Biochemical markers and genetic research of ADHD

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Abstract
ADHD (attention hyperactivity disorder) is a polygenetic disorder with various candidate genes. At this time, more than thirty dopaminergic, noradrenergic, serotonergic and GABA-ergic genes are known. The research of only some candidate genes (DRD4, DAT, DRD5, DBH, 5HTT, HTR1B and SNAP25) brought relatively consistent results confirming the heredity of ADHD syndromes. The results of research of other genes (DRD2, DRD3, MAO, ADR2A, GABA A3, GABA B3) are not clear yet. This paper summarizes the most important genetic data in correlations with biochemical periphery parameters (especially for DBH, HVA, MHPG, serotonin). Hypothetically, certain subgroups of ADHD may be identified by correlation of biochemical characteristics and some candidate genes. The paper discusses some implications for future research. Review.

Introduction
ADHD (attention deficit hyperactivity disorder) is one of the most frequently diagnosed syndromes in the child psychiatry. The incidence is between 3 and 6 per cent of the children population; with boys predominating over girls at a ratio of 3:1 or more [2]. The key symptoms – inattentiveness, impulsivity and hyperactivity – deteriorate the relationship of these children both in the family and with their contemporaries, thus increasing the risk of social isolation. Hyperkinetic disorder (ICD-10 – International Classification Disorders, WHO, 1992) is a narrower diagnosis, and a subgroup of ADHD DSMIV diagnostic criteria, APA (American Psychiatric Association) 1994. Comorbid disorders of ADHD occur in 50% to 80% of patients [44]. These include: conduct disorders in 40% to 90%, depressive disorders in 15% to 20%, anxiety disorders in...
25% and learning disorders: dyslexia, dyscalculia etc. in 20% of all ADHD patients’ cases.

The hyperkinetic syndrome is up to five times more frequent in first-degree relatives of ADHD children than in control group of healthy children’s families [9]. In these ADHD children’s relatives, other diagnostic units were more frequent than in control group (comorbid ADHD disorders). When the occurrence of hyperkinetic disorder in biologically related and unrelated siblings of ADHD patients was compared, the following data were obtained: hyperactivity and conduct disorders were found in 47 to 53 per cent of biologically related and in 9 to 13 per cent of biologically unrelated children [82]. In adoption studies in ADHD, significant genetic role was proved [97 and other authors]. Concordance rates of monzygotic vs. dizygotic hyperkinetic twin pairs were between 50 and 80% for monzygotic twins and between 0 and 33% for dizygotic twins [90, 41, 93]. The heritability of ADHD varies between 0.75 and 0.98, which is highly significant for genetic aetiology of ADHD [27].

At this time, more than thirty dopaminergic, noradrenergic, serotonergic and GABA genes are known. This paper summarizes the most important genetic data in correlations with biochemical periphery parameters (especially for DBH, HVA, MHPG, serotonin). The rise of ADHD phenocopy is most probable caused, for instance, by perinatal hypoxia, influencing the immature dopaminergic and noradrenergic parameters (especially for DBH, HVA, MHPG, serotonin). The high value of dopamine (DA) turnover in children with ADHD respond positively to stimulating treatment [89]. Castel-lanos et al. [11] examined CSF in 29 boys of six to twelve years of age with a hyperkinetic syndrome. He found out that the level of HVA was lower in CSF of ADHD patients and the marked decrease of its level was surprisingly combined with a lower degree of hyperactivity. ADHD in adults is marked by this relation between the decreased plasmatic level of HVA and the presence of symptoms of the hyperkinetic syndrome missing, but the high level of HVA in CSF of ADHD adults predicts a bad response to methylphenidate [28].

MAO is a mitochondrial enzyme, participating in the degradation of neurotransmitters. The high value of plasmatic HVA and DOPAC predicts a bad response to treatment with selegiline in adult ADHD patients [28]. Boix et al. [10] proved inhibition of impulsivity in a clone of hyperactive rats. When MAOB (monoamine oxidase B) was blocked by L-deprenyl, however, hyperactivity and attentiveness disorders were not inhibited. The levels of norepinephrine, dopamine and serotonin in neostriate, nucleus accumbens and frontal cortex decreased. Zametkin et al. [107] administered clorglyline or tranylcypromine (irreversible MAO inhibitors) to fourteen boys with a hyperkinetic syndrome and found a very good clinical response, similar to administration of stimulants. Trott et al. [95, 96] administered moclobemide to hyperkinetic children. The results were less distinct than in administration of stimulants. DRD2 Comings et al. [14, 20] examined the prevalence of the Taq A1 allele of the DRD2 gene. Results suggested that genetic variants at the DRD2 locus played a role in a range of impulsive and compulsive personality disorders, addictive disorders and ADHD. Berman et al. [8] observed that the association of the DRD2 A1 or A2 allele with a given phenotype was dependent upon the presence or absence of childhood stress symptoms. Rowe et al. [80] reported that children with the DRD2 Taq I A2/A2 genotype had a higher mean level of ADHD symptoms than A1 carriers. Todd et al. [94] did not confirm correlation of DRD2 receptor and ADHD. The level of HVA in CSF is connected with the density of the DRD2 receptor and predicts the response to stimulants [17]. The HVA in CSF level rises after stimulant administration [17, 100]. Jönsson et al. [46] found a connection between the concentration of homovanillic acid and the genotype with DRD2 A1 occurrence. Heterozygote A1/ A2 allele had the lowest concentrations of homovanillic acid in CSF. A relation has also been described between the DRD2 A1 genotype and the regional CNS blood flow in children with hyperkinetic syndrome. By using F-deoxyglucose in PET examination, it was found out that the A1 allele carriers have a significant reduction of glucose metabolism in the putamen, frontal, temporal, central, central prefrontal, occipitotemporal and orbital cortex [17, 92]. Genotype and allelic frequency of TaqI A polymorphism of DRD2 gene was statistically different between ADHD group, only boys (n = 49), age 6–13, and control group (n = 40), age 6–13, only boys, p < 0.004 and p < 0.001, respectively [83]. Kirley et al. [51] examined two polymorphisms in 118 ADHD children and their families. No significant associations were identified, though they reported a trend toward significance (p = 0.07) for the Ser311 polymorphism when paternally transmitted. On aggregate, the studies to date suggest little or no association with ADHD [30]. DRD4 is another candidate gene. DRD4 is a gene with a high degree of genetic variability and one of the genes influencing the post-synaptic effects of dopamine [13]. A 48 bp and 16 amino-acid repeat polymorphism is important within the DNA coding for the third cytoplasmic loop, responsible for the binding to guanine-nucleotide proteins [60]. Allele 7, conditioning the inhibition of intracellular adenylyl cyclase and thus suppressing response to dopamine, was found in 41 per cent of ADHD patients in comparison to 21 per cent of controls [54]. Two independent studies [54, 87]
describe, in normal subjects, a relation between the presence of allele 7 DRD4, psychomotoric instability and impulsivity. In patients with ADHD, TS (Tourette's syndrome) and drug addiction, a significantly higher occurrence of 2/2 homozygotes was described [84]. This DNA region is repeated 2 to 11 times; with the most common alleles being the 2, 4, and 7 repeat. There was a modest increase in the prevalence the allele 2 with a decrease in the prevalence of the allele 4. The mean ADHD score, based on the Diagnostic Interview Schedule for Children, was 15.6 for those carrying a 7 allele, versus 13.3 for those not carrying a 7 allele, p < 0.015 [29]. Independent studies of normal subjects [88] have shown an association between the presence of the allele 7 and novelty seeking, a trait associated with impulsivity. Some studies failed to find the association found by Langley et al. [55] who concluded in review and in his own study that DRD4 7 – repeat allele is associated with a hyperactivity and impulsivity. Many other authors supported association of DRD4 and ADHD [26].

**DAT1** gene. The importance of the DAT1 (dopamine transporter) gene is associated to the mechanism of action of stimulants, blocking the dopamine transporter coded by this gene and thus increasing the concentration of dopamine in the synaptic cleft [101]. The changes of the transport of dopamine are probably brought about by the influence on its transmitter coded by DAT1 gene. The dopamine transporter is responsible for the transport of dopamine through the presynaptic membrane back into the nerve cell. Stimulants inhibit the transport of dopamine and these drugs lead to an increase of dopamine in the synaptic cleft. Studies on mice proved a higher degree of hyperactivity in a strain with a mutation of the DAT1 gene, leading to a change in dopamine transporter function in comparison with the normal population of animals [37]. A significantly higher number of homozygotes alleles 10/10 DAT – as compared to heterozygotes – was found in patients with ADHD, conduct disorders and Tourette's syndrome [16]. Cook et al. [21] examined a 3' variable number of tandem repeat (VNTR) polymorphism at the dopamine transporter gene DAT1 in a sample of 49 ADHD patients and their parents, using the haplotype relative risk (HRR) method. A significant association between ADHD and the 480-bp DAT1 VNTR allele was established. Gill et al. [36] found that the 480-bp allele was preferentially transmitted to ADHD probands (p=0.014). Waldman et al. [102] examined the role of the DAT1 gene in ADHD, ODD (oppositional defiant disorder) and CD (conduct disorders) in 123 families, using the TDT technique. A significant association between the DAT1 10 allele and hyperactivity-impulsivity (p = 0.009) was found. In a subsequent report, Waldman et al. [102] examined 74 ADHD probands, 79 siblings and a control sample of 49 twins and confirmed the results. These findings were not confirmed [3, 43, 76, 65]. Maher et al. [62] confirmed the association of DAT1 and ADHD in meta-analysis of DAT1, which included data from 11 studies, with a total of 824 informative meioses. Chen et al. [12] identified the same association of DAT1 and ADHD in Asian populations. When results from the family based studies noted above are pooled the OR is small, but significant, suggesting the dopamine transporter gene merits further investigation but that its effect is modest [30].

**DRD3** gene. Knockout mice (ADHD model), missing the DRD3 gene, are considerably more active than their littermates with normal DRD3 genes [1]. The other common polymorphism is located in intron 5 and results in the change of a restriction site for MspI [66, 73]. Comings et al. [15] observed a significant decrease in DRD3 Msp I heterozygosity in Tourette's syndrome and ADHD comorbidity. Another study, however, did not show the DRD3 gene to be significantly associated with ADHD [6, 50, 30].

**DRD5** gene. The possible role of DRD5 gene in ADHD has been examined using a dinucleotide repeat polymorphism. The 148-bp allele DRD5 was reported to be associated with ADHD [98]. Using the TDT technique, [24] observed a significant increase in the transmission of the 148 bp allele in 160 family sets with ADHD offspring (p < 0.0005). Kirley et al. [50] and Maher et al. [62] observed association between ADHD and 148 bp allele DRD5 in others studies. Consistent with this result, a more recent family-based analysis that combined 14 independent samples identified a significant association of the 148-bp allele with ADHD (OR = 1.2; 95% CI 1.1–1.4) [61].

**MAO** genes. Some authors believe that a deficit of dopamine/norepinephrine in the hyperkinetic syndrome is caused by hyperactive monoamine oxidase (MAO) [95]. Using CA repeat polymorphisms at the MAO-A (monoamine oxidase-A) gene, Gade et al. [32] found an association of this gene with ADHD. Manor et al. [64] examined the MAO-A promoter region polymorphism in 133 triads and observed preferential transmission of the long alleles from heterozygote mothers to ADHD probands (chi (2) = 4.37; p=0.036). Lawson et al. [58] examined MAO-A polymorphisms (the 30-bp VNTR in the promoters and the Fnu4HI 941T-->G) in ADHD children; the results of the study were negative, but case control analysis of the VNTR showed an association with a subgroup of children with co morbid conduct problems. Jiang et al. [45] observed (in a linkage study) significant association between 157-bp allele of the DXS 7 locus of X chromosome and DSM-III-R diagnosed ADHD (n=72, p < 0.001). Study in Caucasian cohort failed to replicate this association [72].

**Noradrenergic system (ADRA2A, DBH)**

**Biochemical markers** of noradrenergic system are closely connected to the dopaminergic system through the dopamine-beta-hydroxylase enzyme. Dopamine-beta-hydroxylase (DBH) is an enzyme responsible for the conversion of dopamine to norepinephrine. In its feedback, it inhibits tyrosine-hydroxylase, which reduces the production of dopamine. It is localized in catecholamine-containing vesicles of adrenergic and noradrenergic cells [48]. When DBH is defective, an alteration of the dopamine/NE levels can result in hyperactivity. DBH protein is released in response to
The above-mentioned polymorphisms of the adrenergic genes, two subsequent family-based analyses, one in 103 families and another in 128 families, showed no evidence of association [7, 25].

**DBH gene.** A Taq I polymorphism B1/B2 in the DBH gene exists; the connection of the B1 allele with the hyperkinetic syndrome and the development of hyperactivity has been described [24]. Roman et al. [77] tested a sample of 88 Brazilian nuclear families and demonstrated an association between the DBH Taq I A2 allele and ADHD. Taq I A1 allele is significant associate with ADHD (p = 0.018) [89]. The B1 allele occurs in 52.9 per cent of patients with drug addiction, in 70.5 per cent of TS patients, and in 73.1 per cent of ADHD patients [23]. Studies using protein-phenotype markers showed strong evidence for linkage between a major locus controlling plasma-DBH activity and the ABO blood-group locus [38, 104]. Zabetian et al. [107] identified a novel polymorphism (-1021C-->T), in the 5' flanking region of the DBH gene that accounts for 35%–52% of the variation in plasma-DBH activity. Despite the mixed evidence for association between DBH and ADHD, when the family-based studies are pooled, they jointly suggest a significant association between ADHD and the 5' Taq1 polymorphism (OR = 1.33; 95% CI 1.11–1.59) [30].

Serotonin system (5-HTR, 5-HTT)

**Biochemical markers.** A decrease of the serotonin level in the serum was also found in ADHD patients and their parents exhibiting symptoms of hyperkinetic disorder. Patients with oppositional defiant disorder and ADHD showed lower serum 5-HT level than patients with only ADHD. The 5-HT level may be a potential biological marker of impulsive behaviour [35]. Urinary excretion (24-hr) of beta-phenyl ethylamine – free and total was significantly lower in ADHD children. Phenyl acetic acid (PAA) and tyrosine were decreased in plasma in ADHD subjects [5].

**5-HTR genes = SERT** (serotonin receptor genes). A gene mutation leads to a decrease of activity in the enzyme converting tryptophan, the precursor of serotonin. The polymorphic alleles of this gene (polymorphism G-T and G/A in introne 6 was identified) occur in ADHD, TS (Tourette's syndrom) and drug addiction in 29 to 33 per cent of cases, that is twice the frequency of control population [17]. HTR(1B) and HTR(2A) polymorphism encode the serotonin receptors of 5HTR(1B) type as well as 5HTR(2A). The authors observed a significant preferential transmission of the allele 861G of the HTR(1B) only [40]. Some findings suggest an association between HTR(1B) and ADHD, with merits future investigation. 5HTR(2A) polymorphism was associated with ADHD in papers by Quist et al. [70] and Levitan et al. [59]. Particularly interesting is an insertion/deletion polymorphism in the promoter region and VNTR within intron 2, both of which appear to have functional effect on 5HTR (SERT) expression [4]. However, in another study of 150 ADHD probands, Langley et al. [56] found no evidence for the association...
with either of the 5HTR (SERT) polymorphism, alone, or combined as a haplotype.

**5-HTT gene** (serotonin transporter). Low serotonin activity has been associated in both animal and human studies with measures of impulsivity, aggression, and disinhibited behaviour. Recently, a common 44-bp deletion in the promoter region of the serotonin transporter (5-HTTLPR) that results in reduced transcription and lower transporter protein levels was described. As for unravelling a possible role of the 5-HTTLPR polymorphism in childhood disruptive behaviour, we examined this gene in attention deficit hyperactivity disorder (ADHD), a heterogeneous childhood disorder in which three phenotypes are recognized by DSM IV criteria: inattentive (type I), hyperactive-impulsive (type II), and combined type (type III). By using the haplotype relative risk design, a group of 98 triads (both parents and proband child) were tested for a possible association between 5-HTTLPR and ADHD. A significant decrease in the short/short 5-HTTLPR genotype was observed in the ADHD type III combined group (10.29% vs. 30.88%) compared to the HRR-derived control group (likelihood ratio = 9.62, P = 0.008, n = 68 triads). Similar results were observed when allele frequencies were compared (likelihood ratio = 3.81, P = 0.05, n = 136 alleles) [63]. Kent et al. [49] examined two other polymorphisms (an SNP in the 3’ untranslated region and a tandem repeat) and identified significant associations for the SNP and for a haplotype including this SNP and 5-HTTLPR. When the 5-HTTLPR studies are combined, the pooled OR for the long allele is 1.31 (95% CI 1.09–1.59) [30].

**Discussion**

The research of only some candidate genes (DRD3, DRD4, DAT, DRD5, DBH, 5HTT, HTR1B a SNAP25) brought consistent results confirming the heredity of ADHD syndromes.

Some candidate genes acetylcholine receptors: (CHRNA4 CHRNA7), glutamate receptors, tryptophan hydroxylase gene, thyrozine hydroxylase gene, catechol o methyl transferase gene, and norepinephrine transporter gene, did not show association with ADHD [30].

The results of research of other genes (DRD2, MAO, ADR2A, GABA A3, GABA B3) are still not clear. Understanding these genes can help comprehending polygenic aetiology of some subgroups of ADHD and aetiology of ADHD with some comorbid disorders.

Hypothetically, certain sub-groups of ADHD may be identified by correlation of some phenotype-characteristics (behavioural, and pharmacogenetic biochemical), with some candidate genes. Changes of biochemical markers were described in some groups of ADHD and in some groups of ADHD comorbid disorders.

Research correlations of periphery and central (cerebrospinal fluid) value of biochemistry markers are limited by ethical regulations. There are only some positive results in HVA, DBH and serotonin. But we presume about some identical or equivalent noradrenergic, dopaminergic or serotoninergic activity, in CF and periphery, what is representing by some biochemical marker's value.

In some ADHD children, the plasma level of MHPG was decreased and plasma level of HVA was increased [105]. Circulating dopamine increased significantly by the exercise in the control subjects (p < 0.016), but no increase was noted in the subjects with ADHD [103]. Children with extremely low MHPG were non-responders to stimulant drugs therapy. The question is whether some connection between very low MHPG and DAT 10/10 genotype (also non responders to stimulant drugs) exists [79, 51]. Plasma levels of noradrenaline and MHPG may be elevated in ADHD patients with comorbid disorders: dyslexia, conduct disorder [39].

A decrease of the serotonin level in the serum was also found in ADHD patients and their parents who had had hyperkinetic syndromes in their childhood. Oppositional defiant disorder, comorbid to ADHD, showed lower serum 5HT level than pure ADHD. 5HT level may be a potential biological marker of impulsive behaviour. [35]
Lower levels of DBH protein may lead to elevated ratios of DA to NE. This model may explain associations between lower plasma DBH activity and vulnerability to psychotic symptoms. Genotype-controlled analysis of plasma DBH holds promise for promoting further progress in research on psychiatric and neurological disorders [22]. Decreased activity of DBH in serum was found in ADHD and in un-socialized conduct disorder. Socialized conduct disorder co-morbid to ADHD was not connected with low DBH activity [71, 53, 75, 33, 34, 81]. The (CA)n repeat polymorphism allele A4 and 19 bp insertion/deletion (5′ non-described area) elevate plasma DBH activity.

Biochemical markers are not used in identification of some ADHD subgroups often. Some biochemical markers (5HT, DBH, MHPG) differ, in some cases, pure ADHD and ADHD with comorbid disorders. However, results introduced here are evidently suitable for possible use in a future genetic research.

There are some difficulties in diagnosis and clinical syndromes connected with changes in ADHD symptoms during development from childhood to adolescents and adults. Some biochemical periphery parameters may change during child development as well [69].

Other possibilities in the study of ADHD aetiology lie in pharmacogenetic markers research and correlation of pharmacogenetic and biochemical markers. The problem of how each transmitter system participates in aetiology of ADHD can be studied on the drugs used in therapy of ADHD with specific mechanism of action. There are dopaminergic drugs (stimulants), nor-epinephrine drug (atomoxetine) and serotonin drugs (serotonin re-uptake inhibitors, especially citalopram, which is the most specific serotonin re-uptake inhibitor). On the other hand, differentiation of some groups by certain candidate genes may help predict responders or non-responders to individual drugs.

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