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The *SEMA5A* gene is associated with hippocampal volume, and their interaction is associated with performance on Raven's Progressive Matrices

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ABSTRACT

The Allen Brain Atlas shows that the semaphorin 5A (*SEMA5A*) gene, which encodes an important protein for neurogenesis and neuronal apoptosis, is predominantly expressed in the human hippocampus. Structural and functional neuroimaging studies have further shown that the hippocampus plays an important role in the performance on Raven's Progressive Matrices (RPM), a measure of reasoning ability and general fluid intelligence. Thus far, however, no study has examined the relationships between the *SEMA5A* gene polymorphism, hippocampal volume, and RPM performance. The current study collected both structural MRI, genetic, and behavioral data in 329 healthy Chinese adults, and examined associations between *SEMA5A* variants, hippocampal volume, and performance on RAPM (the advanced form of RPM). After controlling for intracranial volume (ICV), sex, and age, *SEMA5A* genetic polymorphism at the SNP rs42352 had the strongest association with hippocampal volume ($p = 0.00000552$ and 0.000103 for right and left hippocampal volumes, respectively), with TT homozygotes having higher hippocampal volume than the other genotypes. Furthermore, there was a high correlation between right hippocampal volume and RAPM performance ($r = 0.42$, $p = 0.0000509$) for *SEMA5A* rs42352 TT homozygotes. This study provides the first evidence for the involvement of the *SEMA5A* gene in hippocampal structure and their interaction on RAPM performance. Future studies of the hippocampus–RPM associations should consider genetic factors as potential moderators.

Introduction

Although the hippocampus has traditionally been linked to learning and memory (Squire, 1992), its roles in other mental functions have been documented by recent studies. For example, hippocampal malfunctions have been implicated in several mental disorders (Carlesimo et al., 2012; Groen et al., 2010; Thompson et al., 2011). Of particular relevance to the current study, the hippocampus has recently been found to play an essential role in reasoning ability (Goel et al., 2004; Wendelken and Bunge, 2010; Zeithamova et al., 2012).

One of the widely used measures of nonverbal reasoning ability is Raven's Progressive Matrices (RPM), which depends on visual-spatial processing and is sometimes used to assess general fluid intelligence (Raven et al., 1998). The mental processes measured by RPM include encoding, storing, recalling, and combining detailed information from original items in novel ways to infer unobserved relationships between items. These processes are subserved by the hippocampus and frontal lobe (Zeithamova et al., 2012), the former for relational encoding and the latter for relational integration (Wendelken and Bunge, 2010). For example, an earlier PET (positron emission tomography) study (Esposito et al., 1999) showed that the right hippocampal region was activated when performing RPM.

Subsequent studies attempted to link hippocampal volume to RPM performance and produced somewhat mixed findings. An earlier study (MacLullich et al., 2002) reported a positive correlation between RPM performance and hippocampal volume in healthy elderly men,

Abbreviations: SEMA5A, semaphorin 5A; RPM, Raven's Progressive Matrices; RAPM, Raven's Advanced Progressive Matrices; SNP, single nucleotide polymorphism; ICV, intracranial volume; PET, positron emission tomography; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; LSD, least significant difference; RHPV, right hippocampal volume; LHPV, left hippocampal volume; COMT, catechol-O-methyltransferase; NTSR1, neurotensin receptor 1.

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but this association was not signficant after controlling for intracranial volume (ICV). A recent study found that RPM performance was positively correlated with hippocampal volume after controlling for ICV in healthy elders but not in young adults (Reuben et al., 2011). Most recently, a study of healthy young adult musicians (Oechslin et al., 2013) found a positive correlation between RPM performance and hippocampal volume. One possible cause for such discrepant results is that the association between hippocampal volume and RPM performance might be modulated by age, sex, ICV, and genetic factors (especially genes related to the hippocampus). Genetic factors are important to consider because twin studies showed moderate to high (40%–74%) heritability of hippocampal volume (Kremen et al., 2010; Peper et al., 2007; Stein et al., 2012).

The Allen Brain Atlas shows that the semaphorin 5A (*SEMA5A*) gene, which encodes an important protein for neurogenesis and neuronal apoptosis, is predominantly expressed in the human hippocampus. *SEMA5A* belongs to the semaphorin gene family and encodes membrane proteins containing a semaphorin domain and several thrombospondin type-1 repeats. The semaphorin protein contributes to axonal guidance and neurogenesis, which has been demonstrated in the hippocampus (Zhao et al., 2008). Chivatakarn (2008) reported a significant role of SEMA5A in cortical development and axon in vivo based on studies of SEMA5A conditional mutant mice. Specifically, robust expression of *SEMA5A* was seen in the hippocampus postnatally and continued throughout adulthood, while mice lacking *SEMA5A* had abnormal corticogenesis owing to impaired radial migration (Chivatakarn, 2008). The *SEMA5A* gene has been found to have higher expression in the human hippocampus than in the other human brain structures (see Supplemental Materials Fig. S1, based on data from the Allen Institute Brain Atlas, http://human.brain-map.org/). Among the whole brain structures in human adults, the highest Z score of *SEMA5A* expression (normalized within each subject across brain structures) appeared in the right hippocampus. Similarly, for the human prenatal brain and the rhesus macaque brain, the highest Z score of *SEMA5A* gene expression appeared in the hippocampal granular layer of caudal dentate gyrus (BrainSpan, http://www.brainspan.org/; NIH Blueprint Non-Human Primate Atlas, http://www.blueprintnhpatlas.org/). Therefore, it is plausible that human hippocampal volume would be associated with the *SEMA5A* gene.

Thus far, however, no study has examined relationships between the *SEMA5A* gene polymorphisms, hippocampal volume, and RPM performance. Although there is no direct evidence, their potential relationships have been suggested by several previous studies of clinical samples. For example, several studies reported and replicated significant associations between *SEMA5A* genetic variants and Parkinson's disease (Clarimon et al., 2006; Ding et al., 2008; Lin et al., 2009; Maraganore et al., 2005); and Parkinson's disease patients were shown to have hippocampal atrophy (Tam et al., 2005) and poor performance on RPM but relatively preserved performance on many other problem-solving tasks (Cronin-Golomb and Braun, 1997). Based on a meta-analysis, the *SEMA5A* gene was associated with alcohol dependence (Wang et al., 2011) and alcoholics were reported to have smaller hippocampal volume (Agartz et al., 1999) and poorer RPM performance than normal controls although both groups had similar verbal intelligence (Jones, 1971). Moreover, the expression of the *SEMA5A* gene is reduced in the hippocampus in schizophrenia patients (Altar et al., 2005), who have been found to have reduced hippocampal volume (Harrison, 2004) and to perform poorly on RPM (Caspi et al., 2003). The *SEMA5A* gene is also one of the genes that are lost in the Cri-du-chat gene deletion syndrome, associated with severe mental retardation and small head size (Simmons et al., 1998). The bifunctional protein encoded by *SEMA5A* acts both as attracting and repulsing axon guidance cues. Researchers have demonstrated significant associations between the *SEMA5A* gene and autism, and found that the expression of *SEMA5A* was reduced in the brains of patients with autism (Cheng et al., 2013; Melin et al., 2006; Weiss et al., 2009). Interestingly, patients with autism have been found to show an abnormal enlargement of the hippocampus (Groen et al., 2010), and to do particularly well on RPM (Bolte et al., 2009; Dawson et al., 2007).

In sum, evidence from separate studies has suggested potential relationships among the *SEMA5A* gene, hippocampal volume, and RPM, but no single study has included all three variables. Furthermore, previous studies suggested that it is important to consider the gene–brain interaction on behavior (Alia-Klein et al., 2009; Green et al., 2008). Therefore, in this study, we first performed an association study of hippocampal volume and single nucleotide polymorphisms (SNP) within the *SEMA5A* gene in a sample of healthy Han Chinese college students. Based on the association results, we then selected the *SEMA5A* genetic polymorphism most significantly associated with hippocampal volume and examined potential effects of this genetic variant and hippocampal volume as well as their interaction on Raven's Advanced Progressive Matrices (RAPM, i.e., the advanced form of RPM) performance.

Materials and methods

Participants

Participants were 329 healthy Han Chinese undergraduate students from Beijing Normal University in China (mean age = 20.42 years, *SD* = .88, range 18–22 years old; 59% female, 98.5% right handed), who had valid brain imaging, genotype, and behavioral data. All participants reported having normal or corrected-to-normal vision and no history of psychiatric diseases, head injuries, or stroke/seizure. Individuals in this sample were all unrelated to one another. This study was approved by the Institutional Review Board (IRB) of Beijing Normal University, China. Written consent form was obtained from each participant after a full explanation of the study procedure. Handedness did not influence any result in the current study, therefore we included all subjects in the analysis.

MRI data collection and analysis

Three-dimensional MRI scans were performed with a 3.0 T Siemens Magnetom Trio scanner equipped with a standard head coil at Beijing Normal University Brain Imaging Center. Structural MRI data were acquired with the T1-weighted MPRAGE pulse sequence (TE = 3.75 ms, TR = 2,530 ms, flip angle = 7°; FOV = 256 mm × 256 mm, voxel size = 1 × 1 × 1.33 mm^3, number of partitions = 128). MRI data were analyzed automatically with atlas-based FreeSurfer segmentation software (http://surfer.nmr.mgh.harvard.edu, version 5.0.0) to extract volumetric measures of regions of interest (Fischl et al., 2002). Volumes of bilateral hippocampi were generated according to the standard FreeSurfer segmentation and parcellation procedures, relying upon variations in voxel signal intensities, probabilistic atlas location, and local spatial relationships between the structures (Fischl et al., 2002). Intracranial volume (ICV), including brain tissues and other biological materials such as meninges and cerebrospinal fluid, was taken from the standard output of FreeSurfer analysis as well. Quality control of scan images and segmentation was assured by visual inspection of the whole cortex of each subject. Any inaccuracies in Talairach-transformation, skull stripping, and segmentation were manually corrected, and re-inspected.

Genotyping and quality control

A 4 ml venous blood sample was collected from each subject. Genomic DNA was extracted according to standard methods within two weeks after the blood sample was collected. All samples were genotyped using the standard Affymetrix genotyping protocol (Affymetrix, Inc). As described in Supplemental Materials Table S1, 169 SNPs within the *SEMA5A* gene on chromosome 5 were selected, and the allele

frequencies in our sample were very similar to those of the Chinese in the HapMap dataset. These SNPs covered most of the linkage disequilibrium (LD) blocks in this gene, as defined for the samples of Chinese included in the HapMap Project (http://www.hapmap.org [phase 3], also see Supplemental Materials Fig. S2–S5 for the LD plots of the *SEMA5A* gene in different populations). The *SEMA5A* gene contains 23 exons and 22 introns. All SNPs met the following criteria: minor allele frequency (MAF) > 0.1, Hardy–Weinberg equilibrium (HWE) $p > 0.0001$, and genotype call rate > 0.95.

Behavioral assessment

Raven's Advanced Progressive Matrices is a multiple-choice test of abstract reasoning, which involves visual-spatial processing (Raven et al., 1998). Participants were given 30 min to complete as many items as possible. In each test item, participants were asked to select from eight alternatives the missing segment that would complete a larger pattern. The whole test had 48 items, including 12 easy items and 36 difficult items. Each item was presented in black ink against a white background. Items were arranged in the order of difficulty from the easiest to the most difficult. This test is appropriate for adults and adolescents of above-average intelligence (Raven et al., 1998). It has been used widely in China with good reliability and validity (the split-half reliability was .86) (Zhai, 1999). Cronbach's alpha in this study was .75. The total score on this test was used as the measure of visual-spatial reasoning ability in the current study.

Statistical analysis

Quantitative trait genetic association analysis was carried out by using Plink v1.07 (Purcell et al., 2007), including allelic association tests between individual SNPs and hippocampal volume, and associations between haplotype and hippocampal volume. Linear regression models were used to detect the associations between each SNP and hippocampal volume in each hemisphere. Additive genetic models (i.e., additive effects of allele dosage) were used, with sex and age included as covariates. In order to test genotype group differences in hippocampal volumes, MANCOVA and the Fisher's least significant difference (LSD) post hoc tests were performed with sex and age as covariates in SPSS 16.0. To help disentangle head size effects from those specific to hippocampal volume, associations were assessed with and without adding ICV as one of the covariates. Next, we selected the SNP with strongest association with hippocampal volume for further analysis.

In order to test the effects of hippocampal volume and the genotype of this SNP as well as their interaction on RAPM performance, linear regression models were performed in SPSS 16.0. The score of RAPM performance was the dependent variable, and three independent variables were the composite Z scores of hippocampal volumes (left and right hippocampal volumes separately), the genotype of this SNP (i.e., using this genotype as a continuous [dosage] variable [centered]), and their interaction (the product of standardized (Z) scores of hippocampal volume and the genotype of this SNP). Z scores were used to center the predictors so as to control for multicollinearity (Aiken et al., 1991). Age, sex, and ICV were also included as covariates of no-interest. Potential interactions were further examined by obtaining partial correlations between RAPM performance and hippocampal volume for each genotype of this SNP (i.e., major allele homozygotes, heterozygotes, and minor allele homozygotes), again with age, sex, and ICV as covariates.

Results

The means and standard deviations of right and left hippocampal volumes, intracranial volume (ICV), and performance on RAPM, as well as correlations among these variables are shown in Table 1 (also see Supplemental Materials Fig. S6 for detailed distributions of these variables). Females had significantly smaller bilateral hippocampal volumes than did males ($t = -5.88$ and -5.05, $p < .001$, for right and left hippocampal volumes, respectively). Age had small but statistically significant positive correlations with bilateral hippocampal volumes ($r = .15$ and .11, $p < .05$, for right and left hippocampal volumes, respectively). Therefore, sex and age were used as covariates in all subsequent analyses.

As presented in Fig. 1 and Supplemental Materials Table S2, genetic association analysis using Plink showed that an SNP (rs42352) within the *SEMA5A* gene had the strongest association with hippocampal volumes ($\beta = 113.90$, $t = 4.72$, $p = 3.59 \times 10^{-6}$ for right hippocampal volume, $\beta = 101.80$, $t = 4.09$, $p = 5.53 \times 10^{-5}$ for left hippocampal volume), with sex and age as covariates. T allele was associated with larger right and left hippocampal volumes. In order to test genotype group differences in hippocampal volumes, MANCOVA and the Fisher's least significant difference (LSD) post hoc tests were also performed with sex and age as covariates. Results showed that *SEMA5A* rs42352 TT homozygotes had larger bilateral hippocampal volumes than AA homozygotes ($p = 3.97 \times 10^{-6}$ and 6.09×10^{-5} for right and left hippocampal volumes, respectively) and TA heterozygotes ($p = 7.33 \times 10^{-4}$ and 1.03×10^{-3} for right and left hippocampal volumes, respectively), but there were no significant differences between AA homozygotes and TA heterozygotes in bilateral hippocampal volumes ($p = 0.57$ and 0.20 for right and left hippocampal volumes, respectively). Haplotype analysis of directly genotyped variants near rs42352 in the current sample confirmed that the association was present across SNPs in the haplotype (see Supplemental Note 1 and Fig. S7 for details). After correcting for multiple testing by max(T) permutation approach in Plink (10000 permutation) for individual SNPs within the *SEMA5A* gene, SNP rs42352 remained significantly associated with hippocampal volumes (corrected empirical *p*-values [max(T)/familywise] = 0.0003 and 0.005 for right and left hippocampal volumes, respectively). As shown in Table S3, the associations between rs42352 and bilateral hippocampal volumes remained highly significant after adding ICV as one of the covariates ($\beta = 103.40$ and 91.64, $t = 4.62$ and 3.93, $p = 5.52 \times 10^{-6}$ and 1.03×10^{-4} for right and left hippocampal volumes, respectively; $p = 0.0007$ and 0.01, respectively, after correction for multiple comparisons). We selected the SNP rs42352 for subsequent analysis, because it had the strongest association with bilateral hippocampal volumes. However, complete results for the other SNPs within the *SEMA5A* gene are shown in the Supplemental Materials Table S2 and Table S3.

To explore the joint effect of *SEMA5A* rs42352 genotype and hippocampal structure on the RAPM performance, linear regression analyses were conducted. RAPM performance was the dependent variable, with the *SEMA5A* rs42352 genotype, lateral hippocampal volume, and their interaction as predictors, and sex, age, and ICV as control variables. Results showed a significant interaction between the *SEMA5A* rs42352 genotype and right hippocampal volume ($t = 2.42$, $p = 0.016$). The interaction between rs42352 genotype and left hippocampal volume was not significant ($t = 1.44$, $p = 0.152$).

We further examined the associations between right hippocampal volume and RAPM performance for the three *SEMA5A* rs42352 genotypes separately (90 TT homozygotes, 155 TA heterozygotes, and 84 AA homozygotes). Sex, age and ICV were again used as covariates. Only for the TT homozygotes, a significant positive correlation was found between right hippocampal volume and RAPM performance ($r = .42$, $p = 5.09 \times 10^{-5}$) (see Table 2 and Fig. 2), whereas the correlation between left hippocampal volume and RAPM performance was only marginal ($r = .20$, $p = 0.057$). There was no significant correlation between bilateral hippocampal volumes and RAPM performance for the other two genotype groups ($p > 0.05$, actual *p* values and other details can be seen in Table 2).

Table 1
Descriptive statistics and correlations among phenotypic variables.

	Mean	SD	Right hippocampal volume	Left hippocampal volume	ICV
Right hippocampal volume	4131.00	346.43			
Left hippocampal volume	4002.37	348.38	0.81***		
ICV	1464213.33	230917.82	0.48***	0.44***	
RAPM performance	37.01	4.35	0.19***	0.14**	0.04

Note: ** $p < .01$, *** $p < .001$, the unit of brain structural volume was mm^3. RAPM: Raven's Advanced Progressive Matrices; ICV: Intracranial volume.

Additional analyses were conducted to examine potential confounding variables such as intelligence and working memory (because of the hippocampus's role in memory), mental health-related factors (e.g., depression, anxiety, alcohol and smoking), and other genes related to hippocampal volume in the current dataset (*COMT* and *NTSR1*). Results reported above were not changed when these variables were considered. Detailed results can be seen in the Supplemental Materials (Supplemental Note 2).

Discussion

In the present study, we investigated whether the *SEMA5A* gene was associated with hippocampal volume and whether this gene and hippocampal volume were associated with visual-spatial reasoning as measured by RAPM in normal Chinese adults. We identified an SNP rs42352 within the *SEMA5A* gene that was significantly associated with hippocampal volume and found a significant interaction between *SEMA5A* genotype (rs42352) and right hippocampal volume on RAPM performance. Further analysis revealed that *SEMA5A* (rs42352) TT homozygotes had higher hippocampal volume, and their right hippocampal volume was significantly and positively correlated with RAPM performance. These effects were independent of subjects' sex, age, ICV, and mental health-related covariates (see Supplementary Online materials, Note 2), as well as other genes (i.e., *COMT* and *NTSR1*) and cognitive functions (i.e., intelligence and working memory) related to hippocampal volumes (Li et al., 2012; Wang et al., 2013). These results demonstrated the critical role of the *SEMA5A* gene in the hippocampal structure and its moderating effect on the relation between the hippocampus (especially the right hippocampus) and RAPM performance.

It is worth noting that although the effect of the *SEMA5A* gene on hippocampal volumes was significant for both hemispheres, the gene–brain interaction effect on RAPM was significant only for the right hemisphere. This tendency of laterality (it was not statistically significant when laterality index was used in a separate analysis, see Supplemental Note 2) is intriguingly consistent with the relevant literature that showed a similar tendency, which was also not tested with a strict laterality test. First, the Allen Institute Brain Atlas showed that the

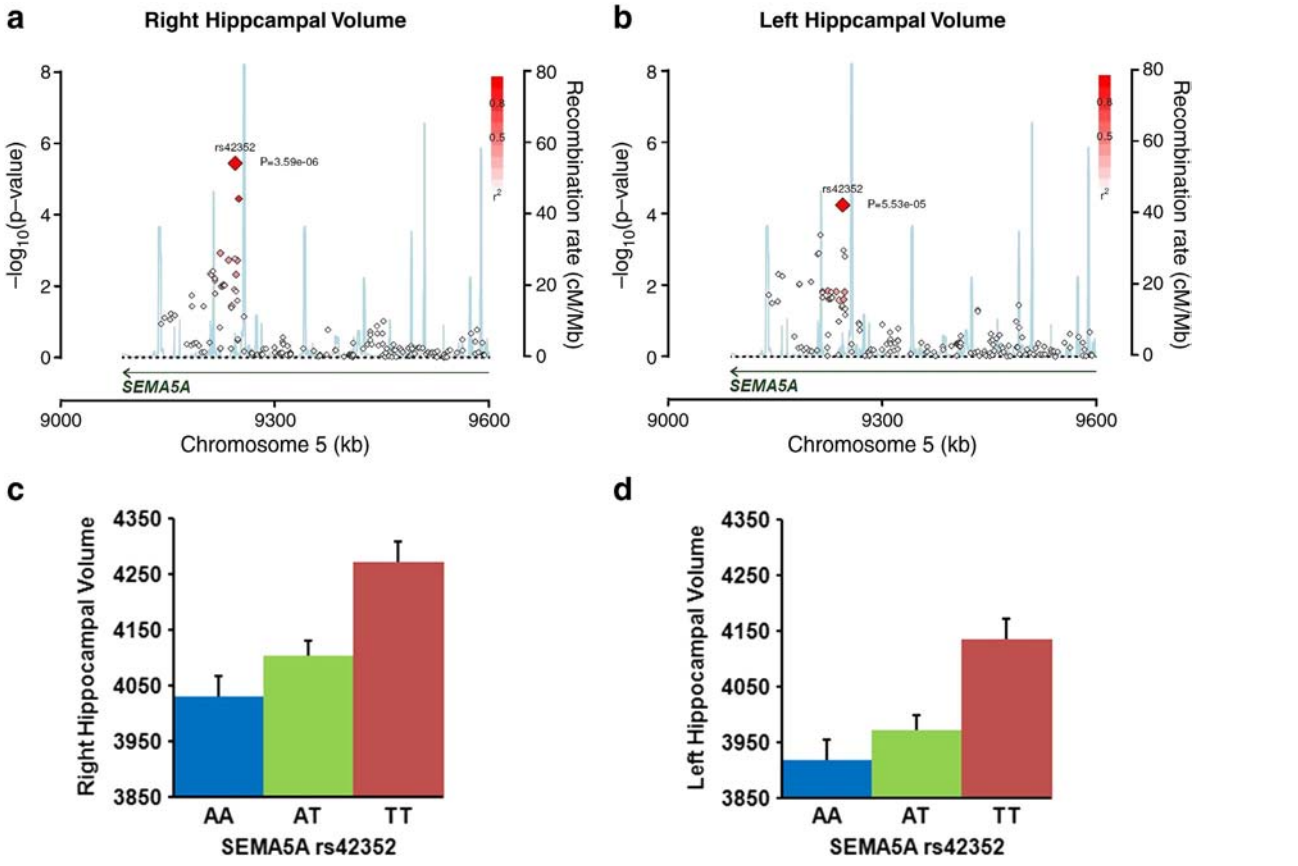

Fig. 1. Associations between 169 SNPs within the *SEMA5A* gene and bilateral hippocampal volumes with sex and age as covariates (a and b at the top) and diagrams of allele-specific hippocampal volumes (means and standard errors) in rs42352 TT homozygotes, AT heterozygotes, and AA homozygotes within the *SEMA5A* gene (c and d at the bottom). For a and b, all SNPs are plotted with their *p* values against their genomic position, with the most significant SNP in the region indicated as a diamond and other SNPs shaded according to their pair-wised correlation (r^2) with the signal SNP. The light blue line represents the estimated recombination rates. Gene annotations are shown as dark green lines. The regional plots were generated using SNAP program (Johnson et al., 2008).

Table 2
Hippocampal volumes and their correlations with RAPM performance for three genotypes of SNP rs42352 within the *SEMA5A* gene.

SEMA5A gene rs42352 genotype	N	Right hippocampal volume (RHPV)		Left hippocampal volume (LHPV)		Correlations between hippocampal volumes and RAPM performance controlling for sex, age, and ICV	
		Mean	SD	Mean	SD	RHPV-RAPM	LHPV-RAPM
TT	90	4272.8	343.9	4135.1	343.7	$r = .42$ ($p = 5.09 \times 10^{-5}$)	$r = .20$ ($p = 0.057$)
TA	155	4103.8	333.1	3971.0	341.1	$r = .12$ ($p = 0.138$)	$r = .08$ ($p = 0.310$)
AA	84	4029.3	332.4	3918.1	328.9	$r = .09$ ($p = 0.418$)	$r = .04$ ($p = 0.702$)

SEMA5A gene had the highest expression in the right hippocampus of human adults. Second, a PET activation study showed that the right hippocampus region was activated by RPM especially for young healthy subjects (Esposito et al., 1999). Future research should use a larger sample size and/or more sensitive tools or measures to study the laterality of neural basis of visual-spatial reasoning.

Our finding of a positive correlation between hippocampal volume and RAPM performance was consistent with previous studies (Oechslin et al., 2013; Reuben et al., 2011). Given that the *SEMA5A* rs42352 TT homozygotes had larger hippocampal volume than TA heterozygotes and AA homozygotes, we hence found that the correlation between hippocampal volume and RAPM performance was higher for the subjects with larger hippocampal volume (i.e., the *SEMA5A* rs42352 TT homozygotes), which would account for the significant interaction between genotype and hippocampal volume on RAPM performance in the current study.

Considering our results and those of previous studies, we speculate that the links between the *SEMA5A* gene, hippocampal volume, and RAPM performance may represent a form of adaptive specialization (Roth and Pravosudov, 2009). Researchers suggested that natural selection has resulted in an increase in the volume of the hippocampus of animals that rely on spatial abilities to solve ecologically important problems (Sherry et al., 1992). For example, birds from harsher climates were found to have larger hippocampal volume than those from milder climates, perhaps because in harsh climates there is an increased need to remember where food is hidden in order to allow animals to survive through the winter (Pravosudov and Clayton, 2002). A similar process might have occurred in human evolution. In other words, natural selection may have produced a specialized endophenotype (i.e., larger hippocampal volume) for a specific cognitive task (i.e., visual-spatial reasoning task) by selecting a particular gene variant. This speculation of course needs to be further investigated in the future studies.

Our results also have potential clinical implications. As described in the introduction, studies have shown that some mental disorders such as autism, Parkinson's disease, and alcohol dependence are associated with abnormal hippocampal volume and abnormal performance on RPM. Therefore, any genes that are associated with hippocampal volume and RPM performance may serve as candidate genes for these mental disorders. As presented in Fig. S8, previous clinical studies have indeed reported that several SNPs of the *SEMA5A* gene were associated with different kinds of mental diseases and substance abuse, such as rs7702187 with Parkinson's disease (Maraganore et al., 2005), rs930076 with alcohol dependence (Wang et al., 2011), rs10513025 with autism (Weiss et al., 2009). Thus far, however, no study has linked *SEMA5A* rs42352 genotype to autism, Parkinson's disease, or alcohol dependence. Future study should explore potential associations between *SEMA5A* rs42352 genotype and hippocampal volume and their interaction effect on RPM performance in clinical samples.

Several limitations of this study and directions for future research need to be noted. First, the biochemical and physiological functions of the identified polymorphism (rs42352 within the *SEMA5A* gene) were not directly explored in the present study. SNP rs42352 is located on the tenth intron of the *SEMA5A* gene. This SNP is very close to the 11th exon (about 1 kb away) of the *SEMA5A* gene. It serves as a marker for a number of polymorphisms in the haplotype block. Any of these SNPs in the haplotype block may have functional consequences on the amount of SEMA5A protein synthesized. Also, it is possible that intron variants can also regulate mRNA expression and slicing (Zhang et al., 2007). Although previous studies suggested that several *SEMA5A* genetic variants were associated with many mental diseases, there was no strong LD between these *SEMA5A* SNPs related to different kinds of phenotypes (supplemental Fig. S8). Therefore, additional molecular functional studies are needed to investigate the detailed biochemical mechanisms of this association.

Second, it should be noted that the current study was based on a healthy Han Chinese sample. Previous studies have shown racial specificity (e.g., Taiwanese versus Finnish population) in the association between the *SEMA5A* gene and phenotype. The SNPs within the *SEMA5A* gene have different minor allele frequencies (MAF) and

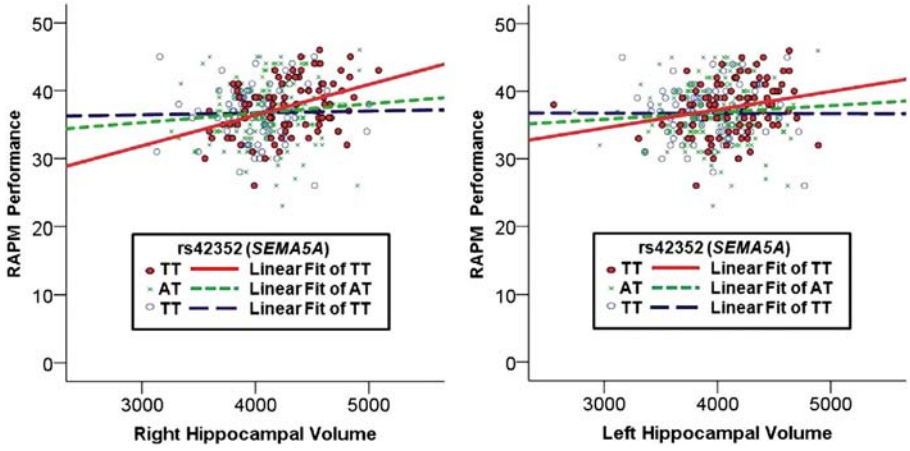

Fig. 2. Scatter plots and regression lines showing the relationship between hippocampal volume and RAPM performance (raw scores) in rs42352 TT homozygotes (filled circles, solid regression line), AT heterozygotes (cross, short-dashed regression line), AA homozygotes (open circles, long-dashed regression line) within the *SEMA5A* gene.

constructed different LD blocks in different ethnic populations based on the HapMap Data (www.hapmap.org, see Table S1 and Figs. S1 to 4). Previous genome-wide association meta-analytic studies of European ancestry samples did not find the association between the *SEMA5A* rs42352 genotype and hippocampal volume ($p > .05$, based on the data from http://enigma.loni.ucla.edu/enigma-vis/, (Novak et al., 2012)). One possible explanation of these inconsistent results is the population-specific genetic effect on hippocampal volume (Lohmueller et al., 2003). Researchers suggested that *SEMA5A* genetic markers had differential effects across populations (Clarimon et al., 2006). For example, Clarimon et al. (2006) found the effect of *SEMA5A* genetic markers in the Taiwanese population, but they did not find such association in the Finnish population. It might explain why previous studies using European ancestry samples did not find the association between *SEMA5A* genetic variants and hippocampal volume; while the current study identified this association using a Han Chinese sample. In order to obtain confirmatory evidence, this association should be explored in future studies using different ethnic samples.

Finally, because this was a college sample, we used only self-report data to screen for neurological and psychiatric disorders. While this is a reasonable approach given the stringent requirements of college admission in China, future research should employ an objective systematic tool to assess such disorders.

Conclusions

In conclusion, this study provided the first evidence of an association between the *SEMA5A* gene and hippocampal volume, and reported a significant interaction between *SEMA5A* genotype (rs42352) and right hippocampal volume on RAPM performance in healthy Chinese individuals. These findings were independent of several potential confounding factors (i.e., sex, age, and ICV), overlapping cognitive functions (i.e., intelligence and working memory), and other relevant genes (*NTSR1* and *COMT*). *SEMA5A* (rs42352) TT homozygotes had higher hippocampal volume, and their right hippocampal volume was significantly positively correlated with RAPM performance. Our findings suggested future studies of the hippocampus–RPM association should consider genetic modulation.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.11.035.

References

Agartz, I., Momenan, R., Rawlings, R.R., Kerich, M.J., Hommer, D.W., 1999. Hippocampal volume in patients with alcohol dependence. Arch. Gen. Psychiatry 56, 356–363.
Aiken, L.S., West, S.G., Reno, R.R., 1991. Multiple Regression: Testing and Interpreting Interactions. Sage, Newbury Park, CA.
Alia-Klein, N., Goldstein, R.Z., Tomasi, D., Woicik, P.A., Moeller, S.J., Williams, B., et al., 2009. Neural mechanisms of anger regulation as a function of genetic risk for violence. Emotion 9, 385–396.
Altar, C.A., Jurata, L.W., Charles, V., Lemire, A., Liu, P., Bukhman, Y., et al., 2005. Deficient hippocampal neuron expression of proteasome, ubiquitin, and mitochondrial genes in multiple schizophrenia cohorts. Biol. Psychiatry 58, 85–96.
Bolte, S., Dziobek, I., Poustka, F., 2009. Brief report: the level and nature of autistic intelligence revisited. J. Autism Dev. Disord. 39, 678–682.
Carlesimo, G.A., Piras, F., Assogna, F., Pontieri, F.E., Caltagirone, C., Spalletta, G., 2012. Hippocampal abnormalities and memory deficits in Parkinson disease: a multimodal imaging study. Neurology 78, 1939–1945.
Caspi, A., Reichenberg, A., Weiser, M., Rabinowitz, J., Kaplan, Z.e, Knobler, H., et al., 2003. Cognitive performance in schizophrenia patients assessed before and following the first psychotic episode. Schizophr. Res. 65, 87–94.
Cheng, Y., Quinn, J.F., Weiss, L.A., 2013. An eQTL mapping approach reveals that rare variants in the SEMA5A regulatory network impact autism risk. Hum. Mol. Genet. 22, 2960–2972.
Chivatakarn, O., 2008. Semaphorin 5A (Sema5A) functions in neocortical development In vivo. (Doctoral dissertation) University of Rochester. ProQuest, UMI Dissertations Publishing 3360879.
Clarimon, J., Scholz, S., Fung, H.C., Hardy, J., Eerola, J., Hellstrom, O., et al., 2006. Conflicting results regarding the semaphorin gene (SEMA5A) and the risk for Parkinson disease. Am. J. Hum. Genet. 78, 1082–1084 (author reply 1092-1084).
Cronin-Golomb, A., Braun, A.E., 1997. Visuospatial dysfunction and problem solving in Parkinson's disease. Neuropsychology 11, 44–52.
Dawson, M., Soulieres, I., Gernsbacher, M.A., Mottron, L., 2007. The level and nature of autistic intelligence. Psychol. Sci. 18, 657–662.
Ding, H., Wang, F., Ding, X., Song, X., Lu, X., Zhang, K., et al., 2008. Association study of semaphorin 5A with risk of Parkinson's disease in a Chinese Han population. Brain Res. 1245, 126–129.
Esposito, G., Kirkby, B.S., Van Horn, J.D., Ellmore, T.M., Berman, K.F., 1999. Context-dependent, neural system-specific neurophysiological concomitants of ageing: mapping PET correlates during cognitive activation. Brain 122 (Pt 5), 963–979.
Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al., 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355.
Goel, V., Makale, M., Grafman, J., 2004. The hippocampal system mediates logical reasoning about familiar spatial environments. J. Cogn. Neurosci. 16, 654–664.
Green, A.E., Munafo, M.R., DeYoung, C.G., Fossella, J.A., Fan, J., Gray, J.R., 2008. Using genetic data in cognitive neuroscience: from growing pains to genuine insights. Nat. Rev. Neurosci. 9, 710–720.
Groen, W., Teluij, M., Buitelaar, J., Tendolkar, I., 2010. Amygdala and hippocampus enlargement during adolescence in autism. J. Am. Acad. Child Adolesc. Psychiatry 49, 552–560.
Harrison, P.J., 2004. The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. Psychopharmacology (Berl.) 174, 151–162.
Johnson, A.D., Handsaker, R.E., Pulit, S.L., Nizzari, M.M., O'Donnell, C.J., de Bakker, P.I., 2008. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics 24, 2938–2939.
Jones, B.M., 1971. Verbal and spatial intelligence in short and long term alcoholics. J. Nerv. Ment. Dis. 153, 292–297.
Kremen, W.S., Prom-Wormley, E., Panizzon, M.S., Eyler, L.T., Fischl, B., Neale, M.C., et al., 2010. Genetic and environmental influences on the size of specific brain regions in midlife: the VETSA MRI study. Neuroimage 49, 1213–1223.
Li, J., Chen, C., Lei, X., Wang, Y., He, Q., Moyzis, R.K., et al., 2012. The NTSR1 gene modulates the association between hippocampal structure and working memory performance. Neuroimage 75C, 79–86.
Lin, L., Lesnick, T.G., Maraganore, D.M., Isacson, O., 2009. Axon guidance and synaptic maintenance: preclinical markers for neurodegenerative disease and therapeutics. Trends Neurosci. 32, 142–149.
Lohmueller, K.E., Pearce, C.L., Pike, M., Lander, E.S., Hirschhorn, J.N., 2003. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat. Genet. 33, 177–182.
MacLullich, A.M., Ferguson, K.J., Deary, I.J., Seckl, J.R., Starr, J.M., Wardlaw, J.M., 2002. Intracranial capacity and brain volumes are associated with cognition in healthy elderly men. Neurology 59, 169–174.
Maraganore, D.M., de Andrade, M., Lesnick, T.G., Strain, K.J., Farrer, M.J., Rocca, W.A., et al., 2005. High-resolution whole-genome association study of Parkinson disease. Am. J. Hum. Genet. 77, 685–693.
Melin, M., Carlsson, B., Anckarsater, H., Rastam, M., Betancur, C., Isaksson, A., et al., 2006. Constitutional downregulation of SEMA5A expression in autism. Neuropsychobiology 54, 64–69.
Novak, N.M., Stein, J.L., Medland, S.E., Hibar, D.P., Thompson, P.M., Toga, A.W., 2012. EnigmaVis: online interactive visualization of genome-wide association studies of the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) consortium. Twin Res. Hum. Genet. 15, 414–418.
Oechslin, M.S., Descloux, C., Croquelois, A., Chanal, J., Van De Ville, D., Lazeyras, F., et al., 2013. Hippocampal volume predicts fluid intelligence in musically trained people. Hippocampus 23, 552–558.
Peper, J.S., Brouwer, R.M., Boomsma, D.I., Kahn, R.S., Hulshoff Pol, H.E., 2007. Genetic influences on human brain structure: a review of brain imaging studies in twins. Hum. Brain Mapp. 28, 464–473.
Pravosudov, V.V., Clayton, N.S., 2002. A test of the adaptive specialization hypothesis: population differences in caching, memory, and the hippocampus in black-capped chickadees (Poecile atricapilla). Behav. Neurosci. 116, 515–522.
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., et al., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575.
Raven, J.C., Raven, J., Court, J.H., 1998. Manual for Raven's Progressive Matrices and Vocabulary Scales. Section 4: Advanced Progressive Matrices. Pearson, San Antonio, TX.

Reuben, A., Brickman, A.M., Muraskin, J., Steffener, J., Stern, Y., 2011. Hippocampal atrophy relates to fluid intelligence decline in the elderly. J. Int. Neuropsychol. Soc. 17, 56–61.
Roth, T.C., Pravosudov, V.V., 2009. Hippocampal volumes and neuron numbers increase along a gradient of environmental harshness: a large-scale comparison. Proc. Biol. Sci. 276, 401–405.
Sherry, D.F., Jacobs, L.F., Gaulin, S.J., 1992. Spatial memory and adaptive specialization of the hippocampus. Trends Neurosci. 15, 298–303.
Simmons, A.D., Puschel, A.W., McPherson, J.D., Overhauser, J., Lovett, M., 1998. Molecular cloning and mapping of human semaphorin F from the Cri-du-chat candidate interval. Biochem. Biophys. Res. Commun. 242, 685–691.
Squire, L.R., 1992. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol. Rev. 99, 195–231.
Stein, J.L., Medland, S.E., Vasquez, A.A., Hibar, D.P., Senstad, R.E., Winkler, A.M., et al., 2012. Identification of common variants associated with human hippocampal and intracranial volumes. Nat. Genet. 44, 552–561.
Tam, C.W., Burton, E.J., McKeith, I.G., Burn, D.J., O'Brien, J.T., 2005. Temporal lobe atrophy on MRI in Parkinson disease with dementia: a comparison with Alzheimer disease and dementia with Lewy bodies. Neurology 64, 861–865.
Thompson, R.M., Weickert, C.S., Wyatt, E., Webster, M.J., 2011. Decreased BDNF, trkB-TK + and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. J. Psychiatry Neurosci. 36, 195–203.
Wang, K.S., Liu, X., Zhang, Q., Pan, Y., Aragam, N., Zeng, M., 2011. A meta-analysis of two genome-wide association studies identifies 3 new loci for alcohol dependence. J. Psychiatr. Res. 45, 1419–1425.
Wang, Y., Li, J., Chen, C., Zhu, B., Moysis, R.K., Lei, X., et al., 2013. COMT rs4680 Met is not always the 'smart allele': Val allele is associated with better working memory and larger hippocampal volume in healthy Chinese. Genes Brain Behav. 12, 323–329.
Weiss, L.A., Arking, D.E., Daly, M.J., Chakravarti, A., 2009. A genome-wide linkage and association scan reveals novel loci for autism. Nature 461, 802–808.
Wendelken, C., Bunge, S.A., 2010. Transitive inference: distinct contributions of rostrolateral prefrontal cortex and the hippocampus. J. Cogn. Neurosci. 22, 837–847.
Zeithamova, D., Schlichting, M.L., Preston, A.R., 2012. The hippocampus and inferential reasoning: building memories to navigate future decisions. Front. Hum. Neurosci. 6, 70.
Zhai, H., 1999. The analysis of Raven's Advance Progressive test in Chinese national public officer test. Psychol. Sci. [Chin.] 22, 169–182.
Zhang, Y., Bertolino, A., Fazio, L., Blasi, G., Rampino, A., Romano, R., et al., 2007. Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. Proc. Natl. Acad. Sci. U. S. A. 104, 20552–20557.
Zhao, C., Deng, W., Gage, F.H., 2008. Mechanisms and functional implications of adult neurogenesis. Cell 132, 645–660.