Genetic Schizophrenia Risk Variants Jointly Modulate Total Brain and White Matter Volume

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Background: Thousands of common single nucleotide polymorphisms (SNPs) are weakly associated with schizophrenia. It is likely that subsets of disease-associated SNPs are associated with distinct heritable disease-associated phenotypes. Therefore, we examined the shared genetic susceptibility modulating schizophrenia and brain volume.

Methods: Odds ratios for genome-wide SNP data were calculated in the sample collected by the Psychiatric Genome-wide Association Study Consortium (8690 schizophrenia patients and 11,831 control subjects, excluding subjects from the present study). These were used to calculate individual polygenic schizophrenia (risk) scores in an independent sample of 152 schizophrenia patients and 142 healthy control subjects with available structural magnetic resonance imaging scans.

Results: In the entire group, the polygenic schizophrenia score was significantly associated with total brain volume ($R^2 = .048$, $p = 1.6 \times 10^{-4}$) and white matter volume ($R^2 = .051$, $p = 8.6 \times 10^{-5}$) equally in patients and control subjects. The number of (independent) SNPs that substantially influenced both disease risk and white matter ($n = 2020$) was much smaller than the entire set of SNPs that modulated disease status ($n = 14{,}751$). From the set of 2020 SNPs, a group of 186 SNPs showed most evidence for association with white matter volume and an explorative functional analysis showed that these SNPs were located in genes with neuronal functions.

Conclusions: These results indicate that a relatively small subset of schizophrenia genetic risk variants is related to the (normal) development of white matter. This, in turn, suggests that disruptions in white matter growth increase the susceptibility to develop schizophrenia.

Key Words: Endophenotype, genome-wide, imaging, psychiatric, SNPs, structural MRI

Schizophrenia is a disabling mental disorder with a heritability of around 80% (1). The Psychiatric Genome-Wide Association Study (GWAS) Consortium (PGC) recently published a large GWAS on schizophrenia (2); this analysis of 17,836 cases and 33,859 control subjects yielded seven loci with common alleles that subtly increase schizophrenia risk. However, there are most likely many more single nucleotide polymorphisms (SNPs) involved in schizophrenia susceptibility: Purcell *et al.* (3) described the additive effects of thousands of disease-associated SNPs combined into a single polygenic schizophrenia score (PSS). This PSS based on >30,000 (mostly independent) SNPs explained around 3% of the variance in schizophrenia in an independent sample. Another recent study estimated that 23% of the variation in liability to schizophrenia is captured by the combined effect of >900,000 SNPs (4). These data support a complex mode of inheritance, with thousands of genetic variants of small effect contributing to disease. This large genetic heterogeneity is further complicated by substantial variation in clinical presentation, disease course, and associated phenotypes. It is likely that subsets of disease-associated SNPs are associated with distinct heritable disease-associated phenotypes (also called endophenotypes [5]). One such phenotype is brain volume and it is well suited to be linked to disease-associated SNPs. Brain volume is robustly associated with schizophrenia, with average reductions in total brain volume of about 3% in schizophrenia patients compared with healthy individuals (6,7). It is highly heritable in healthy subjects, as well as in schizophrenia patients (8–11), and reduced brain volumes are inherited together with illness in families (12). In fact, the largest twin study ($n = 684$) to date recently reported that 77% of the phenotypic overlap between schizophrenia and total brain volume was of genetic origin (11), with white matter loss in schizophrenia patients largely attributable to genetic factors (94%) (although gray matter volume was determined by unique and common environmental factors [11]). Thus, white matter volume is an excellent candidate endophenotype to be linked to schizophrenia-associated SNPs.

The aim of the current study was to investigate the combined effect of schizophrenia-associated loci on brain volume to answer several questions. First, is brain volume in schizophrenia patients indeed determined by disease-associated SNPs, and if so, by which proportion of these SNPs? Second, do disease-associated SNPs affect brain volume in patients only or do they modulate brain volume in general? Finally, is the involvement of genetic factors on white matter volume in particular, as previously

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Received May 8, 2012; revised Jul 17, 2012; accepted Aug 10, 2012.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2012.08.017

suggested by heritability calculations from twin studies, supported by genotype data?

Methods and Materials

Discovery Sample

Data from the Schizophrenia Psychiatric GWAS Consortium was used as a discovery sample to identify the schizophrenia risk variants, their *p* values, and odds ratios. Analysis and quality control were performed according to the consortium standards (2). All subjects from the PGC sample were included, except for the 1342 cases and control subjects from our own schizophrenia genome-wide association study (University of California Los Angeles/University Medical Centre Utrecht).

Target Sample

The target sample consisted of 152 schizophrenia patients and 142 control subjects with available magnetic resonance imaging (MRI) data. Subjects were included for the Genetic Risk and Outcome of Psychosis study ($n = 162$) (13) and a study described previously (14) ($n = 132$) performed in the University Medical Centre Utrecht. For both patients and control subjects, psychopathology was assessed using the Comprehensive Assessment of Symptoms and History (15). Of the target sample, 138 subjects were diagnosed with schizophrenia and 14 subjects were diagnosed with schizoaffective disorder. Unaffected subjects had no history of psychiatric illness, except for four subjects who had a history of depressive disorder, anxiety disorder, obsessive-compulsive disorder, and adjustment disorder, respectively. None of the control subjects had first-degree family members with psychotic illness.

MRI Analysis

Brain images were acquired on either a Philips NT or a Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands) at 1.5 Tesla. Scanner type showed no main effect or interaction effect with disease status on total brain and white matter volume (after correction for age, gender, and intracranial volume). Gray matter volumes were slightly lower with the Philips Achieva scanner (mean 615.0 mL [SD 25.0] vs. 624.3 mL [SD 26.7], $p = .002$). Correcting for scanner type did not influence the results. Magnetic resonance imaging acquisition and processing methods have been previously described (6,16). Postprocessing was done on the neuroimaging computer network of the Department of Psychiatry at the University Medical Centre Utrecht. All images were coded to ensure blindness for subject identification. Scans were put into Talairach frame (no scaling) and corrected for inhomogeneities in the magnetic field (17). Volume measures of the intracranium, total brain, and cerebral gray and white matter were determined. Quantitative assessment of the intracranial volume was performed with use of a fully automated computer program based on histogram analyses followed by mathematical morphological operators in either the dual echo-turbo spin echo image (Philips NT) or a single-shot echo planar imaging scan (as part of a diffusion tensor imaging series) together with a magnetization transfer imaging scan (Philips Achieva). All intracranial segmentations were visually checked and corrected where necessary. Quantitative assessments of the total brain and gray and white matter volumes were performed based on histogram analyses followed by mathematical morphological operators in the three-dimensional fast field echo image, using the intracranial volume as mask (18).

Genetic Analysis

Subjects in the target sample were genotyped at University of California Los Angeles Neurosciences Genomics Core using the Illumina HumanHap550 beadchip (Illumina, San Diego, California). Initial quality control was performed by the PGC, removing individuals with more than 5% missing SNPs or with evidence of more than random genetic similarity (c.q. distant relatedness) and SNPs on chromosomes X and Y and mitochondrial DNA. Only SNPs genotyped in the target sample were included (in the discovery sample, part of the SNPs were imputed due to the use of different genotyping platforms). These SNPs were filtered based on minor allele frequencies of less than .02 (removing 4528 SNPs) and >1% missing genotypes per SNP (7552 SNPs). There was no evidence of deviation from Hardy-Weinberg equilibrium with $p < 1 \times 10^{-6}$, nonrandom genotyping errors with $p < 1 \times 10^{-6}$, such as systematic batch effects. There was a marginally increased call rate in patients compared with control subjects (e.g., for SNPs with a *p* value for association with schizophrenia <.01: mean genotyping rate in patients: 4038/4040 vs. 4036/4040 in control subjects, $p = .02$). To remove all SNPs in linkage disequilibrium, SNPs were pruned based on a pairwise R^2 threshold of .25 and a sliding window of 50 SNPs wide, shifting 5 SNPs at each step, using PLINK (19). In this way, another 341,261 SNPs (74.3%) were removed, leaving 117,924 SNPs for analysis.

Statistical Analysis

Measures of total brain and cerebral gray and white matter volume were corrected for the covariates age, sex, and intracranial volume by taking the unstandardized residuals of the volumes using linear regression in the total group. For each subject, the unstandardized residual was added to the intercept + beta_i * mean_i, where *i* represents the different covariates. Intracranial volume explained a large part of the variation in brain volume, resulting in a correlation between the uncorrected and corrected brain volumes of .36. Intracranial volumes were corrected for age and sex in a similar way. All corrected brain volume measures were normally distributed in the total group and in the patient and control groups separately.

For each individual in the target sample, a PSS was calculated using PLINK (19). For each SNP, the number of risk variants an individual carried (0, 1, or 2) was multiplied by the logarithm of the odds ratio for that particular variant. Risk variants are the alleles (nominally) associated with disease, including both true risk alleles and stochastic variation. Sets of SNPs with *p* values below different cutoffs for effect on schizophrenia (SZ) (*p* value cutoffs for effect on schizophrenia or $p_{\text{cutoff-SZ}}$) were defined. First, the following $p_{\text{cutoff-SZ}}$ were used: .01; .06; .1; .2; .3; .4; and .5. When the largest R^2 values were found at relatively low cutoffs, we added smaller $p_{\text{cutoff-SZ}}$ (.002; .004; .006; .008; .02; .04; and .08). The score was summed over all SNPs in the $p_{\text{cutoff-SZ}}$-SNP sets for each individual in the target sample to obtain the individual polygenic scores.

We first performed a logistic regression with disease status as dependent (outcome) variable and subsequently performed linear regressions using total brain and gray matter and white matter volumes as dependent (outcome) variables. Sex and intracranial volume were analyzed as negative controls. Total brain volume and intracranial volume develop at a similar rate