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Sex Moderates the Effects of the *Sorl1* Gene rs2070045 Polymorphism on Cognitive Impairment and Disruption of the Cingulum Integrity in Healthy Elderly

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The *SORL1* rs2070045 polymorphism was reported to be associated with SorLA expression in the brain and the risk of late-onset Alzheimer's disease (AD). However, the influence of this polymorphism on cognitive functioning is likely to be moderated by sex. This study aimed to examine the sex moderation on the effects of rs2070045 on neuropsychological performance and the cingulum integrity in Chinese Han population. In this study, 780 non-demented older adults completed a battery of neuropsychological scales. Diffusion tensor images (DTI) of 126 subjects were acquired. We adopted the atlas-based segmentation strategy for calculating the DTI indices of the bilateral cingulum and cingulum hippocampal part for each subject. We used a multivariate analysis of variance (MANOVA) to compare the cognitive performance and DTI differences between the rs2070045 genotype. Controlling for age, education, and the *APOE* ε4 status, the influence of sex on the effects of the rs2070045 polymorphism on executive function was observed. We also found an interaction between sex and the rs2070045 polymorphism on the white matter (WM) microstructure of the left cingulum hippocampal part. Furthermore, the mean diffusivity and axial diffusivity of the tract were associated with Trail Making Test performance in T/T men. These results hint that sex moderates the association between the rs2070045 polymorphism and executive function, as well as the WM integrity of the left cingulum hippocampal part. Our findings underscore the importance of considering the influence of sex when examining the candidate genes for cognitive abilities and AD.
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INTRODUCTION

It is well-accepted that in addition to environmental factors, genetic factors have impact on cognitive aging and dementia. For example, the apolipoprotein E (*APOE*) ε4 allele is the best-established genetic risk factor for Alzheimer's disease (AD), which significantly increases the incidence of late-onset AD and decreases the mean onset age of AD (Corder *et al*, 1993). The *APOE* ε4 allele is also related to cognitive impairment and greater cognitive decline in non-demented elderly (Bretsky *et al*, 2003; Small *et al*, 2004). In addition to the *APOE* gene, several other genes have been identified to be associated with AD risk (Tanzi and Bertram, 2005; Bertram *et al*, 2007). The neuronal sortilin-related receptor (*SORL1*) gene, which has a key role in the differential sorting of the amyloid precursor protein (APP) and regulating the amyloid-β (Aβ) production (Offe *et al*, 2006; Rogaeva *et al*, 2007), is one of the most studied putative Alzheimer's disease (AD) susceptibility genes (Lee *et al*, 2007; Meng *et al*, 2007; Kolsch *et al*, 2009).

The synonymous single-nucleotide polymorphism (SNP) rs2070045 is located in the 3′ region of the *SORL1* gene. The SNP genotype alters codon usage for serine in the risk variant, suggesting reduced translation efficiency as the molecular basis of insufficient sorting protein-related receptor with A-type repeats (SorLA) expression (Zeeberg, 2002; Caglayan *et al*, 2012). A brain autopsy study demonstrated the correlation of a *SORL1* haplotype that consists of rs2070045 to SorLA expression in the brain of AD patients (Caglayan *et al*, 2012). The SNP rs2070045 and haplotypes that encompass this SNP have been described to be associated with the risk of late-onset AD in various ethnic populations (Rogaeva *et al*, 2007; Tan *et al*, 2009; Ning *et al*, 2010; Reitz *et al*, 2011; Xue *et al*, 2014). The rs2070045 G allele has been recognized as a risk factor for AD in populations that are mainly of Caucasian origin (Rogaeva *et al*, 2007; Reitz *et al*, 2011). However, a recent study

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showed that the T allele of rs2070045 in the *SORL1* was significantly associated with increased AD risk in the Chinese Han population (Xue *et al*, 2014). These results suggest that the effects of the rs2070045 polymorphism might not be consistent among the different ethnic groups.

Regarding the relationship between the variants in the *SORL1* gene and cognitive abilities, both positive and negative findings have been reported (Seshadri *et al*, 2007; Houlihan *et al*, 2009; Liu *et al*, 2009; Shulman *et al*, 2010). Sex moderation of *SORL1* effects on cognitive abilities could be a source of these discrepant findings. A recent large longitudinal investigation observed sex-moderated associations between the rs2070045 polymorphism and spatial, episodic memory, and verbal trajectories. Specifically, possible protective effects of the rs2070045 rare homozygote (G/G) for men's performance at least before age 75 and detrimental effects for women's performance were both shown (Reynolds *et al*, 2013). Moreover, Cellini *et al* (2009) found an association between *SORL1* variants with sex in Italian patients and late-onset AD and suggested that the *SORL1* could possibly affect AD risks through a female-specific mechanism.

Traditionally, the connection between the micro genotypes and the macro cognitive phenotypes is difficult to establish, and the results of related studies are often irreproducible. The recent introduction and development of imaging genetics, which constructs neuroimaging biomarkers as intermediate phenotypes for gene discovery, would improve the power of genetic association studies and bridge the gap from DNA sequences to human behavior (Meyer-Lindenberg and Weinberger, 2006).

As an important white matter (WM) tract for normal aging and AD development (Yoon *et al*, 2008; Catheline *et al*, 2010), the cingulum bundle is the major median associative WM fasciculus that connects the cingulate subregions and projects to the entorhinal cortex, having a key role in various cognitive abilities (Nestor *et al*, 2007; Schermuly *et al*, 2010). Relative to normal controls, AD patients showed lower anisotropy of cingulum bundles (Xie *et al*, 2005). Greater reduction of the cingulum integrity has also been observed in carriers of the *APOE* ε4 allele compared with non-carriers (Heise *et al*, 2011). It has been demonstrated that *SORL1* induced widespread neuronal expression reduction in the posterior cingulate cortex and influenced the microstructure of fronto-temporal WM tracts with known susceptibility in patients with AD, including the cingulum bundle (Liang *et al*, 2008; Felsky *et al*, 2013).

On the basis of these reported findings, we hypothesized that the effects of the *SORL1* rs2070045 polymorphism on cognitive functioning and WM microstructure are moderated by sex. In addition to further confirming the effects of the *SORL1* variants on cognitive function in the non-demented elderly Chinese Han population, this study aimed to examine whether sex moderates the association between the rs2070045 polymorphism and neuropsychological performance, as well as the cingulum integrity.

MATERIALS AND METHODS

Participants

Subjects were recruited from the Beijing Aging Brain Rejuvenation Initiative Study Group (BABRI), which is a longitudinal study investigating aging and cognitive impairment in urban elderly individuals in Beijing, China. There were 780 participants in this study, and all of the participants had the following properties: scored no less than 24 on the Mini-Mental-Status Examination-Chinese version (MMSE) (Zhang *et al*, 1990); right-handed and native Chinese speakers; 50 to 80 years of age; no less than 6 years of education; had no structural abnormalities; had no history of addiction, neurologic or psychiatric diseases; had no conditions known to influence cerebral function; and had no large vessel disease. Demographic information for each group was presented in Table 1. The study was approved by the Institutional Review Board of the Beijing Normal University Imaging Center for Brain Research. Written informed consent was obtained from each participant.

Cognitive Assessment

All participants underwent a battery of neuropsychological tests to assess cognitive abilities. General mental status was assessed with the MMSE. Episodic memory tests included the Auditory Verbal Learning Test (AVLT) (Guo *et al*, 2009) and Recall component of Rey-Osterrieth Complex Figure Test (ROCF) (Zhou *et al*, 2006). Visual-spatial ability was assessed using the Copy component of ROCF (Zhou *et al*, 2006) and Clock-Drawing Test (CDT) (Ishiai *et al*, 1993). Language ability was assessed using the Boston Naming Test (BNT) (Guo *et al*, 2006) and Category Verbal Fluency Test (CVFT) (Mok *et al*, 2004). Processing speed was assessed using the Symbol Digit Modalities Test (SDMT) (Sheridan *et al*, 2006) and Trail Making Test A (TMT-A) (Lu *et al*, 2006). Finally, executive function was assessed using the Trail Making Test B (TMT-B) (Lu *et al*, 2006), the Trail Making Test BA (TMT-BA) (Lu *et al*, 2006) and Stroop Color-word Test (Stroop) (Guo *et al*, 2005). Neuropsychological characterizations for each group are presented in Table 1.

Genetic Analysis

The participants were prescreened for the rs2070045 genotype using the Custom Taqman SNP Genotyping Assays (Applied Biosystems, Foster City, CA). DNA was extracted from the blood samples of the subjects according to standard procedures to conduct a PCR for characterizing the rs2070045 genotype. An additional two SNPs, rs429358 and rs7412, which collectively form the *APOE* ε2 (with the haplotype of rs429358-rs7412: T/T), ε3 (G/T), and ε4 alleles (G/G), were also genotyped. The sample success rates for all three SNPs were 100%, and the reproducibility of all of the genotyping was 100% according to a duplication of at least 10% of the genotypes. According to the rs2070045 genotyping, all of the subjects were divided into three groups: 234 G/G, 376 G/T, and 170 T/T.

Image Acquisition

Diffusion tensor images of 126 subjects were acquired using a single-shot echo-planar imaging sequence on a Siemens Trio 3.0 Tesla scanner in the Imaging Center for Brain Research at Beijing Normal University (acquisition parameters: 70 axial sections; section thickness, 2 mm; no

Table 1 Demographic and Cognitive Characteristics of all Participants

	Male ($N=289$)			Female ($N=491$)			rs2070045 F/χ^2 (p)[a]	Sex F/χ^2 (p)[a]	rs2070045 × sex F/χ^2 (p)[a]
	G/G ($N=79$)	G/T ($N=138$)	T/T ($N=72$)	G/G ($N=155$)	G/T ($N=238$)	T/T ($N=98$)			
Age	66.7 ± 7.9	66.8 ± 7.6	65.5 ± 7.6	63.5 ± 6.5	63.9 ± 7.1	63.7 ± 7.0	0.89 (0.41)	23.83 (<0.001)*	0.27 (0.76)
Education	12.2 ± 3.4	11.8 ± 3.5	11.3 ± 3.2	11.6 ± 3.0	10.8 ± 3.1	11.1 ± 3.2	2.52 (0.081)	5.30 (0.022)*	0.84 (0.43)
APOE ε4 or not	17/61	18/120	10/61	28/122	31/206	11/85	3.16 (0.21)	0.25 (0.61)	0.26 (0.88)
General mental status									
MMSE	28.1 ± 1.5	28.0 ± 1.6	27.8 ± 1.7	28.2 ± 1.4	27.7 ± 1.7	27.8 ± 1.7	2.36 (0.095)	0.94 (0.33)	0.69 (0.50)
Episodic memory									
AVLT Recall	5.4 ± 2.2	5.3 ± 2.6	5.4 ± 2.8	6.4 ± 2.4	5.6 ± 2.6	6.5 ± 2.5	2.27 (0.10)	13.02 (<0.001)*	1.53 (0.22)
AVLT Total	28.8 ± 8.7	28.5 ± 8.9	29.1 ± 9.9	33.2 ± 8.9	30.6 ± 9.4	32.4 ± 8.9	1.35 (0.26)	15.41 (<0.001)*	0.68 (0.51)
R-O Recall	14.7 ± 6.4	14.6 ± 6.4	12.5 ± 6.2	14.0 ± 6.4	12.5 ± 6.1	13.4 ± 6.3	2.01 (0.14)	4.51 (0.034)*	2.76 (0.064)
Visual-spatial ability									
R-O Copy	33.6 ± 2.5	34.0 ± 2.2	33.3 ± 3.1	33.7 ± 2.9	32.8 ± 4.1	33.0 ± 3.6	0.53 (0.59)	3.86 (0.050)	2.61 (0.074)
CDT	25.4 ± 3.2	25.2 ± 3.0	24.9 ± 3.3	25.1 ± 3.2	24.4 ± 4.2	24.0 ± 3.8	1.42 (0.24)	4.96 (0.026)*	0.38 (0.68)
Language ability									
Fluency Test	43.9 ± 8.5	45.6 ± 8.5	44.3 ± 7.9	46.4 ± 8.1	45.0 ± 8.9	46.2 ± 8.9	0.19 (0.83)	2.47 (0.12)	2.04 (0.13)
BNT	24.5 ± 3.3	24.8 ± 3.1	25.1 ± 2.9	23.1 ± 3.5	22.1 ± 3.5	22.6 ± 3.86	0.47 (0.62)	84.39 (<0.001)*	2.38 (0.09)
Processing speed									
SDMT	33.2 ± 10.8	35.2 ± 10.7	32.8 ± 11.5	36.5 ± 11.0	35.9 ± 11.6	36.4 ± 10.9	1.70 (0.18)	2.30 (0.13)	0.70 (0.50)
TMT-A Time (s)	60.4 ± 19.3	53.7 ± 18.3	60.5 ± 24.5	57.9 ± 20.0	58.0 ± 20.4	58.9 ± 23.1	4.15 (0.016)*	1.36 (0.24)	1.54 (0.22)
Executive function									
TMT-B Time (s)	180.3 ± 68.9	158.9 ± 63.9	179.1 ± 66.8	170.1 ± 65.7	186.5 ± 69.1	175.3 ± 75.9	1.04 (0.35)	5.61 (0.018)*	5.39 (0.005)*
TMT B-A Time (s)	119.7 ± 58.1	105.1 ± 55.7	120.3 ± 57.6	112.1 ± 58.0	128.5 ± 60.3	116.5 ± 63.0	0.26 (0.77)	4.74 (0.030)*	4.92 (0.008)*
Stroop C-B Time (s)	43.5 ± 20.5	39.7 ± 17.8	44.8 ± 20.0	35.8 ± 16.9	38.9 ± 20.2	36.2 ± 15.6	0.47 (0.63)	8.89 (0.003)*	3.37 (0.035)*

Abbreviations: AVLT, Auditory Verbal Learning Test; BNT, Boston Naming Test; CDT, Clock-Drawing Test; MMSE, Mini-Mental State Examination; R-O, Rey-Osterrieth Complex Figure; SDMT, Symbol Digit Modalities Test; TMT, Trail Making Test. Note: data expressed as mean ± s.d. *Significant at $P<0.05$. [a]Comparisons between groups were performed using Wald χ^2-test for gender and *APOE* ε4. Multivariate analysis of variance (MANOVA) was used to determine the main effect of the genotype and sex on the neuropsychological tests, as well as the interaction between them (age, education, and *APOE*ε4 as covariates).

section gap; 30 diffusion directions with a *b* value of 1000 s/mm^2 and an additional image with a *b* value of 0 s/mm^2; field of view, 256 × 256 mm^2; acquisition matrix, 128 × 128; number of signals acquired, three). Further details of the small imaging sample are provided in Supplementary Information Table S1 and S2.

Image Analysis

Image preprocessing and analysis were carried out using FMRIB's Diffusion Toolbox (FDT) (FSL 4.1.4; www.fmrib.ox.ac.uk/fsl). First, we corrected head movement and eddy currents. A brain mask was then created by running the BET procedure on the B0 (no diffusion weighting) images. Last, DTIfit was used to fit the diffusion tensor model. The output of DTIfit yielded voxel-wise maps of the fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (DA), and radial diffusivity (DR). To investigate the diffusion changes in the cingulum, we adopted the atlas-based segmentation strategy. We registered each subject's FA, MD, DA, and DR maps to the JHU ICBM-DTI-81 template (cmrm.med.jhmi.edu/) and calculated the mean values of the bilateral cingulum and cingulum hippocampal part for each subject.

Statistical Analysis

Cognitive assessment. Demographic factors, including age and years of education, of both the rs2070045 genotypes and sex were compared using a multivariate analysis of variance (MANOVA) or logistic regression. For the genotype and sex effects on the cognitive performance,

comparisons were performed using a multivariate analysis of covariance (MANCOVA), with the age, education, and *APOE* ε4 effects as covariates of no interest. All of the statistical analyses were performed with SPSS software. The Hardy–Weinberg test was performed by the software PLINK33.

DTI. For the diffusion alterations in the atlas-based tract ROIs, we performed a MANCOVA to compare the DTI indices differences between the rs2070045 genotype, sex, and interaction between the two (age, education, and *APOE*ε4 effects corrected). The Bonferroni correction was used to adjust for possible spurious findings due to multiple testing. Subsequent *post hoc* comparisons were performed using the Tukey's Test.

Correlation Analyses Between White Matter Integrity and Neuropsychological Scores

Pearson's partial correlation analyses controlling for age, education, and *APOE* ε4 effects were also performed between the DTI indices and the neuropsychological scores to estimate the possible associations between the white matter integrity and cognitive functioning in the six genotype and sex groups separately.

RESULTS

Demographic and Cognitive Characteristics

In the genotype distribution in our samples, no deviation from the Hardy–Weinberg equilibrium was found ($P > 0.05$). In the large samples, we did not find any significant differences between the rs2070045 groups in the demographic results or in the *APOE* status (Table 1). In addition, there was also no significant *SORL1* genotype by sex interactions in the demographic results or in the *APOE* status. We found significant differences between males and females in age and education. All of the subsequent analyses were adjusted for age and education, as well as *APOE* status. For the neuropsychological tests, the effect of the rs2070045 polymorphism was significant only in TMT-A ($P = 0.016$). There were significant interactions between sex and the rs2070045 polymorphisms in the TMT-B ($P = 0.005$), TMT-BA ($P = 0.008$) and Stroop C-B ($P = 0.035$) (Table 1).

The Interaction Between Sex and the rs2070045 Genotype on White Matter Tracts

Sex × rs2070045 showed a significant effect on the MD ($F = 5.089$, $P = 0.008$) and DA ($F = 5.979$, $P = 0.003$) of the left cingulum hippocampal part. Especially, in males, the *post-hoc* results indicated that the rs2070045 G/G carriers had significantly higher MD ($P = 0.007$) and DA ($P = 0.002$) than the G/T carriers in the left cingulum hippocampal part (Figure 1, Table 2).

Correlations Between DTI Indices and Cognitive Performance

Only T/T men showed significant correlations between the DTI indices and neuropsychological scores. We found a significantly positive correlation between the MD values of the left cingulum hippocampal part and the Trail Making Test performance in T/T men (TMT-B: $r = 0.750$, $P = 0.002$; TMT-BA: $r = 0.715$, $P = 0.004$). In the T/T men group, the DA of the left cingulum hippocampal part also showed a significantly positive correlation with the Trail Making Test performance (TMT-B: $r = 0.692$, $P = 0.006$; TMT-BA: $r = 0.677$, $P = 0.008$) (Figure 2).

DISCUSSION

The lipoprotein receptor SorLA has been demonstrated to link to AD pathologically based on its region-specific reduction in vulnerable regions of AD brains, and its potent effects on Aβ production (Offe *et al*, 2006). Evidence showed that inherited or acquired changes in SorLA expression or function are mechanistically involved in causing Alzheimer's disease (Rogaeva *et al*, 2007). Therefore, the *SORL1* gene encoding SorLA has received attention as a major genetic factor for AD. In this study, sex-moderated association between variants in the *SORL1* rs2070045 and executive function performance was observed in non-demented Chinese older adults. We further substantiated the linkage between this genetic risk and cognitive function by demonstrating that the relation of the rs2070045 polymorphism to WM integrity of the left cingulum hippocampal part was also dependent on sex and that the correlations between executive function performance and the DTI indices of the left cingulum hippocampal part were significant in male T/T carriers.

Sex influences the susceptibility to AD, which could be partly due to the sex-specific associations between genetic factors and AD-related pathology (Cahill, 2006). For example, Cellini *et al* (2009) found an association between *SORL1* variants and sex in patients with AD. Their data suggest that *SORL1* could affect AD through a female-specific mechanism . By the same token, it appears that the relationships of genes or gene products to cognitive abilities also differ between the sexes in normal individuals. The interaction between sex and *SORL1* on cognitive functioning was first reported by Reynolds *et al* (2013) . They found that in men homozygous for the rs2070045 risk allele (G/G) initially showed a performance advantage over T/T and G/T carriers in verbal, spatial and memory tests, but their advantage diminished with faster rates of decline. For women subjects, G/G and G/T carriers tended to show lower performance across age compared with T/T carriers. Our current study also observed sex moderation on the effects of SNP rs2070045 on cognitive functioning, but with a different trend: after controlling for age, education, and the *APOE* ε4 status, male homozygous for the G allele and T allele both performed worse than male heterozygous G/T in executive function tests; female T-allele carriers exhibited lower performance than female G/G carriers. This discrepancy could arise from multiple factors such as study designs, cognitive parameters, sample sizes, and even ethnic background. It has been reported that the G allele, which is the minor allele of the rs2070045 for Caucasians and other populations, is a risk factor for AD (Rogaeva *et al*, 2007; Reitz *et al*, 2011; Reynolds *et al*, 2013). In this study, we found that the T allele is the minor allele, which is

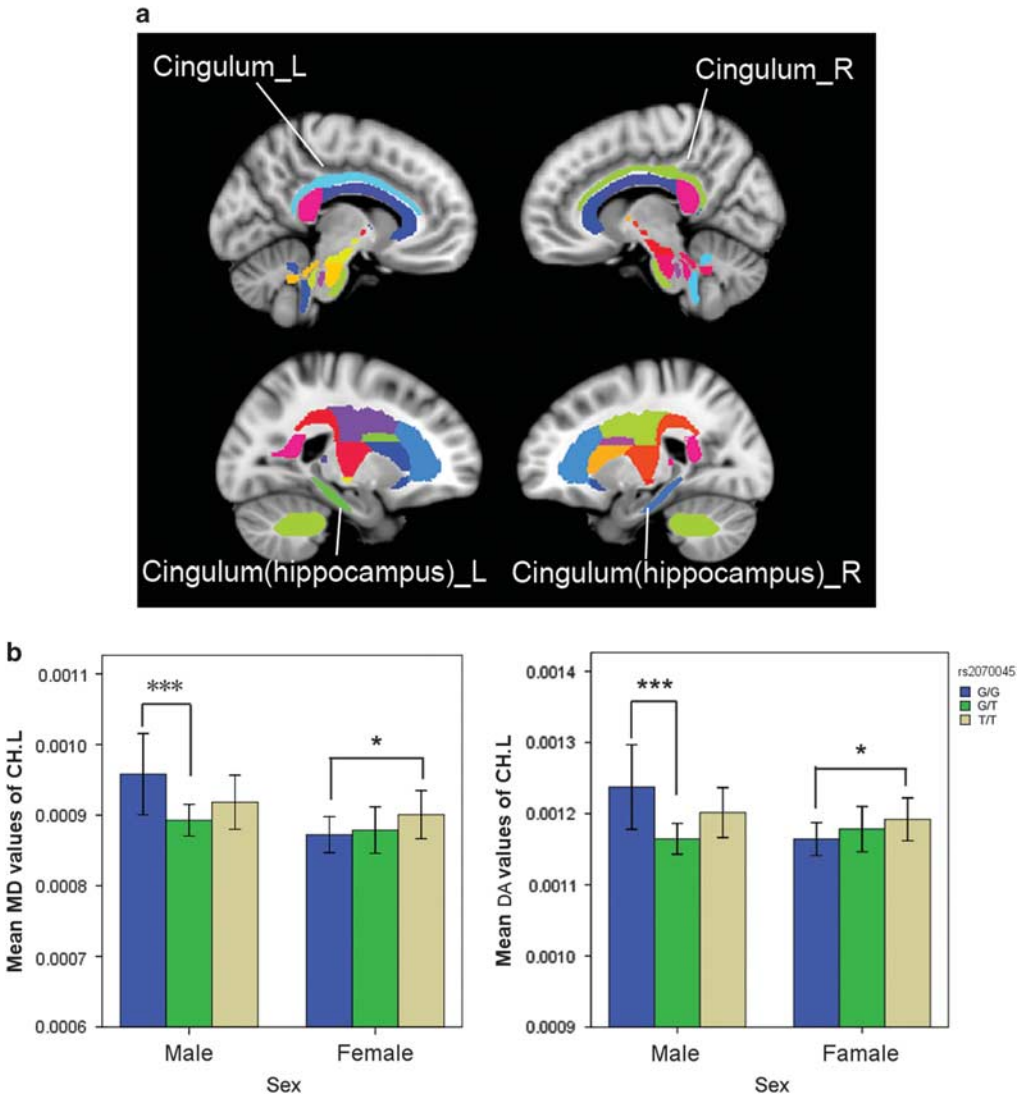

Figure 1 Mean diffusion metrics of the atlas-based tracts in the gene and sex groups. (a) The JHU white matter atlas in the ICBM-DTI-81 space. Colored regions indicate the bilateral cingulum and cingulum hippocampal part. (b) *Post-hoc* differences in the mean diffusion metrics of the atlas-based tracts among groups. $^{*}P<0.05$ uncorrected. $^{***}P<0.05$ in Tukey's test. DA, axial diffusivity; MD, mean diffusivity.

consistent with the findings from previous studies with Chinese Han subjects (Tan *et al*, 2009; Ning *et al*, 2010; Xue *et al*, 2014). Recent evidence has indicated that the T allele of the rs2070045 is associated with AD risk in the Chinese Han population (Xue *et al*, 2014). The differences in the allele and genotype frequencies as well as in the potential risk allele might cause inconsistent results between our study and study by Reynolds *et al* (2013). More interestingly, we observed a heterozygote advantage for the rs2070045 polymorphism in executive function. The condition in which heterozygotes show better functioning than homozygotes for quantitative traits is called positive heterosis. Positive heterosis is common in humans and has been reported for the influence on cognitive function of the catechol O-methyl transferase (COMT) and methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms (Gosso *et al*, 2008; Tsai *et al*, 2011). One possible explanation for this heterozygote advantage for the rs2070045 polymorphism is that the SNP could have both detrimental and beneficial effects on the cognitive function of elderly subjects who carry the T allele. Female T carriers exhibit poorer cognitive functioning than G/G carriers. In contrast, the T allele might also exert positive effects on the aging process through other pathways, which could explain the better cognitive functioning in G/T heterozygotes than in G/G homozygotes in males. Nevertheless, this heterozygote advantage requires further confirmation in other samples, including a larger sample of men. It should be noted that this explanation still needs biochemical evidence for support.

The cingulated fiber tracts have been demonstrated to be susceptible to aging (Yoon *et al*, 2008), and the disruption of the posterior cingulum bundles is associated with AD (Catheline *et al*, 2010). Evidence from human postmortem brain tissue showed that the expression of SorLA protein was reduced in the posterior cingulated cortex, hippocampus, and middle temporal gyrus in AD brains compared with control brains (Liang *et al*, 2008). Diffusion imaging

Table 2 Group Comparisons of Mean Diffusion Metrix of Each ROI

White matter tract	Male			Female			rs2070045 F(*p*)[a]	sex F(*p*)[a]	rs2070045 × sex F(*p*)[a]
	G/G (*N* = 19)	G/T (*N* = 24)	T/T (*N* = 18)	G/G (*N* = 24)	G/T (*N* = 25)	T/T (*N* = 16)			
FA									
Cingulum_R.	0.36 ± 0.03	0.37 ± 0.02	0.35 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.39 (0.68)	2.85 (0.094)	0.39 (0.68)
Cingulum_l.	0.37 ± 0.03	0.37 ± 0.02	0.36 ± 0.02	0.37 ± 0.03	0.38 ± 0.03	0.38 ± 0.03	0.31 (0.74)	3.75 (0.055)	0.65 (0.53)
Cingulum_ hippocampus_R.	0.31 ± 0.02	0.31 ± 0.03	0.31 ± 0.03	0.32 ± 0.03	0.34 ± 0.03	0.33 ± 0.02	0.20 (0.82)	14.36 (<0.001)*	1.10 (0.34)
Cingulum_ hippocampus_l.	0.30 ± 0.02	0.30 ± 0.03	0.31 ± 0.03	0.32 ± 0.03	0.33 ± 0.03	0.32 ± 0.03	0.040 (0.96)	14.25 (<0.001)*	1.15 (0.32)
MD ($\times 10^{-4}$)									
Cingulum_R.	7.78 ± 0.36	7.70 ± 0.36	7.90 ± 0.39	7.67 ± 0.35	7.80 ± 0.32	7.74 ± 0.23	1.57 (0.21)	0.24 (0.62)	3.02 (0.053)
Cingulum_l.	7.96 ± 0.28	7.93 ± 0.30	8.05 ± 0.23	7.85 ± 0.39	7.93 ± 0.30	7.97 ± 0.28	2.42 (0.09)	0.00 (1.00)	1.26 (0.29)
Cingulum_ hippocampus_R.	9.19 ± 0.91	8.75 ± 0.64	8.99 ± 0.77	8.68 ± 0.66	8.56 ± 0.56	8.55 ± 0.50	2.00 (0.14)	3.61 (0.060)	1.17 (0.32)
Cingulum_ hippocampus_l.	9.58 ± 1.20	8.93 ± 0.53	9.18 ± 0.77	8.71 ± 0.62	8.79 ± 0.80	9.01 ± 0.64	2.28 (0.11)	2.28 (0.13)	5.09 (0.008)*
DA ($\times 10^{-4}$)									
Cingulum_R.	10.87 ± 0.37	10.83 ± 0.42	10.97 ± 0.36	10.82 ± 0.35	11.01 ± 0.41	10.98 ± 0.34	1.64 (0.20)	3.07 (0.082)	1.74 (0.18)
Cingulum_l.	11.34 ± 0.29	11.28 ± 0.38	11.34 ± 0.37	11.22 ± 0.36	11.38 ± 0.33	11.45 ± 0.34	1.43 (0.25)	1.72 (0.19)	2.40 (0.096)
Cingulum_ hippocampus_R.	12.07 ± 0.93	11.53 ± 0.59	11.84 ± 0.80	11.63 ± 0.62	11.63 ± 0.51	11.53 ± 0.49	2.00 (0.14)	0.32 (0.57)	2.67 (0.07)
Cingulum_ hippocampus_l.	12.38 ± 1.23	11.65 ± 0.51	12.02 ± 0.70	11.63 ± 0.56	11.78 ± 0.77	11.92 ± 0.56	2.66 (0.075)	0.12 (0.73)	5.98 (0.003)*
DR ($\times 10^{-4}$)									
Cingulum_R.	6.23 ± 0.41	6.14 ± 0.41	6.35 ± 0.46	6.11 ± 0.43	6.19 ± 0.36	6.14 ± 0.31	0.59 (0.56)	1.12 (0.29)	0.76 (0.47)
Cingulum_l.	6.27 ± 0.36	6.27 ± 0.36	6.39 ± 0.26	6.18 ± 0.46	6.21 ± 0.36	6.24 ± 0.37	0.69 (0.50)	2.20 (0.14)	0.093 (0.91)
Cingulum_ hippocampus_R.	7.75 ± 0.91	7.56 ± 1.19	7.53 ± 0.80	7.19 ± 0.71	7.06 ± 0.65	7.10 ± 0.56	0.31 (0.74)	8.91 (0.003)*	0.068 (0.93)
Cingulum_ hippocampus_l.	8.18 ± 1.18	7.75 ± 1.16	7.73 ± 0.86	7.27 ± 0.67	7.34 ± 0.82	7.52 ± 0.70	0.30 (0.74)	8.76 (0.004)*	1.28 (0.28)

Abbreviations: DA, axial diffusivity; DR, radial diffusivity; FA, fractional anisotropy; MD, mean diffusivity. Note: data expressed as mean ± SD. *P*-values are all raw *P*-values. *$P < 0.05$ significant after Bonferroni correction.

[a]Multivariate analysis of variance (MANOVA) was used to determine the main effect of the genotype and sex on the DTI indices, as well as the interaction between them (age, education, and APOEε4 as covariates).

could probe white matter microstructure *in vivo* and indicate the underlying biophysical properties. A recent DTI study of WM microstructure in healthy Caucasian subjects reported that the *SORL1* risk variants (rs689021) predicted lower FA in an age-independent manner in the cingulum bundle and several other fronto-temporal WM tracts. However, the interaction between sex and the *SORL1* variants was not explored (Felsky *et al*, 2013). By performing analysis on a pre-defined ROI that was specially designed for detecting the whole cingulum bundle, we found the interaction between sex and the *SORL1* rs2070045 polymorphism on the MD and DA value of the left cingulum hippocampal part. Increased MD was reported to be caused by decrease in membrane density and DA could reflect the axonal injury (Douaud *et al*, 2011; Wheeler-Kingshott and Cercignani, 2009). Specifically, G/G men showed reduced integrity of the left cingulum hippocampal part compared with G/T men, and T/T women exhibited reduced integrity compared with G/G women, which was consistent with our behavioral observations. Although not significant, there is a similar tendency in the sex differences in the association between the *SORL1* variants and the integrity of the right cingulum hippocampal part. We guess that sex could regulate the relationship between the rs2070045 polymorphism and WM integrity through modulating the allelic differences in the expression of SorLA in the cingulate cortex, which lead to subtle changes in the Aβ concentration.

Some previous DTI evidence suggests that the cingulum bundle could carry anterior-posterior connections that are important for executive function (O'Sullivan *et al*, 2005; Kantarci *et al*, 2011). However, the correlation analyses in these studies have not been separated by sex and by *SORL1* genotypes. In this study, the MD and DA of the left cingulum hippocampal part were found to be associated with TMT performance only in males who were homozygous for the T allele. As shown in Figure 2, G/T men showed the best executive performance and cingulum integrity compared with the other two genotype groups, whose plots fell in the third quadrant. The opposite situation was observed in G/G men. The scatter plots of T/T men were more widely distributed than the G/T and G/G groups. Further studies with larger sample sizes might be necessary to determine the relationship between the cingulum integrity and executive performance. Moreover, we did not find any significant association in the females. Our findings indicate that sex and the rs2070045 polymorphism could affect the relationship between the cingulum integrity and executive function.

There is conflicting evidence regarding the relationships between the *SORL1* gene and cognitive functioning

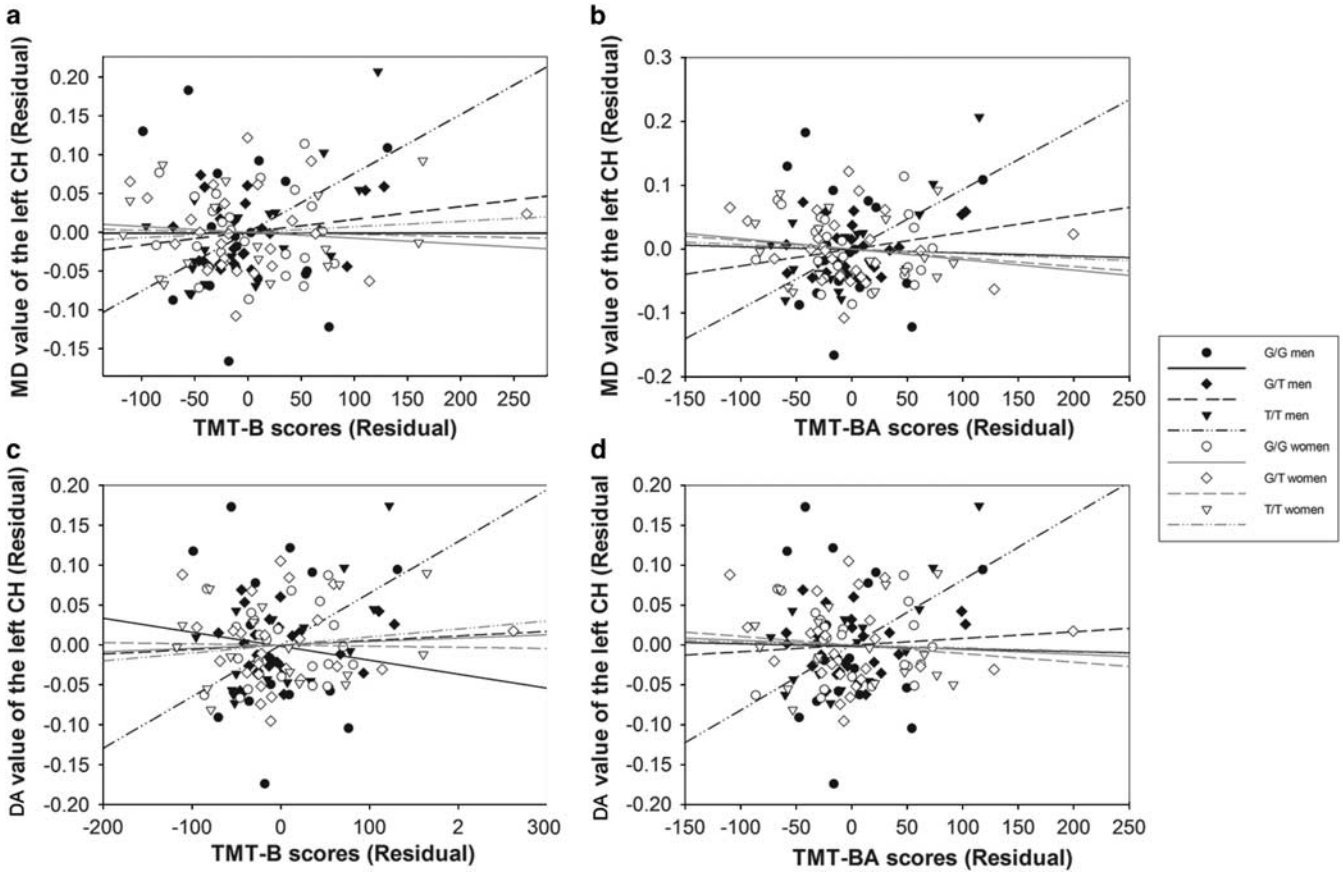

Figure 2 Correlations between the white matter and neuropsychological tests in the six genotype and sex groups, separately. (a) Plots showing the significant correlation between the MD of the left cingulum hippocampal part and Trail Making Test B (TMT-B) scores in T/T men. (b) Plots showing the significant correlation between the mean diffusivity of the left cingulum hippocampal part and TMT-BA scores in T/T men. (c) Plots showing the significant correlation between the DA of the left cingulum hippocampal part and TMT-B scores in T/T men. (d) Plots showing the significant correlation between the DA of the left cingulum hippocampal part and TMT-BA scores in T/T men. No significant correlation was found in the other group.

(Seshadri *et al*, 2007; Houlihan *et al*, 2009; Liu *et al*, 2009; Shulman *et al*, 2010), and the gene variants confer a small-to-modest risk of AD (Lee *et al*, 2008; Reitz *et al*, 2011). In our study, the main effect of the rs2070045 polymorphism was not significant on any of the cognitive measures except on Trail Making Test-A. Nevertheless, the significant interaction of sex with gene was observed on executive function. Thus, the sex differences should be considered when examining the effects of *SORL1* on cognitive function as well as AD.

Several limitations of this study should be acknowledged. First, the male and female subjects were not matched in terms of age and education, although demographic variables have been controlled in all of the analyses. Second, similar to many other genetic association studies, we did not measure SorLA expression in the brain. Such evidence is necessary to clarify the exact mechanisms of how sex moderates the association between the *SORL1* variants and cognition, as well as WM integrity. Finally, in the present study, only the rs2070045 SNP was singled out. The reason is that sex exerts the most evident moderation on this SNP (Reynolds *et al*, 2013). Future studies that examine other *SORL1* SNPs and haplotypes could provide comprehensive descriptions of the interaction between sex and the *SORL1* gene.

In summary, we observed sex-moderated association of the *SORL1* rs2070045 polymorphism and executive function. The relationship of the SNP to the integrity of the left cingulum hippocampal part was also found to be dependent on sex. These results hint that sex moderation of the *SORL1* effects on executive function could be related to its moderation on the *SORL1* effects on the cingulum bundle. Our findings underscore the importance of integrating sex and genetic susceptibility when examining candidate genes for cognitive abilities and AD. A prospective study with a larger sample size and a longitudinal design would permit further clarification on the extent to which the effects of *SORL1* variants on cognitive aging are moderated by sex.

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Author Contributions

Z.J.Z. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Z.J.Z. conceived the original idea for the study and supervised in the conception. Y.L., H.L., C.L.L., X.L., and J.Y.Z. recruited the study population and conducted the neuropsychological tests. Y.L., H.L., and C.L.L. analyzed the data. Y.L., H.L., and C.L.L. drafted the manuscript. N.S., K.W.C. and L.P.H revised the manuscript. All authors read and approved the final manuscript.

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