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COMT Genotype Affects Brain White Matter Pathways in Attention-Deficit/Hyperactivity Disorder

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Abstract: Increased dopamine availability may be associated with impaired structural maturation of brain white matter connectivity. This study aimed to derive a comprehensive, whole-brain characterization of large-scale axonal connectivity differences in attention-deficit/hyperactivity disorder (ADHD) associated with catechol-O-methyltransferase gene (COMT) Val158Met polymorphism. Using diffusion tensor imaging, whole-brain tractography, and an imaging connectomics approach, we characterized altered white matter connectivity in youth with ADHD who were COMT Val-homozygous ($N = 29$) compared with those who were Met-carriers ($N = 29$). Additionally, we examined whether dopamine transporter gene (DAT1) and dopamine D4 receptor gene (DRD4) polymorphisms were associated with white matter differences. Level of attention was assessed using the continuous performance test before and after an 8-week open-label trial of methylphenidate (MPH). A network of white matter connections linking 18 different brain regions was significantly weakened in youth with ADHD who were COMT Met-carriers compared to those who were Val-homozygous ($P < 0.05$, family-wise error-corrected). A measure of white matter integrity, fractional anisotropy, was correlated with impaired pretreatment performance in continuous performance test omission errors and response time variability, as well as with improvement in continuous performance test response time variability after MPH treatment. Altered white matter connectivity was exclusively based on COMT genotypes, and was not

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evident in DAT1 or DRD4. We demonstrated that white matter connectivity in youth with ADHD is associated with COMT Val158Met genotypes. The present findings suggest that different layers of dopamine-related genes and interindividual variability in the genetic polymorphisms should be taken into account when investigating the human connectome. *Hum Brain Mapp 36:367–377, 2015.*

Key words: attention-deficit/hyperactivity disorder; catechol-O-methyltransferase; diffusion tensor imaging; methylphenidate; white matter

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a neurobehavioral condition commonly occurring in children and usually persisting into adulthood, characterized with symptoms of inattention and/or hyperactivity/impulsivity [Polanczyk et al., 2007]. Both alterations in neurotransmitter systems, especially the dopamine system [Froehlich et al., 2010; Swanson et al., 2000], and the brain's structural architecture, such as white matter connectivity [van Ewijk et al., 2012], have been associated with ADHD. Although many studies have explored either the brain's dopamine system or white matter connectivity separately, it is unclear whether and how these two different aspects of the human brain integrate and result in cognitive or behavioral alterations in ADHD patients.

Dopaminergic neurotransmission has been implicated both in the etiology and in the pharmacological treatment response of ADHD, and dopamine transporter gene (DAT1) and dopamine D4 receptor gene (DRD4) are among the most extensively investigated susceptibility genes [Durston, 2010; Froehlich et al., 2010]. DAT1 encodes the major site of action for methylphenidate (MPH) [Volkow et al., 2002] and the variable number of tandem repeat (VNTR) located in the 3′ untranslated region of the DAT1 has been implicated in both ADHD itself [Gizer et al., 2009] and its treatment using MPH [Cheon et al., 2005; Kooij et al., 2008; Roman et al., 2002; Winsberg and Comings, 1999]. DRD4 also encodes a major component of dopamine signaling, particularly involved in the firing rate of dopamine neurons [Froehlich et al., 2010]. Although the 7-repeat allele at the VNTR in exon III of the DRD4 has been the focus of many published studies [Gizer et al., 2009; Hamarman et al., 2004], this allele is very rare in East Asians [Chang et al., 1996], and homozygosity of the 4-repeat allele has been associated with a better response to MPH [Cheon et al., 2007; Froehlich et al., 2011]. However, opposing results have also been observed regarding both DAT1 [Bellgrove et al., 2005; Kirley et al., 2003; McGough et al., 2006; Mick et al., 2006; Stein et al., 2005; Zeni et al., 2007] and DRD4 [McGough et al., 2006; Winsberg and Comings, 1999; Zeni et al., 2007] in relation to ADHD, perhaps suggesting the need to incorporate other contributing factors to ADHD pathogenesis.

It has recently been suggested that high levels of brain dopamine may be associated with reduced myelination of white matter tracts [Thomason et al., 2010]. For example, it was demonstrated in vitro that dopamine receptor activation inhibits myelination [Bongarzone et al., 1998]. In humans, a genetic polymorphism in catechol-O-methyltransferase (COMT) has been associated with altered development of white matter [Li et al., 2009; Thomason et al., 2010; Zhang et al., 2013]. COMT enzyme plays a key role in the metabolism of dopamine, and a single nucleotide polymorphism leading to a valine (Val) to methionine (Met) substitution at codon 158 (Val158Met) of COMT has been estimated to result in an approximately threefold to fourfold decrease in enzymatic activity [Mannisto and Kaakkola, 1999] and thus increased dopamine availability [Tunbridge et al., 2004]. Using diffusion tensor imaging in healthy children and adolescents, the Met allele of this polymorphism, indicating higher dopamine availability, was found to be associated with reduced fractional anisotropy, a localized measure of white matter integrity thought to reflect reduced myelination [Thomason et al., 2010]. In sum, increased dopamine availability is associated with altered maturation of white matter connectivity both in vitro and in vivo. However, it is largely unknown whether variation in white matter connectivity and microstructure is associated with other genes involved in dopamine transmission, such as the VNTR located in the 3′ untranslated region of the DAT1 and the VNTR in exon III of the DRD4, which are among the most studied polymorphisms in relation to ADHD [Froehlich et al., 2010], making COMT relatively unique to white matter development.

We investigated whether variation in white matter connectivity in youth with ADHD is explained by the COMT Val158Met polymorphism. The dopaminergic involvement both in the pathophysiology and pharmacological treatment of ADHD makes these patients ideal candidates for examining the relationship between COMT polymorphism and brain white matter integrity. We hypothesized that specific axonal circuits are more extensively myelinated in ADHD youth who are homozygous for the Val allele compared to Met-carriers, in line with the findings observed in vitro and in healthy children [Bongarzone et al., 1998; Thomason et al., 2010]. We sought to test whether this hypothesized variation in the extent of myelination manifests at the level of the human connectome

and whether it can be localized to white matter connections showing microstructural differences between the two genotypes. By combining diffusion tensor imaging tractography [Zalesky et al., 2011] and an imaging connectomics approach [Li et al., 2012; Sporns, 2012], detailed and comprehensive maps of inter-regional brain connectivity were obtained in a relatively large, clinical sample of children and adolescents diagnosed with ADHD, which enabled us to conduct a stringent test of our hypothesis. We then tested whether any variation in white matter connectivity associated with the COMT polymorphism confers functional effects, as evidenced by correlations between measures of white matter integrity and continuous performance test performance or treatment response to MPH. The latter variables were chosen in light of dopaminergic involvement, as continuous performance test performance is a proposed endophenotype of ADHD that is modulated by variation in dopaminergic genes [Kebir et al., 2009; Kollins et al., 2008] and MPH is one of the most widely prescribed therapeutic agents for ADHD [Santosh and Taylor, 2000; Wilens, 2008] that acts by increasing the amount of dopamine in the synapse [Froehlich et al., 2010; Volkow et al., 2002]. A point of note is that although we hypothesized COMT polymorphism rather than DAT1 or DRD4 to be related with white matter integrity in ADHD patients, we also explored DAT1 and DRD4 considering their importance in the dopamine pathway and in ADHD [Durston, 2010; Froehlich et al., 2010; Gizer et al., 2009]. We consider that our specific focus on white matter integrity in the current study may improve our understanding of ADHD, which is pathologically complex with multifaceted etiologies [Thapar et al., 2013].

MATERIALS AND METHODS

Participants

Given recent evidence suggesting that pharmacological treatment for no longer than 6 months may induce brain structural changes in children with ADHD [Hoekzema et al., 2014], we studied only the stimulant- (including both MPH and amphetamines) and atomoxetine-naïve patients ($N = 61$) among the participants described in detail elsewhere [Hong et al., 2014]. Besides medication history, the exclusion criteria comprised an intelligence quotient below 70, and a past or an ongoing history of tic disorder, obsessive compulsive disorder, language disorder, learning disorder, convulsive disorder, pervasive developmental disorder, schizophrenia, bipolar disorder, or brain damage. The study protocol was approved by the institutional review board for human subjects at the Seoul National University Hospital. Detailed information about the study was given to parents and children, and written informed consents were obtained prior to study entry.

Clinical Evaluations and MPH Administration

We assessed the presence of ADHD and other psychiatric diagnoses using a semistructured diagnostic interview, the K-SADS-PL. The validity and reliability of the original and the Korean versions of the K-SADS-PL have been established [Kaufman et al., 1997; Kim et al., 2004]. The diagnostic interview using the K-SADS-PL was performed by certified child and adolescent psychiatrists. Level of attention was assessed using a standardized visual version of the computerized continuous performance test [Greenberg and Waldman, 1993]. The continuous performance test was standardized for age among Korean children and adolescents, and its reliability and validity as a diagnostic instrument for ADHD has been established [Shin et al., 2000]. In this study, we used two major variables: omission errors (a measure of inattention) and response time variability (a measure of consistency of attention) [Epstein et al., 2003].

The participants were enrolled in an 8-week, open-label trial of MPH. Initial doses of MPH were subsequently adjusted every 2 weeks until sufficient therapeutic effects were achieved, and then the doses were maintained for the remainder of the 8 weeks. In this study, MPH was administered after diffusion tensor imaging scanning, and the continuous performance test scores were measured both before and after the MPH trial.

Genotyping of Dopaminergic Genes

We investigated the effects of three polymorphisms: the COMT Val158Met was the polymorphism of primary interest, and we also explored the effects of the VNTR located on the 3′ untranslated region of the DAT1 as well as the VNTR in exon III of the DRD4 to test the specificity of findings with COMT. For details of the procedures used for genotyping, see Supporting Information. For each polymorphism, participants were divided into two groups based on their genotypes, which was in accordance with the methods in previous studies [Cheon et al., 2005, 2007, 2008].

Among the 61 drug-naïve youth with ADHD, 32 were COMT Val-homozygous, 27 were Val/Met-heterozygous, and 2 were Met-homozygous. The three oldest patients were excluded from the Val-homozygous group to match the age and sample size with the Met-carrier group. The genotype frequencies of the polymorphisms in this final sample ($N = 58$) are presented in Supporting Information Table S1. The distributions of the genotypes were all in agreement with the expected values of the Hardy–Weinberg equilibrium ($P > 0.05$).

Image Acquisition and Processing

The image acquisition and processing implemented herein, including whole-brain tractography, was based on standard protocols and methods used in previous work

[Zalesky et al., 2011] with a few modifications (see Supporting Information for details), and is identical to our recent analysis performed in a similar cohort [Hong et al., 2014]. In brief, for each individual, we seeded streamlines throughout all of white matter and reconstructed the connectome using a parcellation with 116 different cortical and subcortical regions [Tzourio-Mazoyer et al., 2002]. A total of 20 streamlines was generated from random locations within each white matter voxel. Streamlines were propagated using the interpolated streamline method and were terminated as soon as they reached a cortical or subcortical voxel. Streamlines were ignored if they terminated in white matter or if they interconnected only a single region. Streamlines were registered to Montreal Neurological Institute space by applying the rotation matrices resulting from the registration of each individual's fractional anisotropy image to a custom template (see Supporting Information), after which the total number of streamlines interconnecting each pair of regions was enumerated using customized software. The connectivity between each pair of regions was quantified by the number of interconnecting streamlines as well as the average fractional anisotropy over the volume delineated by these streamlines. The tract-averaged fractional anisotropy was calculated by averaging voxel estimates across all voxels intersected by at least one streamline that interconnected the pair of regions associated with the tract.

Data Analyses

The network-based statistic [Zalesky et al., 2010, 2012] (http://www.nitrc.org/projects/nbs/) was used to identify regional brain networks showing a significant between-group difference in inter-regional connectivity strength. Specifically, a *t*-test was performed to test for a between-group difference in the streamline count at each of the $N(N-1)/2 = 6{,}670$ unique regional pairings. Interconnected networks, formally known as graph components, were then identified among the connections with a *t*-statistic exceeding a predefined *t*-threshold of 2, approximately corresponding to an uncorrected *P*-value of 0.05. A graph component represents a set of connections for which the null hypothesis can be rejected at a significance that is not corrected for multiple comparisons. The network-based statistic corrects for multiple comparisons across the family of all regional pairings by testing for evidence against the null at the level of graph components, rather than at the level of individual connections. To this end, a family-wise error-corrected *P*-value was calculated for the size of each graph component using permutation testing (10,000 permutations) [Nichols and Holmes, 2002]. A family-wise error-corrected *P*-value was then estimated for each component as the proportion of permutations that yielded a larger component or one of equal size.

Networks identified with the network-based statistic defined pairs of cortical and subcortical regions interconnected by a significantly different number of streamlines between the two genotypic groups. A pair of regions interconnected by significantly fewer streamlines suggests that the number of interconnecting axons may also be fewer, less well myelinated or affected by pathology. To differentiate between these alternatives, a tract-averaged fractional anisotropy value was also extracted for each fiber bundle, by averaging the fractional anisotropy values over all voxels intersected by at least one streamline associated with the bundle.

Pearson's correlation coefficient was used to evaluate any potential association between fractional anisotropy and the continuous performance test scores or the changes in the continuous performance test scores after MPH treatment (i.e., pre- minus post-treatment continuous performance test scores were used to estimate the MPH response). Fractional anisotropy is a continuous measure of white matter integrity, whereas the streamline counts are integer values. Thus, fractional anisotropy was used in the post hoc correlation analysis to avoid potential binning artifacts associated with an integer scale. To avoid making normality assumptions, bootstrapped *P*-values were obtained with 10,000 samples. Statistical tests were performed using SPSS 20.0 (SPSS, Chicago, IL) and results are reported with a significance threshold of $P < 0.05$ (two-tailed).

RESULTS

Participant Characteristics

Participants either with or without the COMT Val/Val genotype were not significantly different in age, gender distribution, intelligence level, handedness, body weight, parental education, socioeconomic status, maternal age at pregnancy, birth weight, ADHD subtypes, pretreatment continuous performance test scores, comorbidity of oppositional defiant disorder or anxiety disorder, and MPH dose at the end of the eighth week (Table I). In addition, no significant differences were found in the variables above between the participants either with or without the DAT1 VNTR 10/10 genotype (Supporting Information Table S2), or between the participants either with or without the DRD4 VNTR 4/4 genotype (Supporting Information Table S3).

The two groups either with or without the COMT Val/Val genotype did not differ in maximum head translation or rotation as well as mean head translation or rotation along the three [*x*, *y*, *z*] axes ($P > 0.05$), except a significantly greater mean displacement observed in Met-carriers along the *z*-axis (Supporting Information Table S4). No significant difference was found between these two groups in the volume of the binary white matter mask from which streamline seed points were sampled ($P > 0.05$). In addition, no significant difference was found in the head motion parameters as well as in the volume of the binary white matter mask between the participants either with or without the DAT1 VNTR 10/10 genotype, or between the

TABLE I. Demographic and clinical characteristics of the participants

	ADHD ($N = 58$)		Val/Val ($N = 29$)		Met/Val + Met/Met ($N = 29$)		P
Age (years), mean (SD)	8.69	(2.13)	8.54	(1.66)	8.21	(2.57)	0.72
Gender (female), N (%)	13	(22.4)	5	(17.2)	8	(27.6)	0.34
Body weight (kg), mean (SD)	32.86	(11.65)	34.03	(10.93)	31.69	(12.42)	0.44
Intelligence quotient, mean (SD)	105.00	(12.41)	106.97	(11.21)	103.03	(13.40)	0.23
Handedness (right), N (%)	51	(87.9)	25	(86.2)	26	(89.7)	0.99
Continuous performance test, mean (SD)							
Omission errors	66.84	(20.42)	64.55	(20.90)	71.86	(20.70)	0.18
Response time variability	66.45	(17.17)	64.14	(17.56)	66.83	(17.99)	0.56
Social variables							
Paternal education (years), mean (SD)	14.91	(1.90)	14.89	(2.02)	14.92	(1.80)	0.95
Maternal education (years), mean (SD)	14.82	(1.84)	14.62	(1.94)	15.04	(1.74)	0.41
Familial socioeconomic status, N (%)							0.17
High (very or moderately)	14	(25.9)	6	(21.5)	8	(30.8)	
Middle class	32	(59.3)	20	(71.4)	12	(46.2)	
Low (very or moderately)	8	(14.8)	2	(7.1)	6	(23.0)	
Obstetric variables, mean (SD)							
Maternal age at pregnancy (years)	29.84	(3.59)	29.88	(3.50)	29.80	(3.76)	0.93
Child's birth weight (kg)	3.26	(0.47)	3.32	(0.47)	3.20	(0.47)	0.38
ADHD types, N (%)							0.44
Combined	33	(56.9)	18	(62.1)	15	(51.7)	
Inattentive	19	(32.8)	10	(34.5)	9	(31.0)	
Hyperactive-impulsive	1	(1.7)	0	(0.0)	1	(3.4)	
Not otherwise specified	5	(8.6)	1	(3.4)	4	(13.8)	
Oppositional defiant disorder, N (%)	11	(19.0)	3	(10.3)	8	(27.6)	0.09
Anxiety disorder, N (%)	2	(3.4)	0	(0.0)	2	(6.9)	0.49
Final MPH dose (mg), mean (SD)	30.44	(11.49)	31.10	(11.37)	29.61	(11.85)	0.64
Final MPH dose per weight (mg/kg), mean (SD)	0.94	(0.26)	0.92	(0.28)	0.97	(0.24)	0.55

Different number of total respondents for paternal education ($N = 54$), maternal education ($N = 51$), familial socioeconomic status ($N = 54$), maternal age at pregnancy ($N = 52$), child's birth weight ($N = 51$) and final MPH dose ($N = 52$). ADHD, attention-deficit/hyperactivity disorder; MPH, methylphenidate; SD, standard deviation.

participants either with or without the DRD4 VNTR 4/4 genotype ($P > 0.05$).

Differences in White Matter Connectivity According to Genotype

A single network was identified showing significantly ($P = 0.048$, family-wise error-corrected) decreased connectivity in youth with ADHD who were COMT Met-carriers compared to those who were Val-homozygous. The network comprised 19 links, involving 18 different brain regions (Fig. 1, Table II). Figure 1 was visualized with the BrainNet Viewer [Xia et al., 2013] (http://www.nitrc.org/projects/bnv/). We did not identify any network with significantly decreased connectivity in the Val-homozygous group. In addition, we did not identify any network showing significant differences in connectivity between the participants either with or without the DAT1 VNTR 10/10 genotype ($P > 0.05$), or between the participants either with or without the DRD4 VNTR 4/4 genotype ($P > 0.05$).

The network differentiating the COMT Val-homozygous group from the Met-carriers was predominantly confined to the right hemisphere. We performed an additional exploratory analysis by lowering the statistical threshold ($P < 0.10$, family-wise error-corrected). As a result, a second network was identified showing marginally decreased connectivity ($P = 0.073$) in youth with ADHD who were COMT Met-carriers compared to those who were Val-homozygous (Supporting Information Fig. S1 and Table S5). This network was left-predominant.

When we performed the analysis including the three oldest subjects in the Val-homozygous group, both the right- and left-lateralized differences in the network according to COMT genotype were replicated to some extent (Supporting Information Tables S6 and S7, respectively), however, several differences were noted: left- instead of right-predominant network was statistically significant ($P = 0.049$ and 0.096, respectively, family-wise error-corrected); altered subnetwork involving the cerebellum was part of the left- instead of right-predominant network; and new nodes and links were added (28 nodes and 29 links, and 21 nodes and 23 links, in the left- and right-predominant networks, respectively) without eliminating any of the previously included nodes or links. We did not identify any significant ($P < 0.05$) or trend-level ($P < 0.10$)

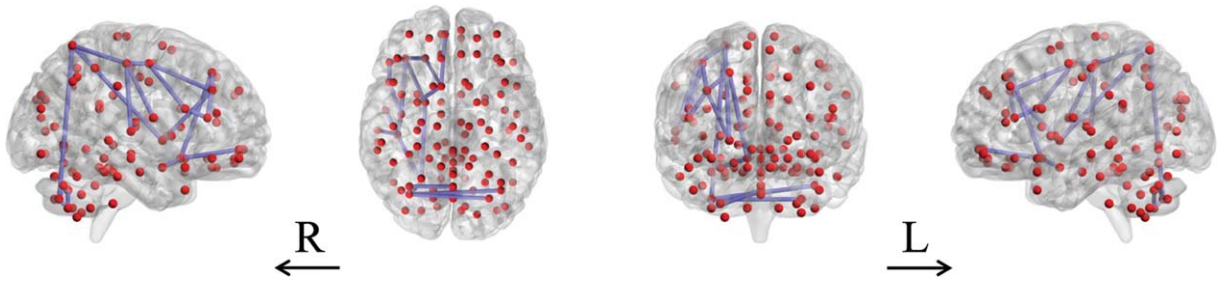

Figure 1.
Significantly decreased white matter connectivity in youth with ADHD who were COMT Met-carriers compared to those who were Val-homozygous. A single abnormal network was identified ($t = 2$). ADHD, attention-deficit/hyperactivity disorder; COMT, catechol-O-methyltransferase; L, left; R, right. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

difference in the network according to DAT1 or DRD4 genotype.

Associations with Attentional Performance and MPH Response

Significant negative associations were found between the tract-averaged fractional anisotropy of each pair-wise connection in the white matter network that was significantly different between COMT genotype groups and the continuous performance test scores. This subnetwork linked right precentral gyrus, right postcentral gyrus, right superior parietal gyrus, and right pallidum (Fig. 2). Specifically, fractional anisotropy in the fibers connecting right superior parietal gyrus and right pallidum was significantly associated with both omission errors ($r = -0.328$, $P = 0.013$) and response time variability ($r = -0.259$, $P = 0.040$); fractional anisotropy in the fibers connecting right superior parietal and right postcentral gyri was significantly associated with omission errors ($r = -0.317$, $P = 0.009$); and fractional anisotropy in the fibers connecting right postcentral and right precentral gyri was significantly associated with response time variability ($r = -0.307$, $P = 0.022$). No significant positive associations were found between fractional anisotropy and the continuous performance test scores.

Significant positive associations were found between the tract-averaged fractional anisotropy of each pair-wise connection in the white matter network that was significantly different between COMT genotype groups and the change in continuous performance test response time variability after the 8-week trial of MPH. This subnetwork linked right precentral gyrus, right middle frontal gyrus, and right inferior frontal gyrus (Fig. 3). Specifically, fractional anisotropy values in the fibers connecting right precentral and right middle frontal gyri ($r = 0.290$, $P = 0.032$), as well as right middle frontal and right inferior frontal gyri ($r = 0.321$, $P = 0.034$) were significantly associated with the change in response time variability. No significant negative associations were found between fractional anisotropy and the change in continuous performance test response time variability. No significant associations were found between fractional anisotropy and the change in continuous performance test omission errors.

When we partialled out the effect of the pretreatment continuous performance test response time variability using partial correlation analysis, the tract-averaged fractional anisotropy in the fibers connecting right precentral

TABLE II. Individual links of the white matter network differentiating youth with ADHD who were COMT Met-carriers from those who were Val-homozygous

Network 1
Precentral gyrus, right ↔ Middle frontal gyrus, right
Superior frontal gyrus, right ↔ Middle frontal gyrus, right
Precentral gyrus, right ↔ Inferior frontal gyrus (operculum), right
Middle frontal gyrus, right ↔ Inferior frontal gyrus (triangular part), right
Inferior frontal gyrus (operculum), right ↔ Inferior frontal gyrus (triangular part), right
Superior frontal gyrus, right ↔ Olfactory gyrus, right
Olfactory gyrus, right ↔ Medial frontal gyrus (orbital part), right
Olfactory gyrus, right ↔ Amygdala, right
Precentral gyrus, right ↔ Postcentral gyrus, right
Rolandic operculum, right ↔ Postcentral gyrus, right
Postcentral gyrus, right ↔ Superior parietal gyrus, right
Middle frontal gyrus, right ↔ Pallidum, right
Superior parietal gyrus, right ↔ Pallidum, right
Postcentral gyrus, right ↔ Superior temporal gyrus, right
Superior parietal gyrus, right ↔ Cerebellar hemisphere (Crus II), right
Cerebellar hemisphere (Crus I), left ↔ Cerebellar hemisphere (Crus II), right
Cerebellar hemisphere (Crus II), left ↔ Cerebellar hemisphere (Crus II), right
Cerebellar hemisphere (Crus II), right ↔ Cerebellar hemisphere (lobule VIIb), right
Cerebellar hemisphere (lobule VIIb), right ↔ Cerebellar vermis (lobule VIII)

ADHD, attention-deficit/hyperactivity disorder.

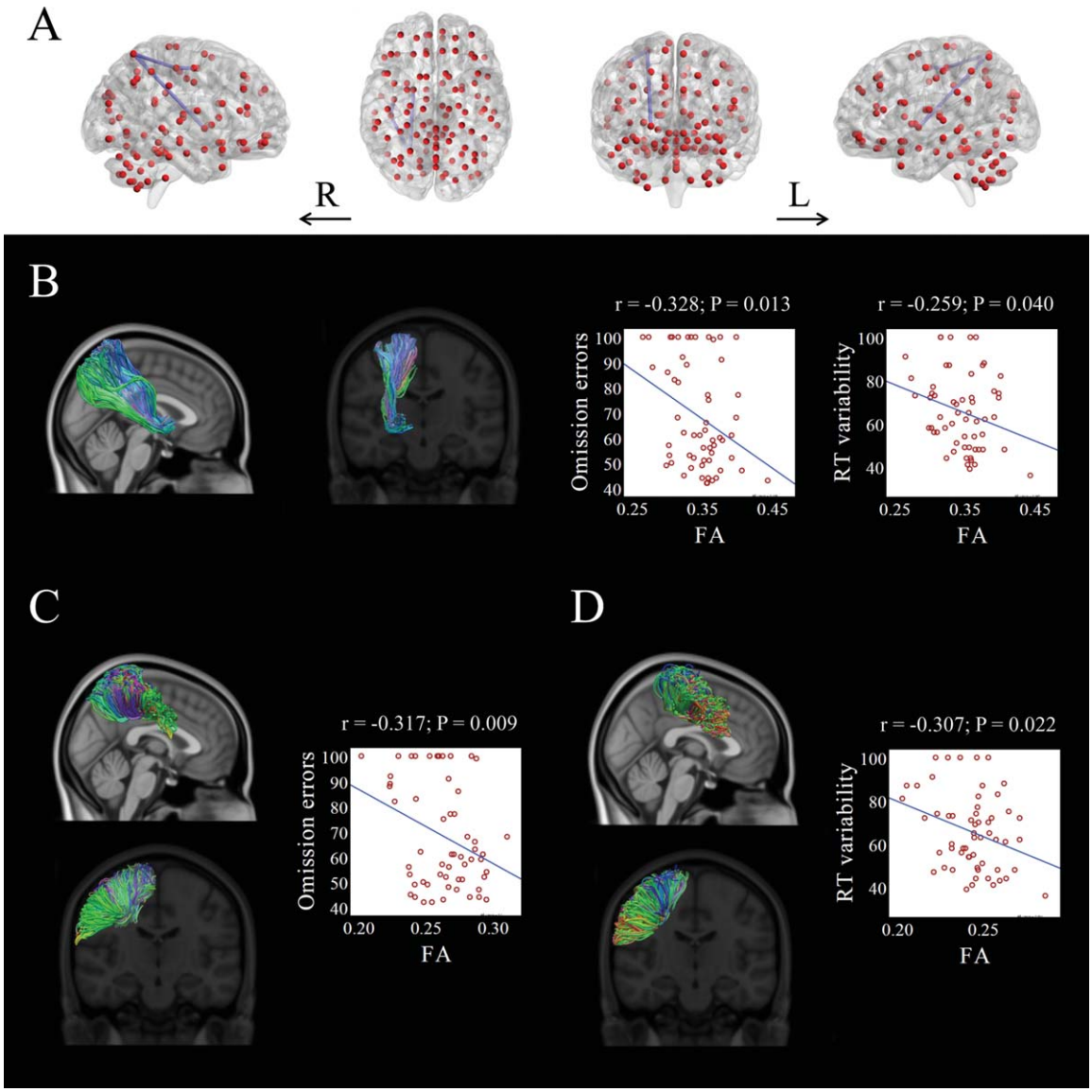

Figure 2.

Significant correlations between the tract-averaged fractional anisotropy value and the continuous performance test scores within the altered white matter network differentiating youth with ADHD who were COMT Met-carriers from those who were Val-homozygous. A single subnetwork was identified (**A**). The fiber tracts linking right pallidum and right superior parietal gyrus (**B**), right superior parietal gyrus and right postcentral gyrus (**C**), and right postcentral gyrus and right precentral gyrus (**D**) are illustrated. ADHD, attention-deficit/hyperactivity disorder; COMT, catechol-O-methyltransferase; L, left; R, right; RT, response time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and right middle frontal gyri as well as right middle frontal and right inferior frontal gyri remained significantly associated with the change in this continuous performance test score ($r = 0.375$, $P = 0.007$; $r = 0.443$, $P = 0.001$, respectively), with the latter being significant after Bonferroni correction for 38 tests (i.e., 19 links $\times$ 2 continuous performance test scores).

DISCUSSION

We found evidence of altered white matter connectivity in youth with ADHD according to their COMT Val158Met genotypes. The network differentiating ADHD patients who were Val-homozygous from those who were Met-carriers showed a distributed pattern of white matter

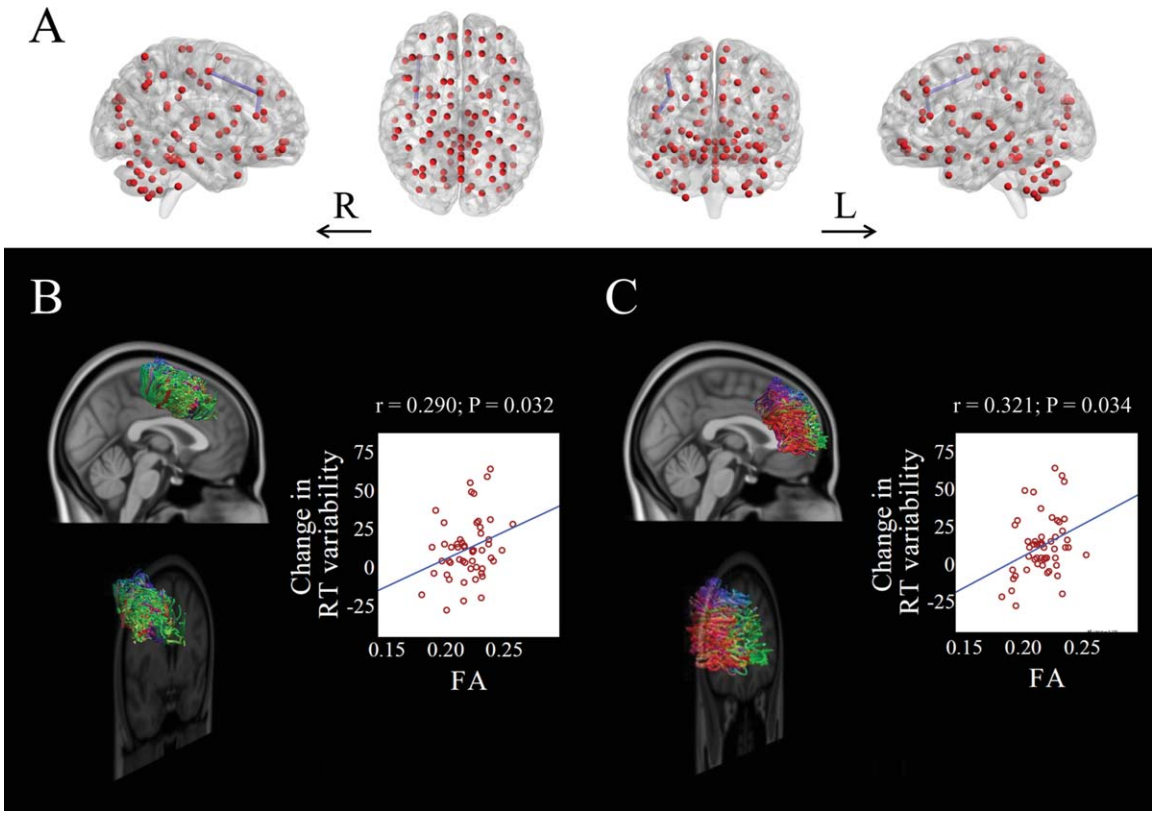

Figure 3.

Significant correlations between the tract-averaged fractional anisotropy value and the changes in continuous performance test response time variability after MPH treatment within the altered white matter network differentiating youth with ADHD who were COMT Met-carriers from those who were Val-homozygous. A single subnetwork was identified (**A**). The fiber tracts linking right precentral gyrus and right middle frontal gyrus (**B**), and right middle frontal gyrus and right inferior frontal gyrus (**C**) are illustrated. ADHD, attention-deficit/hyperactivity disorder; COMT, catechol-O-methyltransferase; L, left; MPH, methylphenidate; R, right; RT, response time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

microstructural alterations involving a range of cortical, subcortical, and cerebellar brain regions. The findings replicate a previous report on healthy children and adolescents demonstrating that the Met allele of the COMT Val158Met polymorphism was associated with impaired structural maturation of brain white matter connectivity [Thomason et al., 2010], supporting the hypothesis that higher dopamine availability inhibits myelination. The network identified was predominantly right-lateralized. Although it was a single interconnected network, the overall configuration included an "anterior" frontal-dominant subnetwork and a "posterior" parietocerebellar subnetwork, which were linked via two brain regions: the globus pallidus and sensorimotor cortex (i.e., precentral and postcentral gyri). The functional significance of these anterior and posterior subnetworks was highlighted by their separate associations with MPH response and continuous performance test performance, respectively. Variation in white matter connectivity according to genotype was specific to COMT, and was not found in DAT1 or DRD4.

Among genes involved in the dopaminergic neurotransmission system, DAT1 has been more strongly implicated than COMT in the neurobiological mechanisms of ADHD [Froehlich et al., 2010]. The COMT enzyme, however, has been postulated to affect the dopamine signal [Matsumoto et al., 2003] especially in brain regions where DAT1 expression is low [Karoum et al., 1994]. COMT expression is widespread and relatively uniform within the human brain [Hong et al., 1998]. COMT is particularly important in regulating cortical dopamine concentrations, whereas DATs regulate dopamine levels in the striatum [Chen et al., 2011]. Moreover, the membrane-bound COMT, which is the main form in the brain, is evenly located in the cell body, axons and dendrites of cortical neurons, and with its C-terminal catalytic domain in the extracellular space, the membrane-bound COMT can inactivate synaptic

as well as extrasynaptic or diffused dopamine [Chen et al., 2011]. Therefore, as white matter is distributed throughout the brain rather than localized to a certain part of it, it is conceivable that the influence of dopamine availability on white matter integrity may be better mediated by COMT rather than DAT1.

In contrast to the functional neuroimaging studies of ADHD pointing to clear abnormalities of frontostriatal regions, research on brain white matter have indicated a distributed pattern of abnormalities involving large fiber tracts [van Ewijk et al., 2012] rather than direct frontostriatal connectivity [Hong et al., 2014]. This implies that a brain-wide causal factor may better account for the white matter abnormalities of ADHD, which is in accordance with the present finding that suggests COMT, but not DAT1 or DRD4, is the culprit. However, COMT and white matter abnormalities may need to be considered in the context of the more complex and multifaceted contributing factors to ADHD pathogenesis.

Besides a single right-lateralized network that differentiated ADHD patients who were Val-homozygous from those who were Met-carriers, we found a second left-lateralized network that marginally differentiated the two genotypic groups. Therefore, COMT appears to affect white matter in a brain-wide manner, although we cannot rule out a more prominent influence on the right hemisphere. Even though COMT is almost uniformly expressed within the human brain, the cerebellum has a relatively higher, and the amygdala, thalamus, and occipital lobe relatively lower levels of COMT expression [Hong et al., 1998]. Interestingly, the altered network found in the present study did not include the thalamus, despite the extensive interconnections between the thalamus and other brain structures [Behrens et al., 2003; Young et al., 2004], nor the occipital lobe, although all three other cerebral lobes were included in the network identified; conversely, the altered network showed a prominent involvement of the white matter tracts within the cerebellum, in line with the known expression levels of COMT in the human brain. Our supplementary analysis including the three oldest subjects in the Val-homozygous group further supports the possibility that COMT affects white matter in a brain-wide manner, rather than favoring either of the cerebral as well as cerebellar hemispheres.

A posterior subnetwork linking the globus pallidus, superior parietal gyrus, precentral gyrus, and postcentral gyrus was identified, where the fractional anisotropy values negatively correlated with the continuous performance test scores before treatment with MPH. In addition, an anterior subnetwork linking the precentral gyrus, middle frontal gyrus, and inferior frontal gyrus was also identified, where the fractional anisotropy values positively correlated with the change in continuous performance test score after MPH treatment. These findings perhaps imply that the COMT-related white matter abnormalities in ADHD are not confined to the prefrontal regions, but treatment response to MPH depends on prefrontal resources, which is similar to the prefrontal compensation strategy adopted by ADHD children in a functional magnetic resonance imaging study of intraindividual response time variability [Suskauer et al., 2008].

Some of the limitations regarding diffusion tensor imaging acquisition and processing, revealed in our recent report [Hong et al., 2014], still apply to this work. In brief, one possible issue is that we used diffusion tensor imaging rather than diffusion spectrum imaging. Although diffusion spectrum imaging was shown to be more successful in resolving the crossing of tracts [Wedeen et al., 2008], acquisition of diffusion spectrum imaging requires individuals to lie motionless in the scanner for a longer period of time, which is particularly challenging for youth with ADHD. In addition, diffusion tensor imaging networks were evidenced with better reproducibility [Bassett et al., 2011]. To minimize acquisition time and the risk of motion-induced artifacts, we used anisotropic voxel dimensions and acquired relatively low angular resolution data, which were at the cost of introducing possible tracking biases in the out-of-plane orientation and of failing to reconstruct white matter pathways with complex geometries, respectively. However, these limitations are common to both genotypic groups that were compared, and are therefore unlikely to introduce spurious between-group differences. The lack of a gold standard for regional parcellation is another limitation of the study. Lastly, we cannot conclude whether our results are general; in particular, the genotypic variation in white matter connectivity identified in this study might not be specific to youth with ADHD, but rather a general effect expressed in other populations. As dopamine is thought to be core to the neurobiology of ADHD, and the levels of this neurotransmitter have been linked to variation in axonal myelination, testing our hypotheses in an ADHD population is likely to have yielded the strongest effects. In addition, considering the high clinical heterogeneity of ADHD, whether this heterogeneity can be explained by genotypic polymorphisms and variability in white matter connectivity is in itself a valid and important research question. However, similar studies need to be conducted in different populations.

CONCLUSION

Our findings indicate that white matter connectivity in youth with ADHD is affected by COMT Val158Met polymorphism, in a way that the Met allele is associated with decreased connectivity of a brain-wide white matter network. The present findings suggest that dopamine-related genes and interindividual variability in the genetic polymorphisms should be taken into account when investigating the human connectome. In addition, our finding provides evidence for the utility of prefrontal white matter connectivity and response time variability as treatment biomarkers for ADHD.

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