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No Association Between Schizophrenia Susceptibility Variants and Macroscopic Structural Brain Volume Variation in Healthy Subjects

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Previous studies have suggested that genetic variants for schizophrenia susceptibility might contribute to structural brain volume variations in schizophrenia patients, including total brain volume, hippocampal volume, and amygdalar volume. However, whether these schizophrenia susceptibility variants are associated with macroscopic structural brain volume (i.e., intracranial volume, total brain volume, and hippocampal volume) in healthy subjects is still unclear. In this study, we investigated the associations between 47 schizophrenia susceptibility variants (from 25 well-characterized schizophrenia susceptibility genes) and cranial volume variation in a healthy Chinese sample (N = 1,013). We also extracted the association between these 47 schizophrenia risk variants and the macroscopic structural brain volume (intracranial volume, total brain volume and hippocampal volume) in a large healthy sample of European ancestry (ENIGMA sample, N = 5,775). We identified several single-nucleotide polymorphisms (SNPs) nominally associated with intracranial volume, total brain volume, and hippocampal volume at $P < 0.05$ (uncorrected). However, after Bonferroni corrections for multiple testing, no SNP showed significant association. Hence, our results do not support previous observations that schizophrenia susceptibility variants are associated with brain structure (e.g., hippocampal volume) in healthy individuals, and indicate that single schizophrenia risk variant may not contribute significantly to macroscopic brain structure (e.g., intracranial volume or hippocampal volume) variation in healthy subjects.

Key words: schizophrenia; brain volume; genetic association; healthy individuals

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INTRODUCTION

The neurodevelopmental hypothesis of schizophrenia (SZ) suggests that the disruption of normal brain development early in life by combinatorial genetic and environmental factors could lead to pathogenesis of SZ eventually [Lewis and Levitt, 2002]. Consistent with this hypothesis, reduced whole brain volume and hippocampal volume have been observed in SZ patients and their unaffected siblings compared with healthy individuals [Cahn et al., 2002; Tepest et al., 2003; Steen et al., 2006; Boos et al., 2007]. Brain volume may, therefore, represent a potential biomarker or intermediate phenotype for SZ, and uncovering the relationship between SZ risk genes and brain volume may help to decipher the genetic basis of structural brain volume abnormalities

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in SZ and to better understand the etiology of SZ. In fact, several SZ genes have been found to play pivotal roles in the proliferation of neural progenitors [Mao et al., 2009; Luo et al., 2012], which may influence the total number of neurons and brain volume variation through regulating neural progenitor pool.

In recent years, owing to the advantages of genotyping platform and utilizes of large-scale samples, accumulating genetic risk variants for SZ have been identified [Shi et al., 2009, 2011; Stefansson et al., 2009; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014]. According to "Common Disease–Common Variant" hypothesis, risk SZ common alleles are also carried by healthy individuals in general populations and may impact brain development of normal subjects. Consistent with this hypothesis, recent genetic studies have also revealed associations between several SZ susceptibility variants and certain brain structures in SZ patients and healthy controls. For example, Bueller et al. [2006] found that *BDNF* Val66Met (rs6265) allele is associated with reduced hippocampal volume in healthy subjects, which was supported by a recent meta-analysis [Hajek et al., 2012]. Other interesting results have also been reported, such as the association between genetic variants in *CACNA1C* gene and brainstem volume [Franke et al., 2010], the association between genetic variants in *NRG1* and gray matter volume [Barnes et al., 2012], the association between genetic variants in *DARPP-32* and neostriatal volume [Meyer-Lindenberg et al., 2007], the association between genetic variants in *AKT1* and ventral prefrontal cortex [Tan et al., 2008], the association between rs2008720 *(*in *PRODH* gene) and bilateral frontal WM density [Zinkstok et al., 2008], and the association between risk variants in *PCM1* and orbitofrontal cortex gray matter [Gurling et al., 2006].

However, the aforementioned studies are limited to only a few SZ candidate variants and were based on a relatively smaller sample size, which may limit the statistical power, especially for variants with small effect. More importantly, it is unknown whether the observed associations between structural brain volume variation and SZ risk variants are disease dependent (i.e., if these associations are only observed in SZ patients). To comprehensively explore whether the reported SZ risk variants are associated with brain volume variation in healthy subjects, we examined the associations between SZ susceptibility variants and macroscopic brain structure (e.g., intracranial volume, total brain volume, and hippocampal volume) variations in healthy individuals in independent samples. We first investigated the associations between 47 SZ susceptibility variants (from 25 well-characterized SZ genes) and cranial volume in a healthy Chinese sample (N = 1,013), and we then attempted to examine the potential associations between these 47 SZ risk variants and intracranial volume in a genetically divergent healthy sample from the Enhancing Neuro Imaging Genetics through Meta-analysis (ENIGMA) consortium (N = 5,775) [Novak et al., 2012; Stein et al., 2012]. Finally, we performed an extended analysis, i.e., we selected those SNPs that significantly associated with SZ in recent genome-wide association studies (GWAS) and explored their associations with intracranial volume, brain volume, and hippocampal volume in ENIGMA sample (N = 5,775).

MATERIALS AND METHODS

Samples

Chinese sample. The detailed information of the Chinese sample was described in our previous study [Wang et al., 2008; Luo et al., 2012]. Briefly, a total of 1,013 unrelated healthy individuals including 460 males and 553 females were included. All the sampled individuals were from Yunnan province of southwestern China. Informed consents for this study were obtained from all the subjects, and the research protocol was approved by the internal review board of Kunming Institute of Zoology, Chinese Academy of Sciences. The ages of the 1,013 individuals range from 19 to 28 years with 98% of them being 21–26 years old.

ENIGMA sample. The ENIGMA consortium performed a large-scale meta-analysis combining genome-wide association study (GWAS) data from 17 separate imaging studies [Stein et al., 2012]. All of included subjects are of European ancestry. Genetic associations between selected SNPs and brain volume were extracted from a healthy subsample (N = 5,775) (mean age of 34.8 years). Detailed information about the sample description, imaging procedure and genotyping methods of this sample have been reported in the original studies [Stein et al., 2012] and ENIGMA website (http://enigma.ini.usc.edu/protocols/) [Novak et al., 2012].

Measurement of Brain Volume

Chinese screening sample. The cranial volume was measured and calculated as described in our previous studies [Wang et al., 2008; Luo et al., 2012]. In brief, three principal dimensions of the cranium were measured, including (i) maximum antero-posterior length (L, measured between glabella and the inion); (ii) maximum breadth (B, biparietal diameter; measured between two parietal eminences); (iii) cranial height (H, basi-bregmatic height, measured between the internal acoustic meatus to the highest point of the vertex). The cranial volumes were computed using the following formula [Manjunath, 2002; Wang et al., 2008; Luo et al., 2012]: male, 0.337 (L-1.1) (B-1.1) (H-1.1) + 406.01 cc; female, 0.400 (L-1.1) (B-1.1) (H-1.1) + 206.60 cc.

Replication sample (ENIGMA). The ENIGMA sample consisted of 17 cohorts of European ancestry. Since we focused on delineating the potential association between SZ susceptibility variants and macroscopic structural brain volume variation in healthy subjects, genetic associations between selected SNPs and macroscopic brain volume variation from a healthy subsample (N = 5,775) were extracted in this study. The intracranial volume, total brain volume, and hippocampal volume were measured separately by magnetic resonance imaging (MRI). The detailed scanners and scan acquisition protocols have been described previously [Stein et al., 2012]. For more details, please refer to ENIGMA's website (http://enigma.ini.usc.edu/protocols/).

Selection of Schizophrenia Susceptibility Variants

Selection of SZ variants was mainly based on previous linkage and association studies of SZ. The inclusion of candidate SZ

susceptibility variants were based on the following criteria: First, we selected those well acknowledged SZ risk variants based on linkage and association studies [Chowdari et al., 2002; Straub et al., 2002, 2007; Hodgkinson et al., 2004; Li et al., 2011a, b, 2012, 2013]. Second, if the biological function of the risk variants were studied, e.g., the Val158Met (rs4680) polymorphism within *COMT* [Chen et al., 2004a,b; Handoko et al., 2005], such SNPs will be given higher priority. Third, we took into account the potential impacts of these SNPs on brain function, if SNPs associated with SZ have also been reported affecting brain function, for example, the *BDNF* val66Met (rs6265) was found to be associated with poor medial temporal lobe-related memory performance [Szeszko et al., 2005; Agartz et al., 2006; Ho et al., 2006], such SNPs were considered. We noticed that seven neurotransmitter system related genes (*GAD1, COMT, GABRB2, DAO, HTR2A, DAOA,* and *TPH1*) were chose in this study. Convergent lines of evidence suggest these genes may play important roles in the pathogenesis of SZ [Spencer et al., 1998; Knickmeyer et al., 2014]. *GAD1* encodes the γ-aminobutyric acid (GABA) synthesizing enzyme glutamate decarboxylase (GAD67), and Uchida et al. [2014] found that heterozygous deletion of *GAD1* disturbed the proliferation of neurons destined to be PV-positive GABAergic interneurons, suggesting this gene may play an important role in neurodevelopment. In addition, Addington et al. [2005] have reported that *GAD1* is associated with childhood-onset SZ and cortical gray matter volume loss. *COMT* encodes catechol-*O*-methyltransferase which degrades catecholamines such as dopamine, epinephrine, and norepinephrine. Recent study revealed that *COMT* plays a role in adolescent cortical development [Raznahan et al., 2011]. Other studies also showed that GABA receptors have pivotal role in neurodevelopment [Caruncho et al., 2004; Polan et al., 2014]. Finally, it has been showed that serotonin neurotransmitter (related to *TPH1* and *HTR2A* gene) [Nakamura et al., 2006; Altamura et al., 2007; Schmitt et al., 2007; Benninghoff et al., 2009] and *DAOA* are also involved in neurodevelopment [Hartz et al., 2009]. These results suggest that neurotransmitter system related genes may exert roles in neurodevelopment and dysfunction of neurotransmitter may result in abnormal brain development.

Based on the above criteria, we first selected 47 SNPs (from 25 well studied SZ genes) (Table S1). Detailed information for the selected variants and genes was shown in Table I and S1. In addition to the above 47 SZ susceptibility variants, we also examined the associations between SZ GWAS risk loci and the macroscopic structural brain volume variation in ENIGMA sample.

Population Stratification Analysis in Chinese Sample

In the Chinese sample, to avoid the impact of population structure on our analysis, we performed population stratification analysis. In addition to the 47 genotyped SNPs, we also genotyped another 17 SNPs (randomly selected from the genome) (data not shown). We then extracted the genotypes of these 64 SNPs from the 270 individuals from the 1,000 Genomes project (85 subjects for CEU [Utah residents with ancestry from northern and Western Europe in the United States], 88 subjects for YRI [Yoruba in Ibadan, Nigeria], and 97 subjects for CHB [Southern Han Chinese]) [Abecasis et al., 2010]. Population stratification analysis was conducted using Structure software (v2.3.3.) [Pritchard et al., 2000; Falush et al., 2003, 2007]. We found that these 64 SNPs worked well to cluster three populations (CHB, CEU, and YRI) from 1,000-human-genome project into three groups by ethnicities (Fig. 1A and C). Using these 64 SNPs, we further tested potential population stratification in our Chinese sample. Admixture model and independent allelic frequency model were used in our analyses. The value of K (the number of potential populations of our tested sample) was set from $K = 2$ to $K = 10$. For all structure runs, we set the parameters with a burn-in of 10,000 iterations and 20,000 MCMC replications after burn-in iterations.

Association Significance Assessment

In the Chinese sample, the cranial volume of 1,013 normal individuals was measured and 47 SNPs (Table S1) were genotyped by SNaPShot method as previously described [Wang et al., 2008; Luo et al., 2012]. The single SNP association was performed using linear regression under an additive genetic model with age and sex as covariates and the *P*-value was obtained by the Wald test as implemented in PLINK [v1.07] [Purcell et al., 2007]. Since both body height and body weight are correlated with brain volume [Ho et al., 1980], to exclude the impact of body height and body weight on our analysis, we carried out the same linear regression association analyses by correcting the brain volume using regressions for body height and body weight. A linear regression formula was inferred before adjusting the brain volume by body height (weight), with the brain volume as the dependent and the body height (weight) as the independent. The adjusted brain volume by height (weight) equal to (brain volume – the constant of the regression formula/body height (weight)). More detailed information about the adjustment of brain volume by body height and weight can be found in our previous study [Wang et al., 2008]. The *P*-value thresholds for significant associations, which correspond to type I error rate of 0.05, were estimated by using the conservative Bonferroni correction according to the number of independent SNPs. Independent SNPs were defined based on the linkage disequilibrium value (r^2) smaller than 0.3. For SZ susceptibility genes with several SNPs (e.g., *GAD1*, *COMT,* and *RELN*), we assessed whether these SNPs represent independent SNPs according to the linkage disequilibrium between them.

In the ENIGMA sample, the intracranial volume, total brain volume, and hippocampal volume were measured by MRI, which have been reported by Stein et al. [2012]. The association results (*P*-values) were extracted from ENIGMA project (please refer to ENIGMA database for details, http://enigma.ini.usc.edu/enigma-vis/) [Stein et al., 2012]. Since the ENIGMA consisted of 17 separate studies, to correct the potential population stratification, each site performed multidimensional scaling (MDS) analyses by comparing their data to the reference populations from the HapMap3. Four MDS for covariates of population stratification, including age, age^2, sex, and the interaction between age and sex and age^2 and sex were obtained. Multiple linear regression were conducted with the brain volume as a dependent variable and the additive dosage of each SNP as an independent

TABLE I. Association Significance Between the 47 Studied SNPs and Cranial Volume in Chinese Sample

Gene	SNP	Polymorphism	Minor allele	MAF	*P*-value	*P*-value[a] (Corrected)
RGS4	rs951439	G/A	A	0.40	0.68	1.0
DISC1	rs821616	T/A	A	0.14	0.18	1.0
GAD1	rs3791878	G/T	T	0.28	0.58	1.0
GAD1	rs2270335	C/T	T	0.24	0.23	1.0
GAD1	rs3791858	G/T	T	0.24	0.39	1.0
DTNBP1	rs1011313	C/T	C	0.20	0.32	1.0
DTNBP1	rs760761	G/A	A	0.09	0.11	1.0
DTNBP1	rs1018381	C/T	T	0.07	**0.02**	0.8
NOTCH4	rs204993	C/T	T	0.41	0.62	1.0
PCM1	rs445422	G/A	A	0.06	0.51	1.0
NRG1	rs35753505	G/A	A	0.42	0.31	1.0
NRG1	rs6994992	C/T	C	0.39	0.27	1.0
BDNF	A-633T	A/T	A	0.29	0.96	1.0
BDNF	rs6265	G/A	A	0.44	0.80	1.0
AKT1	rs2494732	C/T	T	0.30	0.16	1.0
AKT1	rs10149779	G/A	A	0.08	0.09	1.0
AKT1	rs3803300	C/T	C	0.31	0.13	1.0
COMT	rs2075507	C/T	C	0.30	0.61	1.0
COMT	rs2020917	G/A	A	0.30	0.70	1.0
COMT	rs737865	G/A	G	0.29	0.86	1.0
COMT	rs4680	C/T	T	0.26	0.25	1.0
COMT	rs165599	C/T	C	0.48	0.35	1.0
TCF4	rs2958182	A/T	A	0.16	0.73	1.0
GABRB2	rs1816072	G/A	G	0.40	0.98	1.0
PPP3CC	rs10108011	G/A	G	0.25	0.59	1.0
CCKAR	rs1800857	G/A	G	0.21	0.48	1.0
RELN	rs7341475	G/A	A	0.09	**0.02**	0.8
RELN	rs362731	C/T	T	0.44	0.53	1.0
RELN	rs362813	C/T	T	0.48	0.84	1.0
RELN	rs2299356	G/A	G	0.41	0.70	1.0
RELN	rs727709	G/A	A	0.42	0.84	1.0
RELN	rs885995	G/A	A	0.42	0.82	1.0
GSK3B	rs334558	G/A	A	0.38	**0.03**	1.0
GSK3B	rs3755557	A/T	A	0.15	0.87	1.0
GSK3B	rs7431209	G/A	A	0.36	0.06	1.0
CMYA5	rs3828611	G/C	G	0.43	0.98	1.0
CMYA5	rs4704593	G/C	C	0.23	0.26	1.0
ZNF804A	rs1344706	A/C	C	0.49	0.74	1.0
ZNF804A	rs359895	A/T	A	0.22	0.43	1.0
ZNF804A	rs7597593	C/T	T	0.46	0.32	1.0
FZD3	rs2874941	G/T	G	0.35	0.73	1.0
DAO	rs4623951	C/T	C	0.16	0.10	1.0
HTR2A	rs6311	C/T	C	0.40	0.51	1.0
DAOA	rs778293	C/T	C	0.34	0.23	1.0
PDE4B	rs910694	C/T	C	0.20	0.79	1.0
TPH1	rs1800532	G/T	T	0.50	0.96	1.0
PLXNA2	rs841865	C/T	T	0.29	0.84	1.0

MAF, minor allele frequency. The nominally significant *P*-values ($P \leq 0.05$, uncorrected) are shown in bold.
[a]*P*-value is adjusted by Bonferroni correction.

variable, adjusting for the above four MDS for covariates of population stratification. The meta-analysis was then adjusted by following covariates: gender, linear and quadratic effects of age, interactions of gender with age covariates, four MDS components (covariates of population stratification), and dummy covariates for different magnetic resonance acquisitions. Briefly, through using an inverse standard error-weighted meta-analysis protocol implemented in METAL [Willer et al., 2010], meta-analysis was performed for each SNP across all groups based on a fixed-effects model. To adjust for population stratification or

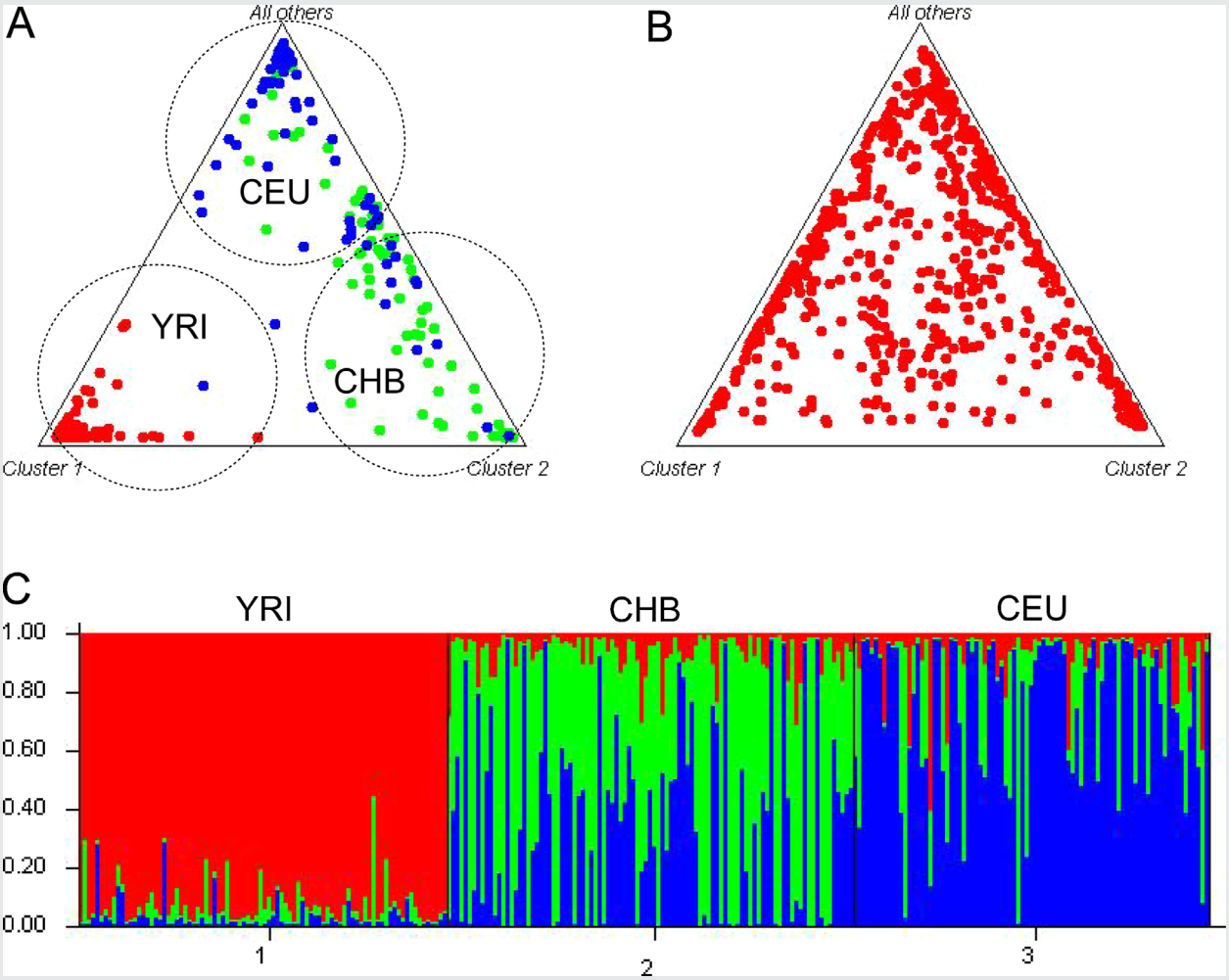

FIG. 1. Population structure analysis revealed that there is no population stratification in our sample. A and C: The genotypes of 64 SNPs (47 studied SNPs in Chinese sample and other 17 random SNPs) were extracted from 1,000-human-genome project and were used to preform population stratification analysis. The 64 SNPs worked well to differentiate three populations into three clusters by ethnicities. A: Triangle plot of the 64 SNPs (genotypes were extracted from 270 individuals from the 1,000 Genomes project). Three clusters (populations) were identified by using these 64 SNPs. Red, YRI; Green, CHB; Blue, CEU. B: The same 64 SNPs were used to detect potential population stratification in Chinese sample. No obvious stratification was observed. C: Bar plot of the 64 SNPs (genotypes were from 270 individuals from the 1,000-human-genome project). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmgb].

cryptic relatedness not accounted for by MDS components [Devlin and Roeder, 1999], genomic control was applied at the level of each study and at the meta-analysis level.

RESULTS

In the Chinese sample, we first performed normality test and found that the cranial volume is normally distributed (Kolmogorov–Smirnov test, $P = 0.90$) (Fig. 2). In addition, we found that the cranial volume of males is significantly larger than the females ($P < 0.0001$, one-way ANOVA). We further performed population stratification analysis and found there is no obvious stratification in the Chinese sample (Fig. 1B and S1).

In Chinese sample, we tested 47 SNPs from 25 well-characterized SZ candidate genes (Table S1). Three SNPs (rs1018381, rs7341475, and rs334558) showed nominal significant associations (uncorrected $P < 0.05$) with cranial volume under the additive genetic model (Table I). Rs1018381 is located in *DTNBP1* gene, rs7341475 is located in *RELN* gene, and rs334558 is located in *GSK3β* gene. However, none of them remained significant (corrected $P > 0.05$) after Bonferroni correction for multiple testing (Table I). Considering that Bonferroni correction is a conservative method for multiple testing correction, we also used Benjamini–Hochberg method (which is less conservative than Bonferroni correction method) to correct the *P*-values. Similar with the results from Bonferroni correction method, we found no SNPs showed

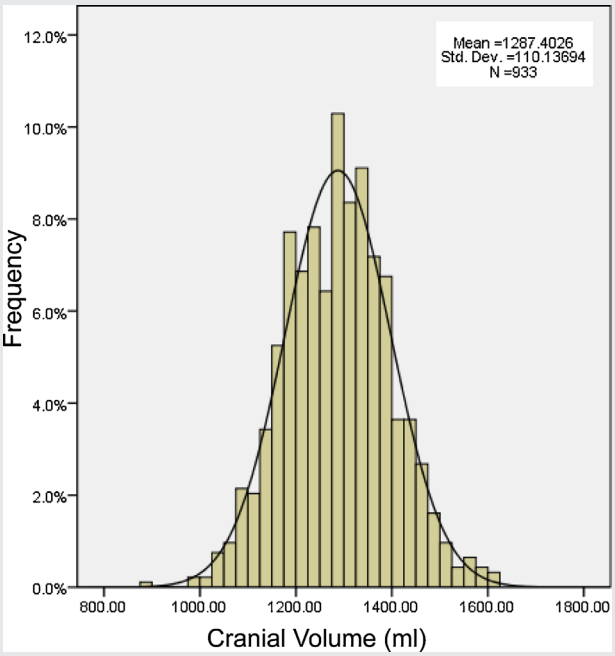

FIG. 2. The cranial volume is normally distributed in Chinese sample. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmgb].

significant association with brain volume when Benjamini–Hochberg correction method was used (data not shown).

To further confirm our results, we then extracted the associations between these 47 SNPs and intracranial volume in ENIGMA sample. We noticed that rs334558 is not available in ENIGMA sample, and rs7341475 and rs1018381, which showed significant association (uncorrected P-value < 0.05) with cranial volume in Chinese sample (Table I), were not associated with intracranial volume in ENIGMA sample ($P = 0.85$ and 0.72, respectively, Table S2). In addition, we found other four SNPs reached nominal significance level (uncorrected $P < 0.05$) in ENIGMA sample (rs2020917 and rs737865 in *COMT* gene, rs4623951 in *DAO* gene, and rs6311 in *HTR2A* gene) (Table S2). Nevertheless, these four SNPs did not survive Bonferroni correction for multiple comparisons (corrected $P > 0.05$) (Table S2). Moreover, contrasting the previous report [Bueller et al., 2006], we found no significant association between *BDNF* (rs6265) and hippocampal volume in healthy subjects (N = 5,775, $P = 0.88$, Table S4) in ENIGMA sample. Taken together, these results suggest that the selected SZ susceptibility variants may not associate with cranial (intracranial) volume in healthy subjects.

Our above results suggested that the well-characterized SZ susceptibility variants may not associate with macroscopic structural brain volume (i.e., intracranial volume or total brain volume) in healthy subjects. However, we noticed that many of the aforementioned SZ susceptibility variants did not show significant associations with SZ in recent GWAS. Considering that SZ susceptibility variants identified by GWAS may represent promising candidate variants for SZ as well. Therefore, assessing the associations between these SZ susceptibility variants and macroscopic structural brain volume will provide additional useful information about whether SZ susceptibility variants are associated with macroscopic structural brain volume (intracranial volume, total brain volume, and hippocampal volume) in healthy subjects. To extend the analyses and validate our results, using ENIGMA sample, we extracted the associations between 39 SNPs (from 33 SZ genes) that reached genome-wide significance level for SZ susceptibility (Table S3) and macroscopic structural brain volume in healthy individuals. We found four of them nominally associated with intracranial volume and total brain volume at P (uncorrected) < 0.05 (Table S3), and two SNPs nominally associated with hippocampal volume at P (uncorrected) < 0.05 (Table S3). However, after Bonferroni correction for multiple testing, no SNPs remained significant. Collectively, our analysis detects no significant association of SZ genes with intracranial volume, total brain volume, and hippocampal volume in healthy individuals.

DISCUSSION

In this study, we tested whether SZ associated SNPs of the well-characterized SZ susceptible genes also correlate with macroscopic structural brain volume (i.e., intracranial volume, total brain volume, and hippocampal volume) in healthy individuals. We first tested 47 SNPs from 25 well-characterized SZ genes in a healthy Chinese sample (N = 1,013). We then examined the association between these SNPs and intracranial volume in ENIGMA sample (N = 5,775, healthy subjects). We also assessed the associations between other well-characterized top SZ susceptibility variants from GWAS studies and macroscopic structural brain volume in healthy subjects, though several SNPs showed nominally significant association with macroscopic structural brain volume in healthy subjects, none of them remained significant after Bonferroni correction for multiple testing. This suggests that single SZ susceptible variant is unlikely associated with cranial (brain) volume in healthy individuals.

There are several methodological limitations in this study. First, we focused on the association between SZ susceptibility variants and macroscopic structural brain volume in healthy subjects, therefore, we cannot exclude potential association between SZ variants and other specific brain regions not tested in this study, such as frontal cortex in healthy subjects. Second, the SNP coverage is not exhaustive, we selected only 47 SNPs located in 25 well-characterized SZ susceptible genes though those SNPs are the most representatives of the candidate genes. Third, in the Chinese sample, the cranial volume was estimated from linear measures, which has limitations. Compared with MRI, cranial volume estimation from linear measures will have greater inaccuracy and variation. Though we noticed that cranial volume estimating from linear measures has its limitations, our previously studies have shown this method is still relatively reliable since our results are supported by independent replications from genetic divergent populations [Luo et al., 2012].

It is well-established that SZ patients and their unaffected relatives have smaller total brain volume compared with healthy subjects [Cahn et al., 2002; Steen et al., 2006; Boos et al., 2007]. Considering common variants conferring risk of SZ are carried by

normal individuals in the general population, SZ susceptibility variants may also affect brain structure such as cranial volume in healthy subjects. However, we did not observe significant association between SZ susceptibility variants and macroscopic structural brain volume (i.e., intracranial volume, total brain volume, and hippocampal volume) in healthy subjects. The absence of significant association between SZ susceptibility variants and macroscopic structural brain volume in healthy subjects can be addressed by following possible explanations: first, as stated above, the SZ susceptibility variants may associate with volumes of specific brain region in healthy subjects, nevertheless, we could not detect such association since we only tested the macroscopic structural brain volume. Second, it is possible that associations between the volumes of brain structure and SZ susceptibility variants are present in both SZ patients and healthy subjects. However, the associations are more readily apparent in patients group where there is greater variance in the brain measures. In contrast, the association is present but not reach statistical significance level in healthy individuals. Third, the single SZ susceptibility variant may not associate with macroscopic structural brain volume variation in healthy subjects, so they do not contribute to macroscopic structural brain volume variation. Fourth, it is possible that each SZ susceptibility variant has a small effect on macroscopic structural brain volume variation and many SZ variants jointly modulate macroscopic structural brain volume, however, we could not detect significant association due to the limited power of this study and we analysed each variant separately.

It should be noted that two different volumes measures were used in two independent populations in this study. However, since we performed the association analysis separately, differences in volumes measurement will not significantly influence our results. In addition, even for the observed nominal significant SNPs, we noticed the *P*-values of these SNPs are range from 0.012 to 0.048. Considering the large number of independent SNPs studies in this study, it is clear that none of these nominal significant SNPs remained significant after Bonferroni correction for multiple testing.

Our results suggest that single SZ variant may not contribute significantly to macroscopic brain structure (e.g., total brain volume or hippocampal volume) variation in normal subjects. Our data are consistent with the recent report by Cousijn et al. [2012] that variants in SZ gene *ZNF804A* does not influence macroscopic brain structure in a large healthy sample. Our results are relatively reliable as the power of this study is relatively high considering the large sample size of this study and independent replication results. Nevertheless, we cannot exclude the potential association between SZ variants and other specific brain regions not tested in this study, such as frontal cortex or medial temporal lobe in healthy subjects.

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