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Haplotypes of catechol-O-methyltransferase modulate intelligence-related brain white matter integrity

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ABSTRACT

Twin studies have indicated a common genetic origin for intelligence and for variations in brain morphology. Our previous diffusion tensor imaging studies found an association between intelligence and white matter integrity of specific brain regions or tracts. However, specific genetic determinants of the white matter integrity of these brain regions and tracts are still unclear. In this study, we assess whether and how catechol-O-methyltransferase (COMT) gene polymorphisms affect brain white matter integrity. We genotyped twelve single nucleotide polymorphisms (SNPs) within the COMT gene and performed haplotype analyses on data from 79 healthy subjects. Our subjects had the same three major COMT haplotypes (termed the HPS, APS and LPS haplotypes) as previous studies have reported as regulating significantly different levels of enzymatic activity and dopamine. We used the mean fractional anisotropy (FA) values from four regions and five tracts of interest to assess the effect of COMT polymorphisms, including the well-studied val158met SNP and the three main haplotypes that we had identified, on intelligence-related white matter integrity. We identified an association between the mean FA values of two regions in the bilateral prefrontal lobes and the COMT haplotypes, rather than between them and val158met. The haplotype-FA value associations modulated nonlinearly and fit an inverted U-model. Our findings suggest that COMT haplotypes can nonlinearly modulate the intelligence-related white matter integrity of the prefrontal lobes by more significantly influencing prefrontal dopamine variations than does val158met.

Introduction

Many studies have identified genetic influences on brain morphology and intelligence (Lewontin, 1975; Mattay and Goldberg, 2004; Plomin and Kosslyn, 2001; Plomin and Petrill, 1997; Thompson et al., 2001, 2002). Twin studies have indicated a common genetic origin shared by both intelligence and variations in brain morphology (Hulshoff Pol et al., 2006; Posthuma et al., 2002; Toga and Thompson, 2005). This common origin suggests that genetic factors may influence cognitive ability by mediating brain structure. In addition to studies by other groups that used structural magnetic resonance imaging (MRI) (Andreasen et al., 1993; Duncan et al., 2000; Haier et al., 2004; Narr et al., 2007; Shaw et al., 2006) to identify correlations between brain structure and intelligence, our previous diffusion tensor imaging (DTI) studies supported an association between the white matter integrity of specific brain regions and the intelligence quotient (IQ) score, a general intelligence measure (Li et al., 2008; Yu et al., 2008). Using voxel-based analysis, we previously found a correlation between IQ scores and the white matter integrity of four brain regions in the bilateral prefrontal lobes and the bilateral hippocampal formations (Li et al., 2008). Also our tractography-based quantitative study reported that, of the five main white matter tracts, only the white matter integrity of the right uncinate fasciculus (UF) is positively associated with IQ score (Yu et al., 2008). However, specific genetic determinants of the white matter integrity of these brain regions and tracts remain unclear.

Catechol-O-methyltransferase (COMT) is a key enzyme responsible for regulating dopamine (DA) flux by inactivating released DA in the brain, especially in the prefrontal cortex (PFC) (Mannisto and Kaakkola, 1999; Mattay et al., 2003). The COMT gene, located on chromosome 22, has received considerable attention in previous imaging genetic studies (Aleman et al., 2008; Mattay and Goldberg, 2004; Weinberger et al., 2001). One recent meta-analysis supported an association of functional variants in the COMT gene with IQ score

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and found that these associations did not differ significantly by ancestry, gender, average sample age, or patient status (Barnett et al., 2008). Previous structural and functional neuroimaging studies have consistently demonstrated that genetic polymorphisms of the human COMT gene can modulate brain morphometry (Cerasa et al., 2008; Honea et al., 2009; McIntosh et al., 2007; Taylor et al., 2007; Zinkstok et al., 2006) and brain activities during cognitive tasks (Goldberg et al., 2003; Mattay et al., 2003; McIntosh et al., 2007; Meyer-Lindenberg et al., 2006). COMT may also influence white matter integrity through regulating DA levels, which can further regulate the proliferation and differentiation of oligodendrocytes and affect myelin formation (Belachew et al., 1999; Bongarzone et al., 1998; Karadottir and Attwell, 2007). In the current study, we investigated the effects of COMT genetic variants on the white matter integrity of four brain regions and five fiber tracts by analyzing the mean FA value, a measure that reflects the directionality of the motion of water molecules (Beaulieu, 2002).

Most previous studies of the various polymorphisms of the COMT gene have focused on a common functional SNP in codon 158 (val158met), because the substitution of valine (val) by methionine (met) can result in 3–4 times lower thermostability and activity of the COMT enzyme (Mannisto and Kaakkola, 1999). However, this SNP can not completely explain the variations in COMT activity. For example, Diatchenko et al. (2005) found three common haplotypes (termed the HPS, APS and LPS haplotypes), of which the COMT LPS haplotype can modulate about 20 times more protein expression than can the COMT HPS haplotypes by altering mRNA secondary structure (Nackley et al., 2006). Thus these COMT haplotypes appear to have a greater impact on the variation in COMT gene function than does COMT val158met. Recent studies have also showed that previously reported effects of the functional val158met SNP on brain structure and function are modified by other functional SNPs and haplotypes in the gene (Honea et al., 2009; Meyer-Lindenberg et al., 2006). Here we investigate whether and how the COMT val158met SNP and the three major haplotypes that we identified in our subjects affect intelligence-related white matter integrity. First, we identified three major COMT haplotypes consisting of five SNPs based on twelve SNPs known to be in this gene. Then, we used imaging genetic techniques to assess the modulation by COMT polymorphisms on the mean FA values of four brain regions and five white matter tracts. The results indicated that the COMT haplotypes were associated with the mean FA values of two regions in the bilateral prefrontal lobes and that the modulations fit an inverted U-model.

Materials and methods

Subjects

We recruited 79 healthy volunteers (mean age: 23.79 + 3.88 years, range: 16.6–33.0, 44 males) for our study. We measured their IQs using the Chinese Revised Wechsler Adult Intelligence Scale (WAIS-RC) (mean IQ: 113.2 ± 19.0, range: 71–145). We obtained written informed consent from all the subjects included in our study under protocols approved by the ethics committee of the Xuanwu Hospital of the Capital Medical University.

COMT genotyping

We extracted genomic DNA from 250 μl of whole blood using a DNA direct kit (Omega Bio-tek, USA) and genotyped all subjects for twelve known COMT SNPs: COMT rs2075507, rs737865, rs174675, rs174690, rs740603, rs4646312, rs6269, rs4633, rs4818, rs4680 (val158met), rs4646316, rs174699 using PCR and restriction digestion techniques. Please see Appendix Table 1 for a summary of the corresponding primers and restriction enzymes for each SNP.

COMT haplotype estimation

In genetics, a haplotype means a combination of alleles at multiple loci that are transmitted together because they are on the same chromosome. In this paper we use haplotype to specifically refer to a set of statistically associated SNPs on a single chromatid. However, currently used genotyping technology cannot provide specific information about the locus of each marker in a haplotype and thus that haplotypes must be estimated if more than one marker locus is heterozygous in unrelated individuals. In this study, we used the software Haploview v4.0 (Barrett et al., 2005) and genotype data from all 79 subjects to calculate the linkage disequilibrium (LD) relationship between SNPs, and then to define the haplotypes blocks with a confidence interval based method using default parameters. Calculations using the Haploview software revealed the major haplotypes and their frequency in our population; however we did not get the haplotypes assignments for each individual. Thus, we further used phase v2.1 (Stephens and Scheet, 2005; Stephens et al., 2001) to determine the most probable haplotype assignments for each individual by assessing the probability of each possible haplotype and determining a degree of confidence for each. We then selected the most probable, if it exceeded a degree of confidence in excess of 90%.

DTI data acquisition

All subjects were examined with a 3.0-Tesla MR scanner (Trio system; Siemens Magnetom scanner, Erlangen, Germany). The DTI scheme included the collection of 12 images with non-collinear diffusion gradients ($b = 1000 s/mm^2$) and one non-diffusion-weighted image ($b = 0 s/mm^2$) employing a single shot echo planar imaging sequence (TR = 6000 ms, TE = 87 ms). The integrated parallel acquisition technique (iPAT) was used with an acceleration factor of 2. Acquisition time was reduced by the iPAT method with less image distortion from susceptibility artifacts. We collected 45 slices from each participant. The field of view was 256 mm × 256 mm; the acquisition matrix was 128 × 128 and zero filled into 256 × 256; the number of averages was 3; and the slice thickness was 3 mm with no gap, which resulted in a voxel-dimension of 1 mm × 1 mm × 3 mm.

Data preprocessing and FA images acquisition

Distortion induced by eddy currents and simple head motion was corrected using FMRIB's Diffusion Toolbox (FSL 4.0: http://www.fmrib.ox.ac.uk/fsl). After correction, three-dimensional maps of the diffusion tensor and the FA were calculated using the DtiStudio software (Jiang et al., 2006). The FA value was used as a measure of the degree of diffusion anisotropy, which varies between 0, representing isotropic diffusion, and 1, representing completely unidirectional diffusion. The SPM-2 software package (http://www.fil.ion.ucl.ac.uk/spm/) was used to normalize the FA images into a standard space. For each subject, the non-diffusion-weighted image was normalized to the SPM stereotactic space, using the EPI template, and then the transfer parameters were applied to the FA images.

Intelligence-related white matter integrity (ROIs selection)

Using a voxel-based whole brain analysis, our previous study found that the FA values of four brain regions located in the bilateral prefrontal lobes (left: peak coordinate (−18, 32, −12), cluster size 509 mm^3; right: peak coordinate (20, 32, −14), cluster size 867 mm^3) and the bilateral hippocampal formations (left: peak coordinate (−34, −14, −20), cluster size 299 mm^3; right: peak coordinate (34, −12, −22), cluster size 867 mm^3) were positively associated with IQ (Li et al., 2008). Therefore, in this research we selected these four brain regions as ROIs. Using DtiStudio (Jiang et al., 2006), our other

DTI study (Yu et al., 2008) implemented the tractography for five major fiber tracts, including the corpus callosum (CC), cingulum, uncinate fasciculus, optic radiation (OR) and corticospinal tract (CST). Here we chose these tracts as tracts of interest. We further evenly divided the CC into 4 quadrants, which roughly corresponded to the genu (CC1), the anterior and posterior part of the truncus (CC2), and the splenium (CC3). From Appendix Figure 1, we can roughly know the location of these regions and tracts of interest. In order to measure the white matter integrity of these regions or tracts, we computed the regional mean FA values of these ROIs and tracts for each subject using the SPM-2 software package (http://www.fil.ion.ucl.ac.uk/spm/).

Statistical analyses

We used Matlab 6.5 and SPSS 13.0 to perform all the statistical analyses in our study. To test for differences in gender and education level, we used a Pearson chi-square test; and to test for differences in age and IQ scores in different groups, we used a one-way analysis of variance (ANOVA). To examine the associations between the COMT functional genetic variants and intelligence-related white matter integrity, we performed a one-way analysis of covariance (ANCOVA), using age, gender and education levels as covariates. We were able to use all 79 subjects to assess the effects of val158met SNP, but only 68 subjects carried any of the five haplotype combinations that we used for haplotype analyses, so we could only use these in those analyses. In order to find the extent to which IQ is a confounding factor on the effects, we further performed ANCOVA by taking age, gender, education level and IQ score as the covariates.

After ANCOVA, we estimated the marginal means for the mean FA of each haplotypes combination by adjusting for covariates. We then performed Sidak *post-hoc* tests on covariate-adjusted factors by doing pairwise comparisons of estimated marginal means.

Results

Genetics

Using the twelve SNPs (rs2075507, rs737865, rs174675, rs174690, rs740603, rs4646312, rs6269, rs4633, rs4818, rs4680 (val158met), rs4646316, rs174699) known to be in the COMT gene, we estimated the haplotype block in 79 healthy subjects. We discarded three SNPs (rs740603, rs6269, rs174699) because they deviated significantly from Hardy–Weinberg equilibrium ($P<0.001$). Two additional SNPs (rs2075507, rs174690) were found to be significantly deviated from Hardy–Weinberg equilibrium under a significance threshold of 0.05. However, we did not discard them for further analyses. Using the other nine SNPs, we found one significant haplotype block consisting of five SNPs (Fig. 1). Three major haplotypes (TCGGT, CCCGC, and CTCAC, respectively corresponding to the extended LPS, HPS, and APS haplotypes (Diatchenko et al., 2005; Nackley et al., 2006)) accounted for 91.2% of all the detected haplotypes (Fig. 2). These three main haplotypes combined to form five haplotype combinations (LPS/LPS, LPS/APS, LPS/HPS, APS/HPS, and HPS/HPS).

Single SNP effects

Subjects with different rs4680 (val158met) genotypes did not show significant differences in age, gender, educational level or IQ scores (Table 1). Single SNP analyses showed a significant association of the rs4680 genotype with the mean FA value of the right CST ($F_{(74,1)}=5.197$, $P=0.026$) but no significant association with the mean FA values of the other four ROIs or other tracts (Table 2). Subjects who were val homozygous ($n=44$) for rs4680 had a worse white matter integrity of the right CST than did subjects who were met carriers ($n=35$) (val/val group: 0.509 ± 0.028; met carrier group: 0.523 ± 0.025). Other individual SNPs had no effect on the mean FA value of regions or tracts of interest, either in the whole group or in subjects with val homozygous for rs4680.

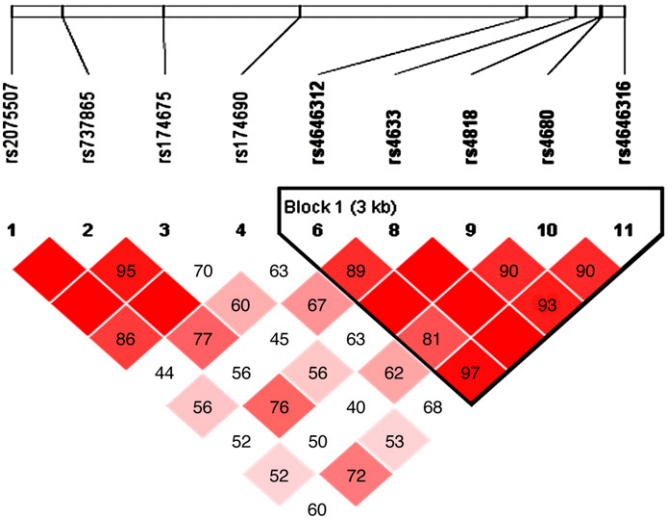

Fig. 1. LD structure of nine SNPs within COMT in our sample. Regions of high LD ($D'=1$ and LOD>2) are shown in bright red. Markers with a lower LD ($D'<1$ and LOD>2) are shown in shades of pink or red with intensity decreasing with a decreased D' value. Regions of low LD and Low LOD scores are shown in white. The numbers indicate the D' statistic value between the corresponding two SNPs. Haplotype blocks were defined by the Haploview program using a confidence interval based block definition with default parameters. One haplotype block across COMT was revealed from the LD data for these nine SNPs.

Haplotype analyses

We only used the 68 subjects who carried one of five haplotype combinations (LPS/LPS, LPS/APS, LPS/HPS, APS/HPS, and HPS/HPS) for subsequent haplotype analyses. We combined APS/HPS and HPS/HPS genotypes into a single group because of the small sample size in the HPS/HPS group ($n=5$). No significant differences were found in age, gender, education levels or IQ of the four groups (Table 1). When we did not consider the effect of IQ score, we found the COMT haplotype combinations affected the mean FA values of two ROIs in the bilateral prefrontal lobes (Left: $F_{(61,3)}=2.789$, $P=0.048$; Right: $F_{(61,3)}=3.581$, $P=0.019$) and the right UF ($F_{(61,3)}=3.507$, $P=0.020$) (Table 2). When IQ score was included as a covariate, we still found that the mean FA values across the haplotype groups are significantly different in the two ROIs of the bilateral prefrontal lobes (Left: $F_{(60,3)}=2.77$, $P=0.049$; Right: $F_{(60,3)}=4.03$, $P=0.011$). However, the mean FA values of the right UF only showed trend-level differences across the haplotype groups ($F_{(60,3)}=2.39$, $P=0.078$) (Table 2). We still did not find any significantly different mean FA values across the haplotype groups in any other ROI. Then we performed a Sidak *post-hoc* analysis to test the differences in mean FA of the two significant ROIs in bilateral prefrontal lobes between individual haplotypes pairs (Appendix Table 2). We found that LPS/APS showed significantly higher mean FA values than the other three haplotypes groups (LPS/LPS, LPS/HPS, APS/HPSorHPS/HPS), and there were no significant differences between the mean FA values of any other pairs of haplotypes groups. So these results revealed that the modulation relationships were nonlinear and resembled an inverted U-model (Fig. 3), which is similar to the inverted U-model that has been established as describing the relationship between dopamine signaling and prefrontal function (Mattay et al., 2003). No significant associations were found between the COMT haplotype combinations and the mean FA values of other ROIs and fiber tracts.

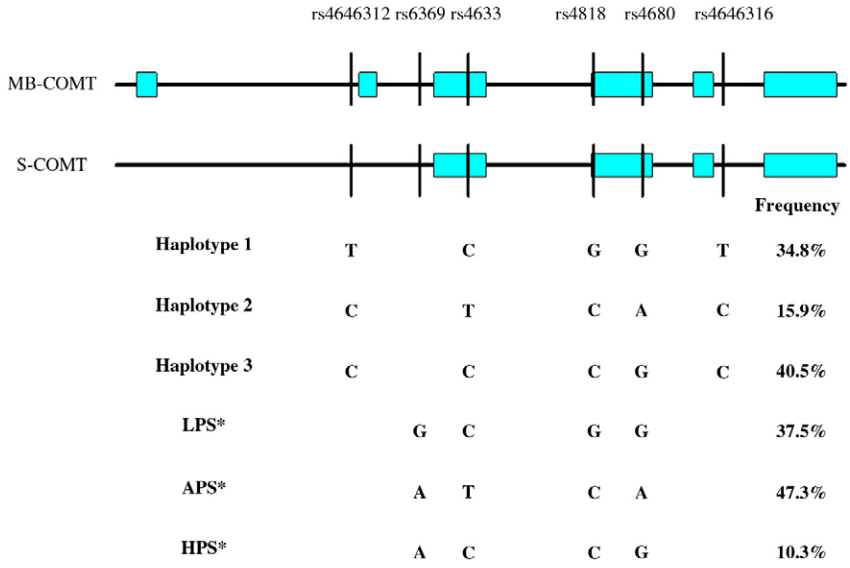

Fig. 2. COMT major haplotypes and their frequency in our study. *Here haplotype HPS, APS and LPS denotes the pain-sensitivity related haplotypes in previous studies (Diatchenko et al., 2005; Nackley et al., 2006). The three major COMT haplotypes in our study correspond to the extended HPS, APS and LPS haplotypes, respectively.

Discussion

In this study, we investigated the potential effects of COMT polymorphisms on intelligence-related white matter integrity. Our previous studies supported a correlation between IQ and the white matter integrity of four regions in the bilateral prefrontal lobes and the bilateral hippocampal formations (Li et al., 2008) and the integrity of the right UF (Yu et al., 2008). Our current findings support the effects of COMT haplotypes on the white matter integrity of the bilateral prefrontal lobes. The modulations are nonlinear and satisfy an inverted U-model, which may indicate that intelligence-related brain white matter integrity is optimal only within a narrow range of dopamine activity, with either too little or too much dopamine having a relatively deleterious effect.

COMT, an enzyme that inactivates released dopamine (DA), may play a unique role in regulating DA flux in the prefrontal cortex (PFC) because of the minimal role of DA transporters in the PFC (Lewis et al., 2001; Mazei et al., 2002; Moron et al., 2002; Sesack et al., 1998). Studies in COMT-knockout mice show increased DA levels in the PFC but no effect on DA levels in the striatum, where DA transporters are abundant (Gogos et al., 1998; Huotari et al., 2002). Prefrontal DA may regulate the proliferation and differentiation of oligodendrocytes and further affect myelin formation (Belachew et al., 1999; Bongarzone et al., 1998; Karadottir and Attwell, 2007). Moreover, convergent evidence supports a lack of significant COMT activity in presynaptic dopaminergic neurons, but some activity in postsynaptic neurons and substantial activity in glial cells (Kaakkola et al., 1987; Karhunen et al., 1995; Rivett et al., 1983). So not surprisingly, our findings indicate that the COMT haplotypes have an effect on the white matter integrity of two ROIs in the bilateral prefrontal lobes. These findings indicate that COMT genetic variations may partially explain the genetic basis of intelligence-related white matter integrity.

Several studies have reported that COMT may contain at least three functional polymorphisms (rs737865, rs4680, and rs165599) that impact its biologic actions and confound its clinical associations (Bray et al., 2003; Mukherjee et al., in press; Palmatier et al., 2004; Shifman et al., 2002). Additionally, structural and functional imaging studies have demonstrated the effects of COMT haplotypes consisting of these three functional polymorphisms on prefrontal structure and function (Honea et al., 2009; Meyer-Lindenberg et al., 2006). Recently other studies have also shown that common synonymous COMT variants modulate the total COMT enzymatic activity by altering the mRNA secondary structure and affecting the expression of the gene (Diatchenko et al., 2005; Nackley et al., 2006). They have reported that the common functional COMT haplotypes, tagged by SNPs rs6269, rs4633, rs4818, and rs4680 and termed HPS, APS and LPS respectively, correspond to the lowest, intermediate, and highest measured COMT enzyme activity (Nackley et al., 2006). We found similar and extended haplotypes, but at different frequencies, in our Chinese Han samples by genotyping twelve SNPs spanning the COMT gene. Therefore, in this study we hypothesized that haplotype analysis could reveal more about the association between COMT and intelligence-related white matter integrity than the val158met polymorphism alone. The resulting associations between the COMT haplotypes and the mean FA of ROIs in the bilateral prefrontal lobes support the rationality of this hypothesis. Single SNP analysis indicates that val158met relates only to the integrity of the right CST. We can not exactly explain why the val158met only affected the integrity of the right CST. However, previous studies have demonstrated that val allele carriers show decreased cognitive performance and lower IQ scores than met allele

Table 1
Subject characteristics with the various genotypes.

	Genotypes	Samples	Age	Gender (M:F)	Education (L:M:H)	IQ score
rs4680	AG/AA (met carrier)	35	24.41(3.90)	19:16	16:6:13	114.23(18.91)
	GG (val/val)	44	23.30(3.84)	25:19	17:16:11	112.43(19.53)
			$P=0.209$	$P=0.822$	$P=0.152$	$P=0.682$
Haplotype combination	LPS/LPS	17	24.18(4.17)	11:6	7:6:4	112.82(18.94)
	LPS/APS	15	24.77(3.52)	8:7	7:4:4	118.00(17.03)
	LPS/HPS	17	22.53(3.47)	10:7	8:4:5	111.76(19.18)
	APS/HPS&HPS/HPS	19	24.65(3.85)	11:8	8:4:7	112.68(19.95)
			$P=0.149$	$P=0.933$	$P=0.962$	$P=0.790$

Table 2
The effects of COMT val158met and haplotype combinations on the mean FA value in four ROIs and five major fiber tracts.

Regions or tracts of interest	val158met ($n=79$)		Haplotype combinations ($n=68$)		
	$F_{(74,1)}$	P	$F_{(61,3)}$	P^a	P^b
ROI[c]	0.285	0.595	2.789	0.048*	0.049*
RO2[c]	1.175	0.282	3.581	0.019*	0.011*
RO3[c]	3.495	0.065	0.554	0.647	0.768
RO4[c]	1.941	0.168	0.384	0.765	0.691
CC	0.109	0.743	0.673	0.572	0.280
CC1	3.365	0.071	1.912	0.137	0.109
CC2	0.001	0.992	1.386	0.256	0.125
CC3	2.490	0.119	0.179	0.910	0.672
Left cingulum	0.184	0.669	0.412	0.745	0.656
Right cingulum	0.546	0.462	0.662	0.578	0.702
Left UF	0.463	0.498	1.805	0.156	0.244
Right UF	0.296	0.588	3.507	0.020*	0.079
Left OR	0.022	0.882	0.647	0.588	0.432
Right OR	0.246	0.621	1.005	0.397	0.752
Left CST	0.393	0.532	0.082	0.970	0.996
Right CST	5.197	0.026*	1.405	0.250	0.164

[a] The P values obtained by ANCOVA where age, gender, and education level were included as covariates.
[b] The P values obtained by ANCOVA where age, gender, education level and IQ score were included as covariates.
[c] Here ROI1, ROI2, ROI3, ROI4 respectively correspond to ROI in the left prefrontal lobe, right prefrontal lobe, left hippocampal formation, and right hippocampal formation.
* $P < 0.05$.

carriers (Barnett et al., 2008). Our results also demonstrated that subjects who were val homozygous had worse white matter integrity of the right CST than did subjects who were met carriers.

We found that the effects of COMT haplotypes on the white matter integrity of regions in the bilateral prefrontal lobes are nonlinear and satisfy an inverted U-model (Fig. 3). Subjects with COMT LPS/LPS are likely to have the lowest dopamine level as a result of their highest COMT activity levels. In contrast, subjects with HPS/HPS or HPS/APS are presumably most deficient in COMT activity and thus most likely to have the highest dopamine level throughout the long-term brain development process. Yet these two groups show similarly low FA values, which mean similarly low white matter integrity in intelligence-related regions or tracts. Although low dopamine is thought to be associated with low prefrontal lobe development and function, several studies have also found that too much dopamine may have adverse effects (Mattay et al., 2003; Seamans and Yang, 2004). These studies would seem to support our findings that subjects with LPS/APS show the highest FA values in the bilateral prefrontal lobes and the right UF and that subjects with HPS/HPS or HPS/APS show decreased intelligence-related white matter integrity.

A previous meta-analysis study supported an association between COMT val158met and IQ score (Barnett et al., 2008). However, we did not find any significant associations between the genotype groups and IQ scores in our population. The brain structural and functional features have been considered to be intermediate phenotypes, called endophenotypes, in the genetic study of complex cognitive problems. These endophenotypes are thought to be more sensitive and nearer to the direct effect of the genotype than is performance at the behavioral level (de Geus et al., 2008; Mattay and Goldberg, 2004; Thompson et al., 2002). Thus, when the sample size is small, we may find significant genetic effects on intelligence-related white matter integrity, but be unable to find the genetic effects on the IQ itself. Of course, it is just this fact that endophenotypes are more sensitive to genetic changes than are behavioral applications that is just the motivation for performing imaging genetics studies.

There are several limitations to this study. First, we know that currently used genotyping technology cannot provide specific information about the chromatids. Thus haplotypes cannot be directly observed in unrelated individuals if more than one marker locus is heterozygous and must, therefore, be estimated. Using phase v2.1 software, we determined the most probable haplotype assignments for each individual by assessing the probability of each possible haplotype and determining a degree of confidence for each. In fact, because of the existence of ambiguous haplotypes, some studies have proposed powerful solutions that incorporating haplotype probabilities into the analysis (Meyer-Lindenberg et al., 2006). However, in our current study, because we found that only three haplotype assignments could be considered to be ambiguous (that is, their degree of confidence is less than 90%) out of the 79 subjects. So we used the most

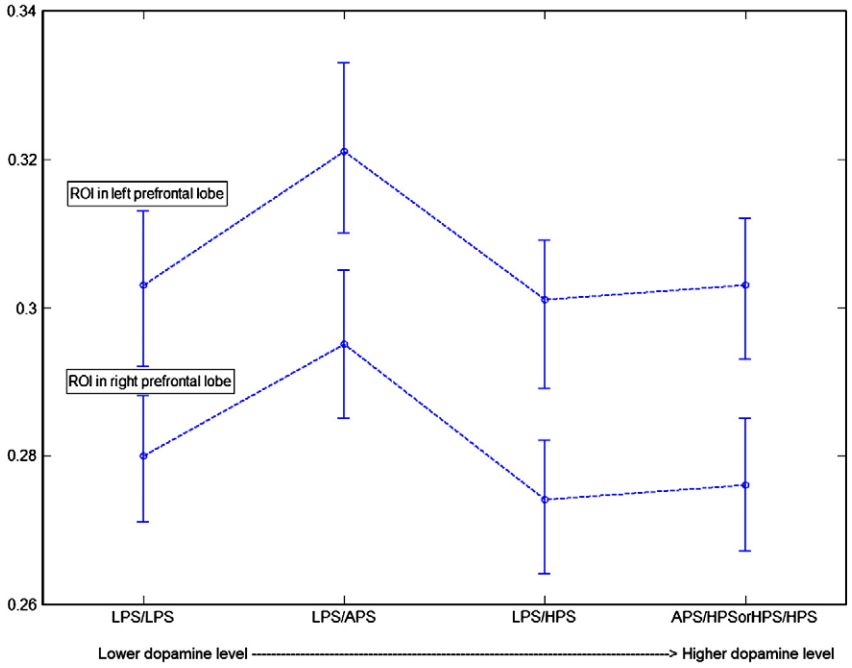

Fig. 3. The relationships between different COMT haplotypes combinations and the mean FA value of two ROIs in the bilateral prefrontal lobes. Bars represent the 95% confidence interval.

likely haplotypes for each subject in the analysis, which may have introduced some relatively minor biases. Second, when we performing ANCOVA by taking age, gender, education level and IQ score as the covariates, we found that the mean FA values of the right UF only showed trend-level differences across the haplotype groups ($F_{(60,3)} = 2.39$, $P = 0.078$), though we found the differences were significant when IQ score was excluded as a covariant. The UF passes across the bottom of the lateral fissure and is the most prominent white matter tract connecting the frontal and temporal brain regions (Ebeling and Cramon, 1992). Thus, the possible association between the COMT haplotypes and the integrity of the right UF may suggest that COMT also influences the integrity of white matter fiber connections between the frontal and temporal regions. However, we need to be cautious in explaining the possible association between the mean FA value of the right UF and the COMT haplotypes since we can not exclude the possibility of IQ as a cofounder in the association. Third, we carried out multiple statistical comparisons and thereby increased the risk of a type I error. Although some studies also argued that correction for multiplicity may not be necessary in some situations (Rothman, 1990), we cannot exclude the possibility that the results are false positive. Independent studies are needed to further validate current findings.

Our study has provided the first evidence for the hypothesis that COMT haplotypes, by more significantly influencing prefrontal dopamine levels than the val158met polymorphism, modulate intelligence-related white matter integrity. Also our study suggests that adopting an inverted U-model of dopamine level and white matter integrity extends the hypothetical model regarding the relationship between dopamine and prefrontal functions (Mattay et al., 2003; Seamans and Yang, 2004). Our evidence further supports the use of the imaging of endophenotypes as an effective bridge from gene to behavior.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2009.12.020.

References

Aleman, A., Swart, M., van Rijn, S., 2008. Brain imaging, genetics and emotion. Biol. Psychol. 79, 58–69.
Andreasen, N.C., Flaum, M., Swayze, V., O'Leary, D.S., Alliger, R., 1993. Intelligence and brain structure in normal individuals. Am. J. Psychiatry 150, 130–134.
Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.
Barnett, J.H., Scoriels, L., Munafo Marcus, R., 2008. Meta-analysis of the cognitive effects of the catechol-O-methyltransferase gene val158/108met polymorphism. Biol. Psychiatry 64, 137–144.
Beaulieu, C., 2002. The basis of anisotropic water diffusion in the nervous system—a technical review. NMR Biomed. 15, 435–455.
Belachew, S., Rogister, B., Rigo, J.M., Malgrange, B., Moonen, G., 1999. Neurotransmitter-mediated regulation of CNS myelination: a review. Acta Neurol. Belg. 99, 21–31.
Bongarzone, E.R., Howard, S.G., Schonmann, V., Campagnoni, A.T., 1998. Identification of the dopamine D3 receptor in oligodendrocyte precursors: potential role in regulating differentiation and myelin formation. J. Neurosci. 18, 5344–5353.
Bray, N.J., Buckland, P.R., Williams, N.M., Williams, H.J., Norton, N., Owen, M.J., O'Donovan, M.C., 2003. A haplotype implicated in schizophrenia susceptibility is associated with reduced COMT expression in human brain. Am. J. Hum. Genet. 73, 152–161.
Cerasa, A.a.c., Gioia, M.C.a., Labate, A.a.b., Liguori, M.a., Lanza, P.a., Quattrone, A.a.b., 2008. Impact of catechol-O-methyltransferase val108/158met genotype on hippocampal and prefrontal gray matter volume. NeuroReport 19, 405–408.
de Geus, E., Goldberg, T., Boomsma, D.I., Posthuma, D., 2008. Imaging the genetics of brain structure and function. Biol. Psychol. 79, 1–8.
Diatchenko, L., Slade, G.D., Nackley, A.G., Bhalang, K., Sigurdsson, A., Belfer, I., Goldman, D., Xu, K., Shabalina, S.A., Shagin, D., Max, M.B., Makarov, S.S., Maixner, W., 2005. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. Hum. Mol. Genet. 14, 135–143.
Duncan, J., Seitz, R.J., Kolodny, J., Bor, D., Herzog, H., 2000. A neural basis for general intelligence. Science 289, 457–460.
Ebeling, U., Cramon, D.v., 1992. Topography of the uncinate fascicle and adjacent temporal fiber tracts. Acta Neurochir. 115, 143–148.
Gogos, J.A., Morgan, M., Luine, V., Santha, M., Ogawa, S., Pfaff, D., Karayiorgou, M., 1998. Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. Proc. Natl. Acad. Sci. U. S. A. 95, 9991–9996.
Goldberg, T.E., Egan, M.F., Gscheidle, T., Coppola, R., Weickert, T., Kolachana, B.S., Goldman, D., Weinberger, D.R., 2003. Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. Arch. Gen. Psychiatry 60, 889–896.
Haier, R.J., Jung, R.E., Yeo, R.A., Head, K., Alkire, M.T., 2004. Structural brain variation and general intelligence. NeuroImage 23, 425–433.
Honea, R., Verchinski, B.A., Pezawas, L., Kolachana, B.S., Callicott, J.H., Mattay, V.S., Weinberger, D.R., Meyer-Lindenberg, A., 2009. Impact of interacting functional variants in COMT on regional gray matter volume in human brain. NeuroImage 45, 44–51.
Hulshoff Pol, H.E., Schnack, H.G., Posthuma, D., Mandl, R.C.W., Baare, W.F., van Oel, C., van Haren, N.E., Collins, D.L., Evans, A.C., Amunts, K., Burgel, U., Zilles, K., de Geus, E., Boomsma, D.I., Kahn, R.S., 2006. Genetic contributions to human brain morphology and intelligence. J. Neurosci. 26, 10235–10242.
Huotari, M., Gogos, J.A., Karayiorgou, M., Koponen, O., Forsberg, M., Raasmaja, A., Hyttinen, J., Männistö, P.T., 2002. Brain catecholamine metabolism in catechol-methyltransferase (COMT)-deficient mice. Eur. J. Neurosci. 15, 246–256.
Jiang, H., van Zijl, P.C., Kim, J., Pearlson, G.D., Mori, S., 2006. DtiStudio: resource program for diffusion tensor computation and fiber bundle tracking. Comput. Methods Programs Biomed. 81, 106–116.
Kaakkola, S., Männistö, P.T., Nissinen, E., 1987. Striatal membrane-bound and soluble catechol-O-methyl-transferase after selective neuronal lesions in the rat. J. Neural Transm. 69, 221–228.
Karadottir, R., Attwell, D., 2007. Neurotransmitter receptors in the life and death of oligodendrocytes. Neuroscience 145, 1426–1438.
Karhunen, T., Tilgmann, C., Ulmanen, I., Panula, P., 1995. Catechol-omethyltransferase (COMT) in rat brain: immunoelectron microscopic study with an antiserum against rat recombinant COMT protein. Neurosci. Lett. 187, 57–60.
Lewis, D.A., Melchitzky, D.S., Sesack, R.E., Whitehead, R.E., Auh, S., Sampson, A., 2001. Dopamine transporter immunoreactivity in monkey cerebral cortex: regional, laminar, and ultrastructural localization. J. Comp. Neurol. 432, 119–136.
Lewontin, R.C., 1975. Genetic aspects of intelligence. Annu. Rev. Genet. 9, 387–405.
Li, J., Yu, C.S., Li, Y.H., Liu, B., Liu, Y., Shu, N., Song, M., Zhou, Y., Zhu, W.L., Li, K.C., Jiang, T.Z., 2008. COMT val158met modulates association between brain white matter architecture and IQ. Am. J. Med. Genet., B Neuropsychiatr. Genet. 150B, 375–380.
Mannisto, P.T., Kaakkola, S., 1999. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol. Rev. 51, 593–628.
Mattay, V.S., Goldberg, T.E., 2004. Imaging genetic influences in human brain function. Curr. Opin. Neurobiol. 14, 239–247.
Mattay, V.S., Goldberg, T.E., Fera, F., Hariri, A.R., Tessitore, A., Egan, M.F., Kolachana, B., Callicott, J.H., Weinberger, D.R., 2003. Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. Proc. Natl. Acad. Sci. U. S. A. 100, 6186–6191.
Mazei, M.S., Pluto, C.P., Kirkbride, B., Pehek, E.A., 2002. Effects of catecholamine uptake blockers in the caudate–putamen and subregions of the medial prefrontal cortex of the rat. Brain Res. 936, 58–67.
McIntosh, A.M., Baig, B.J., Hall, J., Job, D., Whalley, H.C., Lymer, G.K.S., Moorhead, T.W.J., Owens, D.G.C., Miller, P., Porteous, D., Lawrie, S.M., Johnstone, E.C., 2007. Relationship of catechol-O-methyltransferase variants to brain structure and function in a population at high risk of psychosis. Biol. Psychiatry 61, 1127–1134.
Meyer-Lindenberg, A., Nichols, T., Callicott, J.H., Ding, J., Kolachana, B., Buckholtz, J., Mattay, V.S., Egan, M., Weinberger, D.R., 2006. Impact of complex genetic variation in COMT on human brain function. Mol. Psychiatry 11, 867–877.
Moron, J.A., Brockington, A., Wise, R.A., Rocha, B.A., Hope, B.T., 2002. Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. J. Neurosci. 22, 389–395.
Mukherjee, N., Kidd, K.K., Pakstis, A.J., Speed, W.C., Li, H., Tarnok, Z., Barta, C., Kajuna, S.L.B., Kidd, J.R., in press. The complex global pattern of genetic variation and linkage disequilibrium at catechol-O-methyltransferase. Mol. Psychiatry.
Nackley, A., Shabalina, S.A., Tchivileva, I.E., Satterfield, K., Korchynskyi, O., Makarov, S.S., Maixner, W., Diatchenko, L., 2006. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. Science 314, 1930–1933.
Narr, K.L., Woods, R.P., Thompson, P.M., Szeszko, P., Robinson, D., Dimtcheva, T., Gurbani, M., Toga, A.W., Bilder, R.M., 2007. Relationships between IQ and regional cortical gray matter thickness in healthy adults. Cereb. Cortex 17, 2163–2171.
Palmatier, M.A., Pakstis, A.J., Speed, W., Paschou, P., Goldman, D., Odunsi, A., Okonofua, F., Kajuna, S., Karoma, N., Kungulilo, S., Grigorenko, E., Zhukova, O.V., Bonne-Tamir, B.,

Lu, R.B., Parnas, J., Kidd, J.R., DeMille, M.M.C., Kidd, K.K., 2004. COMT haplotypes suggest P2 promoter region relevance for schizophrenia. Mol. Psychiatry 9, 859–870.
Plomin, R., Petrill, S.A., 1997. Genetics and intelligence: what's new. Intelligence 24, 53–57.
Plomin, R., Kosslyn, S.M., 2001. Genes, brain and cognition. Nat. Neurosci. 4, 1153–1154.
Posthuma, D., De Geus, E.J., Baare, W.F., Hulshoff Pol, H.E., Kahn, R.S., Boomsma, D.I., 2002. The association between brain volume and intelligence is of genetic origin. Nat. Neurosci. 5, 83–84.
Rivett, A.J., Francis, A., Roth, J.A., 1983. Distinct cellular localization of membrane bound and soluble forms of catechol-O-methyltransferase in brain. J. Neurochem. 40, 215–219.
Rothman, K.J., 1990. No adjustments are needed for multiple comparisons. Epidemiology 1, 43–46.
Seamans, J.K., Yang, C.R., 2004. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog. Neurobiol. 74, 1–58.
Sesack, S.R., Hawrylak, V.A., Matus, C., Guido, M.A., Levey, A.I., 1998. Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. J. Neurosci. 18, 2697–2708.
Shaw, P., Greenstein, D., Lerch, J., Clasen, L., Lenroot, R., Gogtay, N., Evans, A., Rapoport, J., Giedd, J., 2006. Intellectual ability and cortical development in children and adolescents. Nature 440, 676–679.
Shifman, S., Bronstein, M., Sternfeld, M., Pisant?Shalom, A., Lev-Lehman, E., Weizman, A., Reznik, I., Spivak, B., Grisaru, N., Karp, L., Schiffer, R., Kotler, M., Strous, R.D., Swartz-Vanetik, M., Knobler, H.Y., Shinar, E., Beckmann, J.S., Yakir, B., Risch, N., Zak, N.B., Darvasi, A., 2002. A highly significant association between a COMT haplotype and schizophrenia. Am. J. Hum. Genet. 71, 1296–1302.
Stephens, M., Scheet, P., 2005. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am. J. Hum. Genet. 76, 449–462.
Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68, 978–989.
Taylor, W.D., Züchner, S., Payne, M.E., Messer, D.F., Doty, T.J., MacFall, J.R., Beyer, J.L., Krishnan, K.R.R., 2007. The COMT Val158Met polymorphism and temporal lobe morphometry in healthy adults. Psychiatry Res. 155, 173–177.
Thompson, P.M., Cannon, T.D., Narr, K.L., van Erp, T., Poutanen, V.P., 2001. Genetic influences on brain structure. Nat. Neurosci. 4, 1253–1258.
Thompson, P.M., Cannon, T.D., Toga, A.W., 2002. Mapping genetic influences on human brain structure. Ann. Med. 34, 523–536.
Toga, A.W., Thompson, P.M., 2005. Genetics of brain structure and intelligence. Annu. Rev. Neurosci. 28, 1–23.
Weinberger, D.R., Egan, M.F., Bertolino, A., Callicott, J.H., Mattay, V.S., Lipska, B.K., Berman, K.F., Goldberg, T.E., 2001. Prefrontal neurons and the genetics of schizophrenia. Biol. Psychiatry 50, 825–844.
Yu, C., Li, J., Liu, Y., Qin, W., Li, Y., Shu, N., Jiang, T., Li, K., 2008. White matter tract integrity and intelligence in patients with mental retardation and healthy adults. NeuroImage 40, 1533–1541.
Zinkstok, J., Schmitz, N., van Amelsvoort, T., de Win, M., van den Brink, W., Baas, F., Linszen, D., 2006. The COMT val158met polymorphism and brain morphometry in healthy young adults. Neurosci. Lett. 405, 34–39.