A Neuregulin 1 Variant Is Associated with Increased Lateral Ventricle Volume in Patients with First-Episode Schizophrenia

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Background: Structural brain abnormalities are already present at early phases of psychosis and might be the consequence of neurodevelopmental deviance. Neuregulin 1 gene (NRG1) is a candidate gene for schizophrenia, and its protein has different roles in nervous system development and plasticity. A single nucleotide polymorphism (SNP) within NRG1, SNP8NRG243177, has been associated with brain function among healthy and high-risk subjects and with reduced cell migration among patients with schizophrenia. We examined whether variations in this polymorphism influence brain volumes in first-episode schizophrenia subjects.

Methods: Ninety-five minimally medicated patients experiencing their first episode of schizophrenia underwent genotyping of three SNPs within the NRG1 gene and structural brain magnetic resonance imaging (MRI). A comparison of volumes of lobar gray matter (GM), lateral ventricles, and cortical cerebrospinal fluid (CSF) was made between the groups according to their genotype after controlling for total intracranial volume.

Results: The SNP8NRG243177 risk *T* allele was significantly associated, in an allele copy number-dependent fashion, with increased lateral ventricle volume. Genotype explained 7% of the variance of lateral ventricle volume. No significant differences in GM lobar or cortical CSF volumes were found among subgroups.

Conclusions: Our findings suggest that genetic variations of the NRG1 gene can contribute to the enlargement of the lateral ventricles described in early phases of schizophrenia. These results suggest novel lines of research into potential mechanisms by which schizophrenia susceptibility genes might exert their effect on brain structure.

Key Words: First-episode, genetics, MRI, neuregulin, schizophrenia, ventricles

Schizophrenia is a highly inheritable psychiatric disorder that affects approximately 1% of the population, showing a heterogeneous phenotype mainly characterized by delusions, hallucinations, thought and conduct disorders, and cognitive deficits. Magnetic resonance imaging (MRI) studies have identified various structural brain abnormalities in schizophrenia, confirmed by a number of meta-analyses mainly performed on samples of chronic patients (1–3). Regarding first-episode schizophrenia patients, lateral ventricular enlargement appears to be one of the most consistent findings (4). This increase of ventricular volume at an early age may reflect disturbances of neurodevelopment processes (5) affecting contiguous brain structures and has been related to clinical phenotypes including severity of negative symptoms (6), poor outcome (7), and cognitive abnormalities (8). Its presence among unaffected relatives from multiply affected families (9,10) suggests that it might be a potential morphometric endophenotype for schizophrenia.

Neuregulin 1 (NRG1) is one of the four proteins of the neuregulin family that act on the epidermal growth factor receptor (EGFR) family of receptors performing a wide variety of functions, including some aspects of neuronal development and plasticity (11). Neuregulin 1 gene is localized in chromosome 8p12–21, a region where suggestive linkage to schizophrenia has been shown in several different populations (12,13). The possible role of this gene in schizophrenia susceptibility was first suggested by the Icelandic deCODE Genetics group (14). Following this study, numerous studies have attempted to replicate this finding. While no single causative allele has yet been identified conferring risk to schizophrenia and there have been several negative reports, recent meta-analyses have provided support for the association of NRG1 with schizophrenia, although with a variety of haplotypes located throughout the gene (15–17). Recent studies have reported abnormal expression of NRG1 messenger RNA (mRNA) (18) and NRG1 alpha protein (19,20) in the brain of patients with schizophrenia. Moreover, the Icelandic NRG1 haplotype has been associated with lower hippocampal volumes in schizophrenia patients and in nonaffected family members (21), and one of its polymorphisms (SNP8NRG221533) has been reported to influence subcortical medial microstructure in the human brain (22).

Recently, the main interest has focused on a particular polymorphism, rs6994992 (SNP8NRG243177), in the type IV promoter region of NRG1. This single nucleotide polymorphism (SNP), which is part of the original risk-associated HapICE haplotype (14), has been found to be associated with altered transcription factor binding and increased levels of type IV NRG1 mRNA in postmortem brain tissue (23), alteration in type IV promoter activity in in vitro receptor assays (24), and lymphocyte migration (25).

Clinical studies have found that the *T* allele of SNP8NRG243177 is associated with altered frontotemporal brain function and development of psychotic symptoms in individuals at genetic high risk for schizophrenia (26), increased reactivity to expressed

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emotion among patients with schizophrenia (27), reduction of spatial working memory capacity among random young male conscripts (28), and reduced white matter density and reduced structural connectivity in the anterior limb of the internal capsule (ALIC) of healthy subjects (29). However, a negative result has recently been published in a large sample of chronic schizophrenia patients, where this polymorphism was not found to be associated schizophrenia, age of onset, and neurocognition (30).

In light of this evidence suggesting functionality of the SNP8NRG243177 polymorphism as well as its association with schizophrenia, we sought to examine the possible association of this polymorphism with several brain regions in a sample of minimally medicated patients experiencing their first episode of schizophrenia. Our hypothesis was that the risk *T* allele would be associated with increased lateral ventricle size. Our group has recently found an association between variations in the catechol-*O*-methyltransferase (COMT) valine (Val)158methionine (Met) polymorphism and lateral ventricle size in first-episode schizophrenia (31).

Methods and Materials

Subjects

The subjects formed part of a large prospective longitudinal study on first-episode nonaffective psychosis (Programa Asistencial Fases Iniciales de Psicosis [PAFIP]) (32). Only those patients with a DSM-IV diagnosis of schizophrenia 6 months after inclusion who had had genomic DNA extracted and had undergone MRI scans were included in this study (n = 95). Gender distribution was 59 male subjects (62.1%) and 36 female subjects (37.9%). Mean age of onset in our sample was 27.7 years (SD = 7.9), higher than what has commonly been reported. The mean duration of untreated psychosis (DUP) at initial assessment was 10.9 months (SD = 17.5, median = 3). Clinical assessments were carried out with the Scale for the Assessment of Positive Symptoms (SAPS), Scale for the Assessment of Negative Symptoms (SANS), and the Clinical Global Impression-Severity scale (CGI-S) by a senior psychiatrist (B.C.-F.) who was blind to genotype. At the time of scanning, 28 patients were taking conventional antipsychotics (haloperidol) and 67 patients were on atypical antipsychotics (40 olanzapine, 24 risperidone, 2 quetiapine, and 1 ziprasidone). Mean time from first antipsychotic treatment to the MRI scan was 33.4 days (SD = 26.9). No differences in mean antipsychotic dose or time from the first antipsychotic treatment were observed between different genotypes. Moreover, these variables were not associated with any brain size. Patients who were included in these analyses (n = 95) were not significantly different from those patients from the general study who were not included in these particular analyses (n = 192) with regard to age, gender, age of illness onset, symptom severity, DUP, and frequency of tobacco, alcohol, and cannabis use (data available on request).

In addition, a sample of 16 healthy control subjects (10 male subjects, 6 female subjects) who volunteered to undertake a MRI scan and to provide a blood sample for genotyping was also included. Volunteers had no current or past history of psychiatric illness such as illicit substance use (including cannabis) or neurological or general medical disorders, as determined by using an abbreviated version of the Comprehensive Assessment of Symptoms and History (CASH) (33). The absence of psychosis in first-degree relatives was assessed by clinical records and family interview.

Of the patients, male patients had significantly larger lateral ventricles than female patients (t = 2.05, df = 93, p = .04). However, when total intracranial volume (ICV) was entered as a covariate, this difference disappeared (F = .27, df = 1,92, p = .60). Tobacco, cannabis, or alcohol use did not influence lateral ventricle size, nor did laterality or height. Age and DUP were not significantly associated with lateral ventricle volume.

All subjects gave written informed consent before their inclusion in the study, which was approved by the local ethics committee.

MRI Acquisition and Image Processing

Magnetic resonance images of the whole brain were obtained at the University Hospital Marques de Valdecilla, Santander, Spain, using a 1.5 T General Electric SIGNA System (GE Medical Systems, Milwaukee, Wisconsin). Three-dimensional T1-weighted images, using a spoiled gradient-recalled acquisition in the steady state (GRASS) (SPGR) sequence, were acquired in the coronal plane with the following parameters: echo time (TE) = 5 msec, repetition time (TR) = 24 msec, numbers of excitations (NEX) = 2, rotation angle = 45°, field of view (FOV) = 26 × 19.5 cm, slice thickness = 1.5 mm, and a matrix of 256 × 192. Two-dimensional protein density (PD) and T2 sequences were acquired as follows: 3.0 mm thick coronal slices, TR = 3000 msec, TE = 36 msec (for PD) and 96 msec (for T2), NEX = 1, FOV = 26 × 26 cm, matrix = 256 × 192. The in-plane resolution was 1.016 × 1.016 mm for the three modalities.

The images were processed by using the software BRAINS2 (Imaging Processing Lab, The University of Iowa Hospitals and Clinics, Iowa City, Iowa) (34). A detailed description of image analysis methods has been reported elsewhere (35). A discriminant analysis method based on automated training class selection was used to classify tissue volumes into gray matter (GM), white matter, and cerebrospinal fluid (CSF) (36). We examined GM volumes of cortical regions (parietal, temporal, and frontal lobes), lateral ventricle size, and total CSF volume. These measurements were done blind to genotype.

Molecular Analysis

DNA was extracted from whole venous blood samples. SNP8NRG243177, which is part of the deCODE genetics haplotype (14), was genotyped using SNPlex technology (Applied Biosystems, Foster City, California). Two other single nucleotide polymorphisms within the 5′ region of the NRG1 gene, SNP8NRG221132 and SNP8NRG221533, were also genotyped using the same technique.

Statistical Analysis

Differences in distribution of gender, laterality, and tobacco, alcohol, and cannabis use between the genotype groups were assessed by means of the chi-square test. Differences between genotypes in age, height, and clinical variables were assessed using analysis of variance (ANOVA). The influence of gender, laterality, and tobacco, alcohol, and cannabis use on brain volumes was assessed with ANOVA, and the relationship between brain volumes and age, height, and clinical variables was assessed with Spearman correlation analysis.

The volume of lateral ventricles did not follow a normal distribution, so a logarithmic transformation was conducted. Analyses of the relationship between NRG1 genotypes and brain volumes were performed using analyses of covariance (ANCOVAs). In each general linear model, the respective volume was entered as the dependent measure with genotype as the independent variable and ICV as covariate. In the case of SNP8NRG243177, similar analyses were performed considering patients with *C/T* and *T/T* genotype as