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# Progress and stumbling blocks in the discovery of biological and genetic basis of attention deficit hyperactivity disorder

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**Summary**

Attention deficit hyperactivity disorder, ADHD, is one of the most common neurodevelopmental disorders that affects up to 5% of school-aged children. Despite the defined diagnostic criteria, we are not always able to make a diagnosis as quickly as possible and to implement optimal treatment. Despite different and advanced methods and technologies used to study ADHD, we still not fully understand the biological basis of attention deficit hyperactivity disorder. Therefore, research is continuing to explain genetic and neurobiological background of the disorder. Genetic analysis focuses on the search for risk genes (e.g., mutations, CNV polymorphisms), their transcripts and proteins as well all modifying molecules (epigenetic modifications). Not without significance is the search for non-invasive, simple and cheap peripheral biomarker assays, extremely valuable in the diagnosis, prediction, and monitoring of the disorder. In this review, we summarize current knowledge on a broad range of biological processes underlying ADHD. The results of the presented molecular and neuroimaging studies indicate research challenges and the possibility of clinical application of important genetic and non-genetic biomarkers related to ADHD.

**Key words**: attention deficit hyperactivity disorder, diagnostic approach, genetics

## Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders occurring during childhood. Typical symptoms (inattention, hyperactivity and impulsiveness) occur with various severity in various social situations. Depending on the diagnostic criteria used and the population studied, the prevalence of ADHD in school-aged children ranges from 1–2% (according to ICD-10) to 3–5% (according to DSM-5) [1]. Symptoms of ADHD persist in over 70% of adolescents

and 30–66% of adults. The clinical picture of ADHD differs depending on the age of the patient. Prevalence of ADHD in a population of patients between 18 and 44 years of age is 2.5–4.4% [2]. There is one school-age girl diagnosed with ADHD per 2–10 boys, while in adults, this ratio is 1.6:1. With age, symptoms of hyperactivity become less frequent, while the symptoms of impaired attention and impulsiveness usually persist [3]. However, in girls and women, the symptoms of inattention are more often seen from the very beginning [4]. Most often, in 50–75% of patients, we observe a mixed picture of the disorder (the symptoms from both areas are equally intense). The prevalence of inattention concerns 20–30% of patients, whereas hyperactivity and impulsiveness concern about 15% of patients [5].

The diagnosis of ADHD is based on the observed symptoms using diagnostic criteria for ICD-10 or DSM-5 classification. The DSM-5 classification divides symptoms into two areas – inattention and hyperactivity/impulsiveness. Depending on the dominant symptom, we recognize three presentations of the disorder that change with the patient's age: predominantly inattentive presentation, with the prevalence of hyperactivity and impulsiveness (predominantly hyperactive/impulsive presentation) and mixed (combined presentation). On the other hand, the ICD-10 classification divides the symptoms into three groups (attention disorders, hyperactivity, impulsiveness) and does not distinguish the subtypes of the disorder, which mainly involves patients with a mixed presentation. The impairment of the social, professional, or school functioning of a child with ADHD should be clinically relevant and occur in at least two situations (e.g., at school and home). Symptoms depend on the context in which they appear. Therefore, in some situations, they may not be visible, e.g., in one-on-one contact (in a doctor's office), involvement in an interesting activity. For the diagnosis of ADHD, it is necessary that symptoms persist for at least six months. According to the authors of the ICD-10 classification, the symptoms should occur before the age of 7. In the DSM-5 classification, only a few of them must be present before 12 years of age. Additionally, it allows the diagnosis of ADHD in adolescents and adults (17 years and above). In this case, it is sufficient to have fewer symptoms from the described areas of the disorder (at least 5).

The majority of patients with ADHD (59–87%) are found to have additional disorders, including specific speech development disorders and other specific disorders of development and school skills. Moreover, they are found to have behavioral disorders, oppositional defiant disorder and, during adulthood, anti-social personality disorders or conflicts with the law. Anxiety, mood, obsessive-compulsive disorders, nicotinism, substance abuse, tics, and Tourette's syndrome may also occur. The DSM-5 classification enables the diagnosis of ADHD together with disorders in the autism spectrum, where the symptoms of inattention, hyperactivity and impulsiveness appear very often. Patients with ADHD are more likely to attempt suicide. Girls have a greater risk of comorbidity of anxiety disorders and addiction to psychoactive substances, while boys have a higher risk of depression, behavioral disorders and oppositional defiant disorder [6].

According to some reports, very often (up to 60–90%) ADHD co-occurs with an early-onset bipolar disorder (symptoms before 18 years of age), and especially

with a very early-onset bipolar disorder (symptoms before 13 years of age) [7]. It should be remembered that ADHD or similar symptoms may occur in many somatic disorders, including, for example, neurofibromatosis type I (NF1), generalized lack of tissue response to thyroid hormones, hyperthyroidism, hypertrophy of throat, allergies, asthma, hearing loss, epilepsy, syndrome fragile X chromosome, congenital metabolic diseases. ADHD symptoms may also be the effect of adverse drug reactions (antihistamines, steroids, β2-agonists, theophylline, nootropics) [8, 9]. Also, the occurrence of ADHD-like symptoms is influenced by environmental factors, such as being bored in lessons, exposition to severe stress, physical or sexual abuse. In addition, ADHD-like symptoms may also be a result of the lack of clear rules at home and the consent/ lack of a proper response to unacceptable behavior. That is why it is very important to conduct additional research and obtain information from several environments [10].

## From the neuronal concept to the molecular basis of ADHD

ADHD has multifactorial origins and complex symptomatology. Therefore different methods and technologies are used to study ADHD-related neuronal changes, but it is not yet clear whether the underlying neurobiological factors are associated with symptoms. Magnetic resonance imaging (MRI) is the main method used to study brain structure and function in children and adults.

MRI structural studies have revealed volumetric reductions in the basal ganglia in children with ADHD. Delays (by 2–5 years ) in obtaining peak cortical thickness in frontal, parietal and temporal brain regions of ill children were also observed [11]. The above anomalies may affect the attention and executive function disorders that are observed in children with ADHD. The diffusion MRI (dMRI) studies suggest that volumetric abnormalities in cortical and subcortical regions may result from altered myelination or axonal branching [12]. Research using analyses of large databases proves that in children with ADHD delay and disturbances of myelination occur already in the third trimester of pregnancy. Researchers linked myelination disorders to the *ST3GAL3* gene (ST3 beta-galactoside alpha-2,3-sialyltransferase 3). Mutations in this gene interfere with protein glycosylation processes, making them less stable or recognized as foreign [13]. In turn, intracranial and putamen volume has been linked to the *SEMA6D* gene coding for semaphorin, which determines the targeted growth of neurons [14].

The functional MRI (fMRI) research on ADHD indicates the involvement of specific neural circuits related to, for example, sustained attention, inhibitory control, motivation, and emotional regulation, the importance of which in ADHD has been confirmed by meta-analyzes. Three main circuits are:

– the frontoparietal circuit includes: the frontal lobes (including the supplementary motor area and the frontal eye fields), the temporal-parietal junction, and the inferior parietal sulcus; this circuit is involved in the processes of alerting and orienting of attention,

– the dorsal frontostriatal circuit includes: the dorsolateral prefrontal cortex, the dorsal striatum and the thalamus; the circuit is responsible for inhibitory control including response inhibition and interference control,
– the mesocorticolimbic circuit includes: the orbitofrontal cortex, the ventral striatum, the nucleus accumbens, the ventral tegmental area, and the anterior hippocampus; this circuit is related to the reward system and the regulation of emotions, including motivation, tolerance for frustration and expectations for reward [15].

Functional magnetic resonance imaging (fMRI) at rest informs that people with ADHD have weaker communication results within the default mode network (DMN). During task-based functional neuroimaging, DMN shows increased activity when people rest or engage in introspective tasks such as recovering autobiographical memories [16]. Several independent studies including children, adolescents and adults with ADHD, both with and without exposure to previous drugs, found that the correlations between DMN and the cognitive control network (CCN) are reduced or absent in ADHD [15]. Chen et al. [17] have found that transcranial magnetic stimulation of the cognitive control network (CCN) causes DMN suppression. Examination of functional connectivity in ADHD suggests reduced functional connectivity within the prefrontal cortex, striatum and DMN during tasks engaging executive functions [18, 19]. However, further functional studies are needed to explain the relationship between different regions of the brain and their role in creating a network of connections responsible for executive functions that are disturbed in children with ADHD [20, 21]. Studies on animal model have shown that reducing SERT levels in the rat hippocampus weakens locomotor activity and impulsivity suggesting that increased serotonergic transmission in this area of the brain may alleviate some of the symptoms of ADHD [22]. Pharmacotherapeutic studies suggest that initial abnormalities in the anterior circuits are normalized by psychostimulant therapy versus placebo and that this normalization is associated with improved response inhibition [23].

In summary, neuroimaging studies allowed us to identify the association of specific neuronal circuits with ADHD symptoms. Future research can achieve a greater sensitivity and specificity and thus strengthen causal inference by using a structured protocol of tasks stimulating brain work or comparing pharmacological agents with different mechanisms of action.

## Key ADHD genes

ADHD is one of the most inherited mental disorders with a heritability ranging from 60 to 90% [24, 25]. The polygenic nature of ADHD indicates that many moderate genes are involved in the genetic basis of ADHD [26]. The genome-wide association study (GWAS) turns out to be an important tool to identify common genetic variants with a small effect size in a trait or disorder under study without having to determine the inheritance model in advance [15]. Thanks to the ability to analyze over 1 million single nucleotide polymorphisms (SNPs) throughout the ge-

nome, GWAS is used to explain the genetic basis of polygenic psychiatric disorders [24]. A particularly promising gene identified in the GWAS is cadherin 13 (CDH13) [15, 27]. Also, SNPs in this gene were associated with working memory defects and hyperactivity in people with ADHD [28, 29]. However, due to the necessity to analyze numerous groups of subjects, the candidate gene analysis is still an alternative method. The most frequently analyzed genetic variants in ADHD research concern candidate genes in dopaminergic and serotoninergic systems [30]. A study on the gene-candidate association indicated that both *DRD4* (Dopamine Receptor D4) and *SLCA3* (dopamine transporter) genes are associated with ADHD [31]. However, there are also conflicting results [32].

Most genetic risk factors are derived from research on ADHD in children. In the case of adults with ADHD, an association with the *BAIAP2* (brain-specific angiogenesis inhibitor 1-associated protein 2) gene was indicated that has not been confirmed in children. However, the opposite is true for the *SLC6A3*, *DRD4* and *COMT* (enzyme that breaks down catecholamines) genes. The *SLC6A3* gene is a well-known gene that plays an important role in the pathophysiology of various mental disorders, including ADHD. However, meta-analyzes regarding the relationship of this gene with ADHD and the response to treatment did not confirm the previous reports [33]. Another negative result was obtained by the *DRD4* gene. In the case of the functional *COMT* Val66Met polymorphism, a relationship with overweight [34] or impaired social functioning [35] has also been demonstrated in children with ADHD, while no such relationship has been confirmed in adults. The lack of identifying the same relationships in children and adults with ADHD may indicate that these are genetically distinct subtypes, which need to be analyzed separately [36]. However, considering that the protein products of the above-mentioned genes are significantly related to the etiology of ADHD, many authors indicate the need to continue research in the case of the *SLCA3*, *DRD4* and *COMT* genes [33, 34].

GWAS regularly identifies new genes which are interesting because of their function. Recent reports point to the tenascin R gene (*TNR*). This gene encodes the glycoprotein of the extracellular matrix, which plays a role in neural cell adhesion and neurite outgrowth [37]. Meta-analysis of GWAS showed 12 ADHD risk *loci*. Interestingly, only two genes (*ST3GAL3* and *SEMA6D*) were previously associated with the risk of ADHD in intermediate phenotype studies [38].

The literature review shows that about 105 genes can be associated with ADHD. SNPs are not sufficient to assess their relationship to the risk of ADHD, therefore different analytical approaches are used, e.g., the interaction of protein products to identify ADHD-related pathways. Probably both the list of genes and pathways involved in the development of ADHD is not exhausted [39, 40].

In addition to the SNPs, the analysis of copy number variants – DNA segments from 1 Kbp to several Mbp (copy number variation, CNV) is of unfathomable interest. Increased copy number variation in patients with ADHD was found in genes coding glutamate metabotropic receptor genes [41] as well as in the *CHRNA7* and *NPY* genes [42, 43]. Stergiakouli et al., [44] demonstrated convergence of CNVs with SNPs associated with ADHD on the same biological pathways. These include developmental

pathways associated with cholesterol in the central nervous system. Previous research confirms that both SNP and CNV are important for the risk of ADHD.

Candidate genes studies and GWAS allowed identifying numerous genes with a potential predisposition to the disorder. None of these variants meet the criteria of a genetic biomarker. We hope that the Psychiatric Genomics Consortium (PGC) and ADHD working group, gathering over 100 scientists from 14 countries, will be able to collect a large cohort of patients and select essential genes in ADHD [45]. However, many of these genes and polymorphisms have only a modest effect on individual disease risk. We also have evidence that the same sets of genes can be associated with various mental disorders [46].

## Genetics and imaging

Imaging genetics (IG) is a combination of neuroimaging methodology and genetic analysis that gives the opportunity to increase knowledge about biological mechanisms in the field of neurodevelopment. IG studies are designed to reveal the relationship between specific genetic variants and the function and structure of the brain [45]. Indirect phenotypes come from neuroimaging data based on the assumption that stronger correlations exist between the structure of the brain and genetic variability because the physiology of the brain is etiologically closer to molecular biology than behavioral phenotypes [47]. IG research on ADHD focuses on candidate genes in dopaminergic systems and the structure of basal ganglia using structural magnetic resonance imaging (MRI) as the main brain imaging technique [48]. Neurodevelopmental changes in the glutamatergic frontostriatal circuits in the life span suggested by them may be interesting targets for future studies of imaging genetics [30]. It was found that all studies performed one-factor analyzes of a single variant of the gene and one region of the brain at the same time [48].

Identifying genetic patterns that affect brain development in ADHD can be improve by the use of multidimensional analytical strategies [49]. Pathway-based analysis (PBA) has been shown to increase power to identify genetic factors associated with ADHD [50, 51].

## Intermediate phenotypes in ADHD

Narrowing the phenotype down by using endophenotype or intermediate phenotype allows in complex diseases for targeted genetic markers searching. In the case of ADHD, various potential intermediate phenotypes, such as neurophysiological/neuropsychological, neuroimaging, pharmacogenetic, and biochemical features, were analyzed. Among the most promising were EEG theta/beta ratio [52], reaction time variability [53] or executive function [54]. Unfortunately, further studies have shown that these markers lack sensitivity and specificity in clinical disorders, and thus, they do not meet the criteria for diagnostic markers [55]. Despite this, Pinto et al. [55] decided to analyze selected intermediate phenotypes (hyperactivity/impulsivity, inattention, reading difficulties (RD), reaction time variability (RTV), and commission errors

(CE)), their association with selected polymorphisms and then identify association with ADHD risk. The strongest correlation was observed for the rs7984966 SNP in the serotonin receptor gene (*HTR2A*) and RTV. The authors suggest overlapping genetic associations between ADHD diagnosis and RTV or CE.

However, in the light of previous results and lack of specificity of endophenotype, further confirmations are required [56].

## Pharmacogenetic studies in ADHD

The majority of ADHD pharmacogenetic studies published to date have examined the response to methylphenidate (MPH), which remains the drug of the first choice in the treatment of ADHD at age 6–17. The mechanism of MPH action in ADHD is not fully known. Probably it works by cortical stimulation and stimulation of the activating reticular formation. Methylphenidate inhibits the reuptake of noradrenaline and dopamine and in this way increases the release of these monoamines into the non-neuronal space. About 35% of ADHD patients treated with MPH do not respond to treatment or present adverse effects. The pharmacogenetic studies reported that adverse events were significantly associated with the following polymorphisms: appetite reduction and sadness (*CES1* rs12443580); buccal-lingual movements and irritability (*SNAP25* rs3746544); diastolic blood pressure (*ADRA2A* rs1800544); emotionality and somatic complaints (*SLC6A3/DAT1* 48bp VNTR); social withdrawal (*DRD4* 48bp VNTR); vegetative symptoms (*SLC6A4/5-HTTLPR*); tics (*SLC6A4/5-HTTLRP, SNAP25* rs3746544). Nevertheless, the use of various research protocols does not allow for unambiguous conclusions [57]. Gomez-Sanchez et al., [57] examined the role of 34 genetic variants in response to treatment with MPH. They reported only moderate effects of 4 gees (*SLC6A3/DAT1, DRD4, SNAP25*, and *ADGRL3*) in response to MPH. They also found associations between response to treatment over 12 months and 2 genes (*SLC6A3/DAT1* and *DRD2*). Analyzed polymorphisms allowed explaining around 20% of variance of the response to MPH, which confirms the significant share of other factors the discovery of which requires further research [58]. Other results suggest that the *GRIN2B* rs2284411 C/C genotype is linked with significantly better MPH treatment response and thus may be an important predictor of MPH response in ADHD [59].

Atomoxetine was the first non-stimulant drug to be used in the treatment of ADHD. Atomoxetine is a selective noradrenaline reuptake inhibitor, and it is metabolized by the CYP2D6 enzyme. Individuals who carry two nonfunctional copies of the *CYP2D6* gene are known as poor metabolizers and have higher plasma concentrations of atomoxetine. According to the recommendation, poor metabolizers can be given the standard dose of atomoxetine, but physicians should monitor adverse drug events. Opposite, in ultrarapid metabolizers – individuals who have more than three functional gene copies, physicians should be alert to reduced efficacy or select alternative drug [60]. This seems to be only one result useful in medical practice so far. In ADHD treatment also drugs from other group are used, such us: tricyclic antidepressants (i.e., imipramine, amitriptyline, desipramine, clomipramine), alpha-mimetics (clonidine, guanfacine), other antidepressants (bupropion, moclobemide, reboxetine, venlafaxine). According

to our knowledge, there are currently no pharmacogenetic research results available in ADHD.

Pharmacogenetic studies are essential for the proper and safe pharmacotherapy of children with ADHD. However, the few analyzes to date justify the need for further research.

## Gene expression profiling

Gene expression profiling in the prefrontal cortex found 21 common upregulated genes in ADHD animal model. Among these genes, seven have a known biological function: *Atxn7*, *Kcna2*, *Pbld*, *Per2*, *Rtel1*, *Zfp317*, and *Zfp597*. Thirty-six genes were downregulated. Nevertheless, only 14 have a fixed connection with specific biological processes. Replication studies confirmed higher expression levels of two genes, *Atxn7* and *Per2*, which are involved in transcription and circadian rhythm, respectively. Moreover, these genes were downregulated after amphetamine (AMPH) treatment. The present findings indicate the roles of both genes within the hyperactive phenotype in ADHD [61]. In turn, Grunblatt et al. [61], in the pilot study, identified upregulation of 5 genes (*SLC6A3, DRD4, DRD5, SNAP-25,* and *TPH1*) and downregulation of the *CRHBP* gene in blood samples of ADHD patients compared to healthy control [62].

The process of expression of genes encoding proteins occurs in several stages, each of which is under different regulatory mechanisms. For this reason, these are the most difficult methodological and interpretative studies.

## Peripheral biomarkers in ADHD diagnostics

Biomarkers are biological indicators, the study of which allows qualitative or quantitative assessments of various states, phenomena, or biological characteristics [63]. In psychiatry, the biomarker should additionally allow for screening and diagnostic tests, prediction of illness development, and response to treatment. Despite testing numerous neuropsychological, neurophysiological, neuroimaging, pharmacological, or biochemical indicators, we do not have acknowledged biomarkers. Still, the most prominent field for research is associated with proteome analysis in serum, urine or saliva. In the case of ADHD, such studies with positive findings are very few. Reduced serum level of the MAOA and DBH enzymes have been observed in ADHD compared to the controls. These enzymes are involved in the metabolism of dopamine, serotonin, and norepinephrine [64]. For adiponectin and proteins of oxidative stress, such as: malondialdehyde (MAD), superoxide dismutase (SOD), paraoxonase (PON1), and arylesterase (ARES), there are reports that higher levels of these proteins in the serum of ADHD patients are observed. The levels of total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) were also increased [65]. Brain development proteins such as BDNF, NGF, or NO inspired hope, but conflicting results from different studies do not allow for unambiguous conclusions. The same applies to kynurenine pathway proteins (tryptophan, kynurenine, kynurenic acid, 3-hydroxykynurenine) – no unequivocal results were obtained. In the case of non-

protein biomarkers, such as serum arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and cortisol, lower serum levels in ADHD compared to controls were observed [64]. The serum level of norepinephrine (NE) was higher in ADHD patients, while the level of the main metabolite (3-methoxy-4-hydroxyphenylglycol, MHPG) in the urine of ADHD patients was lower than in the control group [64]. Zinc as an essential cofactor for neurotransmitter metabolism was analyzed according to dopamine metabolism and showed reduced levels in serum, plasma and urine of ADHD patients.

Few and often ambiguous results of the peripheral biomarker analysis obtained to date require further research [66].

## Epigenetics – a link between genes and environmental factors

Epigenetics is a concept of molecular processes that change the expression of genes without modifying their sequences. Epigenetic modifications can be inherited, but they are considered to be flexible and reversible. However, if epigenetic changes occur at critical stages of development, they may be irreversible and cause a disease [67]. Therefore, their importance is emphasized, especially in neuropsychiatric diseases. The existance of the interaction of genetic and environmental factors in ADHD etiology is confirmed by inconsistency in ADHD morbidity in monozygotic twins. This inconsistency suggests that, in addition to genetic predisposition, exposure to environmental factors can influence illness development through epigenetic mechanisms. Researchers have confirmed that toxic exposure, maternal stress during pregnancy, low birth-weight, and psychosocial difficulties are pre and perinatal environmental risk factors linked with ADHD. The evidence of the importance of environmental factors and the effect of epigenetic mechanisms is that with age progression hyperactivity and impulsivity are reduced.

Epigenetic mechanisms occur at several levels and include chromatin modification by histone modification (the posttranslational histone modifications including phosphorylation, acetylation, methylation, and ubiquitination) and methylation of cytosine within CpG dinucleotides. Epigenetic mechanisms also include miRNA-based gene expression modulation. Histone modifications have been so far observed only in the animal model of ADHD. The increased level of histone acetylation was observed in the rat hippocampus after chronic lead exposure. These results indicate that histone acetylation may play essential roles in the toxicant-involved pathogenesis of ADHD. Interestingly, no significant changes were detected in the expression of ADHD-related dopaminergic proteins The vast majority of the studies are focused on the profiles of methylation of gene promoters associated with the etiology of ADHD.

In a prospective study, van Mil et al. [67] observed lower DNA methylation levels of seven genes (*DRD4, 5-HTT, IGF, 2DMR, H19, KCNQ1OT1, MTHFR, NR3C1*) assessed at birth. Lower methylation level of these genes was associated with more severe ADHD symptoms in children at six years of age. Xu et al. [68] observed a different pattern of the *DRD4* gene methylation in ADHD children compared to healthy controls. In turn, Perroud et al. [69] observed a correlation between a different methylation pat-

tern of the *HTR3A* gene in ADHD patients and the experience of childhood trauma and greater severity of illness symptoms. A recent study [70] showed that prenatal maternal stress may affect the *DRD4* gene methylation in the child. The level of methylation affects the level of the protein product, i.e., the density of DRD4 receptors, which is directly related to the effectiveness of MPH treatment and the improvement in executive functions. Unfortunately, at this stage of the research, the mechanism of established dependencies could not be explained.

The methylome extensive associations study (MWAS) in ADHD children indicated decreased CpG methylation level of the *VIPR2* gene. So far the *VIPR2* (vasoactive intestinal peptide receptor 2) gene was not linked with ADHD etiology [71]; however, its duplication was associated with increased risk of schizophrenia [72]. However, the second MWAS conducted by Walton et al. [73] did not confirm previous results. It should be emphasized that few methylome studies in patients with ADHD and divergent results related to the small number of subjects, differences in used methodology, biological material, and subject ethnicity do not allow definite conclusions.

Baykal et al. [74] used a rather obvious but previously unused approach to the analysis of gene interactions and the environment in assessing the risk of developing ADHD. They demonstrated a functional relationship between polymorphism rs1801133 (C677T) methylenetetrahydrofolate reductase (*MTHFR*) in mothers with the risk of developing ADHD in their children. Polymorphism conditioning, i.e., a reduced enzyme in mothers, caused that children during pregnancy were exposed to folate deficiency despite proper supplementation. This study indicates a new direction in the search for both genetic and environmental risk factors for ADHD.

Epigenetic research allows the discovery of mechanisms that control gene expression using environmental factors (healthy lifestyle). These findings represent the incredible potential for 'managing' illness symptoms that awaits further discoveries.

## The role of microRNAs in ADHD etiology

At the epigentic level, apart from histone modifications and DNA methylation, the regulation of mRNA expression is an important mechanism the dysfunction of which may influence the development of mental disorders [75]. In particular, miRNA is considered to be an important pillar of the epigenetic regulation of gene expression. The discovery of microRNA (miRNA) occurred in the 1990s during the study on the larval development of the nematode *Caenorhabditis elegans*. miRNAs are short (18–25 nucleotides), non-coding RNA sequences. Genes encoding miRNA can be found in introns and exons of genes encoding proteins and non-gene sequences [76]. They function in the posttranscriptional regulation of gene expression by binding to the target mRNA, resulting in inhibition or restriction of target mRNA translation [77]. It is suggested that under the control of one miRNA there are hundreds of target sequences, which in practice means that most of the protein-coding genes are under their control and thus every biological process is dependent on miRNA regulation.

On the one hand, It is believed that a slight reduction in gene expression under the influence of miRNA has regulatory functions, may be well tolerated by the organism

and not manifested phenotypically. On the other hand, studies on animal models have shown that even slight repression of multiple targets can have serious phenotypic consequences such as deletion of miR-128 in mice resulting in lethal epilepsy. Therefore, in recent years, intensive research has been conducted on the utility of miRNA as molecular markers of various conditions.

In neuropsychiatric disorders, researchers began to consider the epigenetic potential of miRNA after having confirmed that they are involved in brain development [78]. 70% of known miRNAs have been identified in the brain, where they play a significant role in regulating structural, developmental and functional processes at both cell and tissue levels [79]. The systematic review of the literature made by Srivastaw allowed the identification of 14 miRNAs with altered expression in patients with ADHD [80, 81]. Among the target genes regulated by the selected miRNAs, there were genes previously associated with ADHD etiology such as: *SNAP-25* (miR-641), *HTR1B* (miR-96), *DAT1* (miR – 30b-5p, miR-1301 and miR-6070), *BDNF* (miR-138-1, miR-34c, miR-296, and miR-494), and *HTR2C* (miR – 34c-3p and miR-34b-3p). The above-mentioned genes are involved in a variety of processes, such as neuronal plasticity, migration, adhesion, and cell signaling. Moreover, among the identified miRNAs are also ones potentially involved in regulation processes such as: DNA damage (miR-18a-5p), oxidative stress (miR-24-3p, miR-106b-5p), hypoxia (miR125b-5p) or structural (miR-107) or functional (miR-155-5p) changes in the CNS [80,81].

Presented results highlight the important role of miRNAs in ADHD etiology and confirm the key role of candidate genes associated with ADHD. However, some of the miRNAs associated with ADHD have also been associated with disorders such as schizophrenia, major depression, autism spectrum disorder, and Alzheimer's disease, which significantly hinders the conclusions.

## Research challenges

Taking into account the conclusions of scientists dealing with ADHD research, several principals in future ADHD studies should be included:

1. ADHD studies should be methodologically rigorous regarding the inclusion and exclusion criteria, provided separately for child, adolescent and adult patients.
2. Study design should be performed according to established and respected protocol taking into account study duration, biological material, and pharmacological treatment.
3. Samples large enough to identify interactions of genetic and environmental factors should be obtained.
4. Laboratory quality control should be performed, including cross-laboratory and cross-method reliability checks.
5. Ethnicity should be taken into account due to social stratification effect.
6. Multiple genes should be examined, ideally screened on a global scale, e.g., using GWAS, and confirmed by other methods.
7. The use of integrated analysis of GWAS data, gene expression and gene methylation data to optimally prioritize ADHD-related genes.

## Clinical applications

The study of psychiatric biomarkers is very complex because of the heterogeneous nature of psychiatric disorders. Therefore, a single biomarker is very unlikely to

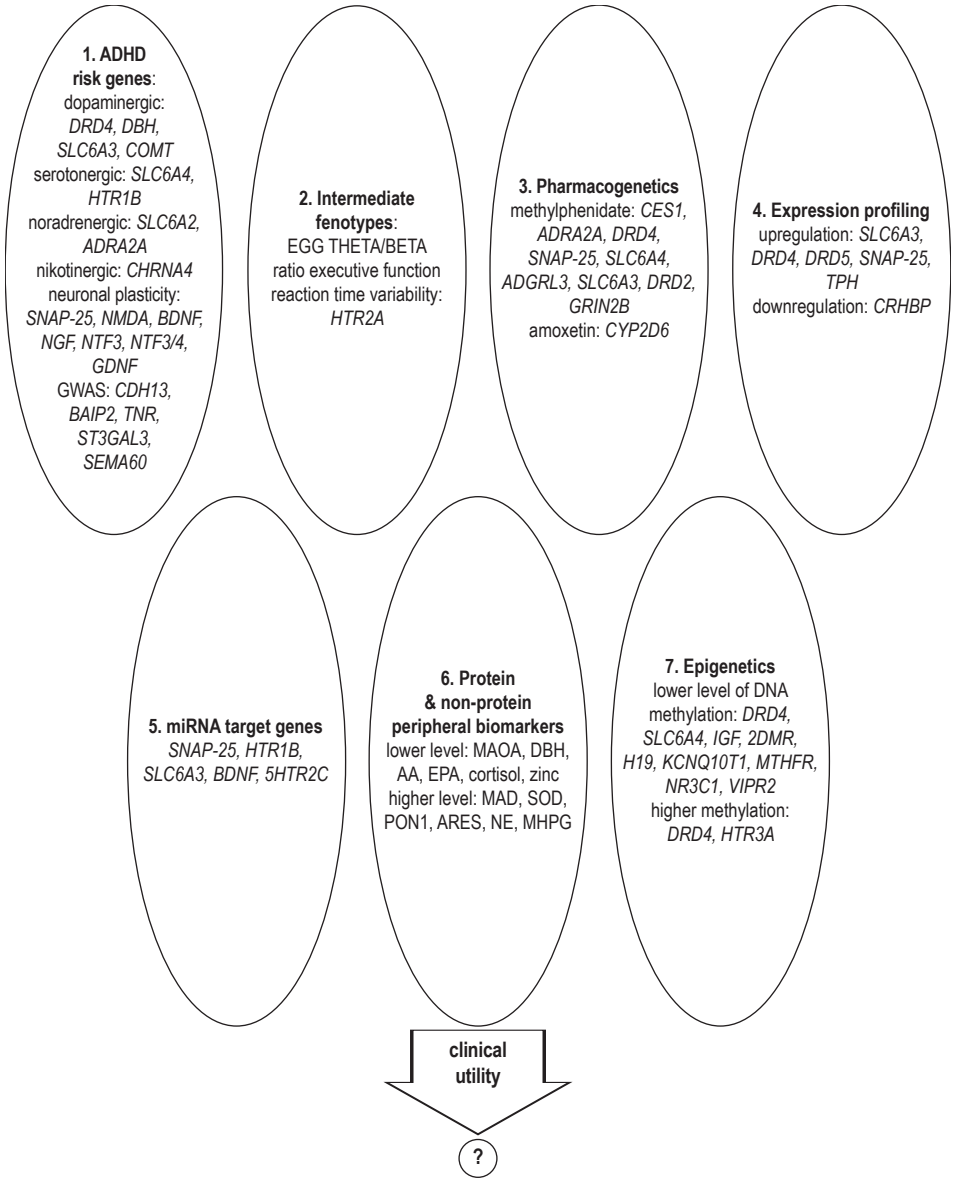


Figure 1. **Summarizing set of significant genetics and non-genetics ADHD biomarkers (explanation of the genetic acronyms in the text)**

allow for unambiguous identification of a disorder, prediction of its development or response to treatment. It seems more likely to identify a set of biomarkers for specific subtypes of disorders, traits or symptoms, each of which is based on specific biological pathways and thus may be associated with a more effective response to treatment. Thus Faraone et al. [55] presented a hypothetical pyramid representation of possible signature sets of biomarkers for ADHD diagnosis. As useful biomarkers, at the top of this pyramid were variants in dopamine transporter (*DAT1, SLC6A3*) and dopamine D4 receptor (*DRD4*) genes, for their associations with neuropsychological tasks, activation in specific brain areas, methylphenidate response, and gene expression levels. A further level is represented by the noradrenergic system (Norepinephrine transporter (*NET1, SLC6A2*), Norepinephrine (*NE*), 3-methoxy-4-hydroxyphenylglycol (*MHPG*), Monoamine oxidase (*MAO*), Neuropeptide Y (*NPY*)) for their altered peripheral levels, their association with neuropsychological tasks, ADHD symptomatology, drugs effect, and brain function. Another level is represented by genetic biomarkers: Dopamine Beta Hydroxylase (*DBH*) and Catechol-O-methyltransferase (*COMT*) are presented. Despite promising results from a wide range of molecular research and built into their predictive models, there are no clinically accepted biomarkers for ADHD diagnosis and prediction of individual treatment response.

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