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An exploratory association study of the influence of dysbindin and neuregulin polymorphisms on brain morphometry in patients with schizophrenia and healthy subjects from South India

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ABSTRACT

Multiple genetic risk variants may act in a convergent manner leading on to the pathophysiological alterations of brain structure and function in schizophrenia. We examined the effect of polymorphisms of two candidate genes that mediate glutamatergic signaling, viz., dysbindin (rs1011313) and neuregulin (rs35753505), on brain morphometry in patients with schizophrenia ($N = 38$) and healthy subjects ($N = 37$) from South India. Patients with schizophrenia showed trend-level ($p < 0.001$ uncorrected, 20 voxel extent correction) volumetric reductions in multiple brain regions when compared to healthy control subjects. Trend-level volumetric differences were also noted between homozygotes of the risk allele (AA) of the neuregulin (NRG1) polymorphism and heterozygotes (AG), as well as homozygotes of the risk allele (CC) of the dysbindin (DTNBP1) polymorphism and heterozygotes (TC), irrespective of diagnosis. Moreover, an additive effect of the risk alleles on brain morphometry was also noted. These preliminary findings highlight the possible influence of polymorphisms of risk genes on brain morphometry in schizophrenia.

1. Introduction

Structural abnormalities in multiple brain regions have been reported in patients with schizophrenia (Shenton et al., 2010). The most consistently replicated of these morphometric findings are reduced whole brain volumes (Harvey et al., 1993; Lim et al., 1996) and increased ventricular volumes (Gaser et al., 2004). However, no regional brain abnormality has been consistently replicated across different samples of patients with schizophrenia (Ioannidis, 2011). This observed variation in brain imaging studies may be due to the influence of confounding factors such as age, medication, illness chronicity, sample heterogeneity, variability of statistical thresholds used etc. More importantly, genetic risk for schizophrenia is likely to be mediated by multiple risk variants acting in a convergent manner (Harrison and Weinberger, 2005); the differential effects of these genetic risk variants on brain morphometry may be a major source of variability of findings across association studies of patients with schizophrenia and healthy subjects.

Schizophrenia is a polygenic condition wherein specific structural abnormalities owing to the influence of multiple genes have been reported (van Haren et al., 2008). The glutamatergic

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hypothesis has recently come to occupy a central position amongst the various theories of schizophrenia pathophysiology (Moghaddam and Javitt, 2012). Genes primarily involved in neurogenesis and/or synaptic transmission have shown significant association with schizophrenia as well as with brain morphometric variations. Several genes mediating glutamatergic neurotransmission are involved in various regulatory functions in schizophrenia (Moghaddam, 2003). For example, genes mediating glutamatergic signaling such as neuregulin (NRG1) and dysbindin (DTNBP1) genes have been linked to risk for schizophrenia by candidate gene studies (Munafò et al., 2006; Straub et al., 2002). In a recent study, Agim et al. (2013) using independent datasets from three genome wide association studies with modest sample sizes, showed an association of the NRG1 haplotypes with genetic risk for schizophrenia. Interestingly, certain polymorphisms of these genes have been linked with brain morphometric variations (Barnes et al., 2012; Narr et al., 2009; Sprooten et al., 2009; Tognin et al., 2011).

We attempted to examine the effect of DTNBP1 (rs1011313) and NRG1 (rs35753505) polymorphisms on brain morphometry in patients with schizophrenia and healthy subjects recruited from South India. From amongst several SNPs of the NRG1 and DTNBP1genes that have been reported to be significantly associated with schizophrenia (Funke et al., 2004; Yang, 2012), we chose the, above-mentioned SNPs of DTNBP1 and NRG1 genes, as they have been shown by previous haplotype analysis and association studies to be core markers of schizophrenia (Bray et al., 2005; Kukshal et al., 2013; Nawaz et al., 2013; Pae et al., 2008; Prata et al., 2009). Surprisingly, however, no previous study has examined the relationship between the above SNPs and brain volumes. An additive model was also used to investigate the cumulative effect of risk alleles of these polymorphisms on regional gray matter (GM) morphometry. We hypothesized that the risk alleles of the above gene polymorphisms will have differential effects on brain volume, individually as well as additively.

2. Methods

2.1. Study sample

The study was carried out at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India, with approval from the Institute Ethics Committee. Subjects with recent - onset schizophrenia ($N = 38$) were recruited from the out-patient department of NIMHANS. The diagnosis of schizophrenia was arrived at using DSM-IV criteria (Diagnostic Statistical Manual for Mental Disorders-Fourth edition) based on the consensus of a research psychiatrist who conducted a semi-structured interview and a trained research assistant who used the Mini International Neuropsychiatric Interview (MINI) Plus (Sheehan et al., 1998). The baseline severity of schizophrenia psychopathology was evaluated using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) by two trained raters who had established good inter-rater reliability. The history of exposure to antipsychotics was ascertained by interviewing the patient and relative/s, and corroborated from available medical records. Twenty three of the thirty eight patients were not on neuroleptics, of which 16 were drug naïve at the time of recruitment into the study. The remaining patients ($N = 15$) were on antipsychotics, the cumulative doses of which were converted to 'risperidone equivalents' (Woods, 2003; Kroken et al., 2009; Taylor et al., 2009) (Table 1). Patients who did not meet criteria for any other Axis I disorder, including substance dependence (other than nicotine) as per MINI-Plus, with an age of first onset of psychotic symptoms at or after 17 years of age and a duration of illness less than or equal to 5 years were recruited into the study. Healthy subjects ($N = 37$) with no history of neurological or psychiatric illnesses, matched for age, gender and education were recruited. The healthy comparison subjects were ascertained to be free from Axis I or II psychiatric disorders using the MINI-Plus. Current use/abuse of psychotropic drugs as well as history of psychiatric illness in first-degree relatives in the healthy comparison subjects were ruled out by an unstructured clinical interview. The presence of any unstable medical/neurological condition was ruled out in both groups of subjects using an unstructured clinical interview, detailed physical examination and baseline laboratory investigations. Written informed consent was obtained from all the participants prior to enrolment into the study. The demographic and clinical characteristics of the study samples are given in Table 1.

2.2. Magnetic resonance imaging acquisition

MRI scans were acquired on Philips Achieva 3.0T scanner using a SENSE-8 head coil. T1 anatomical images were acquired with Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence

Table 1
Demographic and clinical variables of study samples.

Characteristics	Participants ($N = 75$)	
	Control group ($N = 37$)	Schizophrenia group ($N = 38$)
Gender		
Male	30	27
Female	7	11
Age, years: mean (SD)	26.46 (5.942)	27.47 (7.759)
Education:Formal education, years: mean (SD)	13.35 (3.691)	12.45 (3.064)
Diagnosis, *N*		
Paranoid schizophrenia		26
Undifferentiated schizophrenia		7
Schizophreniform disorder		5
Positive and Negative Syndrome Scale (PANSS), psychopathology score: mean (SD)		
Positive		14.94 (5.918)
Negative		14.29 (5.292)
Global		26.19 (7.377)
Total		55.38(13.929)
Age at onset of illness, years: mean (SD)		25.50 (7.914)
Duration of illness, months: mean (SD)		23.53 (16.975)
Medication status[a]		
Antipsychotic naive/free, *N*		16/7
On medication, *N*		15
Life-time cumulative neuroleptic exposure in risperidone equivalent dosages (mg)[a]: mean (SD)		1285.8453 (1959.06995)

[a] Life-time cumulative neuroleptic exposure expressed in risperidone equivalents (mg); SD-standard deviation.

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