MAOA is associated with methylphenidate improvement of oppositional symptoms in boys with attention deficit hyperactivity disorder

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Abstract

The monoamine oxidase A (MAOA) gene has been extensively related to aggressive, impulsive and violent behaviours. Previous studies have documented the improvement of oppositional symptoms in attention deficit hyperactivity disorder (ADHD) patients with methylphenidate (MPH). However, the effect of the MAOA gene in response to MPH has not been investigated. A sample of 85 boys from an ADHD outpatient service was genotyped for the MAOA-uVNTR polymorphism. The outcome measure was the parent-rated oppositional subscale of the Swanson, Nolan and Pelham Scale – version IV. The scale was applied by child psychiatrists blinded to genotype at baseline and in the first and third months of treatment. A significant interaction between the presence of MAOA high-activity genotype and treatment with MPH over time on oppositional scores was detected during the 3 months’ treatment (n=85, F2,136=4.83, p=0.009). These results suggest an effect of the MAOA-uVNTR high-activity genotype on the improvement of oppositional symptoms with MPH treatment.

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Introduction

Attention deficit hyperactivity disorder (ADHD) is a common psychiatric disorder in childhood and adolescence, affecting around 5% of school-aged children worldwide (Polanczyk et al. 2007a). The syndrome is characterized by severe inattention, hyperactivity, and impulsiveness. Antisocial, impulsive and aggressive behaviours are common symptoms of conduct disorder (CD) and oppositional defiant disorder (ODD) that are highly comorbid and related to ADHD (Biederman et al. 1991).

With ADHD being a disorder highly responsive to medication, methylphenidate (MPH) is the most widely used drug to treat this condition (Santosh & Taylor, 2000; Solanto, 1998). MPH improves not only the cardinal symptoms of ADHD, but also symptoms such as aggressiveness found in ODD and CD (Goldman et al. 1998; Sinzig et al. 2007). The main and most important action of MPH in neurotransmission systems is the blockage of transporters, especially the dopamine transporter (DAT1). As a result, extracellular concentrations of dopamine (DA), noradrenaline (NE) and serotonin (5-HT) can be elevated (Arnsten & Li, 2005; Kuczenski & Segal, 1997; Solanto, 1998).

Monoamine oxidase A (MAOA) is one of the main metabolic enzymes for the degradation of catecholamines. Existing evidences suggest that MPH also inhibits MAOA (Solanto, 1998). With regard to MAOA a variable number tandem repeat (uVNTR) functional polymorphism is present at the promoter region with two common alleles (4-repeat and 3-repeat) (Sabol et al. 1998). These are referred to as high and low MAOA genotypes, defined by their significantly different transcriptional activities in human cell lines (Sabol et al. 1998).

The prevalence of the high and low MAOA genotypes in populations has stimulated many studies on
the association of MAOA with impulsivity, inhibitory control, and aggression (Huang et al. 2004; Manuck et al. 2000; Passamonti et al. 2006). There are a number of studies showing that the MAOA genotype influences vulnerability to environmental stress both in humans (Caspì et al. 2002) and animals (Newman et al. 2005), and that this biological process can be initiated early in life (Kim-Cohen et al. 2006). Overall, these investigations above suggest a link between low MAOA genotype and both impulsive and aggressive behaviours.

There are eight studies reporting on the association between the MAOA-uVNTR polymorphism and ADHD. Seven of these studies found an association with ADHD (Mick & Faraone, 2008). Although theoretically the genetic variants involved in susceptibility to ADHD may also be involved in treatment response, to the best of our knowledge no pharmacogenetic study has assessed the role of MAOA in response to MPH treatment.

Considering the effect of the uVNTR polymorphism in the MAOA gene on impulsive/aggressive behaviours, the aim of this investigation was to evaluate the association between the MAOA gene and clinical improvement of oppositional symptoms with MPH treatment in children and adolescents with ADHD.

Methods

The sample for this investigation included children and adolescents who were consecutively evaluated for 2 yr in the ADHD Outpatient Clinic at the Hospital de Clínicas de Porto Alegre. This sample has been fully described by Polanczyk et al. (2007b).

Briefly, the inclusion criteria were: ADHD diagnosis according to DSM-IV criteria, age between 4 and 17 yr, European-Brazilian ethnicity, subjects who were drug-naïve for MPH, and prescribed MPH doses of at least 0.3 mg/kg.d. The diagnostic process relied on the application of semi-structured interviews [schedule for affective disorders and schizophrenia for school-age children – Epidemiological version (KSADS-E); Orvaschel, 1985] by fully trained research assistants. All diagnoses generated were confirmed by experienced child psychiatrists (see Polanczyk et al. 2007b).

The parent-rated oppositional subscale of the Swanson, Nolan and Pelham scale – version IV (SNAP-IV) was selected as the primary outcome measure (Swanson et al. 2001). This measure has been frequently used in ADHD investigations (Polanczyk et al. 2007b; Zeni et al. 2007).

Patients were treated according to the programme’s protocol. Dosages of short-acting MPH were augmented until no further clinical improvement was detected or until there were limited adverse effects (Rohde, 2002). MPH was administered preferentially twice daily (at 08:00 and 12:00 hours), but an extra dose between 17:00 and 18:00 hours was allowed for children needing continuous coverage during evenings. Psychiatrists were blinded to patients’ genotypes. The mean daily dosages of MPH hydrochloride prescribed at baseline and at the 1-month assessment were 0.5 and 0.65 mg/kg, respectively. Clinical assessments were performed by child psychiatrists at baseline prior to medication and after 1 and 3 months of MPH treatment.

The study was approved by the Ethics Committee of the University Hospital. Written informed consent was obtained from parents, and children and adolescents gave their verbal assent to participate.

DNA was extracted from whole-blood lymphocytes by standard procedures. MAOA-uVNTR was amplified by PCR using primers and methods previously described by Sabol et al. (1998).

Comparison among categorical variables was performed using χ² or Fisher’s exact test. All continuous variables showing a normal distribution were compared between groups by Student’s t test; for those variables that did not show a normal distribution, the Mann–Whitney U test was used. Potential confounders evaluated were age, sex, ADHD subtype, IQ, MPH dose prescribed at baseline (mg/kg.d) and comorbidity (mood, anxiety, and disruptive behaviour disorders). Potential confounders to be entered in models were defined based on conceptual analyses of the literature, and by means of a statistical definition (association with the study factor and with the outcome at p ≤ 0.10).

Analyses of SNAP-IV oppositional scores were performed using a mixed-effects model (MEM), as described by Polanczyk et al. (2007b) which provides a flexible framework for the analysis of repeated measures while accounting for missing data (e.g. loss to follow-up). For each analysis, the best covariance structure fitting the data was selected based on the one with the lowest Akaike Information Criterion (AIC) value. Independent factors included in all models were treatment over time, group assignment (defined as the presence of the high-activity allele), and the interaction between these factors. An unbiased estimate of the effect size (ES) was computed for the SNAP-IV oppositional score, according to the method suggested by Cohen (1998).

All analyses were conducted using SPSS version 12.0 software (SPSS Inc., USA). A significance level of 5% was set in all analyses (except for potential
confounders, as indicated above). Tests were two-tailed.

**Results**

A sample of 113 children fulfilled inclusion criteria to participate of the study. Twenty-eight subjects were excluded from the sample. Because the MAOA gene is X-linked, 26 girls were excluded. Two boys were also excluded due to problems in genotyping. Hence, analyses were performed with 85 affected boys. The affected girls were not analysed separately because of the small sample size.

The estimated allele frequencies were 0.38 for the 3-repeat (low-activity) allele, 0.59 for the 4-repeat allele, and 0.02 for the 5-repeat allele. The rare 2- and 3.5-repeat alleles were not observed in this sample. The 4- and 5-repeat alleles were joined because both have high transcriptional activity. Their pooled frequency was 0.62.

Demographic and clinical characteristics of patients are reported in Table 1. No significant between-group differences on potential confounders were found. Moreover, no potential confounder was associated with both the presence of high-activity alleles and oppositional scores in SNAP-IV. The high-activity allele frequency did not differ between individuals lost at follow-up and those that completed the 3 months’ treatment ($\chi^2 = 0.022, p = 0.88$).

Figure 1a shows the model including treatment over time, the presence of high-activity genotype, and their interaction. As expected, an effect of treatment over time for the SNAP-IV oppositional scores during the 3 months of treatment was detected with last-observation carried forward strategy (LOCF) ($n = 85$, $F_{2,136} = 22.94, p < 0.0001$). Although no effect by the presence of high-activity genotype was detected ($n = 85$, $F_{1,83} = 0.33, p = 0.57$), there was a significant interaction effect between the presence of high-activity genotype and treatment over time for the SNAP-IV oppositional scores during 3 months of treatment ($n = 85$, $F_{2,136} = 4.83, p = 0.009$) (Fig. 1). We also performed the same analyses only with patients that completed the 3 months’ treatment, and the results followed the same direction ($p = 0.002$ for interaction; data available upon request). The covariance structures with the lowest AIC value for these analyses were the Toeplitz and compound symmetry, respectively.

Figure 1a also shows that the greatest effect of treatment occurred from baseline to the first month, but no effect occurred from the first to the third month. Thus, we also assessed the effects of treatment over time, the presence of high-activity genotype, and the interaction between these factors during the first month of treatment. As a result, we detected significant effects of treatment over time with MPH ($n = 85$, $F_{1,136} = 35.25, p < 0.0001$) and a significant interaction
effect between the presence of high-activity genotype and treatment over time on SNAP-IV oppositional scores \( (n = 85), F_{1,136} = 6.75, p = 0.01 \). No main effect of genotype was found \( (n = 85), F_{1,83} = 0.01, p = 0.91 \). The covariance structure with the lowest AIC value for these analyses was the Toeplitz. The same significant interaction effect between the presence of high-activity genotype and treatment over time on SNAP-IV oppositional scores was observed \( (n = 83, F_{1,136} = 0.01) \) when the analyses was restricted to the first month (Fig. 1b). The covariance structure with the lowest AIC value for these analyses was the Toeplitz.

**Discussion**

In this investigation, we documented for the first time that a higher improvement on oppositional symptoms with MPH treatment was associated with MAOA-uVNTR high-activity genotypes.

The biological mechanisms to explain our results is yet to be determined, since there is no previous pharmacogenetic study assessing the role of any gene in MPH effects on oppositional symptoms in patients with ADHD. Moreover, even considering the emergent literature documenting the association between uVNTR at the MAOA genotype and impulsivity and aggression (Huang et al. 2004; Manuck et al. 2000; Passamonti et al. 2006), the role of this polymorphism in ADHD is controversial, since findings have suggested an association between ADHD and either the low- and high-transcriptional alleles (Mick & Faraone, 2008). However, it is very important to note the complex interaction among the monoamine systems of neurotransmitters. For instance, in MAOA/5-HTT double knock-out mice, an elevated accumulation of 5-HT has been observed. However, this abnormal accumulation of 5-HT appears not to occur in MAOA/5-HTT/DAT triple knock-out mice (Mössner et al. 2006). The direction of the effect of stimulant medication on DA levels in the brain has been controversial. For example, while Levy (1991) proposed the DA deficit hypothesis, with post-synaptic effects that amplified the DA neural response, Solanto (1998) proposed a DA excess hypothesis, with presynaptic effects of stimulant medication reducing release and reducing the DA neural response. The presynaptic/antagonist and post-synaptic/agonist hypotheses were outlined by Seeman & Madras (1998), who proposed that tonic levels would suppress phasic release, so that stimulants would function as antagonists, thus correcting a DA excess rather than a DA deficit. MAOA is one of the major enzymes responsible for monoamine degradation within the central nervous system; it is plausible to assert that functional variants within the gene might contribute to inter-individual differences in brain monoamine activity balance.

Our study should be understood in the context of some limitations. The sample is of moderate size but it is homogeneous because only boys were included. The rare 2 and 3.5 alleles, not seen in the present study, were observed in another sample from the same population with frequencies < 1% (Contini et al. 2006); this was a naturalistic study. This design might be valuable to better appreciate the role of genetic factors in routine clinical practice beyond the realm of controlled clinical trials. 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 or expectancy bias. The improvement of ADHD symptoms in our sample was
comparable to that previously reported in randomized clinical trials (MTA Cooperative Group, 1999). Although a placebo response was probably 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 SNAP-IV oppositional scores with MPH treatment detected in carriers of the high-activity allele might be attributed to other events because we performed an extensive assessment of potential confounders between groups with and without this allele. Moreover, the frequency of the high-activity allele did not differ between patients that dropped out and those that continued treatment. Second, MPH was administered with no control of adherence by investigators. Although we were able to identify two patients with irregular use of medication, we cannot rule out that lack of adherence occurred to some extent in the remaining sample but there is no reason to expect a preferential compliance to MPH according to the presence of high-activity genotypes at MAOA. Nevertheless, according to the parents, there was an important overall symptomatic reduction during follow-up. We could not adjust our findings for between-group differences in baseline SNAP-IV oppositional scores due to the limited follow-up assessment points (two) and the fact that the main effect of treatment over time occurred in the first month. However, no significant between-group difference was found in baseline SNAP-IV oppositional scores ($p = 0.18$, $ES = 0.29$).

The pharmacogenetic investigation of the effect of MPH on parent-rated SNAP-IV oppositional symptoms in this sample were previously tested for two dopaminergic genes (DRD4, DAT1) and three serotonergic genes (5-HHT, HTR1B, HTR2A) with negative results (Zeni et al. 2007), whereas Polanczyk et al. (2007b) tested only the parent-rated inattentive subscale of SNAP-IV as the primary outcome measure in the association reported with the ADRA2A gene in this sample. In the present study only the parent-rated opposition subscale of SNAP-IV was tested due to previous associations of MAOA-uVNTR with CD and anti-social personality disorder (Huang et al. 2004; Manuck et al. 2000; Passamonti et al. 2006). Although the observed associations might be spurious (type I error) due to multiple statistical comparisons, we considered this work as exploratory because no previous MPH pharmacogenetic study with the MAOA gene was carried out.

Although it is important to study pharmacogenetic effects of reasonable candidate genes in ADHD, their putative effects are small. Thus, candidate gene studies should implement strategies that will provide enough statistical power to detect such small effects. One potential strategy is the examination of specific dimensions of medication response as presented here, since it may reduce heterogeneity. The present investigation is only preliminary and further pharmacogenetic studies should be conducted to replicate our results.

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Statement of Interest

Dr L. A. Rohde was on the speakers’ bureau and/or acted as consultant for Eli-Lilly, Janssen-Cilag, and Novartis in the last three years. Currently, his only industry-related activity is taking part in the advisory board/speakers’ bureau for Eli Lilly & Company. The ADHD Outpatient Programme receives unrestricted educational and research support from the following pharmaceutical companies: Bristol–Myers Squibb, Eli-Lilly, Janssen-Cilag, and Novartis. Guilherme Polanczyk is on the speakers’ bureau of Novartis. Cristian Zeni participates in ADHD and Juvenile Bipolar Disorder Outpatient programmes that receive research support from the following pharmaceutical companies: Abbott Laboratories, Bristol–Myers Squibb, Eli-Lilly, Janssen-Cilag, and Novartis.

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