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ASSOCIATIONS BETWEEN THE *CNTNAP2* GENE, DORSOLATERAL PREFRONTAL CORTEX, AND COGNITIVE PERFORMANCE ON THE STROOP TASK

BI ZHU,[a,b,c*] CHUANSHENG CHEN,[d] GUI XUE,[a,b] XUEMEI LEI,[e] YUNXIN WANG,[f] JIN LI,[g] ROBERT K. MOYZIS,[h] JUN LI,[a,b] QI DONG[a,b] AND CHONGDE LIN[a,b,c]

[a] *State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing, China*

[b] *Center for Collaboration and Innovation in Brain and Learning Sciences, Beijing Normal University, Beijing, China*

[c] *Institute of Developmental Psychology, Beijing Normal University, Beijing, China*

[d] *Department of Psychology and Social Behavior, University of California, Irvine, CA, USA*

[e] *School of Psychology, Beijing Normal University, Beijing, China*

[f] *Capital Institute of Pediatrics, Beijing, China*

[g] *Institute of Automation, Chinese Academy of Sciences, Beijing, China*

[h] *Department of Biological Chemistry and Institute of Genomics and Bioinformatics, University of California, Irvine, CA, USA*

Abstract—The *CNTNAP2* (contactin-associated protein-like 2) gene, highly expressed in the human prefrontal cortex, has been linked with autism and language impairment. Potential relationships between *CNTNAP2*, dorsolateral prefrontal cortex (DLPFC), and cognition have been suggested by previous clinical studies, but have not been directly examined in the same study. The current study collected structural MRI, genetic, and behavioral data in 317 healthy Chinese adults, and examined associations between *CNTNAP2* variants, DLPFC, and cognitive performance (measured by the Stroop task). After controlling for intracranial volume, sex, and age, the *CNTNAP2* genetic polymorphism at SNP rs7809486 had the strongest association with bilateral DLPFC volume ($p = 0.00015$ and 0.00014 for left and right DLPFC volumes, respectively), with GG homozygotes having greater bilateral DLPFC volumes and surface areas than the other genotypes. Furthermore, TT homozygotes of *CNTNAP2* rs4726946 (a nearby SNP that had moderate linkage disequilibrium with rs7809486) had greater left DLPFC volume and surface area, and better cognitive performance than the other genotypes. Subjects with greater left DLPFC surface area had better cognitive performance. Importantly, the left DLPFC surface area mediated the association between the *CNTNAP2* rs4726946 genotype and cognitive performance. This study provides the first evidence for associations among the *CNTNAP2* gene, left DLPFC structure, and cognitive control.

Key words: dorsolateral prefrontal cortex, Stroop, gene, MRI, imaging genetics.

INTRODUCTION

The *CNTNAP2* (contactin-associated protein-like 2) gene, as a member of the neurexin family, is involved in neuron interaction and axon differentiation, and is highly expressed in the human prefrontal cortex (Abrahams et al., 2007). *CNTNAP2* has been associated with many mental disorders (e.g., autism, language impairment, schizophrenia, and depression) (Rodenas-Cuadrado et al., 2014). Patients with these disorders usually have abnormal dorsolateral prefrontal cortex (DLPFC) in structure or function, and show impaired executive function (Hill, 2004).

CNTNAP2 genetic variants have been associated with autism, language impairment, and related brain dysfunctions (Alarcon et al., 2008; Li et al., 2010; Chiocchetti et al., 2015). As illustrated in a recent review, several *CNTNAP2* mutations and common genetic variants have been associated with autism, including SNPs in introns, and mutations in the promoter region and several exons (Rodenas-Cuadrado et al., 2014). In a mixed sample of autistic and typically developing children, a functional magnetic resonance imaging (fMRI) study reported that carriers of the autism risk allele (*CNTNAP2* rs2710102 C allele) had increased activation in the medial prefrontal cortex (mPFC), and stronger functional connectivity between mPFC and DLPFC during reward processing than the TT homozygotes (Scott-Van Zeeland et al., 2010). Subjects with autism have been found to show abnormalities in the DLPFC, including lower fractional anisotropy (Noriuchi et al., 2010), reduced creatine and N-acetylaspartate (Horder et al., 2013), and lower activation during a spatial working memory task (Luna et al., 2002).

The *CNTNAP2* gene has been associated with schizophrenia in Caucasians (i.e., genetic deletion located in exons 9–24 and intron 3) and in Han Chinese

*Correspondence to: B. Zhu, State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing 100875, China. Fax: +86-10-58808470.
E-mail address: zhubi@bnu.edu.cn (B. Zhu).

Abbreviations: DLPFC, dorsolateral prefrontal cortex; ICV, intracranial volume; LD, linkage disequilibrium; RT, reaction time; SNPs, single nucleotide polymorphisms.

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populations (Friedman et al., 2008; Ji et al., 2013; Chen et al., 2015). Moreover, a structural MRI study of a mixed sample of schizophrenia patients and healthy controls suggested that, compared to the TT homozygotes, individuals with the CT heterozygous genotype at rs700281 (located in intron 1 of *CNTNAP2*) had relatively high mean diffusivity in the dorsal cingulum bundle (dCB, as it is one of the major white matter tracts linking the DLPFC and other brain regions) (Clemm von Hohenberg et al., 2013). In a separate study, schizophrenia patients were found to have higher mean diffusivity in the dCB than controls, and moreover, higher mean diffusivity in the dCB of schizophrenia patients was associated with impaired Stroop performance (i.e., longer incongruent and neural reaction times) (Takei et al., 2009). In addition, schizophrenia patients had extensive volume reduction in the DLPFC (Ellison-Wright et al., 2008). Previous studies also reported that both schizophrenia patients and their unaffected relatives had reduced functional activity in the left DLPFC as compared to healthy controls when performing the Stroop task (Becker et al., 2008; Choi et al., 2008).

The *CNTNAP2* genotypes were also associated with depression in Han Chinese samples (Ji et al., 2013; Chen et al., 2015). Depression patients also showed reduced DLPFC gray matter volume as compared with healthy controls (Chang et al., 2011), and hyperactivity in the left DLPFC during the Stroop task (Wagner et al., 2006). To further support the role of left DLPFC in depression and Stroop performance, repetitive transcranial magnetic stimulation (rTMS) at that brain region has been found to lead to a transient improvement on Stroop performance in depression patients (Concerto et al., 2015).

Neuroimaging studies with healthy subjects also have suggested that *CNTNAP2* is involved in the anatomy and function of the frontal cortex. A voxel-based morphometric study of healthy subjects found that *CNTNAP2* rs7794745 TT homozygotes (i.e., T as the risk allele for autism) had a reduction in gray matter volume of the right frontal pole, as compared with the A allele carriers (Tan et al., 2010). A structural brain connectivity study of healthy young adults further found that *CNTNAP2* rs2710102 CC homozygotes (i.e., C as the risk allele for autism) had greater regional efficiency (i.e., the global efficiency computed for each node) and lower eccentricity (i.e., the longest characteristic path length) in the frontal cortex than did the T allele carriers (Dennis et al., 2011). Interestingly, greater regional efficiency and lower eccentricity may be signs of random connections in the brain structural networks (Dennis et al., 2011). *CNTNAP2* rs2710102 CC homozygotes had greater activation in the right inferior frontal gyrus than the T allele carriers during the performance of a language task (i.e., sentence completion) (Whalley et al., 2011). *CNTNAP2* rs7794745 AT heterozygotes had lower activation in the right middle frontal gyrus than AA homozygotes when listening to a story (Koeda et al., 2015). These imaging genetic studies of healthy adults suggested that *CNTNAP2* genetic variations may be linked to altered frontal brain structures and function, which would eventually lead to mental disorders when combined with other genetic and environmental risks.

In sum, the above studies from different lines of research have suggested potential relationships between the *CNTNAP2* gene, DLPFC, and executive function. However, no study thus far has directly examined them in the same study. In this study, we first performed an association study of single nucleotide polymorphisms (SNPs) within the *CNTNAP2* gene with DLPFC morphology (volume, surface area, and cortical thickness) and cognitive performance on the Stroop task. We then examined the potential mediation effect of DLPFC morphology on the relationship between the genetic variant of *CNTNAP2* and cognitive performance on the Stroop task. We selected the Stroop task because it is a well-documented test of executive function and it has been found to be subserved by the DLPFC based on lesion, neuroimaging, and neurostimulation studies (MacDonald et al., 2000; Stuss et al., 2001; Vanderhasselt et al., 2006).

EXPERIMENTAL PROCEDURES

Participants

Participants in this study were 317 healthy Han Chinese college students (128 males; 189 female). The mean age of participants was 20.42 ± 0.89 (range 18–22 years old). They had no history of physical or mental diseases. Only five participants were left-handed. The handedness did not influence any result in the current study. So these left-handed participants were not excluded. Participants signed the written consent and understood the study procedure. An Institutional Review Board approval was obtained for this study.

MRI data collection and analysis

Brain MRI was acquired on a 3.0T Siemens Magnetom Trio scanner using a standard head coil at Beijing Normal University. The whole brain structural 3D T1-weighted images using MPRAGE pulse sequence were acquired (echo time/repetition time/flip angle = 3.75 ms/2530 ms/7°; field of view = 256 mm × 256 mm, voxel size = 1 × 1 × 1.33 mm^3, number of partitions = 128). In order to obtain the intracranial volume (ICV), cortical volume, local cortical surface area and cortical thickness for each subject, we used FreeSurfer 5 to analyze the MRI data (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 2002). The DLPFC was defined based on Freesurfer cortical parcellation and conservative Talairach coordinates as detailed in Rajkowska and Goldman-Rakic (Rajkowska and Goldman-Rakic, 1995). Following the procedure used in a previous study (Ehrlich et al., 2010), DLPFC parcellation was obtained with the following steps. First, we merged the standard Freesurfer parcellation labels of the superior frontal gyrus, rostral middle frontal gyrus, and caudate middle frontal gyrus. Second, we made a cut along the vertex on the inflated cortical surface to divide the medial and lateral components, and removed the medial component. Third, in order to separate the DLPFC from the premotor cortex, we made a coronal cut of the lateral component at Talairach coordinate $y = 26$. The mean and standard deviation

of ICV in the current sample was 1,462,696 ± 228,335 mm^3. Results from segmentation were visually inspected for each participant. Manual corrections and re-inspections were conducted for any inaccuracies in Talairach-transformation, skull stripping, and segmentation.

Genotyping and quality control

DNA was isolated from 4-ml venous blood from each participant. Genotyping was performed on the standard Affymetrix 6.0 genotyping protocol (Affymetrix, Inc). As shown in Fig. 1, 234 SNPs within the *CNTNAP2* gene were selected. The allele frequencies in the current study were very similar to those of the HapMap Chinese samples. Based on the HapMap dataset (http://browser.1000genomes.org), genotyped SNPs in the current study covered most of the linkage disequilibrium (LD) blocks in the *CNTNAP2* gene. The *CNTNAP2* gene contains 24 exons and 23 introns. For all SNPs, their minor allele frequencies were greater than 0.285 (i.e., the number of minor allele homozygotes for each SNP was larger than 20), their p values of Hardy–Weinberg equilibrium were greater than 0.05, and their genotype call rates were greater than 0.95.

Behavioral assessment

Stroop test. This test is commonly used to assess executive function and inhibition abilities (Wagner et al., 2006). In the current study, four colors and their Chinese words were used: red, green, blue, and yellow. There were two conditions, each with 48 trials. For the incongruent condition, each word was printed in a color different from the word's meaning; for the congruent condition, each word was printed in its color. Following the classic procedure, subjects responded by pressing a key (one of four keys on the reaction box) that corresponded with the color in which the word was printed. To familiarize subjects with the keys, there were several practice trials. The differences in reaction time (RT) between incongruent and congruent conditions showed the magnitude of the Stroop effect in RT and were used as an index of executive functions. Greater Stroop effect RT (i.e., greater differences in RT) indicated poorer cognitive performance.

We also measured the response accuracy of the Stroop task. There was, however, a ceiling effect for the accuracy rate (M ± SD = 0.97 ± 0.06), with 21% of the participants scoring 100% accuracy. Therefore, we used response time rather than accuracy rate for exploring individual differences in Stroop performance.

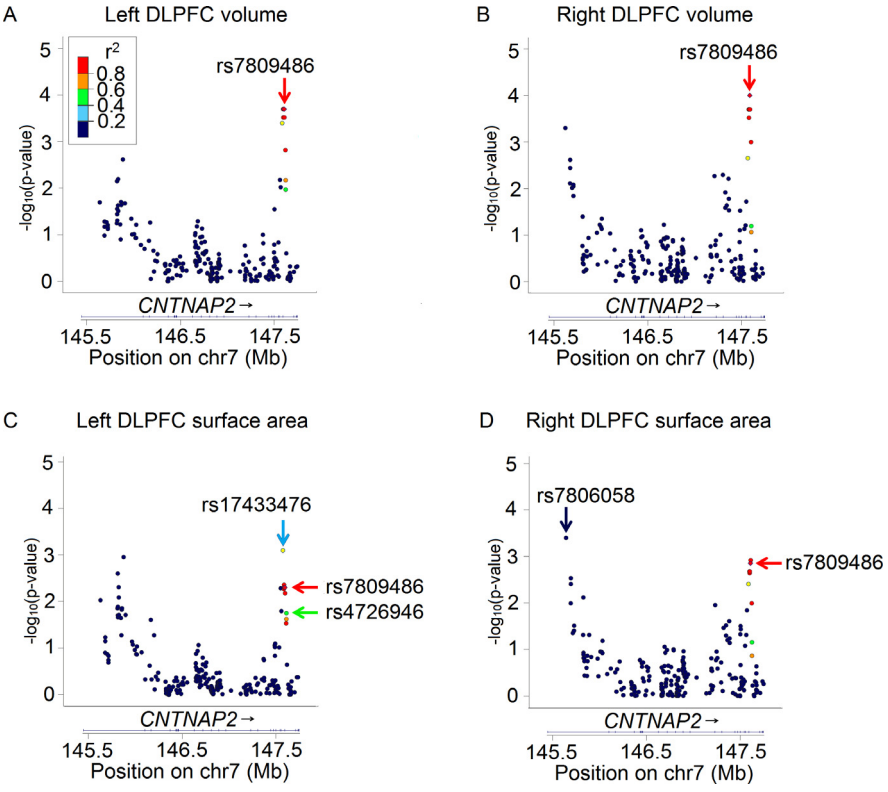

Fig. 1. Associations between 234 SNPs within the *CNTNAP2* gene and bilateral dorsolateral prefrontal cortex (DLPFC) volumes and surface areas with sex, age and ICV as covariates. All SNPs are plotted with their *p* values against their genomic position. For a and b, the strongest associations with bilateral DLPFC volumes were found for rs7809486 (i.e., the leading SNP indicated by a red arrow and plotted as a red diamond). The other genotyped SNPs are plotted as dots, with the color indicating the degree of pairwise LD between the leading SNP rs7809486 and neighboring SNPs. Red indicates strong pairwise LD, with $r^2 > 0.8$; dark blue indicates no LD, with $r^2 < 0.2$. For c and d, the strongest associations with bilateral DLPFC surface areas were found for rs17433476 (indicated by a light blue arrow and plotted as a yellow dot) and rs7806058 (indicated by a dark blue arrow and plotted as a dark blue dot) for the left and right DLPFC surface areas, respectively. For c, rs4726946 was indicated by a green arrow and plotted as a green dot. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Statistical analysis

The software Plink v1.07 was used for genetic association analysis at both SNP and haplotype levels (Purcell et al., 2007). Using linear regression additive genetic models (i.e., additive effects of allele dosage), we explored the associations between each SNP and phenotypic indices, including lateral DLPFC volume, surface area, thickness, and Stroop performance. Covariates were sex, age, and ICV. In addition, MANCOVA and Fisher's least significant difference post hoc test were used to detect genotype group differences in phenotypic indices after controlling for sex, age and ICV. We then obtained partial correlations between Stroop performance and DLPFC structural indices after partialling out sex, age, and ICV.

The gene-brain-behavior links (i.e., the *CNTNAP2* genotype, DLPFC structural index, and Stroop performance) were tested using a

mediation model, with the DLPFC structural index as the mediator [M] between *CNTNAP2* genotype [X] and Stroop performance [Y]. Age, sex, and ICV were also included as covariates. First, we identified SNPs within the *CNTNAP2* gene that had significant associations with Stroop performance. Each significant SNP was used as the independent variable for a mediation analysis (i.e., $Y = i_1 + cX + e_1$, in order to test if "c" is significant). Second, we selected the DLPFC structural indices that had significant associations with these *CNTNAP2* SNPs. Each of these DLPFC structural indices was used as the mediator (i.e., $M = i_2 + aX + e_2$, in order to test if "a" is significant). Third, we tested the mediation effect of a DLPFC structural index on the association between the *CNTNAP2* genetic variant and Stroop performance (i.e., $Y = i_3 + c'X + bM + e_3$, in order to test if "b" and "c′" are significant). If only "b" is significant, while "c′" is not significant (the direct effect of X on Y is not significant after controlling for M), it suggests that there is a complete mediation (i.e., X no longer affects Y after controlling for M). If "b" and "c′" are both significant, it suggests that there is a partial mediation (i.e., the path from X to Y is reduced but it is still different from zero after controlling for M). The indirect effect of X on Y is calculated by "c" minus "c′". To confirm the mediation effect, the significance of indirect effect was established by bootstrapped 95% confidence interval (CI) with 5000 iterations. The software PROCESS macro was used for the mediation analysis (model 4) (Hayes, 2015).

RESULTS

The means and standard deviations of left and right DLPFC volumes, surface areas, thickness, and Stroop effect RT, as well as correlations among these variables are shown in Tables 1 and 2. Females had significantly less bilateral DLPFC volumes, surface areas, and ICV than did males ($t = -9.29$, -8.97, -9.21, -8.93 and -13.46, $p < 0.001$, for the left and right DLPFC volumes and surface areas, and ICV, respectively). There was no sex difference in bilateral DLPFC thickness and Stroop effect RT ($p > 0.05$). Age did not have significant correlation with these indices ($p > 0.05$). ICV was positively correlated with bilateral DLPFC volumes and surface areas ($r = 0.58$, 0.57, 0.61, and 0.59, $p < 0.001$, for the left and right DLPFC volumes and surface areas, respectively). Sex, age, and ICV were used as covariates in the subsequent analyses.

After controlling for sex, age, and ICV, Stroop effect RT had negative correlations with left DLPFC surface area and volume ($r = -0.16$, $p = 0.004$, for the left DLPFC surface area; $r = -0.10$, $p = 0.07$, for the left DLPFC volume), and it had a positive correlation with left DLPFC thickness ($r = 0.13$, $p = 0.02$). This suggested that subjects with greater left DLPFC surface area and less left DLPFC thickness had better cognitive performance on the Stroop task.

As presented in Fig. 1, genetic association analysis using Plink showed that a SNP (rs7809486) within the *CNTNAP2* gene had the strongest association with bilateral DLPFC volumes ($t = 3.84$ and 3.86, $p = 0.0001519$ and 0.0001365 for the left and right DLPFC volumes, respectively), with age, sex, and ICV as covariates. The G allele was associated with greater left and right DLPFC volumes. Because cortical volume is a combination of cortical surface area and cortical thickness, we further examined the *CNTNAP2* genetic associations with DLPFC surface area and cortical thickness. The SNP (rs7809486) G allele was associated with greater left and right DLPFC surface areas ($t = 2.83$ and 3.22, $p = 0.005031$ and 0.001414 for the left and right DLPFC surface areas, respectively), but it was not associated with DLPFC cortical thickness ($p > 0.05$). Moreover, the SNP rs17433476 had the strongest association with left DLPFC surface area ($t = 3.40$, $p = 0.000764$, G allele was associated with greater left DLPFC surface area), while rs7806058 had the strongest association with right DLPFC surface area ($t = -3.60$, $p = 0.0003738$, with the G allele being associated with less right DLPFC surface area).

In order to test genotype group differences in DLPFC structural indices, MANCOVA and Fisher's least significant difference post hoc test were performed with age, sex, and ICV as covariates. After controlling for age, sex, and ICV, the *CNTNAP2* rs7809486 GG homozygotes ($N = 49$) had greater bilateral DLPFC volume and surface area than AG heterozygotes ($N = 147$) ($p = 0.0004$ and 0.0002 for the left and right DLPFC volumes, and $p = 0.015$ and 0.011 for the left and right DLPFC surface areas, respectively) and AA homozygotes ($N = 121$) ($p = 0.00003$ and 0.00002 and for the left and right DLPFC volumes, and $p = 0.002$ and 0.001 for the left and right DLPFC surface areas, respectively), but there were no significant differences between AG heterozygotes and AA homozygotes in bilateral DLPFC volumes and surface areas ($p > 0.05$). For rs17433476, GG homozygotes ($N = 63$) had greater left DLPFC surface area than TG heterozygotes ($N = 143$) ($p = 0.011$) and TT homozygotes ($N = 111$)

Table 1. Descriptive statistics of major brain structural and cognitive indices ($N = 317$)

	Mean	SD
Left dorsolateral prefrontal cortex volume	20,848.43	2111.28
Right dorsolateral prefrontal cortex volume	20,276.85	2180.09
Left dorsolateral prefrontal cortex surface area	7224.29	790.44
Right dorsolateral prefrontal cortex surface area	7233.18	796.01
Left dorsolateral prefrontal cortex thickness	2.47	0.09
Right dorsolateral prefrontal cortex thickness	2.39	0.09
Stroop effect reaction time	0.13	0.07
Stroop accuracy	0.96	0.06

Note: The units of brain structural volume, surface area, thickness, and Stroop effect reaction time are mm^3, mm^2, mm, and second, respectively. Stroop effect reaction time = incongruent condition reaction time – congruent condition reaction time. Higher score in Stroop effect reaction time indicates poorer cognitive performance.

Table 2. Partial correlations among Stroop effect reaction time and major brain structural indices after controlling for age, sex, and intracranial volume (N = 317)

	Stroop effect RT	Left DLPFC V	Right DLPFC V	Left DLPFC SA	Right DLPFC SA	Left DLPFC T	Right DLPFC T
Stroop effect RT							
Left DLPFC V	−0.10#						
Right DLPFC V	−0.01	0.63***					
Left DLPFC SA	−0.16**	0.86***	0.53***				
Right DLPFC SA	−0.06	0.56***	0.88***	0.65***			
Left DLPFC T	0.13*	0.18***	0.11#	−0.29***	−0.20***		
Right DLPFC T	0.06	0.09	0.21***	−0.23***	−0.22***	0.62***	

Note: #p < .10, *p < .05, **p < .01, ***p < .001. DLPFC = dorsolateral prefrontal cortex. The units of brain structural volume (V), surface area (SA), thickness (T), and Stroop effect reaction time (RT) are mm^3, mm^2, mm, and second, respectively. Stroop effect RT = incongruent condition RT – congruent condition RT. Higher score in Stroop effect RT indicates poorer cognitive performance.

(p = 0.0005), but there was no significant difference between TG heterozygotes and TT homozygotes in left DLPFC surface area (p > 0.05). For rs7806058, AA homozygotes (N = 146) had greater right DLPFC surface area than AG heterozygotes (N = 129) (p = 0.0005) and GG homozygotes (N = 26) (p = 0.02), but there was no significant difference between AG heterozygotes and GG homozygotes in right DLPFC surface area (p > 0.05).

After correcting for multiple testing by max(T) permutation approach in Plink (10,000 permutation) for individual SNPs within the *CNTNAP2* gene, SNP rs7809486 and six other SNPs (which were located near rs7809486 and had high LD with it) remained significantly associated with DLPFC volumes (corrected empirical p-values [max(T)/familywise] < 0.05), while the associations between rs17433476 and left DLPFC surface area and between rs7806058 and right DLPFC surface area were marginally significant (corrected empirical p-values [max(T)/familywise] < 0.10).

Haplotype analysis of directly genotyped variants near rs7809486 in the current sample confirmed that the association was present across SNPs in the haplotype. As shown in Fig. 2 and Table 3, 10 SNPs within the 26th haplotype block across the *CNTNAP2* gene (with the leading SNP underlined) were associated with phenotypes, including rs17433476, rs10952742, rs10464460, rs10464464, rs10464426, rs7809486, rs10952747, rs4726942, rs4726945, and rs4726946. Among these 10 SNPs, three of them were also associated with Stroop effect RT (i.e., rs4726942, rs4726945, and rs4726946). As shown in Table 3, rs4726946 T allele, rs4726945 G allele, and rs4726942 T allele were associated with better cognitive performance (i.e., lower score on Stroop effect RT) and greater left DLPFC volume and surface area (p < 0.05). We selected these three SNPs within *CNTNAP2* for subsequent analysis, because they were in the haplotype block across the leading SNP, and because they were associated with both brain structure (left DLPFC volume and surface area) and cognitive performance (Stroop effect). We selected left DLPFC surface area for the subsequent analysis because it had a stronger correlation with Stroop performance than the other DLPFC structural indices (p < 0.01).

Given the associations between the *CNTNAP2* gene, left DLPFC surface area, and Stroop performance, we further examined whether the left DLPFC surface area mediated the effect of *CNTNAP2* (i.e., rs4726942, rs4726945, and rs4726946, one SNP at a time) on Stroop effect RT. As shown in Fig. 3, *CNTNAP2* rs4726946 (GG = 0, GT = 1, TT = 2) was associated with Stroop effect RT (i.e., $Y = i_1 + cX + e_1$, $c = -0.0138$, $p = 0.023$); *CNTNAP2* rs4726946 was associated with the left DLPFC surface area (i.e., $M = i_2 + aX + e_2$, $a = 123.446$, $p = 0.018$); the mediation effect of the left DLPFC surface area on the association between *CNTNAP2* rs4726946 and Stroop performance was significant (i.e., $Y = i_3 + c'X + bM + e_3$, b = -0.00002, $p = 0.006$, $c' = -0.0115$, $p = 0.058$). Only b was significant, while c′ was not significant (i.e., the direct effect of X on Y is not significant after controlling for M). It suggested there is a complete mediation effect (i.e., X no longer affects Y after controlling for M). Mediation analysis showed the left DLPFC surface area mediated the effect of *CNTNAP2* rs4726946 on Stroop effect RT (Indirect effect = −0.0023, 95% confidence intervals (CI) = [−0.0053, −0.0005], SE = 0.0012, p = 0.047). The mediation analyses involving the other two SNPs (i.e., rs4726942 and rs4726945) showed similar results as those for the SNP rs4726946.

DISCUSSION

In this study, we found that *CNTNAP2* rs7809486 GG homozygotes had greater DLPFC volume and surface area than the other genotypes. We also found significant associations between a nearby SNP (rs4726946, which had moderate LD with rs7809486), left DLPFC surface area, and Stroop performance. Furthermore, the association between *CNTNAP2* rs4726946 and Stroop performance was mediated by the left DLPFC surface area.

The leading SNP rs7809486, located in the haplotype block from the 20th intron to the 21st intron of the *CNTNAP2* gene, had the strongest association with bilateral DLPFC volumes. The first SNP rs17433476 in this haplotype block, located in the 20th intron, had the strongest association with left DLPFC surface area. The last SNP rs4726946 in this haplotype block, located in the 21st intron, had significant associations with both the left DLPFC surface area and Stroop performance. Any of these SNPs in the haplotype block could have

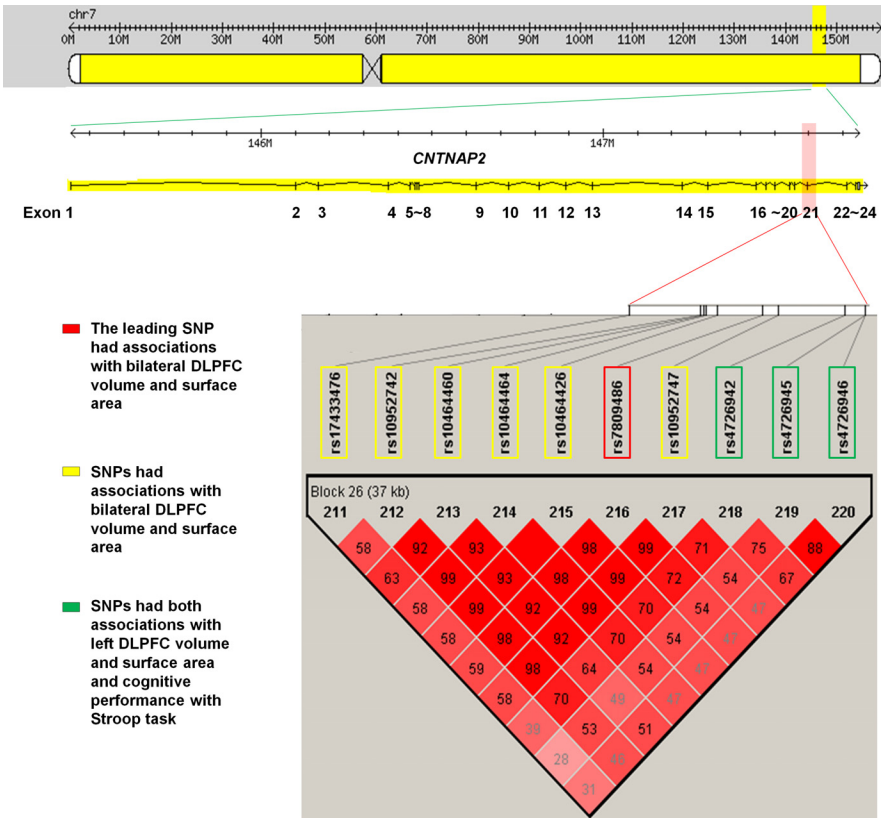

Fig. 2. Schematic representation of SNPs within the 26th haplotype block in the *CNTNAP2* gene associated with DLPFC volume and surface area and cognitive performance on the Stroop task. Regions of high linkage disequilibrium are shown in dark red with the intensity increasing r^2 value in the current study. The numbers indicate the r^2 statistic value between the corresponding two SNPs. The haplotype consisted of ten boxed SNPs located across the 21st exon. It includes one leading SNP rs7809486 boxed in red (which had strong associations with DLPFC volume and surface area), three SNPs boxed in green (i.e., rs4726946, rs4726945, and rs4726942, which had both associations with left DLPFC volume and surface area and Stroop performance), and the other six SNPs boxed in yellow (which also had associations with DLPFC volume and surface area) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

functional consequences on the amount of CNTNAP2 protein synthesized. A previous study suggested that a rare inherited variant D1129H in the 21st exon of *CNTNAP2* contributed to autism, and was found to be deleterious (Bakkaloglu et al., 2008). The 21th exon of *CNTNAP2* encodes a laminin G domain, predicted to play a role in cell adhesion, migration, and differentiation (Rodenas-Cuadrado et al., 2014). A recent study linked three *CNTNAP2* SNPs within this haplotype block to schizophrenia in Han Chinese (Chen et al., 2015). These schizophrenia-associated SNPs had moderate LD with SNPs within this haplotype block ($r^2 = 0.21 \sim 0.48$ in the Han Chinese sample based on the HapMap data). The *CNTNAP2* rs4640984 A allele, rs1547597 A allele, and rs6977567 A allele were identified as plausible risk alleles for schizophrenia (Chen et al., 2015). They had moderate LD with *CNTNAP2* rs17433476 T allele, rs10952742 T allele, rs10464460 C allele, rs10464464 A allele, rs10464426 T allele, rs7809486 A allele, rs10952747 C allele, rs4726942 A allele, rs4726945 C allele, and rs4726946 G allele, all of which were associated with less DPFC volume and surface area as reported in the current study. Since schizophrenia patients had reduced DLPFC volume (Ellison-Wright et al., 2008), future studies should explore potential associations between the *CNTNAP2* gene and DLPFC of individuals with schizophrenia.

SNP rs7806058 in the first intron of the *CNTNAP2* gene had the strongest association with the right DLPFC surface area in our study. Subjects with rs7806058 G allele had less DLPFC volume and surface area. Our finding was consistent with previous studies. A recent study suggested that the rs7806058 G allele was a risk allele for schizophrenia in Han Chinese (Chen et al., 2015), while schizophrenia was associated with volume reduction in the DLPFC (Ellison-Wright et al., 2008). Several SNPs in the first intron of the *CNTNAP2* gene were associated with mental disorders; for example, SNPs rs802524 and rs802568 were associated with schizophrenia and bipolar disorder (Wang et al., 2010), and SNP rs1718101 with autism (Anney et al., 2012). Interestingly, the *FOXP2* gene (i.e., Forkhead Box P2 gene [7q31] involved with speech and language disorder) binds within the first intron of the *CNTNAP2* gene (7q35) to regulate expression of *CNTNAP2* (Vernes et al., 2008).

Our gene–brain–behavior results integrated and expanded several lines of research. Recent neurogenetic research has incorporated mediation analyses by modeling indirect pathways between genetic variants and behavior via the brain (Bogdan et al., 2013). Mutations and common variants of *CNTNAP2* have been implicated in multiple cognitive disorders characterized by poor cognitive control, and altered frontal cortex structure and function (Dennis et al., 2011; Clemm von Hohenberg et al., 2013; Rodenas-Cuadrado et al., 2014; Koeda et al., 2015; Scott-Van Zeeland et al., 2010; Tan et al., 2010; Whalley et al., 2011). Several neuroimaging and neurostimulation studies have directly linked DLPFC to performance on the Stroop task (Vanderhasselt et al., 2009).

As one of the most recently evolved regions of the human brain, DLPFC plays an important role in executive functions, such as the inhibition ability measured by the Stroop task (MacDonald et al., 2000). We found that better cognitive performance on the Stroop task (i.e., smaller Stroop effect RT) was associated with greater left DLPFC volume, greater left DLPFC surface area, and less left DLPFC thickness in healthy young subjects. Since the brain changes over the lifespan, the direction of these correlations may depend on the age of

Table 3. Genetic associations for 10 SNPs within the 26th haplotype block of *CNTNAP2* gene after controlling for age, sex, and intracranial volume

SNP	Position	Min/Maj allele	MAF	Left DLPFC volume		Right DLPFC volume		Left DLPFC surface area		Right DLPFC surface area		Left DLPFC thickness		Right DLPFC thickness		Stroop effect RT	
				t	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*
rs17433476	147,586,327	G/T	0.42	3.55	**0.0004**	3.09	0.0022	3.40	0.0008	2.91	0.0039	0.19	0.8514	0.60	0.5501	-0.47	0.6371
rs10952742	147,597,745	C/T	0.37	3.75	**0.0002**	3.71	**0.0002**	2.81	0.0053	3.10	0.0021	1.25	0.2131	1.18	0.2396	-1.53	0.1283
rs10464460	147,598,256	T/C	0.36	3.67	**0.0003**	3.66	**0.0003**	2.88	0.0043	3.07	0.0023	0.99	0.3241	1.13	0.2590	-1.53	0.1282
rs10464464	147,598,666	G/A	0.37	3.82	**0.0002**	3.75	**0.0002**	2.82	0.0050	3.10	0.0021	1.32	0.1888	1.25	0.2135	-1.60	0.1097
rs10464426	147,600,535	C/T	0.37	3.82	**0.0002**	3.75	**0.0002**	2.82	0.0050	3.10	0.0021	1.32	0.1888	1.25	0.2135	-1.60	0.1097
rs7809486	147,607,703	G/A	0.38	3.84	**0.0002**	3.86	**0.0001**	2.83	0.0050	3.22	0.0014	1.31	0.1899	1.18	0.2407	-1.60	0.1114
rs10952747	147,610,249	T/C	0.38	3.66	**0.0003**	3.83	**0.0002**	2.74	0.0066	3.27	0.0012	1.23	0.2184	1.14	0.2566	-1.66	0.0978
rs4726942	147,620,864	T/A	0.32	3.20	0.0015	3.33	0.0010	2.19	0.0294	2.59	0.0102	1.49	0.1382	1.34	0.1799	-2.15	0.0325
rs4726945	147,624,155	G/C	0.32	2.73	0.0067	1.73	0.0854	2.28	0.0236	1.49	0.1363	0.66	0.5081	0.65	0.5193	-2.51	0.0127
rs4726946	147,624,171	T/G	0.29	2.57	0.0106	1.86	0.0635	2.38	0.0177	1.82	0.0692	0.16	0.8743	0.35	0.7239	-2.28	0.0234

Note: Significant p values after correcting for multiple testing by max(T) permutation approach in Plink (10,000 permutation) are shown in bold. Min/Maj allele = minor allele/ major allele. MAF = minor allele frequency. The direction of the *t* value represents the effect of each minor allele.

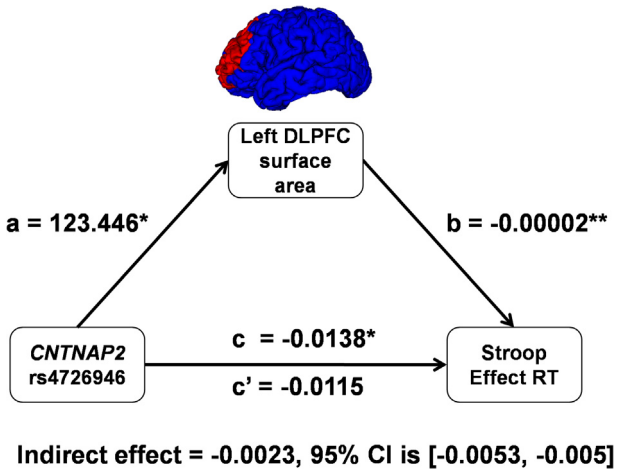

Fig. 3. Mediation analysis showed that left DLPFC surface area (colored in red) mediated the association between the *CNTNAP2* rs4726946 genotype and cognitive performance measured by the Stroop task. Sex, age, and intracranial volume were used as covariates. Bootstrapped for 5000 times. CI: confidence interval. $^{*}p < 0.05$, $^{**}p < 0.01$. The left DLPFC surface area is colored in red (one randomly chosen subject). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

subjects. Our finding is consistent with recent studies using young subjects as samples, which found that better cognitive performance was associated with less cortical thickness and greater surface area (Noble et al., 2015; Schnack et al., 2015). However, using middle-aged or postmortem brain as samples, higher intelligence has been found to be associated with greater cortical volume and greater cortical thickness (Deary et al., 2010).

There were several limitations in the current study, which could be explored in the future research. First, the identified SNPs in the current study have not been linked to neuroimaging data in previous studies. Previous imaging genetic studies identified three other SNPs of the *CNTNAP2* gene (Dennis et al., 2011; Clemm von Hohenberg et al., 2013; Rodenas-Cuadrado et al., 2014; Koeda et al., 2015; Scott-Van Zeeland et al., 2010; Tan et al., 2010; Whalley et al., 2011), which had no LD with SNPs identified in our study. Future studies are needed to investigate the detailed biochemical mechanisms of these imaging genetic associations. Second, we reported the gene-brain-cognition pathway based on structural imaging, rather than functional imaging. Previous studies suggested the *CNTNAP2* gene is involved in both structural and functional brain measures and cognitive performance (Rodenas-Cuadrado et al., 2014). Future studies should examine whether the DLPFC activation mediated the effect of *CNTNAP2* on Stroop performance.

CONCLUSION

In sum, this study provided the first evidence of associations between the *CNTNAP2* gene, DLPFC morphology, and cognitive performance on the Stroop task in healthy Chinese individuals, with *CNTNAP2* rs7809486 GG homozygotes having greater bilateral

DLPFC volumes and surface areas. The left DLPFC surface area mediated the association between the *CNTNAP2* rs4726946 genotype and cognitive performance on the Stroop task.

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