Two candidate gene polymorphisms in ADHD children: a case-control study of catechol-O-methyltransferase (COMT) and monoamine oxidase B (MAOB) genes

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Submitted: 25 September 2006
Accepted: 27 October 2006

Arch Med Sci 2006; 2, 4:235-239

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Abstract

Introduction: Attention-deficit hyperactivity disorder (ADHD) is a common polygenic heritable disorder. We have investigated the relationship between ADHD and polymorphisms of catechol-O-methyltransferase (COMT) and monoaminooxidase B (MAOB) genes. It is well known that COMT and MAOB are metabolising enzymes that degrade biogenic amines and control the levels of these neurotransmitters in the central nervous system. An association has been previously observed between the Val158Met polymorphism of catechol-O-methyltransferase (COMT) gene and ADHD. The gene of monoamine oxidase B (MAOB) has also been suggested to play a role in psychiatric disorders and behavioural traits. Moreover, MAO inhibitors have been shown to be effective in the treatment of ADHD.
Material and methods: 118 boys with ADHD and 153 controls aged from 7 to 13 years were included in this study. PCR methods for the detection of the studied polymorphisms were used. Fisher's exact test was performed to assess the association between the studied groups.
Results: There was no statistically significant difference in the genotype frequency of COMT (p=0.321) or MAOB (p=0.087) gene polymorphisms between the hyperactive group of boys and the control group of boys.
Conclusions: Our study supports the results of the previous studies: these two polymorphisms do not play the main role in the pathogenesis of ADHD.

Key words: ADHD, COMT, MAOB, polymorphism, gene.

Introduction

Attention-deficit hyperactivity disorder (ADHD) is a highly heritable disorder that includes inattention, impulsivity, fractiousness and distractibility. ADHD (using DSM-IV criteria) is the most commonly diagnosed behavioural disorder in childhood.

The hyperactivity syndrome affects approximately 3-6% of school-age children worldwide.

Boys are affected about 8 times more frequently than girls [1]. The symptoms of ADHD may persist into aduldhood. Hyperactive children are sometimes extremely wild and their attention often wanders from one stimulus to another. The symptoms may lead to poor school results or to social maladaptation [2].

The hyperactive disorder is probably caused by dysregulation of levels of dopamine, noradrenaline and serotonine in front-striatal-thalamo-cortical areas of the brain (lateral prefrontal cortex, dorsal anterior cingulate cortex, caudate and putamen). The success of stimulant medications and animal models implicating dopamine pathways were taken as support for this model. The catecholamine hypothesis is also supported by many neuroimaging studies [3, 4].

COMT (EC2.1.1.6) catalyses the transfer of the methyl group from S-adenosylmethionine to catecholamines including the neurotransmitters dopamine, epinephrine and norepinephrine. This O-methylation results in one of the major degradative pathways of the catecholamine transmitters. The COMT gene has been localized on chromosome 22q11. There are two gene variants in codon 158 of the COMT gene variants in humans. In the first one the nucleotide guanine leads to genesis of the amino acid valin; in the second one the nucleotide adenine represents methionine. A polymorphism at codon 158 of the protein sequence (valine to methionine) has been found to be associated with different enzyme activity. A common COMT polymorphism codes a thermolabile low activity enzyme, while the substituted variant codes a high activity enzyme. The homozygote variant Val/Val has a three to four-fold higher enzyme activity level than Met/Met homozygotes. Heterozygotes have an intermediate enzyme activity level [5, 6].

MAOB (amine: O2 oxidoreductases EC1.4.3.4) is a mitochondrial enzyme involved in the degradation of biogenic amines. MAOB preferentially oxidises phenylethylamine and benzylamine, and also oxidises dopamine, tyramine and tryptamine. The role of monoamine oxidase has been shown to be related to some behavioural changes including aggression, cognitive dysfunction and impulsiveness [8-10]. MAOB was found to be strongly expressed in neural crest derivatives [11]. It is suggested that dopamine in the nucleus accumbens shell is transported into MAOB-positive fibres where it is degraded by MAOB [12]. Some studies have shown that MAOB activity in platelets correlates with specific personality characteristics such as sensation seeking and impulsiveness. Low levels of platelet MAO as well as the personality traits associated with these low levels have been associated with type 2 alcoholism, recurrent criminality and antisocial violent behavior. Platelet MAO has a high degree of heritability and the regulation of MAOB gene expression seems to explain most of the inter-individual differences in this enzyme activity [13, 14]. High levels of MAOB are found in astrocytes and serotonergic neurons. There are two distinct MAO isoforms, MAOA and MAOB, which are both encoded in genes on the X chromosome [15]. The genotype at a variable region (A/G dimorphism) in intron 13 of the human gene encoding MAOB with the „A-allele" displays significantly lower enzyme activity than individuals with the „G-allele" [16].

The aim of our case-control study was to determine whether the Val158Met COMT and MAOB polymorphisms are associated with ADHD. We compared genotype and allelic frequencies between a group of ADHD boys and a control group of boys in the Caucasian population of the Czech Republic.

Material and methods

The total sample consisted of 271 boys between the ages of 7 and 13 years. The group of ADHD boys (118 – mean age 9.97) was screened using DSM-IV criteria for ADHD in the Department of Psychiatry of Faculty Hospital Brno Bohunice. Collected blood samples of the ADHD boys were frozen in EDTA and DNA was extracted from blood using the UltraClean Blood Spin kit (Mobio, USA). The control group consisted of 153 (mean age 9.7) psychiatrically normal males. The group was collected in primary schools in the city of Brno. The control group was screened for ADHD using the Connors Parent Rating Scale Questionnaire (CPQ) and Connors Teacher Rating Scale Questionnaire (CTQ). In the control group, the DNA was isolated from bucal tissue using the UltraClean Tissue DNA kit (MoBio, USA). Informed consent was signed by a legitimate guardian before including any boy in this study.

PCR amplification of the Val158Met polymorphism of COMT was performed using the primers described by Mynett-Johnson et al. (1998): forward primer was 5´-ACT GTG GCT ACT CAG CTG TG-3´ and reverse primer was 5´-CCT TTT TCC AGG TCT GAC AA-3´ [17]. The amplification reaction was caried out in a total volume of 50 µl, containing 100 ng genomic DNA, 0.5 M dNTP, 1 µM of each primer, 5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH = 8.4) and 1U Taq-Purple Polymerase (Top-Bio, Czech Republic). After initial denaturation at 94°C for 2.5 min, using the TouchgeneGradient thermal cycler (Techne, England), cycling took place for 32 rounds consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec. The length of the amplified fragment was 169 bp. The PCR product was digested using 5 units of restriction enzyme Hsp92 II at 37°C for 5 hours. The length of digested fragments was 29 bp and 140 bp. The product of 140 bp could be subsequently digested in fragments of 114 bp and 26 bp (140 bp – Val/Val high activity genotype, Val/Met – 140/114/26 bp intermediate activity genotype and 114/26 bp – Met/Met low activity genotype). Digested products were determined by electrophoresis in a 2% agarose gel (Agarose EliPhore, ELISABETH PHARMACON, Czech Republic) containing ethidium bromide at 80 V for 1.5 hour. The gel was visualized under UV light.

The intron 13 A/G polymorphism of MAOB was amplified using three allele-specific primers described in Garpenstrand et al. (2000): MAO1 5´-CACTGG CAA ATA GCA AAA GT-3´, MAO2 5´-CAC

TGG CAA ATA GCA AAA GC-3´ and MAO3 5´-GGA TTT ACT TTG CAG GCA CC-3´. Allele A was identified using MAO1 and MAO3 primers, allele G using MAO2 and MAO3 [16]. PCR conditions were similar to COMT PCR; in brief, mix volume of 50 µl was amplified in 33 cycles, annealing temperature was 64°C. The length of the amplified fragment was 663 bp, separated in a 1.5% agarose gel containing ethidium bromide. The differences of allelic and genotype frequencies between the group of ADHD boys and the control group of boys were compared and analysed using Fisher's exact test. We used the statistical software CSS statistica for the analyses (Statsoft, Tulsa, USA).

Results

Basic data of the assayed subjects are listed in Tables I and II. As shown in the tables, no statistically significant differences in genotype or allele frequencies were found between the control group and the ADHD-affected group of boys in the Caucasian population of the Czech Republic. The frequency of allele Met of the Val158Met polymorphism of COMT was 0.50 in the ADHD-affected group and 0.48 in the control group. The high activity allele Val was more frequent in the control group of boys than in the ADHD group, in contrast with the original hypothesis. The discrepancy between groups was insignificant and the results of Fisher's exact test did not confirm the association (p=0.321). The heterozygote genotype was the most frequent in both groups. Our study suggests that Val158Met polymorphism of the COMT gene is not itself associated with ADHD.

The frequency of allele A of the gene of MAOB was 0.60 in the ADHD group and 0.54 in the control group of boys; however, no differences have been found and all the differences are only numerical. The statistical analysis using Fisher's exact test did not confirm the association of the A/G polymorphism of the MAOB gene and ADHD (p=0.087). The A/G polymorphism of the MAOB gene is not itself associated with ADHD in Czech boys.

Discussion

The results of our study do not suggest an important role of these two functional polymorphisms in susceptibility to ADHD in children. We did not observe a statistically significant difference in the frequency of the high activity allele Val of the Val158Met polymorphism of the COMT gene between the ADHD and control groups of boys, although the association was found in several previous studies.

Rogeness et al. (1982) studied 25 children with behaviour disorders and 20 children in a control group. The affected children had significantly higher plasma levels of COMT [18]. Kuperman et al. (1988) examined the association between psychological parameters and levels of COMT in 31 young people (age 21-23) suffering from hyperactivity [19]. The level of COMT was positively correlated with impulsivity. Eisenberg et al. (1999) examined 48 families with ADHD using family-based methods and found a significant association between ADHD and allele Val of the Val158Met polymorphism (p=0.03) [20]. Reuter et al. (2005) revealed an association between the Met allele of the COMT gene and inattention (p=0.008) and hyperactivity/impulsivity (p=0.039) in 203 healthy subjects [21]. Bellgrove et al. (2005) tested for association of this polymorphism with ADHD and examined its influence on prefrontal cognition in ADHD. Children possessing the methionine variant performed significantly below age-related norms on tests of sustained attention. Nevertheless, they reported no association of the Val158Met COMT gene polymorphism of 179 ADHD cases using a family control design [22]. Sery et al. (2006) found an association between the Val158Met polymorphism of the COMT gene and male alcoholism (p=0.007). A decrease in frequency of the Met allele was

Table I. Genotype and allele frequency of Val158Met polymorphism of COMT gene

Group	Count of genotype			Total	Genotype frequency (%)			Allele Met frequency	Fisher's exact test
	Met/Met	Val/Met	Val/Val		Met/Met	Val/Met	Val/Val		
Controls	36	75	42	153	23.5	49.0	27.5	0.48	p=0.321
ADHD	30	59	29	118	25.4	50.0	24.6	0.50	

Table II. Genotype and allele frequency of intron 13 A/G polymorphism of MAOB gene

Group	Count of genotype		Total	Genotype frequency (%)		Allele A frequency	Fisher's exact test
	A	G		A	G		
Controls	82	70	152	53.9	46.1	0.54	p=0.087
ADHD	71	47	118	60.2	39.8	0.60	

observed in male alcoholics (0.47) when compared with male controls (0.57) [23].

No evidence for an association between COMT and ADHD was observed in other studies. Barr et al. (1999) tested 77 nuclear families using the functional polymorphism at codon 158 that determines COMT activity. They analysed the data with the transmission disequilibrium test (TDT) with negative findings [24]. Hawi et al. (2000), Tahir et al. (2000), Payton et al. (2001), Zhang et al. (2003), Mills et al. (2004), Taerk et al. (2004), Turic et al. (2005) and Bobb et al. (2005) did not find an association between this polymorphism and ADHD [25-32]. Bobb et al. (2005) investigated the association between ADHD and polymorphisms in 12 previously studied candidate genes involving Val158Met polymorphism of the COMT gene. They investigated a group of 163 ADHD probands, 192 parents and 129 healthy subjects. They did not find any association between the COMT polymorphism gene and ADHD using case-control and family-based methods [32]. Futhermore, one of the largest family-based studies of ADHD and COMT (the sample included 176 parent-proband trios and 103 duos), performed by Turic et al. (2005), did not find an association either (p=0.88) [31].

MAOB genes have been previously studied for the association with ADHD in two different studies. Jiang et al. (2001) tested the linkage between MAOB $(GT)_n$ locus and ADHD in 82 nuclear families of the Chinese population. The TDT analysis did not reveal linkage between ADHD and the MAOB $(GT)_n$ locus [33]. The second study did not observe the transmission of T/C alleles of MAOB rs1799836 and $(CA)_n$ rs3838196 polymorphisms of MAOB to affected children [34]. Sery et al. (2006) found a relationship between A/G polymorphisms of the MAOB gene and acute pain perception. Male subjects with G genotype reported higher average intensity of postoperative pain (measured by visual analogue scale (VAS) score = 3.96) than male subjects with A genotype (VAS score = 3.45) [35].

We did not detect any significant difference in A/G polymorphisms of the MAOB gene between the ADHD and control groups of boys (p=0.087). So far there has not been found any association between the MAOB polymorphism and ADHD.

Conclusions

Our results suggest that the Val158Met COMT and MAOB genotypes are not associated with ADHD in the Czech population. We did not exclude the possibility that these polymorphisms play a role in interaction with other polymorphisms of candidate genes. For such studies of interactions, larger groups of studied persons are needed.

Acknowledgements

This study was supported by Grant NF 6520-5/2001 from the Internal Grant Agency of the Czech Ministry of Health. The authors wish to thank all the children, parents, headmasters and teachers from primary schools in the city of Brno who took part in the study.

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