Influence of *ZNF804a* on Brain Structure Volumes and Symptom Severity in Individuals With Schizophrenia

Thomas H. Wassink, MD; Eric A. Epping, MD, PhD; Danielle Rudd, BA; Michael Axelsen, BA; Stephen Ziebell;
Frank W. Fleming; Eric Monson, BA; Beng Choon Ho, MD; Nancy C. Andreasen, MD, PhD

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Context: The single-nucleotide polymorphism rs1344706 in the gene *ZNF804a* has been associated with schizophrenia and with quantitative phenotypic features, including brain structure volume and the core symptoms of schizophrenia.

Objective: To evaluate associations of rs1344706 with brain structure and the core symptoms of schizophrenia.

Design: Case-control analysis of covariance.

Setting: University-based research hospital.

Participants: Volunteer sample of 335 individuals with schizophrenia spectrum disorders (306 with core schizophrenia) and 198 healthy volunteers.

Main Outcome Measures: Cerebral cortical gray matter and white matter (WM) volumes (total and frontal, parietal, temporal, and occipital lobes), lateral ventricular cerebrospinal fluid volume, and symptom severity from the Scale for the Assessment of Negative Symptoms and the Scale for the Assessment of Positive Symptoms divided into 3 domains: psychotic, negative, and disorganized.

Results: The rs1344706 genotype produced significant main effects on total, frontal, and parietal lobe WM volumes ($F = 3.98$, $P = .02$; $F = 4.95$, $P = .007$; and $F = 3.08$, $P = .05$, respectively). In the schizophrenia group, rs1344706 produced significant simple effects on total ($F = 3.93$, $P = .02$) and frontal WM volumes ($F = 7.16$, $P < .001$) and on psychotic symptom severity ($F = 6.07$, $P = .003$); the pattern of effects was concordant with risk allele carriers having larger volumes and more severe symptoms of disease than nonrisk homozygotes. In the healthy volunteer group, risk allele homozygotes had increased total WM volume compared with nonrisk allele carriers ($F = 4.61$, $P = .03$), replicating a previously reported association.

Conclusions: A growing body of evidence suggests that the risk allele of rs1347706 is associated with a distinctive set of phenotypic features in healthy volunteers and individuals with schizophrenia. Our study supports this assertion by finding that specific genotypes of the polymorphism are associated with brain structure volumes in individuals with schizophrenia and healthy volunteers and with symptom severity in schizophrenia.

Arch Gen Psychiatry. 2012;69(9):885-892

I N ONE OF THE FIRST GENOME-wide association studies of schizophrenia, the strongest evidence for association was to rs1344706, an A/C single-nucleotide polymorphism (SNP) in the gene *ZNF804a* (OMIM 612282), with the *A* allele being overrepresented in individuals with schizophrenia.[1] Replication of the association was confirmed within the original study and in multiple subsequent studies,[2-4] although other studies have found no association.[5] The locus was also associated with an expanded definition of the phenotype that included bipolar disorder[4] and psychosis.[1,3] Studies moving beyond diagnostic categories examined the effect of rs1344706 on quantitative phenotypic traits, including brain activation,[6,7] clinical symptomatol-ogy,[8] cognitive abilities,[7,9-11] and brain structure,[9,12] with results generally supporting an effect of the SNP on the trait of interest.

Our research group has a long-standing interest in studying relationships between genetic variants and brain structure volumes in schizophrenia,[13-20] and so we examined these findings in more detail. Lencz et al[9] assessed rs1344706 and brain structure in 39 healthy volunteers (HVs) and found that individuals homozygous for the risk allele (*AA*) had larger total white matter (WM) volumes than individuals with at least 1 nonrisk allele. Donohoe et al,[12] in a sample of 70 individuals with schizophrenia and 38 HVs, found that within the affected group, risk allele homozygotes had relatively larger gray matter (GM) volumes of the superior temporal gyrus, in-

Author Affiliations:
Department of Psychiatry,
University of Iowa Carver
College of Medicine, Iowa City.

sula, and hippocampus, whereas the polymorphism produced no effect on brain structure volumes among the HVs. To carry these findings forward, we performed similar analyses in a substantially larger sample of 335 individuals with schizophrenia and 198 HVs who had undergone magnetic resonance imaging (MRI) of the brain. In addition, we gathered symptom severity data on all individuals with schizophrenia at the time they underwent imaging. Thus, as findings emerged from tests of brain structure, we were able to determine whether rs1344706 was also associated with severity-of-illness measures. Our results support the WM findings of Lencz et al[9] and more precisely delineate the role of rs1344706 in shaping brain structure and the core symptoms of schizophrenia.

METHODS

A total of 335 patients with schizophrenia spectrum disorders and 198 HVs were available for this study through the University of Iowa Mental Health Clinical Research Center. These subjects participated in various Mental Health Clinical Research Center studies approved by the University of Iowa Institutional Review Board, and all subjects gave written informed consent to participate in these protocols.

Patients were evaluated using a semistructured interview instrument, the Comprehensive Assessment of Symptoms and History,[21] from which schizophrenia (n=310), schizoaffective disorder (n=21), delusional disorder (n=2), schizophreniform disorder (n=1), and schizotypal personality disorder (n=1) diagnoses meeting *DSM-III-R*[22] or *DSM-IV*[23] criteria were based. Healthy volunteers (n=198) were recruited from the community through newspaper advertisements. They were initially screened by telephone and further evaluated using an abbreviated version of the Comprehensive Assessment of Symptoms and History instrument to exclude subjects with current or past medical, neurologic, or psychiatric illnesses or with schizophrenia in first-degree relatives.

GENETIC ANALYSES

The DNA was prepared by high-salt extraction from whole blood.[24] One ng/μL DNA was amplified using ABI 2720 thermocyclers, an ABI Taqman SNP Genotyping 5′ nuclease assay for rs1347706, and ABI Taqman Universal PCR Master Mix (all, Applied Biosystems). Alleles were called using ABI Prism 7900 end point–read allelic discrimination software (Applied Biosystems). Replicate samples were included on all genotyping plates to ensure accurate allele calling.

MRI ACQUISITION AND IMAGE PROCESSING

High-resolution brain anatomical MRI data in this study were collected from 1 of 2 imaging protocols. Scans performed before calendar year 2000 (termed MR5) were obtained on a 1.5-T GE Signa MR scanner (General Electric Medical Systems). For the MR5 imaging protocol, 3-dimensional T1-weighted images were acquired in the coronal plane using a spoiled gradient recalled acquisition in steady state sequence. The parameters were echo time (TE)=5 milliseconds, repetition time (TR)=24 milliseconds, numbers of excitations (NEX)=2, rotation angle=45°, field of view (FOV) =26×24×18.8 cm, and matrix=256×192×124. Two-dimensional proton density and T2 sequences were acquired as follows: 3.0- or 4.0-mm-thick coronal slices, TR=3000 milliseconds, TE=36 milliseconds (for proton density) and 96 milliseconds (for T2), NEX=1, FOV=26×26 cm, and matrix=256×192.

Scans from 2000 or later (termed MR6) were obtained on a 1.5-T GE CVMRI scanner (General Electric Medical Systems) using T1- and T2-weighted sequences. For the MR6 imaging protocol, the T1 sequence was gathered as a 3-dimensional volume in the coronal plane using a spoiled gradient recalled acquisition in steady state sequence with the following parameters: TE=6 milliseconds, TR=20 milliseconds, flip angle=30°, FOV=160×160×192 mm, matrix=256×256×124, and NEX=2. The MR6 T2 images were acquired using a 2-dimensional fast spin-echo sequence in the coronal plane. The parameters were TE=85 milliseconds, TR=4800 milliseconds, slice thickness/gap=1.8/0.0 mm, FOV=160×160 mm, matrix=256×256, NEX=3, number of echoes=8, and number of slices=124.

To enhance MR5 and MR6 data compatibility, MR6 scans were resampled into the same resolution and image size as MR5 scans so as to simulate similar amounts of partial volume effects in voxels that border 2 tissue types. To verify our ability to combine data from the 2 MR protocols, we acquired MR5 and MR6 scans on 60 patients.[25] Brain volume differences between the 2 imaging sequences were small (median percent difference, 0.19%). Intraclass correlations were high across regions of interest (median intraclass correlation, 0.97). Hence, MR5 and MR6 data are compatible for combined statistical analyses.

Magnetic resonance images were processed using our locally developed BRAINS2 (Brain Research: Analysis of Images, Networks, and Systems, version 2) software package.[26] Detailed descriptions of image analysis methods have been provided elsewhere.[27-30] In brief, the T1-weighted images were spatially normalized and resampled so that the anterior-posterior axis of the brain was realigned parallel to the anterior-posterior commissure line, and the interhemispheric fissure was aligned on the other 2 axes. The T2-weighted images were aligned to the spatially normalized T1-weighted image using an automated image registration program.[31] These images were then subjected to a linear transformation into standardized stereotaxic Talairach atlas space[32] to generate automated measurements of frontal, temporal, parietal, and occipital lobes.[29] To further classify tissue volumes into GM, WM, and cerebrospinal fluid, we used a discriminant analysis method of tissue segmentation based on automated training class selection that used data from the T1 and T2 sequences.[31] In this study, we examined total and lobar cerebral cortical GM and WM volumes and cerebrospinal fluid volume of the lateral ventricles.

SYMPTOM ASSESSMENT

Symptom severity was assessed at the time of brain imaging using the Scale for the Assessment of Positive Symptoms (SAPS)[33] and the Scale for the Assessment of Negative Symptoms (SANS).[34] 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.[35] The psychotic dimension summed ratings for hallucinations and delusions; the disorganized dimension included ratings for psychotic formal thought disorder, bizarre/disorganized behavior, and inappropriate affect; and the negative dimension included ratings for attention, affective flattening, alogia, avolition/apathy, and anhedonia/asociality.

STATISTICAL ANALYSIS

Genotype frequencies of rs1344706 were compared between patients and HVs using χ^2 tests of differences.

Table 1. Genotype Frequencies

Group	Genotype Group, No. (%)			
	AA	*AC*	*CC*	All
Individuals with schizophrenia	126 (37.6)	165 (49.3)	44 (13.1)	335 (100.0)
Healthy volunteers	69 (34.8)	105 (53.0)	24 (12.1)	198 (99.9)

The relationships between rs1344706 genotype and the quantitative measures were examined using analysis of covariance (ANCOVA). Genotype is a 3-level variable that can be coded in numerous ways. One could presume an additive model in which each copy of the risk allele influences quantitative traits in an incremental fashion, but this would fail to detect recessive/dominant effects. Many studies, because of power limitations due to small sample sizes, combine minor allele homozygotes with heterozygotes and compare these against major allele homozygotes, but this would fail to detect additive effects or dominant effects of the major allele. Both of these approaches would be tests with 1 *df*. Given our larger sample, we chose a more conservative, inclusive approach, coding genotype as a 3-level categorical variable free from a priori assumptions, thereby producing a test with 2 *df* that compared each genotype group against the other 2.

We followed up these analyses with specific tests of replication. Lencz et al,[9] for example, found that HV risk allele homozygotes had increased frontal lobe WM volumes compared with other HVs, so we performed a similar test. Donohoe et al[12] found that risk allele homozygotes with schizophrenia had increased superior temporal GM volumes compared with others with schizophrenia, so we performed a similar analysis.

For tests of brain structure, MRI volumes were the dependent measures, genotype was the independent predictor, and intracranial volume, age at the time of imaging, sex, lifetime antipsychotic treatment, imaging protocol, and diagnostic grouping were entered as covariates. A genotype×diagnostic group interaction term was also included to assess whether genotype relationships with brain volumes differed across patients and HVs. For tests of symptom measures, genotype was the predictor, the 3-symptom summary scores were independent variables, and age and sex were covariates.

The brain structure tests included a treatment variable because we and others have shown that antipsychotic medications can affect brain structure volumes.[25,36] We use a measure that converts all antipsychotic medications into chlorpromazine hydrochloride dose equivalents and calculates a lifetime chlorpromazine equivalent exposure with the unit of measure being years taking chlorpromazine at 100 mg/d.[37] The value for HVs was zero, and because many of our schizophrenia subjects were early in the course of illness, this variable was not normally distributed and so was rank ordered before analysis.

SIGNIFICANCE

Determining an appropriate significance level for tests performed in this study is complicated by a number of factors. First, the brain structure volumes that we test are correlated with each other, and so treating them as independent, as in a Bonferroni correction, would be excessively conservative. Even after removing the variance due to the covariates, the GM measures are correlated with r values of 0.13 to 0.81, while the WM volumes are intercorrelated with r values of 0.41 to 0.90, all of which are highly significant (except for frontal and occipital GM volumes, which are not correlated). This pattern also diminishes enthusiasm for a multivariate ANCOVA test, which

is more appropriate for dependent measures that are not highly intercorrelated. Furthermore, as noted earlier, the primary motivation for this study was to test previously identified associations; although we perform additional tests, these are not numerous. In light of this, we choose $P=.01$ for significance.

RESULTS

Patients and HVs were of comparable mean (SD) age (31.7 [10.1] and 30.7 [9.7] years old, respectively; $t_{531}=1.05$, $P=.29$) and ethnicity (90.3% and 92.9% white, respectively; $\chi^2=1.23$, $P=.26$). A significantly greater proportion of HVs were female (48.0% vs 26.0% in the schizophrenia group; $\chi^2=26.0$, $P<.001$). The median chlorpromazine-equivalent years of treatment was 1.8, with 25.1% of subjects having received less than 3 months of chlorpromazine-equivalent treatment.

TESTING FOR ASSOCIATION OF rs1344706 WITH SCHIZOPHRENIA

Genotype frequencies were not significantly different between individuals with schizophrenia and HVs ($\chi^2=0.21$, $P=.90$) (**Table 1**).

RELATIONSHIPS BETWEEN rs1344706 AND BRAIN STRUCTURE VOLUMES

The rs1344706 genotype produced a significant main effect on frontal lobe WM volume ($F=4.95$, $P=.007$) (**Table 2**). As a measure of effect size, eta[2] showed the rs1344706 genotype to account for 1.6% of the variance in this structure after variance due to the covariates had been removed (**Table 3**). The relationship was in the direction described by Lencz et al,[9] with the *A* risk allele being associated with larger volumes than the nonrisk *C* allele. Temporal and parietal lobe WM volumes showed similar patterns of main effects, and the effect of rs1344706 on total cortical WM volume approached significance ($F=3.98$, $P=.02$). Genotype×diagnostic group interaction terms were not significant.

REPLICATION TESTS OF PREVIOUS BRAIN STRUCTURE FINDINGS

Lencz et al[9] tested for brain structure effects only in HVs, finding that *AA* homozygotes had larger total cerebral cortical WM volumes than combined *AC* and *CC* genotype groups. When we used this same grouping, we found a similar result in our HVs (total WM: $F=4.61$, $P=.03$). A subsequent study by Donohoe et al[12] used voxel-based morphometry to examine brain structure in 70 individu-

Table 2. Genotype Effects on Brain Structure Volumes[a]

| | Adjusted Mean (SD), mL | | | | | | Genotype Main Effects | |
| | Individuals With Schizophrenia | | | Healthy Volunteers | | | | |
Variable	AA (n = 126)	AC (n = 165)	CC (n = 44)	AA (n = 69)	AC (n = 105)	CC (n = 24)	F	P Value
Total GM	791.2 (86.5)	787.3 (76.5)	792.9 (87.2)	793.1 (78.9)	792.9 (77.9)	803.3 (78.2)	1.45	.23
Frontal GM	258.2 (33.1)	255.9 (27.7)	256.7 (33.8)	257.7 (28.9)	257.4 (27.9)	262.5 (28.7)	1.38	.25
Temporal GM	157.5 (17.1)	156.1 (17.2)	158.3 (19.2)	159.4 (17.0)	158.5 (17.7)	159.2 (15.1)	2.91	.06
Parietal GM	135.1 (15.8)	135.5 (14.1)	137.6 (17.2)	136.5 (14.1)	136.6 (15.1)	137.3 (14.8)	0.90	.41
Occipital GM	69.9 (8.4)	70.2 (8.0)	72.2 (7.7)	71.0 (8.4)	71.2 (8.4)	70.8 (8.2)	1.61	.20
Total WM	484.3 (71.8)	490.6 (65.2)	474.5 (67.0)	510.0 (67.8)	499.9 (67.0)	495.6 (69.6)	3.98	.02
Frontal WM	179.9 (29.0)	181.1 (26.1)	172.1 (24.8)	187.1 (27.5)	183.2 (25.6)	184.2 (26.8)	4.95	.007
Temporal WM	70.7 (10.6)	71.2 (10.7)	69.9 (11.0)	73.7 (10.8)	72.6 (11.3)	70.5 (12.0)	1.90	.15
Parietal WM	105.6 (17.1)	107.3 (15.7)	104.5 (16.4)	110.8 (15.4)	109.4 (16.3)	108.1 (17.4)	3.08	.05
Occipital WM	43.6 (8.6)	45.3 (8.6)	44.0 (8.1)	46.3 (7.9)	45.6 (8.2)	44.6 (8.00)	1.52	.22
Ventricular CSF	2.75 (0.45)	2.72 (0.53)	2.80 (0.63)	2.52 (0.48)	2.56 (0.55)	2.54 (0.44)	0.40	.67

Abbreviations: CSF, cerebrospinal fluid; GM, gray matter; WM, white matter.
[a] df for all tests = 523,9.

Table 3. Simple Main Effects and Effect Sizes

| | Simple Main Effects | | | | | | |
| | SZ | | HV | | Eta² | | |
Variable	F	P Value	F	P Value	All	SZ	HV
Total WM	3.93	.02	1.38	.25	1.1	2.0	2.6
Frontal WM	7.16	<.001	0.58	.56	1.6	3.3	1.5
Parietal WM	2.12	.12	1.61	.20	0.5	0.9	0.9

Abbreviations: HV, healthy volunteers; SZ, individuals with schizophrenia; WM, white matter.

als with schizophrenia and 38 HVs. They found GM volumes of the superior temporal gyrus to be enlarged in *AA* homozygotes in the schizophrenia group only, although the significance of the effect did not exceed a multiple testing correction. Because we did not use voxel-based morphometry, our ability to attempt replication of their results is limited; nonetheless, we found significant effects of rs1344706 genotype on GM volumes neither in our initial analyses nor in analyses comparing *AA* homozygotes against all others in the schizophrenia group.

POST HOC TESTS OF BRAIN STRUCTURE FINDINGS

Further examination of rs1344706 and WM volumes showed that the strength of the relationship appeared to arise predominantly from within the schizophrenia group (Table 3). Simple main effects of rs1344706 in the schizophrenia group were significant for total cortical WM and frontal WM volumes (frontal WM: $F=7.16$, $P<.001$; Eta²=3.3). Furthermore, the pattern of genotype group means in the schizophrenia group suggested a dominant effect of the *A* risk allele. This was borne out by post hoc tests showing that for individuals with schizophrenia, *AA* and *AC* least squares means for frontal WM volume were not different from each other ($P=.34$), whereas

both were different from *CC* (*AA* ≠ *CC*, $P=.04$; *AC* ≠ *CC*, $P=.005$). Last, an ANCOVA comparing the combined *AC* and *AA* genotype groups against *CC* homozygotes showed *A* allele carriers to have significantly larger frontal lobe WM volumes ($F=14.33$, $P<.001$).

EFFECTS OF rs1344706 ON SYMPTOM DIMENSION SCORES

The rs1344706 genotype produced a significant effect on psychotic dimension scores ($F=6.07$, $P=.003$; eta²=3.6) (**Table 4**), with the *A* risk allele being associated with more severe psychotic symptoms. When severity of delusions and hallucinations were analyzed separately, they showed similar patterns of genotype effects (Table 4). Neither the negative nor the disorganized symptom dimensions were significantly affected by the rs1344706 genotype (Table 4).

REPLICATION TESTS OF PREVIOUS SYMPTOM FINDINGS

At least 3 previous studies[8,10,38] evaluated the relationship between rs1344706 and symptoms in individuals with schizophrenia. Cummings et al[8] genotyped a large sample of subjects with schizophrenia, schizoaffective disorder, and bipolar disorder and measured symptoms with

Table 4. Genotype Effects on Symptom Severity[a]

| Symptom Domain | Genotype, Adjusted Mean (SD) | | | Effects Tests | | |
	AA (n = 126)	AC (n = 165)	CC (n = 44)	F	P Value	Eta²
Psychotic	6.6 (2.5)	7.0 (2.4)	5.4 (3.1)	6.07	.003	3.6
Delusions	3.7 (1.3)	3.7 (1.3)	3.1 (1.7)	3.83	.02	2.3
Hallucinations	2.9 (1.7)	3.3 (1.6)	2.3 (1.8)	5.56	.004	3.3
Negative	13.0 (4.4)	13.1 (4.4)	12.4 (5.2)	0.38	.68	. . .
Disorganized	5.2 (3.1)	5.1 (3.2)	5.1 (3.2)	0.06	.94	. . .

[a]Values are from the Scale for the Assessment of Negative Symptoms/Scale for the Assessment of Positive Symptoms scores; *df* = 330,4.
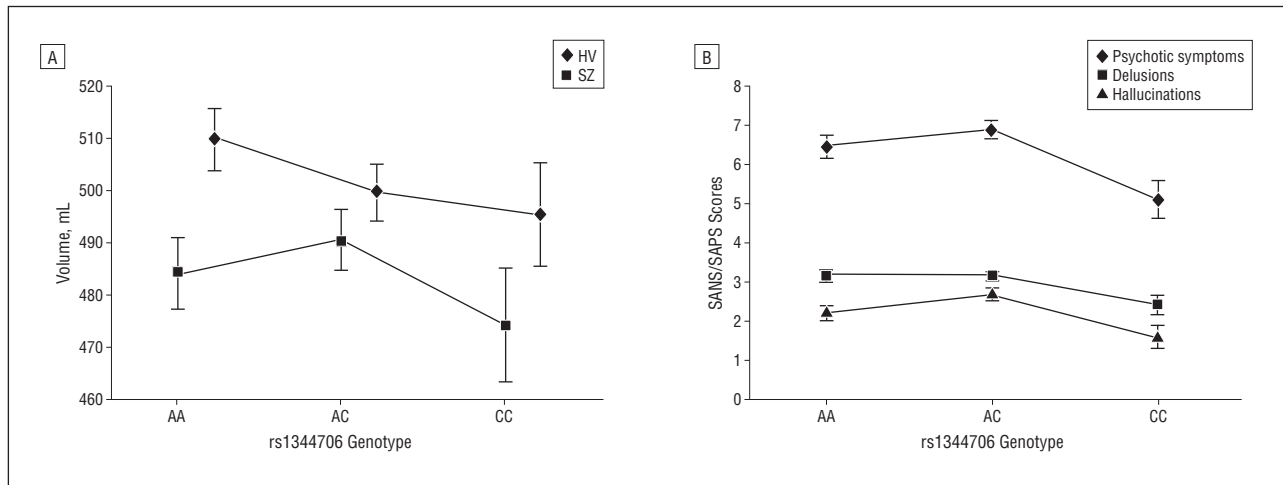

Figure. Brain structure and symptom effects of the rs1344706 genotype. Graphs show adjusted means and SEs of the means. A, Total cortical white matter volume. B, Psychotic dimension symptom severity. Error bars indicate 95% CIs. HV indicates group of healthy volunteers; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; and SZ, group of individuals with schizophrenia.

the Operational Criteria Checklist for Psychotic Illness[39] and the Bipolar Affective Disorder Dimension Scale.[40] They found that *A* allele carriers had more severe mania symptoms than *CC* homozygotes but that genotype was not related to other symptom domains, including psychotic symptoms.[8] We had not gathered a similar measure of mania symptoms and so were unable to test for the replication of this finding. Hashimoto et al[10] genotyped 113 patients with schizophrenia, measuring symptoms using the Positive and Negative Syndrome Scale,[41] and found no effect of genotype on positive or negative symptoms.

Last, Walters et al[38] genotyped 297 schizophrenia patients from Ireland and 251 from Germany. Symptoms in the German sample, evaluated using the Positive and Negative Syndrome Scale, were resolved to 5 factors, none of which were influenced by genotype. Symptoms in the Irish patients were assessed using the SANS/SAPS, as in our study, and were resolved by factor analysis into 3 symptom domains that exactly mirrored our symptom domains. The study reported a trend level effect of genotype on positive symptom severity in these patients (*F* =2.31, *P*=.06), with mean values for *AA* homozygotes being higher than scores from the other genotype groups.

In our data set, risk allele homozygotes also had more severe psychotic symptoms, but this relationship extended to risk allele carriers. Psychotic symptom scores of the *AA* group were similar to those of the *AC* group (*P*=.22 for *AA* ≠ *AC*), both of which were more severe than the *CC* group (*P*=.01 for *AA* ≠ *CC*; *P* < .001 for *AC* ≠ *CC*). An ANCOVA comparing the combined *AC* and *AA* genotype groups against *CC* homozygotes showed *A* allele carriers to have significantly more severe psychotic symptoms (psychotic symptom severity: *F* =10.57, *P*=.001; hallucination severity: *F* =7.32, *P*=.007; and delusion severity: *F* =7.68, *P*=.006). This was similar to the pattern of genotype effects that we observed with cerebral cortical WM volumes.

COMMENT

We tested relationships between the *ZNF804a* SNP rs1344706 and brain structure volumes in a large sample of individuals with schizophrenia and HVs. We replicated the finding that the *A* risk allele is associated with increased cerebral cortical WM volume[9] but did not substantiate previously reported effects of rs1344706 on cerebral cortical GM volume.[9,12] The brain structure results from our data were supported by similarly patterned relationships with psychotic symptom severity in the affected individuals (**Figure**). Thus, our study further strengthens the case for rs1344706 being of relevance to schizophrenia.

BRAIN STRUCTURE VOLUMES

One of the first studies examining the association of rs1344706 with brain structure was by Lencz et al,[9] who found that, in 39 HVs, *AA* risk allele homozygotes had increased total cortical WM volume compared with others (heterozygotes and nonrisk homozygotes). A subsequent study by Donohoe et al[12] examined voxel-based morphometry volumes in individuals with schizophrenia and HVs. They found GM volumes of the superior temporal gyrus and insula to be enlarged in *AA* homozygotes in the schizophrenia group only, although the significance of the effect did not exceed a multiple testing correction. In our sample of 198 HVs and 335 individuals with schizophrenia, rs1344706 produces a significant main effect on frontal WM volume, whereas the effect on cortical WM volume approaches significance; in both instances, the *A* risk allele is associated with increased WM volumes.

Additional analyses show that the *A* risk allele association occurs in HVs and individuals with schizophrenia. In a specific test of replication, when we categorize HVs according to the genotype grouping of Lencz et al,[9] we reproduce their result, showing that HV *AA* homozygotes have increased total cortical WM volume compared with HVs who carry at least 1 nonrisk allele ($P = .03$). We also find that the *A* allele is associated with increased WM volumes in individuals with schizophrenia, but in their case, risk allele carriers (*AA* and *AC*) appear to group together (Tables 2 and 3), both having larger volumes than *CC* homozygotes. Whether this different pattern of associations is biologically meaningful or simply due to chance is unclear.

In the context of this replication, our study produces a smaller estimate of the phenotypic influence attributable to rs1344706 than previous studies. Donohoe et al[12] did not provide effect size estimates, whereas Lencz et al[9] found rs1344706 genotype to account for 15% of total WM volume variance. We find rs1344706 genotype to account for only 1.1% of total cortical WM volume variance in the combined sample and 2.0% in the schizophrenia group. For frontal WM, our estimate is somewhat higher at 1.6% in the combined sample and 3.3% in the schizophrenia group. The difference may be due in part to our testing a 3-genotype model for rs1344706 instead of the 2-group model tested by Lencz et al,[9] but our estimate may also be more reliable given our larger sample size and the low likelihood that a single polymorphism would have such a strong effect on the genetically complex trait of brain structure volume.

SYMPTOMS

We also find a strong association between genotype and psychotic symptom severity that most closely resembles the result in Irish patients from Walters et al.[38] The association exists between both of the symptom measures that constitute the psychotic dimension—hallucinations and delusions—and effect sizes for rs1344706 are similar in magnitude to those for WM volumes, ranging from 2.3% to 3.6% (Table 4). Furthermore, the pattern of the association parallels that seen in cortical WM vol-

umes: the *AA* and *AC* groups have similar levels of psychotic symptom severity, and both of these are greater than the *CC* homozygotes. This concordance of associations is supported by previous studies relating symptoms to WM structure and function. Individuals at high risk for developing psychotic disorders who undergo MRI and who then go on to develop psychosis, for example, have increased WM volume compared with high-risk individuals who do not go on to develop psychosis.[42] Furthermore, diffusion tensor imaging studies have shown that in individuals with schizophrenia, increased cerebral cortical WM integrity, particularly in the frontal lobes, is associated with more severe psychotic symptoms.[43-45]

A growing body of evidence suggests that the risk allele of rs1347706 is associated with a distinctive set of phenotypic features in HVs and individuals with schizophrenia. Our study supports this contention by finding that, in both groups, the schizophrenia risk *A* allele is associated with increased frontal and total cortical WM volumes and, in the individuals with schizophrenia, with more severe psychotic symptoms. Our findings also demonstrate that the way in which symptoms are measured matters. The discrepancies across studies related to the symptom effects of rs1344706 may simply be due to the use of different assessment instruments. Although we cannot assert that our assessment was "correct," the fact that the 2 uses of the SANS/SAPS produced similar findings suggests that this instrument may measure symptoms in a manner more suited to genetic analysis.

In the context of these findings, a number of important questions remain unanswered. It is unclear, for example, whether the phenotype associated with the risk allele is actually "worse" than that associated with the nonrisk allele. Increased WM volume is not, in and of itself, pathologic, and affected individuals with primarily psychotic symptoms may have relatively well-preserved cognitive abilities. In line with this, although some studies have found that the risk allele is associated with poorer cognitive performance or impaired functional brain activity,[6,46-48] others have found the opposite.[12,38] In addition, the specific pattern of genetic influence remains unclear. Some studies[6,46,49,50] have found *A* allele carriers to be different than *CC* homozygotes, and others[9,47] have found *AA* homozygotes to be different than all others; some studies[6,48] have found a dose-dependent relationship with the risk allele. In our study, we find evidence for a carrier effect in HVs and a risk allele homozygote effect in individuals with schizophrenia. It may be that the associations actually are different in these 2 groups or that we still do not have large enough samples to reliably delineate the genotype-phenotype relationships for these quantitative traits.

Last, the question of how rs1344706 produces its effects remains mysterious. The ZNF804a protein is a brain-expressed zinc-finger protein that contains a C2H2-type domain but whose function is currently unknown.[51] Within *ZNF804a*, rs1344706 may be tagging a nearby polymorphism, and some evidence supports the influence of additional *ZNF804a* SNPs,[52] but most data point to rs1344706 itself as the risk variant. The rs1344706 genotype has been shown to have functional effects: the stretch of intron 2 DNA containing the rs1344706 *A* al-

lele, for example, binds to nuclear proteins less intensely than the same stretch containing the *C* allele,[53] and the *A* allele is associated with higher expression of *ZNF804A* messenger RNA in lymphoblasts[2,4] and postmortem brain tissue.[4] But the way in which this translates to effects on brain development, activity, and psychiatric phenotypes is a matter for ongoing and future scientific studies.

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