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Dopamine Transporter 3′-UTR VNTR Genotype and ADHD: a Pharmaco-Behavioural Genetic Study with Methylphenidate

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We sought to test the hypothesis that the variable number of tandem repeat (VNTR) polymorphism in the 3′-untranslated region (3′-UTR) of the *SLC6A3* gene modulates behavior in children with ADHD and/or behavioral response to methylphenidate (MPH). One hundred and fifty-nine children with AHDH (6–12 years) were assessed with regard to the Conners' Global Index for parents (CGI-Parents) and teachers (CGI-Teachers) and the response of these behaviors to MPH (0.5 mg/kg/day) using a 2-week prospective within-subject (crossover) trial. Based on CGI-Parents, the profile of behavioral response to MPH as compared to placebo was not parallel in the three groups of children separated according to their genotype in the 3′-UTR VNTR polymorphism of *SLC6A3,* as indicated by a significant ($p = 0.017$) genotype by treatment two-way interaction. Individuals having the 9/10 and 10/10 genotypes displayed a significant positive response to MPH as opposed to those homozygous for the 9-repeat allele. No genotype or genotype by treatment interaction was observed for CGI-Teachers. These findings support a role for the DAT gene 3′-UTR VNTR polymorphism in modulating the response of some behavioral dimensions to MPH in children with ADHD. They also suggest the presence of genetic heterogeneity that could be indexed by the quality of behavioral response to MPH.
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INTRODUCTION

ADHD, one of the most prevalent conditions in child psychiatry, manifests as an unusually high and chronic level of inattention and/or impulsivity/hyperactivity. ADHD is estimated to occur in 8–12% of children worldwide (Biederman and Faraone, 2005).

There is strong evidence indicating that genetic factors play an important role in the pathogenesis of ADHD, with heritability estimates of approximately 76% (Biederman and Faraone, 2005). It is also well established that dopamine dysregulation is a significant contributor to the pathophysiology of ADHD. This is based mainly, but not solely, on the observation that agents increasing synaptic dopamine, such as methylphenidate (MPH), a drug that acts primarily by blocking the dopamine transporter, are effective in controlling ADHD symptoms (for a review see Biederman and Faraone, 2005). Consequently, the DAT gene (*SLC6A3*) is considered a prime candidate gene that may explain at least part of the susceptibility to ADHD or the variability in therapeutic response to MPH. One putatively functional polymorphism, a variable number of tandem repeat in the 3′ untranslated region of the *SLC6A3* (3′-UTR variable number of tandem repeat (VNTR)) has been the main focus of genetic and pharmacogenetic studies in ADHD.

Studies exploring the 3′-UTR VNTR in increasing risk for ADHD have led to variable results with some, but not all, reporting an association with the 10-repeat allele. The pooled odds ratio derived from family-based association studies was reported to be 1:13 with 95% confidence interval = [1.03–1.24] (Biederman and Faraone, 2005). Four published studies have investigated the relation between the 3′-UTR VNTR alleles/genotypes and therapeutic response to MPH (Winsberg and Comings, 1999; Roman *et al*, 2002; Kirley *et al*, 2003; Stein *et al*, 2005). Although each of these studies reported positive findings, there was no consistency with regard to the effect of each allele/genotype on

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therapeutic response. Briefly, while Winsberg and Comings (1999) and Roman *et al* (2002) reported a better therapeutic response in children with the 9/10 genotype compared to children with the 10/10 genotype, Kirley *et al* (2003) reported that transmission of the 10-repeat allele was significantly greater in children retrospectively judged as very good responders to MPH. Stein *et al* (2005) was the only group that analyzed the 9/9 genotype group separately from the two other genotype groups and found that this group is less sensitive to the effects of MPH, suggesting a recessive mode of action of the 9-repeat allele. Three other groups have studied the association between the response to MPH in children with ADHD and the 3′UTR VNTR in the DAT gene but the details of their results are not publicly available yet, although their design (one prospective and two retrospective open label studies) and findings (essentially negative) were reported by McGough in a recent review (McGough, 2005).

Thus, in spite of the plausibility of the hypothesis implicating the DAT gene in ADHD (Masellis *et al*, 2002), the literature is still not conclusive, possibly because of several limitations inherent to the complex and heterogeneous nature of ADHD, and methodological limitations in the assessment of therapeutic response to MPH, small sample sizes and inappropriate grouping of different genotypes.

Remarkably, to date, all studies investigating the *SLC6A3* in ADHD focused exclusively on its implication, either in the disease process ignoring its role in modulating behavioral response to psychostimulant drugs or vice versa. Because it is plausible that variation in behavioral response to psychostimulant drugs may reflect genetic heterogeneity, it may be crucial to control for this variability in order to better define the role of this gene in ADHD while conducting genetic studies. For example, it is possible that the subgroup of children responding very well to MPH may be related to genetic variations in the *SLC6A3* whereas the group of children responding poorly to MPH may be unrelated to genetic variations in this gene.

The main purpose of the present study is to investigate, concomitantly, the effects of the 3′-UTR VNTR polymorphism in the *SLC6A3* gene on behaviors relevant to ADHD and their response to MPH (0.5 mg/kg/day) using a double-blind placebo-controlled crossover trial. Under this general scheme, we formulated three different hypotheses: (1) *SLC6A3* 3′-UTR VNTR is implicated both in ADHD relevant behaviors and in the response of these behaviors to MPH, (2) *SLC6A3* 3′-UTR VNTR is implicated in ADHD relevant behaviors but not in the response of these behaviors to MPH, and (3) *SLC6A3* 3′-UTR VNTR is implicated in the response of ADHD relevant behaviors to MPH but not in these behaviors.

It is to be noted here that MPH is used in this context to probe behaviors relevant to ADHD and to study dynamically the relation of these behaviors with the *SLC6A3* 3′-UTR VNTR polymorphism. Although the results of this pharmaco-behavioral genetic study may inform pharmacogenetics (therapeutic response as relevant to clinical practice in relation to gene variations) of MPH in ADHD, further trials with different doses, different delivery schedules and longer exposure to MPH are needed to reach pertinent pharmacogenetics' conclusions.

PATIENTS AND METHODS

Patients

One hundred and fifty-nine children were recruited sequentially from the Disruptive Behavior Disorders Program and the child psychiatry outpatient clinic at the Douglas Hospital in Montreal. They were referred to these specialized care facilities by schools, community social workers, family doctors, and pediatricians.

To be included in this study, children were required to be between 6 and 12 years of age and to meet DSM-IV diagnostic criteria for ADHD (Lahey *et al*, 1994). Diagnosis was based on the observation of the child behavior and an interview with at least one parent by a child psychiatrist. This clinical examination was supplemented by a structured clinical interview of parents using the DISC-IV (parental report) (National Institute of Mental Health, 1998) as well as school reports. In majority of the cases, mothers were the primary informants. Parents completed the Child Behavioral Checklist (Achenbach, 1991), a scale that assesses several behavioral domains and the Conners' Global Index for parents (CGI-Parents) (Conners, 1999). Teachers were also asked to complete the Conners' Global Index for teachers (CGI-Teachers) (Conners, 1999). The CGI-Parents and CGI-Teachers are subsets of the original Conners' Rating Scales, widely used for assessing symptoms of ADHD and other psychopathology in children between 3 and 17 years of age, for which normative data have been well established (Conners, 1997). Each CGI scale consists of 10 items representing the Hyperactivity Index of the original Conners' scale. Each item describes a behavior that is rated on a 4-point Likert scale from 0 (not at all true) to 3 (very much true). CGI-Parents and CGI-Teachers are each comprised of two factors: 'Emotional lability' and 'Restless-impulsive behavior'. The raw total and factor scores are transformed into normalized T-scores, with 65 or higher considered to be clinically significant. This rating scale has been recommended for titrating and monitoring treatment with psychostimulant drugs (Conners, 1997). All these assessments were completed during the week preceding the clinical trial (ie, at baseline) while the children were not taking any medication.

Children having an IQ less than 70 or having a history of Tourette's syndrome, pervasive developmental disorder, psychosis or any medical condition, or impairment that would interfere with the ability of the child to complete the study were excluded.

Procedures

Once the children completed the baseline evaluations, a 2-week double-blind, placebo-controlled, within-subject (crossover) experimental design was used to assess the behavioral response to MPH as compared to placebo (hereafter referred to as MPH response). As the primary purpose of the present study was to investigate the effect of the *SLC6A3* genotype on the variability of behavioral response and not to study the efficacy of MPH *per se*, we used only one fixed and moderate dose (0.5 mg/kg/day) of MPH. This dose has been shown to have a significant

effect on behavior, often reaching clinical significance, although it may not be optimal for every subject (Schachter *et al*, 2001). This dose is also in keeping with the recommendations of initiating treatment with MPH at low to moderate dose and titrating to higher doses if the child does not respond adequately (American Academy of Pediatrics, 2001). After 1 week of baseline assessments, which also served as a wash-out period for children previously treated with MPH, subjects received 1 week of treatment with placebo and one week of treatment with 0.5 mg/kg of MPH in a divided dose (0.25 mg/kg morning and noon). The order of administration (placebo and MPH) was determined by random assignment.

Placebo and MPH were prepared individually in opaque gelatin capsules in weekly blister packs by a pharmacist not otherwise involved in the study to maintain the blind allocation of treatments. At the end of each week of treatment, the blister packs were collected and medication adherence checked. At the end of each week, of treatment assistant contacted the child's parents and teacher and asked them to fill the CGI-Parents and CGI-Teachers, respectively, taking into consideration the behavior of the child during the entire week of treatment (including weekends for parents).

The research protocol was approved by the Research Ethics Board of the Douglas Hospital. Parents were explained the study and they provided written consent. Children were explained the study and they gave their assent to participate.

Molecular Genetics

The 3′-UTR-VNTR polymorphism of the *SLC6A3* gene was genotyped using PCR amplification of DNA and resolution of different alleles on agarose gels according to previously published methods (Joober *et al*, 2000).

Statistical Analyses

To test the effects of genotype (9/9, 9/10, and 10/10 genotypes), treatment (placebo and MPH) and genotype-by-treatment interaction on the main outcome variables (CGI-Parents or CGI-Teachers ratings), we used mixed model analyses of variance (SAS MIXED procedure, SAS version 6.12, SAS Institute Inc., Cary, NC) (Littell *et al*, 2005).

Treatment, order of treatment, genotype, and treatment-by-genotype interaction were fixed effects; individuals were random effects. Main effects and any interactions were regarded as statistically significant when $p<0.05$. Baseline value of the outcome scores (CGI-Parents or CGI-Teachers ratings) were included as covariate (Senn, 2002). For significant genotype-by-treatment interaction, pairwise contrasts were carried out to explore how genotype and treatment interact. These contrasts were applied on change scores (CGI scores during placebo week – CGI scores during MPH week) and Tukey corrected.

Demographic and clinical characteristics for the three most common genotypes of *SLC6A3* 3′-UTR VNTR genotype groups were compared using ANOVA or χ^2 tests.

RESULTS

Four alleles each with 3, 9, 10, and 11 repeats were identified in this sample. Observed genotypes (*n*, frequency) were: 9/9 (14, 8.88%); 9/10 (63, 39.62%); 10/10 (74, 46.54%); 10/11 (5, 3.14%); 3/10 (2, 1.25%); and 9/11 (1, 0.6%). This distribution did not depart from the Hardy–Weinberg equilibrium ($\chi^2=3.25$, df = 10, $p=0.97$) and was similar to those reported in previous studies.

Because of the small number of children carrying the 3- and 11-repeat alleles and the absence of homozygous carriers for these alleles, we limited our subsequent analyses to the group of 151 children with 9/9, 9/10, and 10/10 genotypes.

Table 1 presents the clinical and demographic characteristics of children with ADHD separated according to their genotypes in the 3′-UTR VNTR of the *SLC6A3* gene. The three groups were similar with regard to their age, gender, ethnic composition, household income, and the severity of behavioral problems as assessed by the Child Behavioral Check List (CBCL) total, attention, internalization and externalization scores, and IQ. CGI-Parents and CGI-Teachers total and factor T-scores were also similar among the three groups at baseline evaluation. No significant differences between the groups, with regard to previous exposure to psychostimulant medication or diagnostic subtypes of ADHD (inattentive, hyperactive/impulsive, and combined types), were identified. The distribution of the most common comorbid disorders of ADHD (oppositional defiant disorder, conduct disorder, mood disorders, anxiety disorders including panic disorder, agoraphobia, or generalized anxiety disorder) were also similar between the three genotype groups.

Effect of 3′-UTR VNTR Polymorphism on CGI-Parents

The mixed model analysis of variance did not reveal significant main effects of the order of treatment ($F_{1,147}=0.01$, $p=0.94$), genotype ($F_{2,147}=1.78$, $p=0.17$) and treatment ($F_{1,147}=1.25$, $p=0.26$) but revealed a significant genotype by treatment two-way interaction [$F_{2,147}=8.42$, $p=0.017$]. CGI-Parents scores are plotted by treatment among children with the three genotypes to interpret the basis of the interaction (Figure 1a). Whereas CGI-Parents increased from 60.0 (clinically non-significant level) to 65.0 (clinically significant level) [$F_{1,13}=1.58$, $p=0.3$] in children with the 9/9 genotype, parents rating decreased very significantly in children with the 9/10 [$F_{1,62}=16.0$, $p=0.0001$] and 10/10 [$F_{1,73}=7.5$, $p=0.008$] genotypes.

We also calculated the change scores in CGI-Parents between the week of treatment with placebo and the week of treatment with MPH. These scores were subsequently analyzed using mixed model analysis of variance, which revealed a significant genotype effect [$F_{2,148}=4.13$, $p=0.016$] confirming that response to MPH was significantly different between the three genotype groups. *Post hoc* comparisons of the mean change scores using the Tukey honest significant difference test revealed a significantly higher improvement in children with the 9/10 (8.4 ± 16.7, $p=0.005$) and children with the 10/10 (4.8 ± 15.0, $p=0.038$) genotype groups compared to the group of children with the 9/9 genotype (-4.9 ± 17.3). There was no difference

Table 1 Baseline Characteristics of Children with ADHD Separated by their Genotype in the 3′-UTR VNTR Polymorphism of the *SLC6A3* Gene

	9/9 genotype ($n=14$)	9/10 genotype ($n=63$)	10/10 genotype ($n=74$)	Statistic and p-value
Males/females (% males)	13:1 (92.9)	53/10 (84.1)	63/11 (85.1)	$\chi^2=0.71$, df = 2, $p=0.70$
Age (years)	8.6 (1.5)	8.9 (1.7)	9.1 (1.8)	$F_{2,148}=0.79$, $p=0.45$
Household income (% <20.000 $ per year)	42.9%	47.5%	39.4%	$\chi^2=0.84$, df = 2, $p=0.65$
Ethnic origin (Caucasian/non-Caucasian)	14/0	56/7	67/7	$\chi^2=1.69$, df = 2, $p=0.43$
WISQ-III full scale IQ	102.6 (13.7)	95.5 (14.0)	98.9 (13.5)	$F_{2,125}=1.65$, $p=0.20$
CGI-Parents at baseline				
Total score	73.7 (11.0)	75.9 (11.3)	74.7 (8.4)	$F_{2,148}=0.39$, $p=0.67$
RI score	72.5 (11.1)	77.6 (10.3)	75.2 (8.0)	$F_{2,148}=1.24$, $p=0.29$
EL score	70.5 (12.7)	68.6 (13.5)	67.8 (12.0)	$F_{2,148}=0.27$, $p=0.76$
CGI-Teachers at baseline				
Total score	65.3 (11.7)	71.2 (11.6)	68.7 (11.4)	$F_{2,125}=2.32$, $p=0.10$
RI score	64.1 (9.3)	69.6 (10.8)	67.4 (10.5)	$F_{2,125}=1.59$, $p=0.20$
EL score	60.9 (15.5)	70.1 (15.8)	65.9 (15.3)	$F_{2,125}=2.16$, $p=0.11$
CBCL				
Total score	69.5 (10.10)	70.2 (8.30)	69.3 (10.12)	$F_{2,145}=0.16$, $p=0.85$
Attention score	69.0 (14.48)	70.2 (9.26)	70.5 (11.95)	$F_{2,145}=0.11$, $p=0.89$
Externalization score	71.9 (10.10)	71.0 (9.46)	69.0 (10.97)	$F_{2,145}=0.85$, $p=0.43$
Internalization score	64.6 (11.3)	64.2 (9.7)	64.6 (11.3)	$F_{2,145}=0.02$, $p=0.97$
Diagnosis C/I/H	6/6/2	43/14/6	37/27/10	$\chi^2=6.0$, df = 4, $p=0.20$
Comorbidity (%) with				
CD	35.7%	32.8 %	28.8%	$\chi^2=0.58$, df = 2, $p=0.74$
ODD	42.9%	42.6 %	39.7%	$\chi^2=0.13$, df = 2, $p=0.93$
AD	38.5%	29.4 %	30.6%	$\chi^2=0.40$, df = 2, $p=0.81$
MD	23.1%	5.9%	9.7%	$\chi^2=3.55$, df = 2, $p=0.17$
Previously medicated (%)	35.7%	51.7%	52.2 %	$\chi^2=1.33$, df = 2, $p=0.51$

Values are mean (SD). Demographic and clinical characteristics were compared between these groups using the appropriate statistics depending on the nature of the data. WISC = Wechsler Intelligence Scale for Children, 3rd edition; CBCL = child behavioral checklist; C/I/H = combined inattentive hyperactive; ODD = oppositional defiant disorder/conduct disorder; CD = conduct disorder, AD = anxiety disorder, MD = major mood disorders.

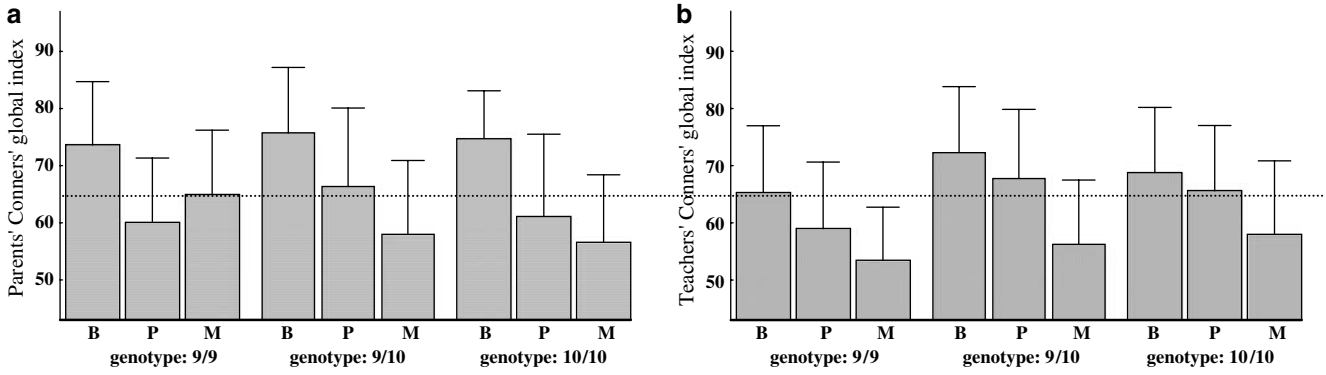

Figure 1 Conners' Global Index scores (±SD) for parents (a) and teachers (b) in children with ADHD separated according to their genotype in the 3′-UTR VNTR of the dopamine transporter gene during baseline evaluation (B), treatment with placebo (P) and treatment with methylphenidate (M). Dashed line represents the threshold for clinical significance on the Conners' scales (⩾65).

between the 9/10 and 10/10 genotype groups ($p=0.18$). The effect sizes (Cohen's d) of response to MPH as compared to placebo are presented in Table 2.

A similar analysis on change scores between baseline scores and scores during the week of treatment with placebo did not identify a genotype effect [$F_{2,148}=1.53$, $p=0.22$],

Table 2 Effect Sizes (Cohen's *d*) of Response to Methylphenidate Compared to Placebo as Measured by Parents' (CGI-Parents) and Teachers' (CGI-Teachers) Global Index in Children with ADHD Separated According to their Genotype in the 3′-UTR VNTR Polymorphism of the *SLC6A3*

	9/9	9/10	10/10
CGI-Parents	−0.43	0.62	0.36
CGI-Teachers	0.54	1.01	0.80

indicating that response to placebo as compared to baseline was not statistically significant, and response to placebo was similar in the three genotype groups.

Similar analyses were carried out for the two factors of CGI-Parents: 'restless-impulsive behavior' and 'emotional lability', and identified significant genotype by treatment two-way interactions for both dimensions (restless impulsive: $F_{2,147} = 3.78$, $p = 0.025$; emotional lability: $F_{2,147} = 3.16$, $p = 0.04$).

Effect of 3-UTR VNTR Polymorphism on CGI-Teachers

For the CGI-Teachers, the mixed model ANOVA revealed no genotype by treatment interaction [$F_{2,121} = 1.15$, $p = 0.31$], a highly significant treatment [$F_{1,121} = 18.69$, $p < 0.0001$] and no genotype [$F_{2,121} = 1.08$, $p = 0.33$] main effects (see Figure 1b). There was no order of treatment effect [$F_{1,121} = 0.54$, $p = 0.46$] (Figure 1b). Parallel results were identified when we analyzed both dimensions of CGI-Teachers separately.

DISCUSSION

Numerous studies have investigated the association between the 3′-UTR VNTR of the *SLC6A3* and ADHD. Overall, the findings follow a pattern of initial positive results that tend to fade overtime. This profile of results suggests that, if the effect of this gene in increasing the risk for ADHD is real, it is rather small and may be confounded by several factors, including the clinical characteristics that may vary from one sample to the other. It is therefore important to use different approaches to improve the detectance, or the predictive value of the observed phenotypes on the underlying genotypes (Terwilliger *et al*, 2002). This could be achieved by considering more refined quantitative phenotypes such as evaluations by parents and teachers separately, and by taking into consideration the modulation of these behaviors with a pharmacological probe, such as MPH, that is known to interact with the dopamine transporter. In contrast to a 'static' genetic investigation of behaviors, this 'dynamic' approach may help to reduce heterogeneity. Indeed, it is possible that, for any particular subject, a deviation of these behaviors from their normal level in the general population may be due to genetic variations in the *SLC6A3* and/or genetic variations in other genes and/or variations in environmental factors. Using MPH, previously shown to modulate these behaviors through the blockade of the dopamine transporter, may help to separate cases that are related to genetic variants in the *SLC6A3,* which are likely to respond to MPH, from those that are not related to this gene, which are likely to respond poorly to MPH. Under this dynamic pharamco-behavioral genetic approach, we investigated the joint effect of the *SLC6A3* 3′-UTR VNTR on CGI (parents and teachers), two well validated and normalized behavioral indices relevant for ADHD and the response of these indices to MPH.

The most important finding of this study is that the profile of behavioral response of children with ADHD to MPH as compared to placebo, and as evaluated by CGI-Parents is clearly distinguishable according to their genotypes in the 3′-UTR VNTR of the DAT gene. While children with the 9/10 and 10/10 genotypes improved their CGI-Parents when treated with MPH, children with the 9/9 genotype did not respond to MPH. These observations were valid for both the 'restless-impulsive' and 'emotional lability' factors of CGI-Parents.

Several studies investigated and reported the relation between therapeutic response to and the 3′-UTR VNTR in the *SLC6A3*. The earliest studies reported an association between homozygosity of the 10-repeat allele and poor response to MPH. In addition to being naturalistic, these studies were based on small numbers of patients and grouped patients with 9/9 and 9/10 genotypes (Winsberg and Comings, 1999; Roman *et al*, 2002). A subsequent retrospective naturalistic study conducted on a sample of 119 patients reported an overtransmission of the 10-repeat allele from parents to children with good response to MPH (Kirley *et al*, 2003). The most recent study included 47 children between 6 and 16 years of age and used a 4-week double-blind placebo-controlled crossover trial with weekly forced dosage change of OROS® MPH (18, 36 and 54 mg). In this study, patients homozygous for the 9-repeat allele ($n = 6$) did not display the usual dose-response curve on several outcome measures derived mainly from parental ratings (Stein *et al*, 2005). Interestingly, Lott *et al* (2005), in a double-blind placebo-controlled crossover designed study, found that healthy volunteers carrying the 9/9 genotype are less sensitive than carriers of the 9/10 and 10/10 genotype to the psychological effects of amphetamine, a drug with similar effects on the dopamine system as MPH. Taken together, results from these three placebo controlled studies, strongly suggest that carriers of the 9/9 genotype have low sensitivity to the effects of psychostimulant drugs.

The biological function of the 3′-UTR VNTR has been explored using several approaches. *In vivo* studies using imaging approaches (Heinz *et al*, 2000; Jacobsen *et al*, 2000; van Dyck *et al*, 2005) led to conflicting results (see Martinez *et al*, 2001 for a detailed discussion), which may be due to several methodological considerations, including the fact that the 9/9 genotype was very underrepresented in all the studies and was either excluded or grouped with the 9/10 genotype. Mill *et al* (2002) reported decreased levels of DAT1 mRNA levels in post-mortem brain tissue of patients having the 9/9 genotype, compared to the 9/10 and 10/10 genotypes. This result was consistent with an *in vivo* expression study, using the luciferase reporter system in COS-7 cells, which showed that the 7- and 9-repeat alleles had lower levels of transcription than the 10-repeat allele (Fuke *et al*, 2001), although opposite results using other

experimental systems were also reported (Miller and Madras, 2002). Further functional studies exploring the three genotypes separately and using homogenous populations are needed to resolve these inconsistencies.

We did not identify a genotype-by-treatment interaction on therapeutic response to MPH as assessed by teachers in the school environment. Although in apparent contradiction with the findings from parents' evaluation, this result could be interpreted by the fact that environmental factors and observer effects have an important impact on the child's behavior and its assessment (Stein, 2004). In fact, genetic epidemiological studies suggest that the genetic factors implicated in ADHD symptoms, as evaluated by parents and teachers are, to a certain extent, different (Sherman *et al*, 1997; Martin *et al*, 2002). Also, it has been well demonstrated that parents and teachers' ratings of improvement to MPH correlate modestly (Faraone *et al*, 2005) and differ in their magnitude (Schachter *et al*, 2001). Moreover, in case worsening, or where no improvement is reported by one reporter (parents or teachers), it is unlikely to be confirmed by the other (Faraone *et al*, 2005). Thus, it is possible that the 3′-UTR VNTR of the *SLC6A3* gene may be specifically modulating behavioral/therapeutic response to MPH as evaluated by parents in the home environment. It is also possible that the absence of genotype effect on teachers' evaluations is due to lack of statistical power. Indeed, as seen in Table 2, the effect size of response to MPH according to teachers' evaluation is, here again, smallest in the group of children with the 9/9 as compared to the two other genotype groups. Increasing the sample size and/or using higher doses and longer treatment may be needed to identify the genotype effect on teachers' ratings.

In this study, we did not identify a genotype effect of the 3′UTR VNTR polymorphism of the DAT gene on CGI-Parents and CGI-Teachers. Although a larger sample size may be needed to reach firm conclusions, this result suggests that this polymorphism does not modulate these behavioral dimensions of ADHD but modulates the response of these behavioral dimensions to MPH.

Finally, although we did not identify statistically significant differences with regard to comorbid disorders and clinical subtypes of ADHD between the three genotype groups, it is interesting to note that children with the 9/9 genotype who display poor response to MPH are 3–4 times more likely to be diagnosed with comorbid mood disorders, they are also more likely to be diagnosed with the inattentive subtype of ADHD. Given that the dopamine transporter gene has been previously associated with mood disorders (Kelsoe *et al*, 1996; Waldman *et al*, 1997; Greenwood *et al*, 2001), the clustering of comorbid mood disorder, inattention, and poor response to MPH in patients with the 9/9 genotype warrants further investigation.

Some limitations may influence the interpretation of the results from our study, particularly in relation to its potential extrapolation to clinical pharmacogenetics. First, this study used a single dose (0.5 mg/kg/day) of MPH, which is in the low to medium range commonly used in clinical practice. As our primary purpose was to use MPH as a pharmacological probe to challenge behaviors relevant to ADHD, we used a dose that is in the low/medium therapeutic range and that is recommended as a starting dose. However, in view of the observation that children with the 9/9 genotype increased, albeit nonsignificantly, their CGI-Parents scores under this dosage regimen, it is unlikely that increasing the dose will be helpful. In addition, Stein *et al* (2005) have shown that increasing the dose does not improve therapeutic response in this group of children. Nonetheless, an experimental design with several doses of MPH will be important to further explore the clinical implications of the present findings. Second, the study was based on evaluation of therapeutic response for a one week period only. Although MPH exerts its effect rapidly after administration, its long-term effects may be different from its shorter term effects (Denney, 2001). It remains to be seen whether children with different genotypes in the *SLC6A3* benefit to the same extent from MPH in the long-term. Third, side effects of MPH, which were systematically collected only in a subgroup of patients, are not presented in this report. These need to be analyzed and extended to a larger group in order to fully comprehend the clinical implications of this study. Fourth, because of the rarity of the 9/9 genotype in the general population, 14 patients with this genotype were recruited in our study. Although this is a relatively small sample, the concordance between our results and those of Stein *et al* (2005) suggests that the association between the 9/9 genotype and poor response to MPH is unlikely to be a chance finding due to small sample sizes. Nonetheless, increasing the sample size will be important in order to fully validate these results. Also, our sample comprised relatively few females. Separate analyses on males and females revealed essentially the same results in males but no significant findings in females. A larger sample needs to be studied in order to validate or refute these results in girls. Finally, the outcome measures used in this study (CGI-Parents and CGI-Teachers) may not assess inattention symptoms adequately compared to other evaluation scales. Future studies investigating the effect of the dopamine transporter 3′UTR VNTR on these symptoms and their response to MPH are warranted.

In conclusion, the data of the present study strongly suggest that children with the 9/9 genotype are less sensitive to the behavioral effects of MPH compared to children with the 9/10 and 10/10 genotypes. This finding has two implications for future research. First, variations in the *SLC6A3* may explain part of the variance of therapeutic response to MPH observed in children with ADHD. Second, children with the 9/9 genotype may represent a distinct group of ADHD where the symptoms are due to variations in other genes or environmental factors. Hence, the genotype in the *SLC6A3* gene may be used as a starting point to resolve the genetic heterogeneity of ADHD.

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