Brief Research Communication

Association of dopamine, serotonin, and nicotinic gene polymorphisms with methylphenidate response in ADHD†

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ABSTRACT

Gene polymorphisms of the 3′ untranslated region (3′-UTR) of the dopamine transporter (DAT1), Dopamine receptor exon 3 D4 variable number tandem repeat (DRD4 VNTR), nicotinic acetylcholine receptor alpha 4 subunit (CHRNA4) and serotonin transporter promoter (SLC6A4 5-HTTLPR) are under consideration as potential risk factors for attention-deficit/hyperactivity disorder (ADHD). A post-hoc attempt was made to investigate the association between the allelic variations of these candidate genes and retrospective parental report of response to methylphenidate in an ADHD-enriched, population-based twin sample. Subjects (N = 243) were selected from the twin sample based on parent report that the child had been treated with methylphenidate for ADHD symptoms. The functional polymorphisms screened were the VNTR located in the 3′-UTR of the dopamine transporter, DRD4 VNTR, CHRNA4 (rs1044396 and rs6090384) and the long (L A and L G) and short (S) forms of the serotonin transporter promoter region. Logistic regression did not demonstrate a significant association between methylphenidate treatment response and the relevant polymorphisms. The sample size had high power to detect effect sizes similar to those reported in some prior methylphenidate pharmacogenetic studies; however, the categorical (yes/no)
Pharmacogenetic investigations of attention-deficit/hyperactivity disorder (ADHD) are an extension of our understanding of the genetic basis of the disorder [McGough, 2005]. It is 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 [Biederman and Faraone, 2005].

Numerous candidate genes are associated with increased risk of ADHD, and many of these genes are related to dopaminergic function [Faraone et al., 2005]. This includes variable number tandem repeat polymorphisms in the 3′ untranslated region of the dopamine transporter gene (DAT1 3′-UTR VNTR), and within the dopamine D4 receptor gene (DRD4 VNTR).

Though less studied, there are several lines of evidence suggesting that the nicotinic system may also be functionally significant in ADHD. Kent et al. [[2001]] conducted the first study to test for linkage and association between the Cfol polymorphism within the nicotinic acetylcholine alpha 4-receptor gene (CHRNA4) and ADHD and found no evidence for association or linkage with ADHD using the TDT ($\chi^2 = 0.89, P = 0.35$). Todd et al. [[2003a]] conducted a TDT study using multiple CHRNA4 markers in a sample of 172 children with DSM-IV ADHD and reported significant evidence of over-transmission of the three SNPs identified in the exon/intron 2 region in ADHD ($\chi^2 = 9.12$, corrected $P = 0.011$), but they did not observe evidence of linkage between ADHD and haplotypes constructed from the exon 5 region polymorphisms.

Many of the ADHD candidate genes have been investigated due to presumed mechanisms of action of psychostimulants and it has been hypothesized that polymorphic variants in these same genes may also influence medication responses in individual patients [McGough et al., 2006]. The majority of published studies in ADHD pharmacogenetics have examined relationships between methylphenidate response and polymorphisms at the dopamine transporter gene (DAT1), although results are inconclusive [McGough, 2005; Levy, 2002]. Four published studies have investigated the relation between the DAT1 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 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, two other studies [Kirley et al., 2003; Stein et al., 2005] found better treatment response in patients homozygous for the 10-repeat allele. Kirley et al. [[2003]] reported that transmission of the 10-repeat allele was significantly greater ($P = 0.005$) 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 the homozygous 9-repeat allele had decreased response to MPH. Several studies found no effect of DAT1 [Hamarman et al., 2004; Langley et al., 2005; Van der Meulen et al., 2005; McGough et al., 2006].

Recently, Joober et al. [[2007]] sought to test the hypothesis that the VNTR polymorphism in the 3′-UTR of the DAT1 gene modulates behavior in children with ADHD and/or behavioral response to MPH in a 2-week prospective within-subject (crossover) trial. They demonstrated that 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.

The role of dopamine genes (DRD4 and DRD5) in treatment response has also been examined in ADHD. Consistent with in vitro studies showing that the 7-repeat (48-base pair) VNTR polymorphism in the coding region of DRD4 produces blunted response to dopamine [Van Tol et al., 1992; Asghari et al., 1995], Hamarman et al. [[2004]] found that one or two copies of the 7-repeat allele necessitated higher methylphenidate doses for optimal
symptom reduction. McGough et al. [[2006]] explored in preschoolers with ADHD whether genetic polymorphisms likely to be involved in stimulant mechanisms and ADHD risk may also underlie variability in medication response. Results indicated a significant association ($P = 0.05$) between symptom response and variants at DRD4. Seeger et al. [[2001]] examined interactive effects of the DRD4*7-repeat allele and the long (L allele) polymorphism of the transcriptional promoter region (5HTTLPR) of the serotonin transporter gene (SLC6A4) on treatment outcome by estimating the CGAS (Children’s Global Assessment Scale) improvement by genotype. Paired comparisons between the different genotypes through post-hoc testing revealed that subjects homozygous for the DRD4 7-repeat allele and the L form of the 5HTTLPR showed a significantly lower improvement in the CGAS compared to each of all other polymorphic combinations, with the exception of the combination non-DRD4*7/SS. The promoter region of the serotonin transporter has subsequently been shown to have a second SNP near or in the repeat region, which confers lower activity [Hu et al., [2004]].

Using retrospective data on methylphenidate treatment response from an epidemiological and clinically relevant naturalistic study of ADHD, the current report describes a post-hoc analysis of the association between methylphenidate treatment response and several gene polymorphisms that have previously shown association with ADHD diagnoses or methylphenidate treatment response. The Missouri Twin study is a large birth records based sample of twins born in the state of Missouri, which was enriched for the presence of ADHD symptoms. The study design and features of the sample are detailed in Neuman et al. [[2005]]. Twin pairs were selected for the study if parent response to a brief screening interview indicated endorsement of three or more present or past inattentive symptoms in at least one twin of the pair. The MAGIC (Missouri Assessment for Genetics Interview for children) interview was used to collect parental report on child psychopathology in 1,647 twins selected for the study. This interview has demonstrated excellent reliability for all DSM-IV diagnoses including ADHD (kappa 0.79-1.0 for both diagnosis and individual symptom endorsement) [Todd et al., [2003b]].

For the current analysis, subjects (N = 243) treated with methylphenidate for ADHD symptoms were selected from the larger sample of 1,647 twins. The twins (N = 243) were predominantly male (83.5%), with an average age of 12.8 ± 3.3 years (range 7-19 years) and mean WISC-III vocabulary scaled score of 8.31 ± 3.1. One hundred and eight of the 243 subjects (44.4%) had a DSM-IV diagnosis of ADHD by the interview. The remaining 135 subjects (55.6%) did not meet criteria for ADHD, but according to parent report were treated with methylphenidate for ADHD symptoms. Among those without DSM-IV ADHD, the mean DSM-IV ADHD symptom count ± SD was 9.45 ± 4.2 (range 1-18), indicating that on average these children had a high number of ADHD symptoms even though they did not meet formal diagnostic criteria (usually due to late age of onset, lack of significant impairment, and/or absence of six symptoms in either the inattentive or hyperactive-impulsive category). As described in a previous publication [Reich et al., [2006]], 85% of methylphenidate-treated children from this sample had improvement of ADHD symptoms after treatment. Response to treatment was based on parent report (yes/no) in the MAGIC-regarding improvement in ADHD symptoms after medication treatment. The Queries related to treatment response in the MAGIC included the following: “Did you ever take her/him to a doctor, a counselor, or some other professional person because of (ADHD) problems?”, “Did (whom child saw) give her/him any medication to help her/him with these (ADHD) problems?”, “Do you remember the name of the medication?”, “After (s)he started taking the medication, did these (ADHD) problems get any better?”. The dose of MPH and treatment related side effects were not included in the MAGIC interview.

Samples of DNA were obtained from families in which at least one twin had DSM-IV ADHD and complete diagnostic information was obtained on both twins. Therefore, genotyping data was not available for all the methylphenidate treated subjects. In the methylphenidate-treated group, 156 subjects were genotyped for DAT1, 159 subjects were genotyped for DRD4, 104 subjects were genotyped for the 5HTTLPR, and 109 subjects were genotyped for CHRNA4 markers.

Written informed consent (and assent for youth under age 18 years) was obtained from participants and legal guardians prior to participation. The Washington University School of Medicine Human Studies Committee approved the study protocol.

Genotyping of the DAT1 and DRD4 polymorphisms was performed as described by Cook et al. [[1995]] for the 3’ VNTR DAT polymorphism and as described by La Hoste et al. [[1996]] for the exon 3 48-base pair repeat of the DRD4 receptor gene, with minor modifications. Full details can be found in Todd et al. [[2001a], [b]]. The polymorphisms of the CHRNA4 (exon 5 SNP rs1044396, CHRNA4 intron 2 SNP rs6090384) described by Todd et al. [[2003a]] and the 5 HTT allelic variants (L4: long allele with ‘A’ nucleotide at the SNP in/near the insertion, L6: long allele with G nucleotide at the SNP, and S: short allele) described by Chorbov et al. [[2007]] were also genotyped.
Logistic regression was used to obtain odds ratios for treatment outcome based on genotype. Standard errors were adjusted to account for family clustering (non-independence of observations within twin pairs) using the “cluster” option available in STATA 9 (College Station, TX).

For the subset of subjects who were genotyped, Table I reflects the percentage of methylphenidate treated subjects responding to treatment by genotype. As seen in Table I, subjects tended to be treatment resistant if they were homozygous for the DAT1 10-repeat allele, homozygous for the CHRNA4 intron two 'G' allele, or heterozygous for the DRD4 7-repeat allele. In these cases, we obtained odds ratios for treatment failure based on homozygosity (or heterozygosity in the case of DRD4). None of these odds ratios were statistically significant (OR = 1.89, \( P = 0.24 \); OR = 1.90, \( P = 0.55 \); OR = 1.18, \( P = 0.76 \), respectively). Subjects homozygous for the 5 HTT-L\(_A\) or CHRNA4 exon 5 ‘C’ allele tended to improve more often with treatment. In these cases, we obtained odds ratios for improvement based on homozygosity, and these odds ratios were non-significant (OR = 1.48, \( P = 0.56 \); OR = 1.78, \( P = 0.48 \)). Due to the prior report of an interaction between DRD4 and 5 HTT genotypes in predicting treatment failure, we also tested a model that included DRD4 7-repeat and 5HTT genotypes plus their interaction, but no significant main or interaction effects were found.

Table I. Percentage of Methylphenidate Treated Subjects Responding to Treatment by Genotype

<table>
<thead>
<tr>
<th>Number of alleles present</th>
<th>DAT1 10-repeat, ( N = 156 )</th>
<th>5 HTT-L(_A), ( N = 104 )</th>
<th>CHRNA4 exon 5 rs1044396 ‘C’, ( N = 103 )</th>
<th>CHRNA4 intron 2 rs6090384 ‘G’, ( N = 109 )</th>
<th>DRD4 7-repeat, ( N = 159 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100% (n = 10)</td>
<td>85% (n = 27)</td>
<td>84% (n = 25)</td>
<td>100% (n = 2)</td>
<td>89% (n = 107)</td>
</tr>
<tr>
<td>1 (homozygous)</td>
<td>90% (n = 63)</td>
<td>85% (n = 48)</td>
<td>87% (n = 54)</td>
<td>100% (n = 8)</td>
<td>88% (n = 48)</td>
</tr>
<tr>
<td>2 (homozygous)</td>
<td>86% (n = 83)</td>
<td>90% (n = 29)</td>
<td>92% (n = 24)</td>
<td>86% (n = 99)</td>
<td>100% (n = 4)</td>
</tr>
</tbody>
</table>

We also looked for any differences in sex, age, or WISC-III vocabulary score (Table II) between the genotype groups that we used for calculation of odds ratios for treatment outcome based on genotype. Age and sex variables were available for all subjects, and the WISC-III vocabulary score was available for the majority of subjects. There was no significant relationship between genotype and sex, age, or IQ except in the case of the CHRNA4 gene. The mean WISC-III vocabulary scaled score in subjects homozygous for the CHRNA4 intron 2 ‘G’ allele was significantly higher than that of subjects with other genotypes (mean 8.42 vs. 5.38, \( P = 0.007 \)), but only 8 of the 10 non-homozygous subjects had scores on this measure. The group that was homozygous for the CHRNA4 exon 5 ‘C’ allele had a lower proportion of male subjects than the non-homozygous group (70.83% vs. 88.61% male, \( \chi^2 = 4.43, P = 0.035 \)).

Table II. Age, Sex, and WISC-III Vocabulary Scores by Genotype Group

<table>
<thead>
<tr>
<th>Genotype as categorized for calculation of OR for treatment outcome based on genotype</th>
<th>Percent male (n)</th>
<th>Mean age ( \text{Mean} \pm \text{SD} ) (n)</th>
<th>Mean WISC-III vocabulary scaled score ( \text{Mean} \pm \text{SD} ) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous for DAT1 10-repeat</td>
<td>78.31 (83)</td>
<td>13.42 ( \pm 3.31 ) (83)</td>
<td>8.57 ( \pm 3.02 ) (76)</td>
</tr>
<tr>
<td>Not homozygous for DAT1 10-repeat</td>
<td>86.30 (73)</td>
<td>12.60 ( \pm 3.30 ) (73)</td>
<td>8.33 ( \pm 3.20 ) (61)</td>
</tr>
<tr>
<td>Homozygous for CHRNA4 intron 2 “G” allele</td>
<td>82.83 (99)</td>
<td>12.82 ( \pm 3.43 ) (99)</td>
<td>8.42 ( \pm 3.00 ) (83)</td>
</tr>
<tr>
<td>Not homozygous for CHRNA4 intron 2 “G” allele</td>
<td>90.00 (10)</td>
<td>12.80 ( \pm 2.90 ) (10)</td>
<td>5.38 ( \pm 2.77 ) (8)</td>
</tr>
<tr>
<td>Heterozygous for DRD4 7-repeat allele</td>
<td>77.08</td>
<td>12.94 ( \pm 3.50 )</td>
<td>8.07 ( \pm 3.50 ) (41)</td>
</tr>
</tbody>
</table>
Not heterozygous for DRD4 7-repeat allele 86.49 (48) 13.00 ± 3.24 (111) 8.62 ± 2.84 (100)

Homozygous for five HTT-L_A allele 89.66 (29) 12.62 ± 3.41 (29) 7.58 ± 3.19 (26)

Not homozygous for 5 HTT- L_A allele 89.66 (75) 12.93 ± 3.31 (75) 8.52 ± 3.13 (64)

Homozygous for CHRNA4 exon 5 “C” allele 70.83 (24) 12.38 ± 3.32 (24) 7.53 ± 2.63 (19)

Not homozygous for CHRNA4 exon 5 “C” allele 88.61 (79) 12.85 ± 3.40 (79) 8.56 ± 3.13 (66)

Bold type indicates that there was a significant difference between the two genotype classes for the indicated gene.

We failed to replicate previously reported significant associations between ADHD treatment response and the studied genes. Our largest odds ratio was for the association between treatment failure and homozygosity for the DAT1 10-repeat allele. As discussed previously, there are opposing results in the ADHD pharmacogenetics literature on association of treatment outcome with the 10-repeat allele of the DAT1 gene. Although non-significant, the direction of our DAT1 finding is in agreement with previously reported data on poor outcome in subjects homozygous for the DAT 10-repeat allele. Two previous studies reported response rates of 31% and 47% for individuals with the 10/10 DAT genotype, compared to, respectively, 86% and 75% for those with other genotypes [Winsberg and Comings, [1999]; Roman et al., [2002]]. The power of our study (with a much larger sample size: 156, compared to 30 and 50 in previous studies) to detect differences of this magnitude is well over 90% at the 0.05 level of significance. The percentage of responders in individuals with the 10/10 genotype found in our study (86%) is highly significantly different from that observed in the other two studies combined (~40%; Chi-square 1 df = 28.7, P < 0.0001). Post-hoc power analyses suggest the present study would have 80% power to detect an association with the 10/10 genotype as long as the associated OR was 2.5-3.0 or higher, much lower than, for example, the OR of 13.7 reported in Winsberg and Comings [[1999]] study.

This study has some limitations. Our sample size was adequate to detect a significant association assuming odds ratios of the magnitude reported by studies such as Winsberg and Comings [[1999]]; however, it may be that our simple retrospective measure of treatment outcome resulted in high methylphenidate response rates in children who would have been non-responders by other outcome measures. These high response rates may have interfered with our ability to detect significant associations between treatment response and genotype. Also, parent reports of treatment response could not be corroborated by physician records, and compliance and medication dosage were not assessed. Similar studies using larger samples, consideration of comorbid disorders, quantitative measures of symptom improvement, and documentation of medication compliance are needed to determine whether true associations are present.

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REFERENCES


