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

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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 ($G \times E$) 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.

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 and 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 and Sutter, 2002) and increase the susceptibility for later development of schizophrenia (Marenco and 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 and Weinberger, 2005). Several of the suggested susceptibility genes are involved in neurodevelopment (Arnold and Rioux, 2001). A high proportion of the genes are regulated by hypoxia–ischemia (Schmidt-Kastner et al., 2006), e.g. neuregulin 1

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, interclass correlation.

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(*NRG1*) and dysbindin (*DTNBP1*), which both affect neural migration and synaptic development (Harrison and 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, has 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 and 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 outpatient 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 min, 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 T 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, and acquisition matrix = 256 × 192. T2-weighted images were acquired with the following parameters; 2.0 mm coronal slices, no gap, TR = 6000 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.

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	$\chi^2 = 0.463$, df 1	0.50
Medication[a]						
None/typical/atypical	3/25/26	6/46/48				
Severe OCs[b]	15	28	12	23	$\chi^2 = 0.37$,df 1	0.54

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