

Association of the Adrenergic α 2A Receptor Gene With Methylphenidate Improvement of Inattentive Symptoms in Children and Adolescents With Attention-Deficit/Hyperactivity Disorder

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Context: Preclinical studies have demonstrated the relevance of adrenergic α 2A receptor on the attentional process and the mechanism of action of methylphenidate hydrochloride. Several molecular genetic investigations suggest a role for the adrenergic α 2A receptor gene (*ADRA2A*) in attention-deficit/hyperactivity disorder (ADHD), especially in the inattentive dimension. However, the effect of *ADRA2A* in the response to methylphenidate in humans has not been previously investigated, to our knowledge.

Objective: To evaluate the association between the *ADRA2A* -1291 C>G polymorphism and the clinical response to methylphenidate treatment in children and adolescents with ADHD.

Design: A pharmacogenomic study was undertaken between November 1, 2002, and May 1, 2004, using a non-random assignment, quasi-experimental design.

Setting: An ADHD outpatient program at a university hospital in Brazil.

Patients: One hundred six patients consecutively diagnosed as having ADHD were genotyped for the *ADRA2A* -1291 C>G polymorphism and were included in the analyses.

Intervention: Short-acting methylphenidate adminis-

tered in increasing dosages until no further clinical improvement was detected or until limited adverse effects occurred.

Main Outcome Measures: The primary outcome measure was the parent-rated inattentive subscale of the Swanson, Nolan, and Pelham Scale version IV. Secondary outcome measures included the Barkley Side Effect Rating Scale and the parent-rated hyperactivity-impulsivity subscale of the Swanson, Nolan, and Pelham Scale version IV. Scales were applied by child psychiatrists blinded to genotype at baseline and at 1 and 3 months of treatment.

Results: A significant interaction effect between the presence of the G allele and treatment with methylphenidate over time on inattentive scores was detected during the 3 months of treatment ($n=106$; $F_{2,198}=4.30$; $P=.02$).

Conclusions: We documented the effect of the G allele at the *ADRA2A* -1291 C>G polymorphism on the improvement of inattentive symptoms with methylphenidate treatment in children and adolescents with ADHD. Our findings provide clinical evidence for the involvement of the noradrenergic system in the modulation of methylphenidate action.

Arch Gen Psychiatry. 2007;64:218-224

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METHYLPHENIDATE HYDROCHLORIDE has been used for the treatment of inattention and hyperactivity for more than 50 years.¹ Subjects with attention-deficit/hyperactivity disorder (ADHD) have clinically significant benefits with methylphenidate treatment in terms of robust decrease of symptoms and improvement of quality of life.² However, its mechanisms of action are not completely understood.³

The effect of methylphenidate on dopaminergic and noradrenergic pathways results in improvement of prefrontal cortex function.^{4,5} The most studied action of

methylphenidate is its blockade of dopamine transporters, which increases levels of synaptic and extracellular dopamine, contributing to the transmission of nervous impulse.³ By raising dopamine levels, methylphenidate may enhance the saliency of events.⁶ The action of methylphenidate on the noradrenergic system has received much less attention. It was demonstrated that the activation of this system, which modulates attentional processes, improves prefrontal cortex function in humans and animals.^{3-5,7} Methylphenidate blocks norepinephrine transporters,³ and low oral doses of this medication have more effect on norepinephrine

than on dopamine in subcortical areas.⁸ Adrenergic $\alpha 2A$ receptor is a key component of the noradrenergic system,⁷ with a putative role on methylphenidate action demonstrated in animal models.^{9,10} Moreover, adrenergic $\alpha 2A$ receptor agonists, such as guanfacine hydrochloride and clonidine hydrochloride, are efficacious in the treatment of ADHD.¹¹

The adrenergic $\alpha 2A$ receptor gene (*ADRA2A*) is located on chromosome 10q24-26. A -1291 C>G single-nucleotide polymorphism (SNP) (rs 1800544) creating an *MspI* site in the promoter region of the gene was identified by Lario et al.¹² Six of 8 studies¹³⁻²⁰ that evaluated the association between *ADRA2A* and ADHD suggest a role for this polymorphism in the susceptibility for the disorder. A specific role for the *ADRA2A* -1291 C>G polymorphism in the dimension of inattention has been demonstrated in several of these investigations.^{15,18,20}

Pharmacogenetic studies^{21,22} have aimed to identify genes associated with variations in efficacy and with adverse effects secondary to a medication regimen. Most ADHD pharmacogenetic investigations have focused on potential genes of susceptibility for ADHD, mainly from the dopamine system.²³ Two studies^{24,25} evaluated noradrenergic genes, but none have studied the effect of *ADRA2A* on methylphenidate treatment outcomes, to our knowledge.

The primary objective of this study was to evaluate the association between the *ADRA2A* -1291 C>G polymorphism and the clinical improvement of inattentive symptoms with methylphenidate treatment in children and adolescents with ADHD. In secondary exploratory analyses, we evaluated the effect of this polymorphism in the improvement of hyperactive-impulsive symptoms and in the occurrence of primary adverse events with methylphenidate use.

METHODS

STUDY DESIGN AND SUBJECTS

This pharmacogenomic study used a nonrandom assignment, quasi-experimental design. Children and adolescents consecutively evaluated during 2 years in the ADHD Outpatient Clinic at the Hospital de Clínicas de Porto Alegre for whom data on response to methylphenidate for at least the first month of treatment were available were invited to join the study. This investigation was approved by the ethics committee of our university hospital. Written informed consent was obtained from parents, and verbal assent was requested from children and adolescents to participate.

The inclusion criteria were as follows: (1) ADHD diagnosis according to *DSM-IV* criteria,²⁶ (2) age between 4 and 17 years, (3) European-Brazilian race/ethnicity, (4) drug naïve for methylphenidate, and (5) prescribed daily dosage of methylphenidate hydrochloride of at least 0.3 mg/kg. Subjects who fulfilled all *DSM-IV* criteria for ADHD except for the age at onset of impairment criterion (symptoms causing impairment before age 7 years) were accepted in this study because recent research does not support the validity of this criterion.^{27,28}

DIAGNOSTIC PROCEDURES AND CLINICAL ASSESSMENTS

The diagnostic procedures in our unit have been described elsewhere.²⁹ In short, diagnoses of ADHD and comorbidities were

achieved through the following 3-stage process: (1) semistructured interviews (Schedule for Affective Disorders and Schizophrenia for School-Age Children—Epidemiologic Version), (2) diagnostic discussion in a clinical committee, and (3) clinical evaluation. When a diagnostic disagreement occurred in the 3-stage process, priority was given to diagnoses derived from clinical interviews.²⁹ Clinical assessments were performed by child psychiatrists at baseline and at 1 month and 3 months of treatment with methylphenidate.

Based on previous investigations in our population showing an association between *ADRA2A* and inattentive scores,^{15,18} we selected the parent-rated inattentive subscale of the Swanson, Nolan, and Pelham Scale version IV (SNAP-IV) as the primary outcome measure. The SNAP-IV is a revision of the original SNAP questionnaire.³⁰ The SNAP-IV items are rated on a scale from 0 to 3. This measure has been frequently used in ADHD investigations, including those designed to assess clinical interventions.³⁰ The internal consistency of the SNAP-IV varies from good to excellent.³¹ In a previous study,³² a Cronbach α coefficient of .74 was obtained for the complete scale (26 items) in a different sample. The scale was applied by child psychiatrists to parents.

Secondary outcome measures included the Barkley Side Effect Rating Scale and the parent-rated hyperactivity-impulsivity subscale of the SNAP-IV. The Barkley Side Effect Rating Scale lists 17 adverse effects associated with the use of stimulants. The severity of each symptom is scored from 0 to 9.³³

PHARMACOLOGICAL INTERVENTION

Patients were treated according to the program's protocol. Dosages of short-acting methylphenidate were augmented until no further clinical improvement was detected or until there were limited adverse effects.²⁹ Methylphenidate was administered preferentially twice daily (at 8 AM and noon), but an extra dose at 5 to 6 PM was allowed for children needing continuous coverage during evenings. Psychiatrists were blinded to patients' genotypes. The mean daily dosages of methylphenidate hydrochloride prescribed at baseline and at the 1-month assessment were 0.5 and 0.65 mg/kg, respectively.

GENOTYPING

High-molecular-weight genomic DNA was extracted from whole-blood lymphocytes by a salting-out procedure.³⁴ The -1291 C>G polymorphism in the promoter region of *ADRA2A* (rs 1800544) was amplified by polymerase chain reaction using primers and protocols as previously reported.¹² The 522-base pair (bp) amplicons were digested with *MspI* for 3 hours at 37°C. The digested fragments were electrophoresed on 10% polyacrylamide gels in 1× Tris/borate/EDTA buffer for 2½ to 3 hours at 160 V. The gels were stained with ethidium bromide and were visualized under UV light. *MspI* digestion at this site resulted in 4 constant fragments (5, 62, 116, and 165 bp). The C allele (formerly M) was identified as a polymorphic band of 174 bp, reflecting the absence of the *MspI* site. The presence of this site produces the loss of the 174-bp fragment, resulting in bands of 121 and 53 bp that determine the presence of the G allele (formerly M).¹²

STATISTICAL ANALYSIS

Patients were compared regarding IQ, ADHD subtype, comorbid conditions, methylphenidate dosage, previous use of medication, demographic characteristics, and baseline scores on measures assessed. The χ^2 test or the Fisher exact test was used for categorical variables, and the *t* test was used for continuous variables with normal distribution.

Table. Demographic and Clinical Characteristics of the Sample According to the Presence of the G Allele*

Characteristic	With G Allele† (n = 66)	Without G Allele‡ (n = 40)	P Value§
Age, y	10.2 ± 2.8	10.4 ± 3.5	.7
Male sex	49 (74.2)	33 (82.5)	.3
IQ	93.5 ± 15.1	95.0 ± 15.2	.6
ADHD subtype			.4
Combined	38 (57.6)	24 (60.0)	
Inattentive	19 (28.8)	9 (22.5)	
Hyperactive	2 (3.0)	4 (10.0)	
Subthreshold	7 (10.6)	3 (7.5)	
Comorbid conditions			
CD	9 (13.6)	8 (20.0)	.4
ODD	31 (47.0)	24 (60.0)	.2
Mood disorder	5 (7.6)	5 (12.5)	.5
Anxiety disorder	16 (24.6)	9 (22.5)	>.99
CGAS baseline score	61.3 ± 10.8	60.7 ± 11.5	.8
SNAP-IV baseline scores			
Total	1.64 ± 0.58	1.66 ± 0.53	.9
Inattentive	2.05 ± 0.50	1.92 ± 0.50	.18
Hyperactivity-impulsivity	1.57 ± 0.82	1.6 ± 0.7	.8
Oppositional	1.3 ± 0.8	1.4 ± 0.7	.4
SERS baseline score	34.6 ± 22.0	34.7 ± 20.0	.9
Previous use of medication	4 (6.1)	3 (7.5)	>.99
Concomitant prescription of another medication	4 (6.1)	6 (15.0)	.17
Methylphenidate hydrochloride prescribed, mg/kg			
At baseline	0.48 ± 0.13	0.53 ± 0.19	.19
At 1 mo	0.66 ± 0.20	0.63 ± 0.17	.4

Abbreviations: ADHD, attention/deficit-hyperactivity disorder; CD, conduct disorder; CGAS, Childrens Global Assessment Scale; ODD, oppositional defiant disorder; SERS, Barkley Side Effect Rating Scale; SNAP-IV, Swanson, Nolan, and Pelham Scale version IV.

*Data are given as number (percentage) or mean ± SD.

†G/G and G/C genotypes.

‡C/C genotype.

§Calculated by χ^2 test or Fisher exact test (categorical variables) or by *t* test (continuous variables with normal distribution).

Analyses of primary and secondary measures of efficacy were performed using a mixed-effects model (MEM), which provides a flexible framework for the analysis of repeated measures while accounting for missing data (eg, loss to follow-up).³⁵⁻³⁷ For each analysis, the best covariance structure fitting the data was selected based on the one with the lowest Akaike information criterion (AIC) value.³⁸ Independent factors included in all models were treatment over time, group assignment (defined as the presence of the G allele), and the interaction between these factors. Potential confounders to be entered in models were defined based on conceptual analyses of the literature^{3,39} and by means of a statistical definition (association with the study factor and with the outcome at $P \leq .10$). We restricted analyses on the SNAP-IV dimensions to patients with baseline scores higher than 1 on the SNAP-IV subscales to allow sufficient room for improvement, as done in previous investigations.^{40,41}

All analyses were conducted using SPSS version 12.0 software (SPSS Inc, Chicago, Ill). A significance level of 5% was set in all analyses (except for potential confounders, as indicated in the previous paragraph). Tests were 2-tailed.

RESULTS

During the study period, 111 children from affected families fulfilled inclusion criteria for analyses of the primary outcome measure (the SNAP-IV inattentive score). Five subjects were excluded from the analyses because of invalid baseline data ($n=2$), irregular use of methyl-

phenidate ($n=2$), and problems in genotyping ($n=1$). Therefore, included in the study were 106 patients for whom data at baseline and at 1 month of treatment were available. After 3 months of treatment, 89 patients were reevaluated. An additional 26 patients were assessed only at baseline (15 patients did not return for the 1-month evaluation, and 11 patients were referred elsewhere after the baseline evaluation because they lived in different parts of the state) and were excluded from the protocol. Regarding baseline characteristics, no significant differences between subjects included ($n=106$) and excluded ($n=31$) were detected in age, sex, IQ, ADHD subtype, global functioning scores, prescribed dosage of methylphenidate, baseline total scores on the SNAP-IV (parent and teacher scores), and comorbidity profile (mood disorders, anxiety disorders, and disruptive behavior disorders) ($P > .1$ for all). In addition, the 106 subjects included in the protocol did not differ in baseline characteristics compared with all subjects evaluated in our unit from October 1, 2002, to February 1, 2006 ($n=457$).

The estimated allele frequencies for the included subjects were 0.62 for the C allele and 0.38 for the G allele. The genotype frequencies were 0.38 for C/C homozygous individuals, 0.49 for G/C heterozygous individuals, and 0.13 for G/G homozygous individuals. These frequencies were under Hardy-Weinberg equilibrium.

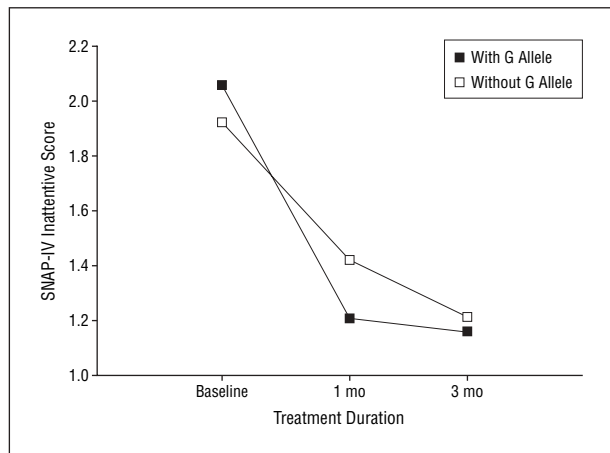


Figure 1. Mean Swanson, Nolan, and Pelham Scale version IV (SNAP-IV) inattentive scores during methylphenidate hydrochloride treatment according to the presence of the G allele in a mixed-effects model (n=106). Treatment over time: $F_{2,198}=79.37$; $P<.001$. Presence of the G allele: $F_{1,118}=0.67$; $P=.41$. Treatment over time \times presence of the G allele: $F_{2,198}=4.30$; $P=.02$.

Because of the low frequency of G/G homozygous individuals, they were grouped with the carriers of the G/C genotype. Therefore, carriers of the G allele were compared with those without this allele (subjects homozygous for the C allele) to explore the effect of the presence of the G allele on outcomes.

Demographic and clinical characteristics of the patients according to the presence of the G allele are given in the **Table**. No significant differences between the 2 groups were found on potential confounders (IQ, ADHD subtype, comorbid conditions, methylphenidate dosages, previous use of medication, demographic characteristics, and baseline scores in measures assessed). In addition, no potential confounder was associated with both the presence of the G allele and the SNAP-IV inattentive score at $P\leq .10$.

In the model that included treatment over time, the presence of the G allele, and the interaction between these factors, an effect of treatment over time was found for the SNAP-IV inattentive scores during the 3 months of treatment (n=106; $F_{2,198}=79.37$; $P<.001$) (**Figure 1**). Although no effect for the presence of the G allele was detected (n=106; $F_{1,118}=0.67$; $P=.41$), there was a significant interaction effect between the presence of the G allele and treatment over time for the SNAP-IV inattentive scores during the 3 months of treatment (n=106; $F_{2,198}=4.30$; $P=.02$). The covariance structure with the lowest AIC value was the first-order autoregressive one. In a secondary analysis, we estimated that this model was able to explain 30.43% of the variance of inattentive scores; 0.24% was explained by the presence of the G allele, 28.85% by treatment over time, and 1.34% by the interaction between these factors.

Data shown in Figure 1 suggest that the greatest effect of treatment occurred from baseline to the first month in subjects with and without the G allele, as expected. In fact, the improvement from baseline to the first month of treatment (n=106; effect size, 1.15) was greater than the improvement from the first month to the third month of treatment (n=89; effect size, 0.20). The effects of treatment over time, the presence of the G allele, and the in-

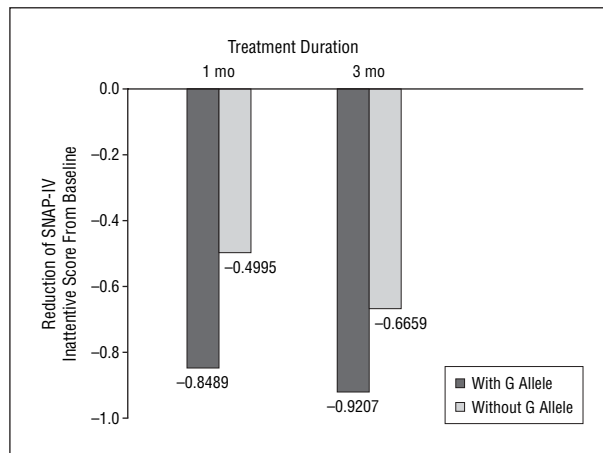


Figure 2. Effect of the presence of the G allele on mean reductions in Swanson, Nolan, and Pelham Scale version IV (SNAP-IV) inattentive scores from baseline in a mixed-effects model (n=106). Presence of the G allele: $F_{1,101.9}=6.6$; $P=.01$.

teraction between these factors during the first month of treatment were also assessed using an MEM. The covariance structure with the lowest AIC value was the compound symmetry. As expected, we detected significant effects of treatment over time with methylphenidate (n=106; $F_{1,104}=126.69$; $P<.001$) and a significant interaction effect between the presence of the G allele and treatment over time on the SNAP-IV inattentive scores (n=106; $F_{1,104}=8.51$; $P=.004$). No effect of the presence of the G allele was found (n=106; $F_{1,104}=0.22$; $P=.64$).

We explored the effects of treatment over time, the presence of the G allele, and the interaction between these factors on the SNAP-IV inattentive scores from the first month to the third month of treatment using the baseline inattentive score as a covariate in an MEM. This was performed because a small between-group difference (although higher than our threshold of $P<.1$) was detected in baseline SNAP-IV inattentive scores. In this MEM analysis, the covariance structure with the lowest AIC value was the compound symmetry. The effects of treatment over time (n=106; $F_{1,96.9}=3.81$; $P=.05$), the baseline inattentive scores (n=106; $F_{1,104}=14.82$; $P<.001$), and the presence of the G allele (n=106; $F_{1,104}=5.46$; $P=0.02$) were determined for the SNAP-IV inattentive scores. As expected, no interaction effect was detected between the presence of the G allele and treatment over time (n=106; $F_{1,96.9}=0.61$; $P=.44$) because the greatest improvement occurred from baseline to the first month of treatment.

The effects of the presence of the G allele on the changes in inattentive scores from baseline to the first and third months of treatment were simultaneously analyzed using an MEM. The covariance structure with the lowest AIC value was the Toeplitz one. This analysis also demonstrated a significant effect of the presence of the G allele on the changes in scores (n=106; $F_{1,101.9}=6.6$; $P=.01$) (**Figure 2**). From baseline to the first and third months of treatment, individuals with the G allele achieved greater reduction of inattentive scores than individuals without this allele.

As expected, an effect of treatment over time was found for the SNAP-IV hyperactive-impulsive scores during the

3 months of treatment ($n=83$; $F_{2,155,6}=62.1$; $P<.001$). However, there was no interaction effect between the presence of the G allele and treatment over time on this SNAP-IV subscale ($n=83$; $F_{2,155,6}=0.61$; $P=.54$). In addition, no effect of the presence of the G allele was detected ($n=83$; $F_{1,81,3}=0.05$; $P=.82$). The covariance structure with the lowest AIC was the compound symmetry.

Regarding adverse events, an MEM analysis demonstrated effects of treatment over time on the Barkley Side Effect Rating Scale scores, as expected ($n=106$; $F_{2,201,2}=5.4$; $P=.005$). However, neither an effect for the presence of the G allele ($n=106$; $F_{1,107,6}=0.15$; $P=.69$) nor an interaction effect between the presence of the G allele and treatment over time ($n=106$; $F_{2,201,2}=0.71$; $P=.49$) on the Barkley Side Effect Rating Scale scores was found during the 3 months of methylphenidate use. The covariance structure with the lowest AIC value was the first-order autoregressive one.

COMMENT

As previously reported by clinical trials,^{42,43} we detected a significantly robust clinical effect of methylphenidate on ADHD symptoms during short-term treatment. Furthermore, we demonstrated greater improvement of inattentive symptoms with methylphenidate treatment in the first month of treatment in children and adolescents with the G allele (*G/G* and *G/C* genotypes) at the *ADRA2A* -1291 C>G polymorphism than in those without this allele. We are unaware of any previous studies investigating the role of *ADRA2A* in the response to methylphenidate treatment.

This finding concurs with recent preclinical studies in animals that provide evidence for the contribution of the blockade of the adrenergic $\alpha 2A$ receptor in producing ADHD-like symptoms and in impairing the response to methylphenidate. Arnsten and Dudley¹⁰ studied the effect of methylphenidate on rats performing a delayed alternation task (an attentional task). The authors demonstrated that methylphenidate significantly improved the performance on the task. Methylphenidate and an adrenergic $\alpha 2A$ receptor antagonist (idazoxan) were subsequently coadministered. The enhancing effect of methylphenidate was blocked by the antagonist, indicating the contribution of this receptor to the positive cognitive effects of methylphenidate.

In the same direction as our findings, previous molecular genetic investigations suggest a relationship between ADHD and the *ADRA2A* -1291 C>G polymorphism. One study¹⁵ demonstrated an association between the *G/G* *ADRA2A* genotype and inattentive scores in a sample of 92 subjects with ADHD. In a subsequent independent sample of children with ADHD, the relationship between inattentive symptoms and the *G/G* genotype was again observed.¹⁸ Similar findings were obtained by Park et al,¹⁶ who investigated a possible role of *ADRA2A* in ADHD by assessing 3 different SNPs, including the -1291 C>G SNP. A significant effect of this polymorphism was detected through quantitative transmission disequilibrium tests in the inattentive and hyperactive-impulsive symptom dimensions, particularly through the

presence of the G allele. Moreover, haplotype analyses showed significant effects of this polymorphism by transmission disequilibrium tests or by quantitative transmission disequilibrium tests. In both cases, the presence of the G allele of the -1291 C>G SNP seemed to contribute to an increased risk, especially when inattentive symptoms were considered. These previous findings and those from the present study suggest that *ADRA2A* may be independently associated with the ADHD phenotype (the inattentive dimension) and with the response to methylphenidate treatment relative to inattentive scores.

It is essential to understand the potential functional significance of the *ADRA2A* -1291 C>G polymorphism. Belfer et al⁴⁴ recently reported that a single haplotype block spanned *ADRA2A*. This haplotype block is composed of 9 different SNPs that are mapped from the 5' end to the 3' end of the *ADRA2A* locus, including the -1291 C>G SNP and a nonsynonymous amino acid change in position 251, known to be of functional relevance for adrenergic $\alpha 2A$ receptor. As noted by the authors, this *ADRA2A* haplotype block was sufficient to capture the information content even when the only known functional locus was excluded. In other words, the -1291 C>G polymorphism can have a role in *ADRA2A* expression or function, or it can be a marker associated with another locus with a functional role. These hypotheses must be further explored.

We demonstrated the positive effect of the presence of the G allele at the *ADRA2A* -1291 C>G polymorphism in promoting greater reduction of inattentive symptoms during methylphenidate treatment. Comings et al⁴⁵ documented a codominant effect of the presence of the G allele (*G/G*>*G/C*>*C/C*) in a group of individuals with ADHD and oppositional defiant disorder diagnostic symptoms. One can speculate whether the presence of 2 G alleles (homozygosity) would confer an additional effect on the reduction of inattentive symptoms. We did not detect additional effects in our preliminary analyses (the mean \pm SE reductions of inattentive symptoms according to genotype from baseline to the first and third months of treatment, respectively, were as follows: *G/G*, 0.69 ± 0.14 and 0.80 ± 0.22 ; *G/C*, 0.89 ± 0.08 and 0.93 ± 0.08 ; and *C/C*, 0.49 ± 0.90 and 0.67 ± 0.09). It is also possible that, once a threshold for action has been achieved with the presence of one allele, a second allele would be irrelevant, compatible with a dominant model. Nevertheless, the small number of subjects with the *G/G* genotype in this sample ($n=14$) limits this analysis. Studies on the functional significance of the -1291 C>G polymorphism are necessary to clarify the nature of its potential effect.

It is important to consider caveats of naturalistic studies. First, we did not have a placebo arm in this trial, so we had no internal control to correct for any effect of time (eg, regression to the mean) or expectancy bias. The improvement of ADHD symptoms in our sample was comparable to that previously reported in randomized clinical trials.³⁹ Although a placebo response was likely present in our study and decreased the power by reducing the measurement precision of drug response, it is unlikely that a placebo response was systematically related to the polymorphism assessed. In addition, we minimized the chance that the higher reduction in inattentive scores with

methylphenidate treatment detected in carriers of the G allele might be attributed to other events because we performed an extensive assessment of potential confounders between groups with and without the G allele, a strategy not usually performed in previous pharmacogenomic investigations of ADHD. Second, methylphenidate was administered with no control of adherence by investigators. Although we were able to identify 2 patients with irregular use of the medication, we cannot rule out that lack of adherence occurred to some extent in the remaining sample. Nevertheless, there was an important overall symptomatic reduction according to the parents during follow-up. Third, although our sample may have been too small to detect significant effects of this polymorphism in the hyperactive-impulsive dimension and on adverse events, we included a larger sample size compared with previous studies.^{25,40,41} This is the first ADHD pharmacogenomic study addressing adverse events, to our knowledge.

Although it is important to study SNPs in reasonable candidate genes, their putative effects are small even if they are related to medication response in ADHD. Therefore, the field needs multisite collaborative efforts to obtain larger samples, such as the sample in development through the ADHD Molecular Genetics Network. In addition, genome scan studies should incorporate response to medication in their analyses.

Previous neurobiological and pharmacological studies^{9,10} documented the importance of the noradrenergic system relative to the action of methylphenidate. Corroborating this hypothesis, we demonstrated the role of an ADRA2A polymorphism in the reduction of inattentive scores associated with methylphenidate treatment in subjects with ADHD. In this regard, further pharmacogenomic randomized controlled trials with full description of sample ascertainment and with control for possible selection bias should be conducted to replicate our results and to address different noradrenergic system genes.

Submitted for Publication: January 3, 2006; final revision received April 13, 2006; accepted May 17, 2006.

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Financial Disclosure: The ADHD program receives research support from the following pharmaceutical companies: Bristol Myers-Squibb, Eli Lilly and Company, Janssen-Cilag, and Novartis Pharmaceuticals, for which Dr Rohde serves on the speakers bureaus or as a consultant.

Funding/Support: This study was supported by grant 471761/03-6 from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Programa de Apoio a Núcleos de Excelência, and Hospital de Clínicas de Porto Alegre.

Previous Presentation: Preliminary results of this study were presented at the 52nd Annual Meeting of the American Academy of Child and Adolescent Psychiatry; October 20, 2005; Toronto, Ontario.

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