Polymorphisms of Dopamine-β-Hydroxylase in ADHD Children

(dopamine-β-hydroxylase / plasma activity / ADHD / polymorphism / G444A / G910T / 1603T / C-1021T / 5'-ins/del / TaqI)

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Abstract. ADHD is a multifactorial disorder clinically characterized by inattentiveness, impulsivity and hyperactivity. The occurrence of this disorder varies between 3 and 6% of the child population, with boys predominating over girls at a ratio of 3 : 1 or more. Dysfunction or imbalance between the dopaminergic and noradrenergic systems of neurotransmitters can play a key role in the ADHD pathophysiology. Alteration of the dopamine/noradrenaline levels can result in hyperactivity. DBH is an enzyme responsible for the conversion of dopamine into noradrenaline. The DBH protein is released in response to stimulation. DBH activity, derived largely from sympathetic nerves, can be measured in human plasma. Patients with ADHD showed decreased activities of DBH in serum and urine. Low DBH levels correlate indirectly with the seriousness of the hyperkinetic syndrome in children (Galvin et al., 1995, 1997). In the DBH gene, the G444A, G910T, C1603T, C1912T, C-1021T, 5'-ins/del and TaqI polymorphisms occur frequently and may affect the function of gene products or modify gene expression and therefore influence the progression of ADHD. This article reviews the DBH itself and polymorphisms in the DBH gene that influence the DBH activity in the serum and the CSF level of DBH. All those are evaluated in connection with ADHD.

Genetics of ADHD

Attention-deficit hyperactivity disorder, ADHD, is one of the most common mental disorders that develop in children. The estimations of prevalence differ with the diagnostic criterion used. The Diagnostic and Statistical Manual of Mental Disorder, 4th edition (DSM-IV), which indicates between 3 and 6% of children with ADHD, is the most common reference today. The International Classification of Diseases, 10th edition (ICD-10) is less strict and indicates 0.5% of afflicted children. A boys’ predominance over girls at a ratio 3 : 1 or more exists (Anderson et al., 1987). The principal characteristics of ADHD are inattention, hyperactivity, and impulsivity. These symptoms appear early in a child’s life, some of them persist to the adult age – approximately about 40–50%, although they tend to diminish with age and social maturing. However, the relationships of these children both in the family and with their contemporaries are affected, increasing the risk of social isolation. Fifty to eighty % of ADHD children are afflicted with another co-morbid disorder (Jensen et al., 1997), including oppositional defiant disorder and conduct disorder, anxiety disorder (25–30%), mood disorder (approximately 15%) and learning disabilities (between 20–30%) (Biederman et al., 1991).

ADHD is a polygenetic disorder with various candidate genes. The multifactorial concept is consistent with high population prevalence of ADHD (3–6%), high concordance in monozygotic twins (68–81%), but modest recurrence risk to first-degree relatives (Kirley et al., 2002). It seems that ADHD is a complex genetic disorder, involving many susceptibility genes with a small effect each (Tannock et al., 1998). Also, as the heritability (h²) of ADHD is less than 1.0 (approximately 0.75–0.91) (Levy et al., 1997), it is likely that environmental factors also play a role in the causation and outcome of ADHD (Kirley et al., 2002). The exact mode of transmission is unknown. Various models of inheritance exist, from major gene effect to polygenic and multifactorial models (Faraoane et al., 1992; Hess et al., 1995), but the differences in statistical average between multifactorial genetic models and single gene
inclusion of only some candidate genes (DRD4, DAT, DRD5, DBH, 5HTT, HTR1B and SNAP25) brought consistent results, confirming the heredity of ADHD syndromes (Faraone, 2005).

It is likely that different neurotransmitter systems and the relative balance between them have varying degrees of influence over these behavioural dimensions. Variation in genes involved in these neurotransmitter systems are likely to mediate this delicate balance and have an effect on the function of these chemicals in the brain (Quist and Kennedy, 2001).

Noradrenergic system

This system is closely connected to the dopaminergic system through the dopamine-β-hydroxylase enzyme converting dopamine into noradrenaline. Shekim et al. (1979) found lower levels of MHPG (metabolite of noradrenaline) in the urine of ADHD boys compared to healthy controls. Yasong et al. (1998) observed 64 ADHD boys and 30 normal controls defining the levels of noradrenaline and the ratio of noradrenaline/MHPG in the serum. Both parameters were decreased in hyperkinetic children while HVA (metabolite of dopamine) was increased. In patients responding positively to administration of stimulants, the values of MHPG were higher than in non-responders. The noradrenaline levels were significantly lower than in the group with a moderate degree of this disorder (Yasong et al., 1998). Dysfunction or imbalance between the dopaminergic and noradrenergic systems of neurotransmitters can play a key role in the ADHD pathophysiology. Gabel et al. (1993) observed the MHPG levels in children with hyperkinetic syndrome and conduct disorders and found out that the MHPG level was higher in children with conduct disorders in prepubertal age. The MHPG values were lower after the 12th year of age. The HVA level was remarkably lower in prepubertal children with conduct disorders than in children over 12.

Other genetic markers implying metabolic changes and altered levels of neurotransmitters in the noradrenergic system are genes PNMT (phenylethanolamine-N-methyltransferase), an enzyme converting noradrenaline into epinephrine, and NT (noradrenaline transporter). The activity of adrenergic receptors, especially of β2A, in the frontal cortex is directly connected with the development of inattentiveness and impulsivity. It is known that clonidine influencing inattentiveness and impulsivity in ADHD treatment acts through the pre-synaptic A2A receptors (inhibiting) and through the increase of the pre-synaptic levels of noradrenaline, and has ten times lower affinity to the post-synaptic A1A receptors (exciting). In clinical terms, clonidine leads to lowering of hyperactivity, increase of attentiveness, improvement of conduct disorders, ticks and anxiety. Comings et al. (1999) published a study observing the additive effect of ADR A2A, ADR A2C and DBH genes. These three genes combined accounted for 3.5% of the variance of ADHD score (P = 0.0005). Individually, the ADR A2C gene accounted for 2.5% of the variance of ADHD score.

Direct proportionality was proved between the ADHD score and the quantity of polymorphisms of noradrenergic genes as well as higher occurrence of learning disorders co-morbid to ADHD. The data mentioned in this study suggest a connection between noradrenergic system genes and ADHD.

Dopamine-β-hydroxylase – biochemistry and physiology

One of the important candidate genes is the DBH gene (Faraone, 2005). The dopamine-β-hydroxylase (DBH) is an enzyme that catalyses the conversion of dopamine to noradrenaline. In its feedback, it inhibits tyrosine-hydroxylase, which reduces the production of dopamine. DBH is found in the brain, in catecholamine vesicles of the noradrenergic neurons of the gray matter in nerve terminals (Lewis et al., 1992), in sympathetic nerves and in the adrenal medula, where it is present in both soluble and membrane-bound forms (Weinshilboum, 1978). The DBH gene, which encodes the DBH protein (OMIM 223360), was cloned, mapped to chromosome 9q34, and shown linked to ABO (Craig et al., 1988). It is approximately 23 kb long, contains 12 exons coding for 603 amino-acid protein (Kobayashi et al., 1989) and exists as a single gene in the genome. The genomic sequence is publicly available (Genbank accession numbers AC000404 and AC001227).

DBH is released into the circulation together with neurotransmitters and other vesicular content during synaptic transmissions (Weinshilboum, 1978; O’Connor et al., 1983) from sympathetic neurons and its enzymatic activity is analysed in plasma or serum (Weinshilboum and Axelrod, 1971) as an indicator of sympathetic noradrenergic tone. DBH found in the cerebrospinal fluid (CSF) predominantly originates in the central noradrenergic neurons (O’Connor et al., 1994), while DBH in the serum originates in the sympathetic nervous system (Weinshilboum 1978). Both forms correlate strongly with each other and are under a strong genetic control, with heritability of serum DBH estimated at 0.98 and that of CSF DBH at 0.83 (Oxenstierna et al., 1986). DBH itself is the major quantitative-trait locus (QTL) for plasma DBH activity (Cubells et al., 1998; Zabetian et al., 2001). Associations with variation in both plasma DBH activity (Wei et al., 1997; Cubells et al., 1998, 2000) and CSF levels of immunoreactive DBH protein (Cubells et al., 1998) have been shown. The difference in measured enzyme activity thus reflects differences in the DBH protein level, rather than in homospecific activity (i.e., activity per mole of enzyme).
Plasma DBH activity varies widely across unrelated individuals (Weinshilboum et al., 1973). However, developmental studies of noradrenergic transmission during the ageing process are conflicting and the investigation of serum noradrenaline, especially in children, is very complicated for methodological reasons. The activity of noradrenergic system increases with age (Freedman et al., 1972; Ziegler et al., 1976). Pacht et al. (2004) examined developmental changes of DBH plasma activity in relation to age in humans in a representative group of children and found that DBH activity rises continually with the exception of puberty period. It increases between 3–10 years of age and then decreases approximately at the age of 10–14 years. At the age of 21 to 60, the DBH level is stable. These findings were confirmed by experiments on animals (rats), showing the same developmental trend of enzymatic activity (Pacht et al., 2004). Weinshilboum and Axelrod (1971) did not find any differences in plasma DBH activity in male and female subjects. Suzuki et al. (1990) described developmental changes of DBH activity in CSF of children, adolescents and adults. The results confirmed a continual rise of DBH activity in CSF in children, with the exception of those aged 10 to 11.

In experimental animals with decreased DBH in the serum, reduced conversion of dopamine to noradrenaline reduced the negative feedback on tyrosine-hydroxylase. These animals showed hyperactivity, aggression, self-stimulation and stereotypic movements (Randrup and Scheel-Kruger, 1966). The DBH gene therefore suggests hyperdopaminergic transmission in ADHD (Kirley et al., 2002). An association was made between different allelic variation at the DBH gene and both plasma DBH activity and CSF levels of DBH in many studies. Polymorphisms G444A, G910T, C1603T, C1912T, C-1021T, 5’-ins/del and TaqI exist.

Polymorphisms of DBH

C-1021T Zabetian et al. (2001) sequenced a total of 6443 bp of DBH, including the proximal 1468 bp of the 5’upstream area, all exons, and 2182 bp of the intronic sequence in groups of individuals with very low, average and high plasma DBH activity to locate a new polymorphism associated with plasma DBH activity. Their experiments identified a C->T substitution located – 1021 bp upstream of the translational start site, within the promoter, as an appropriate candidate (four from eight very low DBH activity individuals were TT homozygous). Subsequently, they examined C-1021T association to plasma activity in samples from African American, European American and Japanese population and showed a strong association of the TT genotype with very low plasma DBH.

Further investigations led to the suggestion that C-1021T could be the major functional DBH polymorphism. The findings of other groups support this contemplation:

1) Dunnette and Weinshilboum (1976) reported that the DBH<sup>L</sup> allele causes lower plasma DBH activity by diminishing the levels of circulating DBH protein, rather than by decreasing the activity of homospecific enzymes. C-1021T resides within the promoter and participates in the regulation of transcription.

2) Hoyle et al. (1994) performed an experiment with the human DBH gene in transgenic mice suggesting that a region between -600 bp and -1100 bp contains elements fundamental for human DBH gene expression in noradrenergic neurons.

3) Kim et al. (1998) observed that general transcription factors Sp1 and CREB, as well as cell-specific factors AP2, Phox2a and Phox2b bind to proximal cis-acting elements and have a critical role in synergic activation of DBH gene transcription.

4) C-1021T is located in a noradrenergic cell type-specific DNase I hypersensitive site of the DBH gene (Ishiguro et al., 1993).

Two years later, Zabetian et al. (2001) published their results of an experiment that investigated the LD structure of the DBH gene. They assumed C-1021T as a true functional polymorphism and examined the LD between C-1021T and another 11 markers, symmetrically distributed around C-1021T, and what is the relationship of each marker to plasma activity. They identified a block of LD at the DBH locus, including C-1021T that spanned across nearly 10 kb of its surroundings. All of these five markers within the LD block (-2124C->T, -1021C->T, IVS1+109G->C, 444A->G and IVS4+601C->T) are strongly associated with the phenotype.

5’-ins/del This polymorphism, named 5’-ins/del, consists of 19 base-pair insertion/deletion approximately 4.7 kb 5’ from the transcriptional start site, -4784-4803del (Cubells et al., 2000). This region resides within the locus that Hoyle et al. (1994) identified as a second positive regulatory element, between -1.5–5.8 kb (first between -600–1100 bp) that confers cell type-specific expression and contains an element responsible for the transient expression. 5’-ins/del is also associated with plasma DBH activity, particularly with deletion of lower and insertion of higher level of plasma DBH (Cubells et al., 2000). These results also showed that 5’-ins/del is in positive LD with another plasma DBH-associated polymorphism G444A and haplotype Del-A is associated with low plasma DBH activity in European American population.

G444A Cubells et al. (1998) studied the relationship between genotypes at this synonymous polymorphism situated in exon 2 and CSF levels of the DBH protein and plasma DBH activity. They observed a significant association between the G444A genotype and both bio-
chemical phenotypes. Furthermore, investigation of European American patients with mood or anxiety disorders suggested that the 444A allele is associated with lower plasma DBH activity and the 444G allele with higher plasma activity. Their results support the hypothesis that DBH is a major locus influencing the plasma DBH activity and the CSF DBH protein levels. Although polymorphism G444A alters the third base of a Gln codon, the primary structure of DBH protein does not alter. The alterations of CSF levels of DBH protein and plasma DBH activity in coherence with this polymorphism may be explained by G444A residing at the splice junction between exon 2 and intron 2 of DBH. Kobayashi et al. (1989) demonstrated that appropriately spliced mRNAs contain either G or A allele. Nevertheless, this substitution could modify the efficiency of the mRNA splicing, thereby affecting levels of mature DBH mRNA and causing the differences in levels of DBH. Cubells et al. (2000) also analysed linkage disequilibrium (LD) between G444A polymorphism and another plasma DBH-associated diallelic variant – 5'-ins/del– and confirmed their positive LD.

**G910T** This single nucleotide polymorphism (SNP) is non-synonymous and is located in exon 5. The difference at the nucleotide 910 causes an amino-acid alteration between Ala (A) and Ser (S) at the amino-acid residue 304 (Ishii et al., 1991). Ishii et al (1991) expressed the two gene variants in COS cells and suggested homospecific activities of DBH. The two forms of protein showed enzyme activities, were immunoreactive, both of them had similar kinetic constants but different homospecific activities. Ishii et al. (1991) found a 13-fold difference in homospecific DBH activity between 910G and 910T alleles, with 910T encoding the lower homospecific active form. Zabetian et al. (2001) examined A304S in groups of individuals representing phenotypic extremes with very low DBH activity levels. The samples were from African American, European American and Japanese population, but there were no deviations from the Hardy-Weinberg equilibrium (HWE) in these cases. Furthermore, Cubells and Zabetian (2004) examined the potential functional consequences of A304S. They used the SIFT software to predict whether an amino-acid substitution affects protein function and established that A304S should be well tolerated. Interestingly, both alleles are presented in most human populations representing all major geographic regions, but only two population samples contained 910T (304S) homozygotes (Danes and Adygei). 910G (304A) is always a more common allele, with frequencies greater than 0.8 in every investigated group (Cubells et al., 1997). Further work will be necessary to evaluate the contribution of this polymorphism to heritable variation in the level and activity of DBH in serum and CSF and eventually to ADHD.

**C1603T** The other non-synonymous SNP is C1603T in exon 2, +1603 base pair from the start site of translation. It encodes a non-conservative difference in the primary amino-acid sequence Arg535Cys and current results suggest that an allelic variance is responsible for a change in homospecificity of the enzyme (Tang et al., 2005). Whereas plasma DBH activity is mostly influenced by the level of circulating DBH protein (O’Connor et al., 1983), in this case the 1603T allele (encoding 535Cys) appears to exhibit an additional effect due to a decline in homospecific activity. The DBH holoenzyme is a homotetramer and Arg535Cys substitution may cause disulphide bridge formation, thus altering the homospecific activity of DBH. Additionally, differences in exocytotic release of the DBH protein or in its clearance from the plasma (Tang et al., 2005) may occur. The research using the SIFT software predicted that SNP C1603T would be weakly tolerated and thus might affect DBH function, because Arg is conserved in all available sequences that include the 3’ end of the gene (Cubells and Zabetian, 2004). Zabetian et al. (2001) supported this prediction. They found a small but significant contribution to the variance of plasma DBH activity of this SNP. Tang et al. (2005) confirmed previous results. They estimated the biological effect of C1603T on plasma DBH activity in a diagnostically heterogeneous group of European population. In this sample, the C-1021T genotype was found, and it was confirmed that no significant LD between both polymorphisms existed. These authors detected a significant additional effect of C1603T in the plasma DBH variance. C-1021T SNP accounts for 35–52% of the variance in the trait across populations of different geographic origin (Zabetian et al., 2001), C1603T may explain additional 2% of variance. The low-activity 1603T allele is relatively rare, with an occurrence of approximately 4% in the European population (Tang et al., 2005).

**C1912T** This SNP, located in exon 12, represents another member of the non-synonymous polymorphism group. It changes the first nucleotide in the codon for Arg, +1912 base pair from the start site of translation, which leads to substitution Arg->Cys. Arg->Cys should be weakly tolerated (Cubells and Zabetian, 2004). Exon 12 encodes the 3’-terminal region spanning from nucleotide +1681 to +2693 of type A cDNA and +1681 to +2393 of type B of the DBH gene. Kobayashi et al. (1989) examined both types of mRNA and showed evidence of inheritance of two polyadenylation sites corresponding to types A and B in exon 12. The A and B types are produced by alternative polyadenylation.

**TaqI** The effect of this DBH SNP on the DBH levels is not completely understood. It is situated in intron 5 (IVS5+192C->T) and is easily genotyped by differential cleavage with the restriction endonuclease TaqI (Cubells and Zabetian, 2004).
ADHD, low DBH activity and genetic polymorphisms

In patients with the hyperkinetic syndrome and non-socialized conduct disorder, reduced DBH activity in serum and urine were recorded (Bowden et al., 1988; Rogeness et al., 1989a; Paclt and Koudelová, 1990; Gabel et al., 1993; Galvin et al., 1995, 1997; Comings et al., 1996, 1999). Zabetian et al. (2001) suggest, on the basis of their results and another hypothesis that low plasma DBH levels result from diminished expression of the DBH gene, that it is strongly associated with allele -1021T. Thus C-1021T, or another polymorphism in very tight LD with it, appears as a variant at DBH controlling plasma DBH levels and accounts for 35–52% of variation in plasma DBH activity. C1603T may explain the additional 2% of variance. The low-activity 1603T allele is relatively rare, approximately 4% in European population (Tannock, 1998). SNP C1912T was not correlated to plasma activity and ADHD disorder. 5′-ins/del is also associated with plasma DBH activity, namely the deletion of lower and insertion of higher level of plasma DBH (Cubells et al., 2000). Wigg et al. (2002) investigated the 5′-ins/del polymorphism (and another two: TaqI and (CA)n STR) in a group of 117 families with ADHD. They observed significant relationships between the genotypes of the three polymorphisms, but no biased transmission for either of the allele of the 5′-ins/del. They also found no significant evidence for biased transmission of the haplotypes. Hawi et al. (2003) observed the G444A polymorphism in connection with ADHD and found a slight increase in the transmission of allele 444A (allele 2), but it was not statistically significant. They also analysed markers creating a high-density map across and flanking this gene and measured inter-marker LD. Strong LD was detected between markers of polymorphisms G444A (exon 2) and TaqI (intron 5). Comings et al. (1996, 1999) investigated whether TaqI B1/B2 may be associated with ADHD in a group of probands with Tourette’s syndrome. They detected that the Taq B1 allele (without TaqI site) was associated with the highest ADHD scores. Research of this problem was also done by Daly et al. (1999). They used a sample of ADHD children and found an association with TaqI (A1/A2) DBH allele A2 (present TaqI site). They both probably examined the same polymorphism, but with another nomenclature and another sample of probands, which may be the explanation for their different results.

Romain et al. (2002) detected an association between the DBH TaqI A2 allele and ADHD in a sample of 88 Brazilian nuclear families with ADHD, thereby confirming the previous report from Daly et al. (1999). The same results were also obtained by Inkster et al. (2004) from their analysis of TaqI polymorphism in two independent samples of adults with ADHD and by Kirley et al. (2002). Wigg et al. (2002) sought to replicate this work, but they found no significant evidence for the linkage of the TaqI A2 allele in the sample of 117 nuclear families with ADHD. Neither did Bhaduri et al. (2005), who implemented the first molecular genetic study on ADHD in an Indian subject, exploring the transmission of G444A and TaqI polymorphisms in the DBH gene. On the other hand, Smith et al. (2003) tested TaqI polymorphism in 105 Caucasian subjects with ADHD and ethnicity-matched controls. They observed that the DBH TaqI A1 allele was more frequently found in the ADHD group than in the control group.

Summary

Lower plasma DBH activity is caused by disappearing levels of circulation of the DBH protein, rather than decreasing the activity of the enzyme. However, which polymorphisms play the main role in this process is not known yet. It could be the ones in the coding region or those in the regulation or non-coding region. Hoyle et al. (1994) suggested an essential domain between -600 bp and -1100 bp. Certain elements fundamental for the human DBH gene are expressed in the noradrenergic neuron. Zabetian et al. (2001) suggested that low plasma DBH levels result from disappearing expression of the DBH gene strongly associated with allele -1021T. 5′-ins/del, located within the second positive regulatory element, may have additional effect on the expression. Allele 5′-del is associated with lower levels of plasma DBH (Cubells et al., 2000). The alteration of CSF levels of DBH protein and plasma DBH activity in coherence with polymorphisms localized in the coding region is influenced by G444A with risk allele 444A, C1603T with relatively rare risk allele 1603T (4% in European population) and G910T. Allele 910T (304S) codes for the lower specific active form of protein. How C1912T contributes to this is not known exactly. Although TaqI is localized in the non-coding region, alterations in this area may play a decisive role for the final protein. Alterations may affect splicing due to the origin or extinction of the artificial splice site, or some enhancer or silencer of splicing exists.

Only some of these polymorphisms were studied in connection with ADHD. Zabetian et al. (2001) found that allele -1021T is associated with a combined subtype of ADHD. Wigg et al. (2002) observed a significant relationship of the genotypes of polymorphisms 5′-ins/del, TaqI and (CA)n STR in the families with ADHD, in particular TaqA2-del-A3 and TaqA2-ins-A4. Hawi et al. (2003) found a slight increase in the transmission of allele 444A in the ADHD families. Association between TaqI and ADHD was also found by Comings et al. (1996, 1999) (allele B1), Smith et al. (2003) (allele A1), Daly et al. (1999), Romain et al. (2002), Inkster et al. (2004) and Kirley et al. (2002) (all with allele A2), but some results of other studies were negative (Wigg et al., 2002; Bhaduri et al., 2005).
difference in the DBH TaqI A polymorphism could be attributed to population stratification, resulting in a false-positive association of the A1 allele with ADHD (Smith et al., 2003).

Which polymorphisms are the most important in the ADHD and low DBH plasma activity? Which of them have the major role and which of them cause additional effects? The role of other DBH polymorphisms is unknown because these polymorphisms were not studied in connection with ADHD. In patients with hyperkinetic syndrome and in non-socialized conduct disorder patients, reduced DBH activity in serum and urine were recorded (Bowden et al., 1988; Rogeness et al., 1989a, b; Paclt and Koudelová, 1990; Gabel et al., 1993; Galvin et al., 1995, 1997; Comings et al., 1996, 1999).

Another question is the correlation between low DBH activity and prenatal hypoxia. Koudelová et al. (1989) found that hypoxia (hyperbaric chamber) decreased the DBH activity in experimental animals (rats), particularly in very young ones (5 days after delivery).

Many conflicting suggestions may emerge as a result of diagnostic problems connected to ADHD with co-morbidity and changes of symptoms in patients under 5 years of age or older than 10. Further investigation of polymorphisms in the DBH gene in connection with ADHD and DBH plasma activity should be done to provide a better understanding of this disorder.

References


