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ORIGINAL ARTICLE

Pharmacogenetics of methylphenidate response and tolerability in attention-deficit/hyperactivity disorder

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Methylphenidate (MPH) is the most frequently used pharmacological treatment in children with attention-deficit/hyperactivity disorder. However, a considerable interindividual variability exists in clinical outcome, which may reflect underlying genetic influences. We analyzed 57 single-nucleotide polymorphisms in 9 dopamine-related candidate genes (*TH*, *DBH*, *COMT*, *DAT1* and *DRD1-5*) as potential predictors of MPH efficacy and tolerability, and we considered prenatal and perinatal risk factors as environmental hazards that may influence treatment effects in a gene-by-environment analysis. Our results provide evidence for the contribution of *DRD3* ($P = 0.041$; odds ratio (OR) = 4.00), *DBH* ($P = 0.032$; OR = 2.85), *TH* ($P = 5.5e\text{-}03$; OR = 4.34) and prenatal smoking ($P = 1.7e\text{-}03$; OR = 5.10) to the clinical efficacy of MPH, with a higher risk for treatment failure in genetically susceptible subjects whose mother smoked during pregnancy. Adverse events after MPH treatment were significantly associated with variation in *DBH* ($P = 6.4e\text{-}03$; OR = 0.28) and *DRD2* ($P = 0.047$; OR = 3.76). This study suggests that the dopaminergic system together with prenatal smoking exposure may moderate MPH treatment effects.

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INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a childhood-onset neuropsychiatric disorder that affects around 5% of children and adolescents worldwide[1] and causes high costs to the health-care system and society.[2]

Among the variety of pharmacological options available in ADHD treatment, methylphenidate (MPH) has shown to be generally effective in reducing ADHD symptoms and improving quality of life.[3] However, a considerable interindividual variability exists in clinical outcome, optimal dosage and duration of effect,[4,5] which may reflect underlying genetic influences.

Most of the pharmacogenetic studies conducted so far in ADHD patients have addressed MPH response, although genetic moderators of tolerability may be clinically more relevant than predictors of efficacy.[6] These investigations have mainly focused on genes related to the catecholamine neurotransmission, as MPH is thought to exert its therapeutic effects by increasing synaptic levels of dopamine through the inhibition of the dopamine transporter (DAT).[7] Thus the *DAT1* gene has long been considered a prime candidate that may contribute to the effectiveness and safety of MPH. Numerous studies have investigated the 40-bp variable number of tandem repeats (VNTR) polymorphism in the 3′-untranslated region of *DAT1* as a possible source of variation in clinical response to MPH, given its putative role in regulating mRNA stability, nuclear transport and protein synthesis.[8] However, the results published to date are controversial,[9–15] and a recent meta-analysis based on 16 studies reported no significant summary effect sizes.[16]

The involvement of the dopamine receptor D4 (DRD4) in treatment outcome has also been extensively examined, as it regulates dopamine synthesis, release and neuron-firing rate.[17] One of the most frequently studied polymorphisms is the 48-bp VNTR in exon 3 of *DRD4*. The seven-repeat allele, although consistently associated with ADHD,[18] has yielded disparate results in pharmacogenetic studies.[9,11,19,20] A second polymorphism in *DRD4*, consisting in a duplication of 120 bp in the promoter region, has been associated with improved response to MPH.[12]

Other investigations have evaluated the catechol-*O*-methyltransferase (COMT), which has a specific role in the catabolism of dopamine in the prefrontal cortex. The p.Val158Met polymorphism reduces the enzyme activity threefold[21] and may moderate MPH effects on ADHD symptoms.[11,12,22]

Few data are available regarding the impact of genetic variation in additional dopamine receptors or enzymes involved in the dopamine synthesis and degradation. Winsberg and Comings[23] attempted to relate the *DRD2* gene to the behavioral outcome of MPH therapy in 30 African–American ADHD children but no evidence of significant main effects was found while Tahir *et al.*[20] reported an association between a 151-bp allele of a microsatellite marker located 5′ from the *DRD5* gene and favorable response to MPH. Only Contini *et al.*[24] have examined the role of *DBH* as a moderator of treatment outcome among adult patients, although the findings were negative.

The majority of studies, however, have focused on a single or few polymorphisms that may not be functional, may be too distant from the true causal variant or may exert an effect too small to reliably detect an association with small sample sizes,[25]

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which could partially explain the absence of clear conclusions regarding MPH response. Distinct environmental influences that act to moderate the effects of genetic factors may also be responsible for discrepancies in the results.

Based on previous reports pointing to the involvement of genetic variants in the effectiveness and safety of MPH, we performed a pharmacogenetic study to evaluate the association of single-nucleotide polymorphisms (SNPs) across nine dopamine-related genes with therapeutic response and risk of side effects in a sample of ADHD pediatric subjects receiving MPH treatment. To our knowledge, this is the first study that (1) assesses multiple SNPs covering, in terms of linkage disequilibrium, the genes encoding the main components of the dopamine neurotransmitter system; (2) considers gene–gene interactions and (3) examines whether environmental risk factors interplay with dopaminergic candidate genes in predicting the response and adverse events to MPH.

MATERIALS AND METHODS

Subjects

The study sample included 107 ADHD pediatric patients for whom MPH was prescribed. Subjects were required to satisfy full Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria for ADHD, be <18 years of age, Spanish of Caucasian origin and have never been exposed to MPH. Patients with an Intelligent Quotient <70 or having pervasive developmental disorders were not eligible for the investigation. Additional exclusion criteria included schizophrenia or other psychotic disorders; adoption; sexual or physical abuse; birth weight <1.5 kg; any significant neurological or systemic disease that might explain ADHD symptoms; and clinical contra-indication to MPH. Comorbid oppositional defiant disorder, conduct disorder, depression and anxiety disorders were allowed unless determined to be the primary cause of ADHD symptomatology. The study was approved by the Ethics Committee of the Hospital Universitari Vall d'Hebron. Written informed consent was obtained from parents/caregivers.

Clinical assessment

Diagnosis of ADHD and comorbidities were established by child psychiatrists blind to patients' genotypes through the Present and Lifetime version of the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children. Furthermore, families were interviewed with the Clinical Global Impression-Severity scale (CGI-S). Additional information on clinical assessment is available in Supplementary Material.

Pharmacological intervention

Patients were treated according to the program's recommendations of initiating treatment with MPH at low-to-moderate dose and titrating to higher doses until no further clinical improvement or limiting adverse effects were observed. The mean daily dose of MPH prescribed was 1.07 mg kg^{-1} (s.d. = 0.30). Risperidone was the most frequent concomitant drug.

Treatment outcome

We considered the Improvement subscale of the Clinical Global Impression scale (CGI-I) as the primary outcome measure of treatment success. The CGI-S scale, applied at baseline and after 8 weeks of treatment, was used as a secondary outcome measure by both categorical and dimensional approaches for those risk variants associated with MPH response according to the CGI-I scale. Stimulants' side effects were elicited through systematic questions at each visit during the first 2 months of treatment. Patients and their parents were asked about the presence or absence of the 17 symptoms listed on the Side Effects Rating Scale developed by Barkley.[26] Any reported side effect was counted as positive, independently of its frequency or severity. Criteria used for therapeutic response evaluation can be found in Supplementary Material.

Environmental screening

Fourteen prenatal and perinatal risk factors were assessed during an interview with the mother in 86 study members (Supplementary Material).

Experimental procedures

The DNA isolation procedure is described elsewhere.[27] We examined nine candidate genes involved in dopaminergic neurotransmission that encode five dopamine receptors (*DRD1-5*), the dopamine transporter (*DAT1*) and three enzymes responsible for dopamine synthesis (*TH*) and degradation (*COMT* and *DBH*). The SNP selection strategy used was published previously.[28] All SNPs were genotyped using the SNPlex Genotyping Platform (Applied Biosystems, Foster City, CA, USA). Of the 69 SNPs initially chosen, 12 were discarded from the analysis (Supplementary Material).

Statistical analyses

The minimal statistical power was estimated using the Genetic Power Calculator software (http://pngu.mgh.harvard.edu/~purcell/gpc). Assuming a significance level of 0.05, the lowest minor allele frequency observed in our study (0.15), and a prevalence of 0.30 and 0.39, we were able to detect odds ratio values >2.5 for treatment failure and adverse events with moderate-to-high statistical power (76% and 89.9%, respectively) in a codominant model of inheritance.[29,30]

The presence of population stratification was previously discarded.[31] Potential confounders were included as covariates based on a statistical definition (association with both the study factor and outcome for $P \leqslant 0.20$).[32]

Genotype frequencies were tested for Hardy–Weinberg equilibrium ($P < 0.01$) using a Chi-square test. The effects of genetic polymorphisms on MPH response or adverse events were examined with the SNPassoc R package (http://www.cran.r-project.org/web/packages/SNPassoc). Those SNPs displaying nominal associations when analyzing genotypes under a codominant model or alleles were also studied under dominant and recessive models. The Bonferroni correction for multiple comparisons, considering 57 SNPs, the analysis of genotype and allele frequencies and two different outcomes (MPH response and adverse effects), establishes the significance threshold at $P \leqslant 2.2e\text{-}04$.

The haplotype-based association study was restricted to genes nominally associated with treatment outcome or adverse events in the single-marker analysis. The best two-marker haplotype from all possible combinations was identified for each of these genes. Additional markers (up to four) were added in a stepwise manner to the initial two-SNP haplotype and specific estimated haplotypes were subsequently assigned to individuals with the PHASE 2.0 software (http://stephenslab.uchicago.edu/software.html#phase). Significance was estimated using 10 000 permutations with the UNPHASED software (https://sites.google.com/site/fdudbridge/software). The best two-, three- or four-marker haplotypes identified were subsequently tested for association with the primary outcome measure of treatment success or adverse events using the statistical package SPSS 15.0 (SPSS, Chicago, IL, USA).

The gene-by-environment (G × E) analysis was restricted to sequence variants identified in the pharmacogenetic study and environmental risk factors associated with either clinical outcome or adverse events after Bonferroni correction (significance threshold at $P \leqslant 3.6e\text{-}03$, considering the 14 prenatal and perinatal risk factors assessed).

Pearson's Chi-square test, Fisher's exact test, Student's *t*-test or non-parametric Mann–Whitney *U*-test were applied, where appropriate. For additional data on statistical analyses, see Supplementary Material.

RESULTS

Subjects were predominantly male (88.8%), with an average age at assessment of 9.36 (s.d. = 2.76) years (range 5–16). Seventy-four percent of participants ($n = 79$) met DSM-IV criteria for ADHD-combined subtype, 20.6% ($n = 22$) had ADHD-inattentive subtype and 5.61% ($n = 6$) were diagnosed with ADHD-hyperactive-impulsive subtype. Comorbid disorders were present in a small number of patients (20.6%), the main ones being disabilities in reading and writing (9.35%), oppositional defiant disorder (6.54%) and anxiety disorders (1.87%).

Seventy-nine percent of subjects ($n = 84$) responded favorably to treatment according to the CGI-I scale, while 21.5% ($n = 23$) failed to show a clinical response to MPH. Responders and non-responders were comparable with regard to age, sex, ADHD subtype, comorbidity, use of concomitant medication, MPH dose and drug formulation ($P > 0.05$). There were significant differences, however, in the severity of symptoms as assessed by the CGI-S

Table 1. (a) Haplotype-based association study between 10 *DRD3* SNPs and methylphenidate response and (b) haplotype distributions of rs2134655 and rs1800828

(a)

	Methylphenidate response[b]		
Haplotype[a]	*Global* P-*value*	*Best haplotype-specific* P-*value (adjusted* P-*value)*	*OR (95% CI)*
2–9	**0.031**	**0.012 (0.028)**	**2.40 (1.20–4.80)**
2-3-9	0.062	9.5e-03 (0.043)	2.47 (1.23–4.95)

(b)

	Methylphenidate response[b]		
Haplotype[a]	*Non-responders*	*Responders*	*Haplotype-specific* P-*value; OR (95% CI)*
2–9			
A-G	7 (15.9)	53 (31.6)	0.040; 0.41 (0.17–0.98)[c]
G-C	8 (18.2)	40 (23.8)	—
G-G	29 (65.9)	75 (44.6)	0.012; 2.40 (1.20–4.80)
$\chi^2 = 6.94$; df = 2; $P = 0.031$			

Abbreviations: CI, confidence interval; *DRD3*, dopamine receptor D3; OR, odds ratio; SNP, single-nucleotide polymorphism. [a]2-rs2134655; 3-rs9880168; 9-rs1800828. Best multiple-marker combination in bold. [b]Defined by a Clinical Global Impression-Improvement score $\leqslant 2$. [c]Under-represented in non-responders.

scale ($P = 0.043$), with children resistant to MPH scoring higher at the baseline evaluation than children showing clinical improvement.

Regarding MPH tolerability, 65.1% of patients ($n = 69$) presented some treatment-related side effect, with insomnia (34.3%) and appetite reduction (25%) being the most prevalent ones. When subjects were divided according to the emergence of adverse events, no significant differences were found in any of the variables mentioned above, except for MPH dose ($P = 0.050$) and the rate of comorbid conditions ($P = 0.030$). MPH dose was significantly higher, whereas comorbid disorders were less frequent in children exhibiting side effects.

MPH response according to the CGI-I scale

The single-marker analysis identified three SNPs in *DRD3*, *DBH* and *TH* displaying a nominal association with the primary outcome measure: rs2134655 ($P = 0.022$; odds ratio (OR) = 3.03 (1.14–8.33)), rs2073833 ($P = 0.030$; OR = 6.25 (0.78–50)) and rs2070762 ($P = 0.020$; OR = 3.03 (1.19–7.69)), respectively (Supplementary Table S1). We included CGI-S baseline scores as a covariate in the analysis of rs2070762, as they were associated with both MPH response ($P = 0.043$) and rs2070762 ($P = 0.033$), and found that subjects homozygous for the rs2070762C allele, who started from lower CGI-S scores than carriers of the rs2070762T variant, had a poorer treatment response ($P = 5.5e\text{-}03$; OR = 4.34 (1.54–12.2)).

Consistent with the single-marker study, the multiple-marker analysis highlighted an association between clinical outcome and a two-marker haplotype in *DRD3* (rs2134655-rs1800828; global *P*-value = 0.031; Table 1a) with an overrepresentation of the rs2134655G–rs1800828G allelic combination in the non-responders group ($P_{adjusted} = 0.028$; OR = 2.40 (1.20–4.80); Table 1b). We then considered the frequency of the rs2134655G-rs1800828G carriers and confirmed the association between *DRD3* and treatment failure ($P = 0.041$; OR = 4.00 (0.87–18.4)). The haplotype-based analysis of *DBH* also revealed a two-marker haplotype associated with MPH response (rs1541332–rs2073833; global *P*-value = 0.042; Table 2a) with an overrepresentation of the rs1541332T–rs2073833C allelic combination among treatment-resistant patients ($P_{adjusted} = 0.049$; OR = 3.43 (1.59–7.40); Table 2b). An increased frequency of this risk haplotype carriers was observed in the non-responders group ($P = 0.032$; OR = 2.85 (1.07–7.62)).

The combined effect of the *TH* rs2070762C/C genotype and the risk haplotypes at *DRD3* (rs2134655G–rs1800828G) and *DBH* (rs1541332T–rs2073833C) accounted for 18.4% of the variability in MPH response. Additionally, we explored possible interactions between the markers identified and found no evidence supporting the existence of epistatic effects in the risk to develop treatment resistance.

MPH response according to the CGI-S scale

In order to demonstrate the robustness of our findings, we expanded our analysis to examine the effect of genetic variants in *DRD3*, *DBH* and *TH* associated with the primary outcome measure on MPH response using the CGI-S scale. In agreement with the results observed in CGI-I scores, subjects homozygous for the rs2070762C allele in the *TH* gene or carriers of the rs1541332T–rs2073833C allelic combination in *DBH* showed an increased risk for treatment failure when we considered an improvement of at least two points on the CGI-S scale at follow-up ($P = 0.017$; OR = 4.33 (1.21–15.5) and $P = 0.011$; OR = 4.58 (1.32–15.9), respectively). Additionally, a significant impact of s2070762 on the variation in CGI-S scores was identified, as patients homozygous for the rs2070762C allele exhibited a smaller symptom reduction than carriers of the T allele ($P = 7.1e\text{-}03$; CC genotype: mean score = 1.92 (s.d. = 0.82); CT/TT genotypes: mean score = 2.36 (s.d. = 0.69).

Adverse events after MPH treatment

Nominally significant differences were found for rs2007153 ($P = 0.016$; OR = 2.07 (1.15-3.73)) and rs2797855 ($P = 0.048$, under a codominant model) in *DBH* and rs2283265 ($P = 0.013$; OR = 4.31 (1.18–15.7)) within *DRD2* (Supplementary Table S1). Patients carrying the rs2283265T allele received a significantly higher MPH dose than subjects homozygous for the rs2283265G variant ($P = 0.061$) and had an increased risk for adverse

Table 2. (a) Haplotype-based association study between 12 *DBH* SNPs and methylphenidate response and (b) haplotype distributions of rs1541332 and rs2073833

(a)

	Methylphenidate response[b]		
Haplotype[a]	*Global* P-*value*	*Best haplotype-specific* P-*value (adjusted* P-*value)*	*OR (95% CI)*
5–10	**0.042**	**9.7e-03 (0.049)**	**3.43 (1.59–7.40)**
2–5–10	0.018	0.014 (0.085)	3.23 (1.47–7.06)

(b)

	Methylphenidate response[b]		
Haplotype[a]	*Non-responders*	*Responders*	*Haplotype-specific* P-*value; OR (95% CI)*
5–10			
C-C	12 (27.3)	73 (43.5)	—
C-G	9 (20.5)	22 (13.1)	—
T-C	15 (34.1)	22 (13.1)	9.7e-03; 3.43 (1.59–7.40)
T-G	8 (18.2)	51 (30.4)	—
$\chi^2 = 8.19$; df = 3; $P = 0.042$			

Abbreviations: CI, confidence interval; *DBH*, dopamine beta-hydroxylase; OR, odds ratio; SNP, single-nucleotide polymorphism. [a]2-rs2797851; 5-rs1541332; 10-rs2073833. Best multiple-marker combination in bold. [b]Defined by a Clinical Global Impression-Improvement score $\leqslant 2$.

Table 3. (a) Haplotype-based association study between 12 *DBH* SNPs and adverse effects and (b) haplotype distributions of rs2007153, rs2797853 and rs77905

(a)

	Adverse effects		
Haplotype[a]	*Global* P-*value*	*Best haplotype-specific* P-*value (adjusted* P-*value)*	*OR (95% CI)*
1–7	0.010	1.5e-03 (5.1e-03)	0.33 (0.18–0.63)
1–7–9	**0.028**	**1.4e-03 (0.027)**	**0.29 (0.14–0.61)**

(b)

	Adverse effects		
Haplotype[a]	*Presence*	*Absence*	*Haplotype-specific* P-*value; OR (95% CI)*
1–7–9			
A-G-C	14 (12.3)	23 (32.9)	1.4e-03; 0.29 (0.14–0.61)[b]
A-G-T	11 (9.6)	7 (10)	—
G-A-C	7 (6.1)	4 (5.7)	—
G-A-T	32 (28.1)	19 (27.1)	—
G-G-C	32 (28.1)	11 (15.7)	—
G-G-T	18 (15.8)	6 (8.6)	—
$\chi^2 = 12.5$; df = 5; $P = 0.028$			

Abbreviations: CI, confidence interval; *DBH*, dopamine beta-hydroxylase; OR, odds ratio; SNP, single-nucleotide polymorphism. [a]1-rs2007153; 7-rs2797853; 9-rs77905. Best multiple-marker combination in bold. [b]Under-represented in patients displaying adverse effects.

effects, considering MPH dose as a covariate ($P = 0.047$; OR = 3.76 (1.02–13.9)).

The multiple-marker analysis revealed a three-marker haplotype in *DBH* associated with the emergence of side effects (rs2007153–rs2797853–rs77905; global *P*-value = 0.028; Table 3a). The rs2007153A–rs2797853G–rs77905C haplotype was significantly more frequent in patients tolerating the medication ($P_{adjusted} = 0.027$; OR = 0.29 (0.14–0.61); Table 3b). In particular, carriers of this allelic combination were 61.9% less likely to present adverse events ($P = 0.020$). As comorbidity was associated with both adverse events ($P = 0.030$) and the *DBH* haplotype ($P = 0.154$), we adjusted the analysis for comorbid conditions and corroborated the protective effect of the rs2007153A–rs2797853G–rs77905C combination ($P = 6.4e\text{-}03$; OR = 0.28 (0.11–0.70)).

The additive effect of the *DBH* rs2007153A–rs2797853G–rs77905C haplotype and the rs2283265T allele in *DRD2* explained 15.7% of the phenotypic variance in MPH tolerability. Epistasis analysis, however, showed no evidence of gene–gene interactions between them.

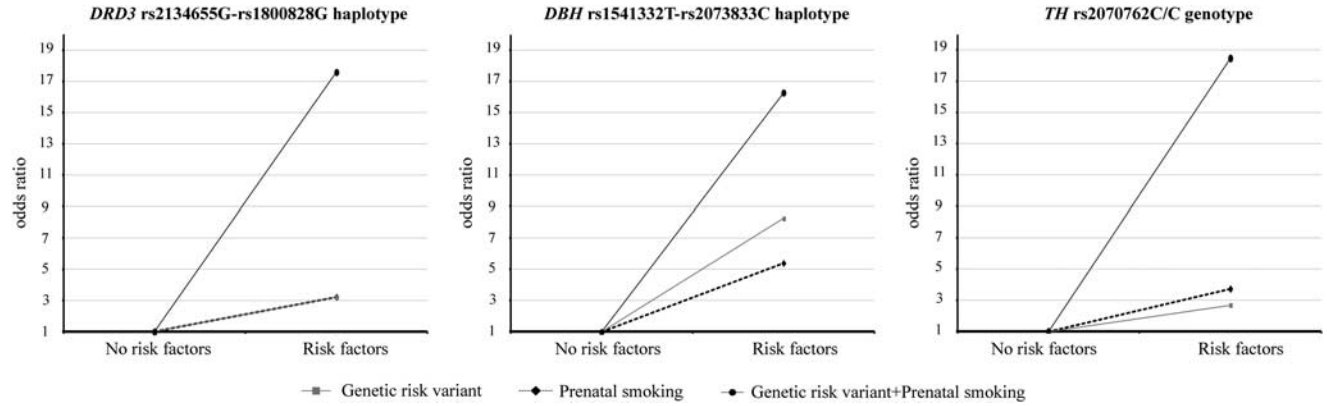

Figure 1. Gene-by-environment analysis between *DRD3*, *DBH*, *TH* and prenatal smoking on methylphenidate response according to the Clinical Global Impression-Improvement scale.

Gene-by-environment interactions

We subsequently examined the effect of 14 prenatal and perinatal risk factors on clinical outcome or adverse events and found association between prenatal smoking and MPH response according to the CGI-I scale ($P = 1.7e\text{-}03$). More than 31% of mothers ($n = 27$) reported smoking cigarettes during pregnancy, and their offspring had a poorer treatment response than those who were not prenatally exposed to nicotine (OR = 5.10 (1.76–14.8)).

Stratified analysis, based on maternal smoking and the presence of the risk variants identified in the pharmacogenetic study, disentangled significant interactions between prenatal smoking and *DRD3*, *DBH* or *TH* on MPH response. The risk for treatment failure was higher for carriers of the risk variants in *DRD3* (rs2134655G–rs1800828G haplotype; $P = 2.2e\text{-}03$; OR = 17.6 (1.96-157.9)), *DBH* (rs1541332T–rs2073833C haplotype; $P = 2.9e\text{-}04$; OR = 16.3 (2.79–94.7)) or *TH* (rs2070762C/C genotype; $P_{adjusted} = 7.5e\text{-}04$; OR = 18.5 (3.40–101.1)) whose mother smoked during pregnancy (Figure 1). The joint effect of *in utero* exposure to tobacco and the *DBH* rs1541332T–rs2073833C haplotype or the *TH* rs2070762C/C genotype on MPH response was also identified when assessed using the CGI-S scale according to the categorical approach. Additionally, the rs2070762 significantly interplayed with prenatal smoking on the variation in CGI-S scores (data not shown).

DISCUSSION

To our knowledge, this is the first study that examines multiple SNPs across genes encoding the main components of the dopaminergic system to identify genetic factors that moderate response variability in ADHD treatment. Specifically, we aimed to (1) identify genetic factors as potential predictors of MPH response; (2) identify genes involved in MPH tolerability; and (3) examine G × E interactions that may influence treatment effects. Our results provide preliminary evidence for the contribution of *DRD3*, *DBH*, *TH* and prenatal smoking to the clinical efficacy of MPH, with a higher risk for treatment failure in genetically susceptible subjects whose mother smoked during pregnancy. Additionally, we identified a significant association between variation in *DBH* and *DRD2* and adverse events after MPH treatment.

The role of *DBH* as a moderator of treatment outcome is supported by the association found with both MPH tolerability and response according to two different scales but contradicts a prior pharmacogenetic study.[24] Although the discrepancy may be attributed to differences in subjects' characteristics, therapeutic response evaluation or the polymorphisms assessed, we cannot exclude spurious positive signals, given our limited sample size. We also provide evidence for the involvement of *DRD3* in MPH response, which is in agreement with McCracken *et al*.,[33] who reported a relationship between *DRD3* and clinical improvement of hyperactive-impulsive behaviors in children with autism spectrum disorders (ASD). This previous investigation examined six SNPs within *DRD3*, including the rs2134655 variation highlighted in the present study, but no overlap between positive signals was identified across studies. As McCracken *et al*.[33] considered a sample of ASD subjects, these differences may be due to underlying disorder neurobiology. Another interesting finding arising from our research is the impact of rs2070762 in *TH* on both the responder status and symptom scores after MPH treatment. Although the current report is the first that examines the contribution of *TH* to the variability in treatment outcome, this polymorphism has previously been associated with ADHD[28] and evidence from *in vitro* assays suggests a potential regulatory activity for this intronic variant.[34] Interestingly, the enzymes encoded by *TH* and *DBH* are involved in the dopamine synthesis and dopamine conversion to norepinephrine, respectively, which suggests that functional variants within these genes may moderate dopamine availability at the synaptic cleft and therefore clinical outcome.

For the most part, pharmacogenetic research has focused on genes presumably related to the mechanism of action of MPH, namely *DAT1*, *DRD4* or *COMT*. The sequence variants assessed in the current investigation were selected to ensure full genetic coverage of candidate genes, not according to their potential functional implications. Thus we did not genotype individuals for previously studied markers such as the 40-bp VNTR in *DAT1* or the 48-bp VNTR in *DRD4*. However, none of the SNPs located in these genes displayed significant associations with either MPH response or side effects. This is in agreement with a prior meta-analysis and several individual studies reporting that the classic candidate genes in ADHD pharmacogenetics do not have an important role in treatment response or adverse events.[11,16,35] Conversely, we propose that variants in genes not extensively examined, but rational candidates given their distribution and regulatory functions in the dopamine neurotransmitter system, may influence the ability of MPH to exert its therapeutic effects.

Additionally, studies conducted so far in ADHD patients have emphasized the efficacy of MPH and have focused little on side effects. Our results suggest that variants in *DBH* and *DRD2* are associated with MPH tolerability. The putative impact of *DRD2* on adverse events is supported by previous findings from McCracken *et al*.,[33] who reported an association between *DRD2* and the rate of treatment discontinuation. Moreover, the rs2283265G allele, which increases mRNA expression of an alternative splicing variant of *DRD2*,[36] has recently been implicated in the emotion dysregulation experienced by children with ASD.[37] This is of particular interest, as increased emotionality and dysphoria occur

in a subgroup of MPH-treated patients with ADHD and there is evidence suggesting that they may be partially moderated by genetic influences.[38]

Considering that evidence of a direct relation between genetic polymorphisms and ADHD treatment has been inconsistent, we hypothesized that clinical response to MPH may be the result of a much more complex matrix of factors, including both genetic and environmental risks. Although epidemiological studies have demonstrated that exposure to various stressors, especially during pregnancy and delivery, such as tobacco smoking, alcohol consumption, stressful life events, and obstetrical complications are associated with an increased risk for ADHD,[39] to date only Grizenko *et al*.[40] examined whether the severity of maternal stress affected the children's response to MPH. The authors did not find significant differences in the child's responder status, suggesting that it may not be related to early environmental factors. In contrast, we revealed a strong association between prenatal smoking and clinical outcome, where children whose mother reported smoking cigarettes during pregnancy showed a poorer treatment response than those who were not exposed. The subsequent G × E analysis unraveled significant epistatic effects between prenatal smoking and *DRD3*, *DBH* or *TH* on MPH response. Similarly, earlier investigations have demonstrated that polymorphisms in dopamine-related genes act in conjunction with maternal smoking to modify the risk and severity of ADHD,[41,42] but no other study has explored G × E interactions between them with regard to MPH response. A prior report, however, assessed the joint effect of SNPs in the *LPHN3* gene and maternal smoking or stress during pregnancy in a double-blind, placebo-controlled, cross-over trial.[43] Choudhry *et al*.[43] provided evidence for a significant association between *LPHN3* and treatment response, which only became apparent in the group where mothers experienced mild or minimal stress. In contrast, a complete lack of interaction was noted with *in utero* exposure to tobacco.

The main strengths of our findings include (1) the recruitment of stimulant-naive patients; (2) the exclusion of confounding population substructures; (3) the full coverage of the dopaminergic genes assessed in terms of linkage disequilibrium; (4) the assessment of both efficacy and side effects and the use of different outcome measures of treatment success; and (5) the evaluation of gene–gene and G × E interactions. Some methodological aspects, however, should also be considered when interpreting the results of the current study: (1) Our estimate of a 20.6% comorbidity rate is in contrast with the high prevalence reported in the literature, where approximately two-thirds of ADHD children have impairing comorbid diagnoses.[44–46] The clinical sample was composed of Spanish ADHD children who had never been exposed to stimulant medication and with an average age at assessment of 9.36 years, which could explain the small number of patients with comorbidity. Although modest, this proportion may influence treatment outcome and thus the presence of comorbid disorders has been considered as a potential confounder. (2) The naturalistic design may have limited the power of our analyses. However, the rate of response to MPH was similar to that generally found in placebo-controlled clinical trials, and we minimized the probability that differences between carriers and non-carriers of the studied alleles might be attributed to other factors by performing a comprehensive assessment of potential confounders. (3) The lack of a strictly standardized medication titration, although patients were treated in a comparable manner by the same experienced psychiatrists. (4) MPH was administered with no control of adherence by clinicians. (5) Information on environmental risk factors was collected retrospectively and may therefore be subject to recall bias. (6) Other prenatal factors, such as maternal stress during pregnancy, or postnatal factors that we did not examine may influence the efficacy or tolerability of MPH treatment. (7) Variability in genes that are known to interact with the dopaminergic system has not been considered. In this sense, *LPHN3* represents a very promising candidate, as it has shown to confer susceptibility to ADHD and moderate MPH response, either alone or in conjunction with environmental factors.[43,47–52]

In conclusion, despite the relatively limited sample size, we identified *DRD3*, *DBH*, *TH*, *DRD2* and prenatal exposure to nicotine as potential predictors of MPH treatment effects. Further pharmacogenetic studies with larger samples are required to fully validate these results and to disentangle the impact of prenatal smoking on clinical response to MPH in genetically susceptible children.

CONFLICT OF INTEREST

Professor Casas has received travel grants and research support from Eli Lilly, Janssen-Cilag, Shire and Laboratorios Rubió. He has been on the advisory board and served as a consultant for Eli Lilly, Janssen-Cilag, Shire and Laboratorios Rubió. Dr Ramos-Quiroga has served on the speakers' bureau and acted as consultant for Eli Lilly, Janssen-Cilag, Novartis, Lundbeck, Shire, Ferrer and Laboratorios Rubió. He has received travel awards from Eli Lilly, Janssen-Cilag and Shire for participating in psychiatric meetings. The ADHD Program chaired by Dr Ramos-Quiroga has received unrestricted educational and research support from Eli Lilly, Janssen-Cilag, Shire, Rovi and Laboratorios Rubió in the past 2 years. The other authors declare no conflict of interest.

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REFERENCES

1 Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA. The worldwide prevalence of ADHD: a systematic review and metaregression analysis. *Am J Psychiatry* 2007; **164**: 942–948.
2 Pelham WE, Foster EM, Robb JA. The economic impact of attention-deficit/hyperactivity disorder in children and adolescents. *J Pediatr Psychol* 2007; **32**: 711–727.
3 Greenhill L, Beyer DH, Finkleson J, Shaffer D, Biederman J, Conners CK *et al.* Guidelines and algorithms for the use of methylphenidate in children with Attention-Deficit/Hyperactivity Disorder. *J Atten Disord* 2002; **6** (Suppl 1): S89–S100.
4 Charach A, Ickowicz A, Schachar R. Stimulant treatment over five years: adherence, effectiveness, and adverse effects. *J Am Acad Child Adolesc Psychiatry* 2004; **43**: 559–567.
5 Wolraich ML, Doffing MA. Pharmacokinetic considerations in the treatment of attention-deficit hyperactivity disorder with methylphenidate. *CNS Drugs* 2004; **18**: 243–250.
6 Polanczyk G, Bigarella MP, Hutz MH, Rohde LA. Pharmacogenetic approach for a better drug treatment in children. *Curr Pharm Des* 2010; **16**: 2462–2473.
7 Wilens TE. Effects of methylphenidate on the catecholaminergic system in attention-deficit/hyperactivity disorder. *J Clin Psychopharmacol* 2008; **28** (Suppl 2): S46–S53.
8 VanNess SH, Owens MJ, Kilts CD. The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. *BMC Genet* 2005; **6**: 55.
9 Froehlich TE, Epstein JN, Nick TG, Melguizo Castro MS, Stein MA, Brinkman WB *et al.* Pharmacogenetic predictors of methylphenidate dose-response in attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 2011; **50**: 1129–1139.e1122.
10 Joober R, Grizenko N, Sengupta S, Amor LB, Schmitz N, Schwartz G *et al.* Dopamine transporter 3'-UTR VNTR genotype and ADHD: a pharmaco-behavioural

genetic study with methylphenidate. *Neuropsychopharmacology* 2007; **32**: 1370–1376.
11 Kereszturi E, Tarnok Z, Bognar E, Lakatos K, Farkas L, Gadoros J *et al.* Catechol-O-methyltransferase Val158Met polymorphism is associated with methylphenidate response in ADHD children. *Am J Med Genet B Neuropsychiatr Genet* 2008; **147B**: 1431–1435.
12 McGough JJ, McCracken JT, Loo SK, Manganiello M, Leung MC, Tietjens JR *et al.* A candidate gene analysis of methylphenidate response in attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 2009; **48**: 1155–1164.
13 Purper-Ouakil D, Wohl M, Orejarena S, Cortese S, Boni C, Asch M *et al.* Pharmacogenetics of methylphenidate response in attention deficit/hyperactivity disorder: association with the dopamine transporter gene (SLC6A3). *Am J Med Genet B Neuropsychiatr Genet* 2008; **147B**: 1425–1430.
14 Stein MA, Waldman ID, Sarampote CS, Seymour KE, Robb AS, Conlon C *et al.* Dopamine transporter genotype and methylphenidate dose response in children with ADHD. *Neuropsychopharmacology* 2005; **30**: 1374–1382.
15 Tharoor H, Lobos EA, Todd RD, Reiersen AM. Association of dopamine, serotonin, and nicotinic gene polymorphisms with methylphenidate response in ADHD. *Am J Med Genet B Neuropsychiatr Genet* 2008; **147B**: 527–530.
16 Kambeitz J, Romanos M, Ettinger U. Meta-analysis of the association between dopamine transporter genotype and response to methylphenidate treatment in ADHD. *Pharmacogenomics J* 2014; **14**: 77–84.
17 Heckers S, Konradi C. Synaptic function and biochemical neuroanatomy. In: Martin A, Scahill L, Charney DS, Leckman JF (eds). *Pediatric Psychopharmacology: Principles and Practice*. Oxford University Press: New York, USA, 2003, pp 20–32.
18 Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA *et al.* Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2005; **57**: 1313–1323.
19 Cheon KA, Kim BN, Cho SC. Association of 4-repeat allele of the dopamine D4 receptor gene exon III polymorphism and response to methylphenidate treatment in Korean ADHD children. *Neuropsychopharmacology* 2007; **32**: 1377–1383.
20 Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. *Mol Psychiatry* 2000; **5**: 396–404.
21 Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996; **6**: 243–250.
22 Cheon KA, Jun JY, Cho DY. Association of the catechol-O-methyltransferase polymorphism with methylphenidate response in a classroom setting in children with attention-deficit hyperactivity disorder. *Int Clin Psychopharmacol* 2008; **23**: 291–298.
23 Winsberg BG, Comings DE. Association of the dopamine transporter gene (DAT1) with poor methylphenidate response. *J Am Acad Child Adolesc Psychiatry* 1999; **38**: 1474–1477.
24 Contini V, Victor MM, Bertuzzi GP, Salgado CA, Picon FA, Grevet EH *et al.* No significant association between genetic variants in 7 candidate genes and response to methylphenidate treatment in adult patients with ADHD. *J Clin Psychopharmacol* 2012; **32**: 820–823.
25 Mick E, Neale B, Middleton FA, McGough JJ, Faraone SV. Genome-wide association study of response to methylphenidate in 187 children with attention-deficit/hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 2008; **147B**: 1412–1418.
26 Barkley RA, McMurray MB, Edelbrock CS, Robbins K. Side effects of methylphenidate in children with attention deficit hyperactivity disorder: a systemic, placebo-controlled evaluation. *Pediatrics* 1990; **86**: 184–192.
27 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215.
28 Ribasés M, Ramos-Quiroga JA, Hervás A, Sánchez-Mora C, Bosch R, Bielsa A *et al.* Candidate system analysis in ADHD: evaluation of nine genes involved in dopaminergic neurotransmission identifies association with DRD1. *World J Biol Psychiatry* 2012; **13**: 281–292.
29 Didoni A, Sequi M, Panei P, Bonati M. One-year prospective follow-up of pharmacological treatment in children with attention-deficit/hyperactivity disorder. *Eur J Clin Pharmacol* 2011; **67**: 1061–1067.
30 Spencer T, Biederman J, Wilens T, Harding M, O'Donnell D, Griffin S. Pharmacotherapy of attention-deficit hyperactivity disorder across the life cycle. *J Am Acad Child Adolesc Psychiatry* 1996; **35**: 409–432.
31 Ribasés M, Ramos-Quiroga JA, Hervás A, Bosch R, Bielsa A, Gastaminza X *et al.* Exploration of 19 serotoninergic candidate genes in adults and children with attention-deficit/hyperactivity disorder identifies association for 5HT2A, DDC and MAOB. *Mol Psychiatry* 2009; **14**: 71–85.
32 Maldonado G, Greenland S. Simulation study of confounder-selection strategies. *Am J Epidemiol* 1993; **138**: 923–936.
33 McCracken JT, Badashova KK, Posey DJ, Aman MG, Scahill L, Tierney E *et al.* Positive effects of methylphenidate on hyperactivity are moderated by monoaminergic gene variants in children with autism spectrum disorders. *Pharmacogenomics J* 2014; **14**: 295–302.
34 Wang L, Li B, Lu X, Zhao Q, Li Y, Ge D *et al.* A functional intronic variant in the tyrosine hydroxylase (TH) gene confers risk of essential hypertension in the Northern Chinese Han population. *Clin Sci (Lond)* 2008; **115**: 151–158.
35 Zeni CP, Guimaraes AP, Polanczyk GV, Genro JP, Roman T, Hutz MH *et al.* No significant association between response to methylphenidate and genes of the dopaminergic and serotonergic systems in a sample of Brazilian children with attention-deficit/hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 2007; **144B**: 391–394.
36 Zhang Y, Bertolino A, Fazio L, Blasi G, Rampino A, Romano R *et al.* Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. *Proc Natl Acad Sci USA* 2007; **104**: 20552–20557.
37 Gadow KD, Pinsonneault JK, Perlman G, Sadee W. Association of dopamine gene variants, emotion dysregulation and ADHD in autism spectrum disorder. *Res Dev Disabil* 2014; **35**: 1658–1665.
38 Gruber R, Joober R, Grizenko N, Leventhal BL, Cook EH Jr., Stein MA. Dopamine transporter genotype and stimulant side effect factors in youth diagnosed with attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol* 2009; **19**: 233–239.
39 Ben Amor L, Grizenko N, Schwartz G, Lageix P, Baron C, Ter-Stepanian M *et al.* Perinatal complications in children with attention-deficit hyperactivity disorder and their unaffected siblings. *J Psychiatry Neurosci* 2005; **30**: 120–126.
40 Grizenko N, Shayan YR, Polotskaia A, Ter-Stepanian M, Joober R. Relation of maternal stress during pregnancy to symptom severity and response to treatment in children with ADHD. *J Psychiatry Neurosci* 2008; **33**: 10–16.
41 Kahn RS, Khoury J, Nichols WC, Lanphear BP. Role of dopamine transporter genotype and maternal prenatal smoking in childhood hyperactive-impulsive, inattentive, and oppositional behaviors. *J Pediatr* 2003; **143**: 104–110.
42 Neuman RJ, Lobos E, Reich W, Henderson CA, Sun LW, Todd RD. Prenatal smoking exposure and dopaminergic genotypes interact to cause a severe ADHD subtype. *Biol Psychiatry* 2007; **61**: 1320–1328.
43 Choudhry Z, Sengupta SM, Grizenko N, Fortier ME, Thakur GA, Bellingham J *et al.* LPHN3 and attention-deficit/hyperactivity disorder: interaction with maternal stress during pregnancy. *J Child Psychol Psychiatry* 2012; **53**: 892–902.
44 Elia J, Ambrosini P, Berrettini W. ADHD characteristics: I. Concurrent co-morbidity patterns in children & adolescents. *Child Adolesc Psychiatry Ment Health* 2008; **2**: 15.
45 Klassen AF, Miller A, Fine S. Health-related quality of life in children and adolescents who have a diagnosis of attention-deficit/hyperactivity disorder. *Pediatrics* 2004; **114**: e541–e547.
46 Larson K, Russ SA, Kahn RS, Halfon N. Patterns of comorbidity, functioning, and service use for US children with ADHD, 2007. *Pediatrics* 2011; **127**: 462–470.
47 Acosta MT, Velez JI, Bustamante ML, Balog JZ, Arcos-Burgos M, Muenke M. A two-locus genetic interaction between LPHN3 and 11q predicts ADHD severity and long-term outcome. *Transl Psychiatry* 2011; **1**: e17.
48 Arcos-Burgos M, Jain M, Acosta MT, Shively S, Stanescu H, Wallis D *et al.* A common variant of the latrophilin 3 gene, LPHN3, confers susceptibility to ADHD and predicts effectiveness of stimulant medication. *Mol Psychiatry* 2010; **15**: 1053–1066.
49 Bruxel EM, Salatino-Oliveira A, Akutagava-Martins GC, Tovo-Rodrigues L, Genro JP, Zeni CP *et al.* LPHN3 and attention-deficit/hyperactivity disorder: a susceptibility and pharmacogenetic study. *Genes Brain Behav* 2015; **14**: 419–427.
50 Labbe A, Liu A, Atherton J, Gizenko N, Fortier ME, Sengupta SM *et al.* Refining psychiatric phenotypes for response to treatment: contribution of LPHN3 in ADHD. *Am J Med Genet B Neuropsychiatr Genet* 2012; **159B**: 776–785.
51 Lange M, Norton W, Coolen M, Chaminade M, Merker S, Proft F *et al.* The ADHD-susceptibility gene lphn3.1 modulates dopaminergic neuron formation and locomotor activity during zebrafish development. *Mol Psychiatry* 2012; **17**: 946–954.
52 van der Voet M, Harich B, Franke B, Schenck A. ADHD-associated dopamine transporter, latrophilin and neurofibromin share a dopamine-related locomotor signature in Drosophila. *Mol Psychiatry* 2015; doi:10.1038/mp.2015.55.

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