the cortical mantle.

The observed discrepancy in the relationship between positive and negative symptoms on brain volumes was not predicted, and further studies are needed to replicate this finding.

The findings may reflect other differences between positive and negative symptoms; e.g. negative symptoms are present at various stages of the disease, while positive symptoms are most severe during acute exacerbations; positive symptoms are more fluctuating than negative symptoms in the course of illness; antipsychotic medication is more efficient in alleviating positive symptoms, but have a negligible effect on negative symptoms; patients with predominantly negative symptoms have a worse prognosis than patients with mainly positive symptoms. The results indicate that positive and negative symptoms have different neural substrates, and they suggest that different pathophysiological processes are present in schizophrenia.

5.1.4 Study IV

Whether schizophrenia represents one or several diseases has been subjected to debate among clinicians and researchers. In study IV, a k-means cluster analysis was performed to determine if patients could be divided into groups based on cortical thickness measurements. Cortical thickness at numerous points throughout the brain was measured on MR images from 96 patients with schizophrenia (70 men, 26 women) and 106 healthy control subjects (73 men, 33 women) using FreeSurfer.

The results demonstrated a generally homogeneous distribution of cortical thickness measurements among both patients and controls when investigated separately. When analyzing the combined group, a bimodal distribution was found within 34 % of the cortex, roughly corresponding to the regions where patients had thinner cortex in study II. Post-hoc analyses confirmed that patients tended to belong to the group with thinner cortex, while healthy subjects tended to belong to the group with thicker cortex. The results provided no evidence for subgroups of patients based on cortical thickness measurements.

One explanation for this finding may be that the patients represent a rather homogeneous group. Most of the patients were treated at a specialist level, and they had to be in a stable phase of the illness to complete the comprehensive protocol of the HUBIN project. The

findings indicate that patients with schizophrenia may be regarded as one group with respect to cortical thickness.

5.2 Summary of results

- As a group, patients with schizophrenia have significantly smaller grey and white matter volumes in the temporal lobe, smaller white matter volumes in the frontal lobe, and larger volumes of CSF in the frontal and temporal lobe, compared to healthy control subjects. Patients also have thinner cortex in prefrontal and temporal regions in both hemispheres of the brain.
- There is a significant correlation between moderate consumption of alcohol and smaller volumes of white matter and larger volumes of CSF in the total brain and in the temporal lobes. There is no significant difference in the effect of alcohol consumption on brain tissue volumes in patients with schizophrenia and in healthy control subjects. Alcohol consumption is not significantly correlated with variation in grey matter volumes and does not explain overall group differences in brain tissue volumes.
- Current or estimated dose of antipsychotic medication has no significant effect on variation in grey and white matter volumes or cortical thickness.
- Severity of negative symptoms is correlated with larger grey matter volumes in the frontal lobe and smaller cortical volumes in all regions of the brain.
- Severity of positive symptoms is correlated with smaller grey matter volumes in the frontal and temporal lobes and smaller cortical volumes in all regions of the brain.
- Patients with schizophrenia do not divide into subgroups based on cortical thickness measurements.

6. Discussion

6.1 Morphological brain abnormalities in schizophrenia

6.1.1 Differences between patients and healthy subjects

A comprehensive review of MRI findings in schizophrenia (Shenton et al., 2001) as well as meta-analyses (Honea et al., 2005; Wright et al., 2000) have provided solid evidence for the presence of morphological brain abnormalities in the disease. The most consistent findings are larger volumes of the lateral ventricles, and smaller grey matter volumes in the prefrontal and temporal lobe, but there is some evidence for smaller cortical volumes in the parietal and occipital regions as well (Narr et al., 2005a). Results from studies focusing on specific brain regions have demonstrated abnormalities in the corpus callosum (Narr et al., 2000), cerebellum (Okugawa et al., 2003), striatum (Tamagaki et al., 2005) and thalamus (Ettinger et al., 2007).

While abnormalities with respect to grey matter volumes are well established, findings regarding white matter volumes have been more mixed. The results in study I demonstrate that patients with schizophrenia have smaller white matter volumes in the frontal and temporal lobes, and smaller grey matter volumes in the temporal lobe. Similar findings have been reported including a larger group of subjects from the HUBIN study (Okugawa et al., 2007). A number of studies using MR diffusion tensor imaging (DTI) have shown reduced fractional anisotropy in white matter bundles connecting the frontal and temporal lobe (Fujiwara et al., 2007; Karlsgodt et al., 2008; Manoach et al., 2007; Price et al., 2008), indicating that smaller grey matter volumes in frontal and temporal regions may be linked to impairment of white matter integrity between these regions. As described in study I, there was no difference between patients and controls in frontal grey matter volumes. Since the frontal compartment in BRAINS encompasses almost half of the intracranial volume, the results in study I may indicate that the group differences are more subtle in the frontal than in the temporal lobe.

In line with previous studies (Kuperberg et al., 2003; Narr et al., 2005b; Wiegand et al., 2004), the results in study II in the present thesis demonstrate that patients with schizophrenia have thinner cerebral cortex in prefrontal and temporal regions of both hemispheres compared to healthy control subjects. These findings provide further evidence for a predilection of prefrontal and temporal regions in the pathophysiological process in schizophrenia.

Although the longitudinal course of brain abnormalities was not eligible for investigation in the studies of the present thesis, the finding of similar age regression slopes in patients and healthy control subjects in study II suggests a lack of progressive volume loss during a stable phase of the disorder. Consistent with this finding, most studies reporting progressive loss in patients relative to control subjects have acquired the baseline data when the patients experienced their first-episode of psychosis (Cahn et al., 2002; Lieberman et al., 2001). In studies of patients in a chronic phase of the disease, less consistent evidence has been found for progression of brain abnormalities (DeLisi et al., 2004; Whitworth et al., 2005). A neurodegenerative model for schizophrenia has been proposed, based on the observed clinical deterioration with time among some patients (Lieberman, 1999). The neurodegenerative process is supposed to imply reduced neuronal size, and reduction in number of synapses and dendrites (Selemon and Goldman-Rakic, 1999), but not neuronal loss (McGlashan, 2006). Such degeneration may occur during exacerbations of the disease, and possibly lead to the deteriorating course of some patients with frequently relapsing or chronic schizophrenia (Hulshoff Pol and Kahn, 2008).

6.1.2 Effects of alcohol consumption

The results in Study I in this thesis demonstrate a significant relationship between level of alcohol consumption and smaller volumes of white matter, even among subjects not being treated for alcohol use disorders. The findings were strongest for global and temporal white matter volumes, but there was a trend level effect on frontal white matter volumes as well. In studies of patients with alcohol use disorder, the amount of alcohol consumption has been associated with smaller volumes of grey and white matter,

predominantly in the frontal lobe (Mann et al., 2001; Pfefferbaum et al., 1997), smaller volumes of the hippocampus (Agartz et al., 1999), and smaller volumes of the vermis region of the cerebellum (Varnäs et al., 2007). Post-mortem studies of subjects with alcohol use disorder have demonstrated gross brain atrophy (Harper, 1998), mainly due to decrease in white matter. Neuronal loss in the frontal cortex, reflected by retraction of dendrites, have also been reported (Harper and Kril, 1990).

There is some evidence for an additional effect of alcohol consumption on morphological brain abnormalities among patients with schizophrenia and co-occurring alcohol use disorder (Mathalon et al., 2003; Sullivan et al., 2000; Sullivan et al., 2003b; Sullivan et al., 2004). Study I of the present thesis shows no significant effect of alcohol on brain tissue volumes when the patients and control subjects were investigated separately. This indicates that the effect of moderate alcohol is not more pronounced in patients with schizophrenia than in healthy subjects.

The brain alterations found in patients with alcohol dependence may to some extent be reversed in periods with prolonged abstinence of alcohol (Agartz et al., 2003; Bartsch et al., 2007), indicating that the effect of alcohol is not entirely neurotoxic. Most studies of alcohol-induced brain changes have focused on patients with an established alcohol use disorder. Some concern has been raised regarding possible neuronal damage of recreational and non-clinical alcohol consumption (Harper and Kril, 1990). The results in study I of the present thesis demonstrate that moderate alcohol consumption may affect brain structure and should therefore be taken into account in MR studies of the brain.

6.1.3 Effects of antipsychotic medication

In study I of the present thesis, a significant correlation between use of antipsychotic medication and larger CSF volumes in the temporal lobe, and a trend level relationship between antipsychotic medications and smaller grey matter volumes in the temporal lobe was found. In study II there was no significant effect of medication on cortical thickness measurements. These results are not consistent with previous reports of a reduction of

global grey matter volumes during long-term treatment with typical antipsychotic medication (Dazzan et al., 2005; Lieberman et al., 2005b). Several studies have demonstrated enlargement of the caudate nucleus in response to typical antipsychotic medication (Dazzan et al., 2005; Keshavan et al., 1994). In a previous report from the HUBIN project, estimated lifetime dose of typical antipsychotic medication was related to larger volumes of the putamen (Tamagaki et al., 2005).

There is solid evidence for an effect of typical antipsychotic medication on volumes of the striatum and basal ganglia, while the effect on cortical grey matter volumes is questionable (Scherk and Falkai, 2006). In post-mortem studies no evidence has been found for neuronal damage due to antipsychotic medication (Harrison, 1999b), supported by results from animal studies (Dorph-Petersen et al., 2005; Konopaske et al., 2006). Longitudinal studies of patients at different clinical states have shown an increase in grey matter volumes during exacerbation of psychosis, and a reduction in grey matter volumes during remission (Garver et al., 2000). Thus, the effects of antipsychotic medication on variation in brain volumes are scarcely due to neurotoxic effects of the antipsychotic agents per se, but may rather be neurobiological markers of the changes in clinical state which accompany the presence or absence of antipsychotic treatment.

6.1.4 Relationships between symptoms and brain morphology

Given the presence of morphological brain abnormalities in schizophrenia, one would expect to find a relationship between brain morphology and severity of symptoms in the disease. Although not all symptoms are specific for schizophrenia, for instance patients with acute mania often exhibit auditive hallucinations and paranoid delusions, the more specific symptoms in schizophrenia, such as bizarre and disorganized behavior, delusions of thought control, and negative symptoms, may be more closely related to morphological brain abnormalities. Most studies of brain morphology in schizophrenia have focused on case-control differences and changes over time, not specifically addressing the relationship between symptoms and brain morphology.

As presented in study III in the present thesis, positive and negative symptoms were differentially related to cortical and lobar grey matter volumes. Positive symptoms tended to be related to smaller volumes, while negative symptoms tended to be related to larger brain volumes. These results are not in full agreement with previous studies on the subject. Symptom severity has mainly been linked to smaller grey matter volumes, e.g. hallucinations and grey matter density (Gaser et al., 2004), apathy and frontal lobe volumes (Roth et al., 2004), and negative symptoms and prefrontal volumes (Wible et al., 2001). However, some studies have found a relationship between larger brain tissue volumes and positive (Kim et al., 2003; Szeszko et al., 1999) as well as negative (Lacerda et al., 2007) symptoms among patients experiencing their first psychotic episode. Volume enlargement has also been found during exacerbation of the illness (Garver et al., 2000; McClure et al., 2006), although these changes may also be a consequence of patients discontinuing their medication.

The observed fluctuations in brain tissue volumes at various stages of the disease argue against a pure neurotoxic process during active psychosis (McGlashan, 2006), supported by the fact that pathological signs of neuronal cell death have not been found in post-mortem studies. An alternative explanation for the observed changes in brain volumes is that active psychosis is related to changes in neuronal size and synaptic plasticity, possibly due to alterations in the expression of specific receptors in the cell membranes. The results presented in study III indicate that specific symptoms have specific neurobiological underpinnings.

Phenomenologically oriented studies have suggested that impairment in the self-consciousness of patients is the fundamental cognitive process in schizophrenia (Sass and Parnas, 2003). The impairment may imply elevated arousal to externally and internally generated stimuli, and reduced ability to recognize thoughts as self generated. This hypothesis may explain the various symptoms of the disorder at a psychological level, but it can not explain which neural processes underlie the disturbances. General modalities of brain functioning, like consciousness, attention, awareness of action, and meta-cognition are supposedly involved. A heuristic model linking phenomenology, pharmacology and

psychopathology suggests that dopamine depletion induce alterations in the attribution of salience to stimuli (Kapur, 2003). In conjunction with studies of neural correlates for cognitive processes in the healthy brain, phenomenological approaches to the subjective experiences in patients with schizophrenia may eventually determine the neural substrate of the disease process itself.

6.1.5 Lateralization

Results of the studies included in the present thesis demonstrate some evidence for lateralized group differences in brain morphology. As presented in study II, the difference in mean cortical thickness was more pronounced in the left frontal and temporal lobe, although the regions with thinner cortex among the patients were fairly equally distributed across the hemispheres. The findings in study III demonstrate a tendency for a left-lateralized relationship between positive symptoms and smaller cortical volumes in the temporal lobe, and a right-lateralized relationship between both positive and negative symptoms and cortical volumes in the occipital lobe. Professor Tim Crow and co-workers at Oxford University in London have developed a model where the faculty of language is central in the pathological process underlying schizophrenia (Crow, 1997; Crow, 2000). A balanced mutation in the X-chromosome is suggested to result in abnormal lateralization of the brain (Crow, 1999), affecting language as a lateralized function in the brain. Findings from studies showing alterations in the dominant ear by dichotic listening among patients with schizophrenia support this theory (Hugdahl et al., 2008; Løberg et al., 2006). In addition to the left temporal dominance for language, subtle left hemisphere dominance in the frontal, and right hemisphere dominance in the occipital cortex is consistently found when examining the healthy brain (Barrick et al., 2005; Luders et al., 2006a). This asymmetry is referred to as the cerebral torque, which according to the lateralization hypothesis may be changed in the brains of patients with schizophrenia. The findings in study II and III provide some support for this hypothesis, although the results are not entirely consistent.

6.1.6 Gender differences

In study II of the present thesis gender was not significantly related to cortical thickness measurements. Although the results indicate that gender plays a minor role for brain morphology in schizophrenia, the small number of women included (26 patients and 34 healthy subjects) may have reduced the power for detecting gender effects. There are striking differences between men and women with respect to the clinical picture in schizophrenia. In general, men have an earlier onset, more negative symptoms, poorer effect of antipsychotic medication, and worse prognosis (Leung and Chue, 2000). The gender differences in prevalence and severity of disease are less prominent in patients with late onset, indicating that estrogen may be a protective factor for developing schizophrenia (Stevens, 2002). There are no significant differences between men and women with respect to signs of abnormal neurodevelopment, such as history of obstetric complications, and occurrence of minor physical anomalies and neurological soft signs. With respect to brain structure, there are some differences between men and women. Men have larger ICV, and women have larger grey matter volumes in the left superior temporal lobe (Good et al., 2001). The difference in ICV may be compensated for by women having higher density of grey matter across the cortical mantle (Luders et al., 2006b). A few studies have investigated the impact of gender on structural brain abnormalities in schizophrenia, with somewhat discrepant findings (Goldstein et al., 2002; Nopoulos et al., 1997). In conclusion, there is limited evidence for a neural correlate for the observed difference in clinical features between men and women with schizophrenia.

6.2 Speculations on the structure-function relationship

In spite of the large amount of studies showing brain abnormalities in schizophrenia, a satisfying explanation for the changes has not yet been found (DeLisi et al., 2006;Malhi and Lagopoulos, 2008). Schizophrenia is no doubt a neurobiological disease, leading to pervasive changes in perception, cognition, and social functioning. The pathological changes in schizophrenia must therefore affect crucial neural networks needed to lead a normal life. Studies in healthy subjects have linked frontal lobe functioning to planning, performing, and critical evaluation of one's actions, and to interpretation of others'

actions and intentions. During evolution the frontal lobe has increased relatively more in humans than in other mammals. Frontal lobe functioning is therefore considered to be the substrate for social behavior (Burns, 2006). Ventral and lateral frontal regions have been linked to the ability to correctly interpret thoughts, actions, and intentions of other people, also referred to as theory of mind (Brunet-Gouet and Decety, 2006). Thus some of the abnormalities in emotional processing and cognitive functioning among patients with schizophrenia may be linked to morphological brain abnormalities in the frontal lobe.

Language is generally processed in the temporal lobe, particularly in the medial aspect of the left temporal cortex. Given the robust finding from MRI studies of morphological brain abnormalities in the left temporal lobe, and a range of the symptoms in the disease being related to language, one may assume that disturbances in the development of language is pivotal to the pathophysiological process in schizophrenia (Crow, 2000). Since development of language is supposed to be human-specific, the neurobiological processes in schizophrenia seem to affect brain functions which define us as human beings.

6.3 Is schizophrenia one or several disease entities?

When schizophrenia first was described, a proposed division of the patients according to symptom profiles was suggested (Bleuler, 1950). Nevertheless, most clinicians and scientists still regard the illness as a unique entity encompassing various patterns of symptomatology, reflected in the provisional subgrouping into paranoid, hebephrenic, and catatonic forms of schizophrenia in DSM-IV and ICD-10, and that most researchers regard patients as a uniform group when comparing with healthy subjects. However, there are major differences in clinical parameters across groups of patients. Crow (1985) suggested a grouping of patients into type I, characterized by acute onset during adulthood, predominantly positive symptoms, good response to antipsychotic medication, and good prognosis, and type II, characterized by an insidious onset during adolescence, more negative symptoms, moderate effect of antipsychotic medication, and a generally poor outcome. MRI-studies have further shown that patients with poor outcome tend to have more brain abnormalities than patients with a better outcome (DeLisi et al., 2004;

Lieberman et al., 2001; Velakoulis et al., 2002), indicating that there may be specific pathophysiological processes for groups of patients.

The results in study II of the present thesis demonstrate widespread regions with a thinner cortex in patients than in healthy control subjects. If there are subtypes of the disease, variation in cortical thickness may be a distinguishing factor. This question was addressed in study IV by performing a cluster analysis of variation in point-wise cortical thickness across the cortical mantle among patients with schizophrenia and healthy subjects, analyzed separately and combined. Both in patients and in controls, there were two clusters in anterior parts of the temporal cortex and in the precentral gyrus. The regional distribution was almost identical across groups. The grouping of cortical thickness measures in these regions are presumably linked to other factors than schizophrenia. When analyzing both groups combined, large regions with a bimodal distribution were found, roughly corresponding to, and extending, the regions where case-control differences were found in study II. From the results of study IV one may conclude that cortical thickness is not the parameter around which to subtype patients with schizophrenia. However, a similar investigation of patients at different stages of the disease, showing different clinical pictures, or subgrouping of patients by a measure other than cortical thickness, would perhaps reach another conclusion.

6.4 Strengths and limitations

The major strength of the studies in the present thesis is the substantial number of subjects who underwent a thorough clinical characterization using standardized and structured interviews for the ascertainment of diagnosis, assessment of symptoms and alcohol consumption. All subjects were investigated in the same MR scanner using identical pulse sequences. MR images were processed using two separate, standardized and validated computer software tools which have been used in a wide variety of settings. By including a large subject sample, acquiring data from many sources of information, and using valid and reliable methods, the results from the studies represents a substantial contribution to the knowledge about the nature of morphological brain abnormalities in

schizophrenia. Results from the HUBIN project have previously been reported in several scientific publications.

Some factors should be considered when interpreting the results presented in this thesis. The assessment of symptoms and alcohol consumption was not subjected to formal reliability testing. The studies were based on cross-sectional data which precludes any conclusions regarding the time course of psychopathological and brain structural measures. To determine if the results are specific to schizophrenia, the inclusion of a clinical comparison group would have been useful. The recruitment of patients from specialized mental health facilities only and some healthy control subjects among hospital staff and students puts some restriction to the generalizability of the subject sample.

6.5 Summary and conclusions

Results from the studies included in the present thesis confirm that morphological brain abnormalities in schizophrenia may be detected using MRI and computer analysis methods. There are robust differences in brain tissue volumes and cortical thickness between patients and healthy control subjects. The abnormalities are not explained by alcohol consumption or use of antipsychotic medication. Gender is not an important factor for the group differences. The similar magnitude of group differences in cortical thickness across the age span argues against a progressive brain tissue loss in the course of the illness. The finding of a differential relationship between positive and negative symptoms and cortical and lobar grey matter volumes indicate that different symptoms may represent different pathophysiological mechanisms. The group differences in cortical and lobar volumes were predominantly found in frontal and temporal regions, while associations between symptom severity and brain volumes were found throughout the cortical mantle, indicating that widely distributed brain circuits are involved in the disease process. There was no evidence for subtypes of schizophrenia based on cortical thickness measurements. This suggests that, with respect to cortical thickness, schizophrenia may be regarded as a unique disease entity in line with Kraepelin's

dementia praecox, and not a syndrome encompassing several disease entities as proposed by Bleuler.

6.6 Implications for research and clinical practice

The search for the etiology and pathophysiological processes underlying schizophrenia continues. Morphological brain abnormalities may serve as endophenotypes, i.e. observable and heritable characteristics intermediate between genetic factors and overt psychopathology. Studies using structural MRI have provided knowledge of the magnitude and localization of morphological brain abnormalities present in the disease. The studies in the present thesis have added to the knowledge by testing for and excluding a number of exogenic factors presumed to affect the brain. Smaller brain tissue volumes and reduced cortical thickness in prefrontal and temporal brain regions among patients compared to healthy control subjects remain core findings in schizophrenia.

To elucidate why and how the morphological brain abnormalities have arisen, more studies are needed. A valuable point of departure would be studies combining structural MRI with genetic and protein markers to determine the relationship between brain structure and genetic factors. Multimodal neuroimaging studies combining structural MRI with DTI, functional MRI, and electrophysiological methods may provide knowledge about the neural circuitry involved. Studies of patients with heterogeneous clinical pictures are needed to establish the link between brain morphology and psychopathology. Longitudinal studies of patients at different clinical states will clarify the temporal pattern of the brain abnormalities in schizophrenia. A five year follow-up investigation of the subjects in the HUBIN project is currently pending, providing an option to study the longitudinal course of the findings presented in this thesis. Finally, studies including patients with other mental disorders are needed to ascertain the specificity of structural brain abnormalities for schizophrenia.

CT and MRI are currently used to exclude other brain pathology in patients with mental illness. No neuroimaging method has the power to determine or substantiate a psychiatric

diagnosis, but the rapid development in imaging techniques may eventually provide this option for clinical psychiatrists.

While waiting for another serendipitous discovery to occur, optimal efficacy for the treatment of patients with schizophrenia will hardly be achieved until the precise pathophysiological mechanisms for the disease are known. Hopefully, another hundred years will not pass for psychiatrists to successfully help their patients gain the abilities needed to fully participate in the social and working society.

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