CACNA1C Genomewide Supported Psychosis Genetic Variation Affects Cortical Brain White Matter Integrity in Chinese Patients With Schizophrenia

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ABSTRACT

Objective: Recent genomewide association studies have implicated the calcium channel, voltage-dependent, L type, alpha 1C subunit (*CACNA1C)* genetic variant in schizophrenia, which is associated with functional brain changes and cognitive deficits in healthy individuals. However, the impact of *CACNA1C* on brain white matter integrity in schizophrenia remains unclear. On the basis of prior evidence of *CACNA1C*-mediated changes involving cortical brain regions, we hypothesize that *CACNA1C* risk variant rs1006737 is associated with reductions of white matter integrity in the frontal, parietal, and temporal regions and cingulate gyrus.

Method: A total of 160 Chinese participants (96 *DSM-IV*–diagnosed patients with schizophrenia and 64 healthy controls) were genotyped by using blood samples and underwent structural magnetic resonance imaging and diffusion tensor imaging scans from 2008 to 2012. Two-way analysis of covariance was employed to examine *CACNA1C*-related genotype effects, diagnosis effects, and genotype × diagnosis interaction effects on fractional anisotropy (FA) of relevant brain regions.

Results: Significant diagnosis-genotype interactions were observed (left frontal lobe mean FA: $F_{1,156} = 6.22$, $P = .014$; left parietal lobe mean FA: $F_{1,156} = 7.14$, $P = .008$; left temporal lobe mean FA: $F_{1,156} = 8.37$, $P = .004$). Compared with patients who were A carriers, patients who were G homozygotes had lower mean FA in the left frontal lobe ($F_{1,93} = 2.504$, $P = .014$), left parietal lobe ($F_{1,93} = 2.37$, $P = .020$), and left temporal lobe ($F_{1,93} = 3.01$, $P = .003$), with standardized effect sizes of −1.43, −1.3, and −1.0, respectively.

Conclusions: *CACNA1C* risk variant rs1006737 affects cortical white matter integrity in schizophrenia. Further imaging genetic investigations on the mediating effect of *CACNA1C* in schizophrenia can uncover brain circuitries involved in schizophrenia and suggest potential novel targets for intervention.

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Previous genomewide association studies have implicated calcium channel, voltage-dependent, L type, alpha 1C subunit *(CACNA1C)* as a genetic risk substrate for affective spectrum disorders, including bipolar disorder[1–3] and major depressive disorder,[4] and neurodevelopmental spectrum disorders such as autism[5] and schizophrenia.[4,6] Recent efforts at studying common genetic factors across psychiatric disorders have consistently pointed to *CACNA1C* as a shared genomewide supported susceptibility gene in schizophrenia and psychotic spectrum conditions. The Psychiatric Genome-Wide Association Study Consortium (PGC)[7] compared the genetic data of 16,374 patients with bipolar disorder and schizophrenia with 14,044 controls and found significant association with *CACNA1C* risk variant, which crosses genomewide significance at $P = 7 \times 10^{-9}$. Another study[8] within the PGC examined 2,640 patients with schizophrenia seen at a clozapine clinic, 2,504 patients with bipolar disorder, and 2,878 controls and found the *CACNA1C* gene as a new locus suggesting neurobiological overlap between schizophrenia and bipolar disorder. A more recent European study[9] by the Cross-Disorder Group evaluated 33,332 cases of 5 disorders (schizophrenia, bipolar disorder, depression, attention-deficit/hyperactivity disorder, and autism) and 27,888 healthy controls highlighted the role of *CACNA1C* and calcium channel signaling genes underlying these disorders along a neurodevelopmental continuum. *CACNA1C* is highly expressed in the central nervous system; encodes α1 subunit of voltage-dependent calcium channel, which is involved in coupling of cell membrane depolarization with transient membrane permeability changes for calcium; and regulates calcium influx for neuronal signaling and communication.[10,11] *CACNA1C* also regulates the expression of genes that are involved in neuronal growth and is thus important for synaptic plasticity, survival of neurons, dendritic development, and learning and memory.[12,13]

Although the mechanism underlying the relationship between *CACNA1C* and psychotic spectrum conditions is not fully known, the putative genomewide psychosis susceptibility *CACNA1C* gene has been associated with specific clinical characteristics,[11,14] cognitive functioning,[15,16] and functional neuroimaging brain substrates[17–20] in healthy individuals. In comparison, there are fewer data on *CACNA1C*-related morphological brain changes in health and illness. The scant genetic-structural neuroimaging studies pertaining to the *CACNA1C* gene suggest either an increase or no increase in gray matter volume,[21,22] an increase in brainstem volume,[23] and an increase in gray matter density in the right amygdala and right hypothalamus[24] in healthy individuals.

A better understanding of the impact of the *CACNA1C* gene on brain white matter integrity in schizophrenia is important for a few reasons. First, it can clarify how the genomewide supported psychosis *CACNA1C* gene affects cortical brain white matter integrity especially involving frontal and temporal regions, which are implicated in extant cognitive

Clinical Points

- The risk gene *CACNA1C* is associated with reductions of cortical white matter integrity involving frontal, parietal, and temporal regions in this study.
- These findings complement earlier *CACNA1C*-related cognitive and functional neuroimaging findings in healthy controls.
- Clarification of the neural effects of *CACNA1C* and other putative genomewide supported psychosis susceptibility genes allows better understanding of the pathophysiology of schizophrenia.

and functional magnetic resonance imaging (MRI) studies of the *CACNA1C* gene in controls as well as separately in patients with schizophrenia. Second, investigations of white matter integrity can complement other investigative modalities to extend and expand our understanding on the effect of genomewide supported psychosis genetic factors on intermediate phenotypic features seen in schizophrenia. This information can shed light on potential neural biomarkers of illness onset, progression, and changes after intervention. Third, there is increasing interest in looking at effects of different genes on brain circuitries in neuropsychiatric conditions.[25,26] Better appreciation of the changes involving these neural networks could potentially provide further insights into the structural basis for functional neuroimaging activation changes and cognitive and clinical factors related to *CACNA1C* in illness. Fourth, common brain white matter regions affected by different putative genes may highlight possible interrelated epistatic effects of multiple and different genes in the pathogenesis and neurobiological basis of schizophrenia.[27–29]

To date and to the best of our knowledge, there is no study on *CACNA1C*-mediated brain white matter integrity in patients and compared with healthy controls. On the basis of prior evidence of *CACNA1C*-mediated changes involving the frontal and temporal regions,[17,18] we sought to examine the effect of *CACNA1C* on brain cortical white matter integrity. We hypothesized that *CACNA1C* risk variant rs1006737 is associated with reductions of white matter integrity in the frontal, parietal, and temporal regions and cingulate gyrus in schizophrenia.

METHOD

Subjects

The study sample comprised 160 Chinese participants (115 men and 45 women) who gave written informed consent to participate in the study after a detailed explanation of the study procedures from 2008 to 2012. Ninety-six participants were patients recruited from the Institute of Mental Health, Singapore, who fulfilled the *DSM-IV*[30] diagnosis of schizophrenia. All diagnoses were made by a psychiatrist (K.S.) using information obtained from the existing medical record, clinical history, mental status examination, and interviews with the patients and their significant others as well as the administration of the Structured Clinical Interview for *DSM-IV* Axis I Disorders-Patient Version (SCID-I/P).[31] There was no history of any significant neurologic illness, such as seizure disorder, head trauma, or cerebrovascular accident, and no subject met *DSM-IV* criteria for alcohol or other substance abuse in the preceding 3 months. The patients were maintained on a stable dose of antipsychotic medications for at least 2 weeks prior to the recruitment and did not have their medication withdrawn for the purpose of the study. Forty-six patients were receiving second-generation antipsychotics, 50 were receiving first-generation antipsychotics, and 8 were taking a combination of first- and second-generation antipsychotics; the mean (SD) antipsychotic dose was 231.37 (207.32) daily chlorpromazine equivalents in milligrams (see Supplementary eTable 1 at Psychiatrist.com). The remaining 64 age-, gender-, and handedness-matched healthy controls were screened by using the Structured Clinical Interview for DSM-IV Axis I Disorders-Nonpatient Version (SCID-I/NP)[32] and deemed not to suffer from any Axis I psychiatric disorder, and they had no history of any major neurologic disorders or medical illnesses, substance abuse, or psychotropic medication use. Healthy controls were recruited from the staff population at the hospital as well as from the community by advertisements. Blood samples were drawn from all participants and subsequently genotyped. Psychopathology and symptom severity of patients were also assessed by using the Positive and Negative Syndrome Scale (PANSS).[33] This study was approved by the Institutional Review Boards of the Institute of Mental Health, Singapore, as well as the National Neuroscience Institute, Singapore.

Genotyping Procedures

Genotyped data (single-nucleotide polymorphism rs1006737) were obtained through an ongoing genetic association study by using the Illumina HumanHap 250K and 317K BeadChips (Illumina Inc, San Diego, California) at the Genome Institute of Singapore, Agency for Science, Technology and Research (GIS/ASTAR). The DNA sample was isothermally amplified to be subsequently fragmented by a controlled enzymatic process that does not require gel electrophoresis. The DNA was consequently alcohol precipitated and hybridized. Allelic specificity was conferred by enzymatic single-based extension reaction followed by fluorescence staining. The intensities of the beads' fluorescence were picked up by the Illumina BeadArray Reader and analyzed by using Illumina BeadStudio software. For quality control, the samples were included for further analysis only if the genotyping rate was >98%, the call rate >90%, a minor allele frequency >5%, and the samples were in Hardy-Weinberg equilibrium (HWE) ($P>.05$). Statistical analyses were performed by using the Haploview v4.2[34] and PASW18 (SPSS Inc, Chicago, Illinois).

Brain Imaging Acquisition

Brain imaging was performed at the National Neuroscience Institute, Singapore, on all participants (96 schizophrenia and 64 healthy controls) by using a 3-Tesla whole body scanner (Philips Achieva, Philips Medical System, Eindhoven, The

Table 1. Demographic and Clinical Features of Subjects

Feature	Healthy Controls (n = 64)	Schizophrenia (n = 96)	Statistic	*P* Value
Age, mean (SD), y	31.7 (10.5)	33.9 (9.4)	$t = -1.39$	.166
Education, mean (SD), y	13.9 (2.0)	11.4 (2.4)	$t = 6.73$	< .001
Age at onset, mean (SD), y	…	26.3 (8.0)	…	…
Duration of illness, mean (SD), y	…	7.5 (7.8)	…	…
Clozapine equivalent, mean (SD), mg/d	…	231.37 (207.324)	…	…
PANSS score, mean (SD)				
PANSS total	…	41.30 (9.720)	…	…
PANSS positive	…	11.12 (4.136)	…	…
PANSS negative	…	9.58 (3.668)	…	…
PANSS general psychopathology	…	20.60 (9.72)	…	…
Gender, n (%)			$\chi^2 = 2.06$	.157
Male	42 (65.6)	73 (76.0)		
Female	22 (34.4)	23 (24.0)		
Handedness, n (%)			$\chi^2 = 0.31$	.783
Left	5 (7.8)	10 (10.4)		
Right	59 (92.2)	86 (89.6)		
Marital status, n (%)			$\chi^2 = 11.1$	.011
Single	45 (70.3)	84 (87.5)		
Married	18 (28.1)	10 (10.4)		
Divorced	0	2 (2.1)		
Widowed	1 (1.6)	0		
Employment status, n (%)			$\chi^2 = 64.4$	< .001
Employed	61 (95.3)	29 (30.2)		
Unemployed	3 (4.7)	67 (69.8)		

Abbreviation: PANSS = Positive and Negative Syndrome Scale.
Symbol: … = not applicable.

Netherlands) with a sensitivity encoding (SENSE) head coil. High-resolution T1-weighted magnetization-prepared rapid gradient echo (MP-RAGE) was required (repetition time [TR] = 7.2 seconds, echo time [TE] = 3.3 milliseconds, flip angle = 8°). Each T1-weighted volume consisted of 180 axial slices of 0.9-mm thickness with no gap (field of view, 230 × 230 mm; acquisition matrix, 256 × 256 pixels). For diffusion-tensor imaging (DTI), single-shot echo-planar diffusion tensor images were obtained (TR = 3,725 milliseconds; TE = 56 milliseconds; flip angle = 90°, $b = 800$ s/mm^2) with 15 different nonparallel directions ($b = 800$ s/mm^2) and the baseline image without diffusion weighting ($b = 0$ s/mm^2). The acquisition matrix was 112 × 109 pixels with a field of view of 230 × 230 mm, which was zero-filled to 256 × 256 pixels. A total of 42 axial slices of 3.0-mm thickness were acquired parallel to the anterior-posterior commissure line. The T1-weighted and DTI data were sequentially acquired in a single session scan time without position change. Stability of a high signal-to-noise ratio was assured through a regular automated quality control procedure.

Image Processing

The structural MRI images were converted from the scanned images into the Analyze format, which were further processed by using the Free Surfer software package (Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard University, http://surfer.nmr.mgh.harvard.edu/). The software reformats each brain volume image into a volume image with 1 mm^3 isovoxels, from which relevant brain structures can be delineated.[35–40] This automated method has been shown to be statistically indistinguishable from manual raters and reduces random errors, rater error, and intersubject variability typical of manual techniques.[38] Fractional anisotropy (FA) maps were acquired from the DTI images from the software DTI Studio[41] and were then coregistered automatically to the MP-RAGE images by using a mutual information cost function and a 12-parameter affine transformation. Eddy current correction was performed prior to registration. As the DTI images were coregistered to the subjects' structural images, FA images were also automatically delineated for the separate brain structures by using the same delineation parameters in the structural images, and FA parameters of relevant brain structures were obtained.

Statistical Analyses

Demographic variables between schizophrenia and healthy controls were compared by using 2-sample Student *t* test and χ^2 test for continuous and categorical variables, respectively. Allelic frequency, genotype frequency, and genotype-diagnosis interactions were obtained by using PASW 18, while the HWE *P* values were obtained by using the Haploview v4.2.[34] The *CACNA1C* genotype effect, diagnosis effects, and genotype-diagnosis interactions on FA were examined by using a 2-way analysis of variance (ANOVA). The genotype effect, diagnosis effect, and genotype-diagnosis interactions were further analyzed by using the 2-way analysis of covariance (ANCOVA) to control for covariates such as age, gender, subject years of education, and intracranial volume. When significant genotype-diagnosis interaction was found, cases and controls were further evaluated separately. The significance level for statistical tests was set at 2-tailed $P < .05$.

RESULTS

Demographic Clinical Characteristics of Subjects

In the whole sample, there was no statistically significant difference in age, gender, or handedness between the schizophrenia and healthy control groups. However, compared to healthy controls, the schizophrenia group had fewer years of education ($P < .001$) and was more likely to be single ($P < .05$) and unemployed ($P < .001$). Table 1 and Supplementary eTable 2 show the sociodemographic and clinical profile of the entire sample.

The Effect of the *CACNA1C* Risk Variant on Cortical White Matter Integrity

Overall, the G allele frequency for the genomewide supported psychosis risk variant rs1006737 of *CACNA1C* among patients in the present study was 97.3%, which was comparable with another recent study[15] of *CACNA1C* in Asian subjects with schizophrenia. The genotype frequencies of the *CACNA1C* risk variant rs1006737 at 12p13.3 in healthy

Table 2. Effects of *CACNA1C* rs1006737 on Brain White Matter Integrity

	Healthy Controls (n = 64), FA				Schizophrenia (n = 96), FA				Analysis of Covariance (adjusted)[a]					
	A Carriers		GG		A Carriers		GG		Diagnosis Effect		Genotype Effect		Interaction	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*	*P*	*F*	*P*	*F*	*P*
Left parietal lobe	0.22	0.039	0.23	0.040	0.30	0.096	0.24	0.044	6.52	.012	3.79	.054	7.14	**.008**
Right parietal lobe	0.23	0.015	0.22	0.028	0.25	0.075	0.23	0.038	0.99	.321	1.23	.270	0.01	.941
Left cingulate gyrus	0.23	0.024	0.25	0.056	0.30	0.175	0.24	0.064	1.19	.277	1.12	.292	3.85	.052
Right cingulate gyrus	0.18	0.025	0.22	0.043	0.28	0.138	0.22	0.066	6.54	.012	0.12	.728	5.87	**.017**
Left temporal lobe	0.18	0.056	0.23	0.048	0.32	0.092	0.25	0.072	3.80	.054	0.85	.359	8.37	**.004**
Right temporal lobe	0.26	0.015	0.26	0.031	0.29	0.060	0.28	0.045	1.62	.205	0.01	.922	0.03	.863
Left frontal lobe	0.23	0.018	0.24	0.023	0.25	0.021	0.22	0.023	0.38	.539	1.77	.185	6.22	**.014**
Right frontal lobe	0.25	0.025	0.26	0.024	0.25	0.274	0.26	0.245	0.12	.73	0.76	.39	0.02	.891

[a]Adjusted for age, gender, subject years of education, and intracranial volume.
Abbreviations: *CACNA1C* = calcium channel, voltage-dependent, L type, alpha 1C subunit; FA = fractional anisotropy; GG = G homozygote.

controls were 7 (10.9%) for A allele carriers and 57 (89.1%) for individuals who were G homozygotes and, in schizophrenia patients, 5 (5.2%) for A allele carriers and 91 (94.8%) for individuals with the GG genotype (HWE $P = 1.000$).

There were significant diagnosis-genotype interactions (left frontal lobe mean FA: adjusted $F_{1,156} = 6.22$, $P = .014$; left parietal lobe mean FA: adjusted $F_{1,156} = 7.14$, $P = .008$; right cingulate gyrus mean FA: adjusted $F_{1,156} = 5.87$, $P = .017$; left temporal lobe mean FA: adjusted $F_{1,156} = 8.37$, $P = .004$). As the diagnosis-genotype interactions were found to be significant for left frontal, parietal, and temporal cortices and right cingulate gyrus (Table 2), we analyzed the genotype effects on these brain regions between the 2 subject groups as well as within patients and controls (Figure 1). Genotype effects were observed in that patients with schizophrenia and G homozygous genotype had lower mean FA when compared to patients who were A allele carriers, in the left frontal lobe ($F_{1,93} = 2.504$, $P = .014$), left parietal lobe ($F_{1,93} = 2.37$, $P = .020$), and left temporal lobe ($F_{1,93} = 3.01$, $P = .003$), with standardized effect sizes of −1.43, −1.3, and −1.0, respectively. Within healthy controls, individuals with GG genotype were noted to have higher mean FA than A-allele carriers in right cingulate gyrus ($F_{1,60} = 2.34$, $P = .023$) and left temporal lobe ($F_{1,60} = 2.17$, $P = .034$).

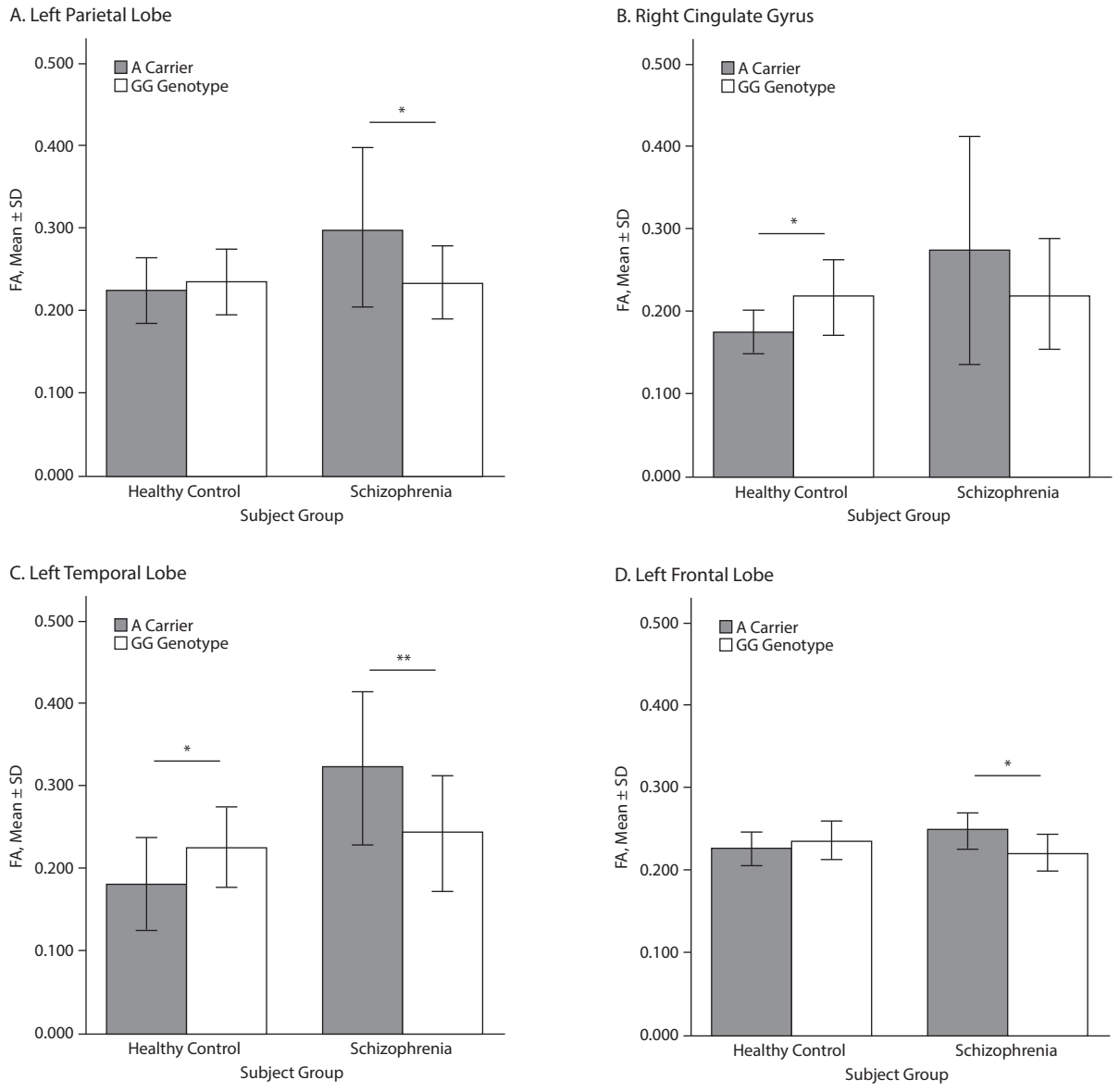
DISCUSSION

In this study, we investigated the effect of *CACNA1C* risk variant on the brain white matter integrity in schizophrenia compared with healthy controls. We found specific *CACNA1C* genotype effects in that patients who were G homozygotes had reduced mean FA in the left frontal, parietal, and temporal cortical regions compared with A carriers. Within healthy controls, we found that G homozygote genotype was associated with nonreduction of mean FA in right cingulate gyrus and left temporal region, highlighting that *CACNA1C* risk variant influences brain white matter integrity in a pleiotropic manner and contributes to morphological changes involving disruptions of white matter integrity within cortical brain regions.

Within this study, the disruptions of white matter integrity in terms of mean FA reductions in frontal, temporal, and parietal cortices within schizophrenia are consistent with previous similar findings in similar brain regions in schizophrenia, although not within the context of association with the putative genomewide supported psychosis susceptibility gene. Szesko et al[42] studied patients with early-onset schizophrenia and found reductions of temporal and frontal FA, which were consistent with the observations of Federspiel et al.[43] In addition, 2 other studies[44,45] documented reductions in parietal FA in patients with first-episode schizophrenia compared with controls. The involvement of white matter within these brain regions occurs within patients with early-onset as well as chronic illness. Studies in patients with chronic schizophrenia found FA reductions in similar frontal, temporal, and parietal cortical regions.[4,46] As such, earlier findings of FA reductions in the frontal-temporal-parietal brain regions together with our findings suggest that underlying genetic factors such as *CACNA1C* may mediate changes in white matter integrity involving these cortical brain regions in schizophrenia. Overall, there are scant studies looking at the effects of putative genetic factors on white matter integrity in schizophrenia. Our earlier study[47] documented that another genomewide supported susceptibility *ZNF804A* gene is associated with lower FA in bilateral parietal lobes and left cingulate gyrus in schizophrenia. However, other combined genetic-neuroimaging studies of schizophrenia have found involvement of cortical white matter volumes. Significant genotype effects influencing frontal, parietal, or temporal regions have been noted in schizophrenia that are related to RGS4,[48] NRG1,[49] COMT,[50] and G72 genes.[51] Further study is needed to examine the epistatic effects between these different genes in affecting brain white matter volumes and integrity and how they, in turn, impact on functional MRI measures in schizophrenia.

Within healthy controls, GG genotype was associated with nonreduction of white matter integrity in right cingulate gyrus and left temporal lobe, suggesting pleiotropic effects of *CACNA1C* gene on brain white matter integrity. Extant structural imaging studies[21–24] found that the met allele of *CACNA1C* was associated with increased gray matter volume, brain stem volume, and subcortical amygdala volumes. Functional MRI studies[19,20] in healthy subjects have found disturbances in frontal-temporal or frontal-

Figure 1. The Association Between *CACNA1C* rs1006737 and the Brain White Matter Regions[a]



[a]Error bars indicate 95% CI.
*$P < .05$.
**$P < .005$.
Abbreviations: *CACNA1C* = calcium channel, voltage-dependent, L type, alpha 1C subunit; FA = fractional anisotropy.

subcortical brain connectivity, which could be mediated by *CACNA1C* genetic variant, although the precise structural basis (volume, white matter integrity) in these healthy controls is less clear. Genetic imaging studies involving other genetic factors implicated in genetic association studies of schizophrenia have found involvement of related cortical white matter volumes studied here. Ohnishi et al[52] found that the catechol-*O*-methyltransferase (COMT) valine (Val) allele was associated with reductions of cingulate gyrus and temporal lobe volumes. Wang et al[53] noted FA changes in anterior cingulum related to neuregulin 1 (NRG1) genotype, and Takahashi et al[54] found disrupted in schizophrenia 1 (DISC1)–related increases in frontal lobe volume within controls. Better appreciation of the influence of relevant genetic factors on these cortical brain structures in controls would clarify gene-gene interactions on brain morphology and allow differentiation of effects on brain white matter in illness.

How is *CACNA1C* relevant to the pathogenesis of cortical white matter abnormalities and dysconnectivity in schizophrenia? This is not entirely clear. *CACNA1C* is highly expressed in the central nervous system, especially the frontal-temporal region,[55] and serves to regulate Ca^{2+} influx for neural communication.[11] This regulation is essential in various processes, including the excitation of neurons and activation of downstream transcription factors necessary for

neuronal cell division and growth and gene expression,[55] as well as maintenance of synaptic plasticity.[12] The *CACNA1C* rs1006737 allele lies within an intronic region of *CACNA1C*, which is involved in the regulation of alternative splicing events. Since the mature protein of *CACNA1C* is formed via alternative splicing,[56] it is possible that the resultant truncated genetic variants could affect the expression level of *CACNA1C* and alter the configuration of the *CACNA1C*-encoded $Ca_V1.2$ channel, thus leading to structural and/or functional neurotransmission abnormalities in the cortical brain regions. In terms of applicability, existing calcium channel drugs were found to have effects on affect, which is affected in patients with psychotic spectrum conditions.[11] Clarification of the neurobiological effects of *CACNA1C* may facilitate discovery of potential promyelinative interventions[57–60] to improve white matter neuroplasticity in patients struck with such a severe and devastating illness.

There are several limitations in this study. First, as this is the first study investigating the effect of *CACNA1C* on brain white matter integrity in patients with schizophrenia, and compared with the healthy controls, these findings need to be replicated in other samples. Second, we did not correlate the structural findings with neurocognitive data, which can offer further insight into the full genetic impact of *CACNA1C* in this study. Third, we did not examine other brain structural parameters such as cortical thickness and specific white matter tracts. Fourth, although a region-of-interest approach was adopted in this study, a better understanding of the biological impact of this putative genomewide psychosis susceptibility variant may be further obtained by complementing the study with whole brain, voxel-based approaches.

Notwithstanding the limitations, we found that *CACNA1C* influences cortical white matter integrity in the frontal, temporal, and parietal regions in schizophrenia. Our data complement findings from previous genomewide association studies and *CACNA1C*-related cognitive and functional neuroimaging studies in healthy controls and patients with schizophrenia to provide a structural basis for earlier findings. Further study is warranted to understand the full impact of this genomewide supported *CACNA1C* risk gene in psychosis and its interrelated, interactional, and integrated role with other genetic and nongenetic factors in the neurobiology of schizophrenia.

Drug names: clozapine (Clozaril, FazaClo, and others).
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Supplementary material follows this article.

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Supplementary Material

Article Title: CACNA1C Genomewide Supported Psychosis Genetic Variation Affects Cortical Brain White Matter Integrity in Chinese Patients With Schizophrenia

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List of Supplementary Material for the article

1. eTable 1 Demographic and Clinical Features of Patients with Schizophrenia, divided by types of medication

2. eTable 2 Differences in Demographic and Clinical Features between Genotypes within Subjects

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Supplementary eTable 1. Demographic and Clinical Features of Patients with Schizophrenia, divided by types of medication

Antipsychotic types		**Typical (n = 41)**		**Atypical (n = 45)**		**Combined (n = 10)**		**Statistical analysis**	
		Mean	SD	Mean	SD	Mean	SD	F	p
Age		36.37	8.09	31.49	9.27	35.9	12.73	3.250	0.043*
Years of education		11.51	2.38	11.49	2.57	11	1.89	0.190	0.830
Age at onset		27.05	6.57	25.36	8.59	27	10.56	0.517	0.590
Duration of illness		9.24	8.59	5.80	5.98	8.60	10.53	2.240	0.112
Duration of untreated psychosis		1.67	3.34	1.62	2.01	1.10	1.07	0.203	0.820
Antipsychotic dose (CPZ)		249.50	206.04	215.11	211.72	280	196.07	0.536	0.587
PANSS total score		42.80	12.26	40.16	7.30	38.60	4.69	1.204	0.305
PANSS positive subscale score		11.10	4.30	11.02	4.06	10	2.46	0.061	0.941
PANSS negative subscale score		10.22	4.42	9.11	2.88	8.50	2.95	1.443	0.241
PANSS general psychopathology subscale score		21.49	5.70	10.02	2.71	19.50	4.25	1.671	0.194
		%		%		%		X^2	p
Gender	Males	82.9		71.1		70		1.868	0.393
	Females	17.1		28.9		30			
Handedness	Left	4.9		11.1		30		5.481	0.065
	Right	95.1		88.9		70			
Genotype	A-carriers	7.5		4.4		0		1.017	0.601
	GG	92.5		95.6		100			
Marital	Single	82.9		91.1		90		1.719	0.787
	Married	14.6		6.7		10			
	Divorced	2.4		2.2		0			

Abbreviations: SD, standard deviation; CPZ, chlorpromazine equivalence; PANSS, Positive and Negative Syndrome Scale
* $p < .05$

Supplementary eTable 2. Differences in Demographic and Clinical Features between Genotypes within Subjects

	Controls (n = 64)				SCZ Patients (n = 96)				ANCOVA[a] (adjusted)					
	A carriers (n = 7)		GG (n = 57)		A carriers (n = 5)		GG (n = 91)		Diagnosis effect		Genotype effect		Interaction	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*	*p*	*F*	*p*	*F*	*p*
Age, mean (s.d.), y	39.71	12.776	30.74	9.832	38.20	7.3621	33.68	9.466	0.019	0.891	5.129	0.025	0.130	0.719
Education, mean (s.d.), y	13.14	2.854	13.98	1.923	12.60	2.510	11.34	2.391	4.240	0.041	0.331	0.566	2.239	0.137
									Statistics				***P-value***	
Age at onset, mean (s.d.), y					23.00	4.416	26.31	8.035	t = -0.911				.365	
Antipsychotic dose, Mean daily CPZ mg/d					240.00	151.658	231.98	210.706	t = 0.084				0.933	

PANSS total score	41.30	15.1921	41.47	9.470	t = 0.030	0.976
PANSS positive subscale score	11.20	4.604	11.14	4.156	t = 0.029	0.977
PANSS negative subscale score	9.60	4.775	9.69	3.680	t = -0.052	0.959
PANSS general psychopathology subscale score	20.80	6.419	20.63	4.161	t = 0.085	0.933

Abbreviations: SCZ, patients with schizophrenia; s.d., standard deviation; y, years; PANSS, Positive and Negative Syndrome Scale.