



## Platelet monoamine oxidase activity in children with attention-deficit/hyperactivity disorder

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### ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is a highly heritable developmental disorder characterized by symptoms of impulsivity, hyperactivity and/or inattention, and associated with structural and biochemical abnormalities in cortical and limbic structures innervated by dopamine, noradrenalin and serotonin. The enzyme monoamine oxidase, type B (MAO-B), is expressed in platelets, and metabolizes endogenous amines. Its activity has been proposed to represent a peripheral marker of various traits and forms of psychopathology. This study evaluated platelet MAO activity with a spectrofluorimetric method in 72 boys and 12 girls with predominantly hyperactive, predominantly inattentive, and combined subtype of ADHD (DSM-IV criteria), and in 64 control children. The results showed significantly lower platelet MAO activity in children with hyperactive, inattentive, and combined subtype of ADHD than in control children. There was no significant association between platelet MAO activity and gender or age. The limitation of the study was in the small sample of girls with ADHD ( $N = 12$ ), and in the determination of only one peripheral marker. In line with hypotheses of lower platelet MAO activity in different types of psychopathology, children with different subtypes of ADHD had significantly lower platelet MAO-B activity than control children.

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### 1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a highly heritable developmental disorder characterized by behavioral symptoms of impulsivity, hyperactivity and/or inattention (Biederman, 2005). The prevalence of ADHD ranges from 4% to 10% (Faraone et al., 2005). Affected children have difficulties in educational, academic, and social functioning and in skill development, and 40% to 60% of children with ADHD are prone to show clinically significant symptoms in adulthood (Biederman, 2005). ADHD is a multifactorial and heterogeneous disorder. The etiology of ADHD involves the complex interplay of different demographic, psychosocial, psychiatric, cognitive, genetic and environmental factors (Biederman, 2005; Faraone and Khan, 2006). The neurobiology of ADHD includes dysfunction of dopaminergic, noradrenergic and serotonergic systems (Comings, 2001; Biederman, 2005; Faraone and Khan, 2006; Chamberlain et al., 2007), and these neurotransmitters regulate forms of behavior that are disturbed in ADHD (Chamberlain et al., 2007).

Monoamine oxidase (MAO) is an enzyme that catalyzes oxidative deamination of monoamines such as dopamine, serotonin and noradrenaline (Oreland, 2004). The MAO-B type is expressed in platelets, astrocytes and serotonergic neurons, catalyzes beta-phenylethylamine, benzylamine, dopamine, tyramine, and tryptamine, and is inactivated by deprenyl (Oreland, 2004). The activity of platelet MAO has been proposed to be a peripheral marker for a variety of personality traits and vulnerability to psychiatric disorders (Schalling et al., 1987; Irving et al., 1989; Oreland and Hallman, 1995; Kirk et al., 2001; Oreland, 2004; Ruchkin et al., 2005; Paaver et al., 2006). Lower platelet MAO activity, found in behaviors such as sensation and novelty seeking, high risk taking, aggression and impulsiveness (Reist et al., 1990; Oreland, 2004; Paaver et al., 2006; Ruchkin et al., 2005), suggested an association between 5-HT function and various pathological behaviors (Reist et al., 1990; Oreland 2004), and supported the proposal that platelet MAO is a genetic marker for the capacity of central serotonergic activity (Oreland 2004). Platelet MAO activity is affected by smoking (Eensoo et al., 2007), gender (Roth et al., 1976; Malmberg et al., 2008), ethnicity and race (Sobell et al., 1997), and medication such as haloperidol (Meszaros et al., 1998), clozapine (Ertugrul et al., 2007), antidepressants (Pivac et al., 2003) or lamotrigine (Muck-Seler et al., 2008). Since early studies, obtained

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on small samples, showed that children with ADHD have low platelet MAO activity (Shekim et al., 1982; Shekim et al., 1986), and platelet MAO activity is under the influence of different factors, the aim of the present study was to evaluate platelet MAO activity in ethnically uniform Caucasian boys and girls of the Croatian origin with ADHD, subdivided into groups with predominantly hyperactive, predominantly inattentive, and combined subtype of ADHD, and in healthy control boys and girls, controlled for the effect of smoking, medication and ethnicity. The hypothesis of the study was that platelet MAO activity would differ between children with subtypes of ADHD and control children.

## 2. Methods

### 2.1. Sample population and study design

The study included 91 children with ADHD ( $9.24 \pm 2.44$  years old, age range 4–14 years), recruited sequentially from the Polyclinic Kocijan/Hercigonja, Zagreb, diagnosed using a psychiatric interview (APA, 1994), a physical examination, and the Conners' Rating Scale for Parents (CRS-P) and Teachers (CRS-T)-short version (Conners, 1998). The children had an average CRS-P raw score of  $53 \pm 19$  (range 10–80). The CRS-T scores were not included in further evaluations, since only a certain number of children were evaluated with the CRS-T. A psychiatrist (DKH) assessed ADHD diagnosis, according to the DSM-IV criteria, and conducted separate interviews with the children and their parents, and asked about smoking habits, while a psychologist performed psychological interview and psychological tests. Children were referred by their parents or teachers as suspected ADHD, but only children who fulfilled the DSM-IV criteria (American Psychiatric Association, 1994) for ADHD were included in the study. Demographic data were uniform for all children: they were Caucasians of urban origin, attending kindergartens or primary schools, and came from undisturbed families, with average intellectual abilities. Exclusion criteria were co-morbid depression, schizophrenia, oppositional defiant disorder, conduct disorder, borderline personality disorder and somatic disorders, all assessed by psychiatric interview based on DSM-IV criteria (APA, 1994). Of the 91 children with ADHD, only 84 medication-free children, without any co-morbidities, were included in the study, while 7 children were excluded due to previous pharmacotherapy with carbamazepine, clomipramine, lamotrigine, or selective serotonin reuptake inhibitors. Based on the predominant symptoms, psychiatrists subdivided 84 children with ADHD into three subgroups: children with an inattentive, hyperactive/impulsive, or combined type of ADHD, and these children had an average CRS-P raw score of  $61 \pm 14$  (range 20–80).

A group of 64 healthy children, matched for age with the children with ADHD ( $10.0 \pm 2.87$ , range 5–15 years old), having their regular physical exams, served as controls. Somatic or other psychiatric co-morbidities were excluded according to DSM-IV criteria and the Child Symptoms Inventory-4 (American Psychiatric Association, 1994). All parents signed written informed consent. The local Ethics committee approved this protocol. The study was carried out in accordance with the Helsinki Declaration.

### 2.2. Blood sampling and determination of platelet MAO activity

Blood samples (4 ml) were taken during routine laboratory tests from forearm vein into a plastic syringe with 1 ml of acid-citrate-dextrose anticoagulant at 08.00 a.m. after overnight fasting. Platelet-rich plasma (PRP) was obtained by centrifugation ( $935 \times g$ ) for 70 s at room temperature. Platelets were sedimented by further centrifugation of PRP at  $10,000 \times g$  for 5 min. The platelet pellet was washed with saline and centrifuged again. Platelets were lysed by sonication (20 kHz, amplitude  $8 \times 10^{-3}$  mm for 30 s). Platelet MAO activity (expressed as nmol 4OHQ/mg protein/h) was determined by the spectrofluorimetric method, using kynuramine as a substrate, by a slight modification of the method of Krajl (1965), as previously described (Pivac et al., 2006). Briefly, specimens of standard, blank (water) and platelet sonicates (100  $\mu$ l) were analyzed in duplicates in phosphate buffer (0.5 M, pH 7.4), incubated 60 min on 37 °C with kynuramine. The reaction was stopped by adding ice-cold 1 N NaOH. The measurement of 4-hydroxyquinoline (4-HOQ) fluorescence, a product of kynuramine, was performed on a Varian Cary Eclipse spectrofluorimeter, with an exciting wavelength of 310 nm and emitted wavelength of 380 nm. The detection limit of the method was 1 nmol/sample, and intra- and inter-assay coefficients of variation were 4.4% and 5.0%, respectively. Platelet protein was determined by the method of Lowry et al. (1951).

### 2.4. Statistical data analysis

The statistical analysis of the data was conducted with Sigmasat 3.5 (Jandell Scientific Corp. San Raphael, California, USA). The results, expressed as means  $\pm$  standard deviations (SD), were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test, and with two-way ANOVA (to evaluate the effect of diagnosis, gender, and the interaction between diagnosis and gender on platelet MAO activity). The correlation between parameters was determined by Pearson's correlation coefficient. To control for multiple testing, Bonferroni correction was applied, using the total number of maximal pair comparisons ( $N=4$ ) as the correction factor, and the level of significance was set to  $P=0.0125$ .

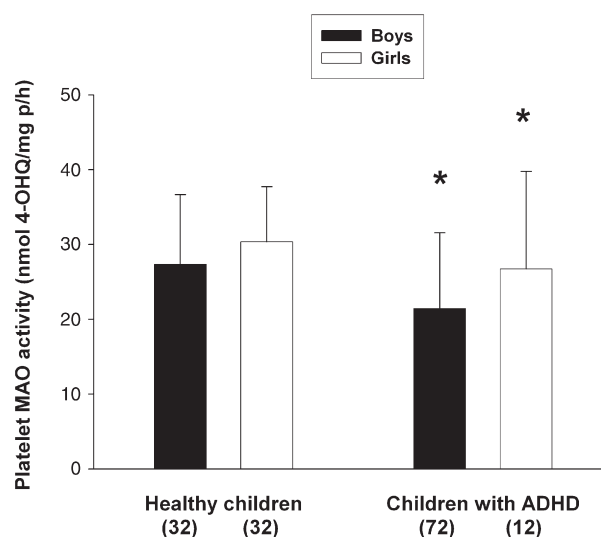


Fig. 1. Platelet MAO activity (mean  $\pm$  S.D.) in boys and girls with ADHD and in healthy boys and girls. The numbers in parentheses represent the number of subjects. \* $P < 0.001$  vs. control children (two-way ANOVA).

## 3. Results

Platelet MAO activity was significantly ( $F=10.710$ ;  $df=1,146$ ;  $P < 0.001$ , one-way ANOVA) different between 64 control children ( $28.84 \pm 8.46$ ) and 84 children with ADHD ( $21.10 \pm 10.13$ ). Children with ADHD had significantly ( $P < 0.001$ , Tukey's test) lower platelet MAO activity than control children. Two-way ANOVA showed a significant ( $F=11.532$ ;  $df=1,144$ ;  $P < 0.001$ ) effect of diagnosis (ADHD vs. control children), no significant ( $F=2.790$ ;  $df=1,144$ ;  $P=0.097$ ) effect of gender (male vs. female) on platelet MAO activity, and no significant interaction ( $F=0.002$ ;  $df=1,143$ ;  $P=0.963$ ) between diagnosis and gender on platelet MAO activity (Fig. 1).

As platelet MAO activity did not differ significantly (two-way ANOVA) between male and female children, the data from boys and girls in the further evaluations were collapsed. Platelet MAO activity was significantly ( $F=8.222$ ;  $df=3,144$ ;  $P < 0.001$ , one-way ANOVA) different, i.e. significantly (Tukey's test) lower in 11 children with

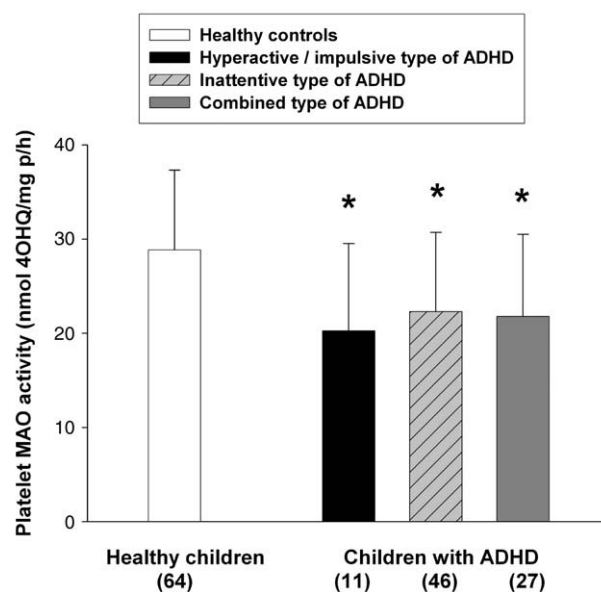


Fig. 2. Platelet MAO activity (mean  $\pm$  S.D.) in children with predominantly hyperactive, predominantly inattentive, and combined type of ADHD, and in control children. The numbers in parentheses represent the number of subjects. \* $P=0.012$ – $0.001$  vs. control children (Tukey's test).

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