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COMT Val$^{108/158}$*Met* Polymorphism and the Modulation of Task-Oriented Behavior in Children with ADHD

Sarojini Sengupta[1,2], Natalie Grizenko[3], Norbert Schmitz[2,3], George Schwartz[2,3], Johanne Bellingham[2], Anna Polotskaia[2,3], Marina Ter Stepanian[2], Yukiori Goto[3], Anthony A Grace[4] and Ridha Joober*[,1,2,3,5]

[1]*Department of Human Genetics, McGill University, Montreal, QC, Canada;* [2]*Douglas Mental Health University Institute, Montreal, QC, Canada;* [3]*Department of Psychiatry, McGill University, Montreal, QC, Canada;* [4]*Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA;* [5]*Department of Neurology and Neurosurgery, McGill University, Montreal, QC, Canada*

It has been suggested that the symptoms of attention-deficit/hyperactivity disorder (ADHD), including inattention and/or hyperactivity/impulsivity, translate into deficits in task-oriented behavior or problem-focused activity. The frontosubcortical dopamine pathway has been implicated in ADHD. One of the key modulators of extracellular dopamine levels in the prefrontal cortex is catechol-*O*-methyltransferase (COMT). The objective of this study was to examine the association of the *COMT Val*$^{108/158}$*Met* polymorphism with (1) task-oriented behavior in children with ADHD, and (2) response of this behavior given methylphenidate (MPH) treatment. Children of Caucasian ethnicity, having ADHD ($n = 188$), were assessed using the Restricted Academic Situation Scale (RASS). The RASS uses a simulated academic environment within the research clinic, to assess the child's ability for independent, sustained orientation to an assignment of math problems. Each child was administered placebo and MPH (0.5 mg/kg in a divided b.i.d. dose), each for a 1-week period, in a randomized, double-blind, crossover trial. On day 3 of the respective treatment week, the child was administered placebo/MPH in the clinic, and the acute change in behavior (before and 1 h after treatment) was evaluated on the RASS. Analysis was carried out using mixed model analysis of variance. Significant main effects of *COMT* genotype ($F_{2,184} = 5.12$, $p = 0.007$) and treatment ($F_{1,184} = 44.26$, $p < 0.001$) on task-oriented behavior were observed. However, no genotype by treatment interaction was observed. These results suggest that the *COMT Val*$^{108/158}$*Met* polymorphism modulates task-oriented behavior, but it does not modulate the response of this behavior with MPH treatment.

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INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common childhood behavioral disorders, affecting 8–12% of school-aged children worldwide (Biederman, 2005). It is characterized by symptoms of inattention and/or hyperactivity/impulsivity, with accompanying cognitive deficits. Neuropsychological studies have converged on the hypothesis that ADHD is associated with deficits in executive function (EF; Willcutt *et al*, 2005). EF encapsulates higher-order neurocognitive control functions required to complete a defined goal. Children with ADHD usually exhibit deficits in response inhibition, working memory and planning, lack attentional and strategic flexibility, and fail to monitor their behavior so as to align themselves with the task set out. Other findings point to key deficits in incentive, motivational, and reward-related processing and suggest that these are largely independent from EF deficits. As described in the dual-pathway model by Sonuga-Barke (2002), the behavioral symptoms associated with ADHD (inattention and/or hyperactivity/impulsiveness), whether they arise from EF or motivational dysregulation, translate into problems with goal-oriented behaviors, affecting the quality and quantity of task or problem-focused activity (Sonuga-Barke, 2002).

Neuropsychological imaging and neuropharmacological studies have suggested that dysregulation of the frontosubcortical circuits involved in EF and emotional/motivational processing of behaviors (reward-based behaviors, error prediction, and the choice between short- and long-term gains) are disrupted in ADHD (Aman *et al*, 1998; Castellanos *et al*, 2006; Durston, 2003; Giedd *et al*, 2001). During performance of goal-oriented behavior, neurons in the prefrontal cortex (PFC) exhibit sustained activity, possibly reflecting the active holding of goal-related information or the preparation of forthcoming actions (Durstewitz *et al*, 2000a). Dopamine strongly modulates both this sustained (delay-period) activity and behavioral performance in working-memory tasks (Durstewitz *et al*, 2000a; Hasegawa *et al*, 1998; Sawaguchi *et al*, 1988, 1990;

*Correspondence: Dr R Joober, Douglas Hospital Research Centre, 6875 LaSalle Blvd., Verdun, Montreal, QC, Canada H4H 1R3,
Tel: +1 514 762 3048, Fax: +1 514 888 4064,
E-mail: ridha.joober@douglas.mcgill.ca
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Sawaguchi and Goldman-Rakic, 1991, 1994; Sawaguchi, 1997; Williams and Goldman-Rakic, 1995). The psychostimulant methylphenidate (MPH), which is widely used for the treatment of ADHD, acts by blocking the dopamine transporter (Krause *et al*, 2000; Volkow *et al*, 1998). Several neuroimaging studies in humans have shown that the administration of MPH at therapeutic doses results in elevated dopamine levels in the brain (Volkow *et al*, 2001), activation of PFC regions involved in emotional processing (Volkow *et al*, 2005), and an increase in the saliency of assigned tasks (Volkow *et al*, 2004).

One of the key endogenous modulators of DA synaptic concentration in the PFC is the enzyme catechol-*O*-methyltransferase (COMT). COMT inactivates catecholamines by transferring a methyl group to the catechol nucleus. COMT is thought to be particularly important for the clearance of dopamine in the PFC (accounting for $>60\%$ of total turnover), given the paucity of dopamine transporter in this region (Karoum *et al*, 1994; Sesack *et al*, 1998). Two transcript isoforms are encoded by the *COMT* gene (mapped to chromosome 22q11), arising from the availability of two transcriptional start sites. The short isoform encodes a soluble protein (s-COMT), which is found in the cytoplasm, whereas the longer isoform codes for a membrane-bound product (m-COMT). Within exon 4 of the *COMT* gene, a common single nucleotide polymorphism (C**G**TG *vs* C**A**TG) results in the presence of methionine or valine at codon 108 (in s-COMT) or codon 158 (in m-COMT). COMT containing valine at position 108/158 has been shown to have higher stability and approximately 2- to 4-fold higher activity than the met variant (Chen *et al*, 2004; Lotta *et al*, 1995). Results of a recent study with healthy volunteers reported that the *COMT* $Val^{108/158}Met$ polymorphism modulates brain activation in the PFC, with the *Val* allele being associated with inefficient prefrontal working-memory response (Meyer-Lindenberg *et al*, 2006). These findings suggest that the *COMT* $Val^{108/158}Met$ polymorphism may be an excellent candidate to investigate for association with task-oriented behavior and response of these behaviors to MPH treatment, in children with ADHD.

We have examined the association between the *COMT* $Val^{108/158}Met$ polymorphism and task-oriented behavior and the response of this behavior to MPH treatment. Task-oriented behavior was measured in the clinic, within a restricted academic situation. This simulated academic environment allows for the assessment of the child's behavior when given an academic task (set of math problems, at a level of difficulty equivalent to the child's ability), in the absence of adult supervision. For each child, the assessment of task-oriented behaviors was performed on four occasions: before, and 1 h after treatment with either placebo or MPH, allowing us to study the association of the *COMT* $Val^{108/158}Met$ polymorphism with task-oriented behavior and its response to MPH. These assessments were part of a 2-week double-blind, placebo-controlled crossover trial with MPH.

PATIENTS AND METHODS

Subjects

A total of 188 Caucasian children (159 boys and 29 girls), between 6 and 12 years with a mean age of 9.1 years ($SD = 1.8$), were recruited from the Disruptive Behaviour Disorders Program and the child psychiatry outpatient clinics at the Douglas Hospital in Montreal. They were referred to these specialized care facilities by schools, community social workers, family doctors, and pediatricians. Each child was diagnosed with ADHD, using DSM-IV criteria, on the basis of a clinical interview, between the child, at least one parent, and a child psychiatrist. This clinical examination was supplemented with a structured clinical interview of parents using the Diagnostic Interview Schedule for Children—version IV (parental report; Shaffer *et al*, 2000). In the majority of cases, mothers were the primary informants.

Exclusion criteria included having an IQ less than 70, as measured with the *Wechsler Intelligence Scale for Children*—III (Wechsler, 1991), Tourette syndrome, pervasive developmental disorder, and psychosis. The research protocol was approved by the Research Ethics Board of the Douglas Hospital. Parents were explained the study and provided written consent. Children were explained the study and gave their assent to participate.

Assessment of Task-Oriented Behavior in the Clinic within a Restricted Academic Situation

The Restricted Academic Situation Scale (RASS) is a coding system, designed to observe and record the behavior of a child when assigned a set of math problems (based on the child's current grade), during a simulated independent academic situation within a clinic setting (Barkley, 1990). It is an assessment of the child's ability for sustained attention to routine, repetitive academic work in the presence of potential distractions, with no adult supervision (Fischer and Newby, 1998). This scale has been used to discriminate between children with ADHD and normal controls as well as from those with conduct problems unrelated to ADHD (Milich *et al*, 1982).

The restricted academic situation was set up in a clinic playroom containing toys, a work table, chair, and an intercom (Barkley, 1990). After allowing the child to play for 5 min, used as a habituation period, the child was given a set of math problems with instructions to complete as many problems as possible, not to leave the seat, and not to play with any of the toys in the room. The child's behavior was then assessed from behind a one-way mirror over a 15 min time period. Behavioral events were recorded at 30 s intervals according to five categories: 'off-task' (looking away from the task sheet), 'playing with objects' (touching any object not directly used in the task), 'out of seat' (lifting buttocks off chair or moving chair), 'vocalizing' (any vocal noise, whether task related or not), and 'fidgeting' (repetitive, purposeless movements). The RASS score is the total number of recorded behavioral events in the 15-min period.

Measuring Response of Task-Oriented Behavior to Methylphenidate Treatment within a Restricted Academic Situation

A 2-week double-blind, placebo-controlled, within-subject (crossover) experimental design was used to assess the behavioral response to a fixed dose of MPH (0.5 mg/kg per

day) as compared to placebo (PBO). However, before the trial was initiated, the child and parents participated in a week of baseline assessments, which also served as a washout period for children previously treated with MPH. During this time, the overall behavior of the child was assessed using the comprehensive (113-item questionnaire) child behavioral checklist (CBCL). The CBCL was completed by the parent(s) of the affected child.

Following this washout period, subjects received 1 week of treatment with PBO and 1 week of treatment with 0.5 mg/kg of MPH in a divided b.i.d. dose (0.25 mg/kg, morning and noon). The order of PBO and MPH administration was determined by random assignment. PBO and MPH were prepared individually in opaque gelatin capsules in weekly blister packs by a pharmacist not otherwise involved in the study to maintain blind allocation of treatments. At the end of each week of treatment, the blister packs were collected and medication adherence was checked.

On day 3 of each week of treatment, the child was asked to come to the clinic and the RASS was conducted both before (pretreatment) and 60 min after (posttreatment) the treatment was administered by the research staff. A different set of math problems were assigned for each assessment. Trained research personnel conducted the assessments. The interrater reliability was high, with intraclass correlation coefficient ranging from 0.97 to 0.99.

Assessment of Motor Activity during the Restricted Academic Situation

Overall motor activity was estimated using Actiwatch actigraphy, while the child was performing the assigned task within the simulated academic situation. Actigraphy is the use of instrumentation sensitive to movement, such as an Actiwatch, to record activity over time. The Actiwatch is a small, rugged electronic device, worn on the nondominant hand, which is sensitive to acceleration. With each subject movement, an accelerometer generates a variable voltage that is digitally processed. Movements $\geqslant$1/100th of *g* are detected and recorded. The signal is integrated over a user-selected epoch (15 s time bin selected in this study) and a value expressed as 'Activity Counts' is recorded in the instrument memory.

Molecular Genetics

DNA was extracted from a blood sample, a buccal swab, or saliva sample, if the child was amenable only to the latter. The *COMT* $Val^{108/158}Met$ polymorphism was genotyped by PCR amplification and digestion of the PCR product with *Nla*III as previously described (Taerk *et al*, 2004).

Statistical Analyses

Demographic and clinical characteristics of the three *COMT* genotype groups were compared using analysis of variance (ANOVA) or χ^2-tests as appropriate. Mixed model ANOVA was used to analyze the data (SAS Mixed procedure, SAS version 6.12; SAS Institute Inc., Cary, NC) (Littell *et al*, 2005). Genotype information for each individual was recoded using a three-class dummy variable (*Met-Met* = 11, *Val-Met* = 21, and *Val-Val* = 22). For the RASS, the dependent variable was the total RASS score (total number of behavioral events measured during a 15-min time period), measured at four time points: before PBO treatment, after PBO treatment, before MPH treatment, and after MPH treatment. Five different main effects were assessed in the mixed model analysis: *COMT* genotype, time of assessment (week 1 or 2), administration of PBO/MPH (before *vs* after treatment), treatment (PBO *vs* MPH), and order of treatment (MPH administered in week 1, PBO in week 2, or reverse order, because the order was randomized in a double-blind trial). Two interaction effects were also investigated in the analysis: *COMT* genotype by treatment, and treatment by administration. *COMT* genotype, time, administration, treatment with PBO and MPH, and order were fixed effects; individuals were random effects.

Identical analysis was conducted for the subscale scores for each of the domains within the RASS: off-task, playing with objects, out of seat, vocalizing, and fidgeting. For motor activity, the dependent variable was the total number of 15 s bins during which at least one movement was recorded by Actiwatch. As with the RASS, motor activity was measured at four different time points: before and after PBO treatment, before and after MPH treatment. Mixed model analysis, as conducted with the RASS, was performed. Where significant association with *COMT* genotype was observed, *post hoc* comparison between the different groups was conducted using the Tukey's Honest Significant Difference Test.

Effect size (ES, Cohen's *d*) was calculated as the difference between the means of each group divided by the pooled standard deviation (SD). To measure ES of placebo treatment, the mean score before and after placebo treatment was divided by the pooled SD for the two treatment conditions. Similar analysis was conducted to calculate ES for MPH treatment. The original SD of each treatment group was used in the calculation, in spite of the correlation between the two groups that results from the repeated-measures design of the study. The choice of using the original SD, rather than the pooled SD corrected for the amount of correlation between the measures, was based on the argument that the latter analysis results in an overestimate of the actual ES (Dunlop *et al*, 1996). For calculation of genotype ES, mean and pooled SD scores of all four treatment conditions (pre-PBO, post-PBO, pre-MPH, and post-MPH) were used to conduct a comparison between the two genotype groups (*Met/Met* + *Val/Met vs Val/Val*). This grouping of genotypes was conducted based on the observation that the *Val* allele shows a recessive effect on task-oriented behavior.

RESULTS

The frequency of the *Val* allele was 52.1% and the *Met* allele was 47.9%. The genotype frequencies (20.2%, *Met-Met*; 55.3%, *Val-Met*; and 24.5%, *Val-Val*) did not depart from Hardy–Weinberg equilibrium ($\chi^2 = 2.21$, d.f. = 2, $p = 0.67$). The three genotype groups did not differ with respect to their demographic characteristics (Table 1). The three groups were also similar with respect to incidence of comorbid disorders and overall number of behavioral symptoms on the CBCL.

Effect of Methylphenidate and Placebo on RASS Scores

Mixed model ANOVA showed a significant treatment by administration interaction with the RASS total score ($F_{1,186} = 134.2$, $p < 0.001$) and with each of the dimensional scores (data not shown). Regardless of genotype, administration of placebo resulted in a significant deterioration in performance ($F_{1,186} = 17.6$, $p < 0.001$; Cohen's d ES = 0.22) whereas administration of MPH resulted in a significant improvement in performance ($F_{1,186} = 70.1$, $p < 0.001$; Cohen's $d = 0.68$) (Figure 1). In spite of the opposing effects of placebo and MPH on the RASS score, a significant main effect of administration of treatment (before *vs* after MPH/PBO treatment) was observed ($F_{1,186} = 11.29$, $p = 0.0009$). This significant administration effect is a reflection of the fact that MPH treatment shows a much stronger effect (~4 times) in improving task orientation, *vis-à-vis* the negative effect of placebo treatment.

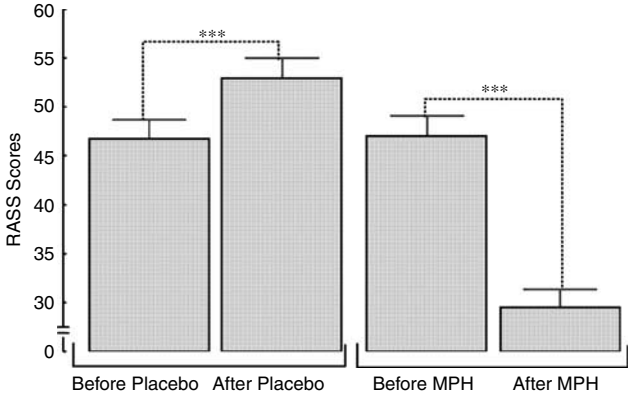

Figure 1 Acute response of task orientation to placebo and methylphenidate (MPH) as measured in the restricted academic situation. Restricted Academic Situation Scale (RASS) scores (mean ± SE), before and 1 h after treatment with placebo (two left bars) or MPH (two right bars). ***$p < 0.0001$. The RASS score is the total number of behavioral events measured over a 15 min time period. Behavioral events were recorded at 30 s intervals according to five categories: off-task, playing with objects, out of seat, vocalizing, and fidgeting.

Association of *COMT Val*$^{108/158}$*Met* Polymorphism with Task-Oriented Behavior

Mixed model analysis showed a significant main effect of *COMT* genotype on the total RASS score ($F_{2,184} = 5.12$, $p = 0.007$). *Post hoc* analysis showed that the *Met-Met* ($p = 0.08$) and *Val-Met* ($p = 0.04$) genotype groups had fewer behavioral events, and were more engaged in the math task, compared to the *Val-Val* group. The *Met-Met* and *Val-Met* genotype groups did not differ significantly from each other ($p = 0.98$), suggesting a recessive effect of the *Val* allele on this phenotype (Figure 2). No significant genotype by treatment interaction was observed ($F_{2,184} = 0.91$, $p = 0.4$), suggesting that *COMT* genotype does not modulate therapeutic response, at least at the dose of MPH tested (0.5 mg/kg).

The RASS score used above is a composite of the total number of behavioral events recorded in each of five categories: off-task, playing with objects, out of seat, vocalizing, and fidgeting. To better understand the relation of each of these behavioral categories to task-oriented behavior, we explored the correlation of each of these behaviors to off-task behavior, as this item likely has the best face validity with regard to orientation to task. The correlation with off-task behavior was very high for playing with objects ($r \geqslant 0.9$), intermediate for out of seat, and vocalization ($0.4 \leqslant r \leqslant 0.5$) and very low for fidgeting ($r < 0.1$; Table 2).

The association between *COMT* genotype and each of the five RASS behavior categories was examined (Table 3). *COMT* genotype showed a strong association with those dimensions that are most representative of task engagement or problem-focused activity; off-task behavior ($F_{2,184} = 5.86$, $p = 0.003$) and playing with objects ($F_{2,184} = 6.1$, $p = 0.003$). The *Val/Val* genotype group had higher scores (significantly

Table 1 Demographic and Clinical Characteristics of Children with ADHD Separated According to their Genotypes in the *COMT Val*$^{108/158}$*Met* Polymorphism

	***Met/Met* (*n* = 38)**	***Val/Met* (*n* = 104)**	***Val/Val* (*n* = 46)**	**Statistic and *p*-value**
M/F (% males)	33/5 (86.8)	85/19 (81.7)	41/5 (89.1)	$\chi^2 = 1.53$, d.f. = 2, $p = 0.47$
Age (years)	9.3 (2.0)	9.0 (1.8)	9.0 (1.7)	$F_{2,185} = 0.4$, $p = 0.65$
Household income (% <$20 000 per year)	41.2	41.7	39.1	$\chi^2 = 0.08$, d.f. = 2, $p = 0.96$
WISQ-III full-scale IQ	97.8 (14.2)	99.8 (14.7)	98.6 (13.7)	$F_{2,169} = 0.3$, $p = 0.74$
Comorbidity (%) with:				
CD	18.4	36.6	28.9	$\chi^2 = 4.4$, d.f. = 2, $p = 0.11$
ODD	36.8	40.8	40	$\chi^2 = 0.18$, d.f. = 2, $p = 0.91$
AD	42.9	45.2	56.4	$\chi^2 = 1.72$, d.f. = 2, $p = 0.42$
MD	14.7	14.3	10	$\chi^2 = 0.51$, d.f. = 2, $p = 0.78$
CBCL total score	69.0 (8.7)	70.8 (9.5)	70.3 (6.9)	$F_{2,179} = 0.56$, $p = 0.57$

Abbreviations: WISC, *Wechsler Intelligence Scale for Children*, 3rd edn; CD, conduct disorder; ODD, oppositional defiant disorder; AD, anxiety disorders; MD, major mood disorders; CBCL, child behavioral checklist; M, male; F, female.
Values are mean (SD) unless otherwise indicated. Demographic and clinical characteristics were compared between these groups using the appropriate statistic depending on the nature of the data.

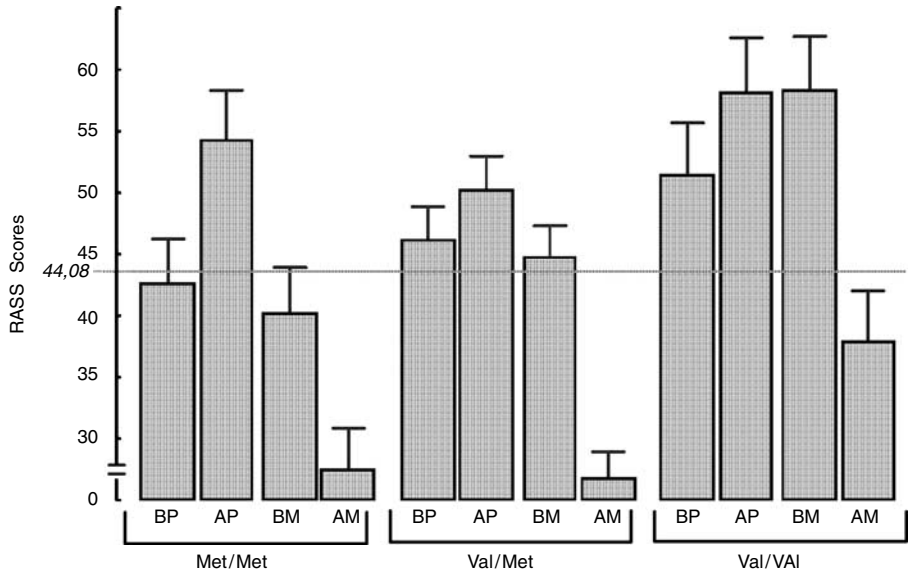

Figure 2 Acute response of task orientation to placebo and methylphenidate (MPH) as measured in the restricted academic situation in children with ADHD separated according to their genotype in the *COMT* (*Val*$^{105/158}$*Met*) polymorphism. Restricted Academic Situation Scale (RASS) scores (mean ± SE) before administration of either placebo (BP) or MPH (BM) and 1 h after administration of either placebo (AP) or MPH (AM) in the three genotype groups. Dashed line indicates the mean of the RASS calculated over the four measurement times (before and after placebo and MPH).

Table 2 Correlation of 'Off-Task' Item of the RASS with the other Four RASS Items

	Playing with object	Out of seat	Vocalization	Fidgeting
Before placebo	0.92	0.51	0.42	0.04
	$p<0.001$	$p<0.001$	$p<0.001$	$p=0.62$
After placebo	0.91	0.53	0.4	0.01
	$p<0.001$	$p<0.001$	$p<0.001$	$p=0.9$
Before methylphenidate	0.90	0.49	0.44	−0.03
	$p<0.001$	$p<0.001$	$p<0.001$	$p=0.64$
After methylphenidate	0.88	0.48	0.48	0.000
	$p<0.001$	$p<0.001$	$p<0.001$	$p=1.00$

Correlation coefficients and *p*-values are provided for each of the four measurement occasions.

higher behavioral problems) than the *Met* carriers on each of these measures. A trend of association was observed between *COMT* genotype and out of seat behavior ($F_{2,184}=3.1$, $p=0.05$). Vocalizing ($F_{2,184}=2.1$, $p=0.12$) and fidgeting ($F_{2,184}=1.23$, $p=0.29$) were not associated with *COMT* genotype.

No Association of *COMT Val*$^{108/158}$*Met* Polymorphism with Motor Activity

To dissect the effect of nonspecific motor hyperactivity from task-oriented behavior (which includes additional important cognitive dimensions), Actiwatch measurements were analyzed. As with the RASS, a main effect of treatment ($F_{1,163}=5.19$, $p=0.02$) and a treatment by administration interaction ($F_{1,159}=25.6$, $p<0.001$) were observed. However, no association was observed between motor activity and *COMT* genotype; there was no main gene effect ($F_{2,173}=1.3$, $p=0.27$) nor a gene by treatment interaction ($F_{2,163}=0.9$, $p=0.4$). These results suggest that the *COMT Val*$^{108/158}$*Met* polymorphism specifically modulates those dimensions of task-oriented behavior or problem-focused activity that are distinct from motor hyperactivity, either in their etiology or in their expression.

DISCUSSION

Previous studies by several independent groups, as well as a recent meta-analysis (including both family-based and case–control studies), have concluded that there is no association between ADHD, considered as a clinical syndrome, and the *COMT Val*$^{108/158}$*Met* polymorphism (Barr *et al*, 1999; Cheuk and Wong, 2006; Hawi *et al*, 2000; Jiang *et al*, 2005; Manor *et al*, 2000; Tahir *et al*, 2000). However, it has been suggested that polymorphisms within candidate genes are more likely to be associated with behavioral dimensions within the disorder (Meyer-Lindenberg and Weinberger, 2006). Indeed, the ADHD syndrome may result from disturbances of various behavioral dimensions

Table 3 Genotype Effect of the *COMT Val*$^{108/158}$*Met* Polymorphism with Each Individual Item of the RASS

	BP	AP	BM	AM	F[a]	p[a]	ES-P	ES-M	ES COMT
Off-task									
Met+	11.6 (8.9)	13.4 (9.5)	10.5 (8.5)	6.5 (8.1)	5.86	0.003	−0.19	0.48	−0.38
Val/Val	14.3 (10.1)	16.0 (9.9)	15.3 (9.6)	10.4 (9.0)					
Playing with object									
Met+	8.8 (8.2)	11.1 (8.9)	8.8 (8.3)	5.2 (7.2)	6.09	0.003	−0.27	0.49	−0.38
Val/Val	11.6 (8.6)	13.9 (9.2)	13.5 (9.5)	8.0 (8.5)					
Out of seat									
Met+	6.2 (7.7)	7.1 (8.1)	5.1 (6.6)	3.9 (6.4)	3.07	0.05	−0.16	0.21	−0.26
Val/Val	7.0 (7.6)	9.3 (8.6)	8.1 (8.1)	5.9 (7.6)					
Vocalization									
Met+	4.7 (7.0)	6.2 (7.7)	5.2 (7.6)	2.2 (4.1)	2.1	0.12	−0.19	0.45	−0.23
Val/Val	5.8 (6.7)	7.1 (7.9)	7.3 (7.7)	4.5 (6.2)					
Fidgeting									
Met+	13.8 (7.9)	13.3 (7.5)	13.9 (7.9)	9.1 (7.8)	1.23	0.29	0.07	0.62	0.08
Val/Val	12.7 (7.3)	11.8 (7.1)	14.1 (7.7)	9.0 (7.9)					

Values are mean (±SD). Cohen's *d* effect size of placebo (ES-P), methylphenidate (ES-M) and *COMT Val*$^{108/158}$*Met* polymorphism (*Met/Met+ Val/Met* genotypes *(Met+) vs Val/Val* genotype).
[a]The statistics is based on the mixed model analysis conducted with the three genotype groups.

that are unique for each affected child and that may be differentially represented in each sample. If a candidate gene is relevant for one or more of these behavioral dimensions, its effect may be difficult to identify, if the between-subject heterogeneity is not taken into consideration.

There is a large body of literature supporting the association between the *COMT Val*$^{108/158}$*Met* polymorphism and neurocognitive functions involving the dorsolateral PFC, in healthy adults as well as adult patients with psychotic disorders (Craddock *et al*, 2006; Tunbridge *et al*, 2006). In contrast, only a limited number of studies have examined the association with specific EF domains in children with ADHD, in spite of compelling evidence implicating dorsolateral PFC dysfunction in the disorder. It is particularly difficult to extrapolate findings obtained in adult studies to complex childhood disorders, as it has been shown that the enzymatic activity of *COMT* in the dorsolateral PFC shows considerable change over the developmental trajectory (Tunbridge *et al*, 2007). Previously, we and others have reported that the *COMT Val*$^{108/158}$*Met* polymorphism is not associated with performance on tests of EF, including the Wisconsin Card Sorting Test, Tower of London, and Self-Ordered Pointing Task (Mills *et al*, 2004; Taerk *et al*, 2004). One other study has reported the association between this polymorphism and two subtests of the Test of Everyday Attention for Children (Walk Don't Walk and Sky Search Dual Task; Bellgrove *et al*, 2005). In this study, the authors report that ADHD children with the *Val/Val* genotype showed better sustained attention than the *Met* carriers. However, as suggested by the authors, a major limitation of this study was that 60% of the children tested were being routinely treated with MPH, which may have been a confound for the assessment of prefrontal cognition.

Here we have examined the association between the *COMT Val*$^{108/158}$*Met* polymorphism, treatment with MPH, and task-oriented or goal-directed behavior, in children with ADHD of Caucasian ethnicity. Task-oriented behavior was measured in a simulated academic situation, which offers the dual advantage that the child's behavior can be assessed objectively within a clinical environment, while simulating a situation similar to homework time or independent study time in the classroom (Fischer and Newby, 1998). Each child was assessed on five behavioral dimensions (off-task, playing with objects, out of seat, vocalizing, and fidgeting). The advantage therefore of using the RASS is that it offers a multidimensional, ecologically relevant evaluation of the child's goal-oriented behavior. Further, coupled with a placebo-controlled evaluation of the effect of MPH, it may have important clinical relevance.

We observed that the *COMT Val*$^{108/158}$*Met* polymorphism modulates task-oriented behavior in children with ADHD. Children in the *Met-Met* and *Val-Met* genotype groups had significantly lower total RASS scores (better behavior in the simulated classroom) than children with the *Val-Val* genotype. Further examination of each of the RASS factors showed that the *COMT Val*$^{108/158}$*Met* polymorphism was specifically associated with 'off-task' behavior. In addition, a significant association was also observed with 'playing with objects', which was highly correlated with 'off-task' behavior. Analysis with each of these factors showed that children with *Met-Met* and *Val-Met* genotypes were more

oriented to the assigned task, and were less distracted than children with the *Val-Val* genotype. To disentangle this association further, we examined the effect of *COMT* genotype on motor activity, as measured by the number of 15 s intervals where there was activity in the nondominant hand. No association was observed, which suggests that the *COMT Val*$^{108/158}$*Met* polymorphism modulates dimensions of task-oriented behavior other than motor hyperactivity.

We model these results on the two-compartment tonic-phasic hypothesis of dopamine regulation (Bilder *et al*, 2004). Tonic DA is the low, background level of extrasynaptic dopamine ($\sim$5–20 nM), which is regulated by baseline firing of the dopamine neurons, which in turn is regulated by inputs from glutamatergic afferents. Phasic DA, on the other hand, is the high-amplitude (μM concentrations), transient burst that occurs in response to a behavioral stimulus. Bilder *et al* (2004) have hypothesized that tonic DA regulates the stability of cortical activation states, by its effect on dopamine receptor D1 stimulation. Tonic D1 stimulation has thus been hypothesized to be important for maintaining stability by preventing 'uncontrolled, spontaneous switches into high-activity states (ie spontaneous activation of task-irrelevant representations)' (Durstewitz *et al*, 2000b). In contrast, phasic DA levels regulate the plasticity of these activation states, by D2 receptor function. Phasic DA levels are believed to be important for 'updating' information into the activation state.

The Val-containing COMT variant has been shown to have 3–4 times higher stability and enzymatic activity compared to the Met-containing variant (Chen *et al*, 2004; Lotta *et al*, 1995). In the PFC, COMT is critical in the methylation of dopamine, thereby regulating extracellular dopamine concentration. It is therefore expected that the Met-containing COMT variant would result in higher tonic DA in the PFC and concurrently higher stability of the activation state compared to the Val-containing COMT variant (Bilder *et al*, 2004). On a task like the RASS, which calls for stability of behavior over the course of the test period, children with the *Met-Met* genotype are therefore expected to have higher tonic DA, therefore performing better than children having the *Val-Val* genotype. This is congruent with our findings.

In this study, task-oriented behavior of the child was assessed in two treatment conditions, given placebo and MPH, administered in a double-blind manner. The acute effect, following administration of placebo or MPH, was measured by conducting the assessment before and 1 h after the treatment. MPH has been shown to increase the level of extracellular dopamine in the brain, by blocking the dopamine transporter (Krause *et al*, 2000; Volkow *et al*, 1998). Positron emission tomography studies using [^{11}C]-labeled MPH have shown that the peak brain concentration of MPH is achieved around 60 min after oral administration (Volkow *et al*, 1995). Hence the time frame (1 h after administration) used in this study should be appropriate for studying the acute effect of MPH treatment. It has also been demonstrated that the median effective dose, ie the dose required to block 50% of the dopamine transporter, is 0.25 mg/kg (Volkow *et al*, 1998). Thus at the dose administered in this study, at least 50% of the DAT is expected to be blocked.

Measurement of task-oriented behavior using the RASS showed a significant two-way interaction between treatment (MPH *vs* placebo) and administration (before *vs* after treatment). Placebo treatment resulted in a significant deterioration, whereas MPH treatment significantly improved task-oriented behavior. It has been previously shown that MPH increases the saliency of a mathematical task in healthy adult subjects (Volkow *et al*, 2004). The rating of a mathematical task as 'interesting', 'exciting', 'motivating', and 'less tiresome' significantly increased with MPH treatment. Together with these findings, our results suggest that in children with ADHD, the saliency of a mathematical task is diminished as a result of administration of placebo, possibly as a result of boredom due to repetition of the task. This boredom effect is dramatically reversed with MPH treatment, possibly as a result of increased dopamine neurotransmission in the PFC and subcortical regions of the brain.

Our results further suggest that the *COMT Val*$^{108/158}$*Met* polymorphism does not modulate response of task-oriented behavior with MPH treatment. Insight into the possible reasons for this lack of interaction may be derived from analysis of the behavior, and the brain regions that appear to be involved. Task-oriented behavior selection is defined as the 'selection of behaviors that are consistent with an organism's goals' (Chadderdon and Sporns, 2006). EF, which encapsulates higher-order neurocognitive control functions, is a key regulator of task-oriented behavior selection, thereby facilitating completion of the defined goal. These top-down cognitive processes maintain and update information about the task, task parameters, and sensory stimuli in working memory, integrate this knowledge into the current context so as to plan and organize the optimal action, facilitate self-initiation, regulate impulse and interference control, and vigilance to execute the task. The second component of task-oriented behavior is the selection of a motor program to generate coherent, coordinated behavior (Chadderdon and Sporns, 2006). Whereas the PFC is the brain region that is important for EF, the main loci for regulation of motor function are in the basal ganglia, including the striatum (Grillner *et al*, 2005).

Dopamine transporter and COMT play different roles for the clearance of DA in the PFC and striatum. DA transporter, which provides the best mechanism for clearance of DA, is widely distributed and strategically located in the striatum (Sesack *et al*, 1998). In contrast, it is far less abundant and less well situated in the PFC. Hence, the PFC is more dependent on secondary mechanisms for inactivating DA such as COMT. COMT accounts for $>$60% of total turnover of DA in the PFC, compared to 15% of the inactivation in the striatum (Karoum *et al*, 1994).

MPH specifically blocks the DA transporter (Krause *et al*, 2000; Volkow *et al*, 1998), and therefore it might be expected that treatment with MPH has a greater effect on the functions of the striatum compared to the PFC. In fact, positron emission tomography studies using [^{11}C]-labeled MPH have shown that the distribution of MPH in the brain is heterogeneous, with the maximum concentration observed in the striatum, and lower levels observed in the cortex, thalamus, and cerebellum (Volkow *et al*, 1995).

Given the findings of each of these studies, it is reasonable that independent effects of *COMT* genotype and MPH treatment on task-oriented behavior, and no interaction

effects, were observed. We hypothesize that COMT regulates one or more dimensions of EF, based on its important role in the PFC. MPH, on the other hand, independently modulates task-oriented behavior, with its action on the dopamine transporter in the striatum. Consistent with this hypothesis, MPH treatment showed an association with motor activity, whereas *COMT* genotype did not.

However, some limitations of the study also need to be considered. First, this study used a single dose (0.5 mg/kg per day) of MPH for the assessment of clinical response, which is in the low to medium range commonly used in clinical practice. Higher doses may be needed to explore fully the role of the *COMT Val*$^{108/158}$*Met* polymorphism in the modulation of behavioral response to MPH. Second, the study was based on evaluation of behavioral response for a 1-week period only. Although MPH exerts its effect rapidly after administration, its long-term effects may be different from its shorter-term effects. It remains to be seen whether children with different *COMT* genotypes benefit to the same extent from MPH in the long term. In addition, the study may be limited by the problem of multiple comparisons. The number of comparisons conducted with *COMT* genotype and the different dimensions on the RASS, as well as with motor activity, total 6. In applying the stringent Bonferroni correction, p would need to be <0.008 for an effect to be considered statistically significant. The association observed between *COMT* genotype and off-task behavior ($F_{2,184}=5.86$, $p=0.003$) and playing with objects ($F_{2,184}=6.1$, $p=0.003$) would therefore be of marginal significance. Therefore, it is imperative that these findings are replicated in an independent sample before definitive conclusions can be reached.

It is also noted that these results were obtained with children diagnosed with ADHD. It would be important to further determine if the association between task-oriented behavior and *COMT* genotype is specific to the ADHD disorder or is true of all children in the school-aged population. It may be predicted that the effect of instability would be greater with ADHD children, given their major deficits in task-orientation, such that small disruptions in DA levels are likely to have multiplicative effects on their capacity to orient to task. On the other hand, the impact of MPH treatment would likely obscure differences related to genotype, as the MPH-induced increase in DA levels would be substantially greater than the differences due to metabolism. Further, this study was conducted in ADHD children with Caucasian ethnicity. It is important to determine if similar effects of *COMT* genotype on task-oriented behavior are observed in other ethnic groups, before generalizations can be drawn.

In conclusion, this is the first study investigating the role of the *COMT Val*$^{108/158}$*Met* polymorphism in task-oriented, ecologically relevant behaviors related to ADHD. It also explores the role of this polymorphism in response of these behaviors to MPH in children with ADHD. To our knowledge, it is the largest study using the double-blind, placebo-controlled, crossover design for the evaluation of behavioral response to psychostimulants. In addition, the assessment tool used in this study was developed for children and was shown to be adequate for the evaluation of therapeutic response to medication in children with ADHD. The results of this study suggest that children with the *Val/Val* genotype demonstrate poor task-oriented behavior. In this study, we did not identify a gene by treatment interaction, suggesting that this polymorphism modulates behavior relevant for ADHD but not the response of this behavior to MPH. If these profiles are confirmed in a larger group of patients, this may help in understanding the pathogenesis of this very common childhood disorder.

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