Effects of obstetric complications on brain morphology in schizophrenia

four MRI studies

Unn Kristin Hansen Haukvik, MD

Department of Clinical Medicine, section Vinderen
Faculty of Medicine
University of Oslo

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Abstract

Magnetic resonance imaging (MRI) studies have shown the brains of schizophrenia patients to have smaller hippocampi, larger ventricles, and reduced cortical thickness and regional brain volumes, as compared to the brains of mentally healthy subjects. The abnormal brain morphology may reflect subtle deviances from normal brain development. Early adverse somatic events, in the current thesis studied in the form of obstetric complications (OCs), in the pre-, peri-, or postnatal periods can cause or influence a deviant neurodevelopment. In scientific studies, it has been demonstrated that occurrence of OCs increase the risk of schizophrenia. Moreover, results from experimental animal studies demonstrate that different OCs cause both abnormal changes in brain morphology and behaviour that parallel what is observed in schizophrenia. In humans, OCs have been related to smaller hippocampi, larger lateral ventricles, and reduced cortical volume in schizophrenia patients with as compared to patients without a history of OCs. Taken together, these findings suggest that early somatic trauma such as OCs may exert an influence on neurodevelopment, detectable in the brain decades later.

The main aim of this PhD thesis was to investigate the relationship between a history of (OCs) and brain morphology in patients with schizophrenia. The subaims were to study 1) if such a putative effect could explain some of the differences in brain morphology observed between schizophrenia patients and healthy controls, and 2) if the effect of OCs on hippocampal volume, if demonstrated, is modified by genetic variation (allele variation in single nucleotide polymorphisms).

The subject sample included in the current four studies comprises 54 schizophrenia patients and 54 healthy control subjects. They all underwent clinical examination, genotyping, and MRI scanning at the Karolinska Institutet and Karolinska University Hospital in Stockholm, Sweden. Automated software tools were used to obtain measures of basal ganglia nuclei and hippocampal volumes, cortical thickness, and cortical folding patterns. Information on OCs was independently collected from original birth records.

The main findings were that OCs are not associated with basal ganglia volumes (study I) or cortical thickness (study II), but significantly associated with reduced cortical folding in the left pars triangularis (Broca's area) (study III) and with altered hippocampal volumes (study IV). The effect of OCs on hippocampal volume appeared to be modulated by allele variation in the hypoxia-regulated *GRM3* gene (study IV). Furthermore, schizophrenia patients did not differ from healthy control subjects with respect to the rate or severity of OCs per se; the effects of OCs on basal ganglia

volumes, cortical thickness, and cortical gyrification; or the gene*OCs interaction effect on hippocampal volume.

In conclusion, while some brain structures (cortical thickness, basal ganglia volumes) were unaffected by a history OCs, OCs influenced other aspects of brain morphology (hippocampal volume, cortical folding) in the same way in both patients with schizophrenia and healthy controls. The differences in brain morphology found between schizophrenia patients and healthy controls were not effects of OCs. Genetic variation may modulate the effect of OCs on hippocampal volume.

List of studies

Study I
Haukvik UK, McNeil T, Nesvåg R, Söderman E, Jönsson EG, Agartz I. No effect of obstetric complications on basal ganglia volumes in schizophrenia. Progress in Neuropsychopharmacology and Biological Psychiatry 2010;34:619-623

Study II
Haukvik UK, Lawyer G, Bjerkan PS, Hartberg CB, Jönsson EG, McNeil T, Agartz I. Cerebral cortical thickness and a history of obstetric complications in schizophrenia. Journal of Psychiatric Research 2009; 43:1287-1293.

Study III
Haukvik UK, Schaer M, Nesvåg R, McNeil T, Hartberg CB, Jönsson E, Eliez S, Agartz I. Cortical folding in Broca's area relates to obstetric complications in schizophrenia patients and healthy controls. Submitted.

Study IV
Haukvik UK, Saetre P, McNeil T, Bjerkan PS, Andreassen OA, Werge T, Jönsson EG, Agartz I. An exploratory model for GxE interaction on hippocampal volume in schizophrenia; obstetric complications and hypoxia related genes. Accepted for publication, Progress in Neuropsychopharmacology and Biological Psychiatry.

Abbreviations

AOS Adolescence onset schizophrenia
BDNF Brain-derived neurotrophic factor
COS Childhood onset schizophrenia
CSF Cerebrospinal fluid
CNS Central nervous system
CT Computer tomography
DODS Different onset, different slope
DOSS Different onset, same slope
DSM Diagnostic and statistical manual (of mental disorders)
DTNBP1 Dystrobrevin binding protein 1
FDR False discovery rate
GI Gyrification index
GRM3 Metabotropic glutamate receptor-3
ICV Intracranial volume
*l*GI Local gyrification index
MRI Magnetic resonance imaging
*NRG*1 Neuregulin1
OCs Obstetric complications
PANSS Positive and negative syndrome scale
PPI Prepulse inhibition
ROI Region of interest
ROIO Region of interest, outer contour
ROIP Region of interest, pial surface
SANS Scale for the assessment of negative symptoms
SAPS Scale for the assessment of positive symptoms
SCID Structural clinical interview for DSM-IV
sMRI Structural magnetic resonance imaging
SNP Single nucleotide polymorphism
VLBW Very low birth weight

Definitions:

Morphology:

a) The branch of biology that deals with the form and structure of organisms without consideration of function. b) The form and structure of an organism or one of its parts: *the morphology of a cell; the morphology of vertebrates.*

(Encyclopaedia Britannica)

Obstetric complications:

"...the broad class of somatic deviations from an expected, normal course of events and offspring development during pregnancy, labour-delivery, and the early neonatal period"

(McNeil, 1999)

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1. Background

1.1 Schizophrenia

Schizophrenia is a severe mental illness with a prevalence of about 0.7-1% worldwide (Gottesman, 1991;Saha et al., 2005). The diagnosis of schizophrenia is descriptive and comprises a broad range of symptoms and clinical manifestations. Schizophrenia as an illness entity was first described by the German psychiatrist Emil Kraepelin (Kraepelin, 1896) as *dementia praecox*; a name implicating a chronic degeneration syndrome with poor prognosis. The term schizophrenia meaning "splitting of the feeling" (Blom, 2003) was introduced by the Swiss psychiatrist Eugen Bleuler to describe the core feature of the illness, the "splitting of psychic functions" (Bleuler, 1911). Today, according to the DSM-IV (American psychiatric association, 1994), a diagnosis of schizophrenia is based on a combination of several symptoms:

a) two or more of the following symptoms have been present for a month or more: hallucinations, delusions, disorganized speech, disorganized behaviour and/or negative symptoms (affective flattening, apathy).

b) decline in function lasting a minimum of 6 months

c) no developmental disorder

d) schizoaffective disorder has been ruled out

e) no organic cause or drugs that cause the symptoms.

Dopamine and glutamate disturbances are considered to be the core underlying pathophysiological mechanisms of the illness (Keshavan et al., 2008;Stone et al., 2007). The locus of dopamine dysregulation appears to be at the presynaptic dopaminergic control level in regionally specific pattern including prefrontal hypodopaminergia and a subcortical hyperdopaminergia (Howes & Kapur, 2009). Brain morphological aberrations in schizophrenia have been reported from *in vivo* (CT and MRI) (Chua & McKenna, 1995;Ellison-Wright & Bullmore, 2009;Glahn et al., 2008;Honea et al., 2005;Malla et al., 2002;Shenton et al., 2001;Steen et al., 2006) and post mortem studies (Benes, 1988;Bernstein et al., 2009). However, neuropathological changes in schizophrenia are subtle, and no pathognomonic lesions have been demonstrated (Keshavan et al., 2008).

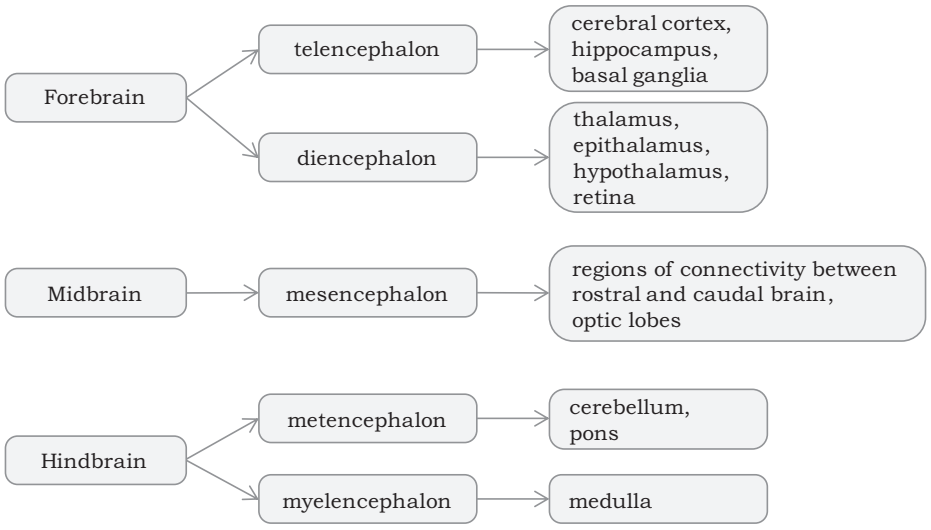
The concise aetiology of schizophrenia is hitherto unknown. The illness has a strong genetic component, with an estimated heritability up to 80% (Sullivan et al., 2003). Multiple environmental effects modify schizophrenia liability. The environmental risk factors include urban birth (Harrison et al., 2003), paternal age (Byrne et al., 2003;Miller et al., 2010;Zammit et al., 2003), migration (Selten et al., 2007;Cantor-Graae, 2007), social disadvantage (Wicks et al., 2005), cannabis use (Hall & Degenhardt, 2008), stressful life events (Miller et al., 2001;van et al., 2008), and pre-

and perinatal adversities (Cannon et al., 2002a;Dalman et al., 2001;Geddes et al., 1999;Hultman et al., 1999;Nilsson et al., 2005;Rosso et al., 2000). The high degree of heritability, combined with findings of several environmental risk factors suggest that gene-environment interactions are of importance to the development of schizophrenia.
Over a hundred years of schizophrenia research have contributed to the understanding of schizophrenia as an illness with biological correlates. Some of these correlates (e.g. brain morphological alterations) have been suggested to have their origin in disturbed neurodevelopment (Fatemi & Folsom, 2009;Lewis & Murray, 1987;Weinberger, 1987).

1.2 Neurodevelopment

Prenatal neurodevelopment follows a highly complex pattern and is under strong genetic influence (Arnold & Rioux, 2001;Hatten, 2002). The development of the neural system starts when the cells in the embryonic ectoderm form the neural plate; the neural plate then folds to form the neural tube (Jain et al., 2001). The cavity of the neural tube is the early rudiment of the cerebral ventricles, and the "walls" of the neural tube is lined with neuroepithelial cells (the ventricular zone) that later develops into neurons and glia (Arnold & Rioux, 2001).
Subdivision of the neuroepithelial cells in the neural tube into different regions give rise to the forebrain, midbrain and hindbrain (Klempan et al., 2004) (see figure 1).

Figure 1. Developmental origin of different brain structures, schematic.



Eight stages in the developing brain has been described (Mayes & Ward, 2003); neural plate induction, neuronal and glial cell proliferation (occurring in the 2nd to 4th foetal month for neurons and in the 5th foetal month to one year postnatally for glial cells), cell migration (third to fifth foetal month), cell aggregation, neuronal maturation, neuronal connectivity including synaptogenesis, cell death, and pruning, from the 6th gestational month and several years postnatally.

Migration from the ventricular zone to the cortex occurs in an inward-out fashion. The first neurons to migrate constitute the inner layer of the mature cortex, and the latest neurons to migrate constitute the outer layer (Angevine, Jr. & Sidman, 1961;Batra et al., 2009). The neural migration to the cortex is position specific (Rakic, 2007). The migration is radially oriented and guided by glial cells to form columnar arrangements (Hatten, 2002;Rakic, 2007). In addition, a different pattern of tangential migration, in a ventral to dorsal fashion, is followed by interneurons and precede their radial organization into columnar shafts (Ang, Jr. et al., 2003;Wichterle et al., 2001). The majority of the interneurons are generated in the subventricular zone (Letinic et al., 2002). Disturbances in the processes of neuronal proliferation or migration lead to malpositioning of the neurons within the cortex which might prevent formation of the appropriate connections and adversely affect mature brain function (Clunic, 2009).

Cortical folding or gyrification develops alongside the cortical migration. The early stages of gyrification appear around gestational week 16, with a rapid increase in cortical gyrification in the third trimester of pregnancy (Armstrong et al., 1995). The human gyrification process appear to be a result of tension based mechanisms. Visco-elastic tension exerted by cortical fibres is considered to draw regions with greater connectivity closer together (forming gyri) and thereby reduces the transit time of the action potentials (Van Essen, 1997;White et al., 2010). The primary sulci appear almost similar in all humans, whereas secondary and tertiary sulci display greater inter subject variety (Armstrong et al., 1995).

Striatal development starts at the 6th gestational week. From the floor of the telencephalic vesicle it bulges into the lateral ventricle (Jain et al., 2001), and divides into the medial and lateral striatum separated by the capsula interna at a later stage. The early rudiments of the hippocampus develop from the pallium at the rostral end of the lateral ventricle, before moving caudally as the di- and telencephalon coalesce (Brodal, 2001).

1.3 The neurodevelopmental hypothesis of schizophrenia

The foetal origin health concept describe a relationship between signs of *in utero* compromise (e.g. low birth weight) and later development of somatic disorders such as coronary heart disease, stroke, and diabetes (Barker et al., 1993;Gluckman et al.,

2008). Findings from epidemiologic and experimental studies are supportive of a foetal origin of mental illnesses such as autism, ADHD, personality disorders, and schizophrenia (Beydoun & Saftlas, 2008;Larsson et al., 2005;Schlotz & Phillips, 2009;Thompson et al., 2010).

The first description of early somatic trauma and neurodevelopmental disturbances as putative risk factors for schizophrenia dates back to Rosanoffs classic twin study "The etiology of the so called schizophrenic psychoses" from 1934. Although the twin concordance rates were the main objective of this study, he discussed the possibility of schizophrenia being a *"decerebration syndrome which may result from birth trauma"* (Rosanoff et al., 1934), based on the finding that birth trauma such as prematurity and the use of forceps were more frequent in the ill than in the healthy twins.

The concept was, however, no focus of research interest before Pasamanick et al's theory of a *"continuum of reproductive causality"* (Pasamanick et al., 1956). According to this theory, pre- and perinatal adversities, depending on their severity, could cause harm ranging from death and severe mental and physical impairment to behavioural deviances and psychiatric illness in the offspring. The theory opened a new research ground (see Cannon et al., 2002a for review). Regarding the impact of pre and perinatal adversities on schizophrenia risk, a "break through" came with the finding of significantly lower birth weight in schizophrenia subjects than in healthy controls (Lane & Albee, 1966). The interaction between genetic (heritability) and environmental factors (OCs) on schizophrenia risk was demonstrated by Mednick & McNeil, who studied the offspring of mothers with schizophrenia (Mednick & McNeil, 1968).

Neuropathological examinations of post mortem brain tissue from schizophrenia patients have revealed alterations such as ectopic grey matter (sign of aberrant prenatal neuronal migration) (Jakob & Beckmann, 1986;Nopoulos et al., 1998;Nopoulos et al., 1995), and absence of gliosis[1] (lack of degenerative processes) (Bogerts, 1999). These findings do not support schizophrenia as a neurodegenerative syndrome (as suggested in the Kraepelinean tradition), but suggest that schizophrenia could be an illness related to disrupted neurodevelopment (Rapoport et al., 2005). Additional support for a neurodevelopmental origin of the illness come from studies that report an increased occurrence of minor physical anomalies (MPA) (Lloyd et al., 2008;McNeil & Cantor-Graae, 2000) and neurological soft signs (Compton et al., 2007) in schizophrenia patents. MPAs such as low set ears and epicanthal eye folds have their developmental origin in the embryonal ectoderm, as has the brain neuronal tissue. The fact that many schizophrenia patients display neurocognitive abnormalities during childhood and adolescence (Sorensen et al., 2010;Woodberry et

[1] Gliosis describe elevated glial cell density as is present in progressive brain diseases

al., 2008;Reichenberg et al., 2006), before illness onset, is further support for a neurodevelopmental origin of the illness.

The conceptualization of schizophrenia as a neurodevelopmental illness proved to be a fruitful framework for further research (Lewis & Murray, 1987;Weinberger, 1987), and the hypothesis has later been supported by numerous register based-, clinical case control-, MRI-, molecular genetic-, and animal model studies. This research provided the fundament for the hypotheses examined in the four studies included in this thesis.

1.4 Studies of pre-and perinatal risk factors in the development of schizophrenia

Many epidemiological but also clinical studies have demonstrated an association between early somatic trauma occurring in the pre- and perinatal period and a later development of schizophrenia. Studies have also investigated the extent to which this increased prevalence may be caused by genetic factors in the offspring, by maternal socio-demographic factors and psychiatric health, or a combination.

1.4.1 Maternal starvation

At the end of World War II, between February and May 1945, severe hunger occurred in a region in the Netherlands. A significantly higher prevalence of schizophrenia was reported among children of women who were pregnant during the starvation period. In particular if starvation was severe and occured during the first trimester, the relative risk increase was 2.0 (Hoek et al., 1998). Similarly, offspring of women who were pregnant during the Chinese starvation between 1959 and 1961 also had a higher rate of schizophrenia (relative risk 2.3) (St Clair et al., 2005). Some evidence from animal models indicate that pro-inflammatory factors may mediate the effect of maternal under-nutrition on foetal brain development (Shen et al., 2008).

1.4.2 Maternal infection

The increased rate of schizophrenia in subjects born during late winter- / spring months has been attributed to maternal infection during pregnancy. Accordingly, evidence for an association between several infectious agents and increased risk for offspring schizophrenia has been put forward. Maternal influenza infection, as determined serologically by the use of frozen maternal plasma, has been associated with a seven fold increase in schizophrenia risk (Brown et al., 2004). *In utero* exposure to other viral infections such as measles (Torrey et al., 1988), rubella (Brown et al., 2001), varicella-zoster (Torrey et al., 1988), herpes simplex virus 2 (Buka et al., 2008), but also toxoplasmosis (Mortensen et al., 2007) and bacterial infections (Sorensen et

al., 2009) has been linked to an increased risk for schizophrenia. Supportive of a gene-environment interaction model, an increased risk of developing schizophrenia after *in utero* exposure to maternal pyelonephritis was associated with a history of familial schizophrenia (Clarke et al., 2009). The variety of pathogens suggests that common underlying inflammatory responses may be of importance (Shi et al., 2005;Smith et al., 2007). Prenatal immune challenge have been demonstrated to cause alterations in the dopamine metabolism (Meyer & Feldon, 2009;Winter et al., 2009).

1.4.3 Obstetric complications

Large register-based studies have provided statistical evidence for a link between a variety of OCs and schizophrenia. Increased risk of schizophrenia in offspring of mothers who had suffered placental- or uterine bleeding, in boys with low birth weight, and in sons of mothers with grand multipara (>3 previous births) was reported from a Swedish study of 3942 subjects (Hultman et al., 1999). In a Finnish birth cohort study (n=25 865), schizophrenia was found to be related to to low birth weight (< 2500g), and to the combination of low birth weight and birth before gestational week 37 (Jones et al., 1998). Further evidence for an association between low birth weight (<2500g) and schizophrenia has been reported from a large twin study of 5680 twin pairs of which 88 were diagnosed with schizophrenia (Nilsson et al., 2005)

Low birth weight is indicative of intra-uterine compromise of the foetus occurring over an extended amount of time (Rehn et al., 2004). However, detrimental events related to birth have also been associated with increased schizophrenia risk. Perinatal asphyxia has been related to schizophrenia with an odds ratio of 4.4, after control for confounding factors such as other obstetric adversities, maternal history of psychosis, and social class (n=1567) (Dalman et al., 2001). The finding was partly replicated in a Danish register study (n=25 865) in which hypoxia, prematurity, and maternal infection were significantly related to schizophrenia after controlling for familial psychiatric illness and social class (Byrne et al., 2007).

The variety of positive findings for specific OCs, taken together with scattered reports of null findings (Kendell et al., 2000;Onstad et al., 1992), have complicated efforts to integrate OCs and schizophrenia in a pathophysiological framework. A large meta-analysis comprising eight studies including 1923 patients with schizophrenia and 527 925 control subjects distinguished three categories of obstetric complications associated with schizophrenia (Cannon et al., 2002a): 1) complications of pregnancy (diabetes> rhesus incompatibility> bleeding> preeclampsia), 2) abnormal foetal development (low birth weight> congenital malformations> reduced head

circumference), and 3) complications of labour and delivery (emergency caesarean section> uterine atony> asphyxia).

Foetal hypoxia may be the common underlying factor of the various complications (Verdoux & Sutter, 2002), as has been demonstrated in animal models, for review see (Boksa, 2004;Rees et al., 2008).

Also pre-disposing socio-demographic factors associated with adverse pregnancy outcome may be confounded with a history of maternal mental illness (Ellman et al., 2007;Jablensky et al., 2005). By this means, the offspring of mothers with schizophrenia may carry the increased risk of schizophrenia from both heritability risk and OCs.

1.4.4 Animal models of obstetric complications in schizophrenia

Animal models have been developed to explore how pre- and perinatal complications affect offspring brain morphology and neurodevelopment. Criteria for animal species to be used in models of human brain damage include the condition that a similar proportion of brain development must occur *in utero*; the insult can be delivered *in utero* at an equivalent stage of development identified to be vulnerable in humans; the volume of white to grey matter is similar to the human brain; the physiological outcome of the insult can be monitored; and neurobehavioural parameters can be tested postnatally (Rees et al., 2008). It is important to note that no animal fulfils all of these criteria. For instance, the rat brain at birth is less mature than the human brain at birth, whereas the maturity of the guinea pig brain is more similar to that of the brain of the human neonate (Boksa, 2004).

Given the limitations of no animal fulfilling all criteria listed above, several experimental animal models of OCs effects have demonstrated resulting abnormalities of *brain morphology* (reviewed by Boksa, 2004); maternal diabetes has caused reduced brain weight in adult rats; intra-uterine growth restriction has caused enlarged ventricles, reduced hippocampal volume, and reduced cortical area in newborn guinea pigs and altered neuronal migration to the cortex in postnatal and postpubertal rats; neonatal viral infection has caused cortical thinning and reduced hippocampal cell number in adult rats; birth hypoxia has been linked to reduced hippocampal cell number in adult rats, reduced neurons in hypothalamus, striatum, and cerebellum as well as hippocampal and hypothalamic neurodegeneration in adult guinea pigs; maternal infection has been linked to hippocampal cell atrophy in adult mice (Fatemi et al., 2008) and reduced cortical grey matter volume in monkeys (Short et al., 2010).

Animal models may also be used to measure alterations in *neurotransmittors* as a response to OCs. Of particular importance for schizophrenia research is the dopamine

metabolism. In different rat models, OCs have been demonstrated to cause altered dopamine-mediated behaviour (caesarean section) (Berger et al., 2000), altered dopamine receptor mRNA in the striatum (perinatal asphyxia) (Gross et al., 2005), decreased striatal dopamine turnover (caesarean section) and nucleus accumbens dopamine turnover (caesarean section + anoxia) (El-Khodor & Boksa, 1997), and greater neuronal spine density in the nucleus accumbens (caesarean section + anoxia) (Juarez et al., 2008)

The nature of foetal neuropathology depends on the severity of the insult and the gestational age of the foetus (reviewed by Rees et al., 2008), as summarized below (1-3).

1. Acute insults in early gestation, when neurogenesis and neuronal migration is at its peak, results in death of e.g. pyramidal cells in the hippocampus, slowing of neuronal migration to the hippocampus, and diffuse white matter damage (Rees et al., 1999).
2. Acute insults in late gestation cause neuronal death in the cortex and striatum, but less severe white matter damage (Loeliger et al., 2003).
3. Chronic insults (placental insufficiency) results in growth restriction, reduced brain weight, enlarged lateral ventricles (Mallard et al., 1999), reduced basal ganglia volume (Rehn et al., 2004), and reduced axonal myelination in the CNS.

To sum up, animal models provide extensive evidence for the propensity of OCs to induce brain morphological alterations and disturbances of dopamine metabolism as observed in schizophrenia patients.

1.5 Neuroimaging

The first *in vivo* neuroimaging studies of schizophrenia patients were pneumoencephalographic studies undertaken in the 1920ies. These studies produced the earliest reports on enlarged ventricles in schizophrenia patients as compared to healthy controls (Jacobi & Winkler, 1927;Keshavan et al., 2008). The use of computer tomography (CT) scans of the brain for research purposes started in the 1970ies (Shenton et al., 2001), but its use was limited by the harm accumulated amounts of x-ray radiation impose on human tissue. This limitation did not apply to the magnetic resonance imaging (MRI) examinations introduced in clinical brain research in the 1980ies. Furthermore, MRI was already then superior to CT in depicting brain anatomy, especially the differences of grey and white matter and the anatomy of the cerebellar fossa.

1.5.1 MRI

MRI images of human tissue are measurements of an induced signal from hydrogen atoms (^1H) (Weishaupt et al., 2006). Hydrogen atoms are widely distributed in all the body tissues. In the atom, the proton possesses positive charge and spin, and the proton rotates about its own axis. When a tissue is exposed to a static magnetic field, the spin of the hydrogen atoms within this tissue aligns with the direction of the magnetic field. By applying a radiofrequency pulse (RF) specific to hydrogen, the spin system gets excited, and some of the spins are tipped away from their original alignment. When the radiofrequency signal is removed, it induces an alternating voltage of the same frequency as the larmor frequency[2] in a receiver coil: the MR signal. This MR signal fades rapidly due to reduction of the transverse magnetization by spin-lattice-interaction (T1-relaxation) and spin-spin interaction (T2-relaxation).

Briefly, in T1-relaxation, the nuclei return to their ground state by dissipating their excess energy to the surroundings (the lattice). The time constant for this recovery is dependent on the strength of the external magnetic field and the internal motion of the molecules. On T1-weighted MRI scans, water (and CSF) appear dark. T2-relaxation reflects loss of phase coherence[3]; the spins do not lose energy to the surroundings but rather exchange energy with each other. On T2-weighted MRI scans water (and CSF) appear bright. The signal intensity is sensitive to the biochemical properties of the tissues and depend on proton density (the number of excitable spins per unit volume), the T1 relaxation time constant (corresponds to the time before the excited spins in a given tissue are recovered and ready for new excitation), the T2 relaxation time constant (determines how quickly a signal fades after excitation), flow and temperature (Agartz, 2008).

The MRI signals are collected in the receiver coils as frequencies and temporarily stored in K-space. After the scanning is finished, the raw data stored in K-space is mathematically transformed (by Fourier transforms) in order to reconstruct the original image in spatial coordinates (McRobbie, 2007). With the currently used pulse sequences, the reconstructed MR-images have a resolution of approximately 1-2 mm[3] (Agartz, 2008).

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[2] The larmor frequency is given by the larmor equation: $\omega_0 = \gamma_0 \cdot B_0$,
where ω_0 signify the larmor frequency in megahertz, γ_0 signify the gyromagnetic ratio (a constant specific to a particular nucleus), and B_0 signify the strength of the magnetic field in tesla.

[3] When spins precess synchronously directly after excitation they have a phase of 0°, they are in phase. Phase coherence is gradually lost as some spins advance and others fall behind their precessional paths. The individual magnetic vectors then cancel each other out instead of adding together and the MR signal is lost as the transverse magnetization gradually disappears

Different image processing methods are used to measure neuroanatomical brain structures (morphometry). With the region of interest (ROI) method, manually or automatically delineated volumes of pre-defined structures are analysed, whereas with the voxel based method (VBM) comparison of the volume of the gray and white matter between groups of interest can be performed for each voxel of the cerebral volume without the need to explicitly define regions of interest in advance. With surface based methods (SBM), cortical complexity can be analysed with great precision (Dale et al., 1999;Fischl et al., 1999a;Schaer et al., 2008).
The structural MRI (sMRI) technique is used when examining neuroanatomy. Other MRI techniques include perfusion imaging, diffusion tensor imaging (DTI) studying connectivity, magnetic resonance spectroscopy (MRS) investigating neurochemistry, and functional MRI (fMRI) examining aspects of brain physiology (Gur et al., 2007).

1.5.2 MRI in schizophrenia

MRI is an invaluable tool for in vivo investigation of brain anatomy. Whereas post mortem examination of brain anatomy allows for description of cytoarchitecture (Benes, 1988;Bernstein et al., 2009;Selemon et al., 1998), in vivo MRI examination allows large scale case-control studies (Falkai et al., 2007;Goldman et al., 2009), longitudinal studies (Ho et al., 2003;van Haren et al., 2007), studies of first-episode cases (Steen et al., 2006) and high-risk samples (Job et al., 2003;Witthaus et al., 2010), as well as childhood- and adolescent-onset schizophrenia studies (Gogtay, 2008;Voets et al., 2008;White et al., 2003) with robust numbers of cases and controls. The larger subject samples facilitate the investigation of e.g. genetic- (Agartz et al., 2006;van Haren et al., 2008), medication- (Smieskova et al., 2009), and cannabis use (Bhattacharyya et al., 2009) effects on brain structure.
The earliest MRI studies on brain anatomy in schizophrenia revealed enlarged ventricles (Andreasen et al., 1990;Kelsoe, Jr. et al., 1988) in line with previous findings from CT studies (Johnstone et al., 1976;Weinberger et al., 1979), and were suggestive of reduced brain tissue (Ward et al., 1996). From MRI studies, smaller hippocampal volumes have consistently been reported in schizophrenia patients (Steen et al., 2006;Stefanis et al., 1999;Velakoulis et al., 2006;Wang et al., 2008). Studies on basal ganglia volumes display more heterogeneity in results (Ballmaier et al., 2008;Glenthoj et al., 2007;Mamah et al., 2007;Tamagaki et al., 2005), some of which may be attributed to the use of antipsychotic medication that acts upon dopamine receptors abundant in the basal ganglia (Scherk & Falkai, 2006;Smieskova et al., 2009). Cortical complexity has been an obstacle to the study of case-control differences in cortical folding and thickness. Prefrontal and temporal volume

reductions have been reported, using a region of interest approach (Suddath et al., 1989;Yamasue et al., 2004;Breier et al., 1992) or voxel-based morphometry (Gur et al., 2000;Honea et al., 2005;Sanfilipo et al., 2000). Advances in MRI post processing tools have facilitated automated surface-based three dimensional analyses of the cortex. With these methods, e.g. widespread cortical thinning has been reported in schizophrenia patients (Kuperberg et al., 2003;Nesvag et al., 2008).

Brain morphological alterations have been reported to be present in first-episode schizophrenia (Crespo-Facorro et al., 2009;Ebdrup et al., 2010;Kubicki et al., 2002;Narr et al., 2005;Schultz et al., 2010;Witthaus et al., 2009), in subjects at high genetic risk (Job et al., 2003;Job et al., 2005;Lawrie et al., 2001;Lawrie et al., 2002) and in subjects with prodromal symptoms of schizophrenia (Witthaus et al., 2010;Jung et al., 2009;Hurlemann et al., 2008;Borgwardt et al., 2007;Pantelis et al., 2003). Also childhood onset schizophrenia (COS) (White et al., 2003) and adolescence onset schizophrenia (AOS) and psychosis (Douaud et al., 2007;Janssen et al., 2009;Voets et al., 2008) patients also demonstrate brain morphological abnormalities compared with healthy control subjects. Heterogeneous findings have been reported from studies on case-control differences in cortical folding; schizophrenia patients demonstrate prefrontal hypergyria (higher GI)(Falkai et al., 2007;Harris et al., 2007;Vogeley et al., 2001), lower prefrontal (Bonnici et al., 2007) and global (Cachia et al., 2008;Sallet et al., 2003) GI, increased metric distortion (an indirect measure of cortical displacement and convolution) in the left pars triangularis (Wisco et al., 2007), and no abnormalities (Highley et al., 2003) when compared to healthy controls. Taken together, the above findings points towards an early origin for the brain morphological abnormalities reported in schizophrenia patients.

Progressive brain morphological abnormalities after illness onset (Ho et al., 2003;van Haren et al., 2007) and around transition to psychosis (Takahashi et al., 2009) have been reported in longitudinal studies, but negative findings have also been reported (Whitworth et al., 2005). In addition, typical antipsychotic medication has been related to reduced cortical volumes and increased basal ganglia volumes, whereas a change to atypical antipsychotics may reverse the basal ganglia enlargement (Scherk & Falkai, 2006;Smieskova et al., 2009). As a consequence, brain abnormalities of putative early neurodevelopmental origin may be confounded.

1.5.3 MRI and obstetric complications in schizophrenia

Enlarged ventricles have been reported to be larger in schizophrenia patients with than without a history of OCs. In monozygotic twins discordant for schizophrenia, enlarged lateral ventricles in the ill twin were associated with prolonged birth (McNeil

et al., 2000). Neonatal OCs has been associated with larger ventricles in schizophrenia patients (Falkai et al., 2003). However, two CT studies from the 1990ies did not find an association between OCs and ventricle volume (Reddy et al., 1990;Smith et al., 1998).

Increased ventricle to brain ratio (VBR) is suggestive of volume reductions in grey and/or white matter tissue. The smaller hippocampal volume in schizophrenia has been reported to be even smaller in schizophrenia patients with than without a history of OCs (Ebner et al., 2008;McNeil et al., 2000;Schulze et al., 2003;Stefanis et al., 1999;van Erp et al., 2002). Schizophrenia patients with OCs (but no family history of schizophrenia) have been reported to have smaller hippocampal volumes when compared to patients with a family history of schizophrenia (but no OCs), a finding that suggests OCs to be of greater importance than heritability risk to the reduced hippocampal volumes in schizophrenia (Stefanis et al., 1999). Bilateral hippocampal reductions related to OCs have been reported in schizophrenia patients and their siblings (Ebner et al., 2008), and left hemisphere hippocampal reductions related to OCs have been reported in schizophrenia patients and in their relatives, at a trend level (Schulze et al., 2003). Empirical evidence for an interaction effect between heritability risk and foetal hypoxia on hippocampal volume has been put forward (van Erp et al 2002); schizophrenia patients tended to demonstrate smaller hippocampi if subjected to foetal hypoxia whereas their siblings and healthy controls did not, these group differences not reaching statistical significance. The findings suggest that genetic liability for schizophrenia may modulate the effect of OCs on hippocampal volume.

Reduced cortical prefrontal and temporal volume has been reported in schizophrenia patients subjected to prenatal hypoxia (Cannon et al., 2002b). Cortical volume is the product of cortical thickness and cortical area (Voets et al., 2008), perhaps also related to cortical folding. Whereas one study of the association between OCs and cortical folding in schizophrenia yielded negative results (Falkai et al., 2007), no studies on the association between OCs and cortical thickness or area have been conducted. It is therefore not as yet possible to conclude whether reduced cortical thickness, -area, or both underpin the volume reductions observed.

In non-clinical samples, severe OCs such as prematurity and intra-uterine growth restriction have been related to brain morphological abnormalities. Cortical thickness reductions have been demonstrated in adolescents with very low birth weight (Martinussen et al., 2005) and cortical volume reductions in premature children (Soria-Pastor et al., 2009). Studies of gyrification in premature (compared to term) infants have demonstrated altered temporal gyrification bilaterally (Kesler et al., 2006), and higher sulcation index (a measure of cortical folding), when related to

brain surface, in preterm intra-uterine growth restriction infants (compared with "normal" preterm) infants (Dubois et al., 2008). Thus, adverse pre- and perinatal events have the propensity to cause morphological alterations that may be detectable in the brain several years after birth. Since cortical folding patterns develop *in utero*, and stay by and large stable postnatally (Armstrong et al., 1995), they may be a valid "window" to study effects of harmful pre- and perinatal events such as OCs.

1.6 Schizophrenia susceptibility genes

Heritability estimates in schizophrenia have been reported to be as high as 80% (Sullivan et al., 2003); the concordance rates of monozygotic and dizygotic twins have been reported to be 40% and 5% respectively (Cardno et al., 1999). The heritability of schizophrenia follows a complex trait; the genetics underpinning the schizophrenia phenotype do not follow classic Mendelian dominant or recessive single locus properties (Kendler & Eaves, 2005). Complex traits result from effects of multiple genetic and environmental influences, where each gene only confers a relatively small risk effect. The genes have low penetrance and relatively high allele frequency in the population. Environmental risk factors are important in complex traits i.e. gene-environment interactions are common (Waldman, 2003). Models for the genetic effect in schizophrenia include the "common illness-common allele" model, where the illness is caused by a combination of modest effects of variation in several alleles, and the "multiple highly penetrant gene variations" model, where individually rare mutations e.g. genetic microdeletions or microduplications (copy number variations) occur rarely but have high penetrance (Prasad & Keshavan, 2008;Walsh et al., 2008).

Over the last years, evidence for several genetic associations to schizophrenia risk has been put forward (Harrison & Weinberger, 2005). From linkage studies[4], *DISC1* emerged as a schizophrenia susceptibility gene (Millar et al., 2000). From association studies[5], genes involved in neuronal migration (e.g. *DISC1* (Saetre et al., 2008;Zhang et al., 2006) and reelin (Liu et al., 2010;Shifman et al., 2008)), dopamine metabolism (e.g. *COMT* (Chen et al., 2004;Wonodi et al., 2003)), and neurodevelopment (*BDNF, NRG1*, *DTNBP1, GRM3*) (Arnold & Rioux, 2001;Harrison & Weinberger, 2005;Harrison et al., 2008;Numakawa et al., 2004;Webster et al., 2006) have been associated with schizophrenia susceptibility. Of particular relevance to the neurodevelopmental hypothesis of schizophrenia, a high proportion of schizophrenia susceptibility genes are involved in prenatal neurodevelopment and regulated by hypoxia-ischemia

[4] In linkage studies, large pedigree samples are analyzed to discover chromosomal regions where genes involved in the illness are likely to be found (Lang et al., 2007)

[5] In association studies, single nucleotide polymorphism (SNP) variation in genes putatively involved in schizophrenia (e.g. after discoveries in linkage studies) are investigated

(Schmidt-Kastner et al., 2006). To date, one study has suggested an interaction effect between severe OCs and SNP variation in seven SNPs from four schizophrenia susceptibility genes (*AKT1, BDNF*, *DTNBP1, and GRM3*) regulated by hypoxia on schizophrenia risk (Nicodemus et al., 2008).

Molecular markers of schizophrenia susceptibility have been associated with brain structural alterations in schizophrenia. Some of the genes are related to neurodevelopment; SNP variation in *BDNF* has been associated with volumes of occipital, temporal, and parietal lobe volumes (Ho et al., 2006), and frontal grey matter and caudate volume (Agartz et al., 2006). Among schizophrenia patients and healthy controls, carriers of the DISC1 leu607phe polymorphism phe-allele have smaller prefrontal grey volumes (Szeszko et al., 2008). Ventricle enlargement has been associated with allele variation in the reelin gene (Gregorio et al., 2009) and *NRG1* (Mata et al., 2009), smaller hippocampal volumes with *NRG1* allele variation (Gruber et al., 2008), and altered prefrontal volumes with variation in AKT1 (Tan et al., 2008). However, if SNP variation in genes of relevance to schizophrenia susceptibility and neuorodevelopment interact with OCs in their effect on brain morphology, has previously not been investigated.

2. Aims

The main aim of this PhD thesis was to investigate the effects of a history of obstetric complications (OCs) on brain morphology in patients with schizophrenia. The sub aims were to determine if such putative effects could explain some of the differences in brain morphology between schizophrenia patients and healthy controls, and to investigate if the effects of OCs on brain morphology are modified by genetic variation (allele variation in single nucleotide polymorphisms).

The theoretical framework for the current aims is the neurodevelopmental hypothesis of schizophrenia, and the empirical foundations for these aims are derived from three areas of research;

1) epidemiological studies of OCs as risk factors to develop schizophrenia,

2) animal models that demonstrate brain morphological alterations as a result of pre- and perinatal adversities alone and in interaction with genetic variation, and

3) neuroimaging studies that show abnormalities of brain morphology in schizophrenia patients as compared to healthy controls,

The following research questions and hypotheses were explored and tested in the four studies included in the thesis. The studies address either hypotheses that have previously not been investigated (study I, II, and IV) or they apply new methods to previously tested hypotheses (study III).

Study I

How do OCs relate to the volume of basal ganglia nuclei known to be of importance to schizophrenia pathology?

Based on the fact that dopamine metabolism is altered in schizophrenia and vulnerable to OCs, we hypothesized that increased number and severity of OCs would be related to the volume of the dopamine fibre rich basal ganglia (nucleus accumbens, nucleus caudatus, globus pallidum, and putamen) in schizophrenia patients but not in healthy controls.

Study II

Do patients with history of OCs and/or foetal hypoxia have thinner brain cortices? Is this found also in healthy control subjects?

Cortical thickness differs in schizophrenia patients versus healthy controls. As OCs affect cortical morphology in animal models, we hypothesized that that higher number and/or severity of OCs and/or foetal hypoxia would be significantly associated with thinner brain cortices, and that this association would be different between

schizophrenia patients and healthy controls, with greater thinning (general or regionally specific) in patients.

Study III

Is increased number and severity of OCs associated with altered cortical folding in schizophrenia? Will applying a surface-based three-dimensional method to calculate the gyrification index detect subtle deviances in cortical folding related to OCs?

Cortical folding occurs prenatally and remains by and large stable postnatally. Differences in cortical folding patterns between schizophrenia patients and healthy controls may reflect aberrant pre-natal neurodevelopment in schizophrenia. The cortex is a complex three dimensional (3D) structure, and by using the two dimensional (2D) gyrification index, information related to buried sulci and sublobar structures may be lost. We hypothesized (1) that OCs would be related to cortical folding as measured by a 3D surface-based local gyrification index, (2) that this relationship would be different in schizophrenia patients and healthy control subjects.

Study IV

Does variation in hypoxia-regulated schizophrenia susceptibility genes modulate the effect of OCs on hippocampal volume?

As hypoxia is a core feature of OCs and a strong modifier of gene expression, we hypothesized that the effect of OCs on hippocampal volume could be modified by variation in hypoxia-regulated genes. Consequently, we explored if 1) there was a statistically significant relation between a history of hypoxia-related severe OCs and hippocampal volume, and 2) if such a putative relationship was modulated by allele variation in four genes that are regulated by hypoxia and associated with schizophrenia (*NRG1, BDNF, GRM3* and *DTNBP1*).

3. Methods

3.1 Subject sample

The four studies in this thesis are conducted on the same subject sample. The subject sample was part of the Human Brain Informatics Project (HUBIN) at the Karolinska Institutet, Stockholm, Sweden. HUBIN is a comprehensive database of genetic, brain morphological, neuropsychological, and clinical information obtained from schizophrenia patients and healthy subjects. The inclusion of subjects who participated in the current study took place between 1999 and 2003. All participants gave written informed consent. The project was approved by the Research Ethics Committee at Karolinska Institutet and the Swedish Data Inspection Board ("Datainspektionen"). The study was performed in accordance with the Helsinki Declaration.

The subject sample consisted of unrelated Caucasian men and women currently resident in the Stockholm Area. Except from one subject born in Finland, all subjects were born in Sweden between the years 1943 and 1982. Demographic and clinical data are listed in Table 1.

Table 1. Demographic and clinical characteristics in schizophrenia patients and healthy control subjects.

	Patients (n=54)		**Controls** (n=54)		**Statistics**	
	Mean	S.E.	Mean	S.E.	Test-value	p-value
Age at MRI (years)	41.9	1.1	41.5	1.2	t=.28	ns
Age at illness onset n=53 (years)	24.9	0.8	na			na
Duration of illness (years)	16.8	1.3	na			na
	Number	%	Number	%		
Gender (male/female)	37/17	68/32	33/21	61/39	χ^2=.65	ns
Handedness n=105 (right/left/ambidextr.)	48/2/2	92/4/4	48/3/2	91/6/4	χ^2=.19	ns
Medication (typical/atypical/none)	25/26/3	46/48/6	na			na

3.1.1 Patients

Invited patients from the out-patients clinic underwent a comprehensive clinical assessment protocol using validated operational instruments (Ekholm et al., 2005;Vares et al., 2006) including symptom severity scores by SANS/SAPS (Andreasen, 1983;Andreasen, 1984). Handedness was ascertained by means of asking the patients which hand they preferred when writing, using scissors, and

throwing/catching a ball. Verification of psychiatric diagnosis was performed by a trained psychiatrist by the use of structured clinical interviews (SCID (Spitzer et al., 1988) and OPCRIT (McGuffin et al., 1991)). Patients fulfilled DSM-III-R and DSM-IV criteria for schizophrenia (n=50) or schizoaffective disorder (n=4). The mean duration of illness was 16.8 years (range 0.4-41.1 years), and mean age of illness onset was 24.9 years (range 15.9-39.5 years).

The antipsychotic medications used by patients in the present study documented at the time of MRI were as follows: typical antipsychotic medication (n=25) included Perphenazine (n=9), Zuclopenthixole (n=5), Haloperidol (n=10), and Flupenthixole (n=1); atypical antipsychotic medication (n=26) included Risperidone (n=8), Clozapine (n=9), and Olanzapine (n=9). Two subjects had both typical and atypical antipsychotic medication and three patients had no medication at the time of MRI.

3.1.2 Control subjects

Control subjects were recruited using three different approaches. The first group (n=25) had previously (2–19 years earlier) served as healthy comparison subjects in biological psychiatric research at the Karolinska Institutet (Damberg et al., 2004). They were reassessed for lifetime psychiatric diagnosis (Jonsson et al., 2000). A second group of controls (n=14) was recruited from hospital staff or their relatives for the present study. A third control group (n=15) was recruited from a population register for the present study. This combined recruitment strategy was used due to the high dropout rates, sometimes approaching 95% (Oxenstierna et al., 1996), when subjects are recruited from the general population for demanding biological psychiatric research. The control subjects were interviewed by a trained psychiatrist and they were found to have no previous or current psychiatric disorders according to a semi-structured diagnostic interview (SCID-non-patient version (Spitzer et al., 1986)). They were matched to the patients by age and gender (on a group level). The blood sample collected from one control subject was insufficient for proper genotyping; 53 control subjects were thus included in study IV.

3.1.3 Exclusion criteria

Exclusion criteria for all subjects were a history of head trauma with loss of consciousness >5 minutes, current treatment for substance abuse, and/or somatic disorders affecting brain function.

3.2 MRI scan aquisition

MR images were obtained at the MR Research Centre at Karolinska Institutet, Stockholm, Sweden, using a 1.5 Tesla GE signa Echo-speed (Milwaukee, Wis., USA) scanner. T1-weighted images were obtained using a three-dimensional spoiled gradient recalled (SPGR) pulse sequence with the following parameters; 1.5 mm coronal slices, no gap, 35° flip angle, repetition time (TR) = 24 ms, echo time (TE) = 6.0 ms, number of excitations (NEX) = 2, field of view (FOV) = 24 cm, acquisition matrix = 256 × 192. T2-weighted images were acquired with the following parameters; 2.0 mm coronal slices, no gap, TR = 6,000 ms, TE = 84 ms, NEX = 2, FOV = 24 cm, acquisition matrix = 256 × 192. All scans included were visually judged to be without obvious motion artefacts. A trained neuroradiologist evaluated all scans to be without gross pathology.

3.3 MRI post processing

MRI post processing by automated software tools was used in all studies. The soft ware suite FreeSurfer was used in all studies and the BRAINS program was used in parts of studies I and IV. In addition, manual delineations of hippocampal volumes were used in
study IV.

3.3.1 FreeSurfer

3.3.1.1 Cortical thickness

The Free-Surfer v.1.2 software package (http://surfer.nmr.mgh.harvard.edu) was used to obtain estimates of cortical thickness (in study II). Cortical thickness was measured by reconstructing representations of the grey/white matter boundary and the cortical surface on T1 weighted images (see Figure 2), and then calculating the distance between those surfaces at each point (vertex) across the whole cortical mantle, a total of approximately 162,000 vertices in each hemisphere (Dale et al., 1999). Topological defects in the grey/white matter boundary were routinely manually corrected by laboratory technicians, under direct supervision of senior researchers, blind to subject identity. The resulting cortical thickness maps were averaged across participants using a non-rigid high-dimensional spherical averaging method to align cortical folding patterns (Fischl et al., 1999b). This procedure results in a mean measure of cortical thickness for each group at each point on the reconstructed surface (Fischl et al., 1999a;Dale et al., 1999). This method has been validated by

histological (Rosas et al., 2002) as well as manual measurements (Kuperberg et al., 2003).

Figure 2. Cortical reconstruction with the FreeSurfer method demonstrated in horizontal, coronal, and sagittal views. The green line represents the border between grey and white matter, the red line represents the pial surface, and the yellow line the outer surface (courtesy by Marie Schaer, (Schaer et al., 2009)).

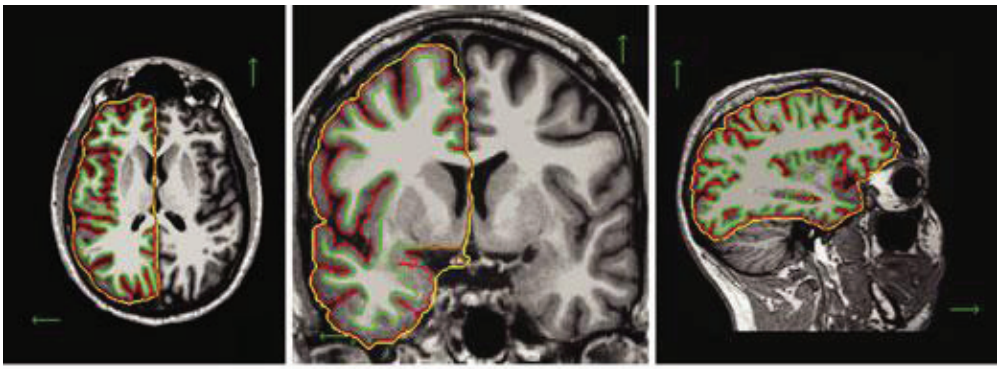
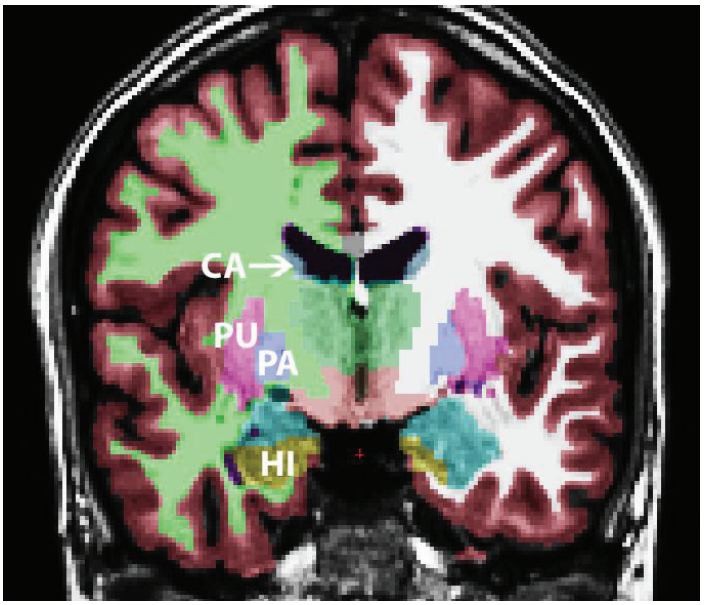


Figure 3. Subcortical segmentation with FreeSurfer, coronal view. CA= nucleus caudatus, PA= globus pallidum, PU= putamen, HI= hippocampus (hippocampal formation). Nucleus accumbens is localized anterior to nucleus caudatus and is not included in this MRI slice (image by Petr S. Bjerkan and Anders Haukvik).



3.3.1.2 Basal ganglia volumes and the hippocampal formation

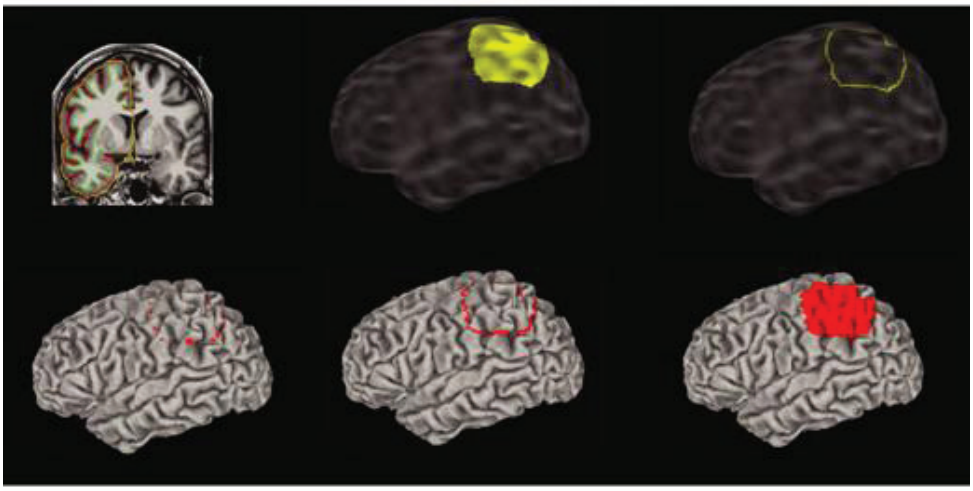
In studies I and IV, MRI post processing was performed using FreeSurfer version 3.0.2. (http://surfer.nmr.mgh.harvard.edu). Automated measurements of total volumes of putamen, globus pallidum, nucleus accumbens, nucleus caudatus, and the hippocampal formation (hippocampus) were obtained from the T1-weighted images (see figure 3). The automated segmentation algorithm combines information on image intensity, probabilistic atlas location, and the local spatial relationships between structures to automatically assign a neuroanatomical label to each voxel in the MRI volume (Fischl et al., 2002). The software does not classify the grey and white matter fractions of the total volumes. The reliability of the automatic volume measurements using FreeSurfer has been tested against manual tracings, and the agreement between the automated FreeSurfer volumes and manual volume measures has been reported to be comparable to that obtained by comparing the manual volume measures of different experts (Fischl et al., 2002). The subcortical segmentation has been reported to be stable across averaging of multiple acquisitions, acquisition sequences, major scanner upgrades and segmentation atlas within the same scanner (Jovicich et al., 2009). The hippocampal segmentation include the fimbria and represent the hippocampal formation (Makris et al., 1999).

3.3.1.3 Gyrification index

In study III, first, cortical reconstructions were obtained from T1-weighted images using the automated computer software FreeSurfer version 3.0.2. Second, we used the *l*GI algorithm (http://surfer.nmr.mgh.harvard.edu/fswiki/LGI) to compute measurements of local gyrification at thousands of vertices across the cortical mantle. From the vertex-wise *l*GI measurements, the average local gyrification index was calculated for 34 pre-defined anatomical cortex parcellations (Desikan et al., 2006) in each hemisphere. The *l*GI method is adapted from the classical gyrification index (2D-GI), which is the ratio of the total pial cortical surface over the perimeter of the brain delineated on coronal sections. The *l*GI method iteratively quantifies GI in circular three-dimensional regions of interest. After the creation of an outer envelope that tightly wraps the pial cortical surface, local measurement of circular GI is computed for each vertex of the outer surface as the ratio of corresponding regions of interest (ROI) on the hull and pial meshes is created. Delineation of the ROI on both the outer surface (ROIO) and pial surface (ROIP) uses a matching algorithm based on geodesic constraints, so that the ROIP takes into account the entire patch of the cortical surface delineated by the ROIO circular perimeter (see figure 4). At the end of the computational process, individual *l*GI cortical maps reflect the amount of cortex

buried within the sulcal folds in the surrounding circular region. The method has been described and validated (Schaer et al., 2008).

Figure 4. Cortical reconstruction and *l*GI-computation with the FreeSurfer method. The circular region of interest on the outer surface (ROI_O) is marked in yellow, and the corresponding region of interest on the pial surface (ROI_P) is marked in red, see text in *3.3.1.3* for details (courtesy by Marie Schaer (Schaer et al., 2009)).



3.3.2 BRAINS

Brain tissue segmentation was also performed using the software suite BRAINS (Andreasen et al., 1993) from both T1- and T2-weighted MRI scans re-sliced to 1-mm^3 voxels in standardized space. Using an automatic tissue classification procedure all brain tissue was classified in the following tissue class volumes: grey matter, white matter, CSF, and venous blood (Agartz et al., 2001;Harris et al., 1999). In the present studies I and IV, measurements of intracranial volume (ICV) obtained using BRAINS were used to correct for inter-individual differences in head size. In addition, in study I measures of nucleus accumbens, nucleus caudatus, and putamen, available for 60 subjects (patients and controls), as obtained automatically (nucleus caudatus and putamen) or by manual delineations (nucleus accumbens), were used to test the reliability of the FreeSurfer measures of the same structures. In study IV, manual delineations of the hippocampus available for 60 subjects were used for a re-analysis, as the main analysis in this study gave unexpected results. The inter-rater reliability, measured by intra class correlation (ICC) for the striatal structures was 0.93 for total striatum, 0.96 for caudate, 0.85 for putamen, and 0.79 for nucleus accumbens

(Agartz et al., 2006). For the hippocampus intra-rater reliability (ICC) was >0.98 for both hemispheres hippocampi (unpublished data).

3.3.3 Correlation between methods

The correlation between the combined left and right hemisphere volume measures of the basal ganglia from FreeSurfer and BRAINS was r=0.56 (p<0.0005) for nucleus accumbens, r=0.86 (p<0.0005) for nucleus caudatus, and r=0.85 (p<0.0005) for putamen. The correlation between hippocampal measures from FreeSurfer (including the white matter of the fimbria and the alveus) and the grey matter fraction of the hippocampus as manually delineated within the BRAINS program was r=0.52 (p<0.001).

3.4 Assessment of obstetric complications

In the studies included in this thesis, OCs were defined in accordance with previous research (McNeil & Cantor-Graae, 1999) as "...the broad class of somatic deviations from an expected, normal course of events and offspring development during pregnancy, labour-delivery, and the early neonatal period". Information on OCs was collected from hospital birth records. Obstetric care in Sweden has been of high quality all this period, and the birth records were very detailed. The information was scored according to the McNeil-Sjöström Scale For Obstetric Complications (McNeil & Sjostrom, 1995) by a physician (UKH) who was blinded to patient/control status and MRI-results. Sixty-two birth records had previously been scored by another physician (MD, PhD), and the intra-class correlation between the two raters (for all 62 records) was 0.93. The McNeil-Sjöström scale rates OCs for severity of probable harmful effect on the offspring according to severity at an ordinal scale from 1-6, where severity level one signifies a "not harmful or relevant" event and 6 signifies "very great harm to or deviation in offspring". The McNeil-Sjöström scale is organized according to the following major sub-categories:

I. Pregnancy complications

a) foetal/foeto-placental conditions, b) maternal disorders, c) medical examinations and interventions, d) maternal toxins/radiation, e) maternal legal medication, f) maternal illicit drugs and other stimulants, g) other maternal trauma

II. Labour-delivery complications

III. Neonatal complications

a) deviations in gestational age/weight/maturation, b) congenital structural malformations c) neoplasm, d) congenital infections, e) neonatal disorders, f)

treatment including offspring medications, g) breast feeding associated with maternal illness, medications and substance abuse.

The scale also divides scores for the given reproduction in total into scores for the specific pregnancy trimesters, total pregnancy, labour-delivery, neonatal period and total reproduction. The scale has been constructed for the use in clinical case-control studies in which individual complications (e.g. low birth-weight, prematurity, or placental abruption) occur too infrequently to be assessed separately (McNeil et al., 1994).

In study I and III, the number of OCs with severity scores of 3 and above was calculated for each individual subject to form one continuous variable. Scores under 3 are considered to be not harmful to the foetus and were therefore not included in the analyses; individuals with "complications" of grade 1 or 2 were classified as having had no complications (and grouped with all individuals with no reported complications).

In study II, analyses were undertaken using the OC scores in different ways. For one analysis the number of OCs with severity level 3 or above was calculated for each subject to form a continuous variable as described above. In the other analyses, the subject sample was divided into groups comprising subjects above or below a set cut-off for OCs. The cut-off level was one or more complications of severity 3 for total pregnancy and trimester scores, and one or more complications of level 4 for labour-delivery scores. For the composite OC-score (Total OCs) the cut-off level was two complications of severity level 4 and above. Furthermore, the cut-off for severe OCs was one or more complications of severity level 5 or 6. A complication of severity level 5 is defined as a complication that is "potentially clearly greatly relevant/harmful" (e.g. severe preeclampsia or foetal asphyxia), and a complication of level 6 is defined as a complication that causes "very great harm to or deviation in offspring" (e.g. eclampsia, offspring hypoxic-ischemic cerebral injury). Finally, to increase comparability to previous studies, a foetal hypoxia variable was constructed according to Cannon et al (2002b) was estimated. Foetal hypoxia was considered present if asphyxia was recorded or if the infant was coded blue at birth, or if two or more of the following occurred: umbilical cord knotted or wrapped tightly around the neck at least once, placental infarcts, third-trimester bleeding, preeclampsia, maternal anaemia, maternal anorexia, foetal heart rate deviations, breech presentation, or premature birth (<week 37).

In study IV, the severe OCs cut-off variable as described under study 2 were utilized alone. Severe OCs were present in 28 of the total 108 subjects, including 15 of the patients and 13 of the controls. Most subjects with severe OCs (25/28) had severity 5 OCs, the remaining 3 subjects with a complication of grade 6 all being born before

gestational week 33. The recorded severe OCs were as follows: Prolonged birth (13 subjects), meconium-stained amniotic fluid (5 subjects), asphyxia (3 subjects), prematurity < 32 weeks (3 subjects), placental abruption (2 subjects), bleeding during first trimester (2 subjects), meconium aspiration syndrome (2 subjects), prematurity < 35 weeks (2 subjects), twin transfusion syndrome (1 subjects), mid-forceps delivery (1 subject), small for gestational age (1 subject), acute caesarean section (1 subject), and low birth weight (1 subject). 17 subjects were exposed to one severe OC, and the maximum number was five complications in one subject. The number of subjects with OCs as described above are listed in Table 2.

Table 2. Obstetric variables and complications in the current subject sample

	Patients (n=54)		**Controls (n=54)**		**p-values**
	Mean (SD)	**Range**	**Mean (SD)**	**Range**	
Birth weight (g)	3494 (635)	1770-5630	3397 (665)	1460-4720	0.44
Head circumference (cm) n=105	33.8 (1.5)	30-37	33.7 (1.6)	28-36	0.77
Maternal age (years)	27.5 (5.6)	17-43	27.9 (5.6)	18-39	0.72
Gestational age (weeks)	39.2 (1.9)	32-42	39.4 (2.2)	31-43	0.52
Continuous OCs score	6.0 (5.3)	0-28	5.5 (4.6)	0-23	0.53
	Number	**%**	**Number**	**%**	
Total OCs	21	39	26	48	0.33
1. trimester	6	11	6	11	1
2. trimester	11	20	10	19	0.81
3. trimester	24	44	21	39	0.56
Total pregnancy	25	46	21	39	0.44
Labour-delivery	28	52	24	44	0.70
Severe OCs	15	28	13	24	0.66
Hypoxia	12	22	7	13	0.21

3.5 Genotyping

For study IV, 32 schizophrenia-related single nucleotide polymorphisms (SNPs), located in four hypoxia-regulated genes, were selected for genotyping. The SNPs were genotyped as part of the Scandinavian Collaboration on Psychosis Etiology (SCOPE) project, a Scandinavian collaborative study on the genetics of psychotic disorders. The SNPs were selected on the basis of previous positive findings from other research groups and independent hypotheses on genes involved in psychosis, neurodevelopmental pathways or CNS function. Genomic DNA was extracted from whole blood samples, and SNPs located in *DTNBP1* (11 SNPs), *GRM3* (5 SNPs), and *NRG1* (12 SNPs) genes were genotyped at the SNP Technology Platform at Uppsala

University and Uppsala University Hospital, Sweden (www.genotyping.se), using the Illumina BeadStation 500GX and the 1536-plex Illumina Golden Gate assay (Illumina Inc., San Diego, CA, USA) (Jonsson et al., 2009). The four SNPs in the *BDNF* gene were genotyped by pyrosequencing (Ahmadian et al., 2000) or cleavage with restriction enzymes (Jonsson et al., 2006). The sample success rate was on average 99.8% for the genotyped SNPs. Hardy–Weinberg (HW) equilibrium was tested in affected and controls using Fisher's exact test as implemented in PEDSTATS (Wigginton & Abecasis, 2005).

3.6 Statistical analyses

The statistical analyses in the four current studies were performed by the use of three different statistical software tools.

1. SPSS version 16.0 (SPSS Inc, Chicago IL), all demographics, study I, and the analysis of the anatomical cortical parcels in study III
2. Statistical functions within the FreeSurfer program (http://surfer.nmr.mgh.harvard. edu), study II and the vertex wise analysis in study III.
3. SAS software (SAS/STAT® software, version 9.1.3, SAS institute Inc., Cary, NC), study IV.

Statistical differences in demographic and obstetric variables between patient and control groups were evaluated using Chi-Square tests, independent sample Student's T-tests and Mann–Whitney non-parametric tests.

Multiple linear regression analyses were used to test the effect of OCs on basal ganglia volumes (study I), local gyrification in 68 anatomical cortical parcels (study III), and corresponding case control differences. The structure (or cortical parcel) was the dependent variable. Age, ICV, diagnosis, and OCs were the independent variables for the basal ganglia analyses; sex was excluded from these analyses due to confounding with ICV. Age, sex, diagnosis, and OCs were the independent variables for the gyrification analyses,. Model fit was assessed by analysing residuals and cook distances.

A general linear model (GLM) was used to test the effect of OCs on each vertex of the cortical mantle (study II), vertex wise local gyrification (study III), and hippocampus (including the gene-OCs interaction) (study IV). In study II, separate analyses for patients and controls for each obstetric variable were conducted, with a DODS (different onset, different slope) model contrast in patients or controls with OCs to patients without OCs respectively. For the continuous OCs variable, separate DOSS (different onset same slope) analyses were conducted in patients and controls. The DODS analyses included cortical thickness as the dependent variable, various

dichotomous OCs scores as fixed factors, and age as a covariate. In the DOSS analysis, cortical thickness was the dependent variable, a continuous OCs measure the predictor variable, and age was co-varied for. In study III analyses of the vertex wise association between OCs and local gyrification were conducted in patients and control separately and in the combined group, with age and sex as covariates.
A mixed linear model was used in study IV. In a first preliminary analysis on the hippocampal volume of the left and right hemisphere without SNP markers, diagnosis, severe OCs, the interaction between diagnosis and OCs, intracranial volume, age at MR scanning, and hemisphere side were treated as fixed factors, whereas individual was treated as a random factor. To test whether the effects of fixed factors differed between hemispheres, all interactions with hemisphere were included in the original model. Since the disease effect did not vary with presence/absence of severe OCs, the interaction between diagnosis and OCs was excluded from the statistical model. Since the effects of diagnosis, OCs, intracranial volume, and age at MR scanning on hippocampal volume did not differ significantly between the right and left hemispheres, the analyses were carried out on the average of left and right hemisphere hippocampal volumes. A linear model was used to carry out single marker allele association of the hippocampal volume including the number of minor alleles and excluding hemisphere in the above model for each SNP separately. A mixed linear model was used to test whether the effects of diagnosis, OCs, and the interaction between OCs and RS13242038 on HCV depended on method of delineation (manual/automatic). In these analyses diagnosis, OCs, intracranial volume, age at MRI, number of minor alleles of RS13242038, and delineation method were considered as fixed factors, and individual as a random factor. To account for differences in reliability of methods, a separate residual variance was used for manually and automated HCV measurements, respectively.

Multiple comparison control was performed with three different methods according to the nature of the statistical analysis performed. The Bonferroni method was applied in study I and the analysis of cortical anatomical parcels in study III. The Bonferroni corrected significance threshold is found by dividing the α-level (in the current studies 0.05) by the number of tests performed (n), α/n. The false discovery rate (FDR) was used to control for multiple tests (>160.000 tests in each hemisphere) in study II and the vertex-wise part of study III. Finally, for the interaction effect of 32 SNPs and OCs on hippocampal volume (study IV), multiple comparison control was done by randomly permuting genotype among individuals 1000 times and re-analyzing the permuted data (Westfall and Young 1993). Thus genotypes and phenotypes were decoupled keeping the original LD structure of the genetic markers and the covariance

structure of phenotypes intact in each permuted data set. The adjusted p-value was defined as the fraction of permutations where the minimum p-value from allele associations, (or from its interaction with severe OC), was less than, or equal to, the minimum p-value in the original data.

4. Summary of studies

Initial analyses showed that 1) there was equal distribution of number or severity of OCs in the patients and the controls, and 2) the full range of OCs from 3 or lower to 6 was present in both groups.

Study 1

"No effect of obstetric complications on basal ganglia volume in schizophrenia"

Based on the fact that dopamine metabolism is both a core feature in schizophrenia and vulnerable to OCs, we hypothesized that number and severity of OCs would be related to basal ganglia volume in schizophrenia patients but not in healthy controls. We thus analyzed and compared the effect of OCs on the volume of four basal ganglia structures (nucleus accumbens, nucleus caudatus, globus pallidum, and putamen) in patients with schizophrenia and healthy control subjects.

A linear regression model was applied to study 1) case control differences in basal ganglia volumes (co-varying for intracranial volumes and age), 2) medication effects of typical vs. atypical vs. no use within the patient group (co-varying for age and ICV), and 3) the association between OCs and each of the basal ganglia volumes (co-varying for age, ICV, and case-control status). For reasons of validity, the basal ganglia volume measurements obtained by FreeSurfer were compared to volume measures previously obtained by a different software (BRAINS) or manually delineated.

Patients demonstrated larger globus pallidum volumes than control subjects ($p<0.0005$), while caudate- ($p=0.18$), putamen- ($p=0.49$), and accumbens ($p=0.20$) volumes did not differ between patients and controls. No statistically significant effects of anti-psychotic medication given at the time of MRI on any of the four measured basal ganglia volumes were found. OCs were not significantly related to any of the basal ganglia structures after proper correction for multiple comparisons, but uncorrected results demonstrated a positive relationship between number and severity of OCs and nucleus accumbens volume ($p=0.034$) independent of diagnostic group. Also, no interaction effects of diagnosis and OCs were demonstrated.

In conclusion, the results did not support the hypothesis. With the current severity of OCs, basal ganglia tissue volumes as studied by MRI appear unaffected by a history of OCs regardless if subjects have schizophrenia or not.

Study 2

"Cerebral cortical thickness and a history of obstetric complications in schizophrenia"

Cortical thickness differs in schizophrenia patients and healthy controls. As OCs affect cortical morphology in animal models, we hypothesized that that number and/or severity of OCs and/or foetal hypoxia would be significantly associated with thinner brain cortices, and that this association would differ between schizophrenia patients and healthy controls.

A general linear model was used to study the effect of nine different measures on OCs and foetal hypoxia on cortical thickness, (for each trimester, pregnancy in total, and labour, a combined OCs score, a continuous OCs score, a severe OCs score, and a hypoxia score). First, analyses of case-control differences (without OCs scores) were conducted with thickness as the dependent variable and age as covariate. Second, analyses were conducted for each of the nine OCs scores in patients and controls separately, with age as a covariate. None of the obstetric variables differed between patients and controls.

Significant cortical thinning in schizophrenia patients, as compared to healthy control subjects, was demonstrated for the temporal pole and superior temporal gyrus bilaterally, in the left hemisphere superior frontal gyrus, and in the right hemisphere planum temporale, insula, and superior orbital gyrus, after multiple comparison control by FDR.

No significant relationships between any of the nine OCs scores and cortical thickness were found in either patients or controls.

In conclusion, even by testing a broad range of OCs measures, no relationships between OCs and cortical thickness were found. This result suggests that OCs, at the current levels of severity, do not explain the thinner cortices found in the present sample of patients with schizophrenia as compared with healthy controls.

Study 3

"Cortical folding in Broca's area relates to obstetric complications in schizophrenia patients and healthy controls."

Cortical folding occurs prenatally and remains by and large stable postnatally. Thus differences in cortical folding between schizophrenia patients and healthy controls may reflect aberrant pre-natal neurodevelopment in schizophrenia. We hypothesized (1) that OCs would be related to cortical folding as measured by a 3D surface-based local gyrification index (*l*GI), (2) that this relationship would differ in schizophrenia patients as compared to healthy control subjects.

First, case-control differences in the *l*GI were analysed, with age and gender as covariates. Two case-control analyses were performed, vertex-wise and as average measures of 34 pre-defined anatomical cortical areas (parcels) in each hemisphere. From the vertex-wise analysis, uncorrected results displayed reduced cortical folding in schizophrenia patients in an area corresponding to parts of the pre- and post-central gyri bilaterally, and the right middle temporal gyrus (at $p<0.01$). No case-control differences were demonstrated in any of the two analyses after proper multiple comparison control.

Then, the relationship between OCs and *l*GI was analysed. Results from the vertex-wise OCs analyses were suggestive of a relationship between a higher number of OCs and lower local GI in lower *l*GI in the left inferior frontal and prefrontal sulci and gyri at p-level of 0.01, but the findings did not survive FDR control for multiple comparisons.

By analysing the average *l*GI in anatomical cortical parcels, a significant relationship between higher number of OCs and lower *l*GI in the left pars triangularis (corresponding to Brodman area 45 and parts of Brocas area) was found ($p<0.0005$, surviving Bonferroni correction). Five additional parcels in the left hemisphere demonstrated similar results; fusiform ($p=0.011$), lateraloccipital ($p=0.037$), parahippocampal ($p=.007$), pars opercularis ($p=0.021$), rostralmiddlefrontal ($p=0.007$). These five p-values did not remain significant after Bonferroni correction for 68 tests. There were no interaction effects between OCs and case-control status.

In conclusion, lower cortical gyrification in the left pars triangularis of both schizophrenia patients and healthy control subjects demonstrated a statistically significant relationship with increased number of OCs after multiple comparison control. That reduced cortical folding in the left pars triangularis was associated with OCs in both patients and control subjects suggest that the cortical effect of OCs may be caused by factors shared between schizophrenia patients and healthy controls rather than factors related to schizophrenia alone.

Study 4

"An exploratory model for G x E interaction on hippocampal volume in schizophrenia; obstetric complications and hypoxia related genes"

OCs and single nucleotide polymorphism (SNP) variation in schizophrenia susceptibility genes have independently been related to hippocampal volume. On account of hypoxia being a core feature of OCs and a strong modifier of gene expression, we hypothesized that the effect of OCs on hippocampal volume could be modified by variation in hypoxia-regulated genes, and tested the hypothesis for 32 SNPs spanning 4 genes (*DTNBP1, NRG1, GRM3, BDNF*).

First, putative case-control differences in hippocampal volume were tested. Patients with schizophrenia demonstrated, on the average, 5% smaller hippocampal volume than healthy control subjects, and this reduction was present in both the left and right hemispheres (p=0.001)

Second, the effect of OCs on hippocampal volume was analysed. The hippocampal volume was on the average larger in individuals with severe OCs, as compared to individuals without (p=0.048); the average effect was 3.6% independent of diagnosis ($p_{diagnosis*OCs}$=0.25).

Third, the SNP x OCs interaction effect was explored. The larger hippocampal volumes observed in individuals with severe OC were significantly related to the *GRM3* rs13242038-T alleles (p=0.014), after adjustment for multiple testing).

We re-analyzed a random sub-sample (n=60) of the study-group, using previous manual delineations of hippocampal grey matter volume (Agartz et al., 2006). In this sample, individuals who had experienced severe OCs (n=17) demonstrated smaller hippocampal grey matter volumes, with the volumes being 5.9% smaller. The mixed model analysis of GxE interaction in manually and automatically derived hippocampal volume confirmed that the effect of severe OC on hippocampal volume was dependent on rs13242038 genotype ($p_{OCs*genotype}$= 0.004), and indicated that the interaction effect between OCs and the GRM3 locus differed between hippocampal volume measurements ($p_{OCs*genotype*method}$ = 0.016). For automatically-derived measurements, the larger hippocampal volume associated with severe OCs were only evident in individuals carrying the rs13242038 T-allele (p=0.026), whereas reduction in hippocampal grey matter associated with severe OC was only evident in individuals who were homozygous for the rs13242038 C-allele (p=0.041).

In conclusion, the effect of severe OCs on hippocampal volume was associated with allele variation in *GRM3* rs13242038 in both schizophrenia patients and healthy controls, but the result must be interpreted with caution due to the limited subject sample size.

5. Discussion

To summarize, the main results from the four studies included in this thesis were:

1. Occurrence of OCs and representativity:

Rate or severity of OCs did not differ between schizophrenia patients and healthy control subjects. The full range of OC were represented in both groups.

2. No evidence of association with OCs:

A history of OCs did not affect basal ganglia volumes (study I) or cortical thickness (study II).

3. Evidence of associations with OCs:

OCs affected cortical folding in the left pars triangularis (study III). The hippocampal volumes (study IV) demonstrated a significant association with OCs.

4. Effect of gene x OCs interaction:

The effect of OCs on hippocampal volume appeared to be modulated by allele variation in the hypoxia-regulated *GRM3* gene (study IV)

5. No specific effects of OCs in schizophrenia patients:

The findings were equal in patients and healthy controls. There was no evidence that brain morphology in patients with schizophrenia was more or differently affected by OCs than in healthy controls.

6. Case-control differences in brain morphology

Schizophrenia patients demonstrated significantly larger globus pallidum volumes (study I), prefrontal and temporal cortical thinning (study II), smaller hippocampal volumes (study IV), and near significantly lower cortical folding in the pericentral cortex (study III), when compared to healthy controls.

In this section I will first address the significance of the results from the four studies included in this thesis by discussing them in the light of previous empirical findings (5.1). Then I will consider theoretical aspects of how the current findings may contribute to the understanding of the relationship between OCs and brain structure in schizophrenia (5.2). Methodological issues, including strengths and limitations in the four current studies, will be discussed (5.3), before I will add some final comments on biological causality (5.4).

5.1. Comparisons to previous empirical findings

5.1.1 Effects of OCs on brain morphology

Contrary to our expectations, two of the current studies reported negative results; no associations were demonstrated between OCs and basal ganglia volume (study I) and

cortical thickness (study II). Both studies are, to the best of our knowledge, the first scientific studies to investigate the hypothesized associations. The current negative results need to be replicated in independent samples.

In study I, no relationship was found between OCs and basal ganglia volumes (accumbens, caudatus, pallidum, and putamen) in either schizophrenia patients or healthy controls. Animal models have demonstrated that OCs such as caesarean section (El-Khodor & Boksa, 2001) and perinatal anoxia (El-Khodor & Boksa, 1997) have the propensity to alter striatal dopamine metabolism (Berger et al., 2000;Winter et al., 2008), and striatal cytoarchitecture (Bernert et al., 2003;Juarez et al., 2008). This study indicate that previously reported alterations of basal ganglia volumes in first-episode schizophrenia (Ebdrup et al., 2010;Ellison-Wright et al., 2008) are caused by other factors than OCs. Such factors may be related to neurodevelopment (Glenthoj et al., 2007), genes (Rajarethinam et al., 2007), or the disease process itself. In accordance with our findings, Bersani and colleagues reported no effect of OCs on the bicaudate ratio (an indirect measure of caudate/ventricle size) in (n=47) male schizophrenia patients (Bersani et al., 2009).

From animal models, the strongest effect of OCs on basal ganglia structure and function was an accumbens effect (El-Khodor & Boksa, 1997;Juarez et al., 2008). The current results demonstrated a relationship between increasing number of OCs and larger volume of nucleus accumbens that was not significant after multiple comparison control. The importance of this almost significant finding is uncertain. The borders of the nucleus accumbens are difficult to delineate on MR images, both automatically and manually obtained measurements have sub-optimal validity (see 5.3.3). This may have affected the findings. In addition, important confounding factors include effects of the long illness duration among the schizophrenia patients (mean duration was 17.8 years), and the long-term use of antipsychotic medication, known to affect basal ganglia volumes (Scherk & Falkai, 2006;Smieskova et al., 2009), that may both have masked subtle volume alterations related to OCs. Basal ganglia volume did, however, not differ between patients using typical, atypical, or no antipsychotic medication.

In study II, no relationships between nine different OCs constructs and cortical thickness were found in either schizophrenia patients or healthy controls. Cannon et al. (2002b) have previously reported that foetal hypoxia predicted smaller grey matter cortical volume, most prominent in the temporal lobe, in 64 schizophrenia patients and their healthy siblings (n=51) but not in healthy control subjects (n=54) in a ROI based study from Finland. An explanation as to why the present findings differ from those of Cannon et al., even though the same definition of foetal hypoxia was used, is

likely to be that cortical thickness and grey matter volume measures address different properties of the cerebral cortex. Reduced cortical volumes may be caused by reduced cortical thickness or cortical area or both (from the equation cortical volume= cortical thickness ∗ cortical area) (Voets et al., 2008). The use of a state-of the-art-method such as FreeSurfer that measures cortical thickness at submillimeter intervals across the whole cortical mantle, makes for high accuracy in anatomical localisation and extent of pathological changes, and cortical thickness effects of OCs should have been found if they existed. The differing results form our and Cannon et als. (2002b) study may reflect cohort effects, or the results form Cannon et als study may have been caused by effects on cortical area and not on thickness.

Other factors that have been reported to influence on cortical volume or cortical grey matter density, such as duration of illness (Sun et al., 2009;van Haren et al., 2007) and medication use (McClure et al., 2006), could potentially have affected the results. Medication use has, however, previously been studied in a larger subject sample (n=203) which included the present (n=108), and did not show any effect on cortical thickness (Nesvag et al., 2008) or cortical volumes (Nesvag et al., 2007).

The current findings indicate that the cortical thinning observed in studies of schizophrenia occurs independently of OCs, and that OCs with the current severity do not cause cortical thinning as reported in adolescents with very low birth weight (VLBW) (Martinussen et al., 2005), or in children exposed to maternal opiate abuse in utero (Walhovd et al., 2007).

The timing of the origin of the cortical thinning in schizophrenia in uncertain. Cortical folding abnormalities may originate from the prenatal period as the pattern of cortical folding is known to be by and large completed at birth (Armstrong et al., 1995). In study III we report a significant relationship between increasing number of OCs and lower cortical folding in the left pars triangularis (Broca's area) in both schizophrenia patients and healthy controls. In addition, associations between OCs ad cortical folding, in the same direction, were found in five other parcels in the left hemisphere, but did not remain significant after strict Bonferroni correction for multiple comparisons.

The relationship between OCs and cortical folding in schizophrenia has previously been studied by Falkai et al. (2007) in a subject sample comprising 29 schizophrenia patients, 21 healthy family members, and 13 family members with other psychiatric illness. By using a two dimensional (2D) method[6] and six coronal MRI slices, no

[6] By using two dimensional methods the gyrification indec (GI) is calculated from coronal MRI slices or post-mortem brains by dividing the inner contour (along the pial surface) on the outer contour (following the brain surface).

relationship between OCs and cortical folding was demonstrated. The 3D surface-based FreeSurfer method measures cortical folding at submillimeter intervals across the entire cortex, and has a greater sensitivity than the 2D method (Schaer et al., 2008). The differing results from study III and Falkai et al's (2007) study may either reflect a cohort effect, or they may reflect relationships between OCs and cortical folding that the use of a more sensitive method allowed us to detect.

Taken together, the cortical findings from studies II and III suggest that cortical folding patterns are a more robust brain morphological correlate to early neurodevelopment (in the current studies in the form of OCs) than cortical thickness. That cortical thickness reductions appear to occur at a later stage than the pre- or perinatal period is in line with the findings from a previous study on cortical thickness and folding patterns in adolescents with psychosis (Janssen et al., 2009). Further support comes from Schaer et al. who have reported that congenital heart disease (presumed to cause lower oxygen delivery to the brain) in patients with 22q11 deletion syndrome was related to altered cortical folding patterns in the brain but not to cortical thickness (Schaer et al., 2009).

The left pars triangularis is of particular interest as it is part of Broca's area. Broca's area is of importance to several cognitive domains including language formation (Bhojraj et al., 2009), semantic encoding (Demb et al., 1995), semantic retrieval (Badre & Wagner, 2007), syntactic processing (Friederici et al., 2003), and syntactic working memory (Fiebach et al., 2005). Disturbances in neurocognitive domains related to Broca's area have been reported in schizophrenia ((Mesholam-Gately et al., 2009) for review) but also in preterm/WLBW children ((Arnoudse-Moens et al., 2009) for review), and in children suffering perinatal asphyxia (Stevens et al., 1999). In line with the present findings on brain morphology but concluded from studies of neurocognitive function, severe pre- and perinatal trauma appear to have adverse effects regardless if the subject develops schizophrenia, other psychiatric disorders, or remains healthy (Soria-Pastor et al., 2009;Nosarti et al., 2008). This emphasizes the importance of studying other factors that may (or may not) interact with OCs to affect brain anatomy in schizophrenia patients differently than in healthy controls. As heredity is an important risk factor for schizophrenia (Sullivan et al., 2003;Tsuang, 2000), it is of interest to study if genetic variation in molecular markers related to schizophrenia and/or neurodevelopment interact with OCs to affect brain anatomy in schizophrenia patients.

To investigate such a model, in study IV, we explored a possible gene-environment interaction effect on hippocampal volume. It is to our knowledge the first study ever to investigate interaction effects of OCs and SNP variation (or any molecular markers) on

hippocampal volume (or any brain volume) in schizophrenia. Contrary to previous studies (Ebner et al., 2008;McNeil et al., 2000;Schulze et al., 2003;Stefanis et al., 1999;van Erp et al., 2002), OCs caused *larger* hippocampal volumes independent of diagnosis when the FreeSurfer measurements were used. The measures include the hippocampal formation along with the fimbria white matter (Fischl et al., 2002;Makris et al., 1999;Morey et al., 2009). The previous studies, in which OCs caused *smaller* hippocampal volumes, used volume measures of the hippocampus proper which *included* mainly hippocampal grey matter and *excluded* the fimbria (Ebner et al., 2008;McNeil et al., 2000;Schulze et al., 2003;Stefanis et al., 1999;van Erp et al., 2002). The contradictory results could be caused by OCs having a differential effect on grey and white matter, which has been suggested by findings from animal models; as a result of perinatal asphyxia, both neuronal degeneration in the hippocampal CA1 region (grey matter) (Boksa, 2004;Rees et al., 2008), and hippocampal gliosis (white matter) (Bernert et al., 2003) have been reported. In addition, prenatal hypoxia has been demonstrated to exert a different effect on grey and white matter depending on the timing as well as the severity of the hypoxic insult (Rees & Inder, 2005). In the subset (n=60) of the current subject sample, OCs were related to smaller volumes of the grey matter in hippocampus proper (as obtained by manual delineations). In this subset, the automated hippocampal formation volume measures (from FreeSurfer) remained larger in subjects with a history of OCs, which is a finding that excludes that a cohort effect would explain the results. This is supportive of a possible differential effect of OCs on grey and white matter that needs to be further investigated.

The suggested modulating effect of SNP variation in the *GRM3* rs13242038 allele on the association between OCs and hippocampal volume was present for both the hippocampal formation (FreeSurfer measure) and the hippocampus proper (manual delineations), regardless of diagnostic group. The finding finds support by results from an animal models in mice where antenatal hypoxia caused a 2-4 fold reduction in mGluR3 expression (the receptor encoded by the *GRM3* gene), and the down-regulation in gene activity was linked to a decreased vulnerability to hypoxia-induced white matter damage (Fontaine et al., 2008). The same response was not found in rats, which may suggest that genetic factors underlie *GRM3* regulation and the susceptibility to white matter damage in rodents. OCs-gene interaction effects on hippocampal volume are supported by a different animal model, where prenatal infection have been demonstrated to alter gene-expression and affect hippocampal volume in rats (Fatemi et al., 2009).

The findings from study IV must be considered to be preliminary because of the relatively limited sample size, and the fact that the rs13242038 polymorphism,

located in the first intron of the *GRM3* gene, has no known function. However, the findings are plausible given the results from the animal models (Fatemi et al., 2009;Fontaine et al., 2008), and the biological fundament for an interaction effect between OCs (with hypoxia) and allele variation in genes regulated by hypoxia (such as *GRM3*) (Nicodemus et al., 2008;Schmidt-Kastner et al., 2006). The *GRM3* gene has been reported to be involved in neurodevelopment (Harrison et al., 2008;Melchiorri et al., 2007). mGluR3 is expressed in the hippocampus (Lyon et al., 2008), and receptor expression has been reported to be altered following transient brain ischemia (Raghavendra Rao et al., 2002).

The fact that the current OCs-gene interaction effect was present in both schizophrenia patients and healthy controls suggests that hippocampal vulnerability to severe OCs could be influenced by factors independent of those related to schizophrenia pathogenesis; the effect may be modified by SNP variation in genes that are associated with schizophrenia risk (e.g. the *GRM3* gene) but also occurs in subjects without the illness. The effect of OCs on hippocampal volume in siblings of schizophrenia patients has been reported to be different from the effect in the schizophrenia patients, but the interaction with diagnostic group was not statistically significant (van Erp et al., 2002). In another study, OCs were associated with reduced hippocampal volume also in the relatives (mothers/fathers/siblings) of schizophrenia patients (Ebner et al., 2008). The effects of OCs on hippocampal volume may be associated with shared genetic underpinnings of both hippocampal volume vulnerability to OCs and of schizophrenia susceptibility.

5.1.2 Differences in brain morphology and frequency of OCs between schizophrenia patients and healthy controls

One of the aims of this thesis was to explore if case-control differences in brain morphology between schizophrenia patients and healthy controls could be attributed to a history of OCs. In all four studies, patients demonstrated brain anatomical abnormalities as compared to healthy controls. Patients demonstrated significantly larger globus pallidum volumes (study I), which is in line with previous studies (Brandt & Bonelli, 2008;Mamah et al., 2007). We also found thinner cortices (study II) in schizophrenia patients in the left and right hemisphere temporal and frontal lobes, the same regions as in a previous study from our group of a larger subject sample (n=203) that included the present (Nesvag et al., 2008), and in line with other previous studies (Goldman et al., 2009;Kuperberg et al., 2003;Schultz et al., 2010). Since no effects of OCs on either basal ganglia volume or cortical thickness were found in study I and II, we can conclude that the case-control differences in basal ganglia volume and cortical thickness appear to be unrelated to a history of OCs with the current severity.

In study III, significant cortical folding differences between patients and controls were present in parts of the pericentral cortex bilaterally, and in the right middle temporal gyrus, before controlling for multiple comparisons. Although no statistically significant case-control differences remained after such control, a larger study of 400 subjects (including the present sample) by our research group demonstrated significantly reduced cortical folding in parts of the left pericentral cortex (Nesvåg et al., in prep) this corresponding to the case-control differences in the current smaller subject sample. The effect of OCs on cortical folding in study III was, however, located in a different brain region (the left pars triangularis) than the case-control differences. The findings from this study indicate that subjects with both schizophrenia and OCs have reduced cortical folding both in the left pars triangularis and in the left pericentral cortex. Accordingly, if (a) schizophrenia disease *per se* is related to abnormal brain morphology independently of OCs, and OCs both (b) increase the risk for schizophrenia (as generally demonstrated in the literature (Cannon et al., 2002a)), and (c) are also independently related to other brain morphological abnormalities, then schizophrenia patients may – in a larger context – tend to be subjected to at least a double "dose" of brain morphological abnormalities, even if the "doses" result from separate factors.

Further evidence for independent effects of OCs and diagnosis on brain morphology can be inferred from study IV. Patients had smaller hippocampal volumes when compared to control subjects, which is a consistent finding in previous scientific studies (Ebdrup et al., 2010;Honea et al., 2005;Shenton et al., 2001). In the current study, OCs were related to larger (when measured by FreeSurfer) and smaller (when measured by manual delineation) hippocampal volumes regardless of diagnostic group. This suggests that case-control differences in hippocampal volume in the current study are not related to OCs. To sum up, all four studies included in the thesis indicate that case-control differences in the investigated brain structures cannot be attributed to OCs.

In the current subject sample, schizophrenia patients and healthy controls had the same frequency of OCs, and the same obstetric characteristics e.g. birth weight, gestational age, or maternal age. This is in contrast with the large epidemiological studies that report significant case-control differences in birth weight (Nilsson et al., 2005), low birth weight in combination with low gestational age (Jones et al., 1998), and obstetric complications such as perinatal asphyxia (Dalman et al., 2001) and maternal bleeding (Hultman et al., 1999). However, in previous clinical studies with subject sample sizes comparable to ours, there were no significant differences in birth

weight (Onstad et al., 1992) or in the frequency a variety of different OCs (Kendell et al., 2000) between schizophrenia patients and healthy controls.

The frequency of OCs in the current schizophrenia group and healthy controls is within variance reported in studies of OCs and brain morphology and OCs-gene interaction (see Table 3). Importantly, within both schizophrenia patients and healthy control subjects, OCs occurred with a high enough frequency to detect their effects on brain morphology, if such effects exist (and they do in study III and IV).

Table 3. Number with OCs in each group in previous studies ingestigating the relationship between OCs and brain morphology or OCs-gene interaction in schizophrenia (scz), siblings and controls. Studies included in the list are those where number of subjects and OCs were clearly stated.

Author	Subject sample	OCs definition	Number with OCs	Comment
Cannon et al, 2002	64 scz patients, 51 siblings, 54 controls	Hypoxia by own definition	15 (23%) of scz patients, 13 (25%) of siblings, and 12 (22%) of controls	Hypoxia definition similar to ours in study II
Ebner et al., 2008	30 scz patients, 34 siblings	McNeil-Sjöström scale	Pregnancy:19 (63%) of patients, 25 (74%) of siblings. Labour: 12 patients (40%) and 15 (44%) siblings Neonatal: 22 (73%) patients and 20 (59%) siblings	OCs cut-off at severity level 3
Falkai et al., 2007	29 scz patients, 13 siblings with psychiatric disorder, 21 healthy siblings	McNeil-Sjöström scale	Average OCs number was 2.10 for scz patients, 3.23 for patients with psych. disorder, and 2.29 for healthy siblings	Number of complications equal or > 3
Nicodemus et al., 2008	116 scz patients	McNeil-Sjöström scale	29 (25%) of scz patients	Cut off at severity level 5, as in our study IV
Schulze et al., 2003	110 subjects in total, comprising scz patients, relatives , and controls	Lewis–Murray scale	18 % of familial scz probands, 62% of non-familial scz probands, 19% of familial relatives, 13% of non-familial relatives, and 19% of controls	OCs recorded as definite.

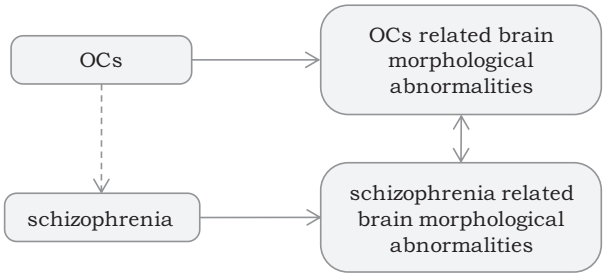
5.2 Theoretical considerations from the empirical findings

The reported findings are internally consistent in that the effects of OCs on brain morphology had equal distribution in schizophrenia patients and healthy controls in all four studies, and when effects of OCs were found, they were not related to case-control differences (study III and IV). Obviously, caution must be exercised when

attempting to infer theoretical conclusions of the empirical findings from one subject sample. The points raised below illustrate how the current findings may be relevant to the understanding of possible relationships between OCs and brain structure in schizophrenia, but they are by no means conclusive.

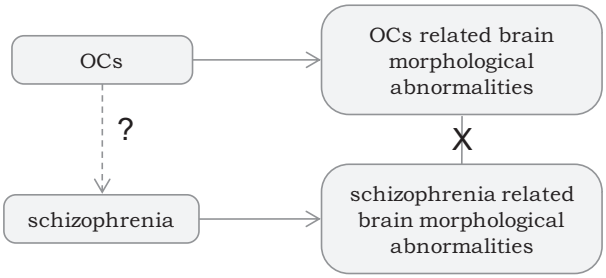
When designing the current studies, our initial hypothesis and model for all studies in this thesis was that the brain morphological abnormalities observed in schizophrenia patients, as compared to healthy controls, could be attributed to abnormal neurodevelopment resulting from OCs (as illustrated in figure 5). If this were true, then OCs would correspondingly be expected to be more frequent or more severe in the schizophrenia group, or possibly cause a negative effect on the brain through an interaction with schizophrenia susceptibility genes or other factor not known.

Figure 5. The hypothesised model of the relationship between OCs, schizophrenia, and brain morphological abnormalities



The empirical findings from the current studies supported, however, a different model (illustrated in figure 6). We found that OCs either had no effects on brain morphology (studies I and II) or affected brain morphology (studies III and IV) in different regions (study III) or in a different direction (study IV) than would fit with causing the case-control brain differences.

Figure 6. The empirical model of the relationship between OCs, schizophrenia, and brain morphological abnormalities, based on the findings from the studies included in the current thesis



When discussing the current findings, the "equifinality" and "multifinality" concept may be of use.

Equifinality describes a phenomenon where several different developmental processes or distinct causes can lead to the same outcome (Waldman, 2003). If "the outcome" is defined as schizophrenia, the current findings of equal distribution of OCs within the patient and control groups do not support OCs acting alone to be part of an equifinality pathway for schizophrenia, although we cannot exclude the possibility that the current studies were underpowered to detect such a putative association (see 5.3.4). If the broader class of brain morphological abnormalities is defined as "the outcome", then the current findings support brain morphological abnormalities in schizophrenia and abnormalities related to OCs to be the results of different equifinality pathways. With both definitions of the outcome, the equifinality concept cannot be meaningfully used to understand or interpret the implications of the current findings.

Multifinality describes a phenomenon where one particular developmental process or distinct cause can lead to a variety of outcomes (Waldman, 2003). Multifinality can be constructed as an example of moderation, where the relation between a cause and the outcome depends on other variables (or moderators). Within this concept, OCs can be defined as "one particular process" involved in the pathogenesis of either schizophrenia (Rapoport et al., 2005), other psychiatric (Verdoux & Sutter, 2002) or somatic disorders (Gluckman et al., 2008), or they may lead to no illness at all (multifinality) if the outcome measures are health or diagnostic status. If the outcome measure is brain morphology, the current findings demonstrate a variety, with no effect of OCs on some of the structures analysed (the cortical thickness and basal ganglia volumes), but an effect on other structures (cortical folding and hippocampal volume). Within this framework, OCs may affect brain structure in both schizophrenia patients and healthy controls, because the variation in outcomes is not defined to include diagnosis. The concept can be used to interpret the current findings as specific with regard to OCs, but non-specific with regard to schizophrenia. Such an interpretation opens the possibility for other genetic and environmental factors, in addition to or in interaction with OCs, to be of importance for the development of schizophrenia or abnormalities of brain morphology as described in schizophrenia.

A distinction between "the deficits model" and "the risk model" for outcomes of early developmental trauma has been postulated from studies in primate species (Schneider, 2003). The "deficits" model describes cause-effect relationships wherein specific early life events result in pathology later in life. In contrast, in the "risk model" specific early life events, along with other events, are considered as probabilistic

contributors to altered developmental outcome, in an dynamic and complex interaction process (Schneider, 2003). The risk model is related to multifinality. It is consistent with a theoretical framework supported by the current findings, where OCs may cause brain morphological alterations, but where the association with schizophrenia risk and pathology, if existent, appears to be a result of interaction with genetic variation or other risk factors, rather than effects of OCs alone. With regards to the theoretical framework for this thesis, the current findings do not directly contradict the neurodevelopmental hypothesis of schizophrenia, as the hypothesis does not postulate OCs to be the sole cause of the brain morphological alterations observed in schizophrenia (Fatemi & Folsom, 2009;Lewis & Murray, 1987;Rapoport et al., 2005;Weinberger, 1987).

5.3 Methodological issues

5.3.1 Pre and perinatal complications

The assessment of OCs in adult schizophrenia faces methodological challenges. First, information on pregnancy and delivery complications as collected from mothers (maternal recall) have been demonstrated to be inaccurate (Buka et al., 2004;Cantor-Graae et al., 1998). Mothers of schizophrenia patients report a higher level of complications than what had been described in the corresponding birth records (McIntosh et al., 2002). This bias has been avoided in the present thesis by using original birth records as the information source on obstetric events. The use of data from historical sources (in this case hospital birth records) distinguishes "follow-back" from "retrospective" studies (Keshavan, 2003). The obstetric journals provide accurate information on diagnosis and medical interventions (including medication) given throughout pregnancy and labour.

Information on environmental factors of putative harm to the foetus may, however, have been missing. Maternal smoking through pregnancy was recorded for two mothers, all others were scored as non-smokers; this may constitute a limitation since it is not certain whether or not these mothers were true non-smokers or not. Prenatal nicotine exposure has been demonstrated to be related to schizophrenia risk in one study (Zammit et al., 2009), but not in others (Ellman et al., 2007;Baguelin-Pinaud et al., 2010). *In utero* nicotine exposure have been demonstrated to decrease total number of cells in the brain (Dwyer et al., 2009). Furthermore, exposure to environmental toxins and alcohol consumption among mothers was not systematically recorded. This constitute a limitation, since *in utero* alcohol exposure has been related to a variety of brain morphological alterations, some of which are hippocampal and basal ganglia volume reductions (Norman et al., 2009). Severe hunger was not present

in Sweden during the period when the study subjects were born; if their mothers had been severely malnourished, this would have been recorded in the medical birth records. Despite the problems of possibly missing data, the use of birth records as the source of obstetric information is generally acknowledged to be superior to maternal information because of the problems of recall bias in the latter.

Second, uncertainty exists as to how one should rate and value the various OCs occurring in any sample (McNeil et al., 1994). As described in chapter 1.4, several different complications, e.g. perinatal asphyxia, maternal infection, and low birth weight, have been demonstrated to increase schizophrenia risk (Cannon et al., 2002a;Dalman et al., 2001;Hultman et al., 1999;Nilsson et al., 2005;Rosso et al., 2000), and different complications may be inter-related. In clinical samples, individual complications occur too infrequently to permit assessing their effect separately. For instance, severe prematurity was present in three subjects in the current sample. To facilitate studies of OCs in clinical samples, scales that add and/or grade different complications have been constructed, e.g. the McNeil-Sjöström scale (McNeil & Sjostrom, 1995) and the Lewis-Murray scale (Lewis et al., 1989). The McNeil-Sjöström scale is the more comprehensive, it allows testing of different stages of the gestational process, and it has been demonstrated to be the most sensitive scale

Third, it may prove difficult to define the optimal or correct cut-off level in dichotomous OCs constructs, or to decide if continuous or dichotomous variables are the most appropriate. By grouping subjects into exposed or non-exposed, potential dose-related effects may be obscured (Mayes & Ward, 2003). By defining cut-offs, one implies that complications above the cut-off threshold have a qualitatively different effect on the studied structure (in the case of MRI studies) or on schizophrenia risk (in the case of risk studies). The biological correlates of such thresholds may be valid, e.g. as presence or absence of a diagnosis of asphyxia, or more uncertain e.g. when a birth weight of 2500 g is categorised as a non-complication and 2499 g is categorised as a complication. Cut-off scores were used in study II and study IV to enable comparisons of our results with results from other studies (Cannon et al., 2002b;Nicodemus et al., 2008). Nevertheless, in study II, the use of cut-off scores was supplemented by a continuous variable that also showed negative results. Thus in studying OCs, it remains to be determined in general whether it is more appropriate to use continuous or dichotomized OC scores, how to decide on valid cut-offs for dichotomized scores, and whether and how different OCs should be combined into larger constructs.

5.3.2 The schizophrenia diagnosis and concept

Schizophrenia is a broad diagnostic entity. The schizophrenia diagnosis is atheoretical, and based on the presence of clinical symptom criteria (American

psychiatric association, 1994). The scientific validity of the schizophrenia diagnosis has been discussed (Blom 2003; Keshavan 2008). Epistemic values, that is values needed for a scientific construct to be valid, include: internal coherence, external consistency, predictive accuracy, fertility, simplicity and validity (Blom, 2003). The operationalisation of diagnostic criteria (i.e. descriptive criteria such as in DSM-IV) has increased the reliability of psychiatric diagnostics (Fulford et al., 2006). Three acknowledged aspects of heterogeneity in schizophrenia are the aetiology, phenotype, and biology of the illness (Keshavan et al., 2008). This challenges the internal coherence of the diagnosis.

The interpretations of the current findings rely on the schizophrenia group to be a valid construct that is distinct from the group of healthy controls. This was, as far as clinically possible, ascertained by the thorough diagnostic procedures in the HUBIN study. The diagnostic procedures have been validated by comparing the structural interviews with psychiatric records and register data (Ekholm et al., 2005;Vares et al., 2006), and the diagnostic concordance rate between three psychiatrists was good to excellent (Ekholm et al., 2005).

The heterogeneity of the illness may account for the fact that effects of various OCs on e.g. brain morphology and schizophrenia risk are small and non-consistent. Of relevance to the current studies, the phenotypic heterogeneity regarding clinical symptomatology may reflect exposure to OCs and genetic variation. There is some evidence that a history of OCs is related to clinical features/sub-groups of schizophrenia. Patients with a high score of negative symptoms (as recorded by PANSS) have been demonstrated to have significantly higher prevalence of OCs when compared to patients with low PANSS scores (Ruiz-Veguilla et al., 2008), and more OCs and lower Apgar score than patients with predominantly positive symptoms (Kotlicka-Antczak et al., 2001). OCs have also been related to earlier age of illness onset (Cannon et al., 2000;Verdoux et al., 1997). Thus the results from the current four studies might have been different if we had studied only the patients with a high prevalence of OCs and an earlier age at onset, and also if we had studied another subject sample with different clinical characteristics and exposure to OCs.

5.3.3 MRI acquisition and post-processing

MR images are digital reconstructions of which the quality is dependent on e.g. scanner properties and pulse sequence parameters. In clinical studies, it is of importance to use the same scanner and scanner parameters for all subjects, and to avoid scanner upgrades during the period of the data collection. By scanning patients and control subjects concurrently potential false case-control differences caused by scanner drifting can be avoided. All these precautions were taken during the

acquisition of the MR images used in the current studies. Motion and flow artefacts, caused by blood flow or CSF circulation, may occur on MR images of the brain. In the present studies we used Gradient echo (GRE) sequences, which to some extent prevent CSF pulsation artefacts (Weishaupt et al, 2003).

The main image post-processing method was FreeSurfer. Cortical reconstruction (in study II and III) by this method is accurate and extremely detailed (Kuperberg et al., 2003;Schaer et al., 2008). For sub-cortical segmentation, reliability has been demonstrated to be good for the hippocampus, caudate, putamen, and pallidum volumes (Fischl et al., 2002;Morey et al., 2009) and across within-scanner multiple acquisitions (Jovicich et al., 2009). The FreeSurfer volume measures of ICV accumbens are more problematic.

The ICV measure in FreeSurfer is obtained from T1-weighted images only. The contrast between CSF and the surrounding tissue is ideal when both T1 and T2 weighted images are used. Accordingly, measurements of ICV from T1 and T2 images as obtained by the software BRAINS was used in studies I and IV. The use of two different MRI post-processing methods may have affected the results from study I and IV.

The borders of nucleus accumbens are difficult to delineate, both automatically and manually, due to its anatomy. In the anterior aspect, artefacts of the lateral ventricles are hard to differentiate from the boundary of the nucleus accumbens, and in the posterior aspect, it is difficult to discriminate the boundary between the nucleus accumbens and the substantia innominata because of their contiguity (Tamagaki et al., 2005). The low correlation between the automated and manual accumbens volumes (see *3.3.3*) indicate that the lack of effect of OCs on accumbens may be less reliable than for putamen and caudate (Study I).

5.3.4 Statistics

In all studies linear associations between OCs and brain structure have been studied, by the use of linear regression (studies I and III), a general linear model (studies II and III), and a mixed linear model (study IV). Thus, we cannot exclude that non-linear relationships may have been overlooked.

In studies I and IV, where subcortical structures were investigated, intra cranial volume (ICV) had to be controlled for. ICV was highly correlated with gender in the current subject sample. To avoid problems of multi-collinearity, gender was excluded as a variable. Gender did, however, not affect the basal ganglia or hippocampal volumes, when ICV was included in the full models.

In order to minimize the risk for false positive findings, the use of a proper method for multiple comparison control is of importance. Multiple comparisons correction in the

current studies was performed with different methods, that reflect the characteristics of the analyses in each study. The Bonferroni method[7] (study I and III) has a strong control of type I errors, but the method has been argued to be too conservative, at least when more than five tests are performed (Altman, 1991). Thus, the use of Bonferroni correction in study I with only four tests appear appropriate, giving a new p-value threshold of 0.05/4= 0.0125. By applying this correction, the relationship between increasing number and severity of OCs and larger accumbens volume did not remain statistically significant. In study III with 68 tests the Bonferroni method of correction may have been too strict, giving a new significance threshold at 0.05/68= 0.00074. Still, the relationship between increasing number of OCs and lower local gyrification index in the left pars triangularis remained significant. Applying a Bonferroni-Holm[8] correction in study III did not alter the number of parcels in which OCs were significantly related to lower cortical folding.

In the cortical surface analyses (studies II and III), as many as 162 000 tests were performed in each hemisphere, that is one test for each vertex across the cortical mantle. The Bonferroni method has been reported to be too sensitive for this kind of analysis (Genovese et al., 2002). The false discovery rate (FDR) for multiple comparison control incorporated in the FreeSurfer analysis algorithms was applied for the FreeSurfer studies (studies II and III). With this method the significance threshold is determined not by number of tests, but by the results, where 5% (at an FDR threshold of 0.05) of the p-values below a given threshold (e.g. 0.01) are expected to be false. Thus the FDR corrected threshold will vary with number of findings.

For the genetic analyses in study IV, multiple comparison control was done by permutation testing, a method that has been recommended for genetic association analyses (Dudbridge, 2008;Westfall & Young, 1993).

In all clinical studies, the risk of type II errors needs be balanced with the risk of type I errors[9]. As we have applied relatively strict multiple comparison corrections, there is a possibility that positive associations between OCs and brain morphology, that would have been found in a larger subject sample, may have been overlooked. Also, the power to detect differences in exposure to OCs between schizophrenia patients and healthy controls was only 37% (given an expected OR of 2 for schizophrenia with a history of OCs (Clarke et al., 2006), and a frequency of OCs of 25%).

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[7] The Bonferroni corrected significance threshold is found by dividing the α level (in the current studies 0.05) on the number of tests performed (n), α/n.

[8] With the Bonferroni Holm method, the corrected threshold is found by reducing n in the original α/n Borferroni correction by 1 for each observed p-value starting from the lowest (the one with the highest significance)

[9] Type I errors: the null hypothesis (H_0) is rejected when it is in fact true, type II errors: the null hypothesis is not rejected when it is in fact false.

5.4 Comments on biological causality

The reported effects of OCs on brain morphology in the current studies are all statistical effects, and do not establish biological causality. The hypotheses in this thesis were partly based on biological knowledge of harmful consequences to neurodevelopment caused by OCs from experimental animal models; OCs have the propensity to cause lasting brain morphological alterations in e.g. sheep (Rees et al., 2008), rats (Fatemi et al., 2008), and monkeys (Short et al., 2010). Disturbed dopamine metabolism and disordered animal behaviour (Boksa, 2004) thought to parallel the disordered behaviour observed in schizophrenia have been reported as an effect of OCs exposure. However, translating findings from animal models explored in a controlled environment to findings in humans, in which environmental conditions are hard to control, is difficult. Even the most robust animal models of neurodevelopment and OCs effects face limitations since human brain development has a far greater complexity (Rees et al., 2008).

In humans, MRI-studies of premature and low birth weight children and adolescents demonstrate a relationship between severe OCs and brain morphology (Kesler et al., 2006;Martinussen et al., 2005). There is a possibility that the current findings are confounded by the relatively high age of the subjects included, and the long illness duration among patients. Nevertheless, age was co-varied for in the statistical analyses, and the findings were the same in healthy controls. The extended time between birth and age at study inclusion allows for exposure to factors that could potentially affect brain morphology, e.g. hormonal and epigenetic changes, or toxin-, drug-, and alcohol exposure.

The gene-OCs interaction effects explored in study IV were based on biologically plausible hypoxia-related mechanisms (Schmidt-Kastner et al., 2006). However, the limitations above apply also to this study. Furthermore, two types of gene-environment interplay exists; the genotype may correlate with the environmental experience (OCs), or the genotype may change the sensitivity to the environmental experience (Eaves et al., 2005). Either way of interplay may be an additional confounder of the biological plausible relationships that were investigated.

6. Conclusion and future directions

From the four studies included in this thesis, it has been demonstrated that OCs affect certain aspects of brain morphology (hippocampal volume and cortical folding) whereas other aspects (cortical thickness and basal ganglia volumes) are unaffected by OCs of the current severity. Genetic variation may modulate the effect of OCs on hippocampal volume.

The effects of OCs on brain morphology were the same in the patients with schizophrenia and the healthy control subjects, and differences in brain morphology between schizophrenia patients and healthy controls could not be attributed to a history of OCs.

Suggestions for future research:

The studies presented in the doctoral thesis raised a number of more spesific questions to be answered in future studies:

Will the examination of single OCs (e.g. asphyxia, maternal infection) reveal effects on basal ganglia volumes and cortical thickness that were not found with the use of a combined OCs measure?

Are there functional consequences of reduced cortical folding in Broca's area in the form of neurocognitive disturbances?

A possible differential effect of OCs on hippocampal grey and white matter needs to be further investigated. Is there a biological correlate?

Does the G x E interaction effect between hypoxia-regulated schizophrenia susceptibility genes and severe OCs on hippocampal volume, replicate in a larger sample? Can interaction effects with genotypes of functional relevance be found?

Is there an G x E interaction effect between severe OCs and genotype on brain structures that were not investigated?

The effects of OCs on brain structure was similar in schizophrenia patients and healthy controls. Are there different OCs effects in the brain in patients with other diagnoses e.g. autism and anorexia (in which OCs may increase risk of disease), bipolar disorder (where OCs are not known to be related to increased risk) or somatic disease (e.g. hypertension or diabetes where adverse foetal events are known to affect disease risk)?

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Cortical folding in Broca's area relates to obstetric complications in schizophrenia patients and healthy controls

Haukvik UK[1], Schaer M[2], Nesvåg R[1,3], McNeil T[4], Hartberg CB[1], Jönsson EG[5], Eliez S[2], Agartz I[1,5,6].

1. Department of Clinical Medicine, section Vinderen, University of Oslo, P.O. Box 85 Vinderen, N-0319 Oslo, Norway
2. Service Médico-Pédagogique, Department of Psychiatry, Geneva University School of Medicine, P.O. Box 50, 1211 Geneva 8, Switzerland
3. Department of Psychiatry, Vinderen DPS, Diakonhjemmet Hospital, P.O. Box 85, N-0319 Oslo, Norway
4. Department of Psychiatric Epidemiology, Lund University Hospital USiL, SE-221 85 Lund, Sweden
5. Department of Clinical Neuroscience, HUBIN project, Karolinska Institutet and Hospital, R5:00, SE-171 76 Stockholm, Sweden
6. Department of Psychiatric Research, Diakonhjemmet Hospital, P.O. Box 85, N-0319 Oslo, Norway

Corresponding author
Unn Kristin Haukvik,
Department of Clinical Medicine, University of Oslo
P.O. Box 85 Vinderen
N-0319 Oslo, Norway
Phone: +47 22029839, Fax: +47 22495861
email: unn.haukvik@medisin.uio.no

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Abstract

Background: The increased occurrence of obstetric complications (OCs) in patients with schizophrenia suggests that alterations in neurodevelopment may be of importance to the aetiology of the illness. Abnormal cortical folding may reflect subtle deviation from normal neurodevelopment during the foetal period. In the present study, we hypothesised that OCs would be related to cortical folding abnormalities in schizophrenia patients corresponding to areas where patients with schizophrenia display altered cortical folding when compared to healthy controls.

Methods: Fifty-four schizophrenia patients and 54 healthy control subjects underwent clinical examination and MRI scanning on a 1.5 T scanner. Information on OCs was collected from original birth records. An automated algorithm was used to calculate a three dimensional local gyrification index (*l*GI) at numerous points across the cortical mantle.

Results: In both schizophrenia patients and controls, an increasing number of OCs was significantly related to lower *l*GI in the left pars triangularis ($p<0.0005$) in Broca's area. Five other anatomical cortical parcellations in the left hemisphere displayed a similar trend. No relationships between OCs and *l*GI were found in the right hemisphere, and no case-control differences in *l*GI were demonstrated.

Conclusion: The reduced cortical folding in the left pars triangularis associated with OCs in both patients and control subjects suggests that the cortical effect of OCs may be caused by factors shared by schizophrenia patients and healthy controls rather than factors related to schizophrenia alone.

1. Introduction

The increased prevalence of pre- and perinatal complications in schizophrenia patients is supportive of a neurodevelopmental origin of the illness (Lewis & Murray, 1987;Marenco & Weinberger, 2000;Weinberger, 1987). Subtle deviances from normal brain development may be reflected in altered brain morphology (Fatemi & Folsom, 2009). In schizophrenia patients, smaller hippocampi, larger ventricles, and reduced cortical thickness and volume have relatively consistently been reported (Glahn et al., 2008;Honea et al., 2005;Steen et al., 2006). In animal models, various obstetric complications (OCs) have been demonstrated to cause both brain morphological alterations and behavioural aberrances that parallel those observed in schizophrenia ((Boksa, 2004) for review). In magnetic resonance imaging (MRI) studies of schizophrenia patients, OCs have been related to smaller hippocampi (Ebner et al., 2008;Schulze et al., 2003;van Erp et al., 2002), larger lateral ventricles (Falkai et al., 2003;McNeil et al., 2000), and reduced cortical volume (Cannon et al., 2002). Taken together, these findings suggest that early somatic trauma such as OCs may exert an influence on neurodevelopment, detectable in the brain decades later. However, the brain morphological alterations reported in schizophrenia may also reflect medication use (Smieskova et al., 2009), illness progression (Tanskanen et al., 2008;van Haren et al., 2007), genetic variation (van Haren et al., 2008), or other illness-related factors.

Alterations in cortical folding patterns may be a brain morphological correlate of aberrant neurodevelopment. The process of cortical gyrification is under genetic control (Piao et al., 2004), but environmental factors have also been demonstrated to be of importance (Bartley et al., 1997). The early stages of gyrification appear around gestational week 16, with a rapid increase in cortical gyrification in the third trimester of pregnancy (Armstrong et al., 1995). The gyrification index (GI), defined as the ratio between the pial and the arachnoideal surface as observed in coronal slices of post-mortem brains or MRI scans (Zilles et al., 1988), demonstrates a steady increase until post-natal week 6, from which the GI remains by and large stable (Armstrong et al., 1995). The human gyrification process may be a result of tension-based mechanisms. Viscoelastic tension exerted by cortical fibres draw regions with greater connectivity closer together (forming gyri) and thereby reduces the transit time of the action potentials (Van, 1997;White et al., 2010). Cortical folding patterns may thus convey information on underlying cortical organization and complexity. As a consequence, gyrification measures demonstrate properties related to both neurodevelopment and cortical organization, aspects that are of importance in schizophrenia.

Several studies on gyrification abnormalities in schizophrenia have been conducted with heterogeneous findings as a result. Both prefrontal hypergyria (higher GI) (Falkai et al., 2007;Harris et al., 2007;Vogeley et al., 2000;Vogeley et al., 2001) and lower prefrontal (Bonnici et al., 2007) and global (Cachia et al., 2008;Sallet et al., 2003) GI, as well as negative findings (Highley et al., 2003) have been reported. Gyrification abnormalities have

been reported to be present before adult illness onset (Harris et al., 2004a;Harris et al., 2004b). A flattening of sulcal curvature along with peaking of gyral curvature has been reported in childhood- and adolescence-onset schizophrenia patients (White et al., 2003). It is however uncertain if early somatic trauma such as OCs influences the gyrification process in schizophrenia. To our knowledge, thus far only one study has examined the relationship between OCs and gyrification in schizophrenia. Falkai et al. found no effect of OCs on a two-dimensional (2D) based gyrification index in schizophrenia patients (n=29) and their relatives (n=21 healthy + 13 with psychosis)(Falkai et al., 2007).

The human cortex is a highly complex three-dimensional (3D) structure. Measuring cortical folding from 2D coronal MRI slices, might lead to loss of information related to buried sulci and gyral anomalies in sublobar regions. In the present study we use a 3D surface-based automated algorithm to calculate the local gyrification index in each vertex across the whole cortical mantle (http://surfer.nmr.mgh.harvard.edu/fswiki/LGI) (Schaer et al., 2008). With this method, subtle localized deviances in cortical folding may be detected with greater precision.

Hypotheses:
Based on the previous literature, in the present study we hypothesised (1) that OCs would be related to cortical folding as measured by a 3D surface-based local gyrification index, (2) that this relationship would differ in schizophrenia patients and healthy control subjects, and (3) that the hypothesised difference would correspond to areas where schizophrenia patients demonstrate altered cortical folding as compared to healthy controls.

2. Methods and materials

2.1 Subject characterization

This study was part of the Human Brain Informatics Project (HUBIN) at the Karolinska Institutet, Stockholm, Sweden. HUBIN is a comprehensive database of genetic, brain morphological, neuropsychological, and clinical information obtained from schizophrenia patients and healthy subjects. The subject inclusion took place between 1999 and 2003. All participants gave written informed consent. The project was approved by the Research Ethics Committee at Karolinska Institutet and the Swedish Data Inspection Board ("Datainspektionen"). The study was performed in accordance with the Helsinki Declaration. The subject sample consisted of unrelated Caucasian men and women currently resident in the Stockholm Area, and has previously been thoroughly described (Haukvik et al., 2009;Jonsson et al., 2006). Briefly; invited patients from the out-patients clinic underwent a comprehensive clinical assessment protocol using validated operational instruments (Ekholm et al., 2005;Vares et al., 2006) including verification of diagnosis by a trained psychiatrist (EGJ). Handedness was ascertained by the means of asking the patients which

hand preference when writing, using a scissor and throwing/catching a ball. Patients fulfilled DSM-III-R or DSM-IV criteria for schizophrenia (n=50) or schizoaffective disorder (n=4).

Control subjects were recruited from hospital staff, their relatives, or from a population register. The control subjects included in the present study were interviewed by the same trained psychiatrist (EGJ) and had no previous or current psychiatric disorders according to a semi-structured diagnostic interview. They were matched to the patients by age and gender (on a group level).

Exclusion criteria for all subjects were a history of head trauma with loss of consciousness > 5 minutes, current treatment for substance abuse, and/or somatic disorders affecting brain function. Demographic characteristics, duration of illness, age at onset, and use of anti-psychotic medication are described in Table 1.

2.2 MRI

2.2.1 MR scan acquisition

MR images were obtained at the MR Research Centre at Karolinska Institutet, Stockholm, Sweden, using a 1.5 Tesla GE signa Echo-speed (Milwaukee, Wis., USA) scanner. T1-weighted images were obtained using a 3D spoiled gradient recalled (SPGR) pulse sequence with the following parameters: 1.5 mm coronal slices, no gap, 35 flip angle, repetition time 24 ms, echo time 6.0 ms, number of excitations 2, field of view 24 cm, acquisition matrix 256x192. All scans included were visually judged to be without obvious motion artefacts. A trained neuroradiologist evaluated all scans to be without gross pathology.

2.2.2 MR scan post-processing

First, cortical reconstructions were obtained from T1-weighted images using the automated computer software FreeSurfer version 3.0.2. Second, we used the *l*GI algorithm (http://surfer.nmr.mgh.harvard.edu/fswiki/LGI) to compute vertex-wise measurements of local gyrification at thousands of vertices across the cortical mantle. In addition, measurements of the average local gyrification index were calculated in 34 pre-defined anatomical cortex parcellations (Desikan et al., 2006) in each hemisphere (Table 2). The *l*GI method is adapted from the classical gyrification index (2D-GI), which is the ratio of the total pial cortical surface over the perimeter of the brain delineated on coronal sections. The present method, *l*GI, iteratively quantifies GI in circular three-dimensional regions of interest. After the creation of an outer envelope that tightly wraps the pial cortical surface, local measurement of circular GI is computed for each vertex of the outer surface as the ratio of corresponding regions of interest (ROI) on the hull and pial meshes is created. Delineation of the ROI on both the outer surface (ROIO) and pial surface (ROIP) uses a matching algorithm based on geodesic constraints, so that the ROIP takes into account the entire patch of the cortical surface delineated by the ROIO circular perimeter. This means that at the end of the computational process, individual *l*GI cortical maps reflect the amount

of cortex buried within the sulcal folds in the surrounding circular region. The method has been thoroughly described and validated (Schaer et al., 2008).

2.3 Assessment of OCs

Information on OCs was collected from hospital birth records. Subjects were born between the years of 1943 and 1982. Obstetric care in Sweden has been of high quality all this period, and the birth records were very detailed. The information was scored according to the McNeil-Sjöström scale (McNeil & Sjostrom, 1995) by a physician who was blinded to patient/control status and MRI-results (UKH). The McNeil-Sjöström scale rates OCs according to severity at an ordinal scale from 1-6, where severity level one signifies a "not harmful or relevant" event and 6 signifies "very great harm to or deviation in offspring". The scale has been constructed for the use in studying the effect of OCs in clinical case-control studies in which individual complications (e.g. low birth-weight, prematurity or placental abruption) occur too infrequently to be assessed separately (McNeil et al., 1994). In the present study, the number of OCs with severity scores of 3 and above was calculated for each individual subject to form one continuous variable. Scores under 3 are considered to be not harmful to the foetus (Haukvik et al., 2010;McNeil & Sjostrom, 1995). Obstetric characteristics are presented in Table 1.

2.4 Statistical analyses

Statistical differences in demographic and obstetric variables between patient and control groups were evaluated using Chi-Square tests, Independent Samples T-tests and Mann-Whitney non-parametric tests in the SPSS version 16.0 (SPSS Inc, Chicago IL.). The main analyses were performed vertex-wise as well as with predefined cortical areas (parcellations)

2.4.1 Vertex-wise analyses

a) At first, vertex-wise analysis of case-control differences in *l*GI across the whole cortical mantle was conducted contrasting schizophrenia patients and healthy controls, with *l*GI as the dependent variable, and age and gender as covariates.

b) Thereafter, vertex-wise analyses with OCs as independent variable (with age and gender as covariates) and *l*GI as the dependent variable were conducted in patients and control subjects both separately and combined.

The vertex-wise analyses were conducted with a general linear model within FreeSurfer, and a false discovery rate (FDR) of 0.05 was applied to correct for multiple comparisons (Genovese et al., 2002).

2.4.2 Parcellation analyses

a) Case-control differences of average *l*GI-values for 34 pre-defined cortical parcellations (41) in each hemisphere (total n=68), were investigated by multiple linear regression analyses

with the parcellation as the dependent variable, and diagnosis, age, and gender as independent variables.
b) Thereafter, the relationship between OCs and average *l*GI in the same 68 parcellations was explored in the combined sample using multiple linear regression analyses with OCs, age, gender, and diagnosis as independent and each parcellation as dependent variables. The diagnosis*OCs interaction term was added to the analysis for the parcellations in which OCs were related to the *l*GI at $p<0.05$.
All parcellation regression analyses were performed in SPSS, and Bonferroni correction was applied to control for the number of multiple regression analyses (α level 0.05/ 68 parcellations).

3. Results

3.1 Demographic and obstetric variables

Demographic variables were similar in patients and control subjects. There were no differences between patients and control subjects regarding number of OCs, or any of the other obstetric variables (Table 1).

3.2.1 Vertex-wise lGI in patients versus controls (2.4.1 a)

Schizophrenia patients displayed lower *l*GI than healthy control subjects in one cluster in the left parietal lobe and two clusters in the right hemisphere (in the temporal-, and frontal lobe) at a significance level of 0.01(Figure1). However, after FDR correction for multiple comparisons, there were no significant differences in *l*GI between patients and controls.

3.2.2.Vertex-wise lGI in relation to OCs in patients and controls (2.4.1 b)

In patients, increasing number of OCs was related to lower *l*GI in a large cluster in the left medial posterior temporal lobe, and in a smaller cluster in the left inferior frontal sulcus and gyrus ($p<0.05$, uncorrected) (Figure 2). In control subjects, OCs were related to lower *l*GI in a larger area in the left inferior frontal sulci and gyri ($p<0.05$); for this area, there was a similar inverse relationship between OCs-scores and *l*GI in patients and control subjects (albeit for a larger area in the control subjects) (Figure 2). When patients and control subjects were analyzed together, the lower *l*GI in the left inferior frontal sulci and gyri was related to increasing number of OCs ($p<0.01$) (Figure 2). After FDR correction for multiple comparisons the findings were non-significant.

3.3.1 Case-control differences in average lGI in cortical parcellations (2.4.2 a)

Patients displayed lower *l*GI values in the rostral middle frontal, precentral, postcentral, and bank of superior temporal gyrus parcellations bilaterally. In addition, patients demonstrated lower lGI in the left hemisphere supramarginal, superioparietal, middle temporal, and inferior temporal parcellations, and the right precuneus, pars opercularis, and isthmus

cingulate parcellations (all p values < 0.05). However, the findings were non-significant after Bonferroni correction for multiple comparisons of 68 parcellations

3.3.2 The relationship between OCs and average lGI in cortical parcellations (2.4.2 b)
In both schizophrenia patients and healthy controls, increasing number of OCs was significantly related to lower *l*GI in the left pars triangularis ($p<0.0005$) (Figure 3). This result remained significant after Bonferroni correction.
Five other parcellations in the left hemisphere (fusiform, lateral occipital, parahippocampal, rostral middle frontal, and pars opercularis) displayed a similar relationship to OCs (Figure 3), but these findings did not remain significant after Bonferroni correction for multiple testing (Table 2). There was no diagnosis*OCs interaction effect in either of 6 parcellations (data not shown). There were no significant relationships between OCs and *l*GI in the right hemisphere (Table 2).

4. Discussion
The main finding in the present study is that increasing number of OCs was significantly related to lower *l*GI in the left pars triangularis in both schizophrenia patients and healthy control subjects. A similar trend was also demonstrated for five other parcellations in the left hemisphere, whereas no relationship between OCs and *l*GI was demonstrated in the right hemisphere. This is the first time the relationship between OCs and a 3D- local gyrification index in schizophrenia has been investigated.

Effects of OCs on cortical folding
Studies of gyrification in premature infants have demonstrated increased temporal gyrification bilaterally as compared to term infants (Kesler et al., 2006), and higher sulcation index (a measure of cortical folding), when related to brain surface, in preterm intra-uterine growth restriction infants compared with “normal” preterm infants (Dubois et al., 2008). Although the present subject sample included three subjects born prematurely, the results are not directly comparable, as the range of obstetric severity is much larger in the present sample. However, the previous findings suggest that gyrification deviances may be related to adverse conditions during foetal brain development.

Only one previous study has investigated the effect of OCs on gyrification in schizophrenia (Falkai et al., 2007). From the two-dimensional gyrification index in six coronal MRI sections (three in the frontal and three in the parietal lobe), Falkai and colleagues did not find any relationship between OCs and gyrification in schizophrenia patients. The methodological differences may explain why the results in the present study differ from those by Falkai and colleagues. In the present study, the 3D surface-based approach allowed searching for multiple local alterations in gyrification across the whole cortical mantle. It is worth noting that the same definition and categorization of OCs were applied, OCs being scored with the

McNeil-Sjöström scale in both studies. This increases the comparability of the studies, and furthermore supports the present use of a surface based local gyrification method to investigate cortical folding patterns.

Smaller prefrontal and temporal cortical volumes have been reported in schizophrenia patients with a history of foetal hypoxia (Cannon et al., 2002). Both cortical folding patterns and cortical thickness affect cortical volume, but their relationship is uncertain. We have previously investigated the present subject sample for association between OCs and cortical thickness and found no association in schizophrenia patients or in healthy controls (Haukvik et al., 2009). Moreover, Schaer et al. have reported that congenital heart disease (presumed to cause lower oxygen delivery to the brain) in patients with 22q11 deletion syndrome was related to altered cortical folding patterns in the brain but not to cortical thickness (Schaer et al., 2009). Janssen et al. reported more widespread cortical thickness reductions than gyrification abnormalities in adolescent onset psychosis, and concluded that the cortical thickness reductions in schizophrenia appear to be caused by factors occurring after cortical folding is finished (Janssen et al., 2009). Taken together, the previous and the current findings suggest that cortical folding patterns may be a more robust brain morphological correlate of early neurodevelopmental aberrances than are measures of cortical thickness.

Pars triangularis

The relationship of OCs and *l*GI in the left pars triangularis is of particular interest, as pars triangularis together with pars opercularis are included under Broca's area. Broca's area is important to different aspects of neurocognitive functioning such as language formation (Bhojraj et al., 2009), semantic encoding (Demb et al., 1995), semantic retrieval (Badre & Wagner, 2007), syntactic processing (Friederici et al., 2003), and syntactic working memory (Fiebach et al., 2005). Aberrations in neurocognitive domains related to Broca's area have been reported in schizophrenia ((Mesholam-Gately et al., 2009) for review) but also in preterm/very low birth weight children ((arnoudse-Moens et al., 2009) for review), and in children suffering perinatal asphyxia (Stevens et al., 1999).

Increased metric distortion (as an indirect measure of cortical displacement and convolution) in the left pars triangularis (Wisco et al., 2007), and reduced sulcal index in Brocas area (Cachia et al., 2008) have been described in schizophrenia patients as compared to healthy controls. In contrast, Janssen et al have reported no relationship between *l*GI and adolescent-onset-psychosis and control status in the pars triangularis by using the same *l*GI algorithm as in the present study (Janssen et al., 2009). In the pars opercularis which is located adjacent to pars triangularis, post-mortem findings demonstrated no abnormalities in laminar neuronal densities, glial density, cortical thickness, or somal size in schizophrenia patients as compared to healthy controls (Selemon

et al., 2003). In the present study, the same relationship between higher number of OCs and lower *l*GI was found in pars triangularis and in pars opercularis, but for pars opercularis the p-value of 0.021 did not remain significant after multiple comparisons control. The cytoarchitecture in pars opercularis and pars triangularis has been reported to be more similar than the other cortical parcellations (Amunts K & Zilles K, 2006), and consequently the post mortem findings (Selemon et al., 2003) may be also transferable to the pars triangularis. We can only speculate that if so, the present findings of no case control differences in the *l*GI in this area may mirror a cytoarchitecture not affected by case control status but, intriguingly, rather by a history of OCs which have a similar untoward effect on both schizophrenia patients and healthy controls.

Parcellation versus vertex-wise analyses

In the present study, the parcellation analysis demonstrated that the reduced *l*GI in the left pars triangularis was not related to schizophrenia diagnosis but to increasing number of OCs in both patients and controls. This corresponds to results from the uncorrected vertex wise analyses on the effects of OCs performed in patients and controls separately. In patients only, the uncorrected vertex-wise analyses demonstrated a relationship between increasing number of OCs and lower *l*GI in the left temporal lobe, corresponding to the parahippocampal, fusiform, and lingual parcellations which might suggest a weak relationship in this cortex region among patients. The findings were however negative after multiple comparisons control, and it is uncertain if they represent a potential differential effect of OCs on cortical folding in patients with schizophrenia and healthy controls in this area. That the relationship between OCs and cortical folding was significant only in the parcellation analysis, may be a result of different methods of correction for multiple comparisons, or it may suggest that subtle differences in *l*GI between groups are easier to detect when measured as average *l*GI over a larger area (such as the pre-defined parcellations).

Case-control differences

No statistically significant differences (after multiple comparisons control) in *l*GI were found between patients with schizophrenia and healthy controls in neither pars triangularis, in any of the other parcellations, or in the vertex wise analyses. Thus the results do not support our hypothesis that OCs would affect *l*GI in areas where patients had a different folding pattern than control subjects, and no definite conclusions as to whether or not the hypothesised case-control differences in cortical folding were attributed to OCs in the current sample could be drawn. However, in a larger study by our group of approximately 200 schizophrenia patients and 200 controls, including the current subject sample, patients demonstrated significantly lower *l*GI in the left pericentral cortex (Nesvåg et al, in prep.). This finding corresponds to one of the areas where case-control differences that did not survive FDR control were found in the current study and provide some support that the

relationship between OCs and cortical folding is independent from putative case-control differences in the current subject sample.

In conclusion, the present study demonstrates a statistically significant relationship between OCs and the local gyrification index with a higher number of OCs related to a lower gyrification index in the pars triangularis of the left brain hemisphere in both schizophrenia patients and healthy control subjects. A similar trend was found for five other cortical anatomical areas in the left hemisphere. The findings suggest that a relationship between OCs and cortical folding may be caused by factors shared by schizophrenia patients and healthy controls rather than factors related to schizophrenia alone.

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Figure 1

Differences in local gyrification between patients and control subjects at p< 0.01 co-varied for age and gender, without FDR correction. Within the coloured areas, patients demonstrate lower cortical folding than healthy control subjects.

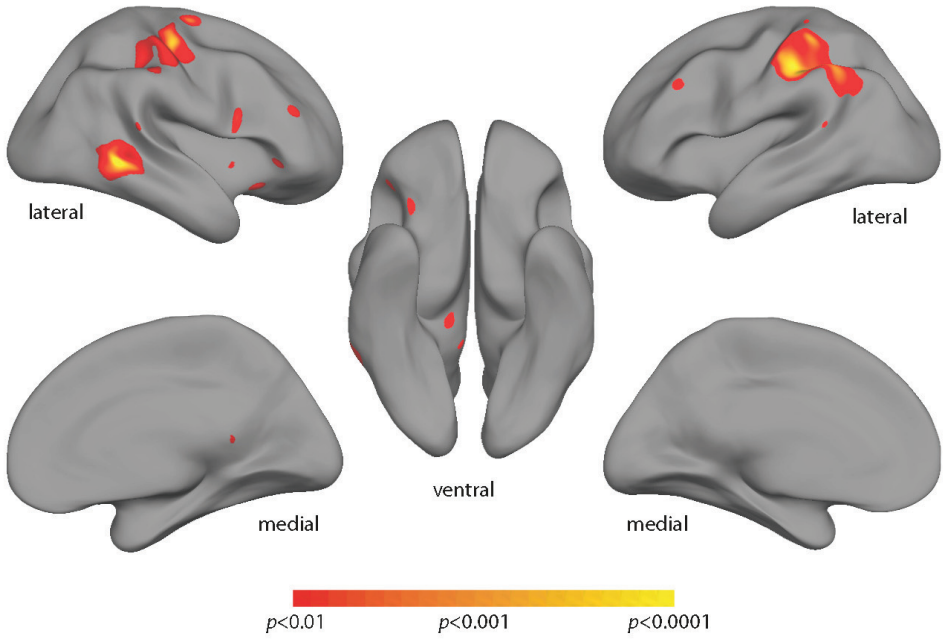

Figure 2

The effect of increasing number of obstetric complications on local gyrification in schizophrenia patients, healthy controls, and the combined sample, co-varied for age and gender at two different p-levels without FDR correction.

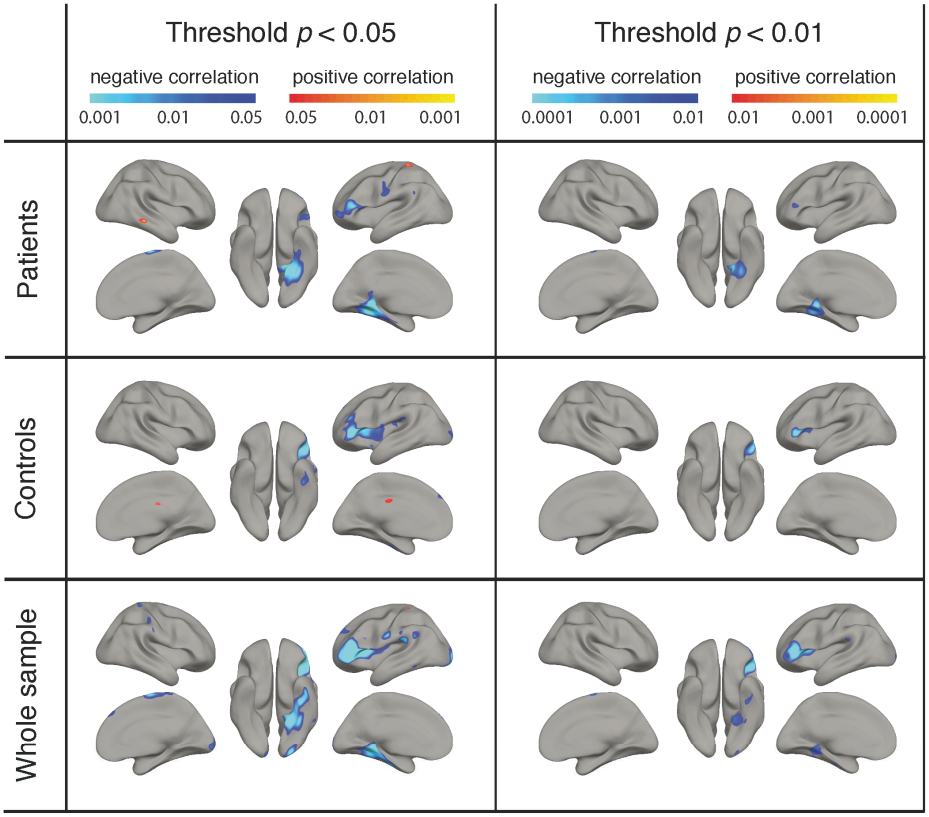

Figure 3

The effect of increasing number of obstetric complications on average local gyrification in pre-defined cortical areas in the left hemisphere, co-varied for age, gender, and diagnosis, significant at p<0.05. The red area remains significant after Bonferroni correction for multiple test. The colour map represents B-values, corresponding p-values are listed in table 2.

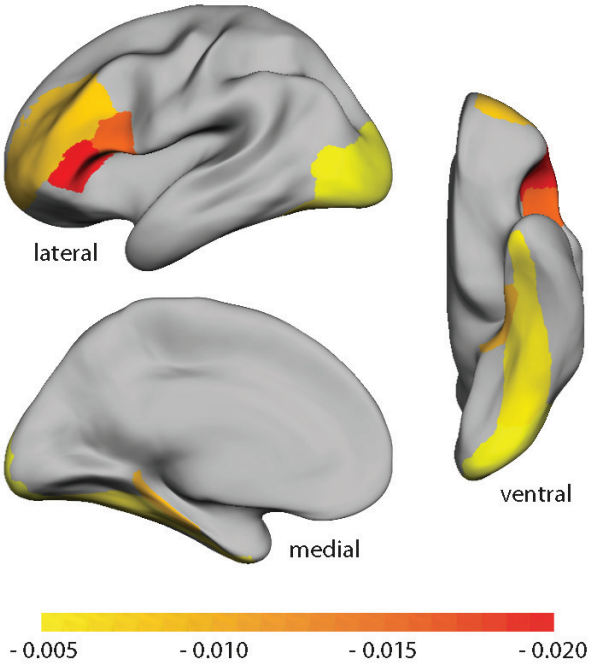

Table 1. Demographic and clinical characteristics in schizophrenia patients and healthy control subjects.

	Patients (n=54)		**Controls** (n=54)		**Statistics**	
	Mean	S.E.	Mean	S.E.	Test-value	p-value
Age at MRI (years)	41.9	1.1	41.5	1.2	t=.28	ns
Age at illness onset n=53 (years)	24.9	0.8	na			na
Duration of illness (years)	16.8	1.3	na			na
	Number	%	Number	%		
Gender (male/female)	37/17	68/32	33/21	61/39	χ^2=.65	ns
Handedness n=105 (right/left/ambidextr.)	48/2/2	92/4/4	48/3/2	91/6/4	χ^2=.19	ns
Medication (typical/atypical/none)	25/26/3	46/48/6	na			na

Table 2. Local gyrification index (*l*GI) of 68 paracellations in 54 schizophrenia patients and 54 healthy controls. Linear regression model with age, diagnosis, gender, and continuous obstetric complications (OCs). Bolded p-values remain significant after Bonferroni control for multiple comparisons.

Parcellation (Desikan)	**Mean *l*GI**	S.E.	**Age** p-value	**Diagnosis** p-value	**Gender** p-value	**OCs** Effect (B), p-value
lh_bankssts	3.3881	.01907	.004	.029	.038	ns
lh_caudalanteriorcingulate*	1.81832	.009010	ns	ns	.027	ns
lh_caudalmiddlefrontal*	3.00211	.014893	.004	ns	ns	ns
lh_corpuscallosum*	1.95189	.010382	ns	ns	ns	ns
lh_cuneus*	2.74741	.017314	ns	ns	.006	ns
lh_entorhinal	2.49633	.014219	ns	ns	**<.0005**	ns
lh_fusiform	2.56096	.012122	.009	ns	**<.0005**	B=-.006, p=.011
lh_inferiorparietal	3.09906	.014355	.001	ns	ns	ns
lh_inferiortemporal*	2.5795	.01137	.008	ns	ns	ns
lh_isthmuscingulate*	2.6629	.01936	ns	ns	.006	ns
lh_lateraloccipital	2.43191	.011829	.001	ns	.005	B=-.005, p=.037
lh_lateralorbitofrontal*	2.44943	.011551	.002	ns	ns	ns
lh_lingual	2.63081	.015502	ns	ns	**<.0005**	ns
lh_medialorbitofrontal*	1.90665	.008340	ns	ns	ns	ns
lh_middletemporal*	3.16071	.016529	ns	.022	ns	ns
lh_parahippocampal	2.75893	.017231	ns	ns	**<.0005**	B=-.009, p=.007
lh_paracentral	2.18237	.010015	.004	ns	.010	ns
lh_parsopercularis	4.10656	.028911	ns	ns	.004	B=-.014, p=.021
lh_parsorbitalis*	2.68776	.017992	.006	ns	ns	ns
lh_parstriangularis	3.50758	.026511	.001	ns	.014	B=-.020, p**<.0005**
lh_pericalcarine*	2.66573	.017317	ns	ns	.001	ns
lh_postcentral	3.38122	.015875	**<.0005**	.010	.006	ns
lh_posteriorcingulate*	2.09632	.012731	ns	ns	.010	ns
lh_precentral	3.33364	.016091	**<.0005**	.022	**<.0005**	ns
lh_precuneus*	2.7341	.01716	ns	ns	.004	ns
lh_rostralanteriorcingulate*	1.90406	.009434	ns	ns	.044	ns
lh_rostralmiddlefrontal	2.67035	.014785	**<.0005**	.048	ns	B=-0.008, p=.007
lh_superiorfrontal	2.0692	.00798	.001	ns	ns	ns
lh_superiorparietal	2.8117	.01269	.001	.040	ns	ns
lh_superiortemporal	3.98287	.022661	ns	ns	**<.0005**	ns
lh_supramarginal	3.45896	.016199	**<.0005**	.018	ns	ns
lh_frontalpole*	1.89652	.009168	ns	ns	ns	ns
lh_temporalpole*	2.21617	.012337	.040	ns	ns	ns
lh_transversetemporal	4.62608	.030140	ns	ns	**<.0005**	ns
rh_bankssts	3.42684	.023447	ns	.006	.029	ns
rh_caudalanteriorcingulate*	1.89577	.008739	ns	ns	ns	ns
rh_caudalmiddlefrontal	2.99039	.017083	.001	ns	.001	ns
rh_corpuscallosum*	2.00781	.008942	ns	ns	ns	ns
rh_cuneus*	2.87261	.017198	ns	ns	.045	ns
rh_entorhinal*	2.47533	.012615	ns	ns	.005	ns
rh_fusiform	2.50782	.010213	.041	ns	**<.0005**	ns

rh_inferiorparietal	3.11019	.013846	**<.0005**	ns	ns	ns
rh_inferiortemporal*	2.49439	.009840	.032	ns	.041	ns
rh_isthmuscingulate*	2.72958	.017948	ns	.041	.027	ns
rh_lateraloccipital*	2.43136	.010828	.019	ns	ns	ns
rh_lateralorbitofrontal	2.38719	.010534	.001	ns	ns	ns
rh_lingual*	2.67593	.014324	.034	ns	.009	ns
rh_medialorbitofrontal*	1.95517	.008255	ns	ns	ns	ns
rh_middletemporal*	3.09317	.017001	ns	ns	.022	ns
rh_parahippocampal	2.73984	.013432	ns	ns	**<.0005**	ns
rh_paracentral*	2.2148	.01033	.019	ns	.028	ns
rh_parsopercularis*	4.10933	.029237	ns	.007	ns	ns
rh_parsorbitalis*	2.63202	.014480	.011	ns	ns	ns
rh_parstriangularis*	3.4628	.02504	.009	ns	ns	ns
rh_pericalcarine	2.76753	.017489	.006	ns	.016	ns
rh_postcentral	3.35444	.017263	**<.0005**	.037	.030	ns
rh_posteriorcingulate*	2.11528	.011755	ns	ns	ns	ns
rh_precentral	3.29687	.016828	**<.0005**	.026	.002	ns
rh_precuneus*	2.8514	.01646	ns	.015	.006	ns
rh_rostralanteriorcingulate*	1.97476	.009784	ns	ns	.023	ns
rh_rostralmiddlefrontal	2.61373	.012540	**<.0005**	.035	ns	ns
rh_superiorfrontal	2.1030	.00780	**<.0005**	ns	.003	ns
rh_superiorparietal	2.8130	.01249	**<.0005**	ns	ns	ns
rh_superiortemporal	3.95814	.029368	ns	ns	.001	ns
rh_supramarginal	3.44792	.018830	**<.0005**	ns	ns	ns
rh_frontalpole*	1.93479	.008813	ns	ns	ns	ns
rh_temporalpole*	2.18840	.010157	ns	ns	ns	ns
rh_transversetemporal*	4.72057	.060423	ns	ns	.013	ns

#Average local gyrification index for each parcellation
*Adjusted R square <0.1 for the linear regression model in this parcellation.

IV

An exploratory model for G x E interaction on hippocampal volume in schizophrenia; obstetric complications and hypoxia-related genes.

Unn Kristin Haukvik MD[a*], Peter Saetre PhD[b], Thomas McNeil PhD[c], Petr S. Bjerkan MSc[a], Ole A. Andreassen PhD[d], Thomas Werge PhD[e], Erik G. Jönsson PhD[b], Ingrid Agartz PhD[a,b,f].

a. Department of Clinical Medicine, section Vinderen, University of Oslo, Norway
b. Department of Clinical Neuroscience, HUBIN project, Karolinska Institute and Hospital, Stockholm, Sweden
c. Department of Psychiatric Epidemiology, Lund University Hospital USiL, Lund, Sweden
d. Department of Psychiatric Research Oslo University Hospital, and Department of Clinical Medicine, Ullevål, University of Oslo, Norway
e. Research Institute of Biological Psychiatry, Mental Health Center Sct. Hans Copenhagen University Hospital, Roskilde, Denmark
f. Department of Psychiatric Research, Diakonhjemmet Hospital, Oslo, Norway

***Corresponding author**
Unn Kristin Haukvik,
Institute of Psychiatry, University of Oslo
P.O. Box 85 Vinderen
N-0319 Oslo, Norway
Phone: +47 22029839, Fax: +47 22495861
E-mail: unn.haukvik@medisin.uio.no

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Abstract

Background: Smaller hippocampal volume has repeatedly been reported in schizophrenia patients. Obstetric complications (OCs) and single nucleotide polymorphism (SNP) variation in schizophrenia susceptibility genes have independently been related to hippocampal volume. We investigated putative independent and interaction effects of severe hypoxia-related OCs and variation in four hypoxia-regulated schizophrenia susceptibility genes (*BDNF, DTNBP1, GRM3* and *NRG1*) on hippocampal volume in schizophrenia patients and healthy controls.

Methods: Clinical assessment, structural MRI-scans, and blood samples for genotyping of 32 SNPs were obtained from 54 schizophrenia patients and 53 control subjects. Information on obstetric complications was collected from original birth records.

Results: Severe OCs were related to hippocampal volume in both patients with schizophrenia and healthy control subjects. Of the 32 SNPs studied, effects of severe OCs on hippocampal volume were associated with allele variation in *GRM3* rs13242038, but the interaction effect was not specific for schizophrenia. SNP variation in any of the four investigated genes alone did not significantly affect hippocampal volume.

Conclusions: The findings suggest a gene-environment (G x E) interaction between *GRM3* gene variants and severe obstetric complications on hippocampus volume, independent of a diagnosis of schizophrenia. Due to the modest sample size, the results must be considered preliminary and require replication in independent samples.

Key words: *GRM3,* hippocampus, hypoxia-regulated genes, obstetric complications, schizophrenia.

Abbreviations

BDNF- brain-derived neurotrophic factor

DTNBP1- dysbindin

GRM3- metabotropic-glutamate-receptor 3

ICV –intracranial volume

MRI- magnetic resonance imaging

NRG1- neuregulin1

OCs- obstetric complications

HCV- hippocampal volume

LD – Linkage disequilibrium

ICC – inter class correlation

1. Introduction

Schizophrenia is a severe mental illness with a prevalence of about 0.7% worldwide (Saha et al., 2005). The illness has a strong genetic component, with an estimated heritability of 80% (Sullivan et al., 2003). However, most patients suffering from schizophrenia have no affected relative, and multiple environmental effects modify schizophrenia liability (Tandon et al., 2008). The exact mechanisms underlying schizophrenia pathology are uncertain, but disturbances of dopamine and glutamate transmission are of importance (Howes & Kapur, 2009;Stone et al., 2007).

Obstetric complications (OCs), occurring during pregnancy, delivery, and the neonatal period, are well documented risk factors for schizophrenia (Cannon et al., 2002;Dalman et al., 2001;Geddes et al., 1999;Hultman et al., 1999). Foetal hypoxia may cause damage to the developing brain (Verdoux & Sutter, 2002) and increase the susceptibility for later development of schizophrenia (Marenco & Weinberger, 2000;Rapoport et al., 2005). In animal models, OCs have been demonstrated to affect both the structure of the brain and the behavior of the offspring ((Boksa, 2004) for review).

Through extensive research efforts over the last years, a number of genes have been suggested to contribute to schizophrenia susceptibility (Harrison & Weinberger, 2005). Several of the suggested susceptibility genes are involved in neurodevelopment (Arnold & Rioux, 2001). A high proportion of the genes are regulated by hypoxia-ischemia (Schmidt-Kastner et al., 2006), e.g. neuregulin 1 (*NRG1*) and dysbindin (*DTNBP1*), which both affect neural migration and synaptic development (Harrison & Weinberger, 2005;Numakawa et al., 2004); brain-derived neurotrophic factor (*BDNF),* which influences pre- and postnatal neuronal survival, differentiation, synaptogenesis, and maintenance (Webster et al., 2006); and the metabotropic-glutamate-receptor 3 (encoded by the *GRM3* gene), which is expressed in neuronal stem cells (Harrison et al., 2008;Melchiorri et al., 2007). Thus variation in hypoxia-regulated genes, in combination with severe OCs leading to hypoxia, have been hypothesized to be of importance to the etiology of schizophrenia, and there is some evidence that the effect of severe OCs on disease risk is modified by SNP variation in *BDNF*, *DTNBP1*, and *GRM3* (Nicodemus et al., 2008).

MRI-studies have consistently shown that schizophrenia patients have a reduced hippocampal volume (HCV) (see (Honea et al., 2005) for review), and nucleotide variations e.g. in genes regulated by hypoxia have been associated with volume changes in the hippocampus (e.g. the *BDNF* gene) and other brain regions (e.g. *NRG1,* catechol-o-

methyl transferase (*COMT*)) in patients, see (van Haren et al., 2008) for review. Interestingly, hippocampal volume has been reported to be smaller in schizophrenia patients with a history of OCs and foetal hypoxia, as compared to patients without such a history (Ebner et al., 2008;McNeil et al., 2000;Schulze et al., 2003;Stefanis et al., 1999;van Erp et al., 2002). This is consistent with results from experimental animal models in which prenatal hypoxic insults have been demonstrated to result in hippocampal CA1-region neuronal damage (Rees & Inder, 2005), and birth related hypoxia to result in reduced hippocampal cell number (Boksa, 2004)

As hypoxia is a core feature of OCs and a strong modifier of gene expression (Schmidt-Kastner et al., 2006), we hypothesized that the effect of OCs on HCV could be modified by variation in hypoxia-regulated genes. Consequently, in the present study of schizophrenia patients and healthy control subjects, we explored if 1) there was a statistically significant relation between a history of hypoxia-related severe OCs and hippocampal volume, and 2) if such a putative relationship was modulated by allele variation in four genes that are regulated by hypoxia and associated with schizophrenia (*NRG1, BDNF, GRM3* and *DTNBP1*).

2. Methods:

2.1 Subject characterization

This study was part of the Human Brain Informatics Project (HUBIN), Karolinska Institutet, Stockholm, Sweden. HUBIN is a comprehensive database of genetic, brain morphological, neuropsychological, and clinical information obtained from schizophrenia patients and healthy subjects. The subject inclusion took place between 1999 and 2003. All participants gave written informed consent. The project was approved by the Research ethics committee at Karolinska Institutet and the Swedish Data Inspection Board ("Datainspektionen"). The study was performed in accordance with the Helsinki Declaration.

The subject sample consisted of unrelated Caucasian men and women currently residents in the Stockholm Area, and has previously been described in detail (Haukvik et al., 2009;Jonsson et al., 2006). Briefly; invited patients from the out-patients clinic underwent a comprehensive clinical assessment protocol using validated operational instruments (Ekholm et al., 2005;Vares et al., 2006) including verification of diagnosis by a trained psychiatrist (EGJ). Patients fulfilled DSM-III-R or DSM-IV criteria for schizophrenia or schizoaffective disorder. Exclusion criteria were a history of head trauma with loss of consciousness > 5 minutes, current diagnosis of substance abuse,

and/or somatic disorders affecting brain function. The healthy control subjects were drawn from a population register or recruited among hospital staff; they were interviewed by a trained psychiatrist and were found to have no current or previous psychiatric illness.

The present analysis included all subjects in the HUBIN-project with a diagnosis of schizophrenia (n = 50) or schizoaffective disorder (n=4) and 53 age-matched healthy controls for whom obstetric records, DNA and high resolution MRI scans were available. Subject characterization and demographics are listed in Table 1.

2.2 MRI

2.2.1 MRI scan acquisition

Magnetic resonance images were obtained at the MR Research Centre at Karolinska Institutet, Stockholm, Sweden, using a 1.5 Tesla GE signa Echo-speed (Milwaukee, Wis., USA) scanner. T1-weighted images, using a three-dimensional spoiled gradient recalled (SPGR) pulse sequence, were acquired with the following parameters; 1.5 mm coronal slices, no gap, 35° flip angle, repetition time (TR) = 24 ms, echo time (TE) = 6.0 ms, number of excitations (NEX) = 2, field of view (FOV) = 24 cm, acquisition matrix = 256 × 192. T2-weighted images were acquired with the following parameters; 2.0 mm coronal slices, no gap, TR = 6,000 ms, TE = 84 ms, NEX = 2, FOV = 24 cm, acquisition matrix = 256 × 192. All scans included were judged visually to be without obvious motion artefacts. A trained neuroradiologist evaluated all scans to be without gross pathology.

2.2.2. *MRI scan post processing using FreeSurfer software*

Hippocampal volume was quantified by automated processing of T1-weighted MR images with the FreeSurfer v 3.0.2 software (http://surfer.nmr.mgh.harvard.edu) (Figure 1a). With this method, a neuroanatomical label is automatically assigned to each voxel in the MRI volume based on probabilistic information estimated automatically from a manually labelled training set (Fischl et al., 2002). The automatic measures define the whole hippocampal formation (including the fimbria) as hippocampus (Makris et al., 1999). HCVs as obtained by FreeSurfer have been reported to be as reliable as manual delineations by experts when compared to different manual delineations by experts (Fischl et al., 2002)

2.2.3 *MRI scan post processing using BRAINS software*

Brain tissue segmentation was also performed using the software suite BRAINS (Andreasen et al., 1993) from T1 and T2-weighted MR images re-sliced to 1-mm^3 voxels in standardized space. Using an automatic tissue classification procedure all brain tissue was classified in the following tissue class volumes: grey matter, white matter, cerebrospinal fluid, and venous blood (Agartz et al., 2001;Agartz et al., 2006;Harris et al., 1999). Measurements of intracranial volume (ICV) obtained using BRAINS were used to correct for inter-individual differences in head size.

To examine to what extent our results depended on the method of quantifying brain volume, and to facilitate comparisons with the results from previous studies (Ebner et al., 2008;McNeil et al., 2000;Schulze et al., 2003;Stefanis et al., 1999;van Erp et al., 2002), we also examined the association between severe OCs and HCV using manual hippocampal delineation (Figure 1b). Our research group had previously delineated hippocampal grey matter (i.e. the hippocampus proper and dentate gyrus) from T1-, T2- and segmented MR images (Agartz et al., 2006), and measurements were available in a randomly chosen subset (n=60, 33 patients and 27 control subjects) of the total subject sample (n=107). All manual delineations were performed by one person. The intra-rater reliability (ICC) for two separate delineations of 10 randomly selected brains was >0.98 for both the left and the right hippocampus. The demographic and clinical characteristics (including obstetric variables) of this group did not significantly differ from the total subject sample (n=107) (data not shown).

2.3 Obstetric complications

Information on OCs was collected from hospital birth records and rated according to the McNeil-Sjöström scale by one physician (UKH) who was blinded to patient/control status and to the results from genotyping and MRI. The inter-rater reliability (interclass correlation) between two physician raters (one of whom was UKH) for 62 of the birth records was 0.93.

The McNeil-Sjöström scale includes several hundred items of potential harm to the foetus, each classified according to severity at an ordinal scale from 1-6. The scale has been validated for use in schizophrenia case-control studies (McNeil et al., 1994). In the present study, severe OCs were considered present in subjects who had experienced one or more complications of grade five or six, whereas subjects with complications of grade four and below were classified as not having had severe OCs, in congruence with the definition used by Nicodemus et al. (Nicodemus et al., 2008). A complication of grade five is defined as “potentially clearly greatly relevant/harmful” (e.g. severe preeclampsia or foetal asphyxia), and a complication of grade six is defined as a

complication that causes “very great harm to or deviation in offspring” (e.g. eclampsia, offspring hypoxic-ischemic cerebral injury). In the present subject sample, the recorded severe OCs were as follows: Prolonged birth (13 subjects), meconium-stained amniotic fluid (5 subjects), asphyxia (3 subjects), prematurity < 32 weeks (3 subjects), placental abruption (2 subjects), bleeding during first trimester (2 subjects), meconium aspiration syndrome (2 subjects), prematurity < 35 weeks (2 subjects), twin transfusion syndrome (1 subjects), mid-forceps delivery (1 subject), small for gestational age (1 subject), acute caesarean section (1 subject), and low birth weight (1 subject). 17 subjects were exposed to one severe OC, and the maximum number was five complications in one subject. The obstetric variables are listed in Table 1.

2.4 Genotyping

Based on previous literature, 32 schizophrenia-related single nucleotide polymorphisms (SNPs), located in four hypoxia-regulated genes, were selected for genotyping (Table 2). Genomic DNA was extracted from whole blood samples, and SNPs located in *DTNBP1*, *GRM3* and *NRG1* genes were genotyped at the SNP Technology Platform at Uppsala University and Uppsala University Hospital, Sweden (www.genotyping.se), using the Illumina BeadStation 500GX and the 1536-plex Illumina Golden Gate assay (Illumina Inc., San Diego, CA, USA) (Jonsson et al., 2009). The four *BDNF* SNPs were genotyped by pyrosequencing (Ahmadian et al., 2000) or cleavage with restriction enzymes as previously described (Jonsson et al., 2006). The sample success rate was on average 99.8% for the genotyped SNPs.

2.5 Statistical analyses

To examine whether the history of severe OCs affects HCV in schizophrenia patients and healthy control subjects, and to what extent such effects depended on allele variation in the four genes, the HCV was analysed with a linear model in Proc MIXED in the SAS software (SAS/STAT® software, version 9.1.3, SAS institute Inc., Cary, NC).

First, we performed a preliminary analysis on the HCV of the left and right hemisphere without SNP markers. In this analysis, diagnosis (schizophrenia vs. control), severe OCs (presence vs. absence), the interaction between diagnosis and OCs, intracranial volume, age at MR scanning, and hemisphere side were treated as fixed factors, whereas individual was treated as a random factor. To test whether the effects of fixed factors differed between hemispheres, all interactions with hemisphere were included in the original model. Residuals were approximately normally distributed, as assessed by the Anderson-Darling test (p-values > 0.25).

Since the disease effect did not vary with presence/absence of severe OCs (p-values for diagnosis*OCs, and diagnosis*OCs*hemisphere were 0.25 and 0.77, respectively), the interaction between diagnosis and OCs was excluded from the statistical model. Moreover the effects of diagnosis, OCs, intracranial volume, and age at MR scanning on hippocampal volume did not differ significantly between the right and left hemispheres (p-values for interaction effects with hemisphere were ≥ 0.42). Consequently, further analyses of HCV were carried out on the average of measurements from the left and right hemisphere.

Second, we used a linear model to carry out single marker allele association of the hippocampal volume (as obtained by FreeSurfer, n=107) including the number of minor alleles (a) and excluding hemisphere in the above model for each SNP separately. To test if the effect of the disease and/or the effect of severe OC on hippocampal volume depended on genetic background, we included the two-way interactions between these factors and the minor allele count. Correction for multiple testing of allele associations, and their interactions with affection and OC status, was done by randomly permuting genotype among individuals 1000 times and re-analyzing the permuted data (Westfall & Young, 1993). Thus genotypes and phenotypes were decoupled keeping the original LD structure of the genetic markers and the covariance structure of phenotypes intact in each permuted data set. The adjusted p-value was defined as the fraction of permutations where the minimum p-value from allele associations, (or from its interaction with severe OC), was less than, or equal to, the minimum p-value in the original data. Hardy–Weinberg (HW) equilibrium of SNPs was tested in affected and controls using Fisher's exact test as implemented in PEDSTATS (Wigginton & Abecasis, 2005).

For a random sub-sample (n= 60) of the study-group, the volume of hippocampal grey matter had been determined by manual delineation. For each individual, HCVs for the left and right hemisphere were combined, and the Pearson correlation and partial correlation coefficients (correcting for the intracranial volume) were calculated between manually and automatically delineated HCVs. In addition, we used mixed linear models to test whether the effects of diagnosis, OCs, and the interaction between OCs and RS13242038 on HCV depended on method of delineation (manual/automatic). In these analyses diagnosis, OCs, intracranial volume, age at MRI, number of minor alleles of RS13242038, and delineation method were considered as fixed factors, and individual as a random factor. The differences in HCV-response due to delineation method were

tested by the interaction effects between method of delineation and the other fixed factors. To account for differences in reliability of methods, a separate residual variance was used for manually and automated HCV measurements, respectively.

3. Results

3.1 Case-control differences in OCs

The frequency of severe OCs was similar in schizophrenia patients and healthy controls (χ^2=0.34, df 1, p=0.54), and there were no differences observed in obstetric variables between the two groups (Table 1). However, the power to detect a true association between OCs and diagnosis, corresponding to an odds-ratio of 2.0 (Clarke et al., 2006), was limited (power<0.40, given a OCs frequency of 23%).

3.2 Automated HCV measures

Patients with schizophrenia demonstrated, on the average, 5% smaller hippocampal volume than healthy control subjects, and this reduction was present in both the left and right hemispheres (p=0.001) (Fig 2a). The HCV was on the average larger in individuals who had experienced severe OCs, as compared to individuals who had no experience of such events (p=0.048) (Fig 2a); the average effect was 3.6% and the response was similar in both hemispheres ($p_{OCs*hemisphere}$=0.42). The effects of diagnosis and of severe OCs on HCV appeared to be independent of each other, and the interaction between the two factors was not statistically significant ($p_{diagnosis*OCs}$=0.25, $p_{diagnosis*OCs*hemisphere}$=0.77). Further analysis was thus conducted on the mean of the left and right HCVs, assuming no interaction effects between diagnosis and OCs on HCV measurements.

3.3 SNP effects from the automated segmentation

We found evidence of the effect of severe OCs on hippocampal volume (n=107) to be dependent on the genotype of the *GRM3* rs13242038 marker (Table 2) and this interaction effect was significant after correction for multiple testing (p=0.014). The larger hippocampal volumes observed in individuals with severe OC largely overlapped with the presence of one or two rs13242038-T alleles (Figure 2b).

HCV was not consistently associated with any of the genotyped SNPs across OC and affection states (a, Table 2). Nor did we find any evidence for allele association varying with diagnosis (a*diagnosis, Table 2). One *DTNBP1* marker (rs1011313) showed a tendency to be associated with HCV, and three additional markers in *DTNBP1, GRM3* and *NRG1* (rs2743852, rs917071, rs2954041) showed a tendency for an allele

association that depended on disease status. Considering the large number of tests conducted, these signals were close to expectations from random variation, and the multiple-test corrected p-values for the most significant allele association (rs1011313) and disease interaction (rs917071) were 0.22 and 0.33, respectively.

3.4 Comparison of automated and manual HCV measures

The manually delineated volume of hippocampal grey matter was approximately three times smaller than the automatically derived measurements (including white matter); the HCVs were (mean± SE) 1.26 ±0.03 and 4.36±0.05 cm^3 respectively. There was an overall correlation of HCV measurements derived by the two methods (r=0.52), which was partly explained by the intracranial volume ($r_{partial\ correlation}$ = 0.41). As in the full sample, the HCV of patients was consistently smaller than that of controls in the sub-sample (4.6%, p=0.037), and the difference was consistent in both automatic and manually derived volumes ($p_{diagnosis*method}$ = 0.51).

The effect of severe OCs on HCV clearly differed between manual and automatic measurements ($p_{OCs*method}$ = 0.02); automatically-derived HCV were larger in individuals who had experienced severe OCs (3.1%) than in individuals who had no such experience, whereas manually-derived volume of hippocampal grey matter was smaller in individuals with severe OCs (-5.9%), compared with the remaining subjects. The joint analysis of manually and automatically derived HCV confirmed that the effect of severe OC on HCV was dependent on rs13242038 genotype ($p_{OCs*genotype}$= 0.004), and indicated that the interaction effect between OCs and the GRM3 locus differed between HCV measurements ($p_{OCs*genotype*method}$ = 0.016, Fig 3). That is, for automatically-derived measurements, the larger HCV volumes associated with severe OCs were only evident in individuals carrying the rs13242038 T-allele (p=0.026), whereas reduction in hippocampal grey matter associated with severe OC was only evident in individuals who were homozygous for the rs13242038 C-allele (p=0.041).

4. Discussion

This is the first study to examine the association of hippocampal volumes with OCs and molecular genetic markers in schizophrenia patients and healthy control subjects. The main finding was that the effect of severe OCs on hippocampal volume is modified by allele variation in the hypoxia-regulated gene *GRM3* in both schizophrenia patients and healthy control subjects. Due to the modest sample size, results must be interpreted with caution.

Glutamate is an excitatory neurotransmitter. It is widely distributed throughout the brain, and has excitotoxic properties when present in high concentrations. *GRM3* encodes the group II metabotropic glutamate receptor mGluR3, which is involved in glutamate transmission, glial function, and neuroprotection, see (Harrison et al., 2008) for review. The receptor is expressed in several neuron populations including the myelin producing oligodendrocytes (Harrison & Weinberger, 2005). mGluR3 is expressed in the hippocampus (Lyon et al., 2008), and receptor expression has been reported to be altered following transient brain ischemia (Raghavendra, V et al., 2002). In mice antenatal hypoxia causes a 2-4 fold reduction in mGluR3 expression, and the down-regulation in gene activity is linked to a decreased vulnerability of hypoxia-induced white matter damage (Fontaine et al., 2008). This response is not found in rats, which may suggest that genetic factors underlie *GRM3* regulation and the susceptibility to white matter damage in rodents (Fontaine et al., 2008). We can only speculate that our results could represent human correlates to the observations from animal models.

In the present study we observed that hippocampal volume, as obtained by the automated FreeSurfer software, was on average larger in individuals who had experienced severe OCs than in individuals without such experience. These results were in contrast with the previous literature, where OCs have been associated with smaller hippocampal volumes in similar clinical samples (Ebner et al., 2008;McNeil et al., 2000;Schulze et al., 2003;Stefanis et al., 1999;van Erp et al., 2002). The discrepancy between the results in this and previous studies could be due to differences in the definition of the hippocampal borders. We used a fully automated procedure to quantify the volume of the hippocampal formation (Fischl et al., 2002;Makris et al., 1999), a procedure that included the white matter (e.g. fimbria) in proximity of the hippocampus proper/dentate gyrus, whereas all previous studies were based on narrower definitions including mainly hippocampal grey matter (Ebner et al., 2008;McNeil et al., 2000;Schulze et al., 2003;Stefanis et al., 1999;van Erp et al., 2002). Thus our results may indicate that severe OCs affect hippocampal white matter differently from hippocampal grey matter. To examine this possibility, we reanalyzed a subset of our sample (60 individuals) for whom we had manual quantifications of hippocampal grey matter volume (Agartz et al., 2006). In this sub-sample, severe OCs were associated with *smaller* hippocampal grey matter volume in individuals who were homozygous for the rs13242038 c-allele. Findings from animal models provide some support for a differential effect of severe OCs on grey and white matter. Both neuronal degeneration in the hippocampal CA1 region (grey matter) (Boksa, 2004;Rees et al., 2008), and hippocampal gliosis (white matter) (Bernert et al., 2003) have been reported

following perinatal asphyxia. In addition, prenatal hypoxia has been demonstrated to exert a different effect on grey and white matter depending on the timing as well as the severity of the hypoxic insult (Rees & Inder, 2005).

The association between OCs and hippocampal volume was similar in the patients with schizophrenia and the healthy control subjects. This is in disagreement with previous clinical studies reporting smaller hippocampal volumes with OCs/hypoxia in patients, but not in healthy siblings (van Erp et al., 2002), co-twins (McNeil et al., 2000) or independent controls (Schulze et al., 2003;Stefanis et al., 1999;van Erp et al., 2002). Our findings suggest instead that hippocampal vulnerability to severe OCs is influenced by factors independent of those related to schizophrenia pathogenesis; the effect may be modified by SNP variation in genes that are associated with schizophrenia risk (e.g. the *GRM3* gene) but also occur in subjects without the illness. This is consistent with the findings by Ebner and co-workers, who reported that the association between OCs and reduced hippocampal volume also was present in relatives of schizophrenia patients (Ebner et al., 2008). Furthermore, severe OCs were equally distributed in schizophrenia patients and healthy controls. This is in contrast to large epidemiological studies (Dalman et al., 2001;Hultman et al., 1999), but in line with previous MRI studies of similar subject sample size as the current study (Ebner et al., 2008;Schulze et al., 2003;van Erp et al., 2002).

Limitations in the current study include the relatively small subject sample for the analyses of gene effects. The combined number of *GRM3* rs13242038 T allele carriers who had experienced severe OC was 9. It is also difficult to interpret possible functional consequences of the observed gene × OC interaction as the rs13242038 polymorphism is located in the first intron of the *GRM3* gene, with no known function. Nevertheless, it is possible that the SNP is in linkage disequilibrium with sequence variation influencing the transcription (or splicing) of the gene. If the minor rs13242038 allele is linked to a functional variant of *GRM3*, our results could lead us to hypothesize that: 1) naturally occurring genetic variants of *GRM3* (in LD with the rs13242038-T allele), causing reduced expression/function of the receptor, decrease the susceptibility to grey matter damage associated with severe OCs, and 2) severe OCs stimulate white-matter growth in hippocampal areas, independently of *GRM3* receptor variation. This could provide an alternative explanation as to why the results in hippocampal volume differ between manually- (only grey matter) versus automatically- (including white matter) obtained volume measures.

In this study, we have observed a statistical gene-environment interaction which is biologically plausible. However, the findings are preliminary and must be interpreted with care. The current approach can be refined and used in studies with better-powered subject samples in future attempts to disentangle genetic and environmental interaction effects on brain structure.

Conclusion

Severe OCs were related to hippocampal volume in both patients with schizophrenia and healthy control subjects, and the effect of severe OCs on hippocampal volume showed an association with allele variation in *GRM3* rs13242038. The present study is the first to explore and report a possible statistical interaction effect between OCs and molecular genetic markers on hippocampal volume. Replication in independent samples is warranted.

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Figure 1. Delineated right hippocampal volume from segmented images from FreeSurfer (A) based on a T1- weighted image, and manually (BRAINS) (B) from T1- and T2-weighted images, from the same slice and scan of one person.

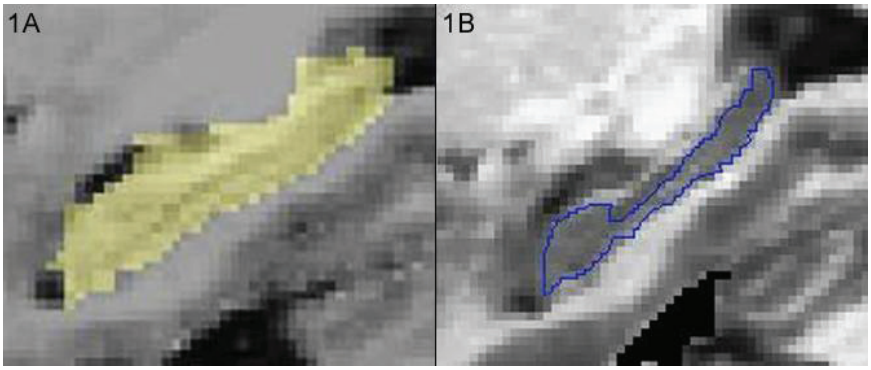

Figure 2. Automatically obtained hippocampal volume (HCV) (cm^3) in schizophrenia patients and healthy control subjects with and without a history of severe obstetric complications (n=107) (A), and mean HCV in the combined group according to allele variation in GRM3 rs13242038 and presence/absence of severe OCs (B).

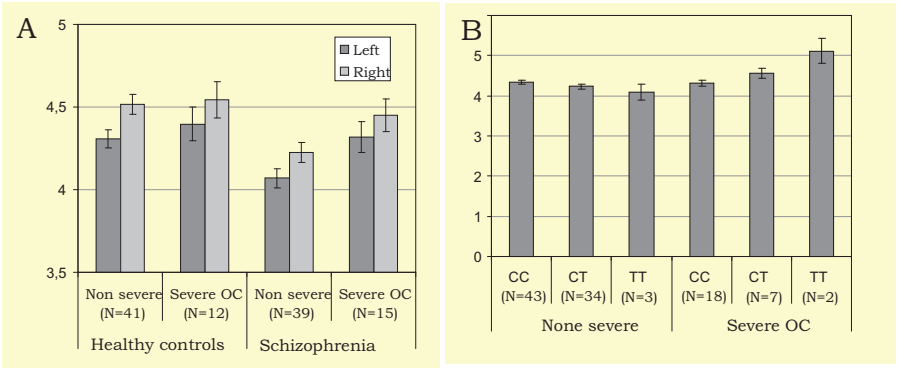

Figure 3. Manually and automatically obtained hippocampal volumes (cm^3) in schizophrenia patients and healthy control subjects combined (n= 60), grouped according to allele variation in GRM3 rs13242038 and presence/absence of severe obstetric complications.

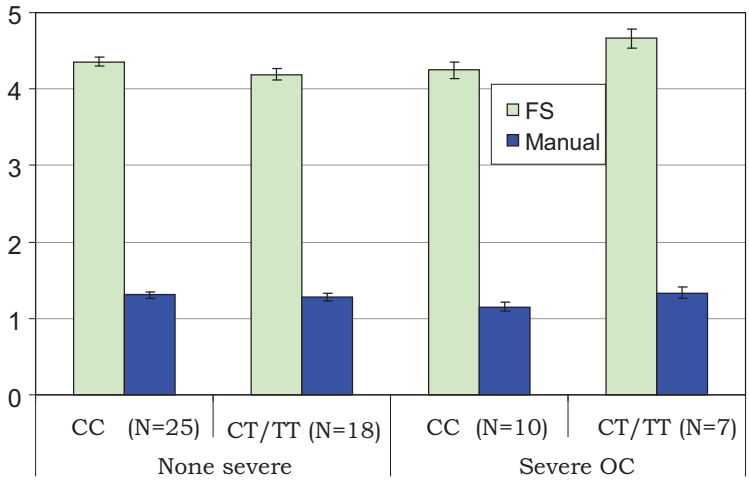

Table 1. Demographic, clinical, and obstetric variables in patients with schizophrenia and healthy control subjects

	Patients (n=54)		**Controls (n=53)**		**Statistics**	
	mean (SD)	*range*	*mean (SD)*	*range*	*test value*	*p-value*
Age at MRI	41.9 (8.0)	25-57	41.4 (9.0)	19-56	t=0.289,df 105	0.77
Age at onset	24.9 (5.6)	15.9-39.5				
Duration of illness	16.8 (9.3)	0.4-41.1				
Birth weight (g)	3494 (635)	1770-5630	3393 (670)	1460-4720	t=0.798,df 105	0.43
Head circumference (cm) (n=104)	33.8 (1.5)	30-37	33.7 (1.6)	28-36	t=0.191,df 102	0.85
Gestational age (weeks)	39.2 (1.9)	32-42	39.3 (2.3)	31-43	t=-0.200,df 105	0.84
Maternal age (years)	27.5 (5.6)	17-43	28.1 (5.4)	19-39	t=0.541,df 105	0.59
	Number	%	Number	%		
Gender -male/female	37/17	68/32	33/20	62/38	χ^2=0.463, df 1	0.50
Medication[1] -none/typical/ atypical	3/25/26	6/46/48				
Severe OCs[2]	15	28	12	23	χ^2=0.37,df 1	0.54

[1] Antipsychotic treatment. Number of individuals receiving each type of medication
[2] Number of individuals with one or more complications of grade 5 or 6.

Table 2. Association between hippocampal volume and SNP variation in four hypoxia-regulated genes. Minor allele frequencies (MAF) and test for Hardy-Weinberg equilibrium (HWE) are listed for patients affected with schizophrenia and control individuals separately. P-values for an allele association (a) and its interactions with severe OCs (a×OC) and affection state (a×Scz) are listed for the combined sample.

			MAF		HWE (p-values)		Allele association (p-values)		
Gene	SNP	alleles	Ctrl	Scz	Ctrl	Scz	a	a*OC	a*diagnosis
DTNBP1	rs12524251	A/G	0.11	0.09	1.00	0.37	0.52	0.36	0.49
	rs760666	G/A	0.23	0.26	0.71	1.00	0.42	0.14	0.74
	rs2619539	C/G	0.49	0.52	0.17	0.18	0.95	0.98	0.82
	rs3213207	T/C	0.15	0.17	0.58	1.00	0.08	0.44	0.53
	rs1011313	C/T	0.10	0.08	0.08	0.30	**0.01**	0.34	0.97
	rs2619528	C/T	0.25	0.26	0.71	0.48	0.10	0.29	0.71
	rs2619522	A/C	0.25	0.26	0.71	0.48	0.10	0.29	0.71
	rs1018381	G/A	0.09	0.09	1.00	1.00	0.85	0.44	0.32
	rs909706	C/T	0.37	0.33	0.14	1.00	0.21	0.67	0.91
	rs2743852	G/C	0.10	0.09	1.00	1.00	0.30	0.43	**0.04**
	rs2619538	T/A	0.49	0.44	1.00	0.42	0.66	0.53	0.97
GRM3	rs187993	T/G	0.35	0.23	0.37	0.44	0.51	0.39	0.18
	rs13242038	C/T	0.21	0.27	1.00	0.73	0.72	**0.001**	0.74
	rs917071	C/T	0.19	0.37	0.67	0.56	0.64	**0.04**	**0.03**
	rs6465084	A/G	0.19	0.30	0.67	0.75	0.16	0.41	0.44
	rs1468412	A/T	0.19	0.32	1.00	0.13	0.68	0.16	0.83
NRG1	SNP8NRG221	T/C	0.40	0.35	0.26	0.77	0.98	0.76	0.53
	SNP8NRG241	G/T	0.40	0.41	0.26	0.57	0.82	0.96	0.53
	rs6994992	C/T	0.43	0.35	0.78	0.77	0.93	0.38	0.56
	rs1354334	C/A	0.38	0.36	0.78	0.14	0.43	0.88	0.32
	rs1503488	G/A	0.28	0.24	1.00	0.71	0.55	0.91	0.49
	rs701955	C/G	0.28	0.24	1.00	0.71	0.68	0.82	0.62
	rs776401	T/C	0.46	0.39	1.00	0.27	0.35	0.98	0.88
	rs1481617	A/T	0.46	0.39	1.00	0.27	0.35	0.98	0.88
	rs3924999	G/A	0.39	0.39	0.77	0.09	0.38	0.18	0.27
	rs2954041	G/T	0.04	0.06	1.00	1.00	0.72	0.83	**0.01**
	NRG1_EXON1	G/T	0.03	0.01	1.00	1.00	0.17	0.48	0.50
	rs10503929	T/C	0.21	0.17	1.00	0.32	0.89	0.25	0.16
BDNF	rs16917204	G/C	0.16	0.17	0.10	0.14	0.81	0.55	0.63
	rs6265	G/A	0.19	0.19	0.36	1.00	0.55	0.21	0.60
	rs11030101	T/A	0.45	0.43	0.11	0.27	0.46	0.63	0.93
	C270T	C/T	0.05	0.05	1.00	1.00	0.51	0.15	0.91