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Differential Association Between MAOA, ADHD and Neuropsychological Functioning in Boys and Girls

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Attention-deficit/hyperactivity disorder (ADHD) is more common in boys than in girls. It has been hypothesized that this sex difference might be related to genes on the X-chromosome, like Monoamine Oxidase A (MAOA). Almost all studies on the role of MAOA in ADHD have focused predominantly on boys, making it unknown whether MAOA also has an effect on ADHD in girls, and few studies have investigated the relationship between MAOA and neuropsychological functioning, yet this may provide insight into the pathways leading from genotype to phenotype. The current study set out to examine the relationship between MAOA, ADHD, and neuropsychological functioning in both boys (265 boys with ADHD and 89 male non-affected siblings) and girls (85 girls with ADHD and 106 female non-affected siblings). A haplotype was used based on three single nucleotide polymorphisms (SNPs) (rs12843268, rs3027400, and rs1137070). Two haplotypes (GGC and ATT) captured 97% of the genetic variance in the investigated MAOA SNPs. The ATT haplotype was more common in non-affected siblings (P = 0.025), conferring a protective effect for ADHD in both boys and girls. The target and direction of the MAOA effect on neuropsychological functioning was different in boys and girls: The ATT haplotype was associated with poorer motor control in boys (P = 0.002), but with better visuo-spatial working memory in girls (P = 0.01). These findings suggest that the genetic and neuropsychological mechanisms underlying ADHD may be different in boys and girls and underline the importance of taking into account sex effects when studying ADHD.

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**KEY WORDS:** attention-deficit/hyperactivity disorder; monoamine oxidase type A (MAOA); sex; neuropsychology; endophenotype


**INTRODUCTION**

Attention-deficit/hyperactivity disorder (ADHD) is a strongly genetically determined disorder, characterized by symptoms of inattention, hyperactivity and impulsivity [American Psychiatric Association [APA], 1994]. The disorder is more common in boys than in girls with estimated sex ratios varying between 3:1 and 9:1 [Arnold, 1996; Gaub and Carlson, 1997]. Given these sex differences, it has been hypothesized that genes on the X-chromosome may be important for the pathogenesis of ADHD [Jiang et al., 2001; Lung et al., 2006; Manor et al., 2002]. In contrast to girls, boys do not have a potentially compensatory spare X-chromosome, making them more vulnerable to X-linked diseases. Even though one X-chromosome is inactivated in girls [Ohno et al., 1959], this inactivation is not complete, since a number of genes escape inactivation [Pinsonneault et al., 2006]. Genetic variants in certain X-linked genes may, therefore, have a different impact on cognition and behavior in boys and girls.

An X-linked gene that may show such an effect and may explain sex ratio differences in ADHD is the gene coding for Monoamine Oxidase A (MAOA). This gene is located on the X-chromosome between p11.23 and p11.4 [Das et al., 2006] and escapes X-inactivation in girls [Pinsonneault et al., 2006]. It has 15 exons and codes for a mitochondrial enzyme involved in the pre-synaptic degradation of the monoamines serotonin, norepinephrine and dopamine [Craig, 2007]. The gene is a candidate for ADHD, because it influences the monoaminergic systems that are also etiologically related to ADHD [Das et al., 2006] and MAOA activity can be inhibited by methylphenidate, which also reduces ADHD symptoms [Solanto, 1998]. Several studies have indeed found various polymorphisms in MAOA (like a 30 bp repeat in the promoter region, a GA repeat in intron 2, and a G/T in exon 8) to be associated with ADHD, with odds ratio’s around 1.31 and 1.94 [Brookes et al., 2006; Das et al., 2006; Domschke et al., 2005; Guan et al., 2008; Manor et al., 2002]. However, since these studies have focused predominantly on boys, the effects of the gene on ADHD in
girls is still unknown. Only one study separately analyzed results for a small sample of girls with ADHD (N = 19) and a larger sample of boys with ADHD (N = 110) and reported that the gene was associated with ADHD in both sexes [Manor et al., 2002]. However, this finding is in need of replication.

Another scarcely investigated issue is the relationship between MAOA and neuropsychological functioning. Neuropsychological functions may serve as ADHD-endophenotypes, or intermediate phenotypes of ADHD. These areheritable, continuously distributed traits that are associated with heightened risk for developing a disorder and act as intermediary between genotype and phenotype [Gottesman and Gould, 2003]. Endophenotypes are proposed to be moreheritable than phenotypes because they are etiologically "closer" to the disease genes than clinical phenotypes and offer the advantage of a quantitative trait instead of dichotomous diagnostic categories [Gottesman and Gould, 2003]. Focusing on neuropsychological functions in relation to MAOA in ADHD may provide insight into the pathways leading from the gene to ADHD. Given that MAOA influences several monoamines, the neuropsychological effects of this gene may also be diverse. Thus far, only the relationship between MAOA and higher cognitive functions has been reported: MAOA genotype and activity have been found to be related to inhibition [Af Klinteberg et al., 1990–1991; Manor et al., 2002; Meyer-Lindenberg et al., 2006] and memory related cognition [Savitz et al., 2007]. In the current study, MAOA genotype was investigated in relation to both cognitive and motor functions, given that ADHD is frequently associated with deficits in these functions [Halperin and Schuls, 2006]. The cognitive and motor measures studied here have been previously tested and consistently associated with ADHD as endophenotypes [Rommelse et al., 2007a,b,c, 2008a,b].

Thus, the current study set out to examine the relationship between MAOA genotype, ADHD and neuropsychological functioning in both boys and girls. To allow for a robust analysis of the MAOA genotypic effect, a haplotype (combination of alleles transmitted together; Sklar, 2005) based on single nucleotide polymorphisms (SNPs) was used in the analysis. This haplotype had shown nominal association with ADHD in the main International Multicenter ADHD Genetics (IMAGE) project sample [Brookes et al., 2006] of which the current study targets a subsample.

### TABLE I. Description of the Neuropsychological Tasks

<table>
<thead>
<tr>
<th>Task</th>
<th>Aim of measurement</th>
<th>Dependent variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Executive/cognitive tasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop task</td>
<td>Inhibition</td>
<td>Stop signal reaction time (SSRT)</td>
</tr>
<tr>
<td>Shifting attentional set</td>
<td>Inhibition and cognitive flexibility</td>
<td>Percentage of errors</td>
</tr>
<tr>
<td>Time test</td>
<td>Time reproduction</td>
<td>Accuracy (total absolute deviation between stimulus and response)</td>
</tr>
<tr>
<td>Visuo-spatial sequencing</td>
<td>Visuo-spatial working memory</td>
<td>Number of correct targets in the correct order</td>
</tr>
<tr>
<td>Digit span</td>
<td>Verbal working memory</td>
<td>Digit span backwards</td>
</tr>
<tr>
<td>Motor tasks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pursuit</td>
<td>Motor control under continuous adaptation</td>
<td>Precision</td>
</tr>
<tr>
<td>Tracking</td>
<td>Motor control without continuous adaptation</td>
<td>Precision</td>
</tr>
<tr>
<td>Tapping</td>
<td>Self-generated motor output</td>
<td>Variability in tapping rate</td>
</tr>
<tr>
<td>Baseline speed</td>
<td>Motor output as response to external cue</td>
<td>Variability in reaction times</td>
</tr>
<tr>
<td>Motor timing</td>
<td>Timing of motor output</td>
<td>Variability in reaction times</td>
</tr>
</tbody>
</table>

*Rommelese et al. [2007a].
*Rommelese et al. [2007b].
*Rommelese et al. [2007c].
*Rommelese et al. [2008a].
*Rommelese et al. [2008b].
DNA Extraction, MAOA Genotyping, and Haplotype Estimation

An elaborate description of methods for DNA extraction is provided elsewhere [Brookes et al., 2006]. Briefly, DNA was extracted directly from blood samples or cell lines at Rutgers Cell line and DNA repository in the US. Three SNPs in MAOA (rs12843268 [intron 5, G/A], rs3027400 [intron 9, G/T] and rs1801291 [exon 14, now known as rs1137070, C/T]) were selected as these had shown nominal association with ADHD in a larger sample of IMAGE (respectively $P = 0.049$, $P = 0.049$, and $P = 0.020$), in which the entire MAOA gene-region had been investigated using tagSNPs [Brookes et al., 2006]. The SNPs were genotyped using the Illumina Golden Gate Assay™ (Illumina Inc., San Diego, CA). Additional families, which had been included in IMAGE at a later stage and had not been described in the paper by Brookes et al. [2006], were genotyped for the three SNPs using ABI SNPlex [Tobler et al., 2005] as part of a replication study (unpublished data). In total 178 (74.8%) of the ADHD families in the current study underwent genotyping, the numbers of samples genotyped for each SNP are shown in Table III.

Linkage disequilibrium (LD) values of the MAOA SNPs were determined using HAPLOVIEW [Barrett et al., 2005] and ranged between 0.873 and 0.99. Haplotypes were estimated using the haplo.em function implemented in the haplo.stats package [Sinnwell and Schaid, 2005], which computes maximum likelihood estimates of haplotype probabilities. Prior probabilities of haplotype pairs for each subject were also computed to account for the fact that there may be more than one pair of haplotypes that are consistent with the observed marker genotypes. Haplotype association analyses were done using the haplo.score function [Schaid et al., 2002]. Briefly, this package computes score statistics to test associations between haplotypes and a wide variety of traits, including binary, and allows adjustment for other determinants. This analysis was corrected for multiple testing by applying the simulate = TRUE parameter in haplo.score which gives simulated $P$ values. These simulated haplotype score statistics are calculated from a permuted re-ordering of the trait (ADHD status) and covariates (in this case MAOA SNPs). We used 1,000 permutations for all the analyses. Finally, missing SNP genotypes were inferred using the observed genotype data from the rest of the sample using the haplo.em function. In this way, the number of missing genotypes was reduced to zero.

Since our sample is composed of family data, we initially estimated the overall haplotype frequencies using the parental data only. Thereafter, we separately estimated the children haplotype frequency in the groups of affected and non-affected children. Six different haplotypes were present in the parental and children samples: GGC, ATT, AGC, ATC, AGT, and GGT (Table II). Haplotypes GGC and ATT captured 97.14% of the genetic variance in the investigated MAOA SNPs. Therefore, further analyses report only on these two haplotypes. Analyses were carried out for the dataset including the imputed genotype data as well as for the dataset without these data.

Data Analysis

Since MAOA is X-linked, we used the genotypes of mothers to test for Hardy–Weinberg equilibrium (HWE) using the Markov-Chain Monte-Carlo approximation of the exact test implemented in the GENEPOP package V 3.3. No deviations from HWE were detected for the three SNPs ($df = 2$, $P$-values between 0.479 and 0.982).

Haplotype frequency was compared between the group of affected and non-affected participants in order to find differences in frequency distribution. The association of MAOA with the neuropsychological measures was analyzed using a linear mixed model with MAOA as factor (two haplotype groups for boys: GGC and ATT; three diplotype groups for girls: GGC_GGC, GGC_ATT, and ATT_ATT) and family structure as random effect. In addition, a possibly moderating effect of age was taken into account by adding the effect of age group (two groups split by median age: children <11.5 years and adolescents >11.5 years) into the model as well as the interaction between MAOA and age. The rationale for this approach was based on previous findings in this sample, showing that associations between the dopamine transporter gene (DAT1) [Rommelse et al., in press] and the dopamine receptor 4 gene (DRD4) [Altink et al., submitted] with neuropsychological measures were different in children and adolescents. Correction for multiple comparisons according to the False Discovery Rate (FDR) controlling procedure was applied to the analyses with a q-value setting of 0.05 [Benjamini and Hochberg, 1995]. Following Cohen’s guidelines [Cohen, 1988], effect sizes were defined in terms of the percentage of explained variance: 1%, 9%, and 25% were used as cut-off to define small, medium, and large effects. These figures translate into $\eta^2$-values of 0.01, 0.06 and 0.14.

Table III. Sample Characteristics

<table>
<thead>
<tr>
<th>N</th>
<th>Mage in years (SD)</th>
<th>NADHD subtype (%)</th>
<th>Genotyped (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls with ADHD</td>
<td>Female non-affected siblings</td>
<td>Boys with ADHD</td>
<td>Male non-affected siblings</td>
</tr>
<tr>
<td>N</td>
<td>106</td>
<td>265</td>
<td>89</td>
</tr>
<tr>
<td>Mage in years (SD)</td>
<td>12.2 (3.1)</td>
<td>11.5 (3.8)</td>
<td>11.9 (2.7)</td>
</tr>
<tr>
<td>NADHD subtype (%)</td>
<td>15 (15.3)</td>
<td>-</td>
<td>15 (5.7)</td>
</tr>
<tr>
<td>Combin</td>
<td>15 (14.1)</td>
<td>-</td>
<td>6 (2.3)</td>
</tr>
<tr>
<td>N genotyped (%)</td>
<td>60 (70.6)</td>
<td>-</td>
<td>244 (92.1)</td>
</tr>
</tbody>
</table>

Table II. MAOA Haplotype Distribution within the Sample

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Parents (N = 477)</th>
<th>Affecte children (N = 350)</th>
<th>Non-affected children (N = 195)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGC</td>
<td>0.63806</td>
<td>0.67455</td>
<td>0.60781</td>
</tr>
<tr>
<td>ATT</td>
<td>0.3278</td>
<td>0.28676</td>
<td>0.3819</td>
</tr>
<tr>
<td>AGC</td>
<td>0.01995</td>
<td>0.02325</td>
<td>-</td>
</tr>
<tr>
<td>ATC</td>
<td>0.00654</td>
<td>0.00759</td>
<td>0.00684</td>
</tr>
<tr>
<td>GGT</td>
<td>0.00446</td>
<td>0</td>
<td>0.00345</td>
</tr>
<tr>
<td>AGT</td>
<td>0.00308</td>
<td>0.00786</td>
<td>0</td>
</tr>
</tbody>
</table>

*Previously known as rs1801291.
boys had more often the combined subtype compared to affected girls (92.1% vs. 70.6%), whereas the inattentive and hyperactive-impulsive subtypes were more common in girls than boys (15.3% vs. 5.7% and 14.1% vs. 2.3%, respectively). The ATT haplotype was more common in non-affected siblings (38.2%) compared to affected participants (28.7%) \( (P = 0.025) \). The frequency of the GGC haplotype was higher in affected participants (67.5%) compared to non-affected siblings (60.8%), though this difference was not significant \( (P = 0.095) \). These effects were apparent in both boys and girls (Fig. 1).

In boys, MAOA haplotype had a significant effect on the Pursuit task, measuring motor control under continuous adaptation \( (F (1, 311.5) = 9.62, P = 0.002, \eta^2_p = 0.03) \), and a nominally significant effect on the Tracking task, measuring motor control without continuous adaptation \( (F (1, 300.8) = 4.66, P = 0.032, \eta^2_p = 0.02) \). Boys having the GGC haplotype performed better than boys with the ATT haplotype (Fig. 2). These effects were comparable in children and adolescents, since the interaction between MAOA and age were not significant \( (F (1, 336.0) = 0.39, P = 0.53 \) and \( F (1, 333.0) = 0.51, P = 0.48, \) respectively).

No main effects of MAOA haplotype or interaction effects between MAOA and age on other neuropsychological measures were found in boys (data available on request).

In girls, MAOA diplotype had a nominal significant effect on the Visuo-Spatial Sequencing task, measuring visuo-spatial working memory \( (F (2, 184.0) = 4.77, P = 0.01, \eta^2_p = 0.05) \). A nominal significant linear effect was present \( (P = 0.01) \) with girls having the ATT_ATT diplotype performing best, girls with the GGC_GGC diplotype performing poorest, and girls with the GGC_ATT diplotype immediately (Fig. 3). This effect was comparable for children and adolescents, since the interaction between MAOA diplotype and age was not significant \( (F (2, 184.0) = 1.85, P = 0.16) \). No additional main effects of MAOA haplotype or interaction effects between MAOA and age on other neuropsychological measures were found in the girls. Findings were similar, when analyses were repeated including only the children for whom haplotype data were available and did not need to be estimated (data available on request). In addition, findings were similar for affected and non-affected children, since post-hoc analysis of the interaction between MAOA haplotype and diagnosis was not significant for any of the measures in boys or girls (data available on request).

**DISCUSSION**

We set out to examine the relationship between MAOA genotype, ADHD and neuropsychological functioning in both boys and girls. Based on three SNPs, six different haplotypes were observed in our sample, of which two were common (GGC in 65.18% and ATT in 31.96%). All other, rare haplotypes (frequencies of 2.3% and below) were excluded from analysis. Both in boys and girls, the ATT haplotype was more common in non-affected siblings compared to affected participants, suggesting that this haplotype may have a protective effect against developing ADHD. The GGC haplotype had a somewhat higher frequency in affected versus non-affected participants, though not significantly. This latter finding is in line with the findings in the IMAGE study sample: The alleles of the SNPs within the haplotype individually as well as part of a haplotype showed
MAOA was associated with visuo-spatial working memory. In girls, the ATT haplotype was associated with motor control. In boys, the ATT haplotype was conversely associated with poorer neuropsychological performance, whereas in girls the ATT haplotype was associated with better performance. This finding may be related to differential effects of serotonin levels on behavior and cognition [Oades, 2007]. Decreased serotonin levels in the cerebrospinal fluid have been associated with poorer aggression control (which may be viewed as decreased behavioral inhibition), yet also with better ability to inhibit on experimental paradigms (which may be viewed as increased cognitive inhibition) [Oades, 2007]. Thus, similar serotonin levels may produce opposite behavioral and cognitive effects [Oades, 2007]. If one would translate this to the current findings, motor control may be viewed as belonging more closely to the behavior domain and visuo-spatial working memory more closely to the cognitive domain.

Differential sex effects of MAOA on brain functions have also previously been reported. For example, only in males, but not females, was an association found between a low expressing variant of MAOA and dysregulated amygdala activation and increased functional coupling with ventromedial prefrontal cortex [Buckholtz et al., 2008]. Furthermore, differential sex effects of MAOA on clinical manifestation have been described for a number of psychiatric disorders, such as for obsessive compulsive disorder [Karayiorgou et al., 1999], panic disorder [Deckert et al., 1999], mood disorders [Lin et al., 2000], and pathological gambling [Ibanez et al., 2000]. The effect of MAOA on brain and behavioral functions may thus be moderated by sex in a wide spectrum of functioning. Our findings of both the target and direction of the MAOA effect on neuropsychological functioning differing between the sexes, suggests that the genetic and neuropsychological mechanisms underlying ADHD may be different in boys and girls and underlines the importance of taking into account sex effects when studying ADHD.

A final point of discussion is the finding that we found an effect of MAOA on visuo-spatial working memory (in girls), but not on other executive functions, such as inhibition and verbal working memory. This might be hypothesized as being related to subtle differences in the underlying neurotransmitter systems mediating the executive functions. Serotonin, amongst other neurotransmitters, relates to visuo-spatial working memory [Luciana et al., 2006], but not, or to a lesser extent, to inhibition [Chamberlain et al., 2008] or verbal working memory [Kugaya et al., 2003]. Given that MAOA mainly influences the metabolization of serotonin [Craig, 2007], this may explain the selective effect of MAOA on visuo-spatial working memory and not inhibition or verbal working memory. However, the current findings need replication before firm conclusions can be drawn on the specificity of MAOA in relation to visuo-spatial working memory.

Our findings should be viewed in the light of several limitations. First of all, group sizes were relatively small, as well as were the haplotype frequency differences between the groups of affected and non-affected siblings. Therefore, our findings need to be replicated before firm conclusions can be drawn. Second, the group of affected boys and girls differed in the distribution of ADHD subtypes: The boys had more often the combined subtype compared to the girls, which may have influenced the differential effect of sex on the findings. However, this is unlikely, since repeating the analyses including only boys and girls with the combined subtype, we observed similar, and in girls even more significant, results. Third, the effect of MAOA on neuropsychological functioning...
appears not to be profound. In only three of ten neuropsychological measures was a small effect found, of which only one survived correction for multiple testing. Importantly, since the female sample was smaller than the male one, the small effect sizes have limited the power to detect effects of genotype in females. Fourth, the neuropsychological measures used here are by no means representative of the full domain of neuropsychological functions and tasks relevant for ADHD. The current findings need replication before firm conclusions may be drawn on the differential effects of MAOA on neuropsychological functioning in boys and girls.

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