G72 Influences Longitudinal Change in Frontal Lobe Volume in Schizophrenia

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Abstract

Schizophrenia is a neurodevelopmental psychiatric disorder characterized by a variety of structural brain abnormalities that appear to progress across the course of illness. Schizophrenia also is highly heritable, and one gene that has emerged as a possible susceptibility factor is *G72*. *G72* influences brain development and activity by an as-yet unclear mechanism, and multiple studies have reported associations between *G72* and schizophrenia. We were interested in linking these domains of investigation by determining whether *G72* also influences the rate of longitudinal structural brain changes in individuals with schizophrenia. As part of the Iowa Longitudinal Study of Recent Onset Psychoses, we genotyped four *G72* polymorphisms previously associated with schizophrenia in 110 subjects with schizophrenia or schizoaffective disorder from whom we had obtained two brain MRI scans an average of three years apart. The four polymorphisms captured three haplotypes, one of which was strongly associated with an increased rate of frontal lobe volume decrement. This same haplotype was also associated with more severe psychotic symptoms at the time of the second scan. These data thus suggest that variation in *G72* modulates the progressive brain changes that characterize schizophrenia.

Keywords

DAOA; MRI; schizophrenia; frontal lobe; psychosis

Introduction

Schizophrenia is a neuropsychiatric disorder that affects 1% of the population. The disease is characterized by positive symptoms such as delusions and hallucinations, negative symptoms such as social withdrawal and affective flattening, and disorganization of speech and behavior. The etiology of schizophrenia appears to be due primarily to genetic factors, as evidenced by a sibling relative risk of approximately 10 and an estimated heritability of 81% (Sullivan et al., 2003). Brain structure volumes are also affected by schizophrenia. Specifically, enlarged CSF volumes, reductions in temporal and frontal lobes, and abnormalities in subcortical structures have been found in cross-sectional studies of individuals with schizophrenia (Shenton et al., 2001). Longitudinal MRI studies show that individuals with schizophrenia experience changes in brain structure volumes over time (Gur et al., 1998), with a progressive reduction in frontal lobe white matter volume (Ho et al., 2003; Mathalon et al., 2001) shown to be modulated by antipsychotic administration (Styner et al., 2005).

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The search for genetic contributors to these pathophysiological processes has recently focused attention on glutamatergic N-methyl-D-aspartic acid (NMDA) receptors (Ghaziuddin et al., 1995; Goff and Coyle 2001). Several studies in the late 1990's showed linkage to schizophrenia on chromosome 13q32-34 (Christian et al., 2002). Two overlapping genes in this region, *G72* and *G30*, were then found to be associated with schizophrenia (Chumakov et al., 2002). Initially, G72 was thought to interact with D-amino acid oxidase (DAO), an enzyme involved in attenuating NMDA receptor-mediated neurotransmission (Chumakov et al., 2002), but more recent data suggest that G72 regulates mitochondrial function without interacting with DAO (Kvajo et al., 2008a). Subsequent studies have evaluated the linkage between *G72* and schizophrenia (Abecasis et al., 2004; Addington et al., 2004; Chen et al., 2004; Detera-Wadleigh and McMahon 2006; Fallin et al., 2005; Korostishevsky et al., 2004; Li and He 2007; Liu et al., 2006; Ma et al., 2006; Mulle et al., 2005; Schulze et al., 2005; Wang et al., 2004; Wood et al., 2006; Yue et al., 2006; Zou et al., 2005). Although some did not find association (Liu et al., 2006; Mulle et al., 2005), multiple others supported the contribution of these genes to schizophrenia susceptibility (Abecasis et al., 2004; Addington et al., 2004; Chen et al., 2004; Detera-Wadleigh and McMahon 2006; Fallin et al., 2005; Korostishevsky et al., 2004; Li and He 2007; Ma et al., 2006; Schulze et al., 2005; Shi et al., 2008; Wang et al., 2004; Wood et al., 2006; Yue et al., 2006; Zou et al., 2005).

Over the past ten years, we have collected DNA from research subjects in the Iowa Longitudinal Study of Recent Onset Psychoses who have undergone serial brain imaging studies. Using these data, we are examining the effect of glutamatergic genetic variation on quantitative traits in individuals with schizophrenia. For this study, we examined whether genetic variations in *G72* affected brain volume change over time in schizophrenia. This represents a unique opportunity to investigate the longitudinal effect of genotype on brain volume in a disorder that shows progressive changes in brain structure.

Materials and Methods

Subjects

Subjects included 110 individuals with schizophrenia or schizoaffective disorder ascertained through the ongoing Iowa Longitudinal Study of Recent Onset Psychoses. The subjects were predominantly Caucasian (107/110) and male (78/110) reflecting the ethnic makeup of Iowa and the gender bias of schizophrenia. The age at the initial MRI scan was 26.7 ± 7.14 years. The longitudinal study design and methods have been previously described (Gupta et al., 1997). In brief, the subjects with schizophrenia or schizophrenia-spectrum disorders all entered the study within 5 years of their first hospitalization. At intake, the subjects were evaluated extensively with standardized clinical rating scales (Comprehensive Assessment of Symptoms and History (CASH)(Andreasen 1985)) and diagnoses were established by the best estimate method at a multi-disciplinary staffing. A neuropsychological battery and magnetic resonance imaging (MRI) of the brain were also obtained. These assessments have since been repeated at 2, 5, and 9 years, and every 3 years thereafter.

Of the 110 study subjects, 99 had DSM-IV schizophrenia while 11 had schizoaffective disorder. All analyses were performed with and without the schizoaffective individuals to determine whether they were associated with different results than the individuals with schizophrenia; in all instances they were not. Thus, given that it is unclear whether schizophrenia and schizoaffective disorder are genetically distinct entities, individuals with schizoaffective disorder were retained in the interest of sample size and power. All subjects had at least two brain MRI scans a minimum of 2 years apart.

Medications

Antipsychotic administration was recorded both prior to the first scan and in between scans. The data was converted to average chlorpromazine daily dose by calculating the total dose of antipsychotic during the time period, converting it to Chlorpromazine equivalents, and dividing by the total number of days. In order to decrease the effect of outliers, these data were then stratified into no antipsychotic, low dose antipsychotic (1–400mg chlorpromazine daily), medium dose antipsychotic (401–800mg chlorpromazine daily), and high dose antipsychotic (> 801 mg chlorpromazine daily). These data showed that study subjects had minimal prior treatment: 33 (30%) were neuroleptic-naïve, while 39 (35%) had received low doses of antipsychotics (<400mg chlorpromazine equivalents daily) across the course of their illnesses.

Genetic Analyses

DNA was prepared by high-salt extraction from whole blood. Four SNPs in the region of G72 (rs3696165, rs3696167, rs2391191, rs778294) were chosen for genotyping based on prior studies demonstrating positive association with schizophrenia (Abecasis et al., 2004; Addington et al., 2004; Chen et al., 2004; Detera-Wadleigh and McMahon 2006; Fallin et al., 2005; Korostishevsky et al., 2004; Li and He 2007; Ma et al., 2006; Schulze et al., 2005; Wang et al., 2004; Wood et al., 2006; Yue et al., 2006; Zou et al., 2005). Genotyping was performed with the fluorogenic 5' nuclease method (TaqMan™, Applied Biosystems (ABI), Foster City, CA) using reagents obtained from ABI, including VIC and FAM labeled probes and TaqMan™ Universal PCR Master Mix. PCR and allele calling were performed on a StrataGene Mx3000P qPCR thermocycler. Replicate samples were included on all genotyping plates to ensure accurate allele calling.

MRI acquisition and image processing

Magnetic resonance images of the whole brain were obtained on a single 1.5-Tesla GE (General Electric Medical Systems, Milwaukee, Wisconsin) Signa MR scanner. Three MR sequences were acquired for each subject: T1-weighted spoiled grass, proton density (PD) and T2-weighted images. The images were processed using the locally developed BRAINS (Brain Research: Analysis of Images, Networks, and Systems) software package; detailed descriptions of image analysis methods and the imaging parameters have been previously described (Andreasen et al., 1996; Magnotta et al., 2002). In short, the T1-weighted images were spatially normalized and re-sampled so that the anterior-posterior axis of the brain was realigned parallel to the anterior-posterior commissure line, and the inter-hemispheric fissure was aligned on the other two axes. Using an automated image registration program, the T2 and PD weighted images were aligned to the spatially normalized T1 weighted image. These images were then linearly transformed into standardized stereotaxic Talairach atlas space, generating automated measurements of frontal, temporal, parietal, and occipital lobes, cerebellum, and subcortical regions (Andreasen et al., 1996). Tissue volumes were further classified into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF), by employing a discriminant analysis method of tissue segmentation (Harris et al., 1999). In this study, total volume, white matter volume, and gray matter volume were examined for each of the following regions: cerebrum, frontal lobe, temporal lobe, parietal lobe, and occipital lobe.

Symptoms

Because symptom severity not likely to be "progressive" in the same manner as brain deterioration, we examined genotype effects only on endpoint symptom severity (symptom severity at second MRI). We also tested only those haplotypes that produced significant effects on brain structure volume change.

Positive and negative symptoms were assessed using the Scale for Assessment of Positive Symptoms (SAPS) (Andreasen 1984) and Scale for Assessment of Negative Symptoms (SANS) (Andreasen 1983). All available sources of information were used to assess symptom severity, including patient reports, informant interviews and medical records. Items corresponding to the SANS/SAPS global items were rated using a score ranging from 0 (none) to 5 (severe), and then grouped into psychotic, disorganized, and negative symptom dimensions that have repeatedly been shown to cluster independently (Andreasen et al., 1995). The psychotic dimension summed SAPS ratings for hallucinations and delusions, the disorganized dimension included SAPS ratings for positive formal thought disorder, bizarre/disorganized behavior, and inappropriate affect, and the negative dimension included SANS ratings for attention, affective flattening, alogia, avolition/apathy, and anhedonia/asociality.

Statistical Analyses

Haploview was used to assess the haplotypes in the sample (Barrett et al., 2005). We first identified haplotypes for subjects who were homozygotes at all four SNPs, and found three haplotypes. Subjects who were heterozygous at one or more of the SNPs were evaluated and no further haplotypes were found. All subjects with genetic data were able to be assigned to the homozygous or heterozygous combinations of the three haplotypes.

Demographics and illness characteristics were stratified by genotype. ANOVA was used to test the difference between the group means for continuous variables, including age at onset, age at first MRI scan, and years between scans. Chi-square tests were used to test the differences between the distributions of the categorical variables, which included gender, neuroleptic use prior to first scan, neuroleptic use between scans, clozapine use prior to first scan, and clozapine use between scans.

We then performed three separate MANCOVAs in which we analyzed the effect of each haplotype jointly on brain structure volumes. Number of haplotype alleles (0, 1, 2), treated categorically, was the predictor variable, and the multivariate dependent variable was the change in brain volume regions between the two scans (including all regions of interest). Covariates included age at onset, age at first MRI scan, years between scans, gender, neuroleptic dose range prior to first scan, and neuroleptic dose range between scans. To follow-up on haplotypes with a statistically significant difference across all brain volumes, univariate ANCOVAs were used to find the regions showing the differences. To ensure that significant results were not due to non-normal distributions or outliers, we also performed non-parametric analogs of the ANCOVAs, which, in all cases, confirmed the parametric tests. Because MANCOVA automatically incorporates p-value correction for multiple testing, adjustment of the type-I error level is unnecessary. A p-value less than 0.05 was regarded as statistically significant.

To determine whether genotypes that influenced brain structure also influenced end-point symptomatology, we performed linear regression with dependent variables of total scores for the three symptom domains, adjusted for age, sex and medication use.

Results

We first tested the 4 SNPs for linkage disequilibrium (LD). The SNPs covered 38 kb, and D' was 1 for all relationships. From these 4 SNPs, we identified three haplotypes which were all relatively common (Table 1).

The data were stratified by haplotype and evaluated. A comparison of the demographics and illness characteristics between the haplotype groups and all other subjects showed no significant demographic or medication use differences between the groups (Table 2).

Relationships between changes in brain structure volumes over time and haplotypes were then investigated. Haplotypes 1 and 3 showed significant differences in brain structure volumes, with MANCOVA p-values of 0.01 and 0.04, respectively. Haplotype 1 produced a significant effect on frontal lobe tissue volume loss (Table 3: $p = 0.008$ for total frontal volume). This effect appeared, based on the allelic group means, to arise primarily from greater volume loss in the haplotype 1 homozygotes. An analysis that compared change in frontal tissue volume in haplotype 1 homozygotes versus all others was confirmatory ($F = 9.75$, $p = 0.002$). The haplotype 1 effect on frontal volume change did not appear to be tissue specific, with both gray and white matter showing patterns of association similar to that for overall frontal volume. The effects for gray and white matter, like that for overall volume, were also stronger when homozygotes were compared against all others (frontal gray: $F = 4.83$, $p = 0.03$; frontal white: $F = 4.03$, $p = 0.05$).

While haplotype 2 did not affect change in brain tissue volume, haplotype 3 produced a significant effect on cerebral cortical white matter volume change ($p=0.0008$). The pattern of effects was similar across all cerebral lobes, with individuals homozygous for haplotype 3 having experienced a significant increase in white matter volume. As with haplotype 1, this effect confirmed by a test comparing haplotype 3 homozygotes against all other subjects (cortical white matter: $F = 14.20$, $p = 0.0003$).

The robustness of these results was evaluated with the non-parametric Wilcoxen two-sample test. The results were unchanged, with $p = 0.008$ for the effect of haplotype 1 on total frontal volume change and $p = 0.0027$ for the effect of haplotype 3 on cerebral white matter volume change.

Given these results, we then tested whether homozygosity for haplotypes 1 and 3 influenced endpoint symptom severity (Table 4). Haplotype 1 homozygotes experienced more severe psychotic symptoms than other subjects ($p=0.006$), while haplotype 3 did not influence endpoint symptom severity. Given the influence of haplotype 1 on two quantitative traits, we tested whether frontal volume change was correlated with endpoint psychotic symptoms while controlling for age and gender. Although change in tissue volume was inversely correlated with increased severity of symptoms, the relationship was not statistically significant (partial $r = -0.14$, $p = 0.17$).

Discussion

Schizophrenia has long been known to be characterized by diffuse brain abnormalities. These manifest, in general, as increased ventricular size and diminished cerebral cortical tissue volume in cross-sectional comparisons with healthy controls. A question that cannot be answered by cross-sectional studies, however, is whether these abnormalities are progressive or static: are they fully expressed at diagnosis, remaining stable thereafter, or do they progress over the life span? And if the latter, is the progression due to medications, innate predisposition, or other factors? The first question has now been answered by longitudinal studies showing that individuals with schizophrenia experience, on average, a gradual loss of cortical tissue across the course of illness (Boos et al., 2007; Farrow et al., 2005; Whitford et al., 2006; Zipparo et al., 2008). We found, for example, that a cohort of individuals with schizophrenia who we have studied with serial MRI scans for up to 20 years have experienced on-going decrement in cortical gray matter volume, particularly in the frontal lobe (Ho et al., 2003). This has occurred at a more rapid rate than in a comparison group of healthy control individuals and during an age period—the twenties and thirties—when brain tissue volume typically remains stable.

The second question, that of causality, has not been answered as clearly. Evidence exists for effects of antipsychotic medication on some brain structure volumes, but the cortex appears to be either unaffected by such medications or protected by them from the on-going deteriorative disease process (Styner et al., 2005; Takahashi et al., 2007). In accord with this, some studies have shown that the degree of cortical volume loss is directly associated with duration of untreated psychosis (Perkins et al., 2005). In a study of twin pairs discordant for schizophrenia, progressive volume changes were found to be at least partially attributable to genetic factors (Brans et al., 2008). In a direct test of this hypothesis, we previously showed that variation in the brain derived neurotrophic factor gene (*BDNF*) influences longitudinal changes in frontal lobe tissue volume in individuals with schizophrenia (Ho et al., 2007).

With the hypothesis, therefore, that genetic factors modulate longitudinal brain changes, we investigated the role of a high interest gene, G72, in producing these changes. Variation in *G72* has been associated with schizophrenia in multiple samples (Abecasis et al., 2004; Addington et al., 2004; Chen et al., 2004; Detera-Wadleigh and McMahon 2006; Fallin et al., 2005; Korostishevsky et al., 2004; Li and He 2007; Ma et al., 2006; Schulze et al., 2005; Shi et al., 2008; Wang et al., 2004; Wood et al., 2006; Yue et al., 2006; Zou et al., 2005). *G72* has been shown to influence, in individuals with schizophrenia, symptom severity (Schulze et al., 2005; Yue et al., 2007) and cognitive processes that depend on frontal lobe function (Donohoe et al., 2007; Goldberg et al., 2006) . *G72* has also been shown to influence hippocampal and prefrontal function in relatives of individuals with schizophrenia (Hall et al., 2008). The mechanisms through which *G72* exerts these effects are unclear. The prevailing hypothesis, suggested by early studies, has been that G72 influences glutamate neurotransmission. G72 was thought to activate D-amino acid oxidase (DAO), which in turn oxidizes D-serine, an important co-agonist for the NMDA glutamate receptor. Individuals with schizophrenia have lower serum levels of D-serine than healthy controls, and use of D-serine as an adjunctive medication has been shown to improve the symptoms of schizophrenia (Boks et al., 2007). Recent data, however, suggests a potential alternative function for G72. Kvajo et al, reported that a specific isoform of G72 that stimulated dendritic arborization did not interact with DAO but rather influenced mitochondrial function (Kvajo et al., 2008b). They identified *G72* as a primate specific, rapidly evolving gene, and hypothesized that it plays a role in supplying the increased energy needs of the primate brain. By either theory, *G72* could plausibly influence brain morphology in individuals with schizophrenia.

We therefore genotyped, in a sample of individuals with schizophrenia who have received serial brain imaging and symptom assessments, four *G72* SNPs that have been associated individually and as haplotypes with both schizophrenia and bipolar disorder. Our analyses of these data showed that individuals with schizophrenia who were homozygous for a specific haplotype (haplotype 1) experienced an accelerated rate of frontal lobe tissue loss compared to subjects with other *G72* haplotypes. When confounds such as age, interscan interval, and medication treatment were removed, the haplotype 1 homozygous state was associated with a loss of 4.1% of frontal lobe tissue volume, while non-homozygous individuals did not experience any change (Figure 1). Progressive decreases in frontal lobe volume have also been shown to be associated with greater symptom severity and impaired cognitive functioning (Ho et al., 2003). We therefore tested whether, at the time of the second scan, haplotype 1 was associated with symptomatology and found that psychotic symptoms were significantly more severe in the haplotype 1 homozygotes than in the rest of the sample (Table 4).

We also found that individuals homozygous for haplotype 3 experienced a larger increase in white matter volume over time in comparison to the rest of the sample. This could indicate

increased myelination and may represent a protective effect, consistent with one prior study that reported a protective variant within the *G72* locus (Abecasis et al., 2004). The haplotype 3 homozygotes also experienced less severe positive and negative symptoms at the time of their second MRI, although the number of haplotype 3 homozygotes was small and the symptom effects were not statistically significant.

Relating these data to previous *G72* studies is challenging because the markers examined and the findings are not consistent. Haplotype 1 has nonetheless been implicated in a number of reports. Of eight studies that evaluated *G72* and schizophrenia and that reported the over-represented alleles (Addington et al., 2004; Chumakov et al., 2002; Hattori et al., 2003; Ma et al., 2006; Mulle et al., 2005; Schulze et al., 2005; Wang et al., 2004; Yue et al., 2006; Zou et al., 2005), six showed consistency with our haplotype 1 finding (Hattori et al., 2003; Mulle et al., 2005; Schulze et al., 2005; Wang et al., 2004; Yue et al., 2006; Zou et al., 2005). Yue et al found that a haplotype overlapping and consistent with our haplotype 1 increased the risk for both early-onset and male schizophrenia (Yue et al., 2006). Meta-analyses by Li & He and Detera-Wadleigh & McMahon found significant over-representation of the A allele in rs3916965 and the A allele in rs2391191, both of which are in haplotype 1 (Detera-Wadleigh and McMahon 2006; Li and He 2007). Thus, prior results found with the four SNPs used in our study implicate haplotype 1, thereby supporting our extension of these findings of a detrimental effect of haplotype 1 on brain structure volumes in schizophrenia

These data must be interpreted in the context of several limitations. First, because we only examined individuals with schizophrenia, we are unable to determine whether the identified associations are specific to schizophrenia or are more generalized. Also, the significant effects were found in the homozygote group samples, with small sample sizes. This diminishes our power to detect more subtle associations, and the actual genetic mechanisms at play are likely to be more complex than what we are modeling. In spite of these limitations, our study suggests that genetic variation in the gene *G72* modulates symptom severity and progressive changes in brain structures that occur in individuals with schizophrenia.

Acknowledgments

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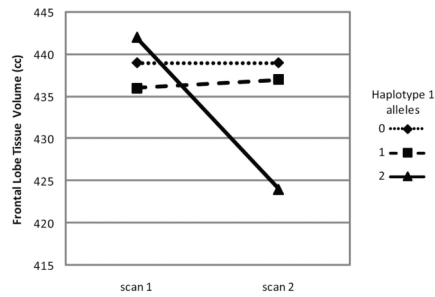
1. . Average frontal lobe volumes at time 1 and time 2 MRI scans for haplotype 1 genotype groups

Scans were obtained a minimum of 2 and an average of 3.0 ± 1.5 years apart. Linear regression was performed with haplotype as the independent variable and change in brain volume between the two scans as the dependent variable. The regression was adjusted for age at onset, age at first MRI scan, years between scans, gender, neuroleptic dose range prior to first scan, and neuroleptic dose range between scans. The decrease in frontal volume over time for haplotype 1 homozygotes is significantly different than for the other groups. In fact, the p-value for the estimated slope of the remaining subjects is 0.49, suggesting that there is no change in frontal lobe volumes for the subjects who are not haplotype 1 homozygotes.

Table 1

G72 haplotype prevalences

| | SNP | | | | Prevalence | |
Haplotype	rs3916965	rs3916967	rs2391191	rs778294	Schizophrenia (n=110)	Controls (n=120)
1	A	G	A	G	0.40	0.35
2	G	A	G	G	0.33	0.35
3	G	A	G	A	0.27	0.30

Table 2

Demographic and Illness Characteristics of Individuals with Schizophrenia

	Total Sample	Haplotype 1 alleles			Haplotype 2 alleles			Haplotype 3 alleles		
		0	1	2	0	1	2	0	1	2
N	110	37	57	16	45	58	7	60	41	9
Males	71%	78%	65%	75%	78%	66%	71%	65%	76%	89%
Age at onset (years)	22.0 ± 5.5	22.6 ± 5.8	21.6 ± 5.5	22.4 ± 5.0	20.7 ± 4.5	22.7 ± 6.1	25.1 ± 4.1	22.7 ± 5.6	21.6 ± 5.4	19.4 ± 4.6
Age at first MRI Scan (years)	26.8 ± 6.9	26.9 ± 7.3	27.1 ± 5.6	25.4 ± 5.7	25.8 ± 6.2	27.6 ± 7.7	26.0 ± 3.5	26.4 ± 6.6	28.0 ± 7.3	23.3 ± 6.2
Years between scans ± sd	3.0 ± 1.6	2.9 ± 1.8	3.1 ± 1.4	2.9 ± 1.8	2.9 ± 1.4	3.0 ± 1.6	3.3 ± 2.6	3.1 ± 1.8	2.9 ± 1.4	2.8 ± 1.4
Years between scans (min, max)	(1.1, 9.0)	(1.1, 9.0)	(1.8, 8.1)	(1.9, 8.2)	(1.9, 8.2)	(1.1, 9.0)	(2.0, 9.0)	(1.8, 9.0)	(1.1, 9.0)	(1.9, 6.4)
Neuroleptic Naïve prior to study	30%	30%	30%	31%	29%	29%	42%	30%	34%	11%
Clozapine use prior to study	8%	11%	7%	6%	11%	7%	0%	7%	7%	22%
Interim Clozapine use	18%	19%	17%	19%	27%	14%	0%	13%	22%	33%
Interim medication treatment[a]										
0 mg	6%	3%	9%	6%	11%	3%	0%	5%	7%	11%
1 mg – 400 mg	17%	22%	19%	0%	7%	26%	14%	15%	24%	0%
401 mg – 800 mg	24%	24%	23%	25%	24%	23%	29%	23%	24%	22%
> 800 mg	53%	51%	49%	69%	58%	48%	57%	57%	44%	67%

[a] average chlorpromazine-equivalent daily dose

Table 3

Changes in brain structure volumes during inter-scan interval (cc ± sd)

Region	Baseline Volume	Haplotype 1 alleles MANCOVA p=0.01				Haplotype 2 alleles MANCOVA p=0.59				Haplotype 3 alleles MANCOVA p=0.04			
		0 (n=37)	1 (n=57)	2 (n=16)	p-value	0 (n=45)	1 (n=58)	2 (n=7)	p-value	0 (n=60)	1 (n=41)	2 (n=9)	p-value
Cerebral cortex	1143 ± 130	−11 ± 6.5	1.7 ± 5.2	−27 ± 9.9	0.03	−9.8 ± 6.0	−7.8 ± 5.3	19 ± 15	0.21	−2.9 ± 5.3	−14 ± 6.4	0.8 ± 14	0.33
Gray	684 ± 79.5	−12 ± 6.8	3.7 ± 5.5	−16 ± 10	0.11	−11 ± 6.2	−1.9 ± 5.5	15 ± 16	0.26	0.1 ± 5.4	−5.3 ± 6.6	−30 ± 14	0.13
White	458 ± 64.7	0.3 ± 4.8	−2.0 ± 3.9	−11 ± 7.4	0.46	0.8 ± 4.3	−5.8 ± 3.8	4.2 ± 11	0.44	−3.0 ± 3.6	−9.1 ± 4.4	31 ± 9.2	0.0008
Frontal lobe	438 ± 56.4	−2.5 ± 3.4	0.8 ± 2.8	−18 ± 5.3	0.008	−7.3 ± 3.2	−1.4 ± 2.8	10 ± 8.2	0.10	−2.3 ± 2.86	−5.0 ± 3.5	0.3 ± 7.4	0.75
Gray	264 ± 35.9	−0.6 ± 3.1	−0.1 ± 2.5	−12 ± 4.8	0.10	−6.3 ± 2.8	0.3 ± 2.5	7.0 ± 7.2	0.10	−2.2 ± 2.5	−0.1 ± 3.1	−8.8 ± 6.5	0.48
White	175 ± 26.4	−1.9 ± 2.0	0.9 ± 1.6	−6.8 ± 3.0	0.07	−1.1 ± 1.8	−1.7 ± 1.6	3.1 ± 4.6	0.61	−0.1 ± 1.5	−4.9 ± 1.8	9.1 ± 3.9	0.005
Temporal lobe	226 ± 25.5	−1.3 ± 2.5	1.5 ± 2.0	−4.1 ± 3.8	0.38	−1.2 ± 2.3	0.1 ± 2.0	2.4 ± 5.8	0.81	0.6 ± 20	−1.7 ± 2.4	0.8 ± 5.1	0.75
Gray	154 ± 18.1	−2.8 ± 2.2	1.2 ± 1.7	−4.5 ± 3.3	0.20	−2.5 ± 2.0	−0.3 ± 1.7	3.0 ± 5.0	0.52	0.3 ± 1.7	−2.0 ± 2.1	−4.7 ± 4.5	0.49
White	372 ± 10.1	1.5 ± 1.0	0.3 ± 0.8	0.3 ± 1.5	0.64	1.2 ± .9	0.5 ± 0.8	−0.7 ± 2.3	0.70	0.3 ± .8	0.3 ± 0.9	5.5 ± 2.0	0.05
Parietal lobe	251 ± 29.6	−1.3 ± 2.2	3.4 ± 1.8	−2.3 ± 3.3	0.14	2.6 ± 2.0	−1.0 ± 1.8	7.2 ± 5.1	0.20	2.1 ± 1.7	−2.0 ± 2.1	7.1 ± 4.5	0.13
Gray	137 ± 17.1	−2.9 ± 1.6	2.3 ± 1.3	−0.4 ± 2.5	0.05	0.6 ± 1.5	−0.7 ± 1.3	4.0 ± 3.9	0.49	1.5 ± 1.3	−1.0 ± 1.6	−3.8 ± 3.4	0.25
White	114 ± 16.2	1.6 ± 1.4	1.1 ± 1.2	−1.9 ± 2.2	0.40	1.9 ± 1.3	−0.3 ± 1.1	3.2 ± 3.3	0.35	0.6 ± 1.1	−1.0 ± 1.3	11 ± 2.8	0.0009
Occipital lobe	124 ± 18.1	−1.0 ± 1.2	−1.9 ± 1.0	−0.8 ± 1.8	0.82	−1.4 ± 1.1	−1.7 ± 1.0	0.5 ± 2.8	0.76	−1.2 ± 1.0	−1.9 ± 1.2	−0.6 ± 2.5	0.84
Gray	69 ± 11.2	−1.4 ± 1.4	1.6 ± 1.1	2.4 ± 2.1	0.15	1.0 ± 1.3	0.3 ± 1.1	2.7 ± 3.2	0.75	2.0 ± 1.1	−0.2 ± 1.3	−3.5 ± 2.8	0.13
White	55 ± 11.4	0.4 ± 1.1	−3.5 ± 0.9	−3.3 ± 1.7	0.03	−2.3 ± 1.1	−2.0 ± 0.9	−2.2 ± 2.7	0.97	−3.2 ± 0.9	−1.7 ± 1.1	2.9 ± 2.3	0.05

Table 3 shows average change in brain structure volumes between time 1 and time 2 MRI brain scans for various haplotype based groups of subjects. The effects of the three haplotypes on the brain structure volumes (taken together) was first tested with MANCOVAs. The independent predictor was number of haplotype alleles, treated as a categorical variable, and covariates included total intra-cranial volume, age, gender, inter-scan interval, anti-psychotic treatment prior to first scan, and anti-psychotic treatment during the inter-scan interval. The group-level p-value is given above the columns and the univariate p-values are given for each region and haplotype.

Table 4

Symptom domain score at follow-up scan

| Symptom Domain | All Subjects (n=110) | Haplotype 1 alleles | | | | Haplotype 3 alleles | | | |
		0 (n=37)	1 (n=57)	2 (n=16)	p-value	0 (n=60)	1 (n=41)	2 (n=9)	p-value
Psychotic	3.0	2.8	2.5	4.5	0.006	2.9	2.8	3.0	0.90
Disorganized	1.9	1.8	1.8	2.0	0.71	2.0	1.8	1.0	0.21
Negative	10.2	9.7	9.6	11.1	0.22	10.6	9.2	8.0	0.17

Scores are adjusted for age, gender, inter-scan interval, and anti-psychotic usage. A higher score indicates increased symptom severity.