

Clinical and molecular-genetic markers of ADHD in children

Ivana DRTELKOVA¹, Omar SERY², Pavel THEINER¹, Alena UHROVA¹,
Marketa ZACKOVA¹, Blanka BALASTIKOVA¹, and Vladimir ZNOJIL³

1. Department of Psychiatry, Faculty Hospital and Masaryk University Brno, Czech Republic
2. Department of Biochemistry, Faculty of Sciences, Masaryk University, Brno, Czech Republic
3. Department of Pathophysiology, Medical Faculty, Masaryk University Brno, Czech Republic

Correspondence to: Prof. Ivana Drtilkova, MD
Psychiatricka klinika FN Brno
Jihlavská 20, 62500 Brno, Czech Republic
TEL.: +420 532 232 056; FAX: +420 532 233 706
E-MAIL: idrtilkova@fnbrno.cz

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Abstract

OBJECTIVES: The objective was to make a contribution to deepening the knowledge of the etiopathogenesis of ADHD.

DESIGN: In an association study design, an analysis of polymorphisms of selected genes was conducted in 119 hyperkinetic boys and a control group of boys, aged 7–13. Furthermore several psychologically determined subgroups were identified. A connection between psychological functions (endophenotypes) and genes were looked for.

RESULTS: There was a statistically significant difference found in allelic and genotype frequencies of the TaqI A polymorphism of the DRD2 gene. The frequency of the allele A1 in hyperkinetic boys and the control subjects was 0.26 and 0.15, respectively ($p < 0.003$). A statistically significant occurrence of atypical genotypes (8/10, 7/10 and 10/11) of the DAT1 gene was also found in hyperkinetic boys and a connection between the M235 polymorphism of the angiotensinogen gene and the positive family history of psychiatric illness was found in probands ($p = 0.031$). Significant correlations between the results of some neuropsychological tests and genes for neuro-/immunomodulators (IL-6, TNF-alpha) and the gene for the brain-derived neurotrophic factor (BDNF) were found.

CONCLUSION: The study showed a statistically significant prevalence of A1 allele of the DRD gene in the hyperkinetic group. We also found a significantly higher incidence of atypical DAT genotypes in the hyperkinetic group. Furthermore we found significant connections with particular gene polymorphisms which may hypothetically represent a neurodevelopmental risk factor in the etiopathogenesis of the disorder (IL-2, IL-6, TNF-alpha, BDNF). We further found a connection of the M235 polymorphism of the AGT (angiotensinogen) gene to positive family history of psychiatric illness ($p = 0.031$). As for cognitive characteristics, we identified three subtypes with different cognitive performance profiles. This finding shows interindividual variability of cognitive style in the group of hyperkinetic boys.

Abbreviations

cdDNA	– complementary DNA - DNA molecule produced by reversal transcription of RNA
COMT	– catechol-o-methyltransferase - enzyme
DBH	– dopamine beta hydroxylase - enzyme
DSM-IV	– 4 th revision of Diagnostic and statistic manual
DAT	– dopamine transporter
DRD2	– D2 dopamine receptor
DRD4	– D4 dopamine receptor
HHR	– haplotype-based haplotype relative risk
5-HTTLPR	– 5-HTT gene-linked promoter region for serotonin transporter
HRR	– haplotype relative risk
IL-6	– interleukin 6 - cytokine
HVA	– homovanillic acid
MAOA	– monoamine oxidase A
MAOB	– monoamine oxidase B
MHPG	– 3-methoxy-4-hydroxyphenylglycol
ICD 10	– 10th revision of International classification of diseases
ADRA1	– alpha-adrenergic (noradrenalin) receptor A1
ADRA2	– alpha-adrenergic (noradrenalin) receptor A2
PCR	– polymerase chain reaction
TDT	– transmission disequilibrium test – statistical method
TDT	– test diskriminace tvaru (shape discrimination test)
TDTp	– test diskriminace tvaru, presnost (shape discrimination test, accuracy)
VNTR	– variable number of tandem repeats – repetitive polymorphism

INTRODUCTION

According to current opinion the cause of ADHD is most probably heterogenous, both genetic and non-genetic factors associated with neuroanatomical and neurochemical insults, predominantly during prenatal development, can play a role in its etiology. The results of molecular genetic studies from recent years have suggested that there is a significantly higher occurrence of specific alleles of some genes in individuals with ADHD, which can be responsible for defects of neurotransmission, or possibly for some of the neurodevelopmental abnormalities of brain structures and functions (Kirley *et al.*, 2002, Bobb *et al.*, 2005, Comings *et al.*, 1991, Hawi *et al.*, 2003, Inkster 2004). It has been also considered that specific gene polymorphisms and their combinations may give rise to certain subtypes of ADHD characterized by specific symptomatology, possibly even with a different prognosis and a different response to medication (Winsberg and Comings, 1999, Roman *et al.*, 2004, Kirley *et al.*, 2003).

The **goal of the study** was to make a contribution to deepening the knowledge of the etiopathogenesis of ADHD by answering the following questions:

- Will genetic testing in the Czech population confirm genotypes specific for this disorder in comparison with the control group?
- Do distinct genetic findings exist within the category of ADHD in particular subtypes (or phenotypes) of hyperkinetic disorders as categorized in DSM-IV (– combined type, – type with predominance of attention deficit, – type with predomi-

nance of hyperactivity/impulsivity) and in ICD-10 (– attention deficit with hyperactivity, – hyperkinetic conduct disorders)?

- Does a distinct phenotype exist for ADHD in the group of children without a hereditary predisposition and in the group of children with the occurrence of these disorders in first-line relatives?
- Will the research confirm recent, sporadic reports indicating the existence of distinct genotypes in respondents and non-respondents to methylphenidate?

STUDY SAMPLE AND METHODSParticipants

Boys treated for the diagnosis of ADHD (in both outpatient and inpatient settings) in the Child Ward of the Department of Psychiatry of the University Hospital in Brno, boys hospitalized in the Children's Psychiatric Institution in Velka Bites and out-patients of some of the psychiatric outpatient facilities in Brno were included into the study group.

Totally, 272 unrelated persons of Czech nationality were included into the study, 119 of them boys with ADHD and 153 control subjects of corresponding age – students of elementary schools in Brno. Boys aged 7–13 were included, the mean age was 9.97 years (SD=1.66). Research was conducted with boys only, with regard to the fact that an impact of estrogens is suspected on the pathogenesis of the disorder (in particular the influence of estrogens on neurogenesis and expression of dopamine- and other receptors). Selection based on age and sex enabled us to obtain relatively homogenous groups for genetic analysis.

Involving each of the boys into the study was preceded by obtaining a written informed consent from their legal guardian.

Excluding criteria for participation in the study for both groups were congenital genetic disorders, epilepsy, mental retardation, schizophrenia, pervasive developmental disorders and serious medical illness.

Methods

In the group of ADHD-patients a detailed pedopsychiatric clinical examination was conducted with a diagnostic assessment based on criteria of the 10th revision of International Classification of Diseases – ICD 10 (WHO, 1996) as well as the DSM-IV (APA, 1994). Questionnaire methods used were: CTQ – Connors Teacher Rating Scale (Connors, 1998b) and CPQ – Children's Parent Questionnaire (Connors, 1998a). The subtype of the hyperkinetic disorder was determined – F90.0 attention deficit with hyperactivity or F90.1 hyperkinetic conduct disorder. A detailed structured objective psychiatric history was taken from parents of children, directed to the course and clinical manifestations of hyperactivity during the phases of the development of the

child. The presence of hyperkinetic symptomatology in parents and siblings was obtained retrospectively.

Psychological Assessment

We used the **Shape Discrimination Test (TDT, Test diskriminace tvaru)** (Svancara, 1976) for assessment of attention. The test is based on determining the level of accuracy and speed of differentiation between shapes in the given template. It is a monotonous task challenging attention, therefore the test is considered an appropriate method for testing it. The test enables to rate two criteria of achievement – speed (TDTr) and accuracy (TDTp).

Impulsivity was tested by the **TE-NA-ZO** test (Test of finding of familiar figures, *Test nachazeni znamych obrazku*) (Müllner, 1984). This test is based on the theoretical model of cognitive style, represented by two components – impulsivity (imp) and efficiency (ef).

In addition to these methods we used four tests from the neuropsychological battery for psychological assessment of the **Neurobehavioral evaluation system (NES2)** (Letz, 1998): Finger Tapping, Hand-Eye Coordination, Visual Digit Span and Switching Attention.

Characteristics of the tests used:

Finger Tapping – Evaluates motor speed during a certain time interval (we chose an interval of 30 seconds). The measurements are carried out for dominant and for non-dominant hand and for alternating of hands.

Hand-Eye Coordination – Evaluates visual-motor coordination. On the computer monitor a sinusoid appears, the task of the child is to keep the cursor pointed on the dot moving to the right on the displayed curve at a constant speed using a joystick.

Visual Digit Span – Evaluates short-term memory and attention. The test is based on the principle of reproduction of a row of visually exposed numbers, in a manner similar to the specific subtest of Wechsler Intelligence Scale.

Switching Attention – The test is considered a measure of selective attention. The principle of the test is evaluation of the capacity to react to the direction of the arrow exposed on the monitor as quickly as possible, number of mistakes (dir) and reaction time (RT) are measured.

Genetic Examination. 3 ml of venous blood were drawn from each boy from the ADHD group into an anti-clotting solution of 0.5 M EDTA, the sample was then frozen to -20°C and transported to the laboratory in a thermobox. Genomic DNA was isolated by the commercially available UltraClean BloodSpin kit (MoBio, U.S.A.), in the control group a swab of buccal mucosa was obtained and DNA was isolated using UltraClean DNA Tissue (MoBio, U.S.A.) The isolated DNA was used for genotyping as template DNA in a classical PCR method followed by restriction analysis or for RealTime PCR detection using marked probes.

Possible connections of ADHD and polymorphisms of these selected genes were tested in the project:

- TaqI A polymorphism of DRD2 (D2 dopamine receptor) gene
- Val158Met polymorphism of COMT (catechol-o-methyltransferase) gene
- I/D polymorphism of ACE (angiotensin-converting enzyme) gene
- -174 polymorphism of IL-6 (interleukin 6) gene
- delta 32 polymorphism of CCR5 (chemokine receptor 5) gene
- -308 polymorphism of TNF-alpha (tumor necrosis factor alpha) gene
- M235T polymorphism of AGT (angiotensinogen) gene
- A/G polymorphism in the 13th intron of MAO-B (monoaminoxidase B) gene
- -330 polymorphism of IL-2 (interleukin 2) gene
- A118G (Asn40Asp) polymorphism of Mu opioid receptor gene
- 196 G/A (val66met) polymorphism of BDNF (brain derived neurotrophic factor) gene
- 48-bp VNTR polymorphism of DRD4 (D4 dopamine receptor) gene
- 40-bp VNTR polymorphism of DAT1 (dopamine transporter) gene

The genotype for “dopamine“ genes (DRD2, DRD4, DAT, COMT, MAO-B), the genes for neuro-/immunomodulators (IL-2, IL-6, TNF alpha), the gene for ACE and the gene for BDNF were determined.

We studied correlations between genotype and the results of neuropsychological testing, the subtypes of the disorder as classified by ICD-10 and DSM-IV, the response to stimulants, heredity (occurrence of ADHD in first-line relatives) and selected comorbid disorders.

RESULTS

Molecular genetic differences between children with ADHD and the control group

A statistically significant difference in allelic and genotypic frequencies between the group of boys with ADHD and the control group was found with the **TaqI A polymorphism of DRD2 gene**. The comparison of molecular genetic findings in 119 hyperkinetic boys and 153 control subjects showed statistically significantly higher occurrence of the A1 allele of the DRD2 gene in the hyperkinetic group. The frequency of A1 allele was 0.26 in hyperkinetic children and 0.15 in the control group ($p < 0.003$). There also exists a statistically significant difference of the genotype frequencies between the studied groups of individuals ($p < 0.008$). In the group of hyperkinetic boys the genotypes **A1A1** and **A1A2** prevailed, whereas in the control group the

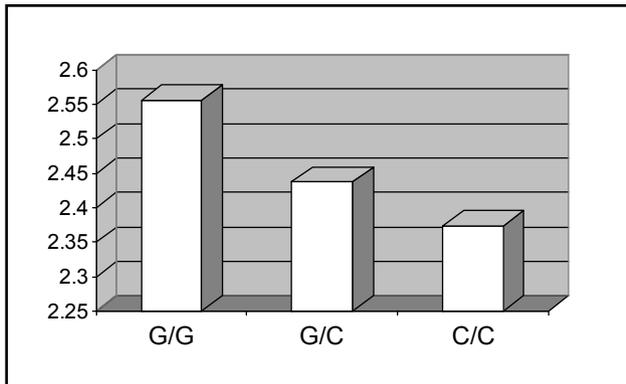


Figure 1. Relationship between the polymorphisms of the gene for IL-6 (alleles C and G) and the results in Hand-Eye Test of the NES2 (logarithm of deviation from the copied line).

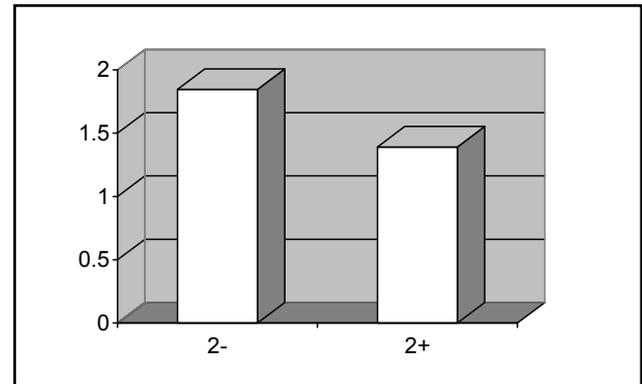


Figure 2. Relationship between the polymorphisms of the gene for TNF-alpha (alleles 1 and 2) and the results in TDT, accuracy subscale. Two groups – allele 2 present in either one or two copies (2+) and allele 2 absent (2-).

typical genotype was A2A2, found in 72 % of individuals from the control group.

We found also a statistically significant occurrence of atypical **genotypes (8/10, 7/10 and 10/11) of the DAT1 gene polymorphism** in boys with ADHD in comparison to control subjects. The Fischer Exact Test showed a statistically significant difference at a probability level of $p < 0.007$. Within the frames of a continuous analysis a statistically significant difference in the allelic and genotypic frequency of **-174 polymorphism of IL-6 gene** was found between the control group and the group of hyperkinetic boys. The frequency of C-allele was dominant in hyperkinetic boys (0.66) in comparison to boys from the control group (0.52).

A marginal connection was found between ADHD and the polymorphisms of the **TNF alpha** gene and the **MAO B** gene, however this relation lacks statistical significance.

Further the relation between interactions of **doublets of polymorphisms** (so called **double-genotypes**) and ADHD was studied. In this context no difference in statistical significance was found between the studied groups of individuals. Certain findings indicated differences between the groups, but these were not statistically significant (the connection between ADHD and MAO B and ACE, MU and DRD2, COMP and ACE and some other double-genotypes). However, for a statistically relevant calculation the size of the study will have to be extended.

On the other hand, a statistically significant correlation ($p < 0.01$) between polymorphisms of the μ -opioid receptor gene and the DRD2 gene was discovered. The incidence of genotype AAA2A2 was significantly higher than the incidence of other genotypes in the control group. There was also a higher incidence of genotype AAA1A2 in individuals with ADHD than in the control group. However, this difference was not statistically significant.

Cognitive functions as endophenotypes of ADHD

Significant correlations were found between the results of Hand-eye test of the NES2 and the **-174 polymorphism of the IL-6 gene** (alleles A and G), where individuals owning two copies of the G allele had major deviation of the line they had had to copy, individuals with A/A genotype performed best and heterozygotes A/D were in between. The differences were statistically significant ($p = 0.05$).

The TNF-alpha gene **-308 polymorphism** (alleles 1 and 2) correlated with the results of the “accuracy” subtest of TDT. The individuals with no allele 2 were more accurate than those owning at least one copy of allele 2 ($p = 0.01$).

The **196 G/A (val66met) polymorphism of the gene for BDNF** correlated with the results of Switching attention (direction) test of the NES2, where individuals owning at least one copy of the A allele made less mistakes than those without the A allele ($p = 0.01$). (Figures 1,2,3)

Molecular-genetic findings in ADHD subtypes according to cognitive style

One of our goals was to divide the group of hyperkinetic children into individual subtypes based on the results of psychological assessments and to test to what extent the combination of listed psychological tests and the genetic analysis can contribute to this categorization.

Based on achievement in the TDT and TE-NA-ZO tests, using cluster analysis, we separated three subtypes inside the studied group. Variables from both tests were included into the analysis (accuracy, speed, impulsivity, efficiency). We used cluster analysis, the *Quick Cluster (k-means)* method. The profiles of the subtypes are described Figure 4.

Profiles of the three specific subtypes:

Subtype A performed with average accuracy and average speed in psychological testing and was neutral in

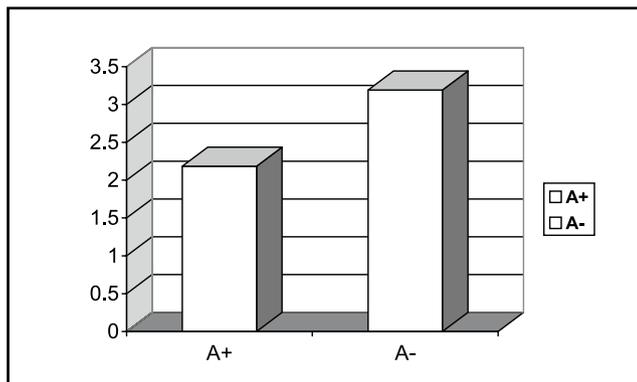


Figure 3. Relationship between the polymorphisms of the gene for BDNF (alleles A and G) and the results in the Switching Attention, direction subtest of the NES2. Two groups – allele A present in either one or two copies (A+) and allele 2 absent (A-). The figures show average number of errors in this subtest.

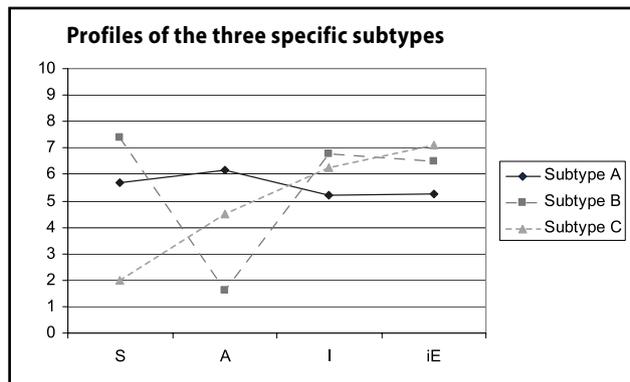


Figure 4. A-Accuracy, S-Speed, I-Impulsivity, iE-inefficiency

terms of impulsivity and efficiency. This subtype represents 33.9 % of the tested individuals.

Subtype B represents 34.7 % of the tested individuals. The performance was less accurate and very quick, manifesting high impulsivity and low efficiency.

Subtype C performed with lower accuracy and lower speed, neutral impulsivity and low efficiency in the tests. It represents 31.4 % of the tested individuals.

The differences of average achievements in tests between the subtypes were statistically significant ($p=0.012$, Mann-Whitney U Test).

We also studied possible connections between the results of neuropsychological testing of cognitive functions of patients with ADHD and the genotype for dopaminergic genes (DAT, DRD2, DRD4) and for neurodevelopmental genes (IL-2, IL-6, TNF-alpha, BDNF). Following significant correlations were found:

- 1) C/G polymorphism of IL-6 gene + hand-eye test from NES2 ($p=0.041$, Spearman rank order correlations).

In the ADHD group a correlation was found between the C/G polymorphism of the IL-6 gene and the performance in hand-eye test from the NES2 neuropsychological battery. GG homozygotes showed a greater deviation than heterozygotes and these showed a greater deviation than CC homozygotes. However, a more exact analysis would require a larger study sample size.

- 2) TNF-alpha 1/2 polymorphism + TDTp ($p=0.013$, Spearman rank order correlations).

We also found a correlation between the polymorphism of the TNF-alpha gene and the average performance in TDTp (accuracy). Carriers of the allele 2 (1/2 heterozygotes and 2/2 homozygotes) performed worse than 1/1 homozygotes.

- 3) BDNF A/G polymorphism + Switching attention, direction ($p=0.005$, Spearman rank order correlations).

The last found correlation is the one between the A/G polymorphism of the BDNF gene and the performance in Switching attention, direction test form NES2 battery. Carriers of the allele A (A/G heterozygotes and AA homozygotes) made fewer mistakes than G/G homozygotes. At once, there was no significant difference in reaction time.

Molecular-genetic findings in children with psychiatric heredity

Hyperkinetic symptoms were present in first-line relatives of 65 boys, not present in 39, not determined in 15. By genetic screening of 15 monitored genes a connection was found between the M235 polymorphism of AGT (angiotensinogene) gene and the incidence of psychiatric heredity (generally) in family history compared to probands with genotype II.

Molecular-genetic differences between clinical subtypes as classified in ICD-10

In the group of children with ADHD we studied possible connections between genotype and the diagnoses F 90.0 or F 90.1. In the group of 119 boys with ADHD 44 probands suffered from attention deficit disorder with hyperactivity subtype (F 90.9), 68 probands from hyperkinetic conduct disorder subtype and in 7 probands it was impossible to determine the subtype with certainty. No correlation between diagnosis (F 90.0 and F 90.1) and genotype was found.

Molecular-genetic findings in children with comorbid disorders

In the studied group **specific developmental learning disorders** (dyslexia, dysorthographia, dysgraphia and dyscalculia) occurred most often – in 69 boys. A connection between the polymorphism of the DRD-2 gene and specific learning disorders was found in probands ($p=0.014$). Specific learning disorders occurred more often in probands with genotype A2A2 in the DRD2

gene. **Enuresis** was present in 20 boys. There might be a connection between the genotype A (i.e. with adenine in the 13th intron) of the MAO-B gene and the occurrence of enuresis, but at this sample size it was not statistically significant. **Tics** were present in 13 boys, connection to genotype was not found.

Molecular-genetic differences in respondents and non-respondents to methylphenidate

In the group of 67 children of main age of 9.97 years, treated in the past or in the present by methylphenidate (Ritalin) we identified 44 non-respondents and 23 respondents to methylphenidate.

Even though our results of genetic screening with the 15 studied genes did not exceed the limit for statistical significance (which can be particularly related to a relatively small number of examined probands), a closer analysis showed some interesting trends. Genotypes whose the occurrence in the project as a whole was statistically significantly higher in the group with hyperkinetic symptoms (as compared with control subjects), also occurred more often (even though non-significantly) in respondents to methylphenidate. It was the case of the occurrence of the allele A1 in the TaqI A of the DRD2 gene and a higher occurrence of -174 polymorphism of the IL-6 gene in the form of the CC genotype.

In the case of Val158Met polymorphism of the COMT gene, the number of respondents to methylphenidate was higher in genotype VV, which is thought to be connected with higher COMT activity, on the contrary, the number of non-respondents was higher in probands with genotype NN, which is thought to be connected with lower activity of COMT.

In the case of A/G polymorphism of the MAO-B gene the occurrence of allele G (thought to be connected with higher enzymatic activity) was also higher in respondents, and the occurrence of allele A, with lower enzymatic activity, was higher in non-respondents.

DISCUSSION

A statistically significant difference in allelic and genotypic frequencies between the group of boys with hyperkinetic disorder and the control group was found in the TaqI A polymorphism of DRD2 gene, where a significantly higher occurrence of A1 allele of DRD2 gene was found in the hyperkinetic group. These results are an evidence of the role of dopamine system in the etiopathogenesis of ADHD. We can hypothesize, that the A1 allele and the A1A1 genotype predispose children for development of ADHD.

In a continuous analysis a statistically significant difference in allelic and genotypic frequencies of the -174 polymorphism of the IL-6 gene was found between the control group and the group of hyperkinetic boys. The frequency of C allele prevailed in hyperkinetic boys (0.66) as compared to boys from the control

group (0.52). Contextualized with the results of other studies (Sery *et al.*, 2003) our results could indicate that the C allele and the CC genotype tend to predispose individuals to alcoholism and boys to ADHD. Since, on the contrary, the GG genotype predominated in boys in the control group, it could be seen as protective against ADHD (Sery *et al.*, 2003).

Cognitive functions can be considered the reflection of functioning of neuronal circuits of the brain, as **endophenotypes**, and it can be presumed that the influence of the environment on them will be smaller than its influence on clinical picture. Deficits in neuropsychological tests are correlates of ADHD and preliminary studies show their connection to candidate genes. In the studied group we found no correlation between the studied polymorphisms of "dopaminergic genes" (DRD2, DRD4 and DAT genes) and the results of neuropsychological tests, however we found *significant connections with particular gene polymorphisms, which hypothetically may represent a risk factor in the etiopathogenesis of the disorder* (IL-2, IL-6, TNF-alpha, BDNF). The -174 polymorphism of the IL-6 gene can influence behavior as a neuromodulator or as a regulator of the development of brain tissue, connected with better survival of dopamine neurons (Juttler *et al.*, 2002). After enlarging the studied group however, the statistical significance of the difference in genotypic and allelic frequencies between the studied groups disappeared. We suppose however, that further enlargement of the studied group in the future will enable obtaining really relevant results about the relationship between the IL-6 gene and ADHD.

The results of this part of our study hypothetically indicate that the genetic basis of the cognitive deficit in ADHD does not necessarily need to be related to dopaminergic system, rather the development and maturation of neuronal circuits may be genetically influenced, as indirectly confirmed by ADHD-studies using structural and functional imaging methods. The occurrence of DD genotype of the ACE gene, found significantly more often in children with a family history of psychiatric illness, might be related to higher mental vulnerability (in the area of cognition, affectivity and personality traits) and be one of the risk factors in the etiopathogenesis of ADHD.

This result is supported by data from literature (Richard *et al.*, 2000, Holla *et al.*, 1999, Amouyel *et al.*, 1996).

Even though we did not succeed in founding a significant relation between the reaction to methylphenidate and the polymorphisms of selected genes, the results indicate some interesting trends. *Enzymes participating in dopamine degradation, such as MAO-B and COMT, were found more often in responders to methylphenidate which indicates their higher enzymatic activity.* In accordance with results of other studies, the study confirmed a higher number of non-respondents to methylphenidate in connection to occurrence of repetitive sequence

of the long allele (9,10,11) in the case of the polymorphism of the DAT gene (Winsberg and Comings, 1999, Roman *et al.*, 2002). Even though several studies with negative results exist, the presence of a 10-fold repeated sequence of the 40 bp repetition may be a risk factor for development of ADHD. Some authors presume that this allele could be connected to the expression and the level of dopamine transporter in vivo, even if the polymorphism is located outside the encoding region. For this reason several researchers studied the influence of this polymorphism on the response to methylphenidate-treatment in ADHD patients. The majority of these studies found a relation between the response to methylphenidate and the 40 bp VNTR polymorphism (Kirley *et al.*, 2003). Winsberg and Comings (1999) and Roman *et al.* (2002) described lower response to methylphenidate in individuals with DAT 10R/10R genotype.

CONCLUSIONS

Comparison of molecular-genetic findings in 119 hyperkinetic boys and 153 control subjects showed a statistically significant prevalence of A1 allele of the DRD gene in the hyperkinetic group. We also found a significantly higher incidence of atypical DAT genotypes in the hyperkinetic group. Furthermore we found significant connections with particular gene polymorphisms which may hypothetically represent a neurodevelopmental risk factor in the etiopathogenesis of the disorder (IL-2, IL-6, TNF-alpha, BDNF). The results of this part of the study indicate that the genetic basis of the cognitive defect in ADHD does not need to be connected only to the dopamine system, *rather the development and maturation of neuronal circuits may be affected in ADHD, as indirectly proven by studies with structural and functional imaging methods.* We further found a connection of the M235 polymorphism of the AGT (angiotensinogene) gene to positive family history of psychiatric illness ($p=0.031$). *The significantly higher occurrence of DD genotype in the ACE gene in probands with positive family history of psychiatric illness might be connected with higher mental vulnerability (in the area of cognition, affectivity and personality traits) and be one of the risk factors in the etiopathogenesis of ADHD.* This result is supported by data from literature.

Even though we did not succeed in our aspiration to find significant correlation between the reaction to methylphenidate and the polymorphisms of the studied genes, we observed that

- enzymes involved in dopamine degradation as MAO-B and COMT are found more often in genotypes of responders to methylphenidate which represents their higher enzymatic activity.

- In accordance with results of studies of foreign researchers our study confirmed a connection between higher number of respondents to methylphenidate and occurrence of repetitive sequence of long allele (9,10,11) in the polymorphism of the DAT gene.

Limitations

Despite the fact that in accordance with our plans we obtained a relatively large and homogenous group of probands (119 boys with ADHD and 153 control subjects), we are aware of the fact that our results will require confirmation and further elaboration in other, even larger groups of patients. However, we consider this first extensive association study in Czech Republic as a very important one, because with regard to the variability of genotype in world population, the results of research carried out in several other countries on individuals from different ethnic groups, are not fully transmittable into our conditions, as confirmed to some extent by the inconsistent results of previous studies.

Statistics

The program of CSS Statistica (Statsoft, Tulsa, USA) was used for statistical analysis. The statistical significance of the difference in genotype frequencies was calculated using X^2 -test and the statistical significance of the difference between allelic frequencies was determined using Fisher Exact Test. Subtypes with different cognitive performance profiles were identified by Quick Cluster (k-means) method. The statistical significance of the difference between subtypes average achievements in other tests was determined using Mann-Whitney U Test.

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