The dynamic human brain

Genetic aspects of brain changes in schizophrenia and health

The studies described in this thesis were performed at the Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, University Medical Center Utrecht, the Netherlands.

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The dynamic human brain

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Het dynamische menselijk brein
Genetische aspecten van hersenveranderingen
bij schizofrenie en gezondheid

(met een samenvatting in het Nederlands)

Proefschrift

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Rachel Gertruida Hendrika Brans

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Promotoren: Prof. dr. H.E. Hulshoff Pol
Prof. dr. R.S. Kahn

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"If we knew what it was we were doing, it would not be called research, would it?"

- Albert Einstein

Voor papa en mama

Contents

Chapter 1

Introduction

1.1 General introduction

The general aim of the studies presented in this thesis is to increase the understanding of human brain development, both in health and in schizophrenia. Human brain research has moved rapidly due to the development of modern imaging techniques. However, since the human brain is very complex and maturation of the brain is a life-time process, doing research is challenging in this fascinating field of science.
To make a valuable contribution and to gain more knowledge of the human brain, we started to explore the possible mechanisms underlying the individual differences that are observed in brain structure and brain structure change. Earlier studies have demonstrated that the brain is in constant development. However, the etiology of these dynamic brain patterns has not been established: Is it a shaping according to environmental demand, or is the process of change under genetic control?
To be able to answer questions regarding the relative contribution of genetic and environmental (possibly disease-related) factors on brain development, twin-pairs and siblings are involved in these studies. Before discussing the specific studies we conducted over the past years, a brief background is provided to put them in perspective.

1.2 Healthy brain development

1.2.1 Brain morphology and dynamics

The brain is a complex organ with substantial variation between individuals. At first sight, the brain looks quite similar across subjects. However, people vary tremendously with respect to their personalities, cognitive capacities and behavioral characteristics. This variation is suggested to be attributable to the fine-tuning of the underlying networks of the brain (Ledoux, 2002).
The brain consists of two hemispheres and both left and right hemisphere can be divided in four lobes: frontal, parietal, temporal and occipital (Figure 1.1). Moreover, a distinction can be made between gray matter, the cell bodies or neurons, and white matter, the connecting fibers between the neurons (myelinated axons).
Maturation of the brain is already starting before birth. Nature is the dominant actor in this period, although the environment, such as maternal malnutrition, exposure to substance abuse (smoking, drugs, and medication) and viral infections can lead to adverse effects on the developing brain (Huttenlocher, 2002, pages 15 and 16). After birth, the brain creates more connections

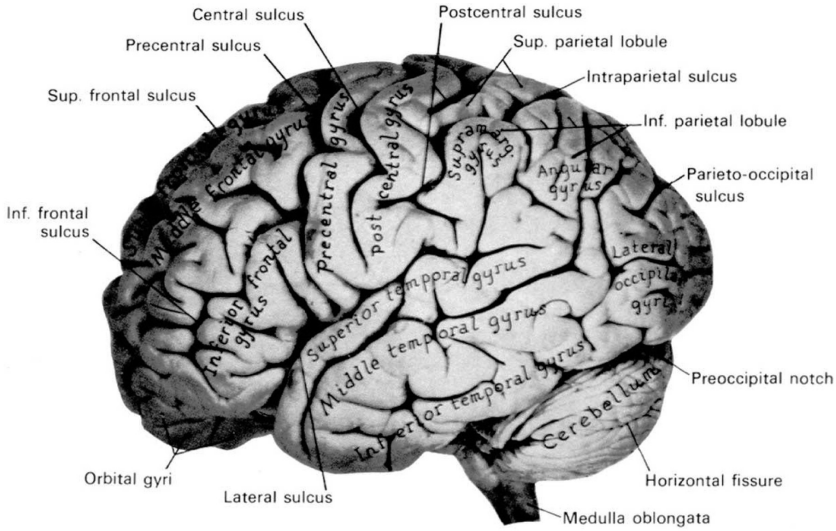

Figure 1.1. Photograph of a lateral view of the human brain. [From Carpenter, Human Neuroanatomy, 1983, reprinted with permission of Lippincott Williams & Wilkins, Philadelphia]

between the neurons. At this point the environment starts to take over the leading role in the process of brain development and learning will take place (Huttenlocher, 1994). When neurons are stimulated, new synapses (functional connections between the neurons) are established or strengthened. For example, when children start to understand and use spoken language (during the first and second year of life), new synapses are established in the areas of the brain that are associated with language comprehension and production (Herschkowitz et al. 1999). The connections of the neurons that are seldom used will die off. The brain's ability to change in response to environmental experience or the ability of the brain to rewire brain areas that are damaged (Konorski, 1948) is called plasticity. During adulthood, plasticity declines, but the brain remains capable of responding to environmental experiences throughout the life span (Stiles, 2002).

While the total size of our brain is already approximately 90% of its adult size around the age of six (Giedd, 2004), substantial changes in brain structure continue to take place after that, reflecting human brain development to be a lifelong process (Bartzokis et al., 2001). In early childhood there is growth of both gray matter and white matter. Different regions of the cortex mature at different rates and the prefrontal cortex (involved in complex cognitive behaviors, personality, decision making and moderating correct social behavior) is the last region to mature (Giedd et al., 1999). In adolescence, gray matter starts to decrease - possibly under the influence of hormonal

changes. In contrast, white matter continues to grow into adulthood and starts to decrease around age 45, thus following a quadratic curve (Bartzokis et al., 2001). The increase of white matter is attributable to axonal myelination. The exact processes underlying the observed changes in gray matter tissue is unknown. It is suggested that a combination of dendritic pruning, myelination and vascular changes are involved (Gogtay et al., 2004).
For years it was assumed that the total number of neurons was established prenatally and would not increase after birth. However, recent studies have now produced evidence for adult neurogenesis in certain brain areas, like the hippocampus and olfactory bulb (Eriksson, 1998; Gould et al., 2007). The hippocampus is involved in short term memory and spatial navigation and the olfactory bulb is involved in olfaction.

1.2.2 Influence of genetic and environmental factors

It is well known that overall head size, brain volume, as well as particular focal gray matter areas, are under considerable genetic control (>80%) in human adults as well as in children and adolescents (Peper et al., 2007). Recently it was suggested that in young as compared to older children and adolescents the influence of genetic and environmental factors vary with region and with age (Peper et al., in press; Lenroot, 2009; Giedd, 2007; Wallace, 2006). However, the extent to which genetic and environmental factors influence brain structure change during adulthood is unknown. Knowledge about the contributions of genetic and environmental factors in human brain development is of profound importance since brain structure has implications for brain functioning. Moreover, the tight coupling of brain structure and genetics may contribute in the search for genes involved in psychiatric diseases that affect the integrity of the brain, such as schizophrenia.

1.3 Schizophrenia

1.3.1 Background

Schizophrenia is a severe and disabling brain disorder characterized by abnormalities in the perception of reality and disruption of thought processes and feelings. This psychiatric disease results in great suffering not only for patients, but also has a large influence on the patient's relatives and friends. Schizophrenia generally manifests during early adulthood and in male on average earlier (around age 20-25 years) than in female (around age 25-30 years). In addition, there is a second smaller peak in female after age 45 (see review Goldstein and Lewine from Castle et al., 2000). The risk to develop

schizophrenia is suggested to be higher in male than in female (Aleman et al., 2003).

The first adequate clinical description of this disorder came from John Haslam (England) and Philippe Pinel (France) in 1809. They independently described what we recognize today as unmistakable schizophrenia. Pinel characterized the detoriation of mental abilities he observed in his chronically ill patients by a démence, which means loss of mind. In 1852, Benedict Morel added précoce (early) to refer to the early onset of symptoms. The definitive categorizer was Emil Kraepelin (1856-1926). He initiated the classification of mental disorders and named the disease dementia praecox. Eugen Bleuler (1857-1939) disagreed with Kraepelin in that the condition was neither dementia, nor did it always occur in young people. He focused more on the splitting of usually integrated psychic functions he observed in these patients and named the condition to the Greek roots 'schizein' (to split) and 'phren-' (mind). An unintended side effect of this term was that in daily life schizophrenia and multiple personality disorder (or split personality) are often confused (Gottesman, 1991).

Schizophrenia is a heterogeneous and complex syndrome. Clinical presentations differ from one patient to another due to variation in combinations of symptoms, severity, course and outcome of the illness. There is debate whether schizophrenia represents a single disorder or a number of syndromes. Thus, an accurate diagnosis requires a multidimensional evaluation of behavior. The most widely used standardized criteria to diagnose mental disorders come from the American Psychiatric Association's Diagnostic and Statistical Manual of Mental disorders, the DSM-IV (1994); and the World Health Organization's International Statistical Classification of Diseases and Related Health Problems, the ICD-10. However, in research, criteria according to the Diagnostic and Statistical Manual of Mental disorders are mostly used (Box 1).

Symptoms can be divided into positive and negative symptoms. Positive symptoms reflect an excess of normal functioning, like hallucinations, delusions and disorganized thinking. Negative symptoms refer to a reduction or loss of normal functioning, like affective flattening, apathy, lack of energy or emotional withdrawal. Moreover, cognitive deficits are also often present, including problems with executive functioning (planning and organization of behavior), attention, memory and concentration.

Treatment of schizophrenia consists of pharmacotherapeutical intervention with antipsychotic medication, psychosocial training and supportive therapies. Antipsychotic medication mainly helps to control the positive symptoms (delusions and hallucinations). However, training or therapies are essential

for coping with the negative symptoms (daily life functioning, social interaction and communication). Providing information to the patient and their immediate environment helps to prevent a relapse or psychosis.

Schizophrenia

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

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

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

C. Duration: Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in criterion A present in attenuated form (i.e., odd beliefs, unusual perceptual experiences).

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

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

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

Box 1.1. Diagnostic criteria for schizophrenia, according to the Diagnostic and Statistical Manual of Mental disorders, fourth edition (DSM-IV)

1.3.2 Etiology

The etiology of schizophrenia is still unknown, but family, twin and adoption studies indicate that genetic factors play an important role. The heritability to develop schizophrenia is estimated to be around 80% (Sullivan et al., 2003). Whereas schizophrenia affects approximately 1% of the general population, the incidence to develop schizophrenia is much higher in first degree relatives of schizophrenic patients. In siblings of schizophrenia patients the risk is up to 10 times higher. In dizygotic patients the risk increases to approximately 17% and for monozygotic twins of patients with schizophrenia this is even around 50% (Figure 1.2; Gottesman, 1991). These numbers indicate that genetic factors are important as predisposition but not sufficient themselves to develop schizophrenia. Environmental factors are also involved in the development of schizophrenia (McDonald, 2000). Viruses (Torrey, 1988) obstetric complications (Cannon et al., 2003), (pre- and perinatal) stress factors (Khashan et al., 2008; Norman et al., 1993) and nutrition (Hulshoff Pol et al., 2000) are suggested environmental factors involved in the development of schizophrenia. However, the specific contribution of these environmental factors remains to be elucidated.

1.3.3 Structural brain change

Kraepelin already considered in 1919 that he was defining a clinical syndrome which represented a disease of the brain. This was supported by early pathological studies describing a lower weight of the brain in schizophrenia patients. In 1927, Jacobi and Winkler observed a high prevalence of cortical and subcortical abnormalities in schizophrenia patients.

The most replicated finding in morphological studies of schizophrenia patients is the enlarged size of the lateral ventricles in schizophrenia patients: on average 20% as compared to healthy control subjects. Furthermore, a volume reduction in both gray (on average 4%) and white matter (on average 2%) volume is demonstrated in several studies. Moreover, lower volumes in the prefrontal and temporal lobes (especially the superior temporal gyrus) and part of the limbic system (including amygdala, hippocampus and parahippocampal gyrus) are reduced in schizophrenia patients as compared to healthy control subjects (Shenton et al., 2001; Wright et al., 2000). An enlargement of nucleus caudate in schizophrenia patients is suggested to be result of antipsychotic medication (Chakos et al., 1994). Although brain deficits are well established in schizophrenia, we have to keep in mind that these changes are subtle and based on quantitative measurements; i.e. based on the comparison of a group of schizophrenia patients with healthy comparison subjects. No diagnosis can be made on a single Magnetic

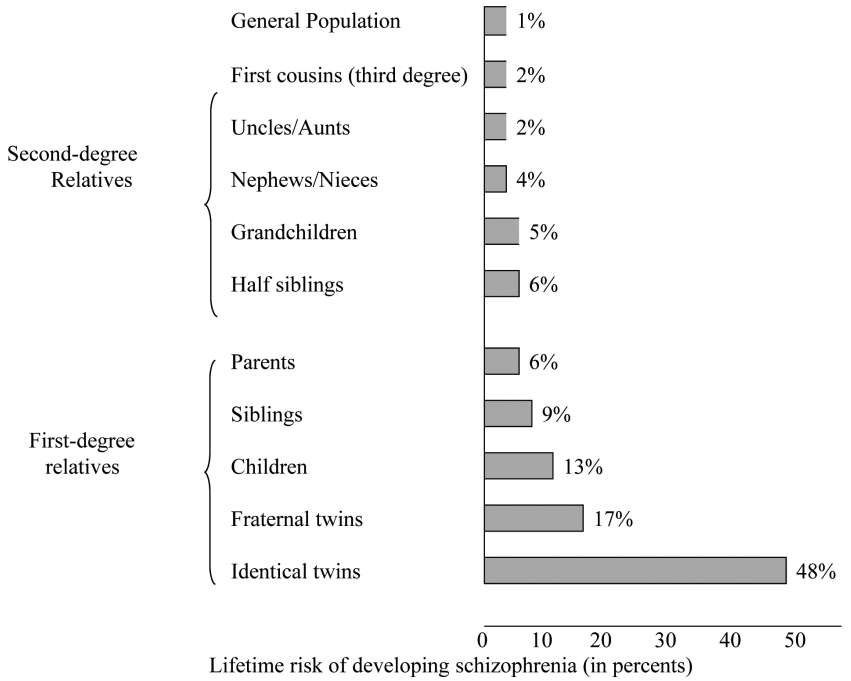

Figure 1.2. Grand average risks for developing schizophrenia compiled from the family and twin studies conducted in European populations between 1920 and 1987; the degree of risk correlates highly with the degree of genetic relatedness.

Resonance Imaging (MRI) scan, since the anatomy of the brains of schizophrenia patients looks normal at first sight.

How can the etiology of schizophrenia be linked to the brain abnormalities present in these patients? One of the theories concerning the etiology of schizophrenia is the neurodevelopmental model (Weinberger, 1987; Murray & Lewis, 1987). This model assumes that normal brain development is disrupted at a critical moment by a combination of environmental and genetic factors, which will lead to schizophrenia (Rapoport et al., 2005). Indeed, brain volume abnormalities are already present before onset of symptoms in high risk subjects (Lawrie et al., 2008). Furthermore, slight behavioral abnormalities are detected in children who later develop schizophrenia, like lower IQ (Reichenberg et al., 2005), deviant social behavior (Watt, 1978), reaching developmental milestones later than healthy controls and being more anxious (Jones et al., 1994). The influence of genetic factors is suggested since brain volume abnormalities (albeit to a less extent) are also demonstrated in first degree relatives of schizophrenia patients (Boos et al., 2007).

Several studies have demonstrated that the brain volume changes in

schizophrenia become progressively severe over time and are not limited to the early stages of the illness. This implicates that an active pathophysiological process is going on in chronic schizophrenia patients (Hulshoff Pol & Kahn, 2008; Pantelis et al., 2005). Therefore it is hypothesized that additively to the developed vulnerability of the brain earlier, a second hit occurs which make the difference in developing the illness or not (McCarley et al., 1999). Suggested factors for this supposed second hit are physical changes resulting from rising hormone levels in puberty, increasing (cognitive) demands or stress (Maynard et al., 2001). While poor outcome, effects of antipsychotic medication and genes involved in schizophrenia are suggested to play a role, the actual cause of these progressive brain volume changes is still unknown.

1.4 Brain Imaging

1.4.1 History

Neuroimaging can be divided into two broad categories: structural and functional imaging. Structural imaging involves brain morphology and functional imaging measures brain function. Studies described in this thesis are all using structural MRI.

In the early 1900s, history of neuroimaging started with techniques called ventriculography and pneumoencephalography. In ventriculography, X-ray images of the ventricular system were obtained by injection of filtered air directly through holes drilled in the skull. In pneumoencephalography, air was injected by lumbar spinal punction to enter the cerebral ventricles and cerebrospinal fluid compartments. Since these techniques were very painful and also resulted in very unpleasant side effects, the American Roentgen Ray Society degreed in 1929 that it was not appropriate to use normal subjects for control purposes in pneumoencephalography. Many studies in schizophrenia bypassed this difficulty by not including any comparison material at all.

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

Computed Tomography (CT) uses a computer to analyze the data from a series of X-ray images. Compared with the older imaging techniques, CT was a great improvement (Lawrie et al, 2004). Several CT studies have been conducted in schizophrenia patients. Johnstone and colleagues (1976) were among the first who reported enlarged ventricles in schizophrenia patients compared with healthy subjects using CT.

1.4.2 Magnetic Resonance Imaging (MRI)

In the early 1980s, Magnetic Resonance Imaging (MRI) was developed. MRI was initially referred to as nuclear magnetic resonance (NMR), since the mechanism is based on the fact that certain atomic nuclei are sensitive to magnetic fields. Approximately 60-70% of the human body consists of water, which means plentiful hydrogen nuclei (protons) that can be aligned in a strong magnetic field (longitudinal magnetization). The MRI scanner consists of a horizontal tube running through a strong magnet. The part of the body to be scanned is placed in the center of the magnetic field and a radio transmitter generates electromagnetic pulses, making the protons start to spin and act as tiny radio transmitters themselves. This radio signal generates an electric current in a receiver coil, which is measured as the MR signal. Different tissue types in the brain lead to differences in MR signal. The MRI scanner is able to determine from which location in the patient's body it receives certain signals. Finally, it integrates all this information to create a three-dimensional (3D) image.

The MRI signal will be in part dependent on magnetic field strength expressed in Tesla (T). In this thesis, subjects were scanned on a 1.5 T scanner. 3 T machines are increasingly becoming available and recently a 7 T scanner operates at the University Medical Centre, Utrecht.

MRI allows for making a distinction between gray and white matter volume and for the measurement of cortical and subcortical structures. Moreover, MRI uses no X-rays and is generally a very safe procedure.

1.4.3 MRI analysis

MR images are composed of 3D voxels. A voxel (a portmanteau of the words volumetric and pixel) is analogous to a pixel, but represents instead of 2D, 3D image data. With specific software, different brain areas can be segmented i.e., partitioned into meaningful structures. Several regions of the brain, such as intracranium, total brain, and gray and white matter (Schnack et al., 2001a) of the cerebrum (total brain excluding cerebellum and stem), and lateral and third ventricles are segmented (Schnack et al., 2001b). Then the number of voxels within a given segment is counted. Since the voxel size is known, in our case 1x1x1.2mm^3, volumes can be calculated. Chapters 3, 4 and 5 describe global brain volume measurements. Additionally, in one study the brain is divided in frontal, parietal, temporal, and occipital lobes to measure each lobe's volume (chapter 5). Recently, methods have been developed to measure cortical thickness (Kim et al., 2005). This method implicates an accurate reconstruction of the inner and outer cortical surfaces of the human cerebrum or the distance between the white matter and gray matter at each

point along the cortical surface. Chapter 2 concerns a study measuring cortical thickness.

1.5 Genetic and environmental influences

1.5.1 Behavior genetics

Since the etiology of brain volume changes in health and in schizophrenia remains largely unknown, it would be of particular interest to explore the sources underlying individual differences in brain volume change and to unravel the genetic mechanisms that underlie schizophrenia.

Most behavioural traits and complex disorders are influenced by heredity: the passing of traits from parents (or ancestors) to offspring. The study of heredity is called genetics. However, in most behavioral traits usually multiple environmental influences are also involved. So, individual differences in a complex trait or phenotype (like brain structure change or schizophrenia), may be due to genetic or environmental variance. This implicates that if genes influence a certain phenotype, variation in these genes can lead to variation in that specific phenotype. Two alleles (one from mother and one from father) make up the individual's genotype. Genetic effects can be additive (effects of different alleles are equal to the sum of their individual effects), dominant (interaction of alleles at a locus) or epistatic (interaction of alleles at different loci). The other main sources of variance in a phenotype are environmental factors (Plomin et al., 2008). Examples of environmental factors are prenatal environment, nutrition, diseases and life events.

Quantitative genetics estimates the extent to which observed differences among individuals are due to genetic differences and to environmental differences, without specifying what these specific genes or environmental factors are. It is focused on individual differences and therefore the variance is important. Accounting for the variance means finding the causes of the deviations from the mean: i.e. which factors make individuals in a population differ from one other?

To be able to estimate the influence of genotype and environment on phenotypic variation, data from groups of individuals who are genetically related is needed. One of the most powerful designs to detect genetic and shared environmental effects is the classical twin design (Boomsma et al., 2002; Martin et al., 1997).

1.5.2 Twin design

In the classical twin design, the resemblance of monozygotic (MZ) twins is compared to the resemblance of the dizygotic (DZ) twins. MZ twin or identical twins are (nearly always) genetically identical since they derive from one fertilized egg (zygote). DZ or fraternal twins are developed from separately fertilized eggs and share like other full siblings, on average 50% of their genes. Both types of twins are usually reared together and consequently share their familial environment (such as parental care and social economical status). However, both of them also experience unique environmental influences (such as life-events).

Resemblance between the twin-pairs is reflected in the twin-pair correlations (respectively rMZ and rDZ for the correlation between MZ and DZ pairs). These correlations provide information about the relative influence of genes or shared and unique environmental factors on the variance of a phenotype.

1. When MZ twins resemble each other more than DZ twins, genetic influence (A) is indicative for that trait (rMZ = 2 * rDZ).
2. The presence of shared environmental factors (C) is suggested when correlations in DZ twins are larger than half the MZ correlation (rDZ = rMZ).
3. When MZ twins resemble each other more than DZ twins and when correlations in DZ twins are smaller than half of the MZ correlation, the influence of both A and C is suggested.
4. Any variance that is not shared between MZ twins can be attributed to unique environmental factors (E: 1 – rMZ). E is always present and also implicates measurement error.

The heritability (h^2) of a phenotype is the relative proportion of variance which can be attributed to additive genetic effects. A first impression of the heritability of the phenotype can be estimated from twice the difference between MZ and DZ correlations: 2(rMZ – rDZ). Moreover, the influence of common environmental factors can be calculated as 2(rDZ) – (rMZ) and the influence of unique environmental effects as 1 – (rMZ) (Boomsma et al., 2002).

Instead of a single variable (univariate), most studies are designed to analyze more than one phenotype per person. In multivariate designs the causes of association between traits can be investigated. Therefore cross-trait/cross-twin correlations are calculated. When for example in a bivariate design the traits schizophrenia and brain volume are investigated, the correlation between schizophrenia in twin 1 with brain structure in twin 2 is obtained. The phenotypic correlation (r_{ph}) indicates whether there is an association

between schizophrenia and brain structure. This correlation can be divided in a genetic correlation (r_g) and an environmental correlation (r_e). The genetic correlation provides the correlation between genetic factors influencing schizophrenia and brain structure and reflects whether the association between these phenotypes has a genetic origin. When the cross-correlation between schizophrenia in twin 1 and brain structure in twin 2 is higher in MZ twins as compared to DZ twins, a common genetic factor influencing both phenotypes is suggested (Plomin et al., 2008).
The bivariate models assume a continuum of risk that is normally distributed with the disorder (i.e. schizophrenia) occurring only when a certain threshold of liability is exceeded. Since twin-pairs are selected for schizophrenia and not coming from a random sample, this would result in an overestimation of the prevalence for schizophrenia. Therefore, the critical threshold for schizophrenia is based on fixed values based on a meta-analysis of twin studies (Sullivan et al., 2003). Heritability (h^2) is fixed at 81% and the influence of family-related environmental factors (c^2) at 11% (rMZ = 0.92, rDZ = 0.52). The prevalence is set to 1%, (resulting in a critical threshold at 2.33), which is based on epidemiological studies (Gottesman, 1991).

1.5.3 Structural Equation Modeling (SEM) or model fitting

As compared to the traditional correlational methods, Structural Equation Modeling (SEM) is a more advanced statistical method that estimates regression coefficients (parameters) between unobserved and observed variables. The advantage of SEM is that it can model multivariate data and incorporate many different types of family structure. It also facilitates the calculation of confidence intervals around the parameter estimates. Moreover, it is possible to test the relative goodness of fit of different models describing the observed data and compare these. For example, the ACE model (family resemblance is due to additive genetic plus shared environmental effects) is compared with the AE model (family resemblance is solely due to additive genetic effects) and the E model (no family resemblance); respectively: ($a > 0$, $c > 0$, $e > 0$) versus ($a > 0$, $e > 0$, $c = 0$) versus ($e > 0$, $a = c = 0$). The observed data that we model are typically the variance-covariance matrices for family members. These matrices are compared with the variances and covariance predicted by the model: the expected variance for MZ and DZ twins equals $a^2 + c^2 + e^2$ and the expected covariance equals $a^2 + c^2$ for MZ twins and $½\ a^2 + c^2$ for DZ twins. When the observed variances and covariance differ only slightly from those predicted by the model, the model will show a good fit according to maximum likelihood χ^2 tests. Aim is to select the best model, which is the model with (1) the fewest parameters and (2) matching

the observed data as closely as possible (Neale et al., 1992).
Path analysis has been widely applied to problems in the genetic field and behavioral sciences. This technique represents graphically the linear 'structural' models of the observed data. Predictions for the variances and covariances of the traits can be derived by this diagram since the arrows represent causal and correlational relations between the traits (Figure 1.3). Moreover, this method permits translation into matrix formulation which is needed for Mx, the statistical package that is applied for SEM (Neale 2003).

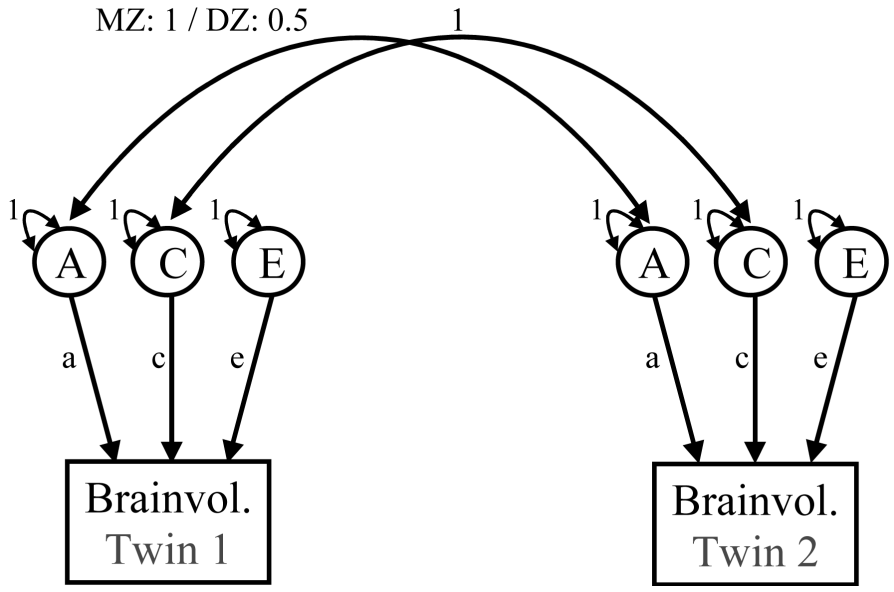

Figure 1.3: Univariate path diagram of the classical twin design.
Phenotypes of twin 1 and twin 2 are influenced by additive genetic (A), common environmental (C) and unique environmental influence (E).The correlation between C of twin 1 and C of twin 2 is 1 and the correlation between A of twin 1 and A of twin 2 is 1 for MZ twins (genetically identical) and ½ for DZ twins (share on average 50% of their segregating genes.

1.6 Aim and outline of this thesis

The general aim of this thesis is to explore the possible mechanisms underlying the individual differences in brain structure and brain structure change in healthy adults and schizophrenia patients. For this purpose we have conducted two lines of research: one study comprises healthy individuals and the other studies comprise schizophrenia patients. All studies are conducted in relatives

to be able to disentangle genetic and environmental influences on the studied phenotypes.

Development of the human brain is a lifelong process. However, the trajectories of structural brain changes, their functional significance and the extent to which genetic and environmental factors are involved during adulthood are unknown. In chapter 2, structural brain change in an epidemiological sample that consists of healthy twin-pairs and their siblings is described. Part of this sample is assessed neuropsychologically using intelligence tests at the Free University in Amsterdam. Results of these tests are used to link changes in brain structure with estimates of IQ.

The second line of research implicates brain structure (change) in schizophrenia. For this purpose brain morphology and changes in brain morphology were studied in two samples: (1) in schizophrenia patients and their healthy siblings, and (2) in twin-pairs discordant for schizophrenia. Earlier measurements in a part of this sample of discordant twin pairs suggested that both genetic and additional disease-related factors are involved in the decreases in whole brain volume observed in schizophrenia patients (Baaré et al., 2001). Whether genetic or environmental factors are involved in gray and white matter volume abnormalities in schizophrenia remains inconclusive. Therefore MRI brain scans of 11 MZ and 11 same-gender DZ twin pairs discordant for schizophrenia are compared with 11 MZ and 11 same-gender DZ healthy control twins. Chapter 3 describes the differences between the twin pairs discordant for schizophrenia and the healthy comparison twin pairs. Moreover, within twin pairs, patients are compared with their co-twins.

Chapters 4 and 5 concern the temporal aspects of brain volume abnormalities in schizophrenia. Earlier studies have demonstrated that at least part of the brain volume change in schizophrenia is progressive over the course of the illness. However, whether these progressive changes are mediated by genetic or disease-related factors remain to be studied. Chapter 4 focuses on the brain volume changes found in schizophrenia patients and their healthy siblings. In this 5 year follow-up study two MRI scans of the brain are acquired in 11 patients, 11 siblings and 33 healthy controls.

Since sibling studies cannot disentangle the extent of genetic and common environmental contributions to familial influences, a follow-up study in discordant twin-pairs is conducted. In chapter 5, a longitudinal measurement of twin-pairs discordant for schizophrenia is presented. In this study 9 MZ and 10 DZ twin pairs discordant for schizophrenia and 14 MZ and 13 DZ healthy twin pairs have completed both scans with an average scan-interval of 5 years. Results of global and lobar brain volume measurements are described.

Chapter 6 summarizes all the studies briefly and gives an overview of the main results. Finally, implications for future research are discussed.

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Chapter 2

How much brain we have and how much brain we keep may be a different matter and is associated with intelligence

Rachel G.H. Brans, René S. Kahn, Hugo G. Schnack,
G. Caroline M. van Baal, Danielle Posthuma, Neeltje E.M. van Haren,
Claude Lepage, Jason P. Lerch, D. Louis Collins, Alan C. Evans,
Dorret I. Boomsma, Hilleke E. Hulshoff Pol

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Abstract

Human brain volume is highly heritable and has been shown to be related to intelligence. Although it is known that brain volume changes throughout adult life, it is unclear whether these changes are under genetic control and related to intelligence. Cortical thickness change was measured using MRI over a 5-year period in 77 monozygotic and 84 dizygotic twins and their 22 siblings from 106 twin families. Here we show that brain volume change through the 3rd to 6th decade of life is heritable. Moreover, the degree of brain loss during that time period (particularly in frontal and temporal cortices) is inversely related to level of intelligence. Interestingly, genes involved in brain loss over time overlap with genes for intelligence and differ from those related to absolute brain volume. Thus, continued brain maturation in adult life promotes our intellectual development and both depend (in part) on the same set of genes.

Introduction

Human brain volume is highly heritable in adults (Baaré et al., 2001; Thompson et al., 2001; Wright et al., 2002; Pfefferbaum et al., 2000) and in children (Peper et al., 2009; Wallace et al., 2006; Lenroot et al., 2009) with heritability estimates exceeding 90% (Peper et al., 2007). This suggests that the genetic influence on overall brain size is established early in life and remains important into adulthood. However, it is also well known that brain volume is far from static and changes throughout life. Having reached approximately 90% of its adult size around the age of six (Giedd et al., 1999), dynamic changes in brain tissue continue to take place in children, adolescents (Giedd et al., 1999; Gogtay et al., 2004) as well as in adults (Bartzokis et al., 2001; Pfefferbaum et al., 2004; Raz et al., 2005; Liu et al., 2003). The most prominent change in adulthood is loss of brain tissue. However, although variation in brain volume is genetic, it is unknown whether the degree of brain volume loss over time is genetically determined. Moreover, it is unknown whether genes that influence brain loss overlap with those for brain volume per se.

Were genes to exert their influence on brain volume change this would provide important implications for both healthy ageing as well as for diseases that are accompanied by progressive brain volume loss such as schizophrenia (Brans et al., 2008; Hulshoff Pol & Kahn, 2008) and depression (Frodl et al., 2008). Several studies have shown that total brain volume (Thompson et al., 2001; Posthuma et al., 2002) and certain focal gray (Thompson et al., 2001; Posthuma et al, 2002; Frangou et al., 2004; Choi et al., 2008; Haier et al., 2004; Hulshoff Pol et al., 2006) and white (Hulshoff Pol et al., 2006) matter densities are positively related to the level of intelligence and that these associations are mediated by common genetic factors (Thompson et al., 2001; Wright et al., 2002; Posthuma et al., 2002; Haier et al., 2004; Hulshoff Pol et al., 2006; Toga & Thompson, 2005; Boomsma et al., 2002). Thus, genes implicated in individual variations in intelligence (Plomin & Spinath, 2004) overlap with genes for brain volume, although the causality underlying this association remains to be determined (Van Leeuwen et al., 2009). In childhood, changes in cortical thickness have been related to intelligence (Shaw et al., 2006). However, whether brain volume change is related to intelligence in adulthood, and whether genes mediate such a possible association, has not been studied. This study was set out to examine if change in brain volume, and particularly in cortical thickness, is related to intelligence and if so, whether this relationship is genetically determined. To this end, we studied the heritability of whole brain volume change and cortical thickness change

and their possible (genetic) link with intelligence in a large longitudinal magnetic resonance imaging (MRI) study in 242 adult individuals from 106 twin families between 19-55 years of age, with a 5-year interval between scans.

Results

Brain volume change

On average, whole brain volume change in subjects between the ages of 19 and 55 was small but the individual differences in extent of change varied considerably (mean (sd) whole brain volume at baseline=1260 (125) ml; change=-3.01 (21.46) ml; percentage change=-.23% (1.71%)). Indeed, in 40% of participants whole brain volume and in 26% gray matter volume increased over the 5-year period. On average, cerebral gray matter volume decreased over the five-year interval (mean (sd) gray matter volume at baseline=645 (67) ml; change=-13.71 (22.75); percentage change=-2.09% (3.49%)).

Heritability of brain volume change

Whole brain volume change was significantly genetically determined. Additive genetic effects accounted for 38% [95% Confidence Interval (CI) = 13 to 55%] of the variance in whole brain volume change. No significant phenotypic and genetic correlations were found between whole brain volume (94% heritable) and whole brain volume change (r_{ph}=-.10 [-.25 to .05]; r_g=-.04 [-.30 to .25]). This implies that different (genetic) systems are involved in determining absolute brain volume and the degree of brain volume change over time. Cerebral gray matter volume change was not significantly influenced by genetic factors, with additive genetic influences accounting for 11% [95% CI=0 to 39%] of its variance, whereas gray matter volume was for 87% heritable. A significant phenotypic correlation was found between gray matter volume change and gray matter volume: a larger initial volume was associated with a more prominent decrease in volume after 5 years (r_{ph}= -.19 [-.32 to -.05]). Bivariate genetic analysis showed that this correlation was environmental (r_e= -.61[-.76 to -.39]) and not due to common genes (r_g=.04; [-1 to 1]).

Cortical thickness change

Focal gray matter volume change was measured as thickening and thinning of the cortex over the 5-year interval. Thickening was found in the parahippocampal gyri, frontal poles bilaterally, right medial frontal, and in

the occipital cortices (Figure 1). When related to age at the first measurement, the increase in the parahippocampal gyrus became more prominent, while in the frontal poles and right medial frontal cortices the increase became less prominent with increasing age. Cortical thinning was primarily found in superior, medial, and inferior frontal, sensory-motor, insula, superior temporal, and lateral and medial parietal cortices.

Heritability of cortical thickness change

Heritabilities for cortical thickness change were up to 56%. Cortical thickening was significantly heritable in the parahippocampal gyri (heritability in percentages with 95% CI: left 48%; 25-66%, right 47%; 24-64%), frontal poles (left 45%; 19-65%, right 43%; 18-63%), and right medial frontal cortex (56%; 31-73%), after Bonferroni correction for multiple comparisons (Figures 2 and 3; Supplementary material Table 1); Cortical thinning was significantly heritable in the left orbitofrontal (41%; 15-63%), superior temporal (left 55%; 32-72%, right 45%; 24-62%), left superior frontal (54%; 30-72%), lateral parietal (left 28%; 11-45%, right 45%; 23-63%), and right lateral (38%, 16-58%) and right medial (35%; 6-62%) occipital cortices, after Bonferroni correction for multiple comparisons. Genetic factors influencing cortical thinning of the left superior frontal and superior temporal cortices were significantly different from genetic factors influencing absolute cortical thickness and survived Bonferroni correction, which indicates that different genetic factors influence change in cortical thickness and absolute cortical thickness in these areas (Figures 2 and 3; Supplementary material Table 1).

Associations between brain structure change and level of intelligence

No significant correlations were found between volume change in whole brain and cerebral gray matter and level of intelligence. Significant positive associations with level of intelligence were found for cortical thickening in the left and right parahippocampal (r_{ph}=.26; r_{ph}=.24) and right medial frontal (r_{ph}=.27) cortices, and for thinning in the left and right inferior frontal (near Broca's area) (r_{ph} =.33 and r_{ph}=.34), left and right superior frontal (r_{ph}= .24; r_{ph} =.32), left superior temporal (r_{ph}=.20), left and right sensory-motor (r_{ph}=.31; r_{ph}=.34), and right occipital (r_{ph}=.34) cortices (Supplementary table 2; Figure 4). All associations indicate that increased thickening and decreased thinning of the cortex is associated with higher IQ scores. There were significant influences of common genes on the associations between cortical thickness change and level of intelligence in these areas. Thus, genes that are implicated in cortical thickness change overlap with those involved in the level of intelligence.

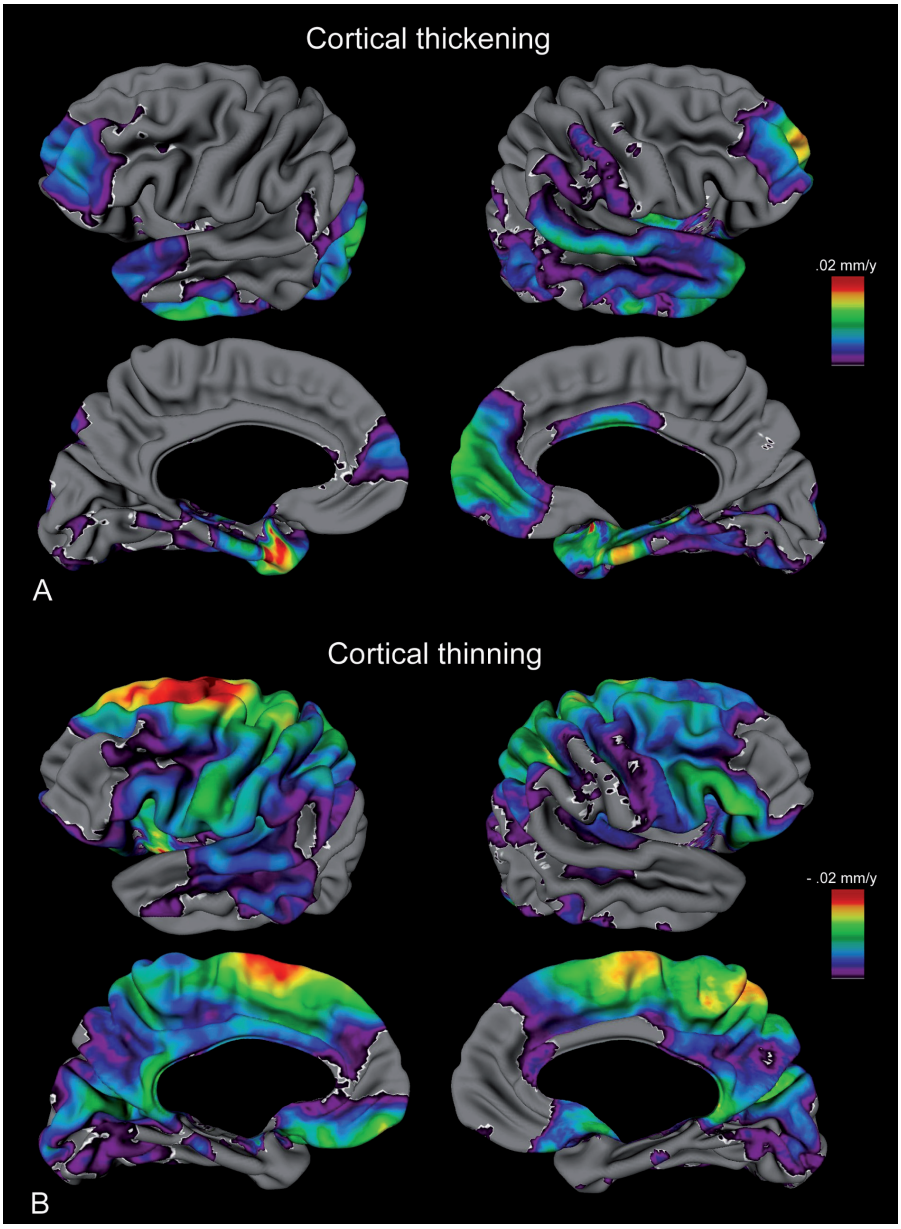

Figure 1. Cortical thickness change in the adult human brain. Mean cortical thickening (A) and thinning (B) in mm change per year between the ages of 19 through 55 years (regressed back to 30 year old right-handed persons; corrected for age, sex, and handedness; the maximum change was -0.026 mm/yr).

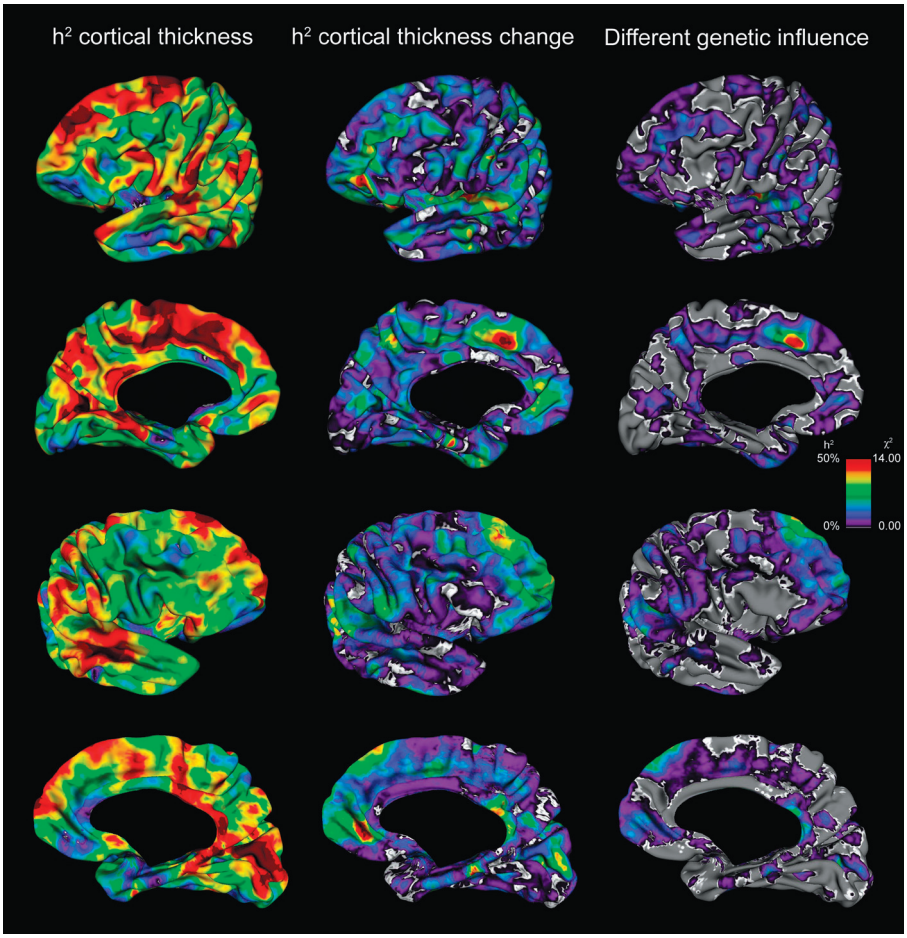

Figure 2. Heritability of cortical thickness and cortical thickness change. The heritability (h^2) of cortical thickness (left), the heritability of cortical thickness change (middle) and the chi-squared test statistics (χ^2) of specific genetic influences on cortical thickness change, which are not associated with genetic influences of absolute cortical thickness (right).

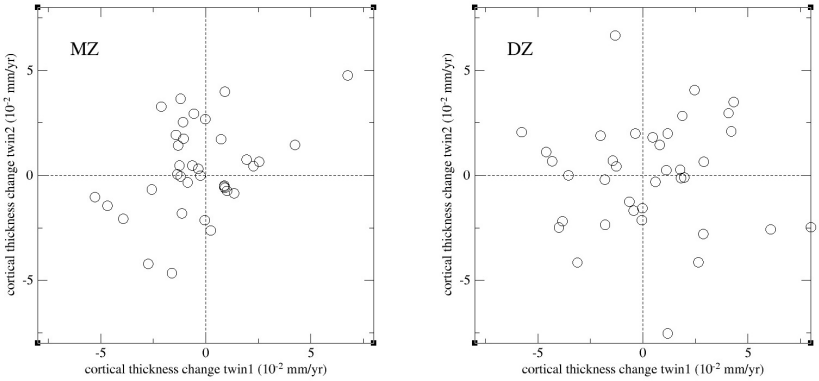

Figure 3. Heritability of cortical thickness change in the left superior frontal cortex. Dots represent values of cortical thickness change for individual monozygotic (left) and dizygotic (right) twin pairs at (x,y,z) (-4,25,38). Twin 1 and twin 2 represent the two individuals of a twin pair. The correlation within MZ twin pairs is much higher than that in DZ twin pairs, indicating that genes influence cortical thickness change in this area (h^2 change=54%).

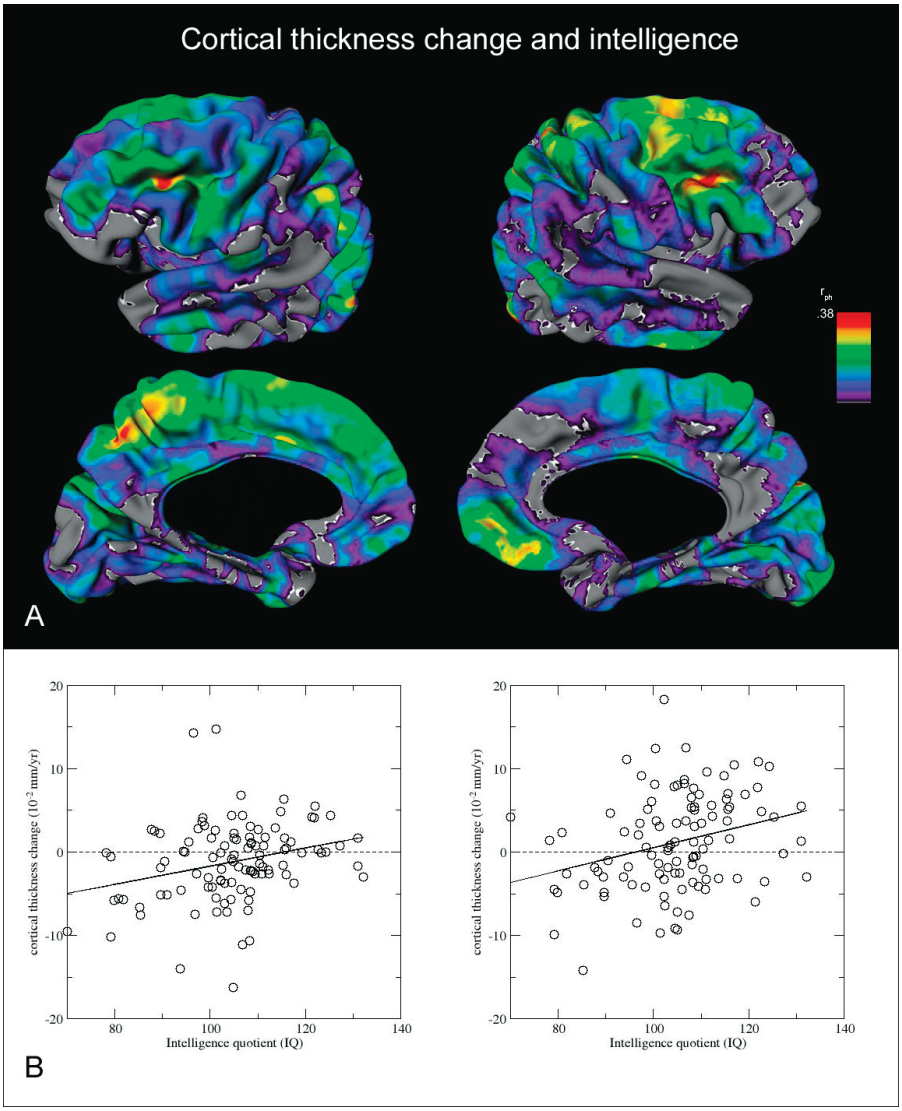

Figure 4. Cortical thickness change and intelligence. The phenotypic correlations, or within-subjects correlations corrected for familiar dependencies, between cortical thickness change and intelligence were positive (A) and mediated through common genes. More cortical thickening and less cortical thinning correlated with higher intelligence in the superior frontal cortex left at (x,y,z) (-4,24,60) (phenotypic correlation r_{ph}=.24) and parahippocampal gyrus left at (x,y,z) (-27,-7,-5) (r_{ph}=.26) (B) as well as in other brain areas.

Supplementary table 1: Estimated influences of additive genetic (h^2) factors on and correlations between cortical thickness and cortical thickness change

		Cortical thickness	Cortical thickness change		Cortical thickness and cortical thickness change				
Cortical area	**X, Y, Z**	**h^2 (%) [CI]**	**h^2 (%) [CI]**	**h^2 (χ^2)**	**h^2_{biv}**	**A-spec (χ^2)**	**r_{ph}**	**r_g**	**r_e**
Thickening									
Frontal pole left	-35, 56, -13	7 [0 to 33]	45 [19 to 65]	15.40	6 [0 to 49]	0.29	-.45 [-.56 to -.32]	-.14 [-1 to 1]	-.59 [-.74 to -.38]
Frontal pole right	13, 69, -3	50 [31 to 65]	43 [18 to 63]	13.81	88 [36 to 100]	4.76	-.31 [-.44 to -.17]	-.59 [-.95 to -.23]	-.07 [-.33 to .20]
Medial frontal right	3, 36, -16	16 [0 to 37]	56 [31 to 73]	17.09	35 [0 to 69]	1.45	-.45 [-.56 to -.32]	-.53 [-1 to 1]	-.48 [-.66 to -.26]
Parahippocampal left	-19, -20, -25	7 [0 to 30]	48 [25 to 66]	19.85	7 [0 to 45]	0.35	-.45 [-.56 to -.33]	-.17 [-1 to 1]	-.61 [-.75 to -.40]
Parahippocampal right	15, -35, -10	47 [25 to 64]	47 [24 to 64]	17.12	68 [34 to 97]	3.43	-.49 [-.59 to -.37]	-.71 [-1 to -.38]	-.29 [-.52 to -.02]
Thinning									
Orbitofrontal left	-41, 25, -18	55 [34 to 70]	41 [15 to 63]	16.36	74 [41 to 100]	0.79	-.56 [-.66 to -.45]	-.87 [-1 to -.57]	-.29 [-.54 to -.00]
Sup. frontal left	-4, 25, 38	58 [37 to 73]	54 [30 to72]	16.99	62 [13 to 95]	14.33*	-.39 [-.52 to -.25]	-.44 [-.71 to -.08]	-.34 [-.57 to -.05]
Sup. temporal/Heschl's left	-52, -13, 7	49 [30 to 64]	55 [32 to 72]	18.37	57 [4 to 94]	15.53*	-.33 [-.46 to -.19]	-.36 [-.65 to -.02]	-.29 [-.51 to -.04]
Sup. temporal right	55, -43, 12	50 [31 to 64]	45 [24 to 62]	17.43	54 [16 to 85]	11.15	-.41 [-.52 to -.27]	-.47 [-.75 to -.13]	-.35 [-.55 to -.11]
Parietal, lateral left	-42, -26, 20	45 [27 to 61]	28 [11 to 45]	19.00	74 [43 to 100]	0.00	-.48 [-.58 to -.36]	-1 [-1 to -.78]	-.20 [-.39 to .01]
Parietal lateral right	59, -52, 35	13 [0 to 35]	45 [23 to 63]	15.02	27 [0 to 66]	0.94	-.42 [-.53 to -.29]	-.48 [-1 to 1]	-.43 [-.62 to -.21]
Lat. occipital right	44, -74, 23	15 [2 to 36]	38 [16 to 58]	20.81	27 [0 to 38]	0.11	-.32 [-.44 to -.17]	.76 [-.03 to 1]	-.68 [-.80 to -.50]
Med. occipital right	5, -83, 18	52 [30 to 68]	35 [6 to 62]	16.96	68 [27 to 100]	0.41	-.56 [-.65 to -.45]	-.90 [-1 to -.56]	-.32 [-.56 to -.00]

Cortical areas with significant heritability for thickness change over time at α=.05 after Bonferroni correction for multiple comparisons (χ^2>14.3), corrected for age and gender. Confidence intervals crossing 0 are not significant for r_{ph}, r_g, r_e. Confidence-intervals including -1 or 1 indicate that there is no evidence different genes are involved in cortical thickness change as compared to cortical thickness in that specific vertex; h^2_{biv}: represents bivariate heritability; the influence of genetic factors on the correlation between cortical thickness and cortical thickness change. I.e. additive genetic effects account for 6% of the covariance of cortical thickness and cortical thickness change in the peak vertex of the left frontal pole. A-spec: reflects the specific genetic influence on cortical thickness change compared with cortical thickness; * Significant at α=.05 with Bonferroni correction (χ^2>14.3); Phenotypic correlations (r_{ph}): reflects the correlation that is found between cortical thickness and cortical thickness change within individuals. I.e. the correlation between cortical thickness and cortical thickness change in the peak vertex of the left frontal pole = -.45, which indicates that there is a significant correlation between cortical thickness and cortical thickness change; Genetic and environmental correlation: phenotypic correlations are decomposed into genetic (r_g) and environmental components (r_e), providing information regarding the possible shared genetic and environmental influences on brain volume and brain volume change.

Supplementary table 2: Cortical thickness change and intelligence: peak values of the r_{ph} maps between thickness change and IQ

		Cortical thickness change	Cortical thickness change and intelligence				
Cortical area	**X, Y, Z**	**h^2 (%) [CI]**	**h^2_{biv} (%) [CI]**	**χ^2 r_{ph}**	**r_{ph}**	**r_g**	**r_e**
Thickening							
Parahippocampal left	-27, -7, -5	22 [9 to 45]	71 [58 to 83]	20.89	.26 [.09 to .42]	1.00 [.49 to 1.00]	-.71 [-.86 to -.36]
Parahippocampal right	24, -25, -27	10 [2 to 29]	82 [62 to 100]	12.06	.24 [.06 to .40]	1.00 [.35 to 1.00]	-.27 [-.55 to .09]
Med. frontal right	3, 39, 28	9 [1 to 23]	94 [71 to 100]	13.01	.27 [.09 to .43]	1.00 [.46 to 1.00]	-.07 [-.39 to .29]
Thinning							
Superior frontal left	-4, 24, 60	5 [1 to 32]	85 [20 to 100]	11.20	.24 [.08 to .39]	1.00 [.10 to 1.00]	.14 [-.26 to .49]
Superior frontal right	25, 13, 53	27 [4 to 54]	92 [73 to 100]	16.89	.32 [.15 to .47]	.70 [.33 to 1.00]	-.13 [-.54 to .33]
Medial frontal left	-9, 36, 10	19 [1 to 44]	60 [11 to 78]	12.75	.08 [-.12 to .27]	.56 [.03 to 1.00]	-.66 [-.84 to -.29]
Inferior frontal left	-41, 24, 23	9 [1 to 25]	88 [48 to 100]	14.42	.33 [.14 to .49]	1.00 [.39 to 1.00]	.18 [-.17 to .49]
Inferior frontal right	47, 21, 23	13 [1 to 38]	82 [31 to 100]	14.44	.34 [.14 to .49]	.81 [.19 to 1.00]	.25 [-.18 to .58]
Sup. temporal/Heschl's left	-40, -19, -3	14 [1 to 37]	76 [47 to 100]	9.31	.20 [-.01 to 39]	.81 [.17 to 1.00]	-.38 [-.69 to .12]
Med. parietal/precuneus left	-10, -58, 54	22 [4 to 47]	94 [73 to 100]	14.00	.35 [.15 to .51]	.83 [.32 to 1.00]	-.12 [-.55 to .38]
Superior sensory left	-29, -27,59	17 [5 to 37]	82 [64 to 100]	15.70	.31 [.12 to .48]	1.00 [.50 to 1.00]	-.40 [-.68 to .08]
Superior sensory right	28, -36, 69	5 [1 to 24]	61 [7 to 93]	20.92	.34 [.17 to .50]	1.00 [.19 to 1.00]	.51 [.10 to .74]
Occipital pole right	28, -99, -9	9 [1 to 22]	84 [46 to 100]	21.18	.34 [.18 to .48]	1.00 [.54 to 1.00]	.22 [-.19 to .56]

Confidence intervals crossing 0 are not significant for r_{ph}, r_g, r_e. Cortical thickness change was corrected for age and gender. *h^2:* Sources of variance on cortical thickness change. This measure reflects the influence of additive genetic factors on cortical thickness change in percentages and 95% confidence interval. E.g., additive genetic effects account for 22% of the variance on change in the peak vertex of the parahippocampal gyrus in the left hemisphere. *h^2_{biv}:* represents bivariate heritability; the influence of genetic factors on the correlation between cortical thickness change in this vertex and IQ. I.e. additive genetic effects account for 71% of the covariance of cortical thickness change in the peak vertex of the parahippocampal gyrus in the left hemisphere and IQ. *Phenotypic correlations (r_{ph}):* reflects the correlation that is found between cortical thickness change in this vertex and IQ within individuals. E.g., the correlation between cortical thickness change in the peak vertex of the left parahippocampal gyrus left and IQ=.26, which indicates that there is a significant correlation between cortical thickness change and IQ. *Genetic and environmental correlation:* phenotypic correlations are decomposed into genetic (r_g) and environmental components (r_e), providing information regarding the possible shared genetic and environmental influences on cortical thickness change in this vertex and IQ.

Implications of the study

Here we present three novel and interrelated findings: genes influence adult human brain changes over time; the degree of this change is related to intelligence; and both are influenced by common genes. We also found that the genetic systems responsible for brain volume change over time in the frontal and temporal cortices are different from those determining absolute brain volumes in these areas, suggesting that genes involved in brain development may differ from those that influence plasticity of the brain. These findings may have important implications in the quest to find genes that affect brain growth and plasticity and will also be relevant for finding the (genetic) causes for diseases characterized by abnormal brain growth and plasticity in adulthood, such as schizophrenia (Brans et al., 2008; Hulshoff Pol & Kahn, 2008), and depression (Frodl et al., 2008).

The heritability estimates that we found for brain change over time, mainly expressed as cortical thickening and thinning of 0.5 to 1.0% per year, were considerable. The highest values (between 50-56%) were found in the frontal and temporal cortices including the parahippocampal gyri. The magnitude of several of these changes, and particularly of the thinning in the left superior frontal and left superior temporal cortices, is determined by genes that are different from those that influence absolute cortical thickness in these areas. The influence of genes on cortical thickening in the parahippocampal gyrus increased over time, with environmental influences on absolute parahippocampal thickness and genes influencing its change. Somewhat lower heritability values were found in the parietal and occipital cortices (between 40-48%), and the magnitude of those changes may overlap with genes for absolute cortical thickness in these areas.

To what extent these findings are specific for cortical gray matter and continue into senescence remains to be studied (Pfefferbaum et al., 2004). We found cortical thinning in vast areas of the cortex up to the 6th decade of life, including in the superior frontal, left superior temporal and parietal cortices. That most cortical gray matter areas become thinner in adulthood extends findings of cortical thinning in childhood and adolescence (Giedd et al., 1999; Gogtay et al., 2004). It is also in agreement with volume decreases in overall gray matter over time in adults (Raz et al., 2005; Liu et al., 2003; Brans et al., 2008; Hulshoff Pol & Kahn, 2008). The finding of cortical thickening in the frontal pole, right temporal, parahippocampal and occipital cortex is consistent with a study in 20 to 77 year olds, where minimal change in the entorhinal cortex (part of the parahippocampus) was found and none in the primary visual cortex despite overall widespread loss of brain volume (Raz et al., 2005).

Importantly, genes loading for higher intelligence were found to overlap with those loading for brain growth (cortical thickening) or attenuated brain loss (cortical thinning) in frontal and temporal cortex. Thus, plastic properties of the cortex are influenced by genes that also affect intelligence. Interestingly, the association between cortical thickness changes and intelligence has also been described in children and adolescents (Shaw et al., 2006), suggesting that the relationship between intelligence and brain development continues throughout life.

One may speculate whether the positive genetic associations between intelligence and cortical thickness change reflect plastic properties of the brain needed for optimal adaptation to an ever-changing environment. The physiological processes underlying the cortical thickness change in the adult human brain remain unclear, but it is interesting to note that the areas that showed cortical thickening in our study, i.e. the parahippocampal gyrus and frontal pole overlap with those that are thought to have plastic properties in adult mammals (Gould, 2007). Cortical thinning with increasing age is not easily explained by decreases in neocortical neuronal cell number (Pakkenberg et al., 1997), but instead may result from a reduction in the complexity of dendrite arborization and dendritic length, a decrease in spine numbers, and change in synaptic densities (Dickstein et al., 2007). Finally, the positive association between cortical change (more cortical thickening and less cortical thinning) and intelligence is consistent with the dependence of learning and memory formation on plasticity of neural circuits (Escobar et al., 2008).

Therefore, genes associated with cortical plasticity may also be relevant for cognitive functioning throughout life. Finding which genes are responsible for cortical thickness change will have implications for healthy adult functioning, as well as for brain diseases that develop their first symptoms in adulthood. Indeed, genes that play a role in synaptic plasticity in adult organisms in concert with environmental influences and experience (Flavel et al., 2008) are starting to be identified. Since the genes involved in the plasticity of the adult cortex have now been shown to differ from those involved in early brain development, both gene sets provide targets for study of brain plasticity and intelligence. Thus, continued brain maturation and intellectual development go hand in hand, and both depend in part on our genetic background well into adulthood.

Methods Summary

Twins and their siblings were recruited from the twin-pair cohort at the University Medical Centre Utrecht (Baaré et al., 2001; Hulshoff Pol et al., 2006), and the Netherlands Twin Registry, Free University Amsterdam (www.tweelingenregister.org/index_uk.html). Of the 242 participants from 106 twin families, 183 from 87 families completed the 5-year follow-up, including 52 monozygotic males, 31 dizygotic males, 25 monozygotic females, 29 dizygotic females, 24 dizygotic opposite sex twins (12 male and 12 female) and 22 siblings (11 male and 11 female). Mean age at baseline was 29.63 years (SD: 7.52). Subjects participated after written informed consent was obtained. DNA testing using polymorphic markers determined zygosity. T1 and T2 weighted MRI brain scans were acquired of the whole head. Intracranial, total brain, and gray matter of the cerebrum (total brain excluding cerebellum and stem) volumes were based on histogram analyses (www.smri.nl/techniques_description.html; Schnack et al., 2001). Cortical thickness was based on the algorithm in CIVET, designed at the McConnell Brain Imaging Centre, Montreal (Kim et al., 2005; Lerch et al., 2008). Following registration to an average surface (ICBM) (Lyttelton et al., 2007), in each subject, cortical thickness and cortical thickness change were calculated for 81,924 vertices. A measure of full scale IQ was obtained of the Dutch adaptation of the WAIS III (1997). Using structural equation modeling, the contributions of additive genetic, common (left out because it did not contribute) and unique environmental factors to the variance on brain structure (change) and IQ were estimated in univariate and bivariate genetic analyses, correcting for age, sex and handedness, using Mx (Neale et al., 2003). By minimizing a goodness-of-fit-statistic between observed and predicted covariance matrices, likelihoods of nested models were compared (-2 Log-Likelihood-difference is Chi-Squared distributed) with a critical level of χ^2>2.71(1 df for h^2 and bivariate h^2_{biv}) (Dominicus et al., 2006) and χ^2>5.99 (2 df for phenotypic correlations r_{ph}) at alpha=.05 for brain volume (change). For cortical thickness change a critical χ^2>14.3 (1 df) was set following Bonferroni correction for multiple comparisons.

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End Notes

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Supplementary Methods

Subjects

DNA testing using the polymorphic markers D06S474, D07S1804, D07S1870, D12S811, D13S119, D13S126, D13S788, D20S119, D22S683, DXS1001, and ELN, or D13S317, VWA, D74520, D35158, TH01, TP0X, CSF1P0, and D55818 determined zygosity. Except for one twin pair, all twins and their siblings were reared together. Two twin pairs were born by caesarean section delivery. Mental and physical health was assessed at baseline and at follow-up by means of the Comprehensive Assessment of Symptoms and History (CASH), and by a medical history inventory, respectively. In addition, at baseline the Family Interview for Genetic Studies (FIGS) was completed. The study was approved by the medical ethics committee for research in humans (METC) of the University Medical Centre Utrecht, the Netherlands and was carried out according to the directives of the "declaration of Helsinki" (amendment of Edinburg, 2000) (Supplementary material table 3).

Supplementary table 3: Demographics of monozygotic and dizygotic twin-pairs and their siblings.[1]

	MZ	DZ	Siblings
N (male/female)	52/25	43/41	11/11
Age at time of the first scan (yr)	31.1 (8.9)	28.7 (6.7)	28.0 (3.1)
Height (cm)	177 (8.9)	177 (8.2)	175 (12.5)
Handedness (r/l/ambidexter)	60/15/2	69/8/7	17/4/1
Level of education (yr)	13.7 (2.9)	13.3 (2.6)	12.7 (3.2)
Parental level of education (yr)	12.2 (2.6)	12.0 (2.7)	12.1 (2.8)
Follow-up duration (yr)	5.1 (.56)	5.5 (.70)	5.4 (.55)
IQ*	104.3 (16.1)	104.4 (8.5)	106.4 (12.6)

[1] Values are total numbers (N), means (standard deviations), unless otherwise indicated

* Available in 36 MZ, 53 DZ and 17 siblings

Brain imaging

Magnetic Resonance Imaging brain scans were acquired on a Philips NT (Best, the Netherlands) scanner operating at 1.5 T in all subjects. T1-weighted three-dimensional fast field echo (3D-FFE) scans with 160–180 contiguous coronal slices scans (TE = 4.6 ms, TR = 30 ms, flip angle = 30°, 1 x 1 x 1.2 mm^3 voxels), and T2-weighted dual-echo turbo-spin-echo (DE-TSE) scans

with 120 contiguous coronal slices (TE1 = 14 ms, TE2 = 80 ms, TR = 6350 ms, flip angle = 90°, 1 x 1 x 1.6 mm^3 voxels) of the whole head were used for quantitative measurements. In addition, T2-weighted dual-echo turbo-spin-echo (DE-TSE) scans (TE1 = 9 ms, TE2 = 100 ms, flip angle = 90°, 0.98 x 0.98 mm^2) with 19 axial 5-mm slices and 1.2-mm gap of the whole head were used for clinical neurodiagnostic evaluation.
Processing was done on the neuroimaging computer network of the Department of Psychiatry at the University Medical Centre Utrecht. All images were coded to ensure blindness for subject identification and diagnoses, scans were manually put into Talairach frame (no scaling) for segmentation purposes, and corrected for inhomogeneities in the magnetic field (Sled et al., 1998). Assessments of white matter/gray matter interface and brain volumes were validated previously (Schnack et al., 2001). All images were checked after measurement and corrected manually if necessary. The interrater reliabilities of the volume measurements, determined by the Intraclass Correlation Coefficient (ICC) were 0.95 and higher.

Cortical thickness extraction was done by hemisphere; each surface consisted of 81,920 polygons and 40,962 vertices. It included fitting of a 3D surface to the white matter/gray matter interface, which created the inner surface of the cortex which was then expanded out to fit the gray matter/cerebrospinal fluid interface, thereby creating the outer cortical surface (Kim et al., 2005). Cortical thickness was estimated by taking the distance between the two surfaces such that each polygon vertex on the outer surface had a counterpart vertex on the inner surface. A vertex-by-vertex analysis was carried out to evaluate the differences in cortical thickness change at each point. Each subject's thickness measurements were smoothed across the surface using a 20 mm (FWHM) surface-based blurring kernel. This method of blurring improves the chances of detecting population differences, but also follows the curvature of the surface to preserve any anatomical boundaries within the cortex. The surfaces of the subjects were registered to an average surface created from 152 subjects (ICBM) (Lyttelton et al., 2007). This registration allowed us to compare cortical thickness locally between subjects. It should be noted that the area defined as the parahippocampal gyrus comprises parts of the entorhinal cortex, perirhinal cortex, parahippocampal cortex, and those parts of uncus and hippocampus proper that are on the cortical surface in the medial temporal lobe (Lerch et al., 2008).

Cognitive assessment

A measure of full IQ was obtained in 132 subjects who had a baseline scan and for 106 participants who also obtained a follow-up scan. Full scale IQ was corrected for age and sex.

Statistical Analyses

To estimate the contribution of genetic and common and unique environmental factors on the variation of brain structure change, the extended twin-sibling model was applied. This model is based on the fact that monozygotic (MZ) twins are (nearly always) genetically identical, dizygotic (DZ) twins share 50% of their genes on average and both types of twins share their familial environment. Therefore, if MZ twins resemble each other more than DZ twins, genetic factors are important for that trait. The presence of shared environmental factors is suggested when correlations in DZ twins are larger than half the MZ correlation (Boomsma et al., 2002). By including siblings, the statistical power to detect the influences of common environmental factors shared by family members is enhanced, because, similar to DZ twins, a twin and its sibling also share 50% of their genes on average and share the familial environment.

Brain volume as well as brain volume change were corrected for age and sex with a linear regression analysis. The relative importance of the genetic factor was estimated, which was expressed as (univariate) heritability (h^2). Because univariate analysis did not show any evidence for influences of shared environment (C), subsequent (bivariate) analysis were based on models containing additive genetic (A) and unique environmental (E) influences only. The covariance between brain volume and brain volume change and between brain volume change and IQ (bivariate genetic analysis), was decomposed into different parts: the percentage covariance attributed to A was expressed as bivariate heritability (h^2_{biv}=|covA|/(|covA|+|covE|)).

The bivariate genetic analysis yielded an estimate of the phenotypic correlations (r_{ph}) between brain structure and brain structure change and between brain structure change and IQ, which can result from a common set of genes or common set of environmental factors depicted in genetic (r_g) and environmental correlations (r_e). These estimates provide information regarding the possible shared genetic and environmental influences of brain structure and brain structure change and brain structure change and IQ. Decomposition of these sources was based on the comparison of cross-trait/cross-twin correlations for MZ and DZ twins, i.e., the correlation between a trait (e.g. whole brain volume at initial measurement) of twin 1 with another trait (e.g. whole brain volume change) of twin 2, where twin 1 and twin 2

represent a twin-pair. If the absolute value of the correlation between brain volume change of twin 1 and brain volume or IQ of twin 2 is larger in MZ twins than in DZ twins, this indicates that genes influencing brain volume (partly) overlap with genes that influence brain volume change. The extent of the overlap is reflected by the magnitude of the genetic correlation (r_g).

It was tested whether an AE model fits as well as an E model, taking the simplest model explaining the data best. A Chi-Squared larger than χ^2 =3.84 (1 df) (Neale et al., 2003) indicates a significant difference at alpha=.05, which means that the reduced model provides a significantly worse fit to the data and indicates that the discarded effect (e.g. additive genetic influence) cannot be left out of the model without seriously deteriorating the goodness of fit. When estimating heritabilities, this Chi-squared can be relaxed because tests of variance components constrained to be non-negative correspond to tests of parameters on the boundary of the parameter space and in such situations the standard test procedure provides too large p-values; these p-values have to be halved, resulting in a critical value of the Chi-squared χ^2=2.71 (1 df) at alpha=.05 (Dominus et al., 2006).

Similar to the analysis of global brain volume (change), genetic model-fitting was carried out in each vertex separately (corrected for age, sex, and handedness), to obtain the same estimates for cortical thicknesses. For cortical thickness measurements a critical chi-squared larger than χ^2=14.3 was set after Bonferroni correction for multiple comparisons based on 81,920 polygons and the 20 mm surfaced based blurring kernel.

Chapter 3

Gray and white matter volume abnormalities in monozygotic and same-gender dizygotic twins discordant for schizophrenia

Hilleke E. Hulshoff Pol, Rachel G.H. Brans, Neeltje E.M. van Haren, Hugo G. Schnack, Marieke Langen, Wim F.C. Baaré, Clarine J. van Oel, and René S. Kahn

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Abstract

Background: Whole brain tissue volume decreases in schizophrenia have been related to both genetic risk factors and disease-related (possibly nongenetic) factors; however, whether genetic and environmental risk factors in the brains of patients with schizophrenia are differentially reflected in gray or white matter volume change is not known.
Methods: Magnetic resonance imaging (1.5 T) brain scans of 11 monozygotic and 11 same-gender dizygotic twin pairs discordant for schizophrenia were acquired and compared with 11 monozygotic and 11 same-gender dizygotic healthy control twin pairs.
Results: Repeated-measures volume analysis of covariance revealed decreased whole brain volume in the patients with schizophrenia as compared with their co-twins and with healthy twin pairs. Decreased white matter volume was found in discordant twin pairs compared with healthy twin pairs, particularly in the monozygotic twin pairs. A decrease in gray matter was found in the patients compared with their co-twins and compared with the healthy twins.
Conclusions: The results suggest that the decreases in white matter volume reflect the increased genetic risk to develop schizophrenia, whereas the decreases in gray matter volume are related to environmental risk factors. Study of genes involved in the (maintenance) of white matter structures may be particularly fruitful in schizophrenia.

Introduction

Twin studies in schizophrenia are particularly informative to examine the relative contribution of genetic and environmental risk factors in the brain volume changes reported in this illness. Environmental factors have been found to influence the often-replicated lateral ventricle enlargement in schizophrenia (Ohara et al., 1998; Reveley et al., 1982; Suddath et al., 1990), although influence of genetic risk factors may also be involved (Baaré et al., 2001a; McDonald et al., 2002). Genetic factors are probably involved in the decreases in whole brain volume with additional decreases in brain volume reflecting disease-related (possibly nongenetic) influences (Baaré et al., 2001a). Indeed, genetic factors were found to be involved in the decreases in the hippocampus (Baaré et al., 2001; Lawrie et al., 1999), although influences of environmental factors have also been reported in relation to decreases in hippocampus (Suddath et al., 1990) and thalamus (Lawrie et al., 1999; Staal et al., 2000) volumes in schizophrenia. Moreover, the frontal pole and dorsolateral prefrontal cortex was influenced by the genetic vulnerability for schizophrenia (Cannon et al., 2002a), whereas nongenetic or illness-related factors were associated with changes in the dorsolateral prefrontal cortex and superior and parietal gyri. Callosal displacements (Narr et al., 2002) and corpus callosum shape (Casanova, 1990) were found to be influenced by genetic risk factors in schizophrenia.

Most volume changes in schizophrenia are reported in gray matter, both cortical and subcortical (Hulshoff Pol et al., 2002a; McCarley, 1999; Wright et al., 2000), although white matter changes have also been found (Cannon et al., 1998; Hulshoff Pol et al., 2002a; Paillère-Martinot et al., 2001; Tibbo et al., 1998). Thus far, however, it is not known whether genetic and environmental risk factors for schizophrenia are differentially reflected in gray or white matter volume changes in schizophrenia.

This study examined gray and white matter volumes in monozygotic (MZ) and dizygotic (DZ) twins discordant for schizophrenia and compared the volumes with those of closely matched healthy MZ and DZ twins.

Methods and Materials

Subjects

Participants in the study included 11 monozygotic (6 male/5 female pairs) and 11 same-gender dizygotic (5/6) twin-pairs discordant for schizophrenia and 11 monozygotic (6/5) and 11 same-gender dizygotic (5/6) healthy control

twin pairs. Written informed consent was obtained from all subjects. Intracranial and total brain volumes of 84 of the twins were reported in a previous paper (Baaré et al., 2001). The study was approved by the medical ethics committee for research in humans (METC) of the University Medical Center Utrecht, the Netherlands.

All subjects underwent extensive psychiatric assessment procedures using the Comprehensive Assessment of Symptoms and History (CASH; Andreasen, 1992) and Schedule for Affective Disorder and Schizophrenia—Lifetime Version (SADS-L; Endicott and Spitzer, 1978) assessed by two independent raters. Diagnostic consensus was achieved in the presence of a psychiatrist. Psychiatric diagnosis was established according to criteria of DSM-IV. All patients met DSM-IV diagnosis for schizophrenia. Diagnoses in nonschizophrenic co-twins included paranoid personality disorder (1 DZ), schizotypal personality disorder (2 MZ), schizoid personality disorder (1 MZ, 1 DZ), (major) depressive disorder (2 MZ, 3DZ), and generalized anxiety disorder (1 MZ). Moreover, 1 MZ and 2 DZ patients and 1 DZ co-twin had histories of substance or alcohol abuse. Five MZ and 5 DZ co-twins of patients were symptom free. All healthy control twins met Research Diagnostic Criteria for "never mentally ill" (Pfohl et al., 1995) and had no first-degree family member with a mental illness or second-degree relatives with a psychotic disorder. All patients were receiving typical (n=13) or atypical (n=8) antipsychotic medication, or both (n=1), at the time of the scan. The dosage of typical antipsychotics taken was expressed in haloperidol equivalents from the Dutch National Health Service (Commissie Farmaceutische Hulp, 2000). For atypical antipsychotics, the respective pharmaceutical companies suggested how to convert the dosage into haloperidol equivalents (clozapine, 40:1; olanzapine, 2.5:1; risperidone, 1:1). Subjects were matched for age, gender, birth order, and handedness. For demographics, see Table 1.

Brain Imaging

Images were acquired on a Philips NT scanner operating at 1.5 T in all subjects. T1-weighted three-dimensional fast field echo (3D-FFE) scans with 160–180 contiguous coronal slices (echo time [TE] = 4.6 msec, repetition time [TR] = 30 msec, flip angle = 30°, 1x1x1.2 mm^3 voxels), and T2-weighted dual-echo turbo-spin-echo (DE-TSE) scans with 120 contiguous coronal slices (TE1 = 14 msec, TE2 = 80 msec, TR = 6350 msec, flip angle = 90°, 1x1x1.6 mm^3 voxels) of the whole head were used for quantitative measurements. Magnetic resonance imaging acquisition and processing methods have been described previously (Hulshoff Pol et al., 2002a).

Table 1. Demograpics for study participants

Group	Male/Female	Birth Order, n firstborn	Age, Mean (SD) (years)	Parental Ed, Mean (SD) (years)	Hand (left/right/amb)	Ed Mean (SD) (years) [a]	Age at First Symptoms, Mean (SD) (years)	Typ/Atyp/Both (n)	Haldol Equivalent mean [b]
MZ Discordant	6/5		39.00 (11.67)	11.20 (3.16)					
Patient		5			9/2	11.40 (3.20)	10.10 (8.58)	7/4/0	7.19/7.12/0
Co-twin					7/3/1	11.80 (2.78)			
MZ Healthy	6/5		37.36 (12.56)	11.72 (2.41)					
C1		6			8/3/0	12.09 (2.43)			
C2					10/1/0	13.00 (2.37)			
DZ Discordant	5/6		34.55 (8.95)						
Patient		6		12.55 (2.50)	10/1/0	10.91 (2.70)	19.82 (6.95)	6/4/1	5.07/4.71/17.8
Co-twin					10/0/1	14.36 (1.57)			
DZ Healthy	5/6		32.55 (9.08)	11.45 (2.34)					
C1		5			10/1/0	14.18 (.98)			
C2					6/4/1	12.73 (2.10)			

Atyp, atypical antipsychotic; C, control subject; DZ, dizygotic; Ed, education; MZ, monozygotic; Typ, typical antipsychotic.

[a] Significantly lower in patients effect (twin X group effect: $F_{(1,39)} = 10.85$, $p = .002$).

[b] Not available in two patients.

Processing was done on the neuroimaging computer network of the Department of Psychiatry, University Medical Center Utrecht. All images were coded to ensure investigators were blind for subject identification and diagnoses; scans were put into Talairach frame (no scaling) for segmentation purposes and corrected for inhomogeneities in the magnetic field. Quantitative assessments of the intracranial, total brain, and gray and white matter of the cerebrum (total brain excluding cerebellum and stem) volumes were performed based on histogram analyses and series of mathematical morphologic operators to connect all voxels of interest. Gray and white matter segmentation procedures have been validated earlier (Schnack et al., 2001). The interrater reliability of the volume measurements determined by the intraclass correlation coefficient in 10 brains was above .95.

Statistical Analysis

Within-twin pair similarities of brain volumes were estimated by calculating intraclass correlation coefficients (ICCs) and their 95% confidence intervals (Bartko et al., 1976) on the unstandardized residuals of the brain volumes after correcting for age and gender for discordant and healthy MZ and DZ twin pairs. Repeated-measures analysis of covariance (ANCOVA) was done for gray and white matter volumes of the cerebrum separately, with TWIN (proband or twin1, co-twin or twin2) as within subjects variable, group (discordant, healthy) and ZYG (monozygotic, dizygotic) as between subjects variables, and age, gender, and intracranial volume as covariates. Interactions for TWIN by GROUP, TWIN by ZYG, and GROUP by ZYG were entered into the model.

To investigate the source of the significant findings in the repeated-measures ANCOVA, the means of the unstandardized residuals of whole brain volume, gray and white matter volume after controlling for intracranial volume, age and gender between GROUP and ZYG and within TWIN were compared.

Results

For mean (SD) brain tissue volumes, see Table 2. For within-twin pair similarities as measured by the intraclass correlation coefficient (lower or higher 95% confidence interval) see Table 3.

Within-twin pair similarities of the brain volumes were higher in monozygotic than dizygotic twin pairs, irrespective of schizophrenia. Moreover, in the monozygotic twins, within-twin pair similarities were lower in discordant twin pairs than in control twin pairs for gray and white matter volumes.

Table 2. Volumes of brain structures in twins in mean (SD) ml

Group	Intracranial	Whole Brain	Gray Matter	White Matter
MZ Discordant				
Patient	1392.98 (147.26)	1180.71 (118.91)	587.80 (66.79)	443.79 (58.28)
Co-twin	1415.61 (170.10)	1210.29 (135.52)	616.91 (75.27)	442.75 (58.64)
MZ healthy				
C1	1446.06 (99.67)	1258.23 (102.62)	633.92 (69.93)	472.91 (44.22)
C2	1433.71 (92.92)	1238.30 (98.35)	619.64 (71.12)	469.27 (51.86)
DZ discordant				
Patient	1410.45 (170.50)	1183.24 (175.75)	600.29 (87.06)	438.23 (79.24)
Co-twin	1430.93 (145.21)	1246.49 (124.64)	631.54 (72.67)	463.90 (56.53)
DZ healthy				
C1	1378.31 (98.02)	1235.99 (87.84)	631.76 (49.92)	452.29 (48.85)
C2	1396.68 (94.45)	1233.02 (111.64)	622.73 (52.41)	460.63 (60.49)

C, Control; DZ, dizygotic; MZ, monozygotic.

Table 3. Within-twin pair similarities

	Intracranial	Whole Brain	Gray Matter	White Matter
Monozygotic Discordant				
ICC	.89[a]	.86[a]	.72[a]	.72[a]
95%L	.66	.58	.27	.25
95%H	.97	.96	.92	.91
Monozygotic Healthy				
ICC	.79 [a]	.83[a]	.83[a]	.82[a]
95%L	.31	.01	.07	.45
95%H	.94	.96	.96	.95
Dizygotic Discordant				
ICC	.17	-.12	.02	-.14
95%L	-.52	-.68	-.60	-.72
95%H	.69	.50	.60	.49
Dizygotic Healthy				
ICC	.33	-.18	-.07	.39
95%L	-.37	-.46	-.63	-.30
95%H	.77	.69	.53	.79

Data are corrected for age and gender.
H, higher; ICC, intraclass coefficient; L, lower; 95%, 95% confidence interval.
[a]$p < .05$

A significant main effect for GROUP on whole brain volume was found [$F_{(1,38)} = 4.99$, $p = .05$], reflecting a decreased whole brain volume in the discordant twin pairs as compared to the healthy twin pairs. Moreover, the TWIN-by-GROUP effect for whole brain volume was significant [$F_{(1,38)} = 6.87$, $p = .001$], reflecting a decrease in the patients with schizophrenia as compared to their co-twins and the healthy twin pairs (Figure 1).

The TWIN-by-GROUP effect for gray matter volume was significant [$F_{(1,38)} = 9.22$, $p = .004$] because of a decrease in the patients compared with their co-twins and compared with the healthy twin pairs (Figure 2). Also, a significant main effect for GROUP was found for white matter volume [$F_{(1,38)} = 4.10$, $p = .05$], reflecting decreased white matter volume in the discordant twin pairs compared with the healthy twin pairs, particularly prominent in the monozygotic twin pairs (Figure 2).

There were no significant main effects for TWIN or interaction effects for ZYG.

Post hoc analysis of the data revealed no significant differences in intracranial, whole brain, gray and white matter volumes of those co-twins of patients with schizophrenia who were diagnosed with a cluster A personality disorder compared with those co-twins who were not.

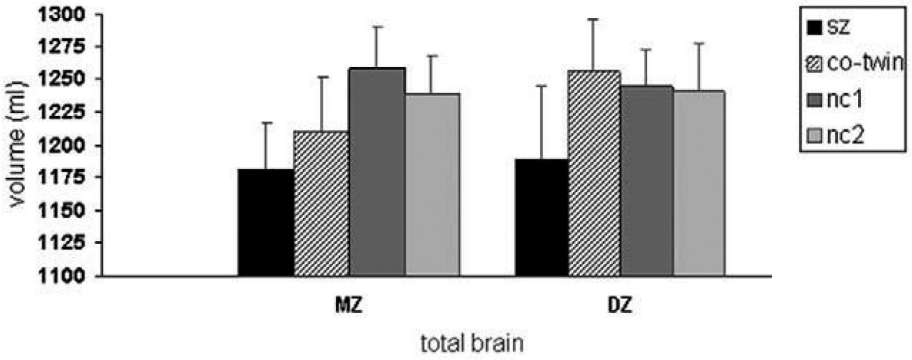

Figure 1. Whole brain volume in monozygotic (MZ) and dizygotic (DZ) twin pairs discordant for schizophrenia compared with healthy twin pairs. SZ: patients with schizophrenia (n=11 MZ, 11 DZ); co-twin: co-twins of patients with schizophrenia (n = 11 MZ, 11 DZ); nc1: first twins of the healthy twin pairs (n = 11 MZ, 11 DZ); nc2: second twin of the healthy twin pairs (n=11 MZ and 11 DZ).

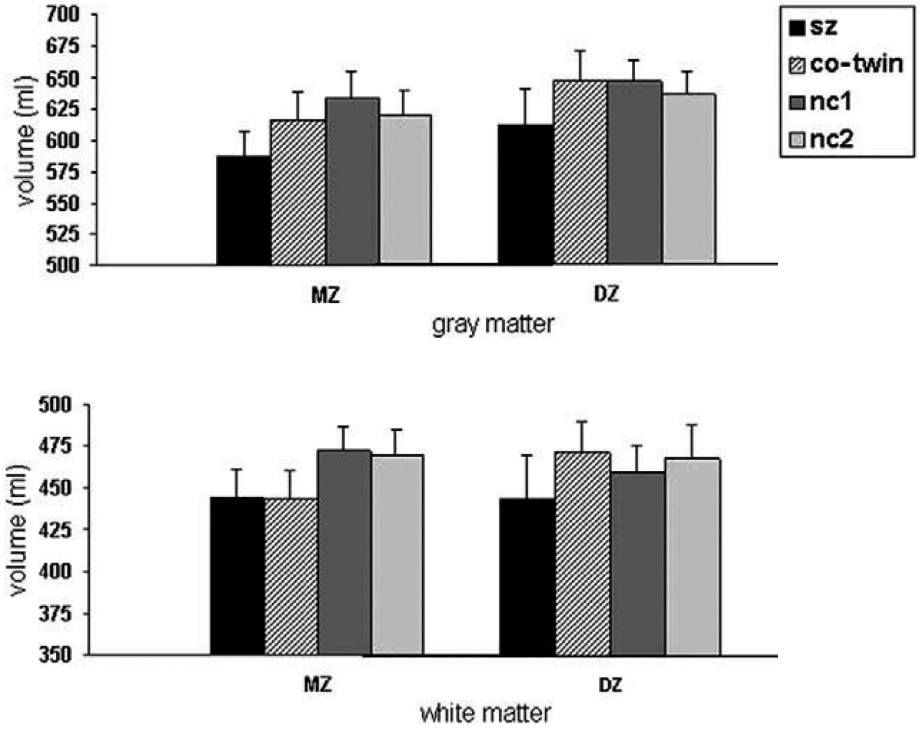

Figure 2. Gray and white matter volumes in monozygotic (MZ) and dizygotic (DZ) twin pairs discordant for schizophrenia compared with healthy twin pairs. SZ: patients with schizophrenia (n = 11 MZ, 11 DZ); co-twin: co-twins of patients with schizophrenia (n=11 MZ, 11 DZ); nc1: first twins of the healthy twin pairs (n = 11 MZ, 11 DZ); nc2: second twin of the healthy twin pairs (n = 11 MZ, 11 DZ).

Discussion

This study compared gray and white matter volumes in 11 monozygotic and 11 same-gender dizygotic twin pairs discordant for schizophrenia to 11 monozygotic and 11 same-gender dizygotic healthy twin pairs. In this study, the influence of genetic and environmental factors on gray and white brain matter volumes in schizophrenia was studied. Gray matter was decreased in the patients with schizophrenia compared with their co-twins and healthy twin pairs. Decreased white matter volume was found in discordant twin pairs compared with healthy twin pairs, irrespective of disease and particularly in the monozygotic twin pairs. Within-twin pair similarities revealed higher similarities in the monozygotic as compared to the dizygotic pairs irrespective of discordance for intracranial volume, total brain volume, and gray and white matter volume. In the monozygotic twins, within-twin pair similarities in discordant twin pairs were lower than in normal twin pairs for gray and white matter of the cerebrum.

The significant decrease in gray matter in the patients compared with their co-twins and compared with the healthy twin pairs suggests that disease-related factors are involved in the decreases in gray matter volume in schizophrenia. The influence of nongenetic or illness-related factors on the decrease in gray matter volume in patients is consistent with the reported environmentally influenced changes in dorsolateral prefrontal cortex and superior temporal and parietal gyri (Cannon et al., 2002). Note however, that changes in the frontal pole and dorsolateral prefrontal cortex were found to be most prominent in monozygotic compared with dizygotic co-twins in discordant pairs and compared with healthy control pairs (Cannon et al., 2002). Therefore, some focal gray matter regions in the brain of patients with schizophrenia may be primarily influenced by genetic risk factors. In contrast, because white matter volume was found to be decreased in the discordant twin pairs (irrespective of disease) compared with the healthy twin pairs, white matter changes may be related more to the genetic vulnerability to develop the disease rather than to the disease process itself. This finding is in agreement with reports on white matter changes such as callosal displacements (albeit not the vertical callosal displacements; Narr et al., 2002) and corpus callosum shape (Casanova, 1990) being influenced by genetic risk factors in schizophrenia; however, global white matter volume changes cannot necessarily be extended to the focal measures of the corpus callosum. Interestingly, in a genomewide expression analysis, genes determined to have altered expression levels in schizophrenia relative to control subjects were involved in the differential expression of myelination-related genes (Hakak et al., 2001).

The high within-twin pair similarities in the monozygotic compared with the dizygotic pairs irrespective of discordance for intracranial volume, total brain volume, and gray and white matter volume suggest that in patients and control subjects, these measures are largely determined by genetic factors, as has been reported previously (Baaré et al., 2001b; Hulshoff Pol et al., 2002b). Lower within-twin pair similarities in discordant twin pairs than in normal twin pairs suggests that, in addition to genetic factors, disease-related changes also influence white (and gray) matter brain volumes in the discordant pairs to some extent.

In summary, our findings suggest that global gray matter decreases in schizophrenia may be secondary to the disease process, whereas global white matter changes may be related to the genetic vulnerability to develop the disease. Indeed, longitudinal studies in schizophrenia have shown gray (but not white) matter to decrease over the course of illness (Cahn et al., 2002; Mathalon et al., 2001) and to be related to outcome (Cahn et al., 2002; Lieberman et al., 2001; Staal et al., 2001), suggesting that gray matter changes are related to schizophrenia and its course. The finding that white matter changes in schizophrenia may be linked to the genetic risk to develop the disorder suggests that the study of genes involved in white matter structures may be particularly fruitful in schizophrenia.

There are limitations to the study that must be taken into consideration when interpreting its findings. First, collecting discordant twin pairs takes considerable time. Indeed, years of collecting discordant twins resulted in the sample size of 11 twin pairs per group. This limited the statistical power of the analyses, despite matching for age, gender, birth order, and handedness between the patient and control groups. Indeed, the group effect for white matter would not have reached statistical significance if a Bonferroni correction for multiple comparisons were added to the statistical analysis. Moreover, the group-by-zygosity effect did not reach significance, whereas comparison of the mean values of the white matter volumes suggested this effect to be due to monozygotic discordant twin pairs only. It also limited our analyses to analysis of variance and did not allow for inferences as to which percentage of brain volume changes could be accounted for by genetic factors in schizophrenia. Second, the findings of our study are limited to whole brain gray and white matter volume measures. Therefore, based on these findings, no further inferences can be made as to which focal gray and white matter structures may be related to the disease and genetic risk to develop schizophrenia. Future study should involve more focal approaches to brain anatomy using voxel-based morphometry.

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Chapter 4

Longitudinal MRI study in schizophrenia patients and their healthy siblings.

Rachel G.H. Brans, Neeltje E.M. van Haren, G. Caroline M. van Baal, Wouter G. Staal, Hugo G. Schnack, René S. Kahn, Hilleke E. Hulshoff Pol.

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Summary

To investigate whether genetic and/or disease-related factors are involved in progressive structural brain changes in schizophrenia, magnetic resonance imaging scans with a 5-year scan-interval were acquired in patients, their same-gender siblings and matched healthy controls. Structural equation modelling was applied to assess disease and familial effects. Whole brain and cerebral gray matter volumes decreased excessively in patients compared with their siblings and the controls, suggesting that the progressive brain loss in schizophrenia may be related to the disease process.

Introduction

Structural brain abnormalities have been reported consistently in patients with schizophrenia (Wright et al., 2000), with less pronounced abnormalities in first-degree relatives of patients (Boos et al., 2007). Thus, the volumetric differences may be related in part to the genetic risk of developing the disease (Hulshoff Pol et al., 2004). At least parts of the morphological brain changes in schizophrenia are progressive over the course of the illness (van Haren et al., 2008; Pantelis et al., 2005), but it is not known for certain whether they are mediated by genetic or disease-related factors.

Methods

Study design

Participants were recruited from the sibling-pair cohort and control sample (Staal et al., 2000; Hulshoff Pol et al., 2002) at the University Medical Center Utrecht. At baseline, 16 patients with schizophrenia (12 male, 4 female), 18 same-gender siblings (14 male, 4 female) and 43 healthy controls (29 male, 14 female) matched for age, gender and parental education participated. Eleven patients (7 male, 4 female), 11 siblings (8 male, 3 female), and 33 controls (22 male, 11 female) completed the follow-up after 5 years (mean=5.02 years; s.d.=0.39). Not all sibling-pairs were complete at follow-up; a total of 7 sibling-pairs were scanned twice. At baseline, mean age of the patients, siblings and controls was 40.9 years (s.d.=8.8), 41.2 years (s.d.=8.8) and 40.2 years (s.d.=8.2) respectively. Mean duration of illness in the patients was 19.6 years (s.d.=11.5). The follow-up sample was representative of the baseline sample in that groups did not differ in mean age, handedness, participants' and parental education, and duration of illness. All individuals underwent extensive psychiatric assessment procedures using the Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992) at baseline and follow-up. Patients met DSM-IV criteria for schizophrenia and all siblings were healthy. Siblings were at least 8 years older than the age the affected sibling developed the first symptoms of schizophrenia; thus, they would be very unlikely to develop schizophrenia in the future. Outcome was assessed using the Global Assessment of Functioning (Hall, 1995), the Positive and Negative Syndrome Scale (Kay et al., 1987) and the Camberwell Assessment of Need (Phelan et al., 1995). Participants with a major medical or neurological illness, IQ below 80, previous electroconvulsive therapy, or history of substance dependence were excluded.

A table from the Dutch National Health Service (Commissie Farmaceutische Hulp van het College voor Zorgverzekeringen) was used to calculate the cumulative dosage of antipsychotics during the 5-year scan interval and to derive haloperidol equivalents (HAL equiv.). During the scan interval, six patients were exclusively on atypical antipsychotics (including clozapine) and two on typical. Two patients switched between typical and atypical antipsychotics; for one patient reliable information on medication was absent. Mean cumulative antipsychotic medication during the scan interval was 14 345.4 HAL equiv. (s.d. = 7984). None of the siblings used antipsychotics. In all, 46% of patients (5 of 11), 18% of siblings (2/11) and 36% of controls (12/33) were smokers. All participants gave written informed consent. The study was approved by the medical ethics committee for research in humans (METC) of the University Medical Center Utrecht.

Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) brain scans were acquired on a Philips NT (Best, the Netherlands) scanner operating at 1.5 T for all participants. A 3D fast field echo and a T2-weighted dual echo–turbo spin echo were acquired. Protocol details and the imaging process are described elsewhere (Hulshoff Pol et al., 2002). Volumes of intracranium, whole brain, cerebral gray and white matter, lateral and third ventricular volumes and cerebellum were measured.

Statistical Analysis

Mixed model analysis was implemented using Structural Equation Modeling with Mx software for Windows (www.vcu.edu/mx/mxhomepage.html). Brain volume change was regressed on intracranial volume, gender, age, disease (patients v. siblings and controls) and familial background (patients and siblings v. controls). Relatedness in the sibling-pairs was accounted for in the covariance structure by allowing dependencies between the residuals in the regression analyses. Effects of disease and familial background were tested by comparing the likelihoods of nested models (-2 log-likelihood), which is Chi-Squared distributed; $\chi^2 > 3.84$ (1 d.f.) indicates a significant difference at $\alpha=0.05$, and depicts that the discarded effect (e.g., disease effect) cannot be left out of the model without seriously reducing the goodness of fit. Using the full model, estimates (including 95% CIs) were obtained that indicated an increase or decrease of the dependent variable (brain volume change) in patients or in siblings.

Results

Over time, whole brain volume decreased excessively in patients (-12.6 ml) compared with siblings (+7.3 ml) and controls (-2.3 ml) (Table 1). A greater decrease in cerebral gray matter volume was observed in patients (-24.9 ml) than in siblings (-9.8 ml) or controls (-16.8 ml). Moreover, patients showed less prominent increases in cerebral white matter volume (+5.3 ml) than siblings (+12.9 ml) or controls (+12.5 ml). Changes in gray and white matter volumes do not add up to changes in total brain volume, because gray/white matter was limited to the cerebrum (i.e. whole brain excluding cerebellum and brain stem).

No associations were found between clinical variables and brain volume changes, except that a larger dose of atypical medication (including clozapine) during the scan interval was positively correlated with less progressive decrease in cerebral gray matter volume (r=0.85; p=0.03).

When comparing patients and siblings as a group with the controls, no familial effects were found in any of the brain volume changes over time.

Discussion

The progressive decreases over time in whole brain and cerebral gray matter volume and less prominent increases in white matter observed in schizophrenia patients but not in siblings may represent a (disease-related) non-genetic risk factor for the disease. Our finding of progressive decrease over time is consistent with those of other longitudinal studies in chronically ill patients with schizophrenia (van Haren et al., 2008; Pantelis et al., 2005). The findings in siblings are consistent with the normalisation of cortical thickness by the age of 20 in siblings of patients with childhood onset schizophrenia (Gogtay et al., 2007).

To our knowledge, this is the first longitudinal MRI study in chronically ill patients with schizophrenia and their healthy siblings. Siblings share on average 50% of their genes. The siblings in this study were all healthy and beyond the age of risk for schizophrenia. Possibly, the disease alleles of schizophrenia-related genes may be underrepresented in these siblings.

This study has several limitations. First, the number of participants was small and genetic factors involved in progressive brain changes in schizophrenia may not have been elucidated owing to a limited statistical power. Second, it is likely that at least some of the disease alleles of schizophrenia-related genes are not present in this sample of healthy siblings of patients. Our findings

have to be considered preliminary and more final conclusions await follow-up studies in monozygotic and dizygotic twin-pairs discordant for schizophrenia that are currently underway.

Table 1. Disease and familial effects of schizophrenia on changes in brain volumes [a]

	Brain volumes at baseline ml: mean (s.d.)			Percentage change per year per group, mean (s.d.)			Disease effects, % change/year		Familial effects, % change/year	
Brain area	**Patients**	**Siblings**	**Controls**	**Patients**	**Siblings**	**Controls**	**Estimates (95% CI)**	**χ^2**	**Estimates (95% CI)**	**χ^2**
Whole brain	1226.0 (154.4)	1267.9 (115.1)	1264.8 (109.7)	-.20 (0.38)	0.15 (0.40)	-0.04 (0.41)	-0.33 (-0.61 to -0.06)	5.18*	0.17 (-0.15 to 0.49)	1.17
Cerebral gray matter [b]	599.8 (78.5)	640.7 (71.0)	636.5 (65.1)	-0.84 (1.09)	-0.18 (1.00)	-0.54 (0.65)	-0.70 (-1.35 to -0.02)	3.97*	0.40 (-0.29 to 1.11)	1.39
Cerebral white matter [b]	468.7 (79.7)	473.9 (51.2)	474.8 (56.0)	0.30 (1.19)	0.51 (0.69)	0.59 (0.90)	-0.36 (-2.54 to -0.00)	3.89*	-0.03 (-0.50 to 0.44)	0.02
Cerebellum	144.2 (19.9)	140.5 (14.6)	140 (10.9)	0.28 (0.37)	0.52 (0.54)	0.19 (0.55)	-0.23 (-0.63 to 0.17)	1.34	0.33 (-0.08 to 0.73)	2.63
Lateral ventricles	29.15 (22.50)	14.33 (5.73)	15.57 (7.68)	2.32 (3.38)	1.13 (1.67)	1.80 (2.16)	0.75 (-2.18 to 3.23)	0.32	-0.80 (-1.68 to 0.09)	3.09
Third ventricles	1.65 (1.13)	1.15 (0.62)	0.89 (0.41)	2.70 (4.35)	-0.29 (5.01)	0.86 (5.73)	2.56 (-2.61 to 7.14)	0.64	-1.38 (-4.56 to 1.87)	0.91

[a] Sample: patients with schizophrenia (n=11), siblings (n=11) and healthy controls (n=33). The estimates are corrected for intracranial volume, age and gender. A significant estimate indicates an increase (positive estimate) or decrease (negative estimate) of the dependent variable in patients or in siblings compared with controls.

[b] Gray matter and white matter volumes of two patients were missing.

* Significant differences at $\chi^2 > 3.84$; $P<0.05$.

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Chapter 5

Heritability of changes in brain volume over time in twin pairs discordant for schizophrenia

Rachel G.H. Brans, Neeltje E.M. van Haren, G. Caroline M. van Baal, Hugo G. Schnack, René S. Kahn, Hilleke E. Hulshoff Pol.

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Abstract

Context: Structural brain abnormalities have consistently been found in schizophrenia, with increased familial risk for the disease associated with these abnormalities. Some brain volume changes are progressive over the course of the illness. Whether these progressive brain volume changes are mediated by genetic or disease-related factors is unknown.
Objective: To investigate whether genetic and/or environmental factors are associated with progressive brain volume changes in schizophrenia.
Design: Longitudinal 5-year follow-up in monozygotic (MZ) and dizygotic (DZ) twin pairs discordant for schizophrenia and healthy comparison twin pairs using brain magnetic resonance imaging.
Setting: Participants were recruited from the twin pair cohort at the University Medical Center Utrecht.
Participants: A total of 92 participants completed the study: 9 MZ and 10 DZ twin pairs discordant for schizophrenia and 14 MZ and 13 DZ healthy twin pairs.
Main Outcome Measures: Percentage volume changes of the whole brain; cerebral gray and white matter of the frontal, temporal, parietal, and occipital lobes; cerebellum; and lateral and third ventricles over time between and within twin pairs were compared using repeated measures analysis of covariance. Structural equation modeling was applied to estimate contributions of additive genetic and common and unique environmental factors.
Results: Significant decreases over time in whole brain and frontal and temporal lobe volumes were found in patients with schizophrenia and their unaffected co-twins compared with control twins. Bivariate structural equation modeling using cross-trait/cross-twin correlations revealed significant additive genetic influences on the correlations between schizophrenia liability and progressive whole brain (66%; 95% confidence interval [CI], 51%-100%), frontal lobe (76%; 95% CI, 54%-100%), and temporal lobe (79%; CI, 56%-100%) volume change.
Conclusion: The progressive brain volume loss found in patients with schizophrenia and their unaffected co-twins is at least partly attributable to genetic factors related to the illness.

Introduction

Structural brain abnormalities have consistently been found in patients with schizophrenia (Wright et al., 2000; Shenton et al., 2001). Interestingly, at least some of these brain volume changes are progressive across the course of the illness (Pantelis et al., 2005; Hulshoff & Kahn, 2008). More specifically, schizophrenic patients show aberrant trajectories of brain volume change during adolescence (Rapoport et al., 1999) and adulthood (van Haren et al., 2008; DeLisi, 1999).

The cause of these progressive brain volume changes in schizophrenia is still unclear. Recently, more pronounced progressive brain volume changes over time have been associated with poor outcomes (van Haren et al., 2008; Davis et al., 1998; Cahn et al., 2002; Ho et al., 2003). Moreover, differential effects of typical vs. atypical antipsychotic medication on progressive gray matter decreases were demonstrated (Lieberman et al., 2005). Thus, both severity of the illness and medication use influence the progressive brain volume changes in schizophrenia. However, genes involved with schizophrenia might also contribute to the progressive brain changes in this illness; the heritability (the percentage of variance explained by genetic factors) of developing schizophrenia is estimated to be around 80% (Sullivan et al., 2003). Indeed, twin and family studies strongly suggest that genetic factors play a role in the decreased brain volumes found in schizophrenia (DeLisi et al., 1987; Boos et al., 2007). A familial influence on the progressive brain changes in schizophrenia was suggested in a study of patients with childhood-onset schizophrenia and their siblings (Gogtay et al., 2007). However, sibling studies cannot disentangle the extent of genetic and common environmental contributions to familial influences.

In this study, we set out to investigate the relative contributions of genetic and environmental (disease-related) factors to the progressive brain volume changes in schizophrenia. Therefore, we conducted a longitudinal magnetic resonance imaging (MRI) study in monozygotic (MZ) and dizygotic (DZ) same-sex twin pairs discordant for schizophrenia and compared them with healthy MZ and DZ twin pairs, with a scan interval of 5 years.

The twin model is a powerful approach for determining the relative contributions of genetic influences and common and unique environmental influences on variation in brain volumes and their common origin with disease liability (Rijsdijk et al., 2005). Moreover, morphologic findings in twins can be extended to the singleton population (Hulshoff Pol et al., 2002a). For genetic influences, the determining factor is the extent to which MZ twin pairs resemble each other more than the DZ twin pairs. The presence of shared

environmental factors is suggested when correlations in DZ twins are larger than half of the MZ correlation (Boomsma et al., 2002). The importance of unique environmental factors can first be obtained from the extent to which MZ twins do not resemble each other. In a similar manner, the extent to which genetic and environmental factors influence brain volume changes and schizophrenia liability can be determined by comparing their cross-trait/cross-twin correlations in MZ and DZ twins. A cross-trait/cross-twin correlation is the correlation of a trait in twin 1 (i.e., liability for schizophrenia) with a second trait (i.e., brain volume change) in twin 2 of the same pair. If the cross-trait/cross-twin correlation is approximately twice as high in MZ as in DZ twins and is comparable with the within-twin/cross-trait correlation (i.e., the association between the 2 traits within the individuals), then it can be inferred that genes common to both traits influence their association.

Methods

Participants

Participants were recruited from the twin pair cohort (Baaré et al., 2001) at the University Medical Center Utrecht. A total of 9 MZ and 10 DZ twin pairs discordant for schizophrenia and 14 MZ and 13 DZ healthy twin pairs completed the longitudinal MRI study (N=92) after an interval of 5 years (mean, 4.86 years [SD, 0.57 years]). The control twin pairs were matched to the discordant twin pairs for zygosity, age, sex, birth order, handedness, their parents' socioeconomic status, and follow-up duration (Table 1). All twins participated after written informed consent was obtained. Zygosity was determined by DNA fingerprinting, using either the polymorphic markers D06S474, D07S1804, D07S1870, D12S811, D13S119, D13S126, D13S788, D20S119, D22S683, DXS1001, and ELN, or D13S317, VWA, D74520, D35158, TH01, TP0X, CSF1P0, and D55818. The study was approved by the medical ethics committee for research in humans of the University Medical Centre Utrecht and was carried out according to the directives of the Declaration of Helsinki (amendment of Edinburgh, 2000).

All participants underwent extensive psychiatric assessments at baseline and at follow-up with the Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992). Age at onset of illness was defined as the first time the patient experienced psychotic symptoms. Duration of illness was defined as the time between age at illness onset and age at first MRI scan. Outcome was assessed using the Global Assessment of Functioning (Hall, 1995). The Camberwell Assessment of Need (Phelan et al., 1995) was used to evaluate

Table 1. Demographics of monozygotic (MZ) and dizygotic (DZ) twin pairs discordant for schizophrenia and healthy control (HC) twin pairs

Characteristic	Mean (SD)							
	MZ Twins				DZ Twins			
	Patient (n = 9)	Co-twin (n = 9)	HC 1 (n = 14)	HC 2 (n = 14)	Patient (n = 10)	Co-twin (n = 10)	HC 1 (n = 13)	HC 2 (n = 13)
Sex, M/F, No.	4/5	4/5	9/5	9/5	6/4	6/4	8/5	8/5
Age at first MRI, y	40.2 (12.2)	40.2 (12.1)	35.5 (11.8)	35.5 (11.8)	37.1 (11.9)	37.2 (11.9)	35.4 (10.6)	35.4 (10.6)
Height, cm	177.9 (11.0)	178.1 (11.7)	175.0 (6.6)	175.6 (6.3)	177.0 (12.1)	178.6 (9.9)	174.7 (11.5)	174.9 (8.9)
Handedness, R/L/A, No.	8/1/0	8/0/1	9/3/2	12/1/1	9/1/0	9/0/1	11/2/0	9/1/3
Level of education, y	11.8 (3.2)	11.9 (3.0)	12.7 (3.1)	12.6 (2.5)	10.4 (2.3)	13.2 (3.0)	12.8 (2.4)	13.0 (2.9)
Parental level of education, y	12.4 (2.7)	12.4 (2.7)	10.9 (2.4)	10.9 (2.4)	11.9 (2.5)	11.9 (2.5)	10.9 (2.5)	10.9 (2.5)
Follow-up duration, y	4.9 (1.0)	4.8 (1.0)	4.8 (0.2)	4.8 (0.6)	5.0 (0.6)	4.9 (0.7)	4.8 (0.4)	4.9 (0.5)
Firstborn, No.	5	4	9	5	5	5	9	4
Smoker, No	5[a]	2	5	6[a]	7	3	6	2
Cigarettes/d at follow-up	19.6 (23.3)[a]	1.0 (2.7)	4.3 (7.8)	4.1 (7.5)[a]	17.8 (14.6) [a]	5.0 (8.5)	3.3 (6.1)	2.1 (5.1)
Alcoholic drinks/wk at follow-up	4.7 (8.2)[a]	6.4 (10.9)	5.4 (5.3)	4.5 (3.8)[a]	4.5 (6.6)	10.8 (7.7)	8.0 (6.7)	4.4 (6.2)
Age at first psychotic symptoms, y	23.8 (5.0)				22.2 (6.2)			
Duration of illness, y	16.6 (11.5)				14.9 (8.8)			
GAF score at follow-up [b]	59.2 (5.9)				60.8 (22.9)			
CAN score at follow-up [c]	4.7 (3.1)				4.8 (3.6)			
Total PANSS score at follow-up	47.6 (11.8) [a]				50.0 (18.5)			
Medication, typical/atypical/both, No. [d]	4/3/2				3/3/3			
Medication intake, haloperidol equivalent/y								
Typical	2006.5				464.4			
Atypical	2264.7				2402.3			
Both	2257.5				2358.6			

Abbreviations: CAN, Camberwell Assessment of Need; GAF, Global Assessment of Functioning; MRI, magnetic resonance imaging; PANSS, Positive and Negative Syndrome Scale; R/L/A, right/left/ambidextrous. [a] Data missing in 1 individual. [b] Available in 6 MZ patients and 6 DZ patients. [c] Available in 6 MZ patients and 5 DZ patients. [d] Data missing in 1 DZ patient. Corrected for scan interval.

the need for care of the patient in daily life functioning. The Positive and Negative Syndrome Scale (Kay et al., 1987) was used to evaluate severity of symptoms. In the co-twins of the schizophrenic patients and the matched healthy twin pairs, the Schedule for Affective Disorders and Schizophrenia–Lifetime version (Endicott & Spitzer, 1978) and the Structured Interview for DSM-IV Personality (Pfohl et al., 1995) were completed. Information about smoking status (number of cigarettes) and alcohol use was collected at follow-up.

Sixteen probands met criteria for schizophrenia and 3 met criteria for schizoaffective disorder. Furthermore, co-twins of the probands met diagnoses of schizoid personality disorder (1 MZ), schizotypical personality disorder (2 MZ), cannabis abuse (1 DZ), conduct disorder (1 MZ), and major depressive disorder (1 MZ and 1 DZ). In 2 healthy participants, diagnoses of adjustment disorder with depressed mood (1 DZ) and major depressive disorder (1 MZ) were made. At follow-up, 2 co-twins of probands had developed a major depressive disorder (2 DZ) and 1 developed a depressive disorder not otherwise specified (1 DZ). Four healthy participants (3 MZ and 1 DZ) developed major depressive disorders. Except for 1 control twin pair that was separated at 12 years of age when both of their parents died, all twins were reared together.

A table from the Dutch National Health Service (Commissie Famaceutische Hulp van het college voor Zorgverzekeringen) was used to calculate the cumulative dosage of typical antipsychotics during the scan interval and to derive the haloperidol equivalents. For atypical antipsychotic drugs, the respective pharmaceutical companies suggested the following conversions into haloperidol equivalents: clozapine, 40:1; olanzapine, 2.5:1; risperidone, 1:1; sulpiride, 170:1; quetiapine, 50:1; sertindole, 2:1, and aripripazole, 2:1. During the scan interval, 6 patients had been taking atypical antipsychotic medication (including clozapine, olanzapine, and aripripazole) exclusively and 7 patients were taking typical medication exclusively. Five patients switched between typical and atypical antipsychotic medications during the scan interval. From 1 patient, we did not have reliable information about medication intake. None of the co-twins (with the exception of 1 co-twin who had used antipsychotic medication for a short period) had ever used antipsychotic medication at the time of the second MRI scan. Five of the co-twins (3 MZ and 2 DZ) as well as 2 of the healthy participants (2 MZ) used antidepressants.

MRI acquisition and analysis

The MRI brain scans were acquired on a Philips NT scanner (Philips Medical Systems, Best, the Netherlands) operating at 1.5 T in all participants. T1-weighted 3-dimensional fast-field echo scans with 160 to 180 contiguous coronal slices (echo time/repetition time, 4.6 milliseconds/30 milliseconds; flip angle, 30°; voxel dimension, 1 x 1 x 1.2 mm^3) and T2-weighted dual-echo turbospin-echo scans with 120 contiguous coronal slices (echo time 1/echo time 2/repetition time, 14 milliseconds/80 milliseconds/6350 milliseconds; flip angle, 90°; voxel dimension, 1 x 1 x 1.6 mm^3) of the whole head were used for quantitative measurements. In addition, T2-weighted dual-echo turbo-spin-echo scans (echo time 1/echo time 2, 9 milliseconds/100 milliseconds; flip angle, 90°; voxel dimension, 0.98 x 0.98 mm^2) with 19 axial 5-mm slices and a 1.2-mm gap of the whole head were used for clinical neurodiagnostic evaluation.

Processing was done on the neuroimaging computer network from our department of psychiatry. All images were coded to ensure blindness of participant identification and diagnoses. Scans were manually put into Talairach frame (no scaling) for segmentation purposes and corrected for inhomogeneities in the magnetic field (Sled et al., 1998). Quantitative assessment of intracranial, total brain, and gray and white matter of the cerebrum (total brain excluding cerebellum and stem), and lateral and third ventricle volumes was performed using histogram analyses and series of mathematical morphology operators to connect all voxels of interest validated previously (Schnack et al., 2001a; 2001b). All images were checked after measurement and corrected manually if necessary. The interrater reliabilities of the volume measurements, determined by the intraclass correlation coefficient, were 0.95 and higher.

The frontal, parietal, temporal, and occipital lobes were segmented based on transformations to a model brain onto which the lobes had been manually demarcated (Hulshoff Pol et al., 2002b; Palmen et al., 2004). The model brain was selected earlier among 200 brain images of healthy individuals between 16 and 70 years of age. Brain images were registered to the model brain through the Automatic Nonlinear Image Matching and Anatomical Labeling algorithm (Collins et al., 1995) to remove global differences in size and shape of individual brains. The inverse of the transformation process registered the manual segmentations of the model brain to all participants' brain images. The segments were visually checked (Figure 1).

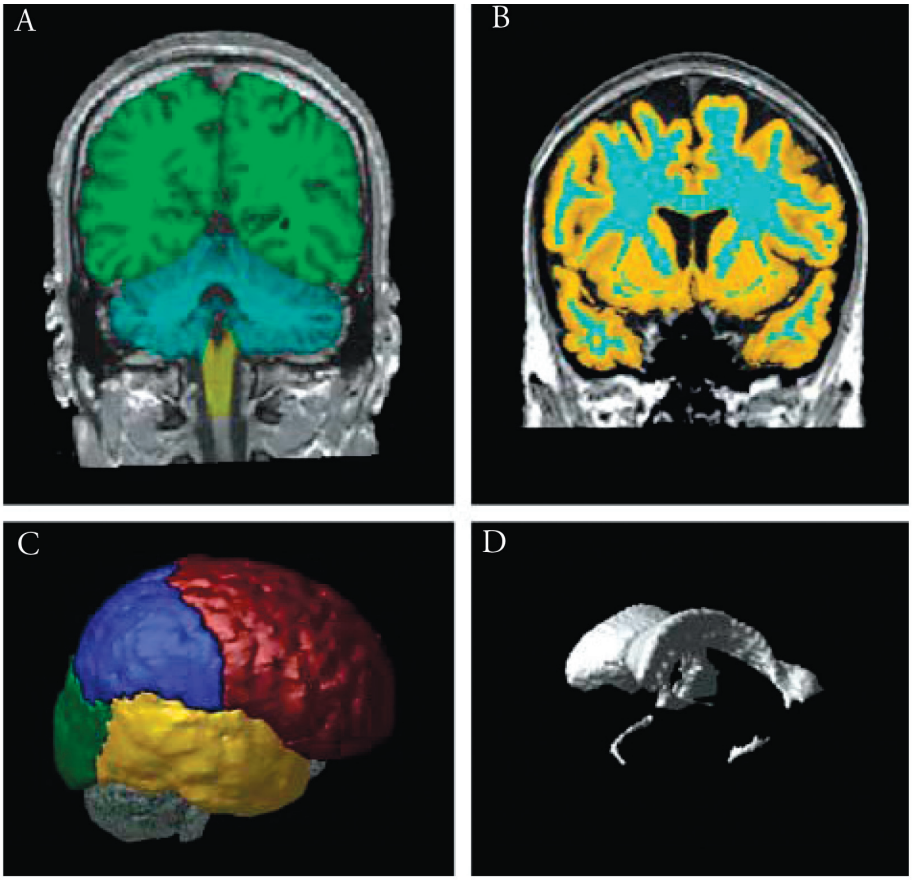

Figure 1. Segmentations of the whole brain and cerebellum (A), gray and white matter (B), the frontal, temporal, parietal, and occipital lobes (C), and the lateral and third ventricles (D). Quantitative assessments of the intracranial, total brain, gray and white matter of the cerebrum (total brain excluding cerebellum and stem) and lateral and third ventricle volumes were performed based on histogram analyses and series of mathematical morphology operators to connect all voxels of interest.

Statistical analysis

Brain volume changes are expressed in percentages: [(follow-up − baseline volume)/baseline volume] x 100. Data were examined for outliers, extreme values, and normality of distribution. No transformations were needed for this or any of the other measures.

For statistical analysis of the data, our approach was 2-fold. Multiple repeated-measures univariate analyses of covariance were applied to initial measurement of brain volume change in the 2 groups. This procedure did not allow for decomposition of the observed variance into genetic and environmental parts, but made the findings comparable with earlier twin studies in which a single MRI scan was made and was readily interpretable. In repeated-measures univariate analysis of covariance, unstandardized residuals of percentage brain volume change were entered as dependent variables. Percentage brain volume change was corrected for age and sex. Twin status (twin 1=patient with schizophrenia or healthy control, twin 2=co-twin of patient or healthy control) was entered as a within-subjects factor. Between-subjects factors were group (discordant or healthy twin pair) and zygosity (MZ or DZ).

For estimating the significance of the relative contributions of genetic and environmental (family-related and unique) factors on variability in (progressive) brain volume changes in schizophrenia, structural equation modeling is the method of choice in twin studies. Implementing bivariate genetic models, structural equation modeling also yields valid tests on whether genetic or family-related factors explain the association between schizophrenia and brain volume changes. To what extent genes and/or environment are responsible for this association was expressed by bivariate heritability: the percentage of covariance that is accounted for by a common genetic factor. This method has been extensively described (Rijsdijk et al., 2002) and applied to schizophrenia before (Rijsdijk et al., 2005; Toulopoulou et al., 2007).

The analyses were done using the statistical package Mx (Neale et al., 2003). Because the Mx software cannot handle ordinal and continuous data simultaneously, the residuals of the percentage brain volume changes (after regression on sex and age) were used to calculate a 5-category ordinal measure for brain volumes. This ordinal scale was created by dividing the residuals, so that each category covers 20% of the data following a normality distribution. Both affected and unaffected individuals were assumed to have an underlying liability to develop schizophrenia (standard normal distribution) (Rijsdijk et al., 2005; Toulopoulou et al., 2007). Thus, it was believed that if a person with a high value on the liability scale crossed a certain threshold, he or she would become ill (patient) or otherwise remain healthy (discordant co-twin of

patient or healthy twin pairs).
The critical threshold for schizophrenia was not based on our sample, as it was approximately 25% schizophrenic patients, which would have resulted in an overestimation of the prevalence (i.e., 1%). Also, our active attempts to find as many discordant twin pairs as possible and exclude concordant pairs would have affected heritability estimates. Instead, we constrained the heritability (h^2) at 81%, the influence of family-related environmental factors (c^2) at 11% (rMZ=0.92, rDZ=0.52) (based on a meta-analysis of twin studies (Sullivan et al., 2003)), and the prevalence at 1% (resulting in a critical threshold at 2.33) (based on epidemiological studies (Gottesman, 1990)). This procedure was found to give valid and reliable estimates for bivariate heritability in such analyses (Rijsdijk et al., 2005).
Using structural equation modeling, the phenotypic correlations (r_{ph}) between schizophrenia liability and percentage brain volume changes were calculated; phenotypic correlations can result from a common set of genes or environmental factors. These phenotypic correlations were then decomposed into genetic (r_g) and environmental components (r_e), thus providing information regarding the possible shared genetic and environmental influences of schizophrenia liability and brain volume changes. Decomposition of these sources was based on the comparison of cross-trait/cross-twin correlations for MZ and DZ twins, i.e., the correlation between a trait (schizophrenia liability) of twin 1 with another trait of twin 2 (brain volume change), where twin 1 and twin 2 represent a twin pair. If the absolute value of the correlation between brain volume change of twin 1 and schizophrenia liability of twin 2 is larger in MZ twins than in DZ twins, this indicates that the genes that influence brain volume change (partly) overlap with genes that influence schizophrenia. The extent of the overlap is reflected by the magnitude of the genetic correlation (r_g). When the cross-trait/cross-twin correlations are similar and, for MZ and DZ twins, differ from 0, this suggests that environmental factors that are shared within families contribute to the phenotypic correlation between brain volume change and schizophrenia. If both correlations are 0, only individual-specific environmental correlations exist.
The contribution of additive genetic (A), common environmental (C), and unique environmental (E) factors to the variance in brain volume changes (univariate heritability) and to the covariance between schizophrenia liability and brain volume changes (bivariate heritability) was expressed as a percentage of the total (co)variance: the percentage A was expressed as h^2 (heritability), C as c^2 (common or shared environment) and E as e^2 (unique environment). Random measurement error is part of e^2. By combining the

information from r_g, r_c, and r_e with h^2, c^2, and e^2, the influence of genetic, common environmental, and unique environmental factors on the total phenotypic correlation between schizophrenia and brain volume change could be established. Specifically, the percentage of covariance between schizophrenia and percentage brain volume change that is accounted for by a common genetic factor is expressed as bivariate heritability: h^2_{biv}=covA/(covA + covC + covE).

By minimizing a goodness-of-fit statistic between observed and predicted covariance matrices, structural equation modeling programs estimate model parameters (a, c, and e). Effects of genetic and family-related factors were tested by comparing the likelihoods of nested models (−2 log likelihood, which is χ^2 distributed) in which it is tested whether, for example, a CE model fits as well as an ACE model, using the most simple model that best explains the data. A χ^2 value greater than 3.84 (1 df) indicates a significant difference at $\alpha = 0.05$, which means that the reduced model provides a significantly worse fit to the data and indicates that the discarded effect (e.g., disease effect) cannot be left out of the model without seriously deteriorating the goodness of fit.

Using the full model, estimates (including 95% confidence intervals [CIs]) were obtained that reflect the increase or decrease in brain volume over time in patients or in individuals with a familial background of schizophrenia.

Results

Repeated-measures analysis of covariance

Over time, a significant group effect was found for percentage whole brain volume change (Table 2 and Figure2). Combined, patients and their co-twins showed a progressive decrease in whole brain volume over time compared with the control twin pairs ($F_{1,42}$=4.60; P=.04). Furthermore, a significant twin x zygosity interaction was found ($F_{1,42}$=8.18; P=.01), which indicates that the MZ co-twins show a more prominent decrease in whole brain volume than the DZ co-twins compared with the patients with schizophrenia. The group X zygosity interaction was significant for lateral ventricle volume ($F_{1,42}$=13.20; P=.01) owing to a more prominent progressive increase in lateral ventricle volume in discordant DZ compared with MZ twin pairs, whereas the controls did not show a difference between MZ and DZ pairs. Moreover, a significant group effect was found in frontal, temporal, and (gray matter) occipital lobes. No significant group effects were found for cerebral white matter ($F_{1,41}$=0.24; P=.63), cerebellum ($F_{1,42}$=2.05; P=.16), or third ventricle ($F_{1,42}$=0.39; P=.54) volumes.

Table 2. Brain volume change after a 5-year follow-up in twins with and without schizophrenia

	Mean (SD) % brain volume change [a]				Discordant compared with healthy twin pairs [b]			
	MZ twins		DZ twins		Discordant vs healthy twin pairs		Schizophrenic patients and co-twins difference vs HCs 1 and HCs 2 difference	
Brain structure	Discordant Twin pairs (n=9 pairs)	Healthy Twin pairs (n=14 pairs)	Discordant Twin pairs (n=10 pairs)	Healthy Twin pairs (n=13 pairs)	$F_{1,42}$	*P* Value	$F_{1,42}$	*P* Value
Whole brain	-1.28 (3.09)	-0.37 (2.18)	-2.39 (2.87)	-0.30 (2.08)	4.60	.04	0.10	.76
Frontal lobe	-2.05 (3.62)	-1.12 (2.16)	-3.29 (3.02)	-1.02 (2.25)	4.79	.03	1.40	.24
Temporal lobe	-0.77 (3.28)	+0.14 (2.43)	-2.01 (2.70)	+0.15 (1.91)	4.64	.04	0.25	.62
Parietal lobe	-1.46 (4.00)	-0.90 (2.74)	-3.07 (3.84)	-0.73 (3.12)	2.36	.13	0.00	.99
Occipital lobe	-1.84 (3.24)	+0.30 (2.45)	-1.53 (2.98)	+0.07 (2.07)	7.13	.01	0.12	.73
Cerebral GM	-3.33 (6.54)	-2.42 (2.77)	-4.16 (5.27)	-1.81 (3.39)	2.34	.13	0.16	.69
Frontal lobe	-3.87 (6.77)	-2.94 (3.08)	-4.82 (4.85)	-2.41 (3.09)	2.36	.13	0.75	.39
Temporal lobe	-1.37 (5.80)	-0.64 (2.98)	-3.34 (4.24)	-0.13 (2.51)	3.50	.07	0.96	.33
Parietal lobe	-3.86 (5.76)	-3.21 (3.48)	-4.68 (6.39)	-2.49 (3.82)	1.27	.27	0.05	.82
Occipital lobe	-5.07 (7.34)	-1.97 (4.66)	-4.52 (6.92)	-0.53 (5.09)	4.55	.04	0.00	.99
Cerebral WM	+1.38 (3.71)	+1.87 (4.03)	+0.26 (4.03)	+1.01 (4.45)	0.24	.63	0.10	.76
Frontal lobe	+0.77 (3.26)	+1.54 (3.07)	-0.29 (3.25)	+1.04 (4.30)	1.22	.28	0.00	.95
Temporal lobe	+0.74 (4.50)	+1.65 (4.35)	+1.49 (4.24)	+0.71 (5.23)	0.00	.99	1.68	.20
Parietal lobe	+2.18 (5.47)	+2.37 (4.04)	-0.04 (4.17)	+1.71 (5.45)	0.50	.48	0.01	.92
Occipital lobe	+3.41 (6.96)	+2.97 (5.05)	+2.85 (5.96)	+0.87 (5.60)	0.76	.39	0.02	.90
Cerebellum	+0.55 (3.02)	+1.01 (3.05)	-0.73 (3.38)	+1.34 (2.35)	2.05	.16	0.01	.91
Lateral ventricle	+2.71 (15.65)	+7.26 (9.06)	+13.26 (13.05)	+0.99 (8.36)	1.53	.22	1.43	.24
Third ventricle	-6.07 (19.44)	+1.03 (14.98)	+5.17 (29.16)	+1.43 (20.55)	0.39	.54	0.00	.96

Abbreviations: DZ, dizygotic; GM, gray matter; HC, healthy control; MZ, monozygotic; WM, white matter. [a] Percentage volume change from baseline to follow-up: [(follow-up - baseline volume)/baseline volume] X 100. Gray matter and WM volumes of 1 DZ co-twin are missing. [b] Multiple repeated measures analysis of covariance of percentage brain volume change with twin as a within-group variable; group (discordant twin pair or healthy twin pair) and zygosity (MZ and DZ) as between-group variables; and age and sex as covariates. Significant main effects for group (discordant vs healthy twin pairs) suggest familiar or genetic influences on percentage brain volume change; significant interaction effects of group x twin (schizophrenic patients and co-twins difference vs HCs 1 and HCs 2 difference) suggest that possible (nongenetic) disease-related factors influence percentage brain volume after the 5-year follow-up.

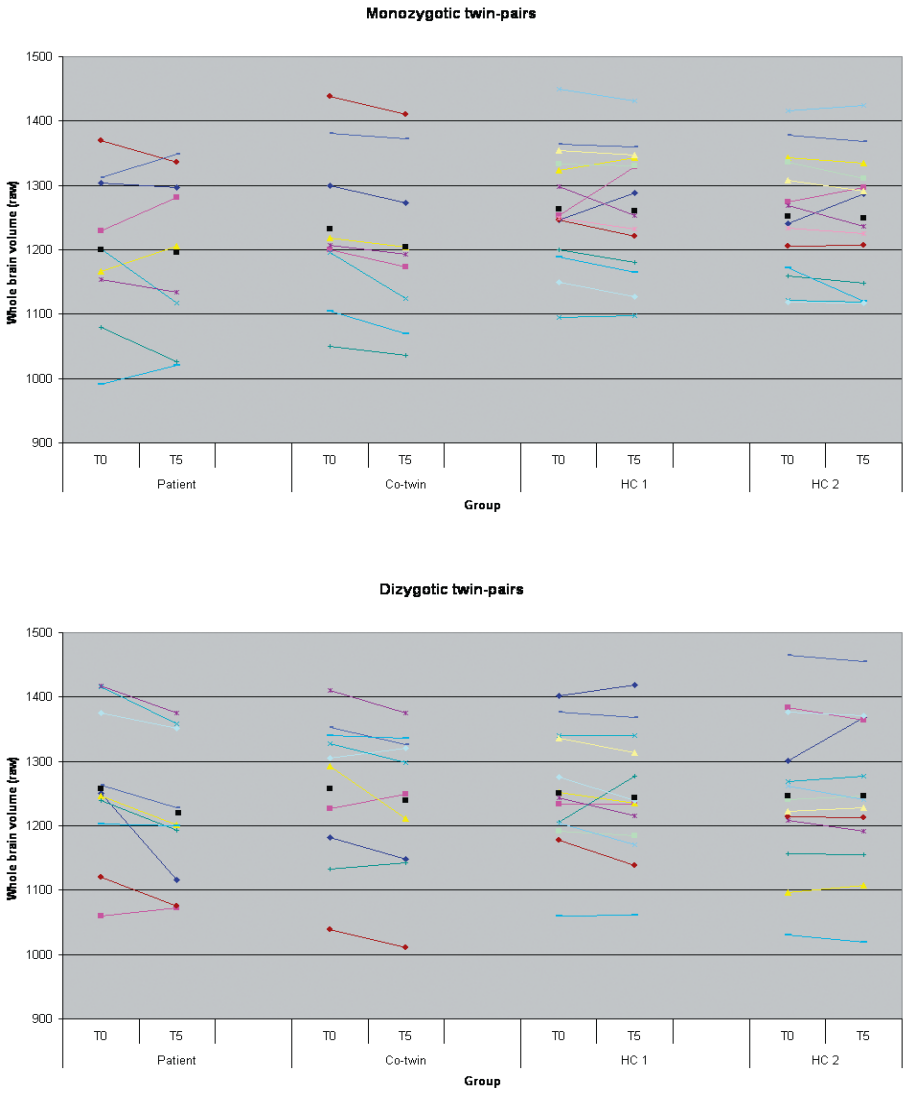

Figure 2. Whole-brain volume at baseline (T0) and follow-up (T5) in twin pairs discordant for schizophrenia and healthy twin pairs. Each line represents an individual. Twins correspond to each other by color; patients are associated with their co-twins, and healthy controls (HCs 1) are associated with their co-twins (HCs 2). The black squares and lines represent mean whole brain volume. For monozygotic twin pairs, percentage volume change was −0.37% for patients and −2.18% for their co-twins; for monozygotic HCs 1, it was −0.23%, and for their co-twins (HCs 2), it was −0.51%. For dizygotic twin pairs, percentage volume change was −3.26% for patients and −1.52% for their co-twins; for dizygotic HCs 1, it was −0.65%, and for their co-twins (HCs 2) it was 0.05%.

Structural Equation Modeling

Over time and irrespective of disease, a decrease in whole brain volume was found, which was moderately correlated within twin pairs. A significant familial effect was found (h^2 and c^2 together, 55%), but it was not possible to disentangle the influence of h^2 and c^2 in this sample. Around half of the variance was accounted for by e^2 (45%) (Table 3).

When adding schizophrenia as a trait in the bivariate analysis (Table 4), the decrease in whole-brain volume became more pronounced, with higher schizophrenia liability due to genetic factors (within-twin/cross-trait correlation, r_{ph}=−0.22). The influence of genes involved in schizophrenia liability and whole-brain volume change was found to be significant and was estimated to account for 66% (95% CI, 51%-100%). This means that at least 51% of the covariance of whole-brain volume change and schizophrenia can be explained by genetic factors. In addition, shared environmental factors, though not of significant influence, explained approximately 23% (95% CI, 0%-40%) of the shared variance between schizophrenia liability and brain volume change. The remaining (nonsignificant) approximately 11% (95% CI, 0%-38%) shared variance between schizophrenia liability and brain volume change was due to unique environmental factors. In addition, a significant influence of additive genetic factors on volume change and schizophrenia was found for frontal (76%; 95% CI, 54-100%) and temporal (79%; 95% CI, 56-100%) lobe volume change.

Association with clinical variables

No associations were found between clinical measurements or level of parental education and brain volume changes. Participants who met diagnosis did not differ from those who were healthy (for whole-brain volume, MZ individuals: $F_{1,35}$=0.14; P=.71; and DZ individuals: $F_{1,34}$=0.01; P=.94). The correlation between whole-brain volume change and number of cigarettes smoked (r=−0.13; P=.22) and amount of alcohol consumed (r=0.12; P=.27) at follow-up was nonsignificant. When repeating repeated measures univariate analyses of covariance with participants who had used drugs, the co-twin who had used antipsychotic drugs briefly, with participants who consumed more than 15 drinks per week at follow-up excluded, the group effect remained significant ($F_{1,27}$=4.36; P=.046).

Table 3. Within-trait/cross-twin correlations and cross-trait/cross-twin correlations on percentage brain volume change

	Correlation (95% Confidence Interval) [a]			
	Within-trait/cross-twin correlation [b]		Cross-trait/cross-twin correlation [c]	
Brain structure	23 MZ twin pairs	23 DZ twin pairs	23 MZ twin pairs	23 DZ twin pairs
Whole brain	0.46 (0.09 to 0.71)	0.35 (-0.18 to 0.69)	-0.32 (-0.51 to -0.10)	-0.05 (-0.31 to 0.21)
Frontal lobe	0.47 (0.07 to 0.73)	0.56 (0.16 to 0.77)	-0.35 (-0.54 to -0.12)	-0.11 (-0.36 to 0.13)
Temporal lobe	0.49 (0.14 to 0.72)	0.48 (-0.06 to 0.77)	-0.38 (-0.56 to -0.16)	-0.16 (-0.41 to 0.09)
Parietal lobe	0.29 (-0.11 to 0.60)	0.21 (-0.31 to 0.62)	-0.22 (-0.42 to 0.01)	-0.14 (-0.40 to 0.14)
Occipital lobe	0.10 (-0.27 to 0.44)	-0.16 (-0.60 to 0.40)	-0.34 (-0.52 to -0.13)	-0.11 (-0.36 to 0.15)
Cerebral GM	-0.03 (-0.42 to 0.37)	0.17 (-0.32 to 0.58)	-0.26 (-0.45 to -0.06)	-0.07 (-0.33 to 0.19)
Frontal lobe	0.29 (-0.14 to 0.62)	0.40 (-0.07 to 0.71)	-0.29 (-0.48 to -0.07)	-0.03 (-0.29 to 0.23)
Temporal lobe	0.30 (-0.12 to 0.62)	0.59 (0.15 to 0.82)	-0.36 (-0.55 to -0.14)	-0.26 (-0.50 to -0.00)
Parietal lobe	-0.03 (-0.47 to 0.42)	0.13 (-0.30 to 0.51)	-0.23 (-0.42 to -0.02)	-0.06 (-0.22 to 0.31)
Occipital lobe	0.35 (-0.08 to 0.66)	0.49 (0.06 to 0.76)	-0.31 (-0.51 to -0.08)	-0.11 (-0.35 to 0.15)
Cerebral WM	0.34 (-0.11 to 0.66)	0.25 (-0.25 to 0.63)	0.01 (-0.22 to 0.24)	0.03 (-0.25 to 0.29)
Frontal lobe	0.01 (-0.45 to 0.46)	0.16 (-0.26 to 0.53)	-0.06 (-0.28 to 0.15)	0.02 (-0.25 to 0.28)
Temporal lobe	0.27 (-0.16 to 0.60)	0.06 (-0.43 to 0.52)	0.01 (-0.22 to 0.23)	0.12 (-0.16 to 0.38)
Parietal lobe	0.53 (0.13 to 0.77)	0.24 (-0.26 to 0.63)	0.08 (-0.15 to 0.30)	-0.03 (-0.30 to 0.24)
Occipital lobe	0.39 (-0.03 to 0.69)	0.02 (-0.48 to 0.40)	0.11 (-0.11 to 0.32)	0.11 (-0.16 to 0.36)
Cerebellum	0.63 (0.33 to 0.82)	0.45 (-0.13 to 0.76)	-0.19 (-0.40 to 0.05)	-0.04 (-0.32 to 0.22)
Lateral ventricle	0.13 (-0.27 to 0.48)	0.29 (-0.33 to 0.68)	0.18 (-0.05 to 0.39)	0.26 (-0.03 to 0.55)

Abbreviations: DZ, dizygotic; GM, gray matter; MZ, monozygotic; WM, white matter.

[a] The 95% confidence intervals including 0 indicate statistical nonsignificance.

[b] Irrespective of disease. Within-trait/cross/twin correlation, or intraclass correlation, is the correlation of twin 1 with his/her co-twin (twin 2) on percentage brain volume change: {[follow-up - baseline volume)/baseline volume] x $100_{twin\ 1}$} - {[follow-up - baseline volume)/baseline volume] x – $100_{twin\ 2}$}.

[c] Associated with schizophrenia. Correlation of percentage brain volume change of twin 1 with genetic liability to schizophrenia (SZ) of his/her co-twin (twin 2): {[(follow-up - baseline volume)/baseline volume] x $100_{twin\ 1}$} - $SZ_{twin\ 2}$. The schizophrenia within-trait/cross-twin correlation ($SZ_{twin\ 1}$ - $SZ_{twin\ 2}$) is constrained to 0.92 in MZ twins and 0.52 in DZ twins based on the genetic point estimates of a meta-analysis and a 1% prevalence.

Table 4. Phenotypic correlations and estimated influences of additive genetic, common and unique environmental factors on brain volume change [a]

	% (95% Confidence Interval) [b]						
	Sources of variance on brain volume change [c]				Sources of covariance between schizophrenia and brain volume change [e]		
Brain structure	h^2	c^2	e^2	Phenotypic correlation [d]	h^2_{biv}	c^2_{biv}	e^2_{biv}
Whole brain	32 (0 to 76)	23 (0 to 59)	45 (23 to 76)	-0.22 (-0.41 to -0.01) [f]	66 (51 to 100) [f]	23 (0 to 40)	11 (0 to 38)
Frontal lobe	17 (0 to 70)	37 (0 to 65)	46 (26 to 74)	-0.26 (-0.44 to -0.05) [f]	76 (54 to 100) [f]	8 (0 to 36)	16 (0 to 38)
Temporal lobe	19 (0 to 71)	32 (0 to 62)	49 (28 to 78)	-0.28 (-0.47 to -0.08) [f]	79 (56 to 100) [f]	3 (0 to 32)	18 (0 to 36)
Parietal lobe	16 (0 to 60)	14 (0 to 51)	71 (40 to 99)	-0.17 (-0.36 to 0.04)	62	20	18
Occipital lobe	13 (0 to 40)	0 (0 to 25)	87 (60 to 99)	-0.21 (-0.37 to 0.01)	74 (52 to 100) [f]	1 (0 to 27)	25 (0 to 47)
Cerebral GM	8 (0 to 45)	7 (0 to 40)	85 (55 to 100)	-0.19 (-0.37 to 0.01)	73	5 (5 to 100)	22 (0 to 51)
Frontal lobe	22 (0 to 61)	16 (0 to 52)	62 (36 to 95)	-0.20 (-0.39 to 0.01)	65 (49 to 89) [f]	21(0 to 40)	14 (0 to 35)
Temporal lobe	3 (0 to 55)	39 (0 to 64)	58 (34 to 86)	-0.25 (-0.43 to -0.04) [f]	32 (0 to 90)	44 (0 to 92)	24 (4 to 42)
Parietal lobe	9 (0 to 41)	3 (0 to 26)	88 (57 to 100)	-0.12 (-0.31 to 0.08)	64	14 (0 to 45)	22 (0 to 77)
Occipital lobe	14 (0 to 65)	31 (0 to 57)	55 (32 to 85)	-0.20 (-0.40 to 0.01)	72 (49 to 100) [f]	8 (0 to 38)	20 (0 to 50)
Cerebral WM	1 (0 to 60)	26 (0 to 54)	73 (40 to 100)	-0.06 (-0.27 to 0.15)	35	38	28
Frontal lobe	3 (0 to 46)	8 (0 to 39)	89 (54 to 100)	-0.12 (-0.32 to 0.08)	50	29	21
Temporal lobe	12 (0 to 56)	12 (0 to 47)	77 (43 to 100)	-0.08 (-0.28 to 0.13)	35	37	28
Parietal lobe	45 (0 to 76)	7 (0 to 62)	48 (24 to 85)	0.01 (-0.20 to 0.22)	51	28	21
Occipital lobe	33 (0 to 66)	2 (0 to 74)	65 (34 to 100)	0.05 (-0.15 to 0.25)	38	27	35
Cerebellum	45 (0 to 83)	21 (0 to 71)	34 (17 to 63)	-0.11 (-0.33 to 0.11)	63	20	17
Lateral ventricle	0 (0 to 47)	41 (1 to 63)	59 (36 to 86)	0.06 (-0.14 to 0.25)	0 (0 to 40)	58 (0 to 80)	42 (20 to 80)
Third ventricle	0 (0 to 44)	6 (0 to 34)	94 (56 to 100)	-0.10 (-0.30 to 0.11)	27	36	37

Abbreviations: biv, bivariate; c^2, shared environmental effects; e^2, nonshared environmental effects; GM, gray matter; h^2, heritability; WM, white matter.

[a] All analyses are for percentage brain volume change and corrected for age and sex. Parameters for schizophrenia are fixed based on a prevalence of 1% and the following genetic model: h^2=0.81, c^2=0.11, e^2=0.08.

[b] The 95% confidence intervals from 0 to 100 are not shown.

[c] Irrespective of disease. Reflects the influence of additive genetic and common and unique environmental factors on brain volume change, ie, additive genetic effects account for 32% of the variance on whole-brain volume change.

[d] Reflects the correlation between schizophrenia liability and percentage brain volume change within individuals, ie, the correlation between schizophrenia and whole-brain volume change = -0.22, which indicates that patients with schizophrenia have a larger reduction in whole-brain volume during the 5-year interval than healthy comparison participants.

[e] Sources of covariance between schizophrenia and percentage brain volume change are represented by h^2_{biv}, c^2_{biv} and e^2_{biv} on the correlation between schizophrenia liability and brain volume change within individuals, ie, genetic influences that are shared by percentage brain volume change and schizophrenia account for 66% of the phenotypic correlation between brain volume change and schizophrenia.

[f] Significant at α=.05.

Comment

This study examined the relative contributions of genetic and environmental factors on percentage brain volume changes over time in schizophrenia. In a longitudinal study with a 5-year interval, MZ and DZ twin pairs discordant for schizophrenia were compared with healthy twin pairs. To our knowledge, this is the first longitudinal MRI study in twin pairs discordant for schizophrenia.

Our main finding is that progressive decreases in whole brain and frontal and temporal lobe volumes were found both in patients with schizophrenia and in their unaffected co-twins compared with the healthy twin pairs. This was largely due to decreases in gray matter volume over time. Furthermore, by applying structural equation modeling, we demonstrated that at least 51% of the correlation of −0.22 between whole-brain, frontal lobe, and temporal lobe volume loss and schizophrenia liability could be explained by genetic factors that are also directly implicated in the disease. Thus, genes that are directly involved in the etiology of schizophrenia may also contribute to the (frontal and temporal) brain volume loss observed in the patients and their co-twins. The results also imply that the genes that play a role in (frontal and temporal) brain volume loss in (healthy) aging may be suitable candidate genes for schizophrenia. Finally, the finding of progressive brain volume loss in the unaffected co-twins of the patients indicates that the progressive brain volume loss in schizophrenia can no longer be explained solely as the result of disease-associated factors, such as antipsychotic medication intake, smoking, or outcome.

The brain volume change over time in the chronically ill patients was approximately 5 times that found in individuals with normal aging (as expressed in the brain volume changes in the control participants during the 5-year interval). This finding is in keeping with longitudinal MRI studies that reported progressive brain changes in chronically ill patients (van Haren et al., 2008; Davis et al., 1998; Mathalon et al., 2001). By including co-twins who were discordant for schizophrenia, we were able to measure the relative contributions of common environmental and genetic factors on these progressive brain volume changes. The extent of the tissue loss in the co-twins of the patients approximated that found in the patients themselves and was considerably more prominent than in controls. This finding strongly suggests that progressive brain volume change in schizophrenia has a familial background and is consistent with the results of a study in siblings discordant for childhood-onset schizophrenia (Gogtay et al., 2007). Additionally, based on the cross-trait/cross-twin comparison of disease liability with brain volume

change in the MZ and DZ twin pairs in our study, the relative influence of genetic, common environmental, and unique environmental factors could be calculated. These results demonstrated that approximately 66%, at least 51%, of the variation in whole-brain volume loss that is associated with schizophrenia could be explained by genetic factors.

That frontal lobe volume loss in particular can be explained by genetic factors (76%; 95% CI, 54%-100%) that also directly contribute to schizophrenia may not be entirely surprising. The frontal lobe has been implicated in the genetic risk for the disease in a cross-sectional study in the current sample (Baaré et al., 2001) and in others (Cannon et al., 2002a). The genetic influences on temporal lobe volume change in schizophrenia (79%; 95% CI, 56%-100%) found in this study are consistent with findings of decreased temporal cortical thickness in family members of patients (Goghari et al., 2007). To what extent genetic risk is associated with cortical thickness change in local areas in the cortex is currently being assessed.

Shared environmental factors implicated in the disease explained another 23% (nonsignificant) of the variation in whole-brain volume loss in schizophrenia (8% of the variation in frontal lobe and 3% in temporal lobe volume). Unique environmental factors did not significantly contribute to the progressive whole-brain volume change found in patients, as the remaining shared variance between schizophrenia liability and brain volume change due to unique environmental factors or measurement error was small, approximately 11% (comparable with the frontal lobe [16%] and the temporal lobe [18%]). Antipsychotic medication intake can be considered such a unique environmental factor. While all the patients were taking antipsychotic medication (except for 1 co-twin who took them briefly), none of the co-twins used antipsychotic medication. Consequently, antipsychotic medication is unlikely to cause the progressive brain volume changes in the patients with schizophrenia.

Our sample size did not allow a statistical analysis to assess possible gene X environment interactions, such as in the case of epigenetic mechanisms, which exert lasting control over gene expression without altering the genetic code (Tsankova et al., 2007). However, even if gene X environment interactions were involved in preventing the co-twins from developing schizophrenia, this does not explain why it would leave the co-twins with a symptom-free progressive brain volume loss later in life. Indeed, there is suggestive evidence that the progressive brain volume loss in co-twins may not be without consequences: studies examining cognitive function in unaffected co-twins of patients with schizophrenia find impairments on cognitive tests and educational attainment (Cannon et al., 2000; Kremen et

al., 2006; Goldberg et al., 1990). Cognitive impairments may thus be associated with the progressive brain volume loss observed in the unaffected co-twins of patients with schizophrenia.

However, in this study, we did not find an association between outcome, level of parental education, and brain volume changes.

As to the (patho)physiological processes that are responsible for the progressive brain changes, we can only speculate. The brain volume loss in the discordant twin pairs may represent altered plasticity in adulthood. It is now clear that the brain continues to show plasticity during adulthood, at least in some areas. Neurogenesis is known to occur in the adult human hippocampus (Eriksson et al., 1998) and olfactory bulb (Bédard et al., 2004). However, adult neurogenesis in several other areas, including the neocortex, striatum, amygdala, and substantia nigra, has also been suggested (Gould et al., 2007). Electron microscopy studies in rodent brains have demonstrated that neural circuits are sculpted by spontaneous activity and sensory experience (Katz et al., 1996). Also, evidence is accumulating that functional rewiring takes place in the adolescent and adult rodent brain, which may involve structural plasticity with synapse formation and elimination (Chkolskii et al., 2004; Zuo et al., 2005). Moreover, action potential firing was found to influence myelination (Ishibashi et al., 2006). Thus, it is tempting to hypothesize that the progressive volume loss associated with the liability to develop schizophrenia represents aberrant plasticity of adult functional neural networks. Indeed, several lines of evidence suggest there is abnormal neurogenesis and aberrant expression of developmental genes in schizophrenia and a role of candidate schizophrenia susceptibility genes in adult neurogenesis (Reif et al., 2006). Some of the candidate schizophrenia susceptibility genes have been associated with brain volumes in healthy subjects (Peper et al., 2007). In schizophrenia, the disrupted in schizophrenia 1 (DISC1), translin-associated factor X (TRAX) (Cannon et al., 2005), and GAD1 (Addington et al., 2005) genes were found to contribute to decreased gray matter volumes.

Common environmental factors implicated in the disease itself explained 23% of the brain volume loss in patients, though this effect was not significant. Possible common environmental factors shared among patients with schizophrenia and their close relatives are stress factors (McEwen, 2000; McDonald & Murray, 2000). The emotional burden of the disease can also be considerable in siblings of patients with schizophrenia (Schmid et al., 2006). For twins, who often have a close emotional relationship with each other, the experience of a co-twin having a severe psychiatric disease like schizophrenia may represent a more pronounced burden. Other possible common environmental factors that patients share with their co-twins that have been

associated with schizophrenia are viral infections (Davis et al., 1995; Torrey, 1988), psychosocial factors (Portin et al., 1997), prenatal environment (Brown et al., 2007), and delivery complications (Verdoux et al., 1997). Delivery complications have been associated with decreased brain volumes in twin pairs discordant for the disease (Cannon et al., 2002b; van Erp et al., 2004). Early (prenatal or perinatal) neurodevelopment lesions that render the brain vulnerable and anomalous late neurodevelopmental processes may interact with other causative factors associated with the onset of psychosis (McDonald & Murray et al., 2000; Weinberger et al., 2002; Cannon et al., 2003; Rapoport et al., 2005; Mathalon et al., 2003).

This study has several limitations that have to be considered. We were able to retrieve 91% of the original twin sample, and no sample bias was present at the time of the second MRI scan. However, the relatively small sample size (N=92) did give room for possible chance variations between the subgroups. The smaller brain tissue loss and ventricular volume increase in MZ compared with DZ patients is counterintuitive. When selecting discordant twin pairs and assuming the genetic liability to have an underlying continuum (as hypothesized here), the MZ patients with schizophrenia may have had a relatively lower genetic liability for schizophrenia. However, this could not have explained our findings since it would have resulted in an underestimation of the genetic liability for progressive brain changes in schizophrenia. The within-trait/cross-twin correlations of the MZ twin pairs were not significantly higher than the within-trait/cross-twin correlations of the DZ twin pairs. Based on the current sample, we cannot conclude that progressive brain volume change (irrespective of disease) is due to genetic factors. However, in a larger sample (approximately 100 healthy twin pairs), we found a comparable and significant heritability for whole-brain volume change (R.G.H.B., unpublished data, 2008). Thus, there indeed appears to be a heritable component to brain volume change. Also, the sample size did not allow for measurement of the effect of interactions between genes and environmental factors on brain volume changes. Confidence intervals were large and therefore the environmental influences should be interpreted cautiously: the extent of common and unique environmental factors common to schizophrenia and progressive brain volume change in the patients remains inconclusive. For the other brain volume changes over time, we were not able to disentangle the influence of genetic, common environmental, and unique environmental factors.

In conclusion, we found progressive brain volume loss during a 5-year interval both in patients with schizophrenia and their unaffected co-twins. A significant proportion of this effect could be attributed to genes that are

implicated in schizophrenia. Localizing and characterizing the genes involved in dynamic brain changes may prove to be a valuable approach in studying the pathophysiology of progressive brain changes in schizophrenia.

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Chapter 6

Summary and discussion

6.1 Summary and discussion

The research presented in this thesis explored the possible mechanisms underlying the individual differences in brain structure and brain structure change in healthy adults and schizophrenia patients. For this purpose we set out two lines of research: one study comprised healthy individuals and the other studies comprised schizophrenia patients. All studies were conducted in relatives (i.e. twin pairs or siblings) to be able to disentangle genetic and environmental influences on brain structure and brain structure change. In this final chapter a summary and discussion of the main findings is provided. Moreover, possible implications and directions for future research are suggested.

6.2 Healthy brain development

Overall head size, brain volume, as well as particular focal gray matter areas, are under considerable genetic control (>80%) in children and adults (Lenroot et al, 2009; Peper et al., 2007 and 2009; Baaré et al., 2001a). Moreover, some of these gray and white matter areas, including the superior occipitofrontal fascicle, corpus callosum and medial frontal and occipital cortices have been positively associated with level of intelligence, and their associations were found to be mediated by common genetic factors (Hulshoff Pol et al., 2006; Posthuma et al., 2002). During life, substantial changes in brain structure continue to take place (Bartzokis et al., 2001). However, the etiology of these dynamic brain patterns and whether this is associated with level of intelligence has not been established. Therefore, a longitudinal MRI study was set up which was described in chapter 2. In 242 adult individuals from 106 twin families, who were 19-55 years of age at first measurement, the heritability of brain structure and brain structure change and its possible link with intelligence was investigated. Hundred eighty-three individuals completed the follow-up measurement after approximately 5 years. Using structural equation modeling, the contributions of additive genetic, common and unique environmental factors to the variance on brain structure, brain structure change and IQ were estimated.

Results indicated that in healthy adults, brain structure continues to be dynamic even resulting in thickening of the cortex in some brain areas. These changes in brain structures are not only heritable, but it also appeared that genes or the expression of genes responsible for brain structure change over time are different from those determining absolute brain structures, particularly

in the left superior frontal and left superior temporal cortices. Moreover, increased thickening and decreased thinning of the cortex is associated with higher IQ scores and this association is mediated by common genes. Thus, genes that are implicated in cortical thickness change overlap with those involved in level of intelligence.

It is well established that the brain has the ability to reorganize itself. In patients suffering from stroke, unaffected regions of the brain can adopt the functions of those parts of the brain that have perished during the insult. Moreover, children who have had a hemisphere completely or partially removed due to brain diseases or drug-resistant epilepsy have been found to develop language in the intact hemisphere (Sperry, 1974).
That the brain is more plastic in children compared with adults was already mentioned in the introduction. In early childhood there is growth of both gray and white matter. Overall gray matter starts to decrease during adolescence, while white matter continues to grow into adulthood and starts to decrease around age 45 (Bartzokis et al., 2001). However, more focal measurements have indicated that different regions of the cortex mature at different rates (Sowell et al., 2003).
In our longitudinal study conducted in healthy subjects we have confirmed that the brain changes constantly during adulthood. However, instead of finding solely decreases in gray matter volume, we also found focal gray matter to increase in certain areas, i.e. the parahippocampal gyri, frontal poles bilaterally, right medial frontal, and occipital cortices. Our finding of cortical thickening in the hippocampal area overlaps with recent findings of neurogenesis in this area. For years it was assumed that the total number of neurons was established prenatally and would not increase after birth. However, there is now accumulating evidence for adult neurogenesis in some brain areas including the hippocampus and olfactory bulb (Gould 2007; Zhao, 2008). In the olfactory bulb ongoing neurogenesis is suggested to be essential for tissue maintenance, while neurogenesis in the hippocampus contributes to growth instead of replacement, which is required for the ability to accumulate new memories throughout life (Imayoshi et al., 2008). However, we have to keep in mind that the actual number of generated neurons during adulthood is a small proportion of the total population of neurons (Gross, 2000).
Furthermore, plasticity of the hippocampus and the influence of learning were demonstrated in a study investigating structural MRIs of the brain of licensed London taxi drivers. London taxi drivers have extensive navigation experience and the hippocampus is known to be involved in spatial memory in the form of navigation (O'Keefe & Nadel, 1978). Compared to control

subjects who did not drive taxis, the posterior part of the hippocampi were significantly larger in the London taxi drivers. Thus, it seems that there is also capacity for local plastic change in healthy adult human brain structure in response to environmental demands (Maguire et al., 2000).
Our study in healthy twin pairs and their siblings has shown positive associations between level of intelligence and cortical thickening in the parahippocampal gyri bilaterally. Environmental factors influence absolute parahippocampal thickness, while genes influence its change. Moreover, we found significant influences of common genes on the associations between cortical thickness change and level of intelligence in these areas. Thus, genes that are implicated in cortical thickness change overlap with those involved in the level of intelligence. The positive association of cortical change with intelligence may be in line with the dependence of learning and memory formation on plasticity of neural circuits in the hippocampal area.
Furthermore, we described that in some brain areas, genetic systems involved in brain structure change differ from the genetic systems involved in brain structure per se. Particularly the magnitude of thinning in the left superior frontal and left superior temporal cortices (both involved in cognitive functioning) is found to be determined by genes that are different from those influencing absolute cortical thickness in these areas. Interestingly, these are also the brain areas that are disturbed in schizophrenia patients as was seen in our study and others (Shenton et al., 2001; Wright et al., 2000).

6.3 Schizophrenia

6.3.1 Cross-sectional study

Brain morphological abnormalities are well established in schizophrenia. Earlier studies have demonstrated that genetic factors are probably involved in the decreases in whole brain volume. However, disease-related (possibly non-genetic) influences are likely to contribute to additional decreases in whole brain volume observed in schizophrenia patients compared to their co-twins (Baaré et al, 2001b). Whether genetic or environmental factors are involved in gray and white matter volume abnormalities in schizophrenia remained inconclusive.
Chapter 3 described the differences in cerebral gray and white matter volume between twin pairs discordant for schizophrenia and healthy comparison twin pairs. MRI brain scans of 11 MZ and 11 same-gender DZ twin pairs discordant for schizophrenia and 11 MZ and 11 same-gender DZ healthy control twins were acquired. Within-twin pair similarities of brain volumes were estimated

by calculating intraclass correlation coefficients (ICCs).
Earlier results, showing both genetic and disease-related factors to be involved in whole brain volume abnormalities in schizophrenia (Baaré et al., 2001b), were confirmed in this study. Furthermore, findings indicated that a global white matter volume decrease reflect the increased genetic risk to develop schizophrenia, whereas the decreases in global gray matter volume may be secondary to the disease. Based on these findings, no further inferences could be made as to which focal gray and white matter structures may be related to the disease and genetic risk to develop schizophrenia. Therefore, voxel-based morphometry was applied in this sample (Hulshoff Pol et al., 2006). Results revealed that change in focal gray and white matter of the left medial (orbito) frontal gyrus and white matter of the left sensory-motor gyrus may reflect the increased genetic risk to develop schizophrenia.
However, whether genes are also involved in the progressive brain volume changes over time in schizophrenia was not known. This can only be measured using a longitudinal design in which two or more brain scans are made in schizophrenia patients and their relatives. Therefore, two longitudinal studies in schizophrenia patients, their relatives and healthy comparison subjects were carried out.

6.3.2 Longitudinal studies

Longitudinal studies have demonstrated that at least parts of the brain volume changes in schizophrenia are progressive over the course of the illness. It is implicated that an active (patho)fysiological process is going on not only in first-episode patients, but also in the chronic schizophrenia patients (Hulshoff & Kahn, 2008). Whether these progressive changes in brain volume are mediated by genetic and/or disease-related factors has not been studied. Chapters 4 and 5 described longitudinal studies addressing the temporal aspects of brain volume abnormalities in schizophrenia.
First we performed a longitudinal sibling study as described in chapter 4. At baseline, MRI scans of the brain were acquired in 16 patients with schizophrenia, 18 same-gender healthy siblings of patients with schizophrenia and 43 healthy comparison subjects. Eleven patients, 11 siblings and 33 healthy comparison subjects completed the follow-up measurement after 5 years. The results demonstrated that whole brain and cerebral gray matter volumes decreased excessively in schizophrenia patients as compared to the healthy siblings and healthy comparison subjects. When structural equation modeling was applied to assess disease and familial effects, it was suggested that the progressive brain volume loss in schizophrenia was related to the disease.

Since only siblings and no twin pairs were included in this study, it was not possible to disentangle the extent of genetic and common environmental contributions to familial influences. Furthermore, these siblings were selected for being completely healthy and beyond the age of risk to develop schizophrenia. Thus, the genetic contribution to schizophrenia and the association disease alleles of genes involved in the risk to develop schizophrenia may be underrepresented in these siblings. A longitudinal follow-up study in MZ and DZ twin pairs discordant for schizophrenia resolved these limitations.

Chapter 5 described a longitudinal study in discordant twin pairs. In this study, 2 MRI brain scans with an average scan-interval of 5 years were acquired in MZ and DZ twin pairs discordant for schizophrenia and healthy comparison twin pairs. In total, 92 participants completed both MRI scans. To our knowledge, this was the first longitudinal MRI study conducted in twin pairs discordant for schizophrenia.
Global brain volumes and lobar volumes were measured. Both in schizophrenia patients and their unaffected co-twins, progressive brain volume decreases were found in whole brain, frontal and temporal lobes, which were largely due to decreases in gray matter volume over time. Bivariate structural equation modeling revealed significant additive genetic influences on the correlations between schizophrenia liability and progressive changes in whole brain volume and frontal and temporal lobe volumes. It was concluded that the progressive brain volume loss found in patients with schizophrenia and their unaffected co-twins could at least partly be attributed to genetic factors that are related to the illness. The results also imply that the genes that play a role in (frontal and temporal) brain volume loss in (healthy) aging may be suitable candidate genes for schizophrenia. Finally, the finding of progressive brain volume loss in the unaffected co-twins of the patients indicates that the progressive brain volume loss in schizophrenia can no longer be explained solely as the result of disease-associated factors, such as antipsychotic medication intake, smoking, and outcome.

Thus, the study conducted in schizophrenia patients and their healthy siblings has shown an excessive decrease in whole brain and cerebral gray matter volume in schizophrenia patients as compared to their siblings and healthy comparison subjects. We suggested that the progressive brain loss observed in schizophrenia patients might be related to the disease process. In contrast, the longitudinal study in twin pairs discordant for schizophrenia has demonstrated that both the schizophrenia patients and their unaffected

co-twins exhibit progressive structural changes in whole brain, frontal and temporal lobe volumes. How can we explain this discrepancy? Owing to a limited statistical power of the sibling-study, genetic factors involved in the progressive brain volume changes in schizophrenia may not have been elucidated. Moreover, since these siblings were selected for being healthy, it indeed appears that at least some of the disease alleles of schizophrenia-related genes are not present in this sample of healthy siblings of patients. This result could possibly reflect an underestimate of the genetic contribution to schizophrenia and the association with progressive brain deficits.
It has been shown in healthy siblings of patients with childhood onset schizophrenia that gray matter deficits are present particularly in the left prefrontal and bilateral temporal cortices. However, these cortical deficits disappeared by age 20 years (Gogtay et al., 2007). Interestingly, a better social and cognitive competence was associated with normalization of gray matter. A relationship between brain plasticity and functional outcome for these non-psychotic, non spectrum siblings was suggested. This study extends findings from both our longitudinal sibling and twin studies. In the sibling study, patients were selected for being healthy, functional outcome in these subjects was good and no progressive brain changes were reported in these siblings compared with healthy comparison subjects. Furthermore, Gogtay et al. (2007) suggested that progressive brain volume change in schizophrenia has a familial background. This finding was demonstrated in our twin study. However, we did not find associations between clinical measurements or level of parental education and brain volume changes.

One of the findings of our study conducted in twin pairs discordant for schizophrenia was that changes in frontal and temporal lobe volumes can be explained by genetic factors that also directly contribute to schizophrenia. This may not be entirely surprising since the frontal lobe has been implicated in the genetic risk for the disease in a cross-sectional study in this sample (Baaré et al., 2001b) and in others (Cannon et al., 2002). The genetic influences on temporal lobe volume change in schizophrenia are consistent with findings of decreased temporal cortical thickness in family members of childhood onset schizophrenia patients (Gogtay et al., 2007).
As to the nature of the pathophysiological processes that are responsible for the progressive brain changes, we can only speculate. It is tempting to hypothesize that the progressive brain volume loss that is associated with the liability to develop schizophrenia represents aberrant plasticity of adult functional neural networks. Furthermore, an abnormality in neurogenesis may be implicated, since reduced rates of neurogenesis have been reported in

schizophrenia patients (Reif et al., 2006).
Although genetic factors play a major role, common environmental factors implicated in the disease may also be of influence since in our longitudinal study conducted in twin pairs discordant for schizophrenia they explained 23% of the brain volume loss in patients. Possible common environmental factors shared among patients with schizophrenia and their close relatives are stress factors (Mc Ewen, 2000; McDonald & Murray, 2000), viral infections (Davis & Phelps, 1995; Torrey, 1988), psychosocial factors (Portin & Alanen, 1997), prenatal environment (Brown et al., 2007) and delivery complications (Verdoux et al., 1997). Early (prenatal or perinatal) neurodevelopment lesions that render the brain vulnerable and anomalous late neurodevelopmental processes may interact with other causative factors associated with the onset of psychosis (McDonald & Murray, 2000; Weinberger & McClure, 2002; Cannon et al., 2003; Rapoport et al., 2005; Mathalon et al., 2003). A small effect was found for unique environmental factors (or measurement error) on progressive whole brain volume change. Possible unique environmental factors are life-events or use of medication.

6.3.3 Cortical thickness change in schizophrenia

Global volume measurements applied in the subject sample described in chapter 5 were extended by measurements of cortical thickness to study which brain areas show the most prominent cortical thickness change in schizophrenia patients and whether or not genes are involved. The results of this study are reported here and have to be considered preliminary.
Abnormalities in cortical thickness have been demonstrated mainly in prefrontal and temporal areas in patients with childhood onset schizophrenia (Greenstein et al., 2006; White et al., 2003) as well as in first-episode schizophrenia patients (Narr et al, 2005a; 2005b; Wiegand et al., 2004) and in chronic patients (Kuperberg et al, 2003, Lawyer et al., 2008). Moreover, in high risk subjects who convert to psychosis, excessive changes were demonstrated particularly in medial temporal and prefrontal cortical regions (Wood et al., 2008).
Longitudinal analyses of cortical thickness change are limited to childhood onset schizophrenia patients. Patients with childhood onset schizophrenia show a wave of ‘‘back-to-front’’ tissue loss with early gray matter loss in parietal cortices followed by gray matter loss in frontal and temporal cortices (Thompson et al., 2001; Vidal et al., 2006). By adult years, the pattern of cortical thickness abnormalities resembles the pattern of abnormalities seen in adult onset schizophrenia (Greenstein et al., 2006). Regarding the influence of genetic and environmental factors on cortical thickness change in

schizophrenia, a study conducted in siblings of childhood-onset schizophrenia patients has demonstrated a loss in prefrontal and temporal gray matter in unaffected siblings of these patients. This decrease appeared to be a familial/ trait marker, however it disappeared during adolescence (Gogtay et al., 2007). Global volume measurements in the same sample of twin pairs discordant for schizophrenia demonstrated progressive changes in whole brain, frontal and temporal lobe volumes both in the patients and their unaffected co-twins, which were suggested to be at least partly attributable to genetic factors related to the illness. However, whether cortical thickness volume change in adult onset schizophrenia is mediated by genetic or environmental factors remains to be elucidated.

One of the purposes of the current study was to establish the relative contributions of genetic and environmental (disease-related) factors to cortical thickness change in schizophrenia. To that end, we conducted a longitudinal magnetic resonance imaging study in twin pairs discordant for schizophrenia and healthy comparison twin pairs with a scan-interval of 5 years. Global volume measurements applied in the subject sample described in chapter 5 were extended by measurements of cortical thickness volume. To our knowledge, this is the first longitudinal study on cortical thickness change in twin pairs discordant for schizophrenia.

Since we are still analyzing these data, preliminary findings of this measurement are only reported here in the discussion. Preliminary results of this study indicate that patients with schizophrenia show a difference in the extent of cortical thickness change particularly in the superior frontal and frontal pole right, inferior frontal left, superior temporal left and right, and left lateral and medial occipital cortices, which is illustrated in Figure 1. This figure reflects the chi-squared statistics (χ^2; 2 df) of the phenotypic correlation (r_{ph}), i.e. the significant correlations between schizophrenia liability and cortical thickness change. In the superior frontal and frontal pole right, superior temporal left and lateral occipital left cortices, the change in cortical thickness may be attributed to familial (possibly genetic) factors. Cortical changes in the right superior temporal and temporal-parietal cortices seem to be influenced by environmental (possibly disease-related) factors. However, further analyses are needed to establish these findings and to estimate the specific contributions of genetic and environmental influences on cortical thickness volume change in schizophrenia.

Our finding that cortical thickness change in the frontal areas seem to be explained by familial (possibly genetic) factors, is consistent with the findings of the study conducted in family members of patients with childhood onset schizophrenia (Gogtay et al., 2007) and the global volume measurements of the current sample as described in chapter 5.

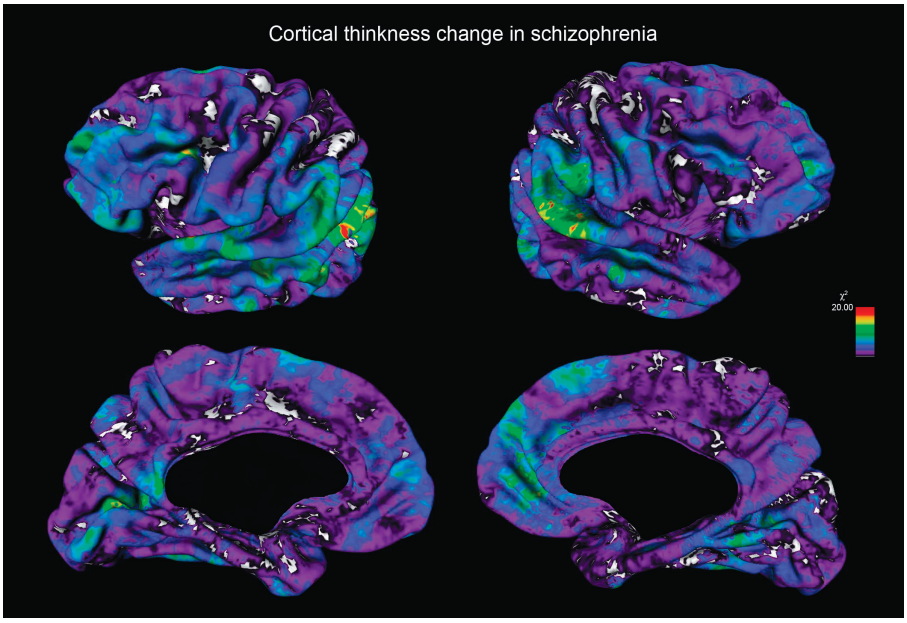

Figure 1. The chi-squared test statistics (χ^2; 2 df) of the phenotypic correlations (r_{ph}) between schizophrenia liability and cortical thickness change.

6.4 Genetic influence on brain structure change in schizophrenia and health

Genetic factors play an important role in brain volume (change), intelligence, the etiology of schizophrenia and the progressive brain volume changes observed in schizophrenia patients. However, the interrelationship between these traits (phenotypes), the interaction between genes and environment throughout life in health and disease, as well as the dynamics of brain structure and its association with brain functioning remain inconclusive. Moreover, up till now, no single genes with major risk effects for schizophrenia have been found. Using family and twin studies combining with newly evolving genetic approaches start to give us a glimpse as to which genes and environmental influences are shaping our brains and are involved in brain disorders such as schizophrenia.

Since schizophrenia is a complex disorder, caused by multiple genetic and environmental factors, finding schizophrenia susceptibility genes is like searching for a needle in a haystack. Therefore, several studies have focused on testing specific endophenotypes of schizophrenia (van Haren et al., 2008). An endophenotype is a characteristic of a disorder that is assumed to be closer to the action of a gene than the disorder itself. In schizophrenia for example,

the overt symptom could be psychosis. However, the underlying phenotype could be a deficit in structural brain morphology. Since brain abnormalities are well established in schizophrenia, state-independent, heritable, found at a higher rate in non-affected family members than in the general population, and co-segregate within families, they are assumed to be useful endophenotypes for schizophrenia research (Gottesman & Gould, 2003; Braff et al., 2007; Glahn et al., 2007).

Some of the candidate schizophrenia susceptibility genes have been associated with brain volumes in healthy subjects (Peper et al., 2007). For example, presence of a polymorphism (naturally occurring variation in the DNA at allele level) of the Brain Derived Neurotrophic Factor (BDNF) gene was associated with reduced gray matter volume of the prefrontal cortex (Pezawas et al., 2004) and hippocampus in healthy subjects (Bueller et al., 2006; Szeszko et al, 2005). Moreover, expression of BDNF in the prefrontal cortex (Weickert et al., 2003) and hippocampus (Szesko et al., 2005) has been implicated in schizophrenia. Another suggested candidate gene is the Disrupted in Schizophrenia 1 gene (DISC1), which increases the risk of developing schizophrenia and is associated with structural and functional alterations in the prefrontal cortex (Cannon et al., 2005) and hippocampus (Callicott et al., 2005). Defects in glutamatergic neurotransmission have been linked to the psychiatric manifestations of schizophrenia. Neuregulin 1 (NRG1) has a clear role in the expression and activation of neurotransmitter receptor, including the glutamate receptors and is therefore identified as a candidate gene for schizophrenia (Bakker et al., 2004; Stefansson et al., 2002). Furthermore, NRG1 is suggested to play a role in the enlargement of lateral ventricular volume in patients with first-episode schizophrenia (Mata et al., 2008). The Catechol-O-methyltransferase (COMT) gene contributes to normal variation in cognitive functioning. Polymorphisms in COMT are also associated with adult brain volumes in schizophrenia patients (Ohnishi et al., 2006) and poor functioning of the prefrontal cortex both in normal individuals and patients with schizophrenia (Egan et al., 2001).

However, although some of these findings are promising, the associations reported so far represent only minor genetic contributions. The many ways that schizophrenia manifests itself may have hampered progress in the search for schizophrenia genes. Finding genes involved in healthy brain change over time may contribute in the search for genes involved in the progressive brain volume change in schizophrenia. Moreover, the tight coupling of brain structure and genetics may contribute in the search for genes involved in psychiatric diseases that affect the integrity of the brain, such as schizophrenia.

6.5 Methodological considerations

There are some methodological considerations in this thesis which need to be addressed. First, the twin design in general is criticized for some of its assumptions. The 'equal environment assumption' implies that MZ and DZ twins are treated alike, although it has been shown that MZ twins do experience more similar environments than DZ twins (Martin et al., 1997). However, studies investigating twins rearing apart or cases of mistaken zygosity diagnosis have indicated that the more similar treatment of MZ twin pairs is a consequence of their greater phenotypic similarity instead of the cause (Kendler et al., 1993). Furthermore, concerns regarding generalization of findings towards the singleton population have been raised. However, studies comparing MZ and DZ twin pairs with their own siblings have demonstrated that they provide reliable estimates of heritabilities, at least with respect to intellectual abilities (Posthuma et al., 2000) and brain structure (Hulshoff Pol et al., 2002)

MZ or identical twin pairs share a common genotype and are assumed to be genetically identical. However, epigenetics or the assumption that there is something selectively activating or inhibiting certain genes, imply that some genes might be active in one twin but not in the other (Fraga et al., 2005). During the early years of life, MZ twin pairs are genetically and epigenetically identical, while in older MZ twin pairs epigenetic differences are found. There is mounting evidence that experience affects the way genes are expressed (turned on and off) in the developing brain. Thus, epigenetics may also play a role in brain volume changes both in schizophrenia and health.

Unfortunately, due to small sample size, we were not able to analyze statistically a possible gene X environment interaction, such as in the case of epigenetic mechanisms. When gene X environmental interaction is present, this is comprised in the estimate of unique environmental influences. However, when taking the longitudinal study in the twin pairs discordant for schizophrenia, even if gene X environment interactions would be involved in preventing the co-twins from developing schizophrenia, this does not explain why the co-twins of the schizophrenia patients show progressive brain volume loss later in life. There is suggestive evidence that the progressive brain volume loss in co-twins may not be without consequences: studies examining cognitive function in unaffected co-twins of patients with schizophrenia find impairments on cognitive tests and educational attainment (Cannon et al., 2000; Kremen et al., 2006; Goldberg et al., 1990). Cognitive impairments may thus be associated with the progressive brain volume loss.

Another problem of a relatively small sample size is that it gives room for

possible chance variations between the subgroups. Furthermore, it was not always possible to disentangle the influence of genetic, common environmental and unique environmental factors on a given trait. When confidence intervals were large, the genetic or environmental influences should be interpreted cautiously.

At last, the term 'heritability' is sometimes wrongly interpreted. Heritability is a statistic that describes the contribution of genetic differences to observed (phenotypic) differences among individuals in a particular population at a particular time. Thus, it refers to the genetic contribution to individual differences (variance) and not to the phenotype of a single individual (Plomin et al., 2008). In our healthy twin study for example, the estimated heritability for whole brain volume was 94%. This means that most of the differences among individuals in whole brain volume are due to the genetic differences among them. This also implicates that variance in a trait is essential to be able to estimate heritability. As was already mentioned in the introduction: behavioral genetics is concerned with the study of individual differences: detecting the factors that make individuals in a population different from one other.

6.6 Clinical implications and future directions

Knowledge about the contributions of genetic and environmental factors in human brain development is of profound importance since brain structure and even brain structure change have implications for brain functioning. Considering that most brain structures are highly genetic, the quest to find genes involved in brain morphology, brain structure change and aging, is important. Furthermore, insight in the connections between these processes can help us understand normal brain development and age-associated changes in brain functioning. Moreover, knowledge about healthy brain development is essential when trying to interpret morphological changes found in psychiatric disorders such as schizophrenia.

Our longitudinal twin study in twin pairs discordant for schizophrenia revealed that genes involved in schizophrenia are also involved in the progressive brain volume changes in these patients and their family members. This implicates that progressive brain tissue loss in schizophrenia can no longer be considered to be solely due to medication intake, smoking or outcome. Finding the (patho)physiological processes underlying these progressive brain changes in schizophrenia is of importance because this knowledge may ultimately enable us to halt or even reverse the disease

process. In searching for the gene systems to halt these progressive brain changes, it becomes important to look for genetic systems or gene expressions that may be particularly implicated in brain structure change. Localizing and characterizing the genes involved in dynamic brain changes may prove to be a valuable approach in studying the pathophysiology of progressive brain changes in schizophrenia. Nevertheless, the progressive brain volume changes in schizophrenia per se also warrant further study.
Further studies into longitudinal brain changes in healthy subjects and schizophrenia patients using high-field and ultrahigh field (3T and 7T) MRI are of great value. These new methods may allow us to zoom in on areas of progressive brain changes, as well as on neural networks. Moreover, new imaging acquisition procedures that may aid in getting closer to the (patho) physiological processes taking place in the brains of patients with schizophrenia are diffusion tensor imaging fiber tracking and resting-state functional MRI. Meanwhile, a multicenter extension of the MRI study in twin pairs discordant for schizophrenia has started on 3T scanners financially supported by the European Union (EUTwinsS). Furthermore, protocols are processed concerning measurements on 7T scanner in healthy twin pairs and twin pairs discordant for schizophrenia.
With these new methods and (ultra)high-field scanners, we can be carefully optimistic about the future progress in finding pathophysiological processes in schizophrenia.

6.7 Concluding words

Taken together, the studies presented in this thesis show that in adulthood the brain continues to be dynamic. In the longitudinal study of healthy participants we demonstrated that brain volume change during a 5-year period through the 3rd to 6th decade of life is heritable. Moreover, the degree of brain loss during that time period is inversely related to the level of intelligence. Interestingly, genes involved in brain loss over time overlap with genes for intelligence and differ from those related to absolute brain volume. Thus, it appears that continued brain maturation in adult life and intellectual development go hand in hand, and both are mediated by common genes.
Regarding the progressive brain volume changes in schizophrenia, we conclude that a significant proportion of the progressive brain volume loss that we observed both in schizophrenia patients and their unaffected co-twins during a 5-year interval is at least partly attributable to genes that are implicated in the disease.

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Nederlandse samenvatting

Het menselijke brein: een zeer complex en fascinerend orgaan, wat voortdurend in ontwikkeling is. Ondanks dat er, mede dankzij de ontwikkeling van moderne imaging technieken, veel onderzoek is verricht, blijf het voor een groot deel nog onbekend terrein. Om meer inzicht te verkrijgen in het menselijk brein zijn we gaan kijken naar mogelijke mechanismen die ten grondslag liggen aan de verschillen die we zien tussen mensen in hersenstructuren en de continue veranderingen die er optreden in het brein. Daarnaast hebben we gekeken in hoeverre genetische en/of omgevingsfactoren (mogelijk ziektegerelateerde factoren) betrokken zijn bij de progressieve hersenveranderingen die we zien bij schizofreniepatiënten. Om dit te kunnen onderzoeken zijn deze studies uitgevoerd bij familieleden (tweelingen en broers en zussen).

Gezonde hersenontwikkeling

De ontwikkeling van het menselijk brein is een proces wat gedurende het gehele leven doorgaat. In de vroege kindertijd is er voornamelijk groei te zien in zowel de witte als de grijze stof van de hersenen. Wanneer we naar verschillende gebieden binnen het brein kijken, dan zien we dat de ontwikkeling in deze gebieden in aanvang en snelheid van elkaar verschillen. De prefrontale cortex, het hersengebied dat onder andere betrokken is bij complexe cognitieve taken, persoonlijkheid, het maken van beslissingen en het moduleren van sociaal gedrag, ontwikkelt zich als laatste. In de adolescentie begint de grijze stof af te nemen, terwijl de witte stof doorgroeit tot een jaar of 45 en dan een afname laat zien.

Het is bekend dat de genetica een belangrijke rol speelt in verschillende hersenstructuren. Wanneer jonge kinderen worden vergeleken met oudere kinderen en adolescenten, dan lijkt het er op dat de invloed van genetische en omgevingsfactoren per hersengebied en ook met leeftijd verschilt. Bovendien wordt er bij een aantal hersengebieden een positieve associatie gevonden met intelligentie die door dezelfde genetische factoren tot stand gebracht wordt. Met andere woorden, er bestaat een overlap in de genen die een rol spelen bij bepaalde hersenstructuren en mate van intelligentie.

Zoals al eerder gezegd is het menselijk brein voortdurend in ontwikkeling. Echter, de oorzaak van deze dynamiek is onbekend: wordt dit veranderingsproces voornamelijk bepaald door omgevingsfactoren of wordt het genetisch gestuurd? En in hoeverre speelt intelligentie hierbij een rol? Om dit te onderzoeken zijn we gestart met een longitudinaal onderzoek in een grote groep tweelingen en hun broers en zussen, wat staat beschreven in

hoofdstuk 2. Er is een MRI scan van de hersenen gemaakt en er zijn intelligentietests afgenomen. Na een periode van ongeveer 5 jaar is een groot deel van deze mensen teruggekomen voor een tweede meting. Resultaten van dit onderzoek laten zien dat het brein dynamisch blijft in de volwassenheid en zelfs een verdikking van de cortex laat zien in bepaalde hersengebieden, zoals de parahippocampale gyri, de frontale polen, rechts mediaal frontaal en occipitaal. Bovendien zijn deze veranderingen niet alleen voor een deel erfelijk bepaald, maar het blijkt ook dat genen (of de expressie van genen) verantwoordelijk voor deze hersenveranderingen, verschillen van de genen die van invloed zijn op de betreffende breinstructuur op zich. Dit is voornamelijk het geval in de linker superior frontale en temporale cortex. Daarnaast wordt een verdikking en een afgenomen verdunning van de cortex geassocieerd met hogere IQ scores. Deze associatie wordt door gemeenschappelijke genen bewerkstelligd. Dus, genen die betrokken zijn bij de verandering in corticale dikte van het brein zijn voor een deel ook betrokken bij intelligentie.

Schizofrenie

Schizofrenie is ernstige hersenziekte die zich binnen een populatie bij 1 op de 100 mensen openbaart. Deze psychiatrische aandoening kenmerkt zich door afwijkingen in het waarnemen van de werkelijkheid en een verstoring in gedachten en gevoelens. De etiologie van schizofrenie is onbekend, maar familieonderzoek heeft aangetoond dat genetische factoren een belangrijke rol spelen. De meest gerepliceerde hersenafwijkingen bij schizofrenie zijn een vergroting van de laterale ventrikels, een afname in de grijze en witte stof, afname van volume in de frontale en temporale kwab en delen van het limbisch systeem (inclusief de amygdala, hippocampus en parahippocampale gyrus). Echter, deze veranderingen in het brein zijn subtiel en een diagnose is niet op basis van een MRI scan te stellen.

Onderzoek heeft aangetoond dat genetische factoren een rol lijken te spelen in een verkleining van het totale brein volume bij schizofreniepatiënten. Echter, wanneer er binnen een tweelingpaar wat discordant is voor schizofrenie (d.w.z. de een heeft de ziekte wel en de andere niet) wordt gekeken naar het totale breinvolume, dan laat de patiënt een kleiner breinvolume zien dan zijn niet aangedane tweelingbroer of -zus. Ziektegerelateerde (mogelijk niet genetische) factoren lijken bij te dragen aan een extra afname van het totale brein volume. In hoeverre genetische en omgevingsfactoren een rol spelen bij een verminderd volume van grijze en witte stof bij schizofrenie staat beschreven in hoofdstuk 3. De afname in witte stof die we zien bij schizofreniepatiënten lijkt toegeschreven te kunnen worden aan genetische

factoren, terwijl een afname in grijze stof veroorzaakt lijkt te worden door ziekte (gerelateerde factoren).
Recenter onderzoek heeft aangetoond dat de hersenafwijkingen bij schizofrenie een progressief karakter hebben en niet alleen plaatsvinden bij eerste episode patiënten. Echter, in hoeverre deze progressieve veranderingen toe te schrijven zijn aan genetische en/of omgevingsgerelateerde factoren, was tot dusver onbekend. Om dit te kunnen onderzoeken hebben we longitudinale studies opgezet die zijn uitgevoerd bij schizofreniepatiënten en hun familieleden.
In hoofdstuk 4 wordt een longitudinale studie gepresenteerd die is uitgevoerd bij schizofreniepatiënten en hun gezonde broers en zussen. In deze studie laten we zien dat er bij schizofreniepatiënten ten opzichte van hun gezonde broers en zussen een progressieve afname over tijd plaatsvindt in totaal brein volume en cerebrale grijze stof. Deze progressieve afname lijkt toegeschreven te kunnen worden aan de ziekte. Echter, aangezien de broers en zussen van de schizofreniepatiënten geselecteerd zijn op gezond zijn, zou het kunnen dat de genen die betrokken zijn bij het risico om schizofrenie te ontwikkelen, niet of minder aanwezig zijn in deze familieleden.
Om deze beperking het hoofd te bieden hebben we ook een longitudinale studie uitgevoerd in tweelingen die discordant zijn voor schizofrenie. Deze studie, die staat beschreven in hoofdstuk 5, toont aan dat er zowel bij schizofreniepatiënten als hun tweeling broer of -zus, progressieve afnamen plaatsvinden in het totale breinvolume, de frontale en temporale kwabben. Dit wordt voornamelijk veroorzaakt door een afname in grijze stof. Additief genetische invloeden lijken verantwoordelijk te zijn voor deze afname in breinvolumes. Bovendien werd gevonden dat tenminste voor een deel dezelfde genen die betrokken zijn bij het risico om schizofrenie te ontwikkelen ook van invloed zijn op deze progressieve afname in breinvolumes. Dat er ook progressieve veranderingen worden gevonden bij de tweelinghelft die geen schizofrenie heeft, wijst er op dat deze progressieve veranderingen in het brein niet meer alleen toegeschreven kunnen worden aan ziektegerelateerde effecten, zoals medicatie, roken en mate van functioneren.
Echter, hoewel genetische factoren een belangrijke rol spelen in de progressieve hersenafwijkingen die we zien bij schizofrenie, zouden gedeelde omgevingsfactoren ook nog van invloed kunnen zijn. Mogelijke omgevingsfactoren die schizofreniepatiënten delen met hun tweelingbroer of –zus zijn de prenatale omgeving, geboortecomplicaties, virussen, stressfactoren en psychosociale factoren.
In deze tweelinggroep worden de globale hersenmetingen uitgebreid met corticale dikte metingen. De eerste voorlopige resultaten laten zien dat

schizofreniepatiënten een grotere verandering in corticale dikte laten zien in voornamelijk de frontale, temporale en linker occipitale cortex. De veranderingen in de frontale en in de linker temporale en occipitale hersengebieden lijken toegeschreven te kunnen worden aan familiale (mogelijk genetische) factoren. Corticale veranderingen in de rechter temporale gebieden lijken beïnvloed te worden door genetische (mogelijk ziektegerelateerde) factoren. Echter, verdere analyses zijn nodig om deze resultaten te bevestigen en om de specifieke bijdrage van genetische en omgevingsinvloeden op veranderingen in corticale dikte te kunnen schatten.

Genetische invloed op de verandering van hersenstructuren bij schizofrenie en gezondheid

Inzicht in de bijdrage van genetische en omgevingsfactoren aan de ontwikkeling van het menselijk brein zijn van zeer groot belang aangezien hersenstructuren en een verandering ervan invloed hebben op het functioneren van het brein. Rekening houdend met het feit dat de meeste hersenstructuren in hoge mate door genen wordt bepaald, is het belangrijk op zoek te gaan naar de genen die hierbij een rol spelen. Wanneer er meer bekend is wat er in het brein gebeurt gedurende het normale verouderingsproces, kunnen morfologische veranderingen die optreden bij psychiatrische ziekten ook beter geïnterpreteerd worden. Een aantal genen die gelinkt kunnen worden aan gezonde hersenontwikkeling, zouden ook een rol kunnen spelen bij een verstoorde hersenontwikkeling zoals deze optreedt bij schizofrenie.
Meer kennis betreffende de (patho)fysiologische processen die ten grondslag liggen aan de progressieve hersenveranderingen die we zien bij schizofrenie, zou mogelijkerwijs kunnen bijdragen aan de ontwikkeling van een methode om het ziekteproces af te remmen of te stoppen.

Kort samengevat laten de studies die in dit proefschrift staan beschreven zien dat het brein gedurende het gehele leven dynamisch is en in grote mate door genen wordt beïnvloed. Bovendien laten mensen die een hogere score behalen op intelligentie, minder hersenafname gedurende de volwassenheid zien. De ontwikkeling van het brein en de mate van intelligentie lijkt deels beïnvloed te worden door dezelfde genen.
Ten aanzien van de progressieve breinveranderingen die we zien bij schizofrenie, kan gesteld worden dat deze ook voor een groot deel door genen worden bepaald. Bovendien zijn dit deels dezelfde genen die verantwoordelijk zijn voor het ontwikkelen van deze ziekte.

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Curriculum Vitae

Rachel Brans werd op 6 april 1975 geboren te Nijmegen. In 1994 behaalde zij het VWO diploma aan het Elshof College te Nijmegen. In datzelfde jaar startte ze met de opleiding Hogere Europese Beroepen Opleiding aan de Haagse hogeschool. Na het behalen van haar propedeuse, koos ze voor de studie psychologie aan de Radboud Universiteit Nijmegen. In 2000 voltooide ze deze opleiding met als richting neuro- en revalidatiepsychologie. Gedurende de laatste studieperiode werkte zij in dienst van het Trimbos Instituut in het kader van een grootschalig onderzoek naar de omvang, kenmerken en zorgbehoefte van dak- en thuislozen in ‘s-Gravenhage. Na het afstuderen ging zij werken als onderzoeksassistente bij de neuroimaging groep van de afdeling volwassenen psychiatrie van het Universitair Medisch Centrum Utrecht. In 2002 is zij bij het Rudolf Magnus Instituut voor Neurowetenschappen onder supervisie van Prof. Dr. Hilleke Hulshoff Pol en Prof. Dr. René Kahn begonnen met haar promotieonderzoek. Sinds mei 2009 is zij daarnaast als psycholoog werkzaam bij het mobiel diagnostisch team van het Universitair Medisch Centrum te Utrecht.