GENETIC INFLUENCES ON GYRIFICATION OF THE CEREBRAL CORTEX IN SCHIZOPHRENIA: A TWIN STUDY

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Index of Abbreviations

AMC	Absolute Mean Curvature
ANCOVA	Analysis of Covariance
Ant Cingulate	Anterior cingulate gyrus
BMS	Between-people mean squares
C	Control
CPM	Cortical pattern matching
DSM-IV-TR	Diagnostic Statistical Manual IV Text Revision
DSM	Diagnostic Statistical Manual
DTI	Diffusion tensor imaging
EMS	Residual mean square
EUTwinsS	European Twin Study on Schizophrenia network
FDR	False Discovery Rate
FES	First episode schizophrenia
fMRI	Functional magnetic resonance imaging
FWHM	Full Width Half Maximum
GI	Gyrification Index
GM	Grey matter
HR	High risk for schizophrenia
ICC	Intra Class Coefficients
ICD	International Classification of Diseases
IFG	Inferior frontal gyrus
J	Jena
JMS	Between-scanners mean square
L	London
MFG	Middle frontal gyrus
Mm	Millimeter
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy.
Msec	Milliseconds
MTG	Middle temporal gyrus
MZ	Monozygotic
N	Number of subjects
neg	Negative
OW	Ontogenic Weeks
PET	Positron Emission Tomography
PHG	Parahippocampal gyrus
pos	Positive
PANSS	Positive and Negative Symptoms Scale
rmANOVA	Repeated measures Analysis of Variance
s.d	Standard Deviation
SANS	Scale for the Assessment of Negative Symptoms
SAPS	Scale for the Assessment of Positive Symptoms
STaR	Schizophrenia Twins and Relatives Consortium
STG	Superior temporal gyrus
Sz	Chronic schizophrenia
T	Tesla
T_E	Echo Time
T_R	Repetition Time
U	Utrecht
VBM	Voxel-based Morphometry
WM	White matter

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Summary

Abnormality of cortical gyrification, the process of formation of gyri and sulci in the brain, has been documented in schizophrenia. We used Absolute Mean Curvature to quantify regional cortical gyrification index (GI). First, we demonstrated that this method allows for pooling of MRI data from different research centers to study GI reliably with a multi-centre design. We collected structural MRI data from (a) one subject scanned 12 times on two scanners at two different centers and (b) six unrelated healthy adult volunteers scanned on four MRI scanners at four centers. Cortical surfaces were extracted from each hemisphere and the AMC calculated at each vertex. Intra-class correlation coefficients (ICC) were high for both single subject and multi-center data. For the second study, MRI data from 104 monozygotic (MZ) twins i.e. 27 MZ healthy twin-pairs and 25 MZ twin-pairs discordant for schizophrenia, from three research centers, were pooled. We found higher correlations in GI among discordant twins than among healthy twins. This indicated that discordant twins were more similar to each other suggesting that genes for schizophrenia may have an important role in regulating development of gyrification. We found increased gyrification in the left hemisphere in frontal pole, middle temporal, parahippocampal and fusiform regions in both affected and unaffected co-twins compared to healthy twins. This indicated that increased left fronto-temporal-GI might represent a structural phenotype for genetic vulnerability to schizophrenia. Affected co-twins differed from their unaffected co-twins in right parahippocampal-GI indicating a possible effect of disease status. In addition, affected co-twins had significantly dissimilar parahippocampal GI In left and right hemispheres. Thus our results indicate that although increased left fronto-temporal-GI might represent a genetic vulnerability to schizophrenia, divergent developmental trajectories of left and right parahippocampal-GI might represent a structural phenotype for expression of the disease phenotype of schizophrenia.

Zusammenfassung

Veränderungen in der kortikalen Gyrifizierung, d.h. in der Formation der Windungen und Furchen im Gehirn, wurden bei Patienten mit Schizophrenie wiederholt berichtet. In der vorliegenden Arbeit wurde der Parameter der Absoluten Mittleren Kurvatur (engl. „Absolute Mean Curvature“, AMC) herangezogen, um den regionalen kortikalen Gyrifizierungsindex (GI) zu quantifizieren. In einer ersten Studie konnte hierbei festgestellt werden, dass diese Methode zur Untersuchung der Gyrifizierung die kombinierte Auswertung von Magnetresonanztomographie (MRT) Daten unterschiedlicher Untersuchungszentren erlaubt. Hierfür wurden strukturelle MRT Messungen an (a) einem gesunden Probanden, der insgesamt 12 mal in MRT Scannern zweier unterschiedlicher Untersuchungszentren und (b) sechs gesunden Probanden, die in vier MRT Scannern unterschiedlicher Untersuchungszentren gemessen wurden, durchgeführt. Im Anschluss wurde die kortikale Oberfläche jeder Hemisphäre sowie die Absolute Mittlere Kurvatur für jeden Vertex im Gehirn berechnet. Die Koeffizienten der Intra-Klassen-Korrelation (engl. ”intra-class correlation”, ICC) erwiesen sich sowohl für die Daten desselben Probanden als auch die Daten der verschiedenen Untersuchungszentren als hoch. In einer zweiten Studie wurden sodann strukturelle MRT Daten von 104 monozygoten Zwillingen, d.h. 27 monozygoten gesunden Zwillingspaaren und 25 monozygoten, im Hinblick auf Schizophrenie diskordanten Zwillingspaaren aus drei verschiedenen Untersuchungszentren kombiniert. Hier ergaben sich höhere Korrelationen in der Gyrifizierung innerhalb der diskordanten Zwillingspaare im Vergleich zu gesunden Zwillingpaaren. Dieser Befund deutet darauf hin, dass monozygote Zwillinge mit einer Diskordanz für die Erkrankung diesbezüglich eine größere Ähnlichkeit aufweisen und eine genetische Veranlagung zur schizophrenen Erkrankung möglicherweise eine relevante Rolle im Rahmen der Gyrifizierung spielen könnte. Zudem ließ sich eine erhöhte linksseitige Gyrifizierung im Bereich des frontopolaren Kortex sowie in mittleren temporalen, parahippokampalen und fusiformen Arealen sowohl bei erkrankten als auch nicht affizierten Co-Zwillingen im Vergleich zu den gesunden Zwillingen feststellen. Dies wiederum deutet darauf hin, dass eine erhöhte linksseitige frontotemporale Gyrifizierung einen strukturellen Phänotyp

für eine genetische Veranlagung zur Schizophrenie darstellt. Darüber hinaus unterschieden sich die betroffenen Zwillinge von ihren nicht betroffenen Zwillingsgeschwistern in der Gyrifizierung im rechten Gyrus parahippocampalis Gyrus, sodass dieser Befund möglicherweise einen Effekt der Krankheitsmanifestation darstellt. Zudem fand sich bei den betroffenen Zwillingen ein signifikanter Unterschied in der Gyrifizierung zwischen rechtem und linkem parahippokampalen Gyrus. Diese Befunde weisen darauf hin, dass – wenngleich eine erhöhte linksseitige frontotemporale Gyrifizierung möglicherweise eine genetische Vulnerabilität für die Schizophrenie repräsentiert – divergente Trajektorien in der Gyrifizierung des rechten und linken Gyrus parahippocampalis vermutlich einen strukturellen Phänotyp für die Expression eines Erkrankungsphänotyps darstellen.

1. Introduction

1.1 Concept of schizophrenia

Schizophrenia is a disease of the mind. A disease of the mind manifests itself as oddness of behavior, cognition and affect.

The earliest medical document that describes mental illnesses is the *Ebers Papyrus* dated 1550 BC.[1] Although certain presentations that may resemble modern concept of depression or dementia, are found in the *Book of Heart* in the *Ebers papyrus* (Nasser 1987, Okasha A 2000), it does not contain explicit descriptions of psychosis. It is possible that hallucinations, delusions and abnormal behavior were considered to be caused by spirits and hence were treated by sorcerers (Nasser 1987) instead of physicians.

The earliest mention of 'madness' in ancient India is found in *Atharva-Veda*, which was composed between 3000-1000BC. The earliest authoritative written medical document that presents the first composite structure of Ayurveda (the practice of medicine in ancient India) describing diseases and treatments was believed to be compiled around 1000BC and given its final form, known as *Caraka-Samhita*, between 400-200BC.[2] Caraka-Samhita describes '*Unmada*' which most closely corresponds to modern definitions of psychosis (Bhugra 1992). '*Unmada*' and its subtypes were considered to be diseases of the mind and were treated by physicians. Correspondence between diagnostic criteria of 'Unmada' (Caraka-Samhita) and 'schizophrenia' (ICD-9) has been established

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[1] The Ebers papyrus dates from 1550 BC and was discovered by a German Egyptologist Georg Ebers in 1873-74, who donated the papyrus to the University of Leipzig. In 1890 Heinrich Joachim, who was a physician and Egyptologist, provided the first comparison with modern medicine. First available English translations became available from the 1930s.

[2] Vedic and many subsequent writings such as Caraka-Samhita do not exist in their original form. The earliest inscription of ancient India dates back 3000BC but the earliest decipherable inscription dates back to about 3rd century BC. The oldest preserved medical manuscript called the Bower manuscript consisting of birch-bark leaves, which dates to 500-550AD. (Dani, Ahmad Hasan. *Indian Palaeography.* (2nd edition New Delhi: Munshiram Manoharlal, 1986; Sander, Lore, "Origin and date of the Bower Manuscript, a new approach" in M. Yaldiz and W. Lobo (eds.), Investigating the Indian Arts (Berlin: Museum für Indische Kunst, 1987).

(Dube 1979) and included in the Cochrane Schizophrenia Group Trials Register (Agarwal et al. 2007).

In ancient China, Chao Yuanfang's Aetiology and Symptomatology of Diseases in 610 CE, describes schizophrenia-like symptoms among other forms of psychosis (Liu 1981). Although psychotic symptoms were well recognized, the classificatory systems in ancient China did not recognize 'schizophrenia' as a specific symptom cluster representing a disease entity. Modern Chinese medicine has, however, revised their classificatory system that is compatible with ICD and DSM (Zheng et al. 1994, Chen 2002).

Ancient Greeks (400-200BC) recognized symptoms of 'madness'. Hippocrates is the first physician to have discarded supernatural causes for mental illness and proposed that physical factors contributed to imbalance of 'humors' that resulted in mental illness. The available literature indicates that even though psychotic symptoms were recognized, schizophrenia did not exist as a conceptual disease entity (Evans et al. 2003).

The ancient Romans perpetuated Greek knowledge and understanding of mental illness. Initially an attempt was made to discard a humoral basis of diseases, such as by Asclepiades (124 – 40 BC) but Galen (129 -199 AD) revived the humoral theory and emphasized a symptom-based approach (Pilgrim 2007). Galen's interest in anatomy and the humors led him to become the most influential physician in Ancient Rome to contribute to the advancement of understanding in anatomy, physiology, pathology and neurology. Nevertheless an understanding of mental illnesses did not see a conceptual sea change. The symptom-based approach continued and there was no disease entity such as schizophrenia (Evans et al. 2003).

In Islamic medicine, psychosis (*Al Quatrat* or *Al Qutrub*) is recognized as a syndrome combining ancient Greek and Indian medical traditions. The most famous book *Al Quanun*, written by Ibn Sina (dated 1037 AD), has a clear description of non-affective psychosis. 'Ibn Sina defined madness (*Junun*) as a condition in which reality is replaced by fantasy that could be associated with

agitation, behavioral and sleep disturbance, and giving inappropriate answers to questions. He considered all madness (Junun) to be disorders of reason, with their origin in the middle part of the brain' (Youssef und Youssef 1996).

Mental illnesses were considered to be diseases of the mind and the first Islamic hospitals to treat mental illnesses were established in the 9th century. Although psychotic symptoms were well recognized and many historical accounts of patients are comparable to modern notions of schizophrenia (Youssef und Youssef 1996), it has been argued that schizophrenia perhaps did not exist as a classificatory entity in Islamic medicine (Higgins und Kose 2007).

Thus the ancient civilizations vacillated between a symptom-based approach and a syndrome-based approach towards mental illness. We either find accounts and analyses of psychotic symptoms, or descriptions of a syndrome that approximates the current conception of schizophrenia as a disease entity. Advancement in psychiatry stalled in the Middle Ages owing to political and religious upheavals.

In Europe, the first hospital to take mentally ill patients was Royal Bethlehem hospital in 1350 AD. With the European Renaissance, demonological and religious explanations of psychiatric symptoms fell into disfavor. The period from the 16th to 18th century saw a rise in asylums for the mentally ill. Psychiatry developed as a branch of neurology in the 18th and 19th centuries.

Advancement of knowledge in neurology almost fuelled a revolution in psychiatric thinking, notably in German psychiatry, which laid the foundation of modern psychiatry and also firmly established schizophrenia as one of the major psychiatric disorders.

The term 'Psychosis' was first mentioned in 1847 by Ernst von Feuchtersleben in his book Principles of Medical Psychology although the concept had been introduced by Canstatt (1841) (Burgy 2008). Beer (1995) argues that the new term, originating from the Greek word 'psyche', was an attempt by Feuchtersleben to imply that mental disorders represented a complex interplay

of body *and* mind (Beer 1995). From 1847 to 1933, '*psychosis*' was a synonym for mental illness.

In the early 19th century, owing to advancement of knowledge of neurology, psychiatric illnesses were being considered as diseases of the brain, a view especially advocated by Wilhelm Griesinger (1840-1870) (Beer 1995). A disease entity needed to have distinctive etiology, symptomatology and pathology as Flemming (1859) had pointed out (Beer 1995). Karl Kahlbaum (1863-1874) differentiated between a 'group of symptoms' and a 'symptom complex arising out of a disease process' (Kahlbaum und Berrios 1996). Kahlbaum added the notion of 'disease course' and Emil Kraepelin (1909) included 'prognosis'. In 1863 Kahlbaum identified the disease entity 'catatonia' in his textbook *Classification of the Mental Illnesses*[3]. Later Kahlbaum's student, Ewald Hecker, similarly identified 'Hebephrenia' using Kahlbaum's case notes and Hecker's own approach in 1871. Kraepelin was much influenced by Kahlbaum's classification of disease, who had been one of the first to renounce the purely symptomatic method of classification.

Kraepelin remarked in his *Memoirs* that the idea of dementia praecox gradually 'dawned' on him *"when observing that many patients, who initially present with mania melancholia or amentia, develop progressive dementia and, in spite of individual differences, begin to resemble one another"* (Jablensky 2007). This led him to the insight that: (a) one uniform disease process must be affecting them all; and (b) the process might be slow or quick, sometimes accompanied by delusions, hallucinations and excitement, but always leading to a loss of 'intellectual' capacity' (Jablensky 2007).

Kraepelin first distinguished between acute affective and chronic paranoid states. He then differentiated *Dementia Praecox*[4] (formerly Hebephrenia) and *Dementia Paranoides* from other forms of paranoia.

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[3] The original German title is Gruppierung der psychischen Krankheiten und die Einteilung der Seelenstörungen, Danzig 1863

[4] The term *Dementia Praecox* was inspired by Morel's *Demence Precoce* in 1852-53 for a condition similar to Hecker's Hebephrenia. Bleuler E. 1950. Dementia praecox or The Group of Schizophrenias. New York: International Universities Press. In 1891

In 1911, Eugen Bleuler published *Dementia Praecox or the Group of Schizophrenias* (translated to English in 1950), in which Bleuler argued that 'Schizophrenia' was a better term, as the disease neither represented an early onset nor a dementing/deteriorating course. It instead represented 'splitting of different psychic functions'(Bleuler 1950). Bleuler defined Schizophrenia as:
"a group of psychoses whose course is at times chronic, at times marked by intermittent attacks, and which can stop or retrograde at any stage, but does not permit a full restitutio ad integrum. The disease is characterized by a specific type of alteration of thinking, feeling and relation to the exterior world which appears nowhere else in this particular fashion" (Bleuler 1950).

Bleuler attempted a causal explanation for symptoms constituting schizophrenia. He stated that 'integration of different psychic complexes' are inadequate such that 'associations' are made between 'fragments of ideas' in an 'illogical way' (Bleuler 1950); thinking may stop in the middle of a thought and/or a new thought may follow with no connection with the previous stream of thought; affective expressions may be completely lacking or have an inappropriate degree of intensity' (Bleuler 1950).

In addition to these 'accessory symptoms', hallucinations, delusions, catatonic symptoms may be present (Bleuler 1950). Bleuler divided symptoms into 'fundamental' and 'accessory' symptoms. 'Fundamental' symptoms were further classified into three 'simple' (association, affect and ambivalence) and six 'compound' functions, of which only four were considered important (relation to reality or autism, attention, will, activity or behavior). The eight groups of 'accessory' symptoms comprised hallucinations, delusions, the person (referring to self or ownership), speech and writing, somatic symptoms, catatonic symptoms and the acute syndromes (which included mood, catatonic, delirious states and others).

Bleuler argued that the concept of Schizophrenia is a disease concept in which the disease process is assumed to "*directly produce the primary symptoms*" whilst,

"*the secondary symptoms are partly psychic functions operating under altered conditions, and partly the results of more or less successful attempts at adaptation to the primary disturbance"* (Bleuler 1950).

Bleuler's schizophrenia is a disease that has an insidious onset, varied courses of illness, and results in deterioration[5], although this deterioration is not a dementia. Bleuler further subdivided Schizophrenia into paranoid, catatonia, hebephrenic and schizophrenia simplex, following the Kahlbaum-Kraepelian nosology.

Kurt Schneider published his book Clinical Psychopathology[6] in the 1950s, in which he presented 'first rank' symptoms for schizophrenia. The first rank symptoms were voices arguing or commenting, voices commanding, thought echo, thought broadcasting, thought withdrawal, made volition, made affect, and delusional perception[7]. However this advance in phenomenology led to neither any reclassification of the Kahlbaum-Kraepelian nosology, nor of Bleuler's concept of schizophrenia.

The British and other European countries agreed on a concept of schizophrenia based on Bleuler's fundamental and accessory symptoms (Jablensky 1986). A serious attempt was made to standardize definitions and criteria in the 8th revision of International Classification of Diseases (ICD) in 1974. This was followed by the International Pilot Study in Schizophrenia in 1978 to establish the validity of diagnostic concepts across cultures (Sartorius 1978). A high concordance was observed when criteria of ICD-9 and Kraepelin's classification were compared (Jablensky et al. 1993).

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[5] Bleuler in his book *Dementia Praecox or the Group of Schizophrenias* shows that after the first acute episode, 65% of patients have mild deterioration while 22% have severe deterioration ibid.

[6] This book was originally written in German with the title Klinische Psychopathologie. It was translated into English in 1959 and has greatly influenced present definitions of schizophrenia.

[7] For an in-depth discussion on the interpretation of 'first rank symptoms' by the English speaking world, one may refer to an article by Koehler K. 1979. First rank symptoms of schizophrenia: questions concerning clinical boundaries. Br J Psychiatry, 134:236-248.

The criteria for Schizophrenia in ICD-10 (WHO 2004) are:

Schizophrenia in ICD-10 (WHO 2004)	
Although no strictly pathognomonic symptoms can be identified, for practical purposes it is useful to divide the symptoms into groups that have special importance for the diagnosis and often occur together, such as:	
	(a) thought echo, thought insertion or withdrawal, and thought broadcasting;
	(b) delusions of control, influence, or passivity, clearly referred to body or limb movements or specific thoughts, actions, or sensations; delusional perception;
	(c) hallucinatory voices giving a running commentary on the patient's behaviour, or discussing the patient among themselves, or other types of hallucinatory voices coming from some part of the body;
	(d) persistent delusions of other kinds that are culturally inappropriate and completely impossible, such as religious or political identity, or superhuman powers and abilities (e.g. being able to control the weather, or being in communication with aliens from another world);
	(e) persistent hallucinations in any modality, when accompanied either by fleeting or half-formed delusions without clear affective content, or by persistent over-valued ideas, or when occurring every day for weeks or months on end;
	(f) breaks or interpolations in the train of thought, resulting in incoherence or irrelevant speech, or neologisms;
	(g) catatonic behaviour, such as excitement, posturing, or waxy flexibility, negativism, mutism, and stupor;
	(h) "negative" symptoms such as marked apathy, paucity of speech, and blunting or incongruity of emotional responses, usually resulting in social withdrawal and lowering of social performance; it must be clear that these are not due to depression or to neuroleptic medication;
	(i) a significant and consistent change in the overall quality of some aspects of personal behaviour, manifest as loss of interest, aimlessness, idleness, a self-absorbed attitude, and social withdrawal.

Diagnostic Guidelines

The normal requirement for a diagnosis of schizophrenia is that a minimum of one very clear symptom (and usually two or more if less clear-cut) belonging to any one of the groups listed as (a) to (d) above, or symptoms from at least two of the groups referred to as (e) to (h), should have been clearly present for most of the time during a period of 1 month or more.'
Schizophrenia is further categorized into paranoid, hebephrenic, catatonic, undifferentiated, residual, simple schizophrenia and post schizophrenic depression (WHO 2004)

In the 1960s and 1970s, there was a growing dissatisfaction with the predominantly psychoanalytic approach to psychiatry. Psychiatrists at Washington University, Eli Robins, Samuel Guze, George Winokur and one of their students, John Feighner, believed that only empirical psychiatric research with a strong focus on biology held any hope for the treatment and improvement of the mentally ill. They were later named as neo-Kraepelians (Decker 2007). They stated 'Classification is diagnosis', and set out five steps thought necessary to develop a valid classification: (1) clinical description; (2) laboratory studies (which they admitted did not exist for 'the more common psychiatric disorders'); (3) exclusion criteria to weed out patients with other illnesses; (4) follow-up studies; (5) family studies(Decker 2007, Robins und Guze 1970). They extensively reviewed literature and attempted a reclassification that came to be known as Feighner's criteria.

Feighner's criteria influenced Robert Spitzer, who went on to develop Research Diagnostic Criteria (RDC) in the 1970s, followed by Diagnostic and Statistical Manual III (DSM-III) in 1980. DSM-III was a major advancement as it ensured inter-rater reliability for diagnoses using its 'operational' criteria, as well as providing a multi-axial system of diagnosis. This consisted of the primary psychiatric diagnosis as Axis I, personality as Axis II, associated medical conditions as Axis III, any psychosocial factors as Axis IV, and overall level of functioning as Axis V. This favored a bio-psychosocial model for diseases.

The DSM undergoes regular reviews and the current diagnostic criteria for schizophrenia in DSM–IV-TR (APA 2000) are :

Schizophrenia in DSM–IV-TR (APA 2000)
1. Characteristic symptoms: Two or more of the following, each present for much of the time during a one-month period (or less, if symptoms remitted with treatment). - Delusions - Hallucinations - Disorganized speech, which is a manifestation of formal thought disorder - Grossly disorganized behavior (e.g. dressing inappropriately, crying frequently) - Catatonic behavior - Negative symptoms: Blunted affect (lack or decline in emotional response), - Alogia (lack or decline in speech), or avolition (lack or decline in motivation) If the delusions are judged to be bizarre, or hallucinations consist of hearing one voice participating in a running commentary of the patient's actions or of hearing two or more voices conversing with each other, only that symptom is required above. The speech disorganization criterion is only met if it is severe enough to substantially impair communication.
2. Social or occupational dysfunction: For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care, are markedly below the level achieved prior to the onset.
3. Significant duration: Continuous signs of the disturbance persist for at least six months. This six-month period must include at least one month of symptoms (or less, if symptoms remitted with treatment).
Schizophrenia is further subdivided into paranoid, disorganized, undifferentiated, catatonic, and residual schizophrenia(APA 2000).

The current definition of schizophrenia has received many criticisms. Jablensky has summarized criticism of Kraepelinian nosology as follows (Jablensky 2007):

“The various objections raised by its critics fall into several groups (Jablensky 2007):

(i) It is impossible to infer anything about the etiology and cerebral localization from the symptoms and course of the illness.

(ii) Similarity in outcomes does not necessarily imply that the same disease is present; the same illness may result in deterioration in some cases and in recovery in other cases.

(iii) A categorical distinction within the group of major psychoses is impossible to sustain, as there are many inter-forms and transitions in symptomatology, as well as in the individual course of illness over time.

(iv) The diagnostic categories of schizophrenia and manic-depressive disorder do not define genetically distinct biological entities."

Although the validity and inter-rater reliability for ICD-10 and DSM-IV criteria are good for schizophrenia, it is the lack of success of genetic studies in schizophrenia that fosters the doubt whether current operational criteria yield suitable phenotypes for genetic research (Jablensky 2007).

The DSM-IV criteria have been criticized as making schizophrenia a 'diagnosis of exclusion'; thus introducing heterogeneity such that it would interfere with any research aimed at understanding etiology (Maj 1998). It has also been argued that 'operationalization' of the definition of schizophrenia may have improved inter-rater reliability at the cost of construct validity of the definition (Jansson und Parnas 2007).

A recent review (Tandon et al. 2009) of the conceptualization of schizophrenia emphasized that the current concept of schizophrenia comprises a heterogenous group of diseases. However the authors also point out that the current concept of schizophrenia as a diagnostic entity *does* distinguish a specific clinical profile, is stable over lifetime, and has high concordance across cultures. The authors suggest that the various subtypes of schizophrenia represent an attempt to organize this heterogeneity but that these subtypes are i) not stable over the course of illness and ii) they do not provide etiological or pathophysiological explanations. The authors propose that the current concept of schizophrenia needs to be 'deconstructed' by adopting a dimensional approach to the psychopathology and a clinical staging approach to the course

of illness. Moreover they recommend that 'endophenotypes'[8] need exploration to study development of psychopathology, and 'markers of disease progression' require development for an understanding of aetio-pathology. They do not discard the Kraepelinian tradition but emphasize the need for refinement.

Other subdivisions for schizophrenia have been attempted. A *Two-syndrome* concept for Schizophrenia was suggested by Crow based on positive and negative symptomatology (Crow 1985). A deficit syndrome was suggested by Carpenter in patients diagnosed with schizophrenia using DSM-III-R criteria (Carpenter et al. 1988). It is beyond the scope of this thesis to discuss these in further detail however it may suffice to say that at the moment the ICD and DSM criteria are used world wide to diagnose schizophrenia.

[8] Gottesman and Gould have defined five criteria of endophenotypes. Gottesman, II, Gould TD. 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry, 160 (4):636-645.
(1) The endophenotype is associated with the illness; (2) The endophenotype is heritable; (3) The endophenotype can be detected in remitted patients who do not suffer from active illness (state-independence); (4) The endophenotype and the illness co-segregate in affected families; (5) The endophenotype found in ill family members is found in unaffected family members at a higher rate than in the general population.

1.2 Neuropathology of schizophrenia: insights from structural deficits in schizophrenia

Over the years schizophrenia has been recognized as a disease entity although attempts are still ongoing to elicit characteristic features of the disease process. As it has been conceived to be a disease of the brain, exploring and understanding morphological changes in the brain has been one of the predominant methods of investigation to uncover neuropathological abnormalities in schizophrenia.

Kraepelin stated that different manifestations of an illness might not represent a particular disease process. He believed that the 'disease process however may have a preference for 'some organ' (or perhaps some parts of the brain)' and that 'in general, the same illness tends to affect the same areas in the same way and to the same extent' (Kraepelin 1992).

Kraepelin noted that *schizophrenic* symptoms were not confined to Dementia Praecox. He also observed that many 'primitive responses' can be seen in patients with Dementia Praecox and suggested they were 'release' signs owing to extensive brain damage (Kraepelin 1992). These ideas of Kraepelin have been linked to Guislain's theory that the various developmental stages of a mental disorder correspond to the extension of a morbid process to more and more areas of the brain (Kraepelin 1992).

Thus owing to Kraepelin and other eminent psychiatrists, towards the end of 19th century, it was thought that mental disorders could be related to alterations in the anatomical substrate. This led to the hypothesis that the mental processes governing everyday life (thought, consciousness, intelligence, imagination, memory, etc.) must also be located in a particular anatomical substrate (Lopez-Munoz et al. 2008). For example, in 1894, Cajal had suggested that the increase in number of connections between neurons (pyramidal cells which he called as 'psychic cells') in the cerebral cortex may underlie a phenomenon of neuronal plasticity associated with the process of learning and development of intellectual and other abilities (Lopez-Munoz et al.

2008). This implied that structural alterations could lead to dysfunction of mental processes, which might manifest as symptoms of mental disorder.

Bleuler noted that "*pathological anatomical findings exist (in schizophrenia) although the nature of their relation to the psychosis remain enigmatic*"(Bleuler 1950). He argued that the concept of schizophrenia as a disease can be understood if one assumed the presence of an 'anatomical or a chemical' disturbance in the brain such that the disease remains latent until an "*acute pathological thrust produces prominent symptoms, or until a psychic shock intensifies the secondary symptoms*" (Bleuler 1950). He reviewed contemporary anatomical studies in Dementia Praecox and concluded that "*we do not know what the schizophrenic process actually is*" (Bleuler 1950). Bleuler remarked further that some had considered schizophrenia to be a "*congenital pathological disposition of the brain*" (Bleuler 1950) although he did not find much evidence for it.

In the thoughts of late 19th century and early 20th century psychiatrists we find seeds being planted for the following

- Schizophrenia may have anatomical substrates
- Anatomical substrates may be present prior to the development of schizophrenia
- Progression of the disease process of schizophrenia may incorporate more anatomical substrates in due course
- Alterations in anatomical substrates may neither be specific nor sufficient for schizophrenia

Exploring brain anatomy continues to be an active field of research in understanding etiopathogenesis of schizophrenia.

1.2.1 Structural changes in schizophrenia: neurodevelopmental, progressive or degenerative

Although the modern concept of schizophrenia had initially emerged as Dementia Praecox, absence of features of neurodegeneration, such as gliosis in brain tissue, has led to the current view that schizophrenia is not a neurodegenerative illness by classical standards (Harrison 1999).

Studies of neurons and other cytoarchitectural markers do support the possibility of schizophrenia being a neurodevelopmental illness. Cytoarchitectural changes seen in patients with schizophrenia are also seen in other congenital disorders of the brain (Kalus et al. 1999).

Altered distribution of Cajal-Retzius cells in prefrontal cortex, altered migration of neurons in entorhinal cortex, altered distribution of microtubule associated proteins, and impaired migration of subplate neurons in prefrontal and temporal cortices in schizophrenia may imply altered corticogenesis as an etiopathological factor in schizophrenia (Kalus et al. 1999).

An extensive review summarizes the cytoarchitectural changes in schizophrenia as 1) altered neuronal shape, neuronal number, neuronal density, density of interneurons in hippocampal, entorhinal cortex, various Brodmann areas in frontal lobes, thalamus and other subcortical regions, and 2) altered synapses in hippocampal formation, temporal and frontal cortex, as well as in striatum, corpus callosum and thalamus (Harrison 1999). This review argues that the most compelling cytoarchitectural changes are evident in the hippocampus, prefrontal cortex and dorsal thalamus, suggestive of a disturbance of connectivity within and between these regions, most likely originating during brain development.

However none of these cytoarchitectural abnormalities have been unequivocally established to be a feature of schizophrenia, and it is still unknown whether neurodevelopmental deviance is either necessary or sufficient for development of schizophrenia (Harrison 1999).

Magnetic resonance imaging studies indicate that structural alterations in schizophrenia may be both neurodevelopmental in origin as well as representing progressive changes during the course of illness (Pantelis et al. 2005). The salient points of previous structural imaging studies are summarized in Table 1 regarding to studies that explore anatomical or structural deficits in schizophrenia using the operationalization of criteria to diagnose schizophrenia (ICD and DSM)[9].

The reviews and meta-analyses in Table 1 indicate that structural changes are present prior to the onset of schizophrenia. The changes that exist prior to the onset of illness have been assumed to reflect changes that point towards a neurodevelopmental abnormality. The meta-analyses also suggest that structural changes may represent progression of disease process. Meta-analysis of region-of-interest based longitudinal MRI studies comparing 928 patients and 867 healthy adults showed a greater reduction in patients over time in frontal grey and white matter, parietal white matter, and temporal white matter volume (Olabi et al. 2011). These findings indicate that schizophrenia may be associated with progressive structural brain abnormalities. Grey matter reductions in chronic schizophrenia are more widespread than in patients with a first episode of schizophrenia and those at high-risk of developing schizophrenia (Smieskova et al. 2010, Pantelis et al. 2005). The prefrontal and medial temporal lobes are implicated both in pathophysiology of schizophrenia as well as in transition to psychosis (Smieskova et al. 2010, Pantelis et al. 2005). Fronto-temporal and thalamo-striatal regions are accepted to play an important role in the pathophysiology of schizophrenia (Andreasen et al. 1998).

[9] For the rest of the paper and all other chapters, the term 'schizophrenia' would always indicate the diagnostic status following ICD (9,10) or DSM (III, IIIR, IV, IV-TR) criteria or both as appropriately mentioned.

Table 1: Meta-analysis studies of grey matter and white matter in schizophrenia

Group	N	Finding	Left Hemisphere	Right Hemisphere	Study
Chronic schizo - phrenia	390 Sz 364 C	Reduced GM; Sz < C	STG		VBM (Honea et al. 2005)
	1195 Sz 1262 C	Reduced GM; Sz < C	Insula Ant Cingulate PHG MFG Post central Thalamus		VBM (Glahn et al. 2008
	407 Sz 383 C	Reduced WM Sz < C	Frontal lobe Temporal lobe		DTI (Ellison-Wright und Bullmore 2009)
First episode schizo - phrenia	170 FES 809 Sz 986 C	Reduced GM; FES > C SZ < C	Amygdala Insula IFG	Thalamus Insula Ant Cingulate	VBM (Ellison-Wright et al. 2008)
		Reduced GM; FES < Sz	Caudate	Caudate	
		Reduced GM; Sz < FES	Fusiform MFG	Hippo-campus STG MTG	
High risk for schizo - phrenia	601 HR 466 FES 808 Sz 1902 C	Reduced GM; HR < C	Amygdala Ant Cingulate	Insula Ant Cingulate	VBM (Chan et al. 2011)
		Reduced GM; HR < FES	Subcallosal gyrus Amygdala IFG		
		Reduced GM; Sz < FES	Ant cingulate Amygdala	Insula	
		Reduced GM; Sz < C	MTG Amygdala Thalamus Insula Frontal lobe Post Cingulate	MTG PHG	
Transition to psychosis	1169 HR	Predict transition	Prefrontal Ant Cingulate Medial temporal Cerebellum (regions reported in right, left or both hemispheres)		VBM, CPM, fMRI, PET, MRS (Smieskova et al. 2010)

Abbreviations: C: Control; Sz: Chronic schizophrenia; FES: First episode schizophrenia; HR: High risk for schizophrenia; GM: Grey matter; WM: White matter; STG: Superior temporal gyrus; MTG: Middle temporal gyrus; PHG: Parahippocampal gyrus; Ant Cingulate: Anterior cingulate gyrus; MFG: Middle frontal gyrus; IFG: Inferior frontal gyrus; VBM: Voxel-based morphometry; DTI: Diffusion tensor imaging; CPM: Cortical pattern matching; fMRI: Functional magnetic resonance imaging; PET: Positron emission tomography; MRS: Magnetic resonance spectroscopy.

A meta-analysis comparing 679 patients with schizophrenia, 1065 independent first-degree relatives of patients and 1100 healthy control subjects, found that first degree relatives had reduced overall grey matter, and significantly reduced hippocampal volumes as seen in the patients (Boos et al. 2007). This indicates that structural changes in the brain may represent a heritable factor, increasing the risk of developing schizophrenia. Twin studies have found significant heritability effects for the hippocampus, a significant genetic correlation of whole brain volume (Rijsdijk et al. 2005) and cerebral white matter volume (van Haren et al. 2012) with schizophrenia. Thus both family studies and twin studies suggest a genetic basis for neurodevelopmental structural brain changes seen in schizophrenia. Genetic polymorphisms have been found to correlate with structural brain changes in schizophrenia. This shows that alterations in the brain structure can provide an insight into genetic and neurodevelopmental etiopathogenesis of schizophrenia (Prasad und Keshavan 2008).

An important aspect of structural deficits in schizophrenia is the overlap with other illnesses, which makes interpretations of these findings difficult. Meta-analysis of VBM studies of patients with schizophrenia and bipolar illness has revealed that there are overlapping areas of grey matter reductions although the reductions in schizophrenia are more widespread involving frontal, temporal, cingulate, insular cortex and thalamus (Ellison-Wright und Bullmore 2010). A recent twin study investigating genetic liabilities of structural deficits in schizophrenia and bipolar disorder concluded that overlapping smaller white matter volume and common areas of thinner cortex in right (and left) parahippocampus, and right orbitofrontal cortex, suggest that both disorders share genetic and hence neurodevelopmental etiopathology (Hulshoff Pol et al. 2012).

From the above discussions one can conclude

- Structural brain changes are present prior to the onset of schizophrenia
- Presence of structural brain changes may not be a sufficient condition to develop schizophrenia
- Structural brain changes occur following onset of illness and become more widespread as the disease progresses.
- Fronto-temporal regions as well as thalamo-striatal regions are implicated in schizophrenia.

1.2.2 Need for novel brain structural markers to investigate neurodevelopmental etiopathology

It is unclear how the macroscopic structural deficits as seen in MRI studies relate to the microscopic alterations seen in brain tissue in schizophrenia. Genetic studies, although inconclusive, point towards abnormality of formation and functioning of synapses in schizophrenia (Harrison und Weinberger 2005). There is currently a concerted effort to decipher the genetic neuropathology of schizophrenia (Kleinman et al. 2011). Over the last decades there is increasing evidence of a neurodevelopmental component in the pathogenesis of schizophrenia. It might be useful to view neurodevelopmental changes as early and late, with the latter occurring around adolescence and early adulthood. It has been suggested that early neurodevelopmental abnormalities may predispose the late neurodevelopmental processes to further insults, which may lead to the onset of schizophrenia as well as to further structural abnormalities as the disease progresses (Pantelis et al. 2005).

Whole brain volume, regional grey matter volume and thickness, white matter volume and white matter structural integrity change with age from childhood to adulthood (Lange 2012, Tamnes et al. 2010), during adulthood (Taki et al. 2012) and normal aging (Thambisetty et al. 2010, Michielse et al. 2010). Although healthy siblings of patients with schizophrenia show abnormality of cortical grey matter, these abnormalities normalize as the siblings reach adulthood (Mattai et al. 2011, Gogtay et al. 2007). Thus both grey matter and white matter do not remain stable over adulthood and hence do not provide a

feature that primarily reflects a deviation of neurodevelopmental trajectory. Such a measure would be useful to differentiate structural abnormality that coexists with schizophrenia from structural abnormality that may play an etiopathological role in schizophrenia. Cortical gyrification, which is a measure of foldedness of the cortical surface, has been proposed as a relatively stable brain structural feature to investigate neurodevelopmental etiopathogenesis of schizophrenia (Jakob und Beckmann 1986).

1.3 Cortical gyrification as a neurodevelopmental marker in schizophrenia

1.3.1 Concept of gyrification

The human brain has a folded cortex as seen in other mammals. Gyrification is the process of folding of the cortex into *gyri* and *sulci*. It begins at around 16 weeks of gestation in humans, with most rapid growth in the third trimester, and most gyri and sulci are present at birth. Gyrification index has been conceived as a ratio of the surface of brain including sulci, to a smooth surface enveloping the brain (Zilles et al. 1988). Gyrification index reaches it's maximum at the age of three years reaching adult values around the age of 23 years, although temporal aspect of this process varies in different regions of the brain (Armstrong et al. 1995). Thus gyrification provides an important window to study neurodevelopmental delays preceding neuro-psychiatric illnesses.

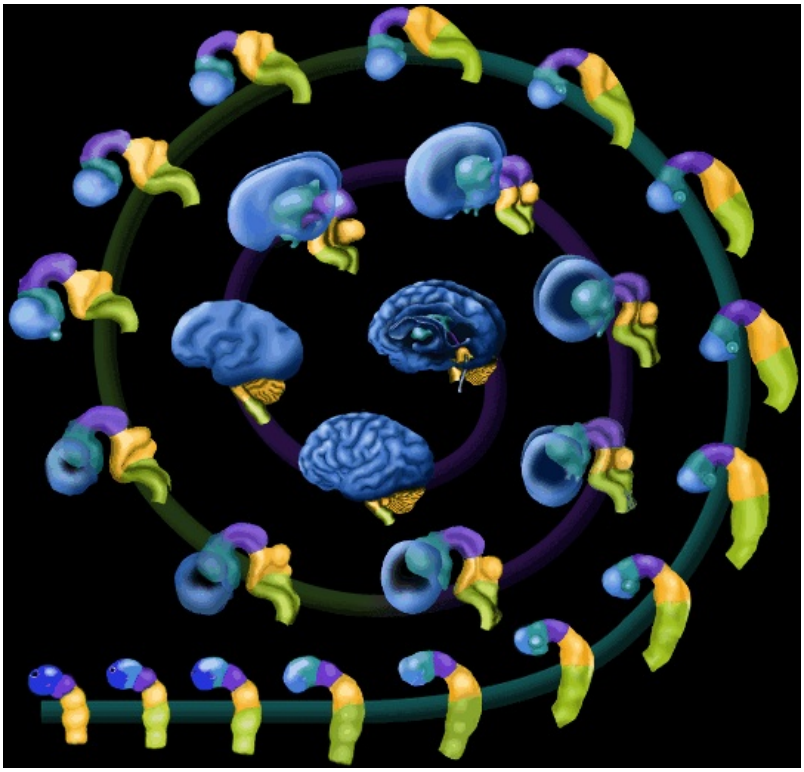

Figure 1: Schematic for cortical gyrification in a human embryo. Modified from http://www.visembryo.com/baby/NewsArchive31.html

Humans have a particularly expanded neocortex that separates them from primates. During evolution the increase in size of cortical surface and pattern of cytoarchitechtonic areas have resulted in a highly folded cortical surface in humans, comprising of bulges (gyri) and furrows (sulci), while the changes in cortical thickness and cellular organization within the cortex have undergone relatively minor changes (Rakic 2002) . Thus deviation in the folding pattern or gyrification could serve as an indication for an altered neurodevelopmental trajectory.

The human cortex has distinct layers with the neocortex having 6 layers. During development, cortical neurons are generated from 6.5 to 17 gestational weeks, and thereafter produced at a slower rate till 25 gestational weeks (Rakic 2000). During the development of the brain, the cells in the ventricular zone start dividing and then migrate to form the cortex in an inside out manner. This occurs in two stages.

In the first stage each cell (progenitor cell) divides into two, thus exponentially increasing the cell numbers at the ventricular zone with each cycle of cell division. This is called the stage of symmetrical division. The second stage is called the stage of asymmetrical division, during which each progenitor cell divides into one neuron cell and a progenitor cell. This stage therefore marks the beginning of neurogenesis. The neuronal cell migrates to form the cortex. This migration occurs along radially arranged processes of another cell type called *glia*. The neuronal cells arrange themselves vertically in the cortex in the reverse order of their arrival. Rakic (Rakic 2000) states that this glial scaffolding is an essential prerequisite for formation of the cerebral cortex.

Rakic supports the minicolumn (Mountcastle 1997) as the basic unit of cortex and asserts that the cortex comprises of “arrays of radial columns intersected by horizontal layers of isochromously generated neurons”. Based on this Rakic proposes a Radial Unit Hypothesis for increased cortical surface for humans and argues that i) a slight increase in the duration of symmetrical division phase (i.e slight delay in the onset of asymmetrical division stage or neurogenesis) would lead to an increase in cells at the ventricular zone and hence an

increased number of radial units while a slight increase in duration of the asymmetrical division phase would lead to an increased number of neurons in one radial unit. Comparing macaque monkeys and humans, Rakic shows that neurogenesis in monkey is from the 40th day to 100th day of gestation while in humans neurogenesis is from the 42nd day of gestation to 120th day of gestation.

In addition ii) a delay in the onset of asymmetrical division that allows 3 to 4 extra rounds of cell division (mitosis) would result in 2^3-2^4 fold increase in progenitor cells that would generate an 8-16 fold larger number of columns and hence proportionally a larger cortex while a 20 day delay in the end of asymmetrical division would add only 10 more neurons to the ontogenic column, which would be only a 10% increase in thickness assuming that each column had about 100 cells (Rakic 2000). Even though this model does not provide exhaustive explanation for cortical morphology of humans it has been shown that such an increase in cortical surface without a corresponding increase in cortex thickness may be sufficient for the convoluted cortical morphology (Chenn und Walsh 2002).

The six cortical layers are grouped as supragranular (I-III) and granular and infragranular (IV-VI) layers. Richman proposed that differential growth of these layers would lead to ‘buckling’ of the cortical surface (Richman 1975) resulting in the appearance of a folded cortex. The model assumes the brain to be an elastic sphere consisting of two layers and bound to an inner core. The two layers represent the cortical layers while the inner core represents the white matter along with subcortical nuclei.

In this model, the supragranular layer grows at a slightly faster rate than the infragranular layer, while the core grows very slowly. Consequently different ‘stresses’ appear on the surface, which lead to ‘buckling’ or folding of the cortex. They showed that if the growth of the infragranular layer is sufficiently slower than the supragranular layer, it leads to microgyria with the intersulcal distances almost similar to the cortical thickness. If on the other hand the growth of both

layers of cortex were sufficiently slowed while the growth of the inner core was sufficiently accelerated, this would result in a lissencephalic brain.

They corroborated their proposed model with histological measurements of normal, lissencephalic brains, and brains with microgyria. This model explained the random pattern of tertiary convolutions or gyrification but failed to explain the constant pattern of primary gyrification. The authors acknowledged that mechanical stress from 'external constraints' or those focusing on cytoarchitectonic boundaries might be more significant in the development of convolutions thus leading to a stable pattern of primary gyri and sulci.

White matter has been also proposed to be an important source of mechanical tension during the development of the cortex. Van Essen (Van Essen 1997) highlights two characteristics of brain white matter:

1) Dendrites, glial processes and axons are orientated in different directions and thus the mechanical tension would be greatest along the predominant orientation

2) Axons of interconnected areas should pull these areas together during cortical growth to keep 'wiring length short and overall neural circuitry intact'.

It is proposed that as neurons establish long distance connections early during development, axonal length is constantly modified throughout development in order to adjust to cortical growth, as well as to keep synaptic contacts intact. It has been observed in various species with folded brains that during development the cortical surface increases disproportionately to the brain size. In addition, both cortico-cortical *and* cortico-subcortical connections are established early, ensuring that cortical mantle is wrapped around the subcortical core. The resulting mechanical tension could lead to two types of folding. It is proposed that tension would pull two strongly interconnected regions together producing an outward fold, thus resulting in a gyrus. Geometrical constraints require an inward fold in between two outward folds. It is proposed that sparsely connected areas will not be able to resist this force on the elongation of their axons, thus resulting in a sulcus.

Van Essen demonstrated such folding with computer simulation for the visual cortex of the macaque. This model accounted both for the standard pattern of primary gyrification as well as the variability of tertiary gyri. The latter was ascribed to the fact that there is marked individual variability in size and shape of cortical areas and thus possibly also in white matter connections between areas. It has been shown in a recent study in cats that myelination and structural changes in thalamo-cortical and cortico-cortical white matter tracts in a developing brain coincides with formation of gyri (Takahashi et al. 2010), thus lending support to the mechanical model of cortical gyrification.

In contrast to the above theories, which propose that gyrification results due to the growth of cortex, 'gyrogenesis' proposes that formation of the gyrus is an important developmental goal during cortical growth. This active process of 'gyrogenesis' has been suggested to involve diverse processes such as cytoarchitectonic differentiation, in-growth of thalamic and cortical afferents, selective neuronal death and progressive myelination that move the gyral crowns outward (Rakic 1988, Kostovic und Rakic 1990, Welker 1990). These processes implicated in gyrogenesis are partially genetically determined (Rubenstein et al. 1999, Rubenstein und Rakic 1999). The processes act together to produce a gyrus and none of the processes in isolation provide a sufficient explanation for gyrification.

In a recent review of developmental processes in human cortical development, Bystron et al (2008) conclude that molecular studies have shown that cortical size, lamination and connectivity are largely genetically regulated; that changes in brain related genes evolved much faster in human lineage; that changes in gene expression, modification of sequence, and appearance of novel genes may have all contributed to the unique features of the human cerebral cortex (Bystron et al. 2008). However this review focuses primarily on cortical layer formation rather than gyrification. Although it is not clear whether axons influence cortical shape by mechanical tension, it is accepted beyond doubt that cortical connectivity is an important aspect of the final form of the human cortex and represents functional efficiency. It is not only intracortical connections, but also local synapses that are believed to influence local cortical shape.

The primate cortex undergoes a biphasic neuronal and axonal elimination. The first phase involves neuronal elimination in the second half of gestation. Neuronal elimination from the superficial layers of cortex is larger than neuronal loss in the deeper cortical layers. The second phase involves axonal elimination. The majority of axonal elimination in cortico-cortical fiber tracts occurs during the first 3 post-natal weeks and continues slowly until adult level is reached at puberty (in monkeys). Loss of inter-hemispheric axons coincide with increased synaptic production and thus this biphasic elimination has been suggested to be causative for synaptic remodeling at the local level (Rakic 2002).

In addition, synaptic density increases from the first few post-natal months to adolescence, when synaptic elimination occurs, which primarily involves loss of asymmetric synapses on dendritic spines. Synaptic density increases from before birth to 8 months of age in the visual cortex (Huttenlocher et al. 1982b, Huttenlocher et al. 1982a), to about three years of age in the auditory cortex, and to between one and three years of age in the prefrontal cortex (Huttenlocher 1979). It then progressively declines to adult values, which are obtained between 12 years of age and mid-adolescence. It has been observed that synaptogenesis and formation of neurotransmitter maturation occurs concurrently in anatomically and functionally diverse areas of the cortex. As these significantly precede the phase of synaptic elimination, it has been suggested that the phase of synaptogenesis provides an “enormous window of opportunity for the generation of cortical diversity beyond genes” (Rakic 2002).

Other aspects of cortical growth may simultaneously affect gyrification. Development of cortical thickness is interrupted by maturational troughs at 2 and 6 years, followed by accelerated growth between years 2 to 4 and 6 to 10 (Rabinowicz 1979). While myelination of most tracts is completed at or before 2 to 3 years of age, myelination of the intracortical association areas continues into the second and third decades (Yakovlev PI 1967). Myelination in hippocampal areas show two fold increase between first and second decades and further increase between fourth and sixth decade (Benes et al. 1994).

Differential myelination has been confirmed by recent DTI studies (Lebel et al. 2008). It is not clear how these processes affect gyrification, however they provide evidence that neurodevelopment in humans continues for a fairly long time, which may reflect itself in various morphological parameters of the brain, e.g. changes in cortical shape.

1.3.2 Functional significance of cortical gyrification

Cortical gyrification does not have a precise positional relationship with Brodmann cytoarchitechtonic areas. It has been suggested that as development of cortical gyrification and cytoarchitechtonic areas are not interdependent, they might represent developmentally distinct processes with partially overlapping genetic and epigenetic mechanisms (Rademacher 2002). Variability of gyri and cytoarchitechtonic areas is more pronounced in the association cortices.

Longitudinal studies exploring changes in cortical gyrification across the life span in healthy human adults is limited. In a post mortem study (Armstrong et al. 1995) of 97 human brains ranging in age from 11-95 years, it was found that gyrification starts early in the rostral third of the neocortex. 5% of the total change in GI occurs at 21.6 weeks in the rostral third, at 22.4 weeks in the intermediate, and at 24.9 weeks in the caudal third of the brain. It was also noted that the frontal third also took longer to reach its adult degree of cortical folding compared to the intermediate or caudal thirds (95% of the change in GI is at ontogenic weeks (OW) 48.3, 44.4, and 43.0, respectively).

The author had concluded that gyrification is relatively stable over adult life (Armstrong et al. 1995). However this conclusion was based on a 2-dimensional method of measuring gyrification. It remains an open question whether white matter changes in late adulthood (Yakovlev PI 1967) could lead to age related changes in cortical gyrification, and whether these can be detected using the improved available imaging techniques and methods of cortical surface analysis.

It has been proposed that the purpose of gyrification is to achieve optimal efficiency of neural information transfer (Welker 1990, Chklovskii 2004). Design efficiency models have been suggested for neurons (Wen und Chklovskii 2008), dendritic arborization(Wen et al. 2009) and cortical organization (Murre und Sturdy 1995, Wen und Chklovskii 2005). These theories suggest a deterministic developmental framework for gyrification, as well as leaving room for any possible modification in gyrification owing to alterations in underlying cortical connections.

The magnitude of cortical folding has been associated with gender differences (Luders et al. 2004), IQ (Zhang et al. 2010), cognitive function (Luders et al. 2012), and various neuropsychiatric conditions (Gaser et al. 2006). It is unclear whether it is the *increased* or the *decreased* gyrification that is an indication of the underlying neurodevelopmental abnormality.

1.3.3 Gyrification in schizophrenia

Various theories of gyrification suggest that it is a morphological feature reflecting changes in underlying connectivity. Significant changes in synaptic connectivity occur prior to birth and then again in mid-adolescence. Therefore any abnormality of gyrification may reflect an early neurodevelopmental insult or a late neurodevelopmental insult. Neuropathology of schizophrenia suggests that either a neurodevelopmental deviance in-utero and/ or a neurodevelopmental deviance during adolescence may play an important role in etiopathology of schizophrenia. This makes gyrification a good candidate to explore neurodevelopmental etiopathogenesis of schizophrenia.

Altered gyrification has been observed in schizophrenia. Post mortem studies of schizophrenia have reported increased gyral folding of temporal lobes in patients (Highley et al. 1998), and increased gyrification of the frontal lobe in patients and their unaffected relatives (Falkai et al. 2007). Studies have also reported reduced posterior cingulate folding (Wheeler und Harper 2007), and reduced gyrification in cerebeller vermis (Schmitt et al. 2011) in patients with schizophrenia.

Altered gyrification has also been observed in MRI studies of schizophrenia. In patients with chronic schizophrenia, MRI studies have shown increased gyrification of the right frontal lobe (Vogeley et al. 2001), left pars triangularis (Wisco et al. 2007), and bilateral visual areas (Schultz et al. 2011). In patients with chronic schizophrenia, MRI studies have also reported reduced gyrification in left hemisphere (Kulynych et al. 1997), in bilateral hemispheres (Sallet et al. 2003), reduced global and temporal sulcal index (Penttila et al. 2008), and reduced folding of prefrontal cortex (Palaniyappan et al. 2011).

MRI studies in patients with first episode schizophrenia have reported increased gyrification of right temporal lobe (Harris et al. 2004a), right parahippocampal and lingual gyri (Schultz et al. 2010). MRI study of individuals with early onset schizophrenia have shown flattened sulci and peaked gyri (White et al. 2003). MRI studies of individuals at high risk for developing schizophrenia have shown increased prefrontal gyrification (Harris et al. 2007, Harris et al. 2004b, Stanfield et al. 2008) and reduced cortical folding in the left hemisphere (Jou et al. 2005).

One reason for the heterogeneity of results in MRI studies of gyrification in schizophrenia may be due to the differences in the methods. Firstly, gyrification index in MRI studies has been measured either by using two-dimensional (Zilles et al. 1988) or three-dimensional methods (Luders et al. 2006, Schaer et al. 2012). There are no studies that have established comparability of these different available methods. Secondly, gyrification index (GI) has been studied for either an entire hemisphere subdivided into two parts (Kulynych et al. 1997, Sallet et al. 2003), an entire lobe (Harris et al. 2004a, Stanfield et al. 2008), specific subdivisions of a lobe (Schultz et al. 2011) or an entire gyrus and its subdivisions (Wisco et al. 2007). GI for an entire hemisphere has been found to be reduced in schizophrenia while GI for a lobe, subdivisions of a lobe, gyrus or its subdivisions is reported to be increased in schizophrenia.

Despite the varied findings, most studies indicate that increased gyrification may represent neurodevelopmental deviance in schizophrenia. Altered fronto-temporal gyrification predominates the findings in schizophrenia. As altered

gyrification is found in high-risk individuals as well as in unaffected relatives, it appears that there may be a genetic underpinning for abnormality of gyrification in schizophrenia.

Twin studies offer an opportunity to further delineate the genetic influence on gyrification in schizophrenia. Twin studies have shown less than 50% concordance for schizophrenia (Gottesman et al. 1972) i.e. chances of both twins having schizophrenia among monozygotic (MZ) twins is less than 50%. Gyrification abnormalities may provide useful insight into discordance of schizophrenia in MZ twins. There are no studies of gyrification in twins discordant for schizophrenia.

It has been demonstrated that MZ twins differ in post-zygotic genetic, epigenetic and prenatal environments (Gringras und Chen 2001, Singh et al. 2002, Kato et al. 2005). These differences that may affect developmental trajectories or the differences that arise owing to the onset of the disease process, may explain disease discordance among monozygotic twins.

Epigenetic events may explain phenotype and disease discordance among MZ twins. A Two-Hit hypothesis has been proposed for some disorders (e.g. tumor suppressor genes (Sapienza 1990)), which involves an initial imprinting of gametes, followed by an imprinting of somatic cells. This may result in variable expressivity of the imprinted genes leading to a difference in penetrance and hence discordance between MZ twins. It is important to note that as the initial imprinting is of the gametes, there is a clear 'parent-of-origin' effect on the disease. There is no known 'parent-of-origin' effect for the disease manifestation of schizophrenia. Yet one cannot rule out such a phenomena for structural and functional alterations during development of the brain that might increase the vulnerability to schizophrenia.

Epigenetic mechanisms involving non-imprinted genes in somatic cells may also contribute to MZ discordance. It has been suggested that as abnormal cleavage resulting in twinning varies from the 2-cell stage to the 6-7 day old embryo stage, the difference in the number of cells in each of the twin embryos,

as well as differences in methylation specification, lead to MZ discordance. A Two-Hit hypothesis has been proposed for schizophrenia, where the first hit involves the immediate post-zygotic epigenetic differences following the event of twinning. The second hit involves an epigenetic mechanism silencing genes and altering the epistatic effects of a group of genes (Singh et al. 2002). Research into the genetic and epigenetic basis of discordance for schizophrenia among MZ twins favors epigenetic causation, though substantial proof is still elusive (Kato et al. 2005).

If susceptibility genes for schizophrenia affect gyrification then both affected and unaffected co-twin will show altered gyrification. This gives the advantage of seeing the cumulative effect of susceptibility genes on gyrification without any prior knowledge of such susceptibility genes or any assumptions regarding their 'risk-genotypes'. On the other hand such an abnormality of cortical gyrification, which is shared by both discordant twins, may provide useful a phenotype to explore genetic underpinning of vulnerability to schizophrenia.

If abnormal cortical gyrification were only seen in the affected twin and not the unaffected co-twin, such an abnormality could be intrinsically related to the manifestation of the illness. This could result from epigenetic effects prior to the onset of illness. Alternatively, such an abnormality may be an effect of the disease process. Thus it may provide a useful phenotype to explore epigenetic underpinning of the pathophysiology of schizophrenia.

Neurodevelopmental theory for the pathophysiology of schizophrenia has prompted large-scale genetic studies as well as Imaging-genetic studies in the last decade. Thus co-twin control studies, that compare MZ healthy twins and MZ discordant twins, remain crucial as they provide a relatively cost-effective way of differentiating genetic effect from disease effect.

1.3.4 Methods to measure gyrification: Absolute Mean Curvature

The gyrification index (GI) is a measure of cortical complexity traditionally estimated in 2-dimensional images of post mortem brains and *in vivo* structural

MRI data. GI can be expressed as the ratio between the contours of inner and outer cortex in coronal section (Zilles et al. 1988). Originally, GI was developed as a global measure across an entire hemisphere or lobe. In order to explore more subtle regional cortical characteristics, refined GI methods have been developed delivering local quantification of curvature across the entire brain while preserving 3-dimensional morphological data (Luders et al. 2006, Schaer et al. 2008b, Toro und Burnod 2005).

Various methods have been developed to study cortical folding in 3D. Some methods reflect gyrification index (GI) while others measure cortical shape characteristics. Absolute Mean Curvature (AMC) is an extrinsic measure of the cortical shape that reliably yields a 3-dimensional quantification of the cortical gyrification index (GI) (Luders et al. 2006). Another method of quantifying GI is called local gyrification index (LGI) (Schaer et al. 2008a) that computes GI using the folded cortex and a local convex hull. Sulcal morphology has been proposed to reflect gyrification (Kochunov et al. 2005, Mangin et al. 2004, Molko et al. 2003) assuming that 'sulcal pits' play a deterministic role in sulcal and gyral formation. Other methods of local cortical complexity (Yotter et al. 2010), including 'Fractal dimension' (Blanton et al. 2001, Lee et al. 2004), have been proposed to reflect the complexity of cortical convolutions and thus reflect a property comparable to GI. Yet fractal dimension is difficult to explain from a biological point of view (Im et al. 2006).

These various approaches to measure gyrification or cortical folding may capture subtle differences in surface morphology of the brain. The various approaches are all valid approaches to study cortical folding or gyrification. However future work is needed to establish cytoarchitectural characteristics of the different available methods to study gyrification.

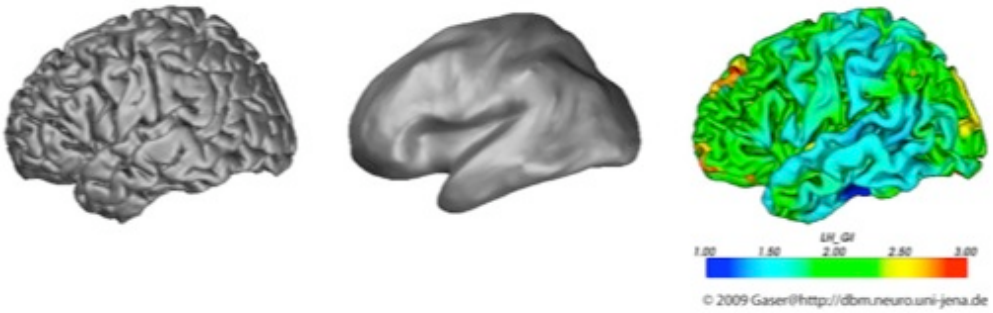

Figure 2: Calculation of Gyrification Index (GI) based on Absolute Mean Curvature (AMC) of folded cortical surface (left) and unfolded cortical surface of the same subject. Image on the far right shows local GI values across the entire 3-D cortical surface Modified from http://dbm.neuro.uni-jena.de

The definition of Gyrification index (GI), when extrapolated to 3D, is that it is the ratio of the cortical surface including the sulcal depths to the convex hull i.e. the unfolded cortical surface. Absolute Mean Curvature (AMC) is an extrinsic measure of the cortical shape (Fig 2). It reliably reflects spatial frequency and amplitude of a complex folded surface yielding a 3-dimensional quantification of the cortical gyrification index (GI) (Luders et al. 2006). As the convex hull is the same for all subjects after being aligned to the template, the difference in GI is essentially the differences in the magnitude of their cortical folding. This is exactly what is reflected by AMC (Luders et al. 2006) and thus differences in AMC are a good approximation of differences in GI. AMC has already been used successfully to study gyrification with respect to gender (Luders et al. 2004) and William's syndrome (Gaser et al. 2006), while mean curvature has been used to study schizophrenia (Schultz et al. 2010).

2. Aims and objectives

In this study, we aimed to analyze cortical gyrification measure from high-resolution MR images in schizophrenia and healthy control twins to assess genetic vs. disease-related effects. Gyrification was chosen as a morphometric marker, since it might reflect neurodevelopmental deviance more specifically than other morphometric parameters.

Monozygotic co-twin design that compares disease-affected and disease-unaffected co-twins, distinguishes effects related to genetic liability from those related to the expression of the disease phenotype. Monozygotic (MZ) twins share the same DNA sequence and hence 100% shared genetic make-up. However it has been demonstrated that MZ twins differ in post-zygotic genetic, epigenetic and prenatal environments (Gringras und Chen 2001, Singh et al. 2002, Kato et al. 2005). As monozygotic twins share 100% genes, their similarity reflects the effect of shared genes and may reflect genetic liability for a disease such as schizophrenia. Any dissimilarity between monozygotic twins reflects either epigenetic differences or an effect of the disease process. Thus an MZ co-twin design offers useful insight into neurodevelopmental genetic etiopathology in schizophrenia. So far, there are no studies of gyrification in twins discordant for schizophrenia.

We opted for a multi-center co-twin control design to study genetic influences on gyrification of the cortex in schizophrenia.

Given the multi-center design, a preceding reliability study was conducted for the measure of gyrification, i.e. absolute mean curvature.

Our hypotheses for Study 1, Reliability study for Absolute Mean Curvature, a curvature based estimation of local cortical gyrification, were:

1. Absolute Mean Curvature is a reliable measurement of curvature-based GI,
2. Absolute Mean Curvature measurements can be pooled across different research centers.

Based on the results of the above study, we combined appropriate data and conducted the co-twin control study to differentiate between effects of genetic liability from effects of expression of disease phenotype.

Our hypotheses for Study 2, Co-twin control study to investigate genetic influences on cortical gyrification in monozygotic twins discordant for schizophrenia, were:

1. Both affected and unaffected co-twins would show similar abnormality of regional gyrification in fronto-temporal areas compared to the normal MZ twins indicating genetic vulnerability to develop schizophrenia,
2. Affected and unaffected co-twins would show differences in regional gyrification within fronto-temporal areas that have been affected by the disease process.

3. Study 1: Reliability study of Absolute Mean Curvature, a curvature based estimation of local cortical gyrification

3.1 Method

3.1.1 Study design

Reliability, in short, establishes repeatability, consistency and reproducibility of the measure being studied. One can establish reliability in several ways. One may establish if the measure is repeatable, i.e. whether measures of the same person studied more than once are fairly similar. One may also measure consistency, i.e. when a group of individuals is measured, whether their measurements are comparable with each other.

Our study investigated reliability of a surface-based curvature method. Firstly, the repeatability and consistency was estimated of measurement of curvature-based GI using single subject data from two different scanners in a longitudinal study design. This was followed by a cross-sectional study estimating the reliability of measurement of GI using multi-subject data from four different scanners.

In this study, the reliability of local cortical GI across four different MRI acquisition protocols was measured at four different research centers, with the aim of establishing the feasibility of pooling data for latter cortical GI quantification, based on the absolute mean curvature approach (Luders et al. 2006). Data in this study was analyzed as part of EUTwinsS (European Twin Study on Schizophrenia network), and mostly used data acquired by the Schizophrenia Twins and Relatives (STaR) Consortium.

3.1.2 Ethics approval

Informed consent was obtained from one healthy adult volunteer for the single subject reliability study, after approval by local ethics committees. Six healthy adult volunteers were recruited as a part of the Schizophrenia Twin And

Relatives (STAR) consortium. Informed consent was obtained from six healthy adult volunteers for the multi-center reliability study, after approval by the relevant ethics committees.

3.1.3 Demographics

A 34-year old healthy male volunteer participated in the single subject reliability study. Six healthy volunteers (two males, four females, aged 20-35 years) participated in the multi-center reliability study.

3.1.4 MRI acquisition

Single subject two-center reliability study

Twelve MRI scans of a healthy male volunteer were obtained at two research centers over the course of one year. 3D T1-weighted structural MR images of the entire head were obtained using the following MR parameters:

Table 2: MRI acquisition parameters for single subject two-center reliability study

Center	Jena, Germany	Boston, USA
Scanner name	*Scanner 1*	*Scanner 2*
Magnetic strength	1.5T	1.5T
Scanner	Siemens Vision scanner	Siemens Vision scanner
Sequence	3D T1-weighted sagittal MPRAGE scan	3D T1-weighted sagittal MPRAGE scan
T_R (msec)	15	11.4
T_E (msec)	5	4.4
Flip angle (degrees)	30°	15°
Slices and slice thickness	192 contiguous 1-mm slices	160 contiguous 1-mm slices
In-plane voxel size (mm^2)	1 X 1	1x1

Abbreviations: T = Tesla, T_R =Repetition Time, T_E = Echo Time, msec = Milliseconds, mm = Millimeter

Multi-subject multi-center reliability study

Six healthy volunteers were scanned once at each of the four research centers. 3D T1-weighted structural MR images of the entire head were obtained using

the following MR parameters:

Table 3: MRI acquisition parameters multi-subject multi-center reliability study

Center	Jena, Germany	London, UK	Utrecht, Netherlands	Heidelberg, Germany
Scanner name	*Scanner J*	*Scanner L*	*Scanner U*	*Scanner H*
Magnetic strength	1.5 T	1.5 T	1.5 T	1.5 T
Scanner	Philips Gyroscan ACS-II scanner	General Electric Signa System scanner	Philips Gyroscan NT scanner	Picker Edge scanner
Sequence	3D T1-weighted 3D-FFE scan	3-D T1-weighted coronal spoiled gradient recalled echo (SPGR) scan	3D T1-weighted coronal spoiled gradient echo scan	3D T1-weighted sagittal 3D-FLASH scan
T_R (msec)	13	35	30	30
T_E (msec)	5	5	4.6	3
Flip angle (degrees)	25°	35°	30°	30°
Slices and slice thickness	256 contiguous 1 mm slices	124 contiguous 1.5-mm slices	170 contiguous 1.2-mm slices	135 contiguous 1.5-mm slices
In-plane voxel size (mm^2)	1x1	0.781 X 0.781	1 X 1	1 X 1

Abbreviations: T = Tesla, T_R =Repetition Time, T_E = Echo Time, msec = Milliseconds, mm = Millimeter

3.1.5 Measurement of local cortical gyrification

We implemented a pre-processing pipeline for surface extraction in FreeSurfer v4.1.0 (http://surfer.nmr.mgh.harvard.edu). This involved registering the T1 scan to standard Talairach space, bias correction, skull stripping, and tissue segmentation, prior to surface extraction. Reliable bias correction and segmentation are the key steps for latter surface extraction as demarcation between grey and white matter is crucially dependent on the signal intensity.

Cortical surfaces for each hemisphere were extracted separately. Each hemispheric surface consisted of a triangulated mesh of approximately 170,000 vertices. Two surfaces were extracted: a pial surface (grey matter-cerebrospinal fluid border) and a white matter surface (grey matter-white matter border) from

each hemisphere. A central surface, lying between the pial and white matter surfaces, is considered to offer analytical advantage over the pial and white matter surfaces (Liu et al. 2008). The central surface is quantified by averaging the pial and white matter surfaces.

Absolute mean curvature (AMC) was then calculated at each vertex over a 3mm radius in native space of the extracted central surface. AMC reliably reflects the spatial frequency and amplitude of a folded surface, while remaining relatively stable to prior tissue normalization (Luders et al. 2006). The data was smoothed using a Full Width Half maximum (FWHM) filter of 25 mm for statistical analyses across groups. AMC were obtained across cortical regions delineated according to the Desikan atlas (Desikan et al. 2006). These measures (hereafter referred to as Regional GI) were smoothed by a nearest neighbor iterative procedure (Han et al. 2006), equivalent to smoothing of 25mm FWHM, and then analyzed using SPSS 18 Statistics (http://www.spss.com).

3.1.6 Statistical analysis

Single subject two-center reliability study:
Correlation coefficient:
Pearson's correlation coefficient establishes how two data sets are linearly related to each other. If a person is measured twice for a measurement of interest, a high correlation coefficient between the two measurements will indicate that the two measurements are highly similar.

We calculated correlation coefficient of Vertex-wise GI across all vertices in the surface data extracted from scans from each research center separately. Eight scans from Research center 1 (*Jena*) and four scans from Research center 2 (*Boston*) were used to establish correlation coefficient matrices for Research centers 1 and 2, respectively. All 12 scans from both centers were then combined to explore the change in the correlation coefficients when data are pooled. The correlation coefficients were represented as a correlation matrix.

Multi-subject multi-center reliability study:

Intra-Class Correlation Coefficient:

When data is acquired from more than one person, Intra Class Correlation Coefficient provides a more robust method to estimate similarity based on a linear relationship compared with Pearson's Correlation. In the method proposed by Shrout and Fleiss, one compares the *variance between people* to the *total variance in measurement*, which is the variance between people and the variance between scanners (Shrout und Fleiss 1979). If the variance between the scanners is zero, then ICC is maximum, i.e. 1; if the variance between people is much higher than the variance between scanners, then ICC is high, and this is our aim for reliability in the real world. However, if variance between people is markedly less than variance between scanners, one would expect negative values for ICC (Parsey et al. 2000).

Following Shrout and Fleiss' model (Shrout und Fleiss 1979) Case 2, k random judges (scanners) , we considered the scanner as a random factor and implemented the model as follows:

$$ICC(2,k) = \frac{BMS - EMS}{BMS + (JMS - EMS)/n}$$

Here, ICC (2,k) is the intra-class correlation for all k scanners and n people, BMS is the between-people mean squares, JMS is the between-scanners mean square, and EMS is the residual mean square.

Spearman-Brown Prophecy

For Shrout and Fleiss Case 2, one random judge (scanner), ICC is given by:

$$r^* = ICC(2,1) = \frac{BMS - EMS}{BMS + (k-1)EMS + k(JMS - EMS)/n}$$

$$m = \frac{r_{req}(1 - r^*)}{r^*(1 - r_{req})}$$

If r* is the ICC value assuming one random scanner, and the desired ICC value r_{req} is 0.9, then the Spearman-Brown prophecy uses the formula 0.9 (1 – r*) /r* (1-0.9) to give the number of additional scanners needed to achieve an ICC of 0.9 on the given sample.

Cronbach's alpha

For Shrout and Fleiss, Case 3 the ICC is given by the following formula, also known as the Cronbach's Alpha (α):

$$\alpha = \frac{BMS - EMS}{BMS}$$

A different number of fixed scanners will give different Cronbach's alpha values. Thus one can evaluate the Cronbach's Alpha when all scanners are considered and the change in alpha when any scanner is excluded, and providing an indication of which scanners together have the least amount of between-scanner variance. Thus Cronbach's alpha helps us decide on the exclusion or inclusion of data from scanners helping us to minimize the variance and achieve the best ICC in our given sample.

Repeated measures ANOVA (rmANOVA)

ICC expresses the agreement between two scanners while rmANOVA establishes scanner effect, i.e. if the mean measurements across subjects, at a vertex or a region, differ markedly between the scanners. Thus scanner effects are not necessarily correlated to either high or low ICC and need to be explored separately.

For Vertex-wise ICC, we followed Shrout and Fleiss' method (Shrout und Fleiss 1979). We calculated ICC of the local gyrification assuming the scanners as random. ICC values were then obtained, assuming that the measures of local

gyrification came from a random single scanner randomly selected. The Spearman Brown Prophecy was used to establish how many additional scanners were required to deliver an ICC above 0.90 at each vertex when pooling the data from six subjects (Brown 1910, Spearman 1910). Vertex-wise, Cronbach's alpha was calculated to determine data from which scanner could be excluded. In addition a Vertex-wise rmANOVA was used to explore areas that show a scanner effect for Vertex-wise GI at the threshold of $P < 0.001$ (uncorrected) as well as after a correction for multiple comparisons using false discovery rate (FDR). All analyses on Vertex-wise GI were performed using MATLAB version 7.5 (http://www.mathworks.com).

Furthermore, similar analyses were carried out on regional gyrification index (Regional GI). Regional GI measures were extracted based on the Desikan atlas parcellating the cortex according to the conventional anatomical definition of a gyrus(Desikan et al. 2006). All analyses on Regional GI were performed using SPSS version 18 (http://www.spss.com).

3.2 Results

3.2.1 Single subject two-center reliability study:

Vertex-wise GI was compared across all the scans from a single subject. The ranges of correlations were 0.85-0.89 (center 1, *Jena*) and 0.78-0.88 (center 2, *Boston*) for the left hemisphere; 0.86-0.91 (center 1, *Jena*) and 0.74-0.88 (center 2, *Boston*) for the right hemisphere. When data from both centers were combined, the correlation for the left hemisphere was 0.78-0.89, and 0.74-0.91 for the right hemisphere (Fig 3).

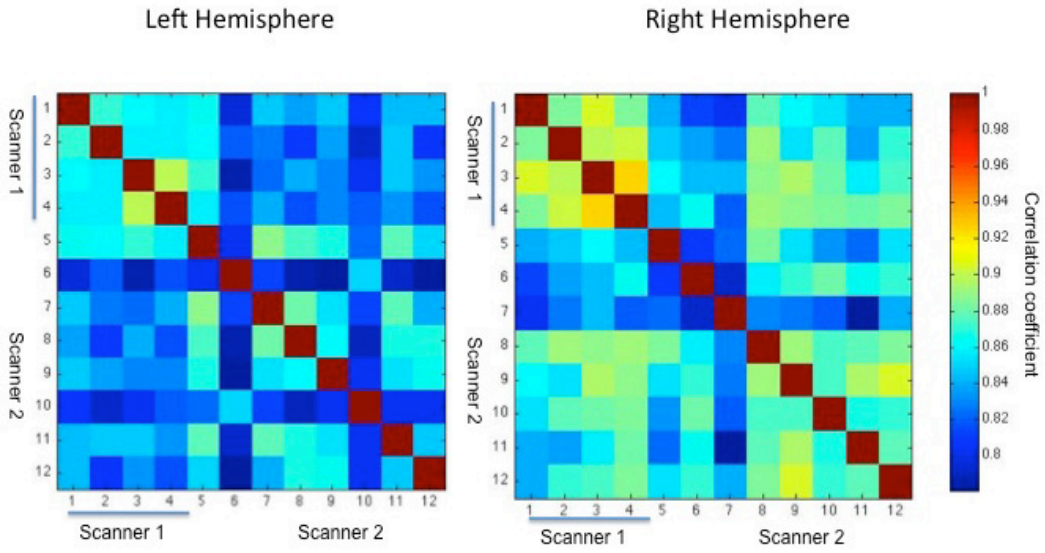

Figure 3: Correlation matrix for absolute mean curvature (AMC) of single subject data from two research centers

3.2.2 Multi-subject multi-center reliability study:

Vertex-wise gyrification index

Vertex-wise ICC was calculated for each hemisphere separately. For pooled data from four centers, ICC on the lateral surface of each hemisphere was >0.8; however, on the medial surface ICC was markedly lower. The ICC of the pooled data improved on the medial surface without compromising the high ICC on the lateral surfaces after excluding data from *Scanner H* (Fig 4).

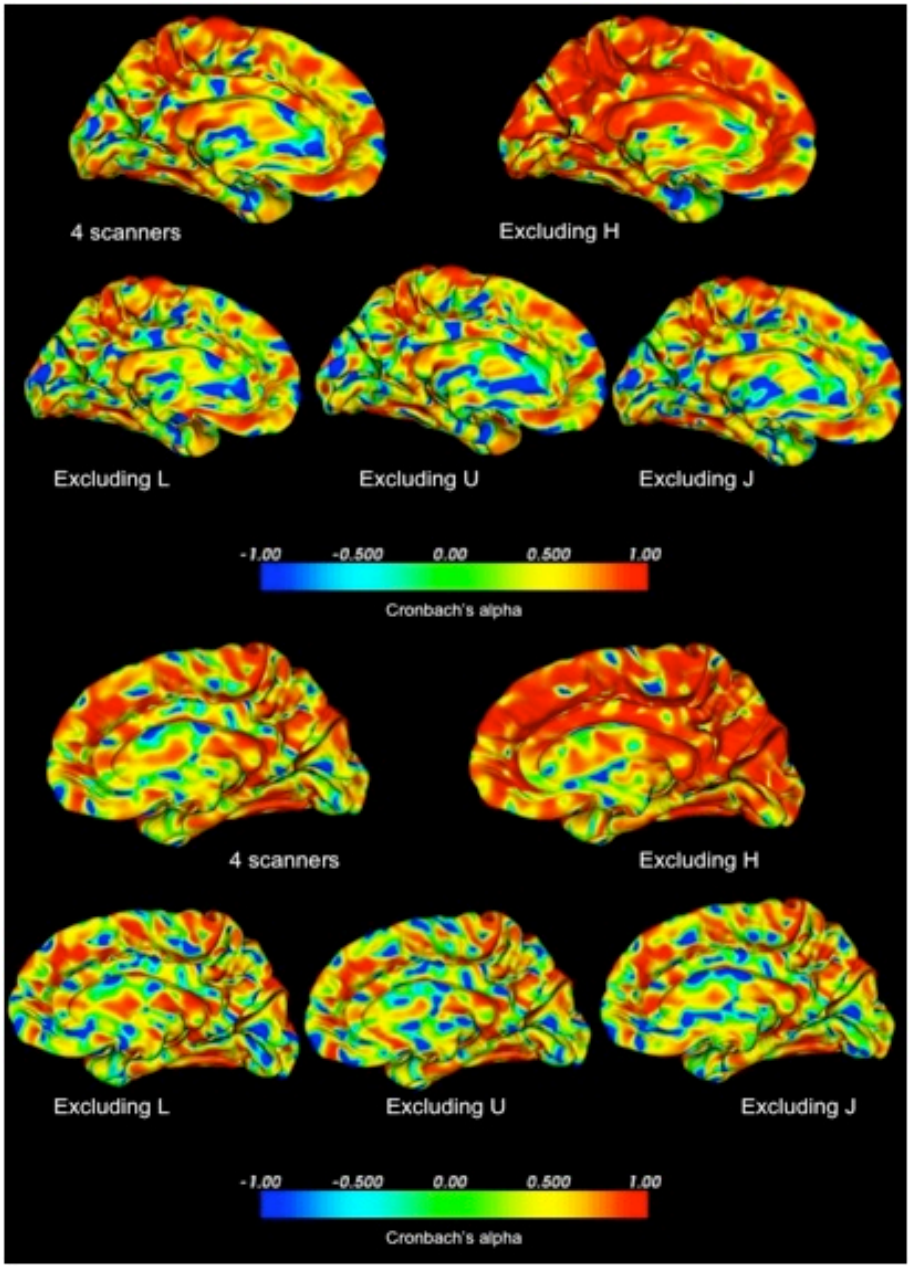

Figure 4: Intra class coefficients (ICC) of absolute mean curvature (AMC) of six subjects and change in Cronbach's alpha in the left hemisphere (left) and right hemisphere (right)

The Spearman Brown prophecy from three-center pooled data established that for most of the cortex in both hemispheres, three scanners were sufficient to establish a reliability of 0.9 among six subjects.

Repeated measures ANOVA showed no regional scanner effects at $p<0.05$ corrected for multiple comparisons using false discovery rate (FDR). However there were scattered scanner effects for Vertex-wise GI measurements at the threshold of $p<0.001$, uncorrected for multiple comparisons (df 2,10; $F=14.905$).

Regions implicated included the cuneus, precuneus, temporal pole, Heschl's gyrus, superior frontal gyrus in the left hemisphere, and temporal pole and superior temporal gyrus on the right (Fig 5).

Regional gyrification index

Regional gyrification index (Regional GI) were extracted according to the Desikan atlas and ICCs calculated. The effect of each research center contributing data on the Cronbach's alpha for Regional GI was calculated to establish pooling data from which centers contributed to the least variance in the pooled sample. Data from *Scanner H* led to negative ICC and removing that center's data markedly improved the ICC (Fig 6). Spearman Brown prophecy confirmed that data from the three most compatible centers were sufficient for regions that had a high Regional ICC. Repeated measures ANOVA ($p<0.001$, uncorrected for multiple comparisons) on Regional GI measures corroborated with the 'Scanner effects' noticed with the Vertex-wise GI analyses.

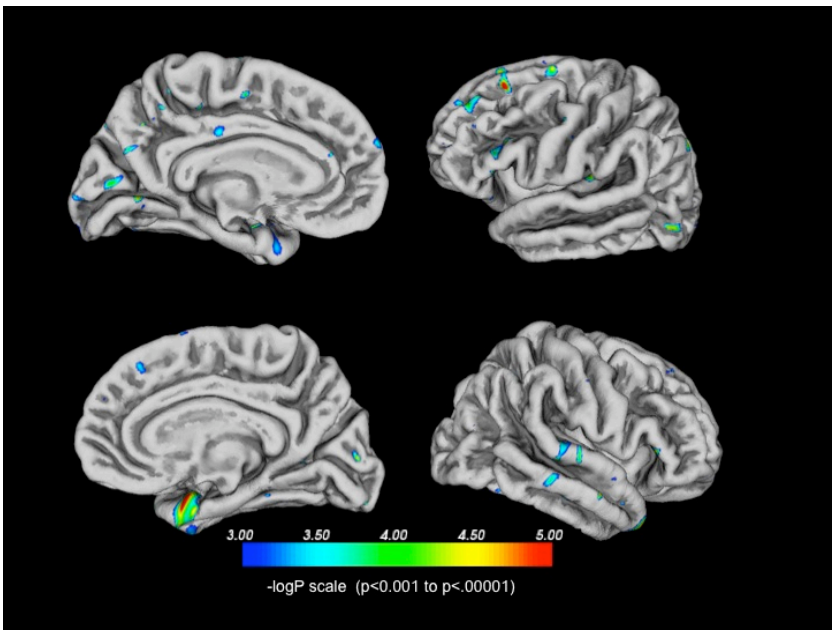

Figure 5: Regions showing scanner effects in the left hemisphere (top) and right hemisphere (bottom) in repeated measure ANOVA of absolute mean curvature of six subjects scanned in three scanners at p<0.001

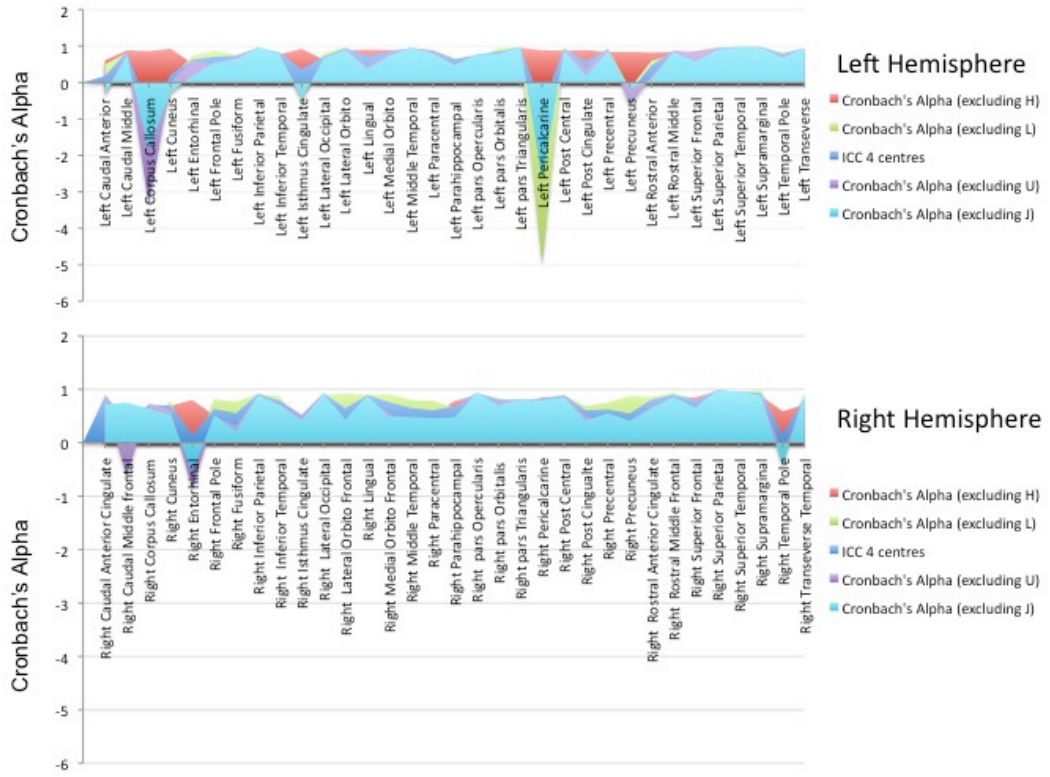

Figure 6: Cronbach's alpha showing effect of research center on Intra Class Correlation Coefficient of Regional GI of six subjects scanned in four scanners

3.3 Discussion

Multicenter neuroimaging studies are increasingly seen as a vital approach to maximize sample sizes and statistical power, especially in heterogeneous samples and rare conditions (Schnack et al. 2010). As data sets grow, analytical processes have focused on automated rather than labour-intensive manual techniques. Our study has demonstrated that an automated and simple measure to quantify reliability of an automated cortical gyrification index can be applied across structural MR data from four different scanners and sequences.

It was demonstrated in the single subject study that a) within the same scanner, gyrification reproducibility was high, and b) that this was maintained when data from the same subject across two scanners was introduced. The scanners were identical and the MR acquisition parameters were very similar, possibly contributing to the high data correlation.

In the second part of this study it was demonstrated that the absolute mean curvature reliability is high both for Vertex-wise GI and for Regional GI. In our sample, data from one center compromised data reliability when combined with the other three centers for local cortical GI measurement and was excluded. An earlier study on the same subjects that examined the same MR data but different cortical parameters detected similar findings (Schnack et al. 2010, Schnack et al. 2004). In our multi-subject study we demonstrate that reliability only improves when compatible data are combined, emphasizing the importance of establishing reliability before final data pooling.

In addition, it was found that despite high ICC, at a lower statistical threshold ($p<0.001$), a proportion of data variability could be explained by a scanner effect, which was not significant at more commonly used stringent thresholds. This implies that scanner effects should be modeled when investigating group differences in local cortical GI at low statistical thresholds. Taken together, we have shown that MR data from three research centers can be effectively pooled to investigate local cortical gyrification. It would be fair to generalize that MR data from centers using similar scanning parameters can be pooled for cortical GI measurement using AMC.

Reliability studies on voxel-based morphometry (Schnack et al. 2004) and cortical thickness (Schnack et al. 2010) of the same MRI scans from the participating centers of this network (and one additional center) had shown the feasibility of combining such data. The volumetric study demonstrated that owing to differences in scanner parameters, data from *Scanner H* adversely affected data pooling. This concurs with our findings. For the reliability of cortical thickness, the authors have calculated a 'group effect' measure that incorporates effect size (Cohen's d) for ICC measurement. This necessitates an assumption about the expected 'effect size'. As there are already several studies of cortical grey matter and cortical thickness in healthy twins, healthy individuals and patients with disease such as schizophrenia, it is possible to derive the 'expected effect size' for these measures based on meta-analysis. Since there are very few studies of cortical gyrification, we were unable to assume the 'expected effect size'. However, in our study Cronbach's alpha

establishes the effect of adding data from a center without necessitating an assumption of the 'expected effect size'.

The ICC has been used to establish reliability in neuroimaging studies. ICC for pooling MRI data of voxel-based volumetric analysis (Schnack et al. 2004) was used to assess modification of segmentation algorithm to improve pooling of data. Test-retest reliability has been shown to improve voxel-based intensity following correction of site-specific image distortion (Jovicich et al. 2006). ICC was used to establish reliability of both volumetric and surface-based cortical thickness using Freesurfer (Wonderlick et al. 2009). This demonstrated that pulse sequence could significantly affect surface-based measure. Wonderlick et al. suggested a careful selection of sequence parameters rather than modifying any pre- or post-processing algorithms for the data. Finally, a modification of ICC, incorporating effect size (*Cohen's d*), has been proposed for reliability of surface-based cortical thickness data (Schnack et al. 2010).

Methods other than ICC have also been used to establish reliability of structural neuroimaging data. Mixed effects general linear modeling has been advocated for reliability analysis of subcortical segmentation using Freesurfer (Fennema-Notestine et al. 2007). Fennema-Notestine et al. emphasized detecting and incorporating the scanner differences at different sites without any modification of either the processing pipeline or the method of calculation of the measure of interest (Fennema-Notestine et al. 2007). Measurement of standard error following registration by Freesurfer has been proposed for establishing reliability of surface- based cortical thickness (Han et al. 2006). Han et al. showed that smoothing with 30mm *Gaussian kernel* markedly reduces within-scanner measurement error for surface-based cortical thickness data(Han et al. 2006). This study also demonstrated that differences in field strength, pulse sequences, pre-processing algorithms, and post-processing smoothing, significantly affect reliability of surface-based cortical thickness measures. Our study was the first study to establish reliability of AMC as a surface-based curvature method.

Limitations

There are a few limitations of our study. Ideally, in order to incorporate neuroimaging data from multiple centers, scanning protocols should be prospectively standardized and optimized across centers, and intra and inter site quality control standards should be established. As scanning protocols differed across centers we used a single analytical pipeline to extract the brain surfaces and a single method to calculate local gyrification for data from all centers. This approach to combine multi-center neuroimaging data has been previously validated (Coffey et al. 2001).

We did not manually edit the extracted surfaces. It is possible that manual editing might have resulted in better estimation of the central surface and therefore improved our local cortical GI measurements. Manual editing is often unrealistic for very large studies and hence we aimed to optimize reliability in a fully automated approach. The power of our study is limited as we had only 24 MRI scans from six subjects at four research centers.

When ICC is measured it is important to note that the intra-subject reliability estimation is not based on intra-subject alignment but on alignment to the template. Hence the impact of the scanner specificity on the spatial normalization performed by Freesurfer may have an impact on the estimation of the geometry of the cortical surface leading to the GI. This consideration motivated the additional repeated measures ANOVA that incorporated scanner effect as a factor while accounting for any discrepancy in the estimation of the geometry of the cortical surface in the residual error.

One might have expected higher ICCs if the scanning sequences had been the same across research centers. In the single subject study, we found that the ICCs differed between the two hemispheres. This is unlikely to be related to cortical asymmetry and it remains unclear whether this might be related to the nature of surface extraction in Freesurfer. This would imply that the sensitivity for detecting group differences might vary by side. Thus it additionally highlights the importance of a reliability study, as suggested in this paper for interpretation of group comparisons, without making any *a priori* assumption of the effect size.

We have not compared other methods of calculating GI with AMC or their reliability in comparison to AMC, as we were primarily interested in establishing reliability for AMC. Our proposed structure for establishing reliability can be applied by those who are interested in comparing the reliability of other methods of calculating GI.

Thus in this study, we have demonstrated that data from multiple scanners and centers can be pooled reliably for estimation of local cortical gyrification, based on absolute mean curvature measurements of extracted cortical surfaces, with modest variance attributable to scanner and sequence. ICC, Spearman Brown Prophecy and Cronbach's Alpha offer an easily implementable method to evaluate variance within the pooled dataset and indicate which data should and should not be included. In this study it has been shown that a reliability study with a modest number of subjects can establish the feasibility of pooling of MR data.

4. Study 2: Co-twin control study to investigate genetic influences on cortical gyrification in monozygotic twins discordant for schizophrenia

4.1 Method

4.1.1 Study design

Monozygotic (MZ) twins who are discordant for disease offer an opportunity to study the effect of genetic influence from the effect of expression of the disease phenotype. Monozygotic co-twin control design compares disease-affected and disease-unaffected co-twins. In this design the unaffected co-twin is the control for the affected co-twin. In addition we also included healthy twins as additional controls. Comparison of affected and unaffected co-twins of the discordant pair with the healthy twins elicits whether both affected and unaffected co-twins have similar abnormalities. As monozygotic twins share 100% genes, their similarity reflects the effect of shared genes and may reflect genetic liability for the disease such as schizophrenia. Comparison of affected twin with the unaffected co-twin elicits any dissimilarity between them, which may reflect either epigenetic differences or an effect of the expression of the disease phenotype.

Our study is the first study to explore cortical gyrification in monozygotic twins discordant for schizophrenia. Data in this study was analyzed as part of EUTwinsS (European Twin Study on Schizophrenia network), and mostly used data acquired by the Schizophrenia Twins and Relatives (STaR) Consortium, which represents the largest structural MR datasets in twins with schizophrenia.

4.1.2 Ethics approval

The participants had been recruited as a part of the Schizophrenia Twin And Relatives (STAR) consortium. The research protocols had been approved by the respective ethics committees and written consent forms had been obtained from the participants. Appropriate consent had been obtained from the collaborating centers.

4.1.3 Demographics

Data in this study was analyzed as part of EUTwinsS (European Twin Study on Schizophrenia network), and mostly used data acquired by the Schizophrenia Twins and Relatives (STaR) Consortium, which represents the largest structural MR datasets in twins with schizophrenia. Both are multi-center collaborations pooling data from twins affected by schizophrenia and healthy twins across multiple European centers.

Data had been collected from 104 monozygotic twins who were recruited at 3 research sites at Jena (J), London (L), and Utrecht (U). 54 monozygotic healthy twins (i.e. 27 pairs same sex healthy MZ twins) and 50 monozygotic twins discordant for schizophrenia (i.e. 25 pairs same sex discordant MZ twins), in the age range of 20-50 years, were included for this study. Mean ages for MZ healthy twin pairs were 29.7 years, 29.6 years and 30.5 years, while the mean ages for MZ discordant twins were 31.1 years, 25.9 years, and 31.2 years at the 3 research sites (J, L and U respectively).

DSM-IV diagnostic criteria were used to establish diagnosis. Any co-morbid neurological, psychiatric or other medical conditions were ruled out for either group. In addition, the healthy MZ twins had no family history for mental illness. Psychopathology was measured by Scale for the Assessment of Positive Symptoms (SAPS) and Scale for the Assessment of Negative Symptoms (SANS) by research centers L and J and by Positive and Negative Symptoms Scale (PANSS) by research center U.

4.1.4 MRI acquisition

3D T1-weighted structural MR images of the entire head were obtained using the following MR parameters from three participating centers:

Table 4: MRI acquisition parameters for co-twin control study for monozygotic twins discordant for schizophrenia

Center	Jena, Germany	London, UK	Utrecht, Netherlands
Magnetic field strength	1.5 T	1.5 T	1.5 T
Scanner	Philips Gyroscan ACS-II scanner	General Electric Signa System scanner	Philips Gyroscan NT scanner
Sequence	3D T1-weighted 3D-FFE scan	3-D T1-weighted coronal spoiled gradient recalled echo (SPGR) scan	3D T1- weighted coronal spoiled gradient echo scan
T_R (msec)	13	35	30
T_E (msec)	5	5	4.6
Flip angle (degrees)	25°	35°	30°
Slices and slice thickness	256 contiguous 1 mm slices	124 contiguous 1.5 mm slices	170 contiguous 1.2 mm slices
In-plane voxel size (mm^2)	1x1	0.781 X 0.781	1 X 1

Abbreviations: T = Tesla, T_R =Repetition Time, T_E = Echo Time, msec = Milliseconds, mm = Millimeter

4.1.5 Measurement of regional cortical gyrification

Cortical surfaces from the MRI scans were obtained by implementing a pre-processing pipeline for surface extraction using FreeSurfer v4.1.0 (http://surfer.nmr.mgh.harvard.edu). This involved registering the T1 scan to standard Talairach space, bias correction, skull stripping, and tissue segmentation prior to surface extraction. Cortical surfaces for each hemisphere were extracted separately. Absolute mean curvature (AMC) was then calculated at each vertex over a 3mm radius in native space of the extracted central surface. Regional GI AMC measures were obtained across cortical regions delineated according to the Desikan atlas (Desikan et al. 2006). AMC measures were smoothed by a nearest neighbor iterative procedure, equivalent to smoothing of 25mm FWHM, and were used for the statistical analyses.

4.1.6 Statistical analysis

Sample characteristics were evaluated using 2 sample T-tests for age, chi-square tests for gender and handedness, paired T tests for within group psychopathology, one-way ANOVA for age of onset, psychopathology scores and duration of illness among patients across research sites.

Paired T-tests was used to compare negative and positive symptoms within subjects for each center separately. To compare psychopathology across the centers, we converted the scores into categories of severity as suggested in a recent review (Lyne et al. 2011). Thus for SANS and SAPS which have a range of scores of 0 to 25, a score of 0 was recoded as absent (bridged scale score of 0), a score of 1 to 8 recoded as mild (bridged scale score of 1), a score of 9 to 17 recoded as moderate (bridged scale score of 2), and a score of 18 to 25 recoded as severe (bridged scale score of 3). For the PANSS positive and negative scores, a score of 7 was recoded as absent (0), a score of 8 to 21 recode as mild (1), a score of 22 to 35 recoded as moderate (2), and a score of 36 to 49 recoded as severe (3). However as such categorization of severity of symptoms across psychopathology scales, have not been validated we did not use this beyond demographic description.

Similarities of regional cortical GI within monozygotic twin pairs was measured by Partial correlations controlled for research center, age, gender and concordance or discordance of handedness. Partial correlations were statistically compared using Fisher's z transformation.

To study whether both unaffected and affected co-twins differ markedly from MZ healthy twins, we used a repeated measures ANCOVA design. Research center, gender, age and concordance or discordance for handedness, were considered as covariates of no interest for the repeated measures ANCOVA. Main effect of group (i.e. MZ healthy versus MZ discordant) indicated if mean cortical GI of discordant twins were markedly different from that of the healthy twins. Twins were randomly assigned Twin 1 or Twin 2 status to randomize for birth order among the MZ healthy twins. In the MZ discordant group, twins with

schizophrenia were assigned the status Twin 2. Group X twin interaction effects indicated whether the differences in mean cortical GI between affected and unaffected co-twins were larger or smaller compared to within-pair differences in healthy twins. This interaction effect was further confirmed by post hoc paired T-test between affected and unaffected twin as described below. Interpretations of results were restricted to main effect of group in the repeated measures ANCOVA and the post hoc paired T-test analysis.

To test whether the Regional GI of the affected twin was significantly different from the unaffected twin, we performed paired T-test within MZ discordant pairs using research center, gender, age and concordance for handedness as covariates of no interest.

We defined a threshold of $p<0.05$ not corrected for multiple comparisons for this hypothesis-led analysis of rather subtle differences. However in order to limit false positives, we have controlled for research center, age, gender and handedness for all comparisons.

Further analyses were conducted for one region with abnormal gyrification in both hemispheres. This region showed abnormal gyrification in the left hemisphere for the main effect of group in the repeated measures ANCOVA. The same region showed abnormal gyrification in the right hemisphere both in the group X twin interaction in the repeated measures ANCOVA and the paired T-test between affected and unaffected co-twins. Thus we wanted to explore the difference in GI in the left and the right hemisphere for this region within each twin category, i.e. control twin 1s, control twin 2s, unaffected twins and affected twins, separately. For this analysis we used paired T-test without any covariates at a statistical threshold of $p<0.05$. All statistical analyses were computed using SPSS 18 Statistics (http://www.spss.com).

4.2 Results

4.2.1 Demographics

The demographic and clinical profile is provided in Table 5.

Table 5: Demographic and clinical profile of MZ healthy twins and discordant twins.

	MZ healthy twins	MZ discordant twins
N	54 (27 twin pairs)	50 (25 twin pairs)
Twin pairs	27	25
Mean age (years)	29.9	29.4
Gender		
Male twin pairs	15	12
Female twin pairs	12	13
Handedness		
Right handed	83%	84%
Discordant handedness	33%	24%
Psychopathology		
SAPS (mean, s.d)		L - 4.4 (2.5) J – 12.8 (7.8)
SANS (mean, s.d)		L – 6 (2.5) J – 27.8 (13.3)
PANSS pos (mean, s.d)		U – 12.8 (4.7)
PANSS neg (mean, s.d)		U – 12 (4.5)
Severity of positive symptoms		One way ANOVA (p=0.002)*
Severity of negative symptoms		One way ANOVA (p=0.000)*
Duration of illness (mean, s.d in years)		L - 5 (2.7) U - 8.5 (5.6) J - 8.7 (7.4)
Age of onset (mean, s.d in years)		L - 20.7 (3.4) U - 21.8 (4.5) J - 22.7 (5.9)

Abbreviations: N: Number of subjects; MZ: Monozygotic; L: London; J: Jena; U: Utrecht; SAPS: Scale for Assessment of Positive Symptoms; SANS: Scale for Assessment of Negative Symptoms; PANSS : Positive and Negative Syndrome Scale; pos : Positive; neg : Negative; s.d : Standard deviation.

Information on age of onset and duration of illness was unavailable for one subject out of 25 MZ twins with schizophrenia. All patients were on medication. Information on psychopathology was not available for 4 subjects among the 25

twins with schizophrenia.

4.2.2 Results for regional gyrification index

Mean regional gyrification index (Regional GI) measurements were higher for the discordant group compared to the normal MZ twins (Fig 7a and Fig 7b).

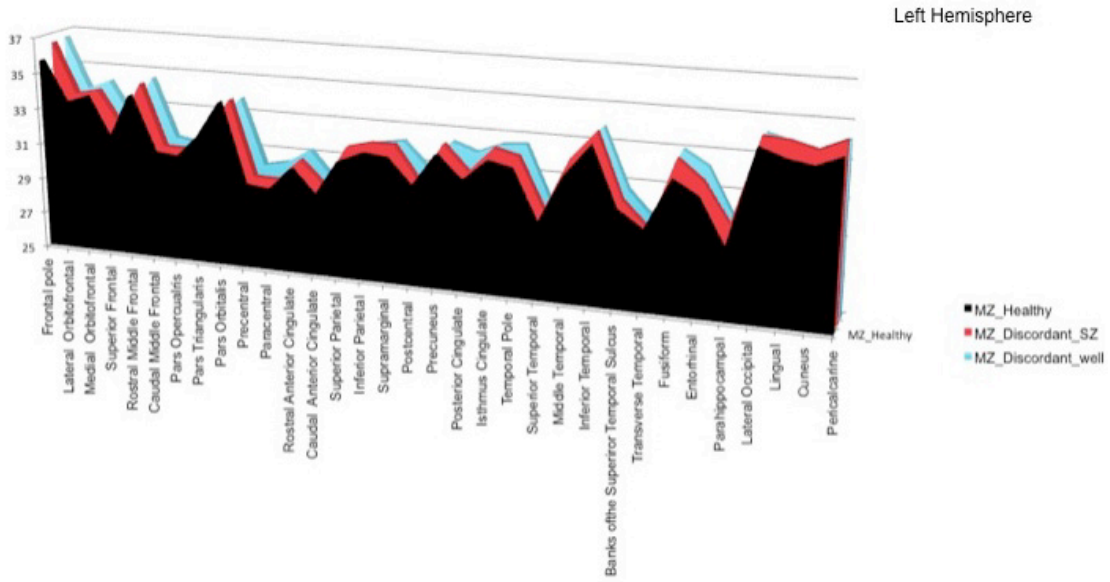

Figure 7a: Mean regional cortical gyrification in the left hemisphere in monozygotic (MZ) healthy twins and twins discordant for schizophrenia.

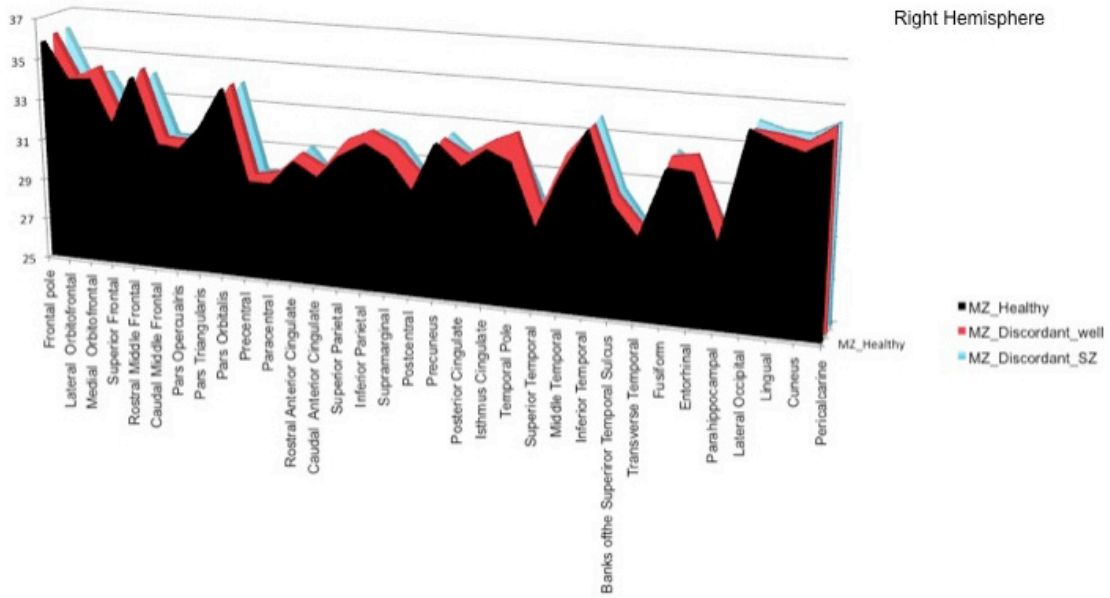

Figure 7b: Mean regional cortical gyrification in the right hemisphere in monozygotic (MZ) healthy twins and twins discordant for schizophrenia.

MZ discordant twins showed higher correlations of Regional GI measurements

compared to the MZ healthy twins in 8 regions in the left hemisphere, and 11 regions in the right hemisphere. This was confirmed by statistical analysis of Fisher's z transformation of the correlation coefficients. Thus in 27% of regions, affected and unaffected twins were more similar to each other than healthy twins (Fig 8).

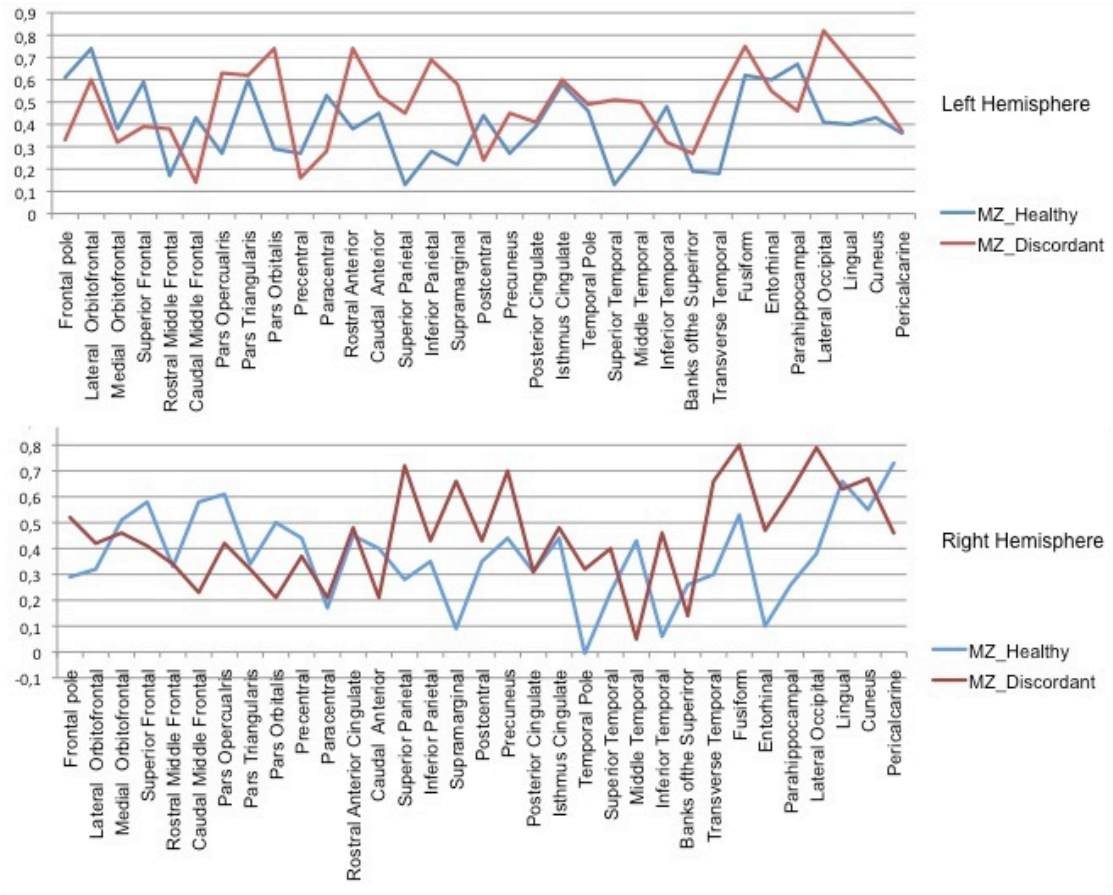

Figure 8: Correlation coefficient of mean regional cortical gyrification among monozygotic (MZ) healthy twin pairs and twins discordant for schizophrenia

Repeated measures ANCOVA showed main effect of group only in the left hemisphere in the frontal pole, middle temporal, fusiform, and parahippocampal gyrus at $p<0.05$. In these areas both the affected and unaffected twins had significantly increased Regional GI compared to the healthy twins (Fig 9).

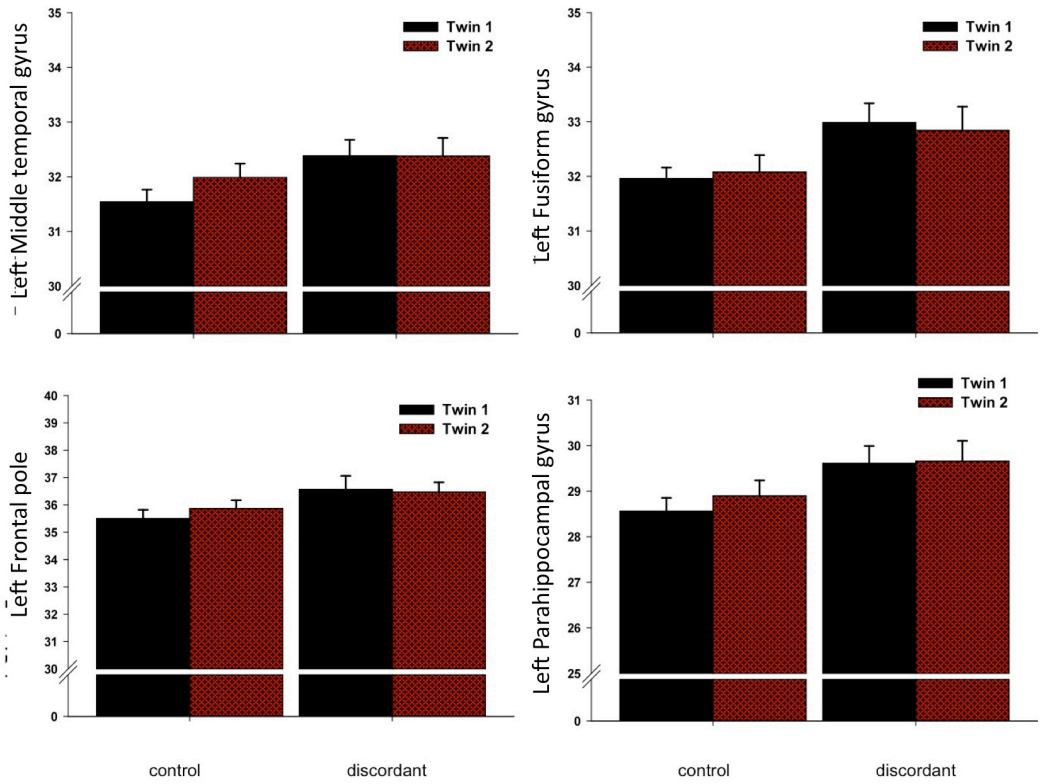

Figure 9: Mean regional cortical gyrification of monozygotic (MZ) healthy twins and MZ discordant twins depicting main effect of group in repeated measures ANCOVA at p <0.05. Center, age, gender and handedness were used as covariates of no interest.

Group X twin interaction was significant in six areas: paracentral and posterior cingulated in the left hemisphere, caudal anterior cingulate, post-central, temporal pole, and parahippocampal gyrus in the right hemisphere at p< 0.05. In these areas the difference in mean Regional GI between unaffected and affected twins was greater than the difference observed within healthy twins. However, post-hoc paired T-test between unaffected and affected co-twins was significant for right superior parietal GI and right parahippocampal gyrus. Hence although the group X twin interaction was significant in six areas, it was only for right parahippocampal GI that the affected twin was significantly different from the unaffected twin and this difference was significantly larger than that observed within healthy twins. Owing to the lenient threshold used in our analyses, we chose to restrict further analysis and interpretation only to the right parahippocampal gyrus as it featured in both the repeated measures ANCOVA as well as paired T-test within the discordant pair. When each group was considered separately, paired T-test comparing right and left parahippocampal

GI was significant only for affected twins at p <0.05 (Fig 10).

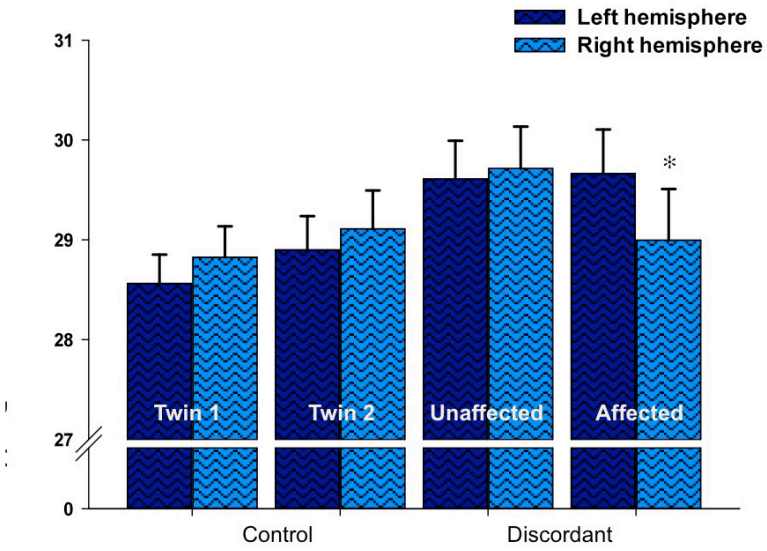

Figure 10: Mean cortical gyrification in left and right parahippocampal gyrus in monozygotic (MZ) twins

4.3 Discussion

Our study demonstrates that monozygotic (MZ) twins discordant for schizophrenia have increased gyrification compared to monozygotic healthy twins. Increased gyrification in both the affected and unaffected co-twins probably reflect an effect of shared genes, especially those genes that are involved in schizophrenia.

We have also shown that in 27% of areas of the brain, the affected and unaffected co-twins have a greater similarity, i.e. higher correlation in Regional GI, which exceeds that seen in healthy twins. This might appear counter-intuitive, as one would expect the affected and unaffected twins to be more dissimilar to each other than healthy twins, as one developed the illness and the other did not. However if genes for schizophrenia play an important deterministic role in the process of gyrification, then discordant twins could be expected to be more similar (in some brain areas). There is some evidence that genes implicated in schizophrenia play a role in neurodevelopment such that

they may affect development of cortical folding (Gregorio et al. 2009, Kalus et al. 1999).

In our study, both affected and unaffected co-twins showed increased fronto-temporal gyrification in the left hemisphere. Gyrification in both affected and unaffected twins was markedly increased in the left frontal pole, middle temporal, fusiform, and parahippocampal gyrus, compared to the healthy MZ twins. Fronto-temporal pathology has often been implicated in schizophrenia (Andreasen et al. 1998, Woodruff et al. 1997, Fletcher et al. 1999) and increased gyrification in fronto-temporal areas have also been reported in previous studies in schizophrenia (Harris et al. 2004a, Vogeley et al. 2001, Wisco et al. 2007). The middle temporal gyrus, parahippocampal gyrus and fusiform gyrus have been found to be abnormal in patients with schizophrenia both in post mortem as well as MRI studies (Highley et al. 1998, Highley et al. 2002, McDonald et al. 2000, Takahashi et al. 2006). As schizophrenia genes may play a role in cortical folding, it is possible that inheriting schizophrenia genes leads to altered gyrification. Both the affected and unaffected twins had similar magnitude of fronto-temporal gyrification. This might imply that increased fronto-temporal gyrification in frontal pole, middle temporal, fusiform and parahippocampal regions in the left hemisphere might be important but not sufficient to develop schizophrenia.

Our study also demonstrates differences in gyrification between affected and unaffected co-twins. Compared to the healthy MZ twins, MZ discordant twins had larger differences in GI between each other in six regions, such as paracentral and posterior cingulate in the left hemisphere, and caudal anterior cingulate, post-central, temporal pole, and parahippocampal gyrus in the right hemisphere. Although these differences were greater than that seen within healthy MZ twins, they failed to reach a statistical significance on post-hoc comparisons apart from the right parahippocampal GI. Thus the affected co-twin had significantly different right parahippocampal GI compared to the unaffected co-twin.

Although unaffected and affected co-twins significantly differed from each other for right parahippocampal GI, when considered together as a group i.e. as MZ discordant twins, they did not significantly differ from the MZ healthy twins for right parahippocampal GI. However both MZ discordant twins had significantly increased left parahippocampal GI compared to the MZ healthy twins. Thus both affected and unaffected twins had similar parahippocampal GI in the left hemisphere but they differed in the right hemisphere. This suggested that parahippocampal gyrification in each hemisphere might be different in the affected twins. This was confirmed by a significant difference between the left and right parahippocampal GI that was found only among the affected twins (see Fig 10). This discrepancy between the left and right parahippocampal GI seemed to differentiate the affected twins from their unaffected co-twins, as well as other healthy twins. This indicated that dissimilarity of left and right parahippocampal GI may play an important role in the pathophysiology of schizophrenia.

It has been suggested that the genetic liability to schizophrenia may be mediated through structural alteration of medial temporal lobe areas and a tendency for prefrontal cortex dysfunction (Lawrie et al. 2008, Seidman et al. 2003). Although the nature of such a genetic liability is unclear, the fronto-temporal areas implicated in our study subserve important neuropsychological functions that may underlie etiopathogenesis of schizophrenia.

Parahippocampal areas subserve the formation of long term memories associating information from various sensory domains, recognition of faces, objects and emotional stimuli, while the parahippocampal gyrus along with the fusiform gyrus are important in processing of emotional expression (Squire et al. 2004, Dougal et al. 2007, Pourtois et al. 2010). Post mortem studies in patients with schizophrenia have demonstrated reduced left parahippocampal and fusiform volume(McDonald et al. 2000), thinner parahippocampal gyrus (Brown et al. 1986) and reduced white matter in the parahippocampal gyrus (Colter et al. 1987). In addition, temporal lobe epilepsy with psychotic features has been associated with decreased left parahippocampal grey matter on MRI (Sundram et al. 2010). Left parahippocampal dysfunction has also been implicated in

auditory hallucinations in schizophrenia, a key symptom of the disorder (Diederen et al. 2010). Reduced left parahippocampal grey matter has been reported in ultra high-risk individuals who later experience a transition to psychosis (Mechelli et al. 2011). In our study the affected twin had a significantly different right parahippocampal GI from the unaffected co-twin. Gyrification of this region has been found to be abnormal in first episode schizophrenia patients (Schultz et al. 2010) while grey matter volume changes in this region have been implicated in the onset of psychosis in individuals at high risk of developing schizophrenia (Job et al. 2005). Also, glioma in the right parahippocampus has been associated with psychotic symptoms (Acioly et al. 2010).

Fusiform gyral abnormalities are also known in schizophrenia in both post mortem studies (McDonald et al. 2000, Di Rosa et al. 2009) and MRI studies(Lee et al. 2002, Takahashi et al. 2011). Abnormalities in fusiform gyrus in schizophrenia have been associated with deficits in face processing (Onitsuka et al. 2003, Quintana et al. 2003, Mancini-Marie et al. 2004, Pinkham et al. 2005) and facial memory (Nestor et al. 2007, Walther et al. 2009). However, there is comparably little information on the role of this area in the established network models of the disease.

The middle temporal gyrus is an auditory association area that plays a role in perceptual and conceptual processing of sound (Trumpp et al. 2012). Post-mortem studies in schizophrenia have found decreased volume of left temporal lobe (Highley et al. 1999). Reduction in middle temporal gyral volumes has been reported in schizophrenia (Kuroki et al. 2006, Onitsuka et al. 2004, Takahashi et al. 2006, Takahashi et al. 2011).

Although prefrontal structural deficits are widely reported in schizophrenia, limited information exists in particular relation to the frontal pole. The frontal pole is the single largest cytoarchitectonic region of prefrontal cortex (Ramnani und Owen 2004). It projects to superior temporal gyrus, amygdala and anterior cingulate (Petrides und Pandya 2007) and has a protracted developmental trajectory (Dumontheil et al. 2008). It plays an important role in executive

functions especially as a gateway that biases the priority of information from environment and the information from self generated or maintained representations (Burgess et al. 2007b, Burgess et al. 2007a) Lack of age-associated decreases in frontal pole have been reported in schizophrenia(John et al. 2009), indicating a possible deviation of maturational processes. This is consistent with post mortem findings in schizophrenia.of reduced interneurons (Benes et al. 1991, Beasley und Reynolds 1997) and increased neuropil (Vogeley et al. 2003) in frontal pole.

Thus in our study alteration of parahippocampal, fusiform, middle temporal and frontal pole gyrification in the left hemisphere in both affected and unaffected twins concur with widely reported deficits in these areas in schizophrenia.

The gyrification abnormalities found in our study could have a neurodevelopmental origin. One of the earliest post-mortem studies exploring gyrification in schizophrenia had found ‘abnormal sulco-gyral pattern of the temporal lobe’ and had reported that ‘disturbed structure’ of specific layers of cytoarchitecture in the parahippocampal gyrus suggested a ‘*disturbance of neuronal migration in a later phase of cortical development*’ (Jakob und Beckmann 1986).

As myelination and therefore possibly gyrification of some of the implicated areas continues until late in development, it is possible that early neurodevelopmental abnormalities render it more susceptible to later insults. The parahippocampal gyrus is an important part of the limbic cortex, receiving input from sensory association areas and then transmitting it to the entorhinal cortex. The entorhinal cortex in turn sends input to the subiculum of the hippocampal region (Burwell 2000). During development, the parahippocampal region undergoes a rapid growth between 16 to 18 weeks (Sitoh und Tien 1997). Myelination of the parahippocampal gyrus may continue until the second decade (Benes 1989). Myelination of subiculum also occurs until the second decade (Benes 1989), but may continue for much longer into adulthood (Benes et al. 1994). It is conceivable that these changes may contribute to changes in gyrification of medial temporal lobe structures.

Gyrification shows considerable regional variability even among healthy twins. One of the early studies in healthy twins exploring sulci found that left hemisphere sulci were less variable, and that twin similarity was greatest for deep sulci - usually the primary sulci (Lohmann et al. 1999). The same pattern of similarity was elicited among healthy twin pairs concordant and discordant for handedness, although twins concordant for right handedness were more similar for shallow sulci (presumably later developing sulci), than the discordant pairs (Lohmann et al. 1999). Assessment of shape of central sulcus has shown more similarity for healthy monozygotic (MZ) twins compared to healthy dizygotic (DZ) twins (Le Goualher et al. 2000). A recent analysis of sulcal patterns demonstrated significant similarity in frontal, temporal, parietal and occipital lobes in each hemisphere among healthy monozygotic (MZ) twins (Im et al. 2011). However, previous studies investigating gyral patterns have argued in favor of considerable influence of non-genetic factors for gyrification in healthy individuals (Bartley et al. 1997, Mohr et al. 2004). It might be possible that schizophrenia genes affect the process of gyrification such that it is less susceptible to later non-genetic influences. This could lead to less variation in gyrification among monozygotic discordant twins both of whom have the schizophrenia genes, compared to variation in gyrification seen among monozygotic healthy twins.

There is some preliminary evidence that genes implicated in schizophrenia are involved in neurodevelopment and may also be associated with altered brain morphology in schizophrenia. A recent study has reported associations between the RELN gene and ventricular size (Gregorio et al. 2009). The RELN gene encodes for a protease that guides neurons in the developing brain as well as influencing neurotransmission and synaptic plasticity in adults. Cajal-Retzius cells that secrete Reelin are reported to have altered distribution in the prefrontal cortex in post-mortem studies in schizophrenia (Kalus et al. 1999). p73, a protein expressed by the Cajal-Retzius cells, has been found to play a significant role in cortical folding, especially in the medial temporal lobe in mice (Meyer et al. 2004). Protocadherin 12, also implicated in schizophrenia, is a cell

adhesion molecule involved in axonal guidance and synaptic specificity that has been reported to affect cortical folding (Gregorio et al. 2009).

It cannot be deduced at this stage whether the gyrification abnormalities in the different regions found in our study are isolated occurrences or related to each other. Neurons and axons in the developing limbic system, such as prefrontal and perirhinal cortices, express the limbic-system-associated membrane protein (LAMP) (Horton und Levitt 1988). Neurons seem to have a critical period following which LAMP is expressed, and neurons are committed to become limbic cortex neurons (Barbe und Levitt 1991). Thus it is possible that development of the regions implicated in our study i.e. medial temporal lobe structures and frontal pole, may be inter-related. Overexpression of LAMP has been found in patients with schizophrenia (Iwamoto et al. 2005, Behan et al. 2009). LAMP has also been found to be overexpressed in dorsolateral prefrontal cortex in both patients with schizophrenia and bipolar disorder (Behan et al. 2009).

It is not entirely clear whether the various structures implicated in our study increase the vulnerability of developing schizophrenia, or they merely increase the likelihood of neuropsychological deficits thereby increasing the vulnerability of certain symptoms. As our study is of a cross-sectional co-twin control design, we do not know whether the abnormalities seen are specific to schizophrenia alone or might be found in other illnesses. It is also not possible to comment whether dissimilarity in left and right hemispheric findings for parahippocampal gyrus represent separate abnormalities or a failure of compensation for abnormalities in one hemisphere.

Our study has demonstrated that schizophrenia genes probably influence cortical gyrification, such that increased left hemispheric fronto-temporal GI involving frontal pole, middle temporal, fusiform and parahippocampal gyrus, may be a structural phenotype indicating a genetic vulnerability to schizophrenia. Our study has also demonstrated that parahippocampal GI may have an important role in the etiopathogenesis of schizophrenia. The pattern of gyrification in the parahippocampal region differed in the unaffected and

affected twins such that the twins who remained well had bilaterally increased parahippocampal GI while the twins who developed schizophrenia had divergent development of parahippocampal gyri. Thus dissimilar left and right parahippocampal GI may be a structural phenotype for the expression of the disease phenotype in schizophrenia.

Limitations

One of the limitations could be the restriction to cortical subdivisions according to automatic parcellation, as this limits us to evaluate other anatomically relevant areas, such as dorsolateral prefrontal cortex. Atlases specifically exploring the parahippocampal region may further improve the accuracy in future studies.

Furthermore, It has been demonstrated in one study that gyrification may be slower in twins compared to singletons (Dubois et al. 2008). Hence it is possible that a selection bias is inherent in twin studies measuring gyrification.

Another limitation of our study is the problem of multiple statistical comparisons. While we refrained from corrections like the Bonferroni method, which might have been too conservative, we did correct for a number of confounders in our statistical analyses. Correcting for confounders as well as relevant post hoc analyses improve the credibility to the results despite the lack of multiple corrections.

The present study being of a cross-sectional design does not provide definitive information regarding temporal causative association between gyrification abnormalities and schizophrenia. Thus the inferences derived from our study require replication in a longitudinal study in a cohort of individuals at-risk for schizophrenia.

5. Conclusion

Schizophrenia is a syndrome-based disease entity. As a disease concept, it encompasses heterogeneity in both presentations as well as outcomes. Understanding its etiology therefore rests on finding structural, functional, biochemical and physiological abnormalities, which may represent vulnerability to a specific group of symptoms, vulnerability to development of the disease or they may indicate expression of disease phenotype and disease progression. There is an implicit assumption that vulnerability to a group of symptoms that are characteristic of schizophrenia essentially indicates vulnerability to develop schizophrenia.

Twin studies offer a unique advantage to investigate the genetic etiology of a disease. Gottesman and Shields (Gottesman et al. 1972) state in one of the earliest overviews of twin studies in schizophrenia, that genetic contribution is necessary but not sufficient to develop schizophrenia; that genetic contribution may or may not be the largest contributor of variance of liability to schizophrenia; that if the specific genetic etiology is missing then it may prevent other factors (genetic or environmental or both) to cause schizophrenia. Over the last four decades twin studies as well as large genome wide association studies in schizophrenia have identified many possible genetic etiological factors although it still remains unclear which of these factors or their combinations are essential for the development of schizophrenia.

Cortical gyrification provides a useful insight into neurodevelopment as it is established long before adulthood and is considered not to be influenced by post-developmental events (Weinberger et al. 1992). Abnormalities of cortical gyrification are often observed in schizophrenia (Kulynych et al. 1997, Sallet et al. 2003, White et al. 2003, Harris et al. 2004a, Harris et al. 2004b, Falkai et al. 2007, Harris et al. 2007, Penttila et al. 2008). Research work presented in this thesis is the first study investigating cortical gyrification in monozygotic (MZ) twins discordant for schizophrenia. Twin data from three research centers were combined and reliability of the measure of gyrification across different research centers was first established prior to the co-twin control study. Our study

showed that left fronto-temporal gyrification was increased in both affected and unaffected MZ twins compared to the healthy MZ twins. This implied that a strong genetic influence of shared schizophrenia genes altered cortical gyrification in both affected and unaffected discordant twins. Unlike the unaffected co-twin, the affected co-twin had significantly dissimilar parahippocampal gyrification in left and right hemispheres. Thus dissimilarity in parahippocampal gyrification may represent a structural phenotype for expression of the disease phenotype of schizophrenia.

It is important to consider whether the structural abnormality being measured i.e. cortical gyrification, relate to the syndrome of schizophrenia, or to a symptom. MZ twins concordant for schizophrenia have been reported to have similar prodromal symptoms and onset of schizophrenia (Kendler und Tsuang 1982). Concordant MZ twins have shown schizophrenia subtype similarities (Gottesman 1968, Onstad et al. 1991), which might indicate that when the twins develop schizophrenia, they are more likely to manifest similar symptomatology. Our study cannot differentiate whether abnormalities in cortical gyrification represent vulnerability to develop certain symptoms or schizophrenia per se. It is possible that multiple vulnerability factors interact to lead to development of schizophrenia and that structural abnormalities may be one of such factors.

Differences between MZ discordant twins are often attributed to non-genetic influences. An epigenetic basis of gyrification and its subsequent role in the development of schizophrenia is currently not known. The results of our study could suggest that shared schizophrenia genes affect fronto-temporal gyrification in a similar manner in both affected and unaffected twins while a possible epigenetic mechanism may underlie dissimilarity in the development of parahippocampal gyrification that may play a role in the development of schizophrenia.

Our results may provide an important structural phenotype for genetic and epigenetic studies aimed at an etiological understanding of schizophrenia and also help to clarify the specificity of cortical gyrification abnormalities in schizophrenia.

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Ehrenwörtliche Erklärung

Hiermit erkläre ich, dass mir die Promotionsordnung der Medizinischen Fakultät der Friedrich-Schiller- Universität bekannt ist,

ich die Dissertation selbst angefertigt habe und alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen in meiner Arbeit angegeben sind,

mich folgende Personen bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskripts unterstützt haben:.

- Prof. Dr. Heinrich Sauer (Jena), Prof. Sir Robin Murray (London), Prof. Hilleke Hulshoff Pol (Utrecht), Prof. Rene S. Kahn (Utrecht), Prof. M. Weisbrod (Heidelberg) (Auswahl und Bereitstellung von MRT-Daten)
- Dr. Christian Gaser (Jena), Dr. Igor Nenadic (Jena) (Datenauswertung)

die Hilfe eines Promotionsberaters nicht in Anspruch genommen wurde und dass Dritte weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen,

dass ich die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe und

dass ich die gleiche, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung nicht bei einer anderen Hochschule als Dissertation eingereicht habe.

Jena, den 21.06.2012 Raka Maitra, MBBS, DPM

Curriculum Vitae

	Name	:	Raka Maitra		
	Date of Birth	:	18th March 1976	Gender	Female
	Nationality	:	Indian		
	Personal Address	:	68, Broadmead road, Woodford Green Essex IG8 0AZ UK		
	Telephone No. (home)	:	+44 208 5044197		
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	Education	:	Degree	Institution	Year
			Diploma in Psychological Medicine (DPM)	Christian Medical College, Vellore, Tamil nadu, India	2001-2003
			Bachelor of Medicine and Surgery (MBBS)	Lady Hardinge Medical College, New Delhi, India	1994-1998
	Other training		4th International Summer School in Biomedical Engineering - Brain connectivity and information transfer Structural connectivity	Max Planck Institute, Leipzig, Germany	2009
			SGDP Twin Modelling Summer School	Institute of Psychiatry, London	2009
			Visiting academic for training in neuroimaging	Howard Florey Institute, University of Melbourne, Australia	2005-2006
			Neuroscience coursework	National Brain Research Centre, Manesar, Haryana, India	2004-2005
	Awards and fellowships		First prize poster presentation	Higher trainee conference, East of England Deanery, UK	2012
			Marie Curie Fellowship for Early stage researcher in Neuroimaging,	This fellowship is part of EUTwinsS, EU-funded Research Training Network	2008-2010

				under FP6 EUTwinsS node: Jena, Germany	
			Highest scorer in neuroscience coursework with Distinction in each subject	National Brain Research Centre, India	2005
			Florence Nichols prize for best DPM student of the year.	Dept of Psychiatry, Christian Medical College, Vellore, India	2003
			Highest scorer in DPM part II exam	Dept of Psychiatry, Christian Medical College, Vellore, India	2003
			Distinction in DPM part I exam	Dept of Psychiatry, Christian Medical College, Vellore, India	2002
			Annie McKenzie prize for student corporate life (in the capacity of President, Student Union)	Lady Hardinge Medical College, New Delhi, India	1999
	Other qualifications		MRCPsych Paper 3	Passed.	2012
			MRCPsych Paper 2	Passed.	2009
			MRCPsych Paper 1	Passed.	2009
			GRE General test	Verbal 630/800; Math 720/800; Analytical 5.5/6	2007
			PLAB	Passed Part I in 2004 and Passed Part II in 2006.	2006
			IELTS	Score 8.5 (Max score 9.0)	2004
	Membership in Professional Societies		General Medical Council (UK) (reg no: 6100247) Full Registration. Medical Council of India (Registration No: 20226 International Early Psychosis Association (IEPA) (MAIR06IN) Society for international Schizophrenia Research (SIRS) (MEM no 525) European Forum for Good Clinical Practice (EFGCP)		

	Publications	1	Nenadic I, Maitra R, Scherpiet S, Gaser C, Schultz CC, Schachtzabel C, Smesny S, Reichenbach JR, Treutlein J, Mühleisen TW, Deufel T, Cichon S, Rietschel M, Nöthen MM, Sauer H, Schlösser RG. Glutamate receptor delta 1 (GRID1) genetic variation and brain structure in schizophrenia. J Psychiatr Res. 2012 Dec;46(12):1531-9.
		2	Mingoia G, Wagner G, Langbein K, Maitra R, Smesny S, Dietzek M, Burmeister HP, Reichenbach JR, Schlösser RG, Gaser C, Sauer H, Nenadic I. Default mode network activity in schizophrenia studied at resting state using probabilistic ICA. Schizophr Res. 2012 Jul;138(2-3):143-9.
		3	Nenadic I, Langbein K, Weisbrod M, Maitra R, Rzanny R, Gussew A, Reichenbach JR, Sauer H, Smesny S. 31P-MR spectroscopy in monozygotic twins discordant for schizophrenia or schizoaffective disorder. Schizophr Res. 2012 Feb;134(2-3):296-7.
		4	Smith KM, Mecoli MD, Altaye M, Komlos M, Maitra R, Eaton KP, Egelhoff JC, Holland SK Morphometric Differences in the Heschl's Gyrus of Hearing Impaired and Normal Hearing Infants. Cereb Cortex. 2010 Sep 13. [Epub ahead of print]
		5	Maitra et al,"Structural Variability of Human Auditory Cortices: Does it Matter?". Article 04 Advances in Developmental Neuroscience and Imaging , 2009, edited by Hari Eswaran and Nandini Chatterjee Singh, Pub Anamaya Publishers,New Delhi, India
	Oral presentations	1	East Of England Deanery Conference 2nd Nov 2012 –First prize Nenadic, Maitra et al Glutamate receptor delta 1 (GRID1) genetic variation and brain structure in schizophrenia
		2	Marie Curie Fellows presentation Closing Symposium EUTwinsS , Institute of Psychiatry, London 2010
			Marie Curie Fellows presentation EUTwinsS UMC Utrecht 2009
		3	Oral presentation at ICOSR (International Congress on Schizophrenis Research) San Diego, 2009 Raka Maitra et al, Interleukin-1beta gene modulates prefrontal and temporal brain structure in schizophrenia
		4	Oral presentation at WFSBP, (World Federation of Societies of Biological Psychiatry) Paris, 2009 Maitra et al, Cortical gyrification in twins discordant for Schizophrenia
		5	Oral presentation at ECSR (European Conference on schizophrenia Research), Berlin, 2009 Maitra et al,Cortical gyrification in monozygotic twins discordant for Schizophrenia

		6	'Early Investigator presentation' at fMRI Experience 8, Melbourne, 2006 Raka Maitra et al, Does the anatomy have a say? Comparing group level analysis with region of interest based analysis for fMRI data	
	Posters in conferences	1	Nenadic, Maitra et al, Effects of Glutamate Receptor Delta 1 (GRID1) Genetic Variation on Brain Structure in Schizophrenia: A VBM Study	XXth World Congress of Psychiatric Genetics, Hamburg, Germany,2012
		2	Maitra et al, Reliability of curvature based estimation of local cortical gyrification : a multicentre study	Human Brain Mapping Conference, Barcelona, Spain, 2010
		3	Nenadic, Maitra et al, Glutamate receptor delta 1 (GRID1) genetic variation and brain structure in schizophrenia	Schizophrenia International Research Society conference, Florence, Italy, 2010
		4	Nenadic et al, Gyrification in twins discordant for schizophrenia	Schizophrenia International Research Society conference, Florence, Italy, 2010
		5	Maitra et al, Heritability of cortical gyrification and implications for schizophrenia: preliminary results from the STAR / EUTwinsS studies	Schizophrenia International Research Society conference, Florence, Italy, 2010
		6	Maria Gavrilescu et al, A dissociation of structure and function in the auditory cortex of patients with Schizophrenia	Human Brain Mapping Conference, Chicago, USA, 2007
		7	Maitra et al, Structural variability of auditory cortices	A Joint Indo-US Symposium/ Workshop on Developmental Neuroscience and Imaging, NBRC, India, 2007
		8	Raka Maitra et al, Relationship of auditory hallucination with volume of auditory cortex of patients with schizophrenia	3rd International NBRC conference, India, 2006

		9	Anusha Sritharan et al, Abnormal inter-hemispheric white matter integrity in psychotic patients with auditory hallucinations: a DTI study	Human Brain Mapping Conference, Florence, Italy, 2006

	Research activities	:	
	From/To		Feb 2008 to Jan 2011, Aug2011-June2012
	Institution		Department of Psychiatry and Psychotherapy, Friedrich-Schiller-University of Jena, Jena, Germany
	Position held and description		Marie Curie Fellow- Early Stage Researcher in Neuroimaging EUTwinsS Research Training Network EUTwinsS (European Twin Study Network on Schizophrenia) is an international study of twins affected by schizophrenia in the world and involves a collaboration between nine mental health research centres in six European countries: Germany, the UK, The Netherlands, Spain, Hungary and Switzerland. My research focused on measuring cortical gyrification in normal twin volunteers and Discordant twins with Schizophrenia. I used Freesurfer software for this work. I was also involved in another project that is exploring genetic contribution to grey matter changes in patients with schizophrenia compared with healthy normal volunteers using Voxel Based morphometry.
	From/To		August 2006- Jan 2008
	Institution		National Brain Research Centre, Manesar, India
	Position held and description		Senior Research fellow Designed an fMRI paradigm using EPRIME investigating central executive function in working memory. Have collected fMRI data from volunteers and analysed the preliminary data using SPM 2 and also Brain Voyager.
	From/To		Oct 2005- June 2006
	Institution		Neuroimaging and neuroinformatics lab, Howard Florey Institute, University of Melbourne, Australia

	Position held and description		Visiting PhD student Collaborated on the Central Auditory Processing in Auditory Hallucinations (CAPAH) Project. The CAPAH project involved Magnetic Resonance (MR) imaging measurements in a group of patients with schizophrenia who experienced auditory hallucinations, in comparison to a group of non-hallucinating schizophrenic patients and to a group of control subjects. Trained in image analysis procedures such as region of interest analysis (ROI) of structural MR imaging by manual tracing using ANALYZE vs7.5; group level fMRI data analysis using SPM 2; Voxel based morphometry (VBM) analysis of structural MR as well as diffusion MR images.
	From/To		August 2004 –July 2006
	Institution		National Brain Research Centre, Manesar, India
	Position held and description		Junior Research fellow Completed one year compulsory coursework in Neuroscience. The coursework comprised of the following subjects: neuroanatomy, developmental neuroscience, neurochemistry, clinical neuroscience, cellular and molecular neuroscience, systems neuroscience, cognitive neuroscience, biophysics, biostatistics, and computational neuroscience. The coursework was a prerequisite for registering for PhD. Each subject was a module of one month duration. Module consisted of lectures, written assignments, review of research papers and oral presentations. Each module ended with a written assessment, which contributed to internal marks towards the two semester exams.
	Software skills		Neuroimaging: ANALYZE, SPM, FSL, MRIcro, EPRIME, Freesurfer Statistics : SPSS, Microsoft Excel.

	Clinical experience	:	
	From/To		Aug 2012 - present
	Deanery		East of England
	Hospital		SEPT South Essex Partnership Trust Dept of Psychotherapy, Basildon Mental Health Unit, Basildon Mental Health Unit Nethermayne, Basildon, Essex SS16 5NL Grays Child and family Consultation Services, 62 Maidstone Road, Grays, Essex RM17 6NF
	Position held and description		CT3 Trainee in Psychotherapy and CAMHS
	From/To		Feb 2011-Aug 2011

	Deanery		East of England
	Hospital		CPFT Cambridgeshire and Peterborough NHS Foundation Trust Learning Disability Partnership, Block 7, Ida Darwin Hospital, Cambridge, CB21 5EE
	Position held and description		Specialty Registrar, LAT ST3 in Learning Disability Clinical responsibilities involved management of inpatients at IASS Unit, Ida Darwin Hospital, assessment and follow up of outpatients. Nature of work involved working closely with multidisciplinary teams comprising of nurses, social workers, and occupational therapists. Management of patients essentially adopted a multidisciplinary approach. Tier 1 rota for junior doctors involved On Call duties covering Fulbourn hospital, Ida Darwin hospital, A&E at Addenbrooke's Hospital and medical and surgical wards at Addenbrooke's hospital. Academic commitments within the post involved attending lectures, attending MRCPsych course, attending psychotherapy group called the Balint group, participating in Journal clubs and Case Presentations. E-Portfolio was maintained for all Work Place Based Assessments.
	From/To		Mar 2001-Mar 2003
	Hospital		Dept of Psychiatry, Mental Health Centre, Christian medical College, Vellore, Tamil Nadu, India
	Position held and description		Post Graduate Registrar Underwent two year training programme in psychiatry and was awarded Diploma in Psychological Medicine. The course was accredited by Medical Council of India and examinations were conducted by the state university. The course comprised of two examinations. First year examination tested neuroanatomy, neurophysiology, neurochemistry, general psychology, abnormal psychology and social psychology. Second year examination tested Psychiatry and Neurology. Trainees are offered placements throughout the academic year with the main clinical specialty units including 18 months in general adult psychiatry, 4 months in child and adolescent psychiatry and learning disability, 3 month rotation in Neurology. Trainees attended once a week out patient clinics for liaison psychiatry throughout the training period.

			Trainees were expected to carry out full time clinical responsibilities. Training was provided in interviewing and communication skills, including psychotherapeutic techniques. Logbook was maintained with 75 inpatient treatment under supervision and 350 new patient workup on an out-patient basis. Each work up and treatment was supervised. Trained in emergency management, out patient management, in patient management as well as in eclectic psychotherapeutic intervention. Weekly journal clubs were held and trainees were also required to give regular seminars. Research work was not included in the curriculum.
	From/To		Aug 2000-Feb 2001
	Hospital		Dept of Psychiatry, Mental Health Centre, Christian medical College, Vellore, Tamil Nadu, India
	Position held and description		Non PG Registrar Full time clinical responsibilities and academic activities as described above for a PG Registrar.
	From/To		Jan 1999-Dec1999
	Hospital		Lady Hardinge Medical College, New Delhi, India
	Position held and description		Internship Successfully completed one year compulsory internship, training in skills and taking up responsibilities as per the requirements of Medical Council of India.

	Teaching experience	:	Year	Institution	Responsibility
	Teaching		2010	Friedrich-Schller University	Undergraduate students. One lecture for the course Computational neuroscience CNS016
	Supervision		2009-2011	Friedrich-Schller University	Project assistants and doctoral students

	Community work	:	
	Public awareness talks		1. ‘What we must remember about forgetting-the Alzheimer’s story’ on World Alzheimer’s day celebrations organized by the Delhi Chapter of Alzheimer's & Related Disorders Society of India (ARDSI) at India international Centre, 21 Sept 2005. 2.’Strengthening memory capacity and

			understanding memory loss' during World Alzheimer's day celebration organised by Development, welfare and research foundation (DWARF) at India Habitat centre, 22 Sept 2005 3. 'Progress and research on Alzheimer's disease' during Alzheimer's disease centenary celebrations organised by the Delhi Chapter of Alzheimer's & Related Disorders Society of India (ARDSI) at India Habitat Centre, 25 Sept 2006.
	Extra curricular activities		Medical Committee Member, NBRC 2004 – 2007 Library Committee Member, NBRC 2006 - 2007 President Students Union, LHMC 1998-1999 Editor and Literary Secretary, LHMC 1996-1997 Head girl, Lady Irwin School 1992-1993

I, the undersigned, confirm that
To the best of my knowledge, this CV correctly describes myself, my qualifications, and my experience, and

Signature	Date of signing	Day/Month/Year
Raka Maitra		01 / July / 2013