ORIGINAL ARTICLE

Some ADHD polymorphisms (in genes DAT1, DRD2, DRD3, DBH, 5-HTT) in case-control study of 100 subjects 6–10 age

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Abstract

BACKGROUND: Pharmakological approach is the most effective way of treatment of ADHD and its early application prevents from the progress of secondary disorders. The study on present neurotransmitter systems in pathology of ADHD can be helpful in selecting appropriate drug, since there are used various substances with different mechanisms of functioning in treatment of the hyperkinetic syndrome. **METHOD:** Within our study there were selected the genes of dopaminergic (DRD2, DRD3, DAT1), noradrenergic (DBH) and serotoninergic (5-HTT) systems. With the use of molecular-genetic methods based on association strategy "case-control" there were analysed genes including 11 polymorphisms. The presence of risk alleles was examined in comparison of the sample of 100 ADHD children to a control group of another 100 subjects, who were checked by child psychiatrists and examined with the Conners test in order to exclude eventual cases with ADHD symptoms. **RESULTS:** Our research suggests the association of some genes with ADHD. It could be concluded: 1) the risk of ADHD is significantly increased in the presence of one risk allele in genes DRD2 (O.R.= 7,5), 5-HTT (O.R.= 2,7) and DAT1 (O.R.= 1,6). 2) The risk of ADHD is significantly increased at homozygotes for risk alleles in genes DRD2 (O.R.= 54,8), 5-HTT (O.R.= 6,7) and DAT1 (O.R.= 6,6). For polymorphisms G444A and C1603T in DBH, which were detected by univariant analysis, haplotype analysis was performed and resulted in conclusion that: 3) the risk of ADHD is significantly increased in the presence of allele DBH +444A as well as in the presence of allele DBH +1603T (O.R.= 15).

Abbreviations

5-HTT	– serotonin transporter
ADHD	– Attention-deficit/hyperactivity Disorder
DAT1	– dopamine transporter
DBH	– dopamine-beta-hydroxylase
DRD(1-5)	– genes for dopamine receptors (1-5)
DSM-IV	– The Diagnostic and Statistical Manual of Mental Disorder, 4th edition
EDTA	– ethylenediaminetetraacetic acid
GABA	– gamma-aminobutyric acid
HTR1B	– 5-hydroxytryptamine (serotonin) receptor 1B
HWE	– Hardy-Weinberg equilibrium
LD	– linkage disequilibrium
MR	– magnetic resonance
O.R.	– odds ratio
PET	– positron emission tomography
RFLP	– restriction fragment length polymorphism
SNAP 24	– synaptosomal-associated protein 24
SNP	– single nucleotide polymorphism
UTR	– untranslated region
VNTR	– variable number of tandem repeats

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a clinical disorder characterized by an impulsivity, hyperactivity and inattentiveness. The symptoms appear early in a child's development, some of them persist to the adult age (40–50%), although they subside with age. The comorbides are associated at 50–80% of the patients [22], the most often behavioral deficiency, anxiety, depression, learning disabilities and problems with sleeping [5]. ADHD children have also frequently additional health problems in particular asthma and allergy. Estimations of prevalence are different due to diagnostic criteria. Nowadays, The Diagnostic and Statistical Manual of Mental Disorder, 4th edition, DSM-IV (3–6% afflicted children) distinguishes between three types of disorder: 1) combined type, 2) ADHD with dominance of inattentiveness, 3) ADHD with dominance of hyperactivity and impulsivity.

In neuro-imagines studies (MR, PET) there were described: decrease volume of basal ganglia, frontal and prefrontal cortex and decrease of metabolic activity in frontal cortex, together with compensatory increase of metabolic activity in basal ganglia. ADHD is a complex genetic disorder. The heredity and prenatal pathology are dominant theories of ADHD etiology with a multifactorious pattern of inheritance [11], involving many susceptibility genes with a smaller or greater effect [38]. More than thirty genes of dopaminergic, noradrenergic, serotoninergic and GABA-ergic systems are studied with connection of this disorder. These genes may take part in the development of the hyperkinetic disorder, and thus also of the whole range of comorbide psychiatric diseases in children, adolescents and grown-ups [10]. Following genes probably determine partially basal ganglia and prefrontal and frontal cortex function. For instance: Durston et al., 2005 showed that DAT1 gene expressed predominantly in the basal ganglia, preferentially influences caudate volume, whereas the DRD4 gene, a gene expressed predominantly in the prefrontal cortex, preferentially influences prefrontal grey matter volume [13]. Some consistent results were obtained in genes: DRD4, DRD5, DAT1, DBH, 5-HTT, HTR1B and SNAP 24 [1, 13, 16, 19, 27, 32, 34, 40, 41] in other are more conflict results in DRD3, DRD2 [6, 43], but in Czech population were described some positive results especially in DRD2 [36].

In our study we selected genes of dopamine receptors DRD2 and DRD3, dopamine transporter DAT1, enzyme dopamine-beta-hydroxylase DBH and serotonin transporter 5-HTT. In these genes we examined 11 polymorphisms (Table 1). The dopamine transporter is localised in the presynaptic membrane and its function consist in reuptake of released dopamine back into the presynaptic neurons. The importance of the DAT1 gene is connected with the mechanism of stimulants effect, that blocking the dopamine transporter coded by this gene and thus increasing the concentration of dopamine in the synaptic gap [9, 42]. 5-HTT is gene, which codes serotonin transporter, its function is similar as dopamine transporter only with difference in transferred neurotransmitter respectively serotonin. Dopamine receptors DRD2 and DRD3 are localised in the post and presynaptic membrane, these play a crucial role via inhibition of the adenylate cyclase and production of cAMP.

DBH is an enzyme responsible for the conversion of dopamine into norepinephrine. In its feedback, it inhibits an enzyme tyrosine-hydroxylase, which reduces the production of dopamine. Decreased activities of DBH in serum and urine were found in the patients with hyperkinetic syndrome [18, 32, 33]. The association between allelic variations in DBH gene and ADHD were approved in some studies [16, 25]. There were observed polymorphisms G444A, G910T, C1603T, C1912T, C-1021T, 5´-ins/del and TaqI, what were examined in our study. However, what polymorphisms play the main role in this process is not known yet.

Hypotheses of our study: 1) Relatively exhausting sampling design of patients and controls (excluding comorbid disorders) results in more exact results [37]. 2) Study of more polymorphisms of DBH elevated possibility of the positive results (higher in carriers of the some polymorphic alleles) [2, 3]. 3) We anticipate repetition of positive results of some study in DRD2 gene [36] in Czech population.

METHODS AND MATERIAL

Experimental groups

We examined two experimental groups. Two blind independent graduated children and adolescent psychiatrists examined both groups of children. Both child psychiatrists confirmed DSM IV diagnose of combine type in ADHD group: age 6–11, IQ > 80, comorbid disorders (conduct disorder, dyslexia, and others) were excluded and Conners Scale for Parents, Czech version Paclt, 1998 [31] ≥ 30 (this means ≥ 2 sigma). Number of

Table 1: View of examined polymorphisms, their localisation, SNP ID, molecular matter, risk alleles and manifestation.
Grey: polymorphisms in DBH.

polymorphisms	localisation	refSNP ID	molecular matter	risk allele	manifestation
DBH C-1021T (RFLP)	-1021 promotore	rs1611115	C>T	-1021T (without restriction site)	↓expression of mRNA
DBH 5´-ins/del	-4784-4803 promotore		19bp inzerce/delece	delece	↓ expression of mRNA
DBH G444A (RFLP)	Exon 2	rs1108580	G>A Glu148Glu	444A (Glu148) (without restriction site)	alteration in splicing →↓ amount of maturated mRNA
DBH G910T (RFLP)	Exon 5	rs4531	G>T Ala304Ser	910T (Ala304) (without restriction site)	↓ homospecifity of enzyme
DBH C1603T (RFLP)	Exon 11	rs6271	C>T Arg535Cys	1603T (Arg535) (without restriction site)	↓ homospecifity of enzyme
DBH C1912T (RFLP)	Exon 12	rs129882	C>T Arg638Cys	1912T (Arg638) (without restriction site)	↓ stability of mRNA
DBH TaqI (RFLP)	Intron 5	rs2519152	T>C	TaqI B1 (C) (without restriction site)	? alteration in splicing
DRD2 TaqI (RFLP)	9,5 kb downstream from 3´-UTR	rs1800497	T>C	TaqI A1 (C) (without restriction site)	↓ stability of mRNA ↓ translation
DRD3 Ser9Gly (RFLP)	Exon 1	rs6280	C>T Ser9Gly	456T (Ser9) (without restriction site)	↓ affinity to dopamine
DAT1 (VNTR)	Exon 5		40bp VNTR	alela 10	↑ expression of mRNA
5-HTT (VNTR)	5´-UTR		20-23bp VNTR	alela *l* (16)	↑ expression of mRNA

Table 2: View of primers, restriction enzymes, PCR conditions and cleaving conditions of each polymorphism.
Grey: polymorphisms in DBH.

	primers	PCR conditions	Restriction enzymes
DBH C-1021T RFLP	F: 5´-GGA GGG ACA GCT TCT AGT CC-3´ R: 5´-CAC CTC TCC CTC CTG TCC TCT CGC-3´	Zabetian et al., 2003	HhaI (37°C; 0,2U;20µl) Cleaved allele C
DBH 5´-ins/del VNTR	F: 5´-GCA AAA GTC AGG CAC ATG CAC C-3´ R: 5´-CAA TAA TTT GGC CTC AAT CTT GG-3´	Cubells et al., 2000	
DBH G444A RFLP	F: 5´-CCT GGA GCC CAG TGC TTG TC-3´ R: 5´-ACG CCC TCC TGG GTA CTC GC-3´	Cubells et al., 1998	EcoNI (37°C;0,5U;15µl) Cleaved allele G
DBH G910T RFLP	F: 5´-GCC CTC TCA GGA CAC ACC-3´ R: 5´-ACA CAG CTG AGT CCT AGG G-3´	Hot start 95°C 5min, 3+3+25 cycles 95°C/1min, 66, 64, 62°C/1min, 72°C/1min, final extension 72°C/10min.	MwoI (60°C; 2U; 20µl) Cleaved allele G
DBH C1603T RFLP	F: 5´-CCA GGG ACA GGA CTC GAG TTG-3´ R: 5´-AGC AGT TTG GAG TGC AGA CCC-3´	Zabetian et al., 2003	BstUI (60°C; 1U; 20µl) Cleaved allele C
DBH C1912T RFLP	F: 5´- CCC ATG GAA CAG CCG TGC AC-3´ R: 5´-ACA CCC TCT CAG CCA TGC AG-3´	Zabetian et al., 2003	ApaLI (37°C; 1U; 20µl) Cleaved allele C
DBH TaqI RFLP	F: 5´-CTG GAA GTT CAC TAC CAC-3´ R: 5´-GTC GTT TCG TCC TGG GAG-3´	Hot start 95°C 5min, 30 cycles 95°C/1min, 62°C/1min, 72°C/4min, final extension 72°C/10min.	TaqI (60°C 0,5U; 20µl) Cleaved allele B2
DRD2 TaqI RFLP	F: 5´-ACG GCT GGC CAA GTT GTC TA-3´ R: 5´-ACC CTT CCT GAG TGT CAT CA-3´	Hot start 94°C 5min, 35 cycles 95°C/1min, 62°C/1min, 72°C/1min, final extension 72°C/10min.	TaqI (65°C; 1U; 20µl) Cleaved allele A2
DRD3 Ser9Gly RFLP	F: 5´-GCT CTA TCT CCA ACT CTC ACA-3´ R: 5´-AAG TCT ACT CAC CTC CAG GTA-3´	Hot start 95°C 5min, 32 cycles 95°C/1min, 56°C/1min, 72°C/1min, final extension 72°C/10min.	BalI/MscI (37°C;1U; 20µl) Cleaved allele A2
DAT1 VNTR	F: 5´-TGT GGT GTA GGG AAC GGC CTG AG -3´ R: 5´-CTT CCT GGA GGT CAC GGC TCA AGG -3´	Hot start 95°C 5min, 30 cycles 95°C/1min, 66°C/1,5min, 72°C/1,5min, final extension 72°C/10min.	-
5-HTT VNTR	F: 5´-ATG CCA GCA CCT AAC CCC TAA TGT-3´ R: 5´-GGA CCG CAA GGT GGG CGG GA-3´	Hot start 95°C 5min, 30 cycles 95°C/1min, 64°C/1min, 72°C/2min, final extension 72°C/15min.	-

patients: 100, boys: girls ratio 89:11. In control group: age 6–11, IQ > 80, by DSM IV criteria, were excluded all psychiatric disorder in two child psychiatric independent examination and Conners Scale for Parents, scale for Teachers: Czech version Paclt, 1998 [31] ≤ 1 sigma. Number of controls: 100, boys: girls ratio 63:37.

Examined polymorphisms

We have studied 11 polymorphisms in the dopaminergic genes DRD2, DRD3, DAT1; serotoninergic gene 5-HTT and noradrenergic gene DBH (Table 1). DNA was extracted from peripheral blood with EDTA by Gentra kit or from saliva by kit OraGene according to routine protocol. The target sequences of DNA were amplified by PCR using the thermostabile polymerase TaqI, dNTP´s, buffer and specifically designed primers. This method was applied for both polymorphisms: variable number of tandem repeats VNTR (DAT1, 5-HTT) and DBH 19 bp ins/del and restriction fragment length polymorphism RFLP (DBH C-1021T, DBH G444A, DBH G910T, DBH C1603T, DBH C1912T, DBH TaqI, DRD2 TaqI and DRD3 Ser9Gly). Afterwards the PRC products were digested (only in RFLP method) and separated on 2% agarose gels. Bands were visualised using ethidium bromide and UV transluminator. Accurate conditions are presented in table 2.

Statistical analysis

Statistical analysis was performed for two models, allelic model, when the risk of disease is in the presence of one risk allele, and recessive model, when only two alleles represent the risk of disease. The analysis of Hardy-Weinberg equilibrium (HWE) and comparisons between the ADHD children and the group of control children was performed using chi-square test. Consequently, the sex correction was executed in all the polymorphisms. A regression analysis was applied to testing two polymorphisms G444A and C1603T in DBH, which were detected by univariant analysis.

RESULTS

Tables 3 and 4 present observed data of the molecular-genetic analysis. The results of our research suggest an association of the genes DRD2, DAT1, DBH and 5-HTT with ADHD (P<0,05) (Table 5). Sex correction did not exclude any of polymorphisms (P<0,05).

Table 3: Observed genotypes in the group of ADHD patients.

polymorfisms		genotype 1 (alleles 11)	genotype 2 (alleles 12)	genotype 3 (aleles 22)	total
DBH	C-1021T	69 (CC)	25 (CT)	6 (TT)	100
	19 bp ins/del	39 (ins)	37 (i/d)	24 (del)	100
	G444A	18 (GG)	55 (AG)	27 (AA)	100
	G910T	85 (GG)	12 (GT)	3 (TT)	100
	C1603T	85 (CC)	15 (CT)	0 (TT)	100
	C1912T	78 (CC)	19 (CT)	3 (TT)	100
	TaqI	20 (22)	49 (12)	31 (11)	100
DRD2	TaqI	24 (22)	40 (12)	36 (11)	100
DRD3	Ser9Gly	2 (22)	55 (12)	43 (11)	100
DAT1	VNTR	2 (9/9)	39 (9/10)	59 (10/10)	100
5-HTT	VNTR	16 (ss)	55 (sl)	29(ll)	100

Table 4: Observed genotypes in the control group.

polymorfismus		genotype 1 (alleles 11)	genotype 2 (alleles 12)	genotype 3 (alleles 22)	total
DBH	C-1021T	77 (CC)	23 (CT)	0 (TT)	100
	19 bp ins/del	40 (ins)	41 (i/d)	19 (del)	100
	G444A	28 (GG)	57 (AG)	15 (AA)	100
	G910T	83 (GG)	17 (GT)	0 (TT)	100
	C1603T	98 (CC)	2 (CT)	0 (TT)	100
	C1912T	86 (CC)	14 (CT)	0 (TT)	100
	TaqI	21 (22)	51 (12)	28 (11)	100
DRD2	TaqI	73 (22)	25 (12)	2 (11)	100
DRD3	Ser9Gly	17 (22)	32 (12)	51 (11)	100
DAT1	VNTR	11 (9/9)	40 (9/10)	49 (10/10)	100
5-HTT	VNTR	48 (ss)	39 (sl)	13 (ll)	100

Table 5: View of *P values* (values which define significance of results, for significant results P<0,05), and *O.R.* (odds ratio; define**s** risk of disease when the risk allele – allelic model - or alleles – recessive model - are presented).

gene	polymorphisms	model	*P* value	O.R.
DRD2	TaqI	allel	3,74e-18	7,50
DRD2	TaqI	recess	1,83e-13	54,75
5-HTT	VNTR	allel	1,37e-06	2,70
5-HTT	VNTR	recess	7,20e-06	6,70
DAT1	VNTR	allel	3,08e-02	1,64
DAT1	VNTR	recess	7,50e-03	6,62
DBH	G444A	allel	2,78e-02	1,57
DBH	G444A	recess	1,84e-02	2,80
DBH	C1021T	recess	1,37e-02	13,71
DBH	C1603T	allel	1,27e-03	8,03
DBH	C1603T	recess	1,00e+00	1,15

The logistic regression for polymorphisms G444A and C1603T in DBH showed, that risk with presence of both polymorphisms is higher than in presence either of polymorphisms (G444A singly O.R.= 1,5; C1603T singly O.R.= 8,03; both in common O.R.= 15). This means that effect of polymorphisms is additive.

DISCUSSION

The results of this study note the polymorphisms in genes DRD2, 5-HTT, DAT1 and DBH as associated with ADHD. ADHD risk is significantly higher in carriers of the risk allele TaqI A1 in the DRD2 gene (7,5x), allele l in 5-HTT (2,7x) and allele 10 in DAT1 (1,6x); 2) ADHD risk is significantly higher in homozygous for above-mentioned alleles in genes DRD2 (54,8x), 5-HTT (6,7x) and DAT1 (6,6x); 3) ADHD risk is significantly higher in carriers of the polymorphism allele DBH +444A that are carriers of the polymorphism allele DBH +1603T at the same time (15x).

These results reasserted some conclusions of previous association studies and also studies in vivo and in vitro. Duan et al., 2003 [12] found out that polymorfism TaqI in DRD2 is in strong LD with SNP C957T, that in vitro study showed 50% decrease of translation activity in presence of allele 957T, probably owing to decreased stability of mRNA; Hirvonen et al., 2005 [21] in vivo examined the significant interference at binding potential of striatal DRD2 receptors by polymorfism C957T. It is probable that polymorfism TaqI (or another one in LD) contributes to decrease in translation and also in lower availability of DRD2 receptors. In DAT1 the coherence between allele 10 and increased activity was detected in experiments in vitro in substantia nigra [28], in COS cells [17], and also in vivo [29]. Also allele *l* in 5-HTT leads to higher transcriptional activity [20, 26, 14]. Zabetian et al., 2001 [45] showed that lower gene expression is strong associated with -1021T allele in promotore region in DBH gene. G444A polymorphism may alter efficiency of splicing and thus may alter amount of matured mRNA and final DBH protein [24]. These results correspond with some variability in DBH genes studies [1, 8, 23]. This is important to study more DBH alleles. However, some other data are still conflicting in various polymorphisms [6, 43, 44].

We confirmed the importance of exhaustive design of study groups – patients and controls [37]. More detailed analysis, elaborating some results of this paper, will be published in future. Research in candidate gene studies of ADHD like association studies (case-controls) and family-based designs are still important method for replicated results [15].

The study of various neurotransmitter systems might help to choose the right pharmacology drug in the future with regard to utilization of drugs with different operation mechanism in treatment of ADHD [27].

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REFERENCES:

1 Barkley RA, Smith KM, Fischer M, Navia B. An Examination of the Behavioral and Neuropsychological Correlates of Three ADHD Candidate Gene Polymorphisms (DRD4-7, DBH TaqI A2, and DAT1 40 bp VNTR) in Hyperactive and Normal Children Followed to Adulthood. American Journal of Medical Genetics Pard B. 2006; **141B**:487–498.

2 Bhaduri N, Mukhopadhyay K. Lack of significant association between – 1021C > T polymorphism in the dopamine beta hydroxylase gene and attention deficit hyperactivity disorder. Neuroscience letters. 2006; **402**: 12–16.

3 Bhaduri N, Sinha S, Chattopadhyay A, Gangopadhyay PK, Singh M, Mukhopadhyay KK. Analysis of polymorphisms in the dopamine Beta hydroxylase gene: association with attention deficit hyperactivity disorder in Indian children. Indian Pediatr. 2005; **42**(2):123–9.

4 Biederman J, Mick E, Faraone SV, Brašen E, Doyle A, Spenser T, Wilens TE, Frazier E, Johnson MA. Influence of gender on attention deficit hyperactivity disorder in children referred to a psychiatric clinic. American Journal of Psychiatry. 2002; **159:**36–42.

5 Biederman J, Newcorn J, Sprich S. Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. Am J Psychiatry. 1991; **148**(5): 564–77.

6 Bobb AJ, Castellanos FX, Addington AM, Rapoport JL. Molecular genetic studies of ADHD: 1991 to 2004. Am J Med Genet B Neuropsychiatr Genet. 2005 Jan 5; **132**(1): 109–25.

7 Brookes K, Xu X, Chen W, Zhou K, Neale B, Lowe N, Aneey R, Franke B, Gill M, Ebstein R, Buitelaar J, Sham P. The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. Mol Psychiatry. 2006 Oct; **11**(10): 934–53.

8 Carrasco X., Rothhammer P, Moraga M, Henrıq H, Chakraborty R,. Aboitiz F, Rothhammer F. Genotypic Interaction Between DRD4 and DAT1 Loci Is a High Risk Factor for Attention-Deficit/Hyperactivity Disorder in Chilean Families. American Journal of Medical Genetics Pard B. 2006; **141B**:51–54.

9 Comings DE, Wu S, Chiu C, Ring RH, Gade R, Ahn C. et al. Polygenic inheritance of Tourette syndrome, stuttering, attention deficit hyperactivity, conduct, and oppositional defiant disorder: The additive and subtractive effect of the three dopaminergic genes – DRD2, D beta H, and DAT1. Am J Med Genet Neuropsychiatr Genet. 1996; **67(**3):264–288.

10 Comings D. Genetics of ADHD, konference IBC, April 28–29, Boston. 1997.

11 Doyle AE, Willcutt EG, Seidman LJ, Biederman J, Chouinard VA, Silva J, Faraone SV. Attention-deficit/hyperactivity disorder endophenotypes. Biol Psychiatry. 2005; **57**(11): 1324–35.

12 Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. Hum Mol Genet. 2003 Feb1;**12**(3): 205–16.

13 Durston S, Fossella JA, Casey BJ, Hulshoff-Pol HE, Galvan A, Schnack HG, Steenhuis MP, Minderaa RB, Buitelaar JK, Kahn RS, van-Engeland H. Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls. Mol Psychiatry. 2005; **10**(7): 678–85.

14 Eddahibi S, Humbert M, Fadel E, Raffestin B, Darmon M, Capron F, Simonneau G, Dartevelle P, Hamon M, Adnot S. Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. J Clin Invest. 2001; **108**(8): 1141–50.

15 Faraone SV, Khan SA. Candidate gene studies of attention-deficit/hyperactivity disorder. J Clin Psychiatry. 2006; **67** (8): 13–20.

16 Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, Sklar P. Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005; **57**(11):1313–23.

17 Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, Ishiura S. The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. Pharmacogenomics J. 2001; **1**(2): 152–6.
18 Galvin M, TenEyck R, Shekhar A, Stilwell B, Fineberg N, Laite G, et al. Serum dopamine-beta-hydroxylase and maltreatment in psychiatrically hospitalized boys. Child Abuse and Neglected. 1995; **19**(7):821–83.
19 Hawi Z, Segurado R, Conroy J, Sheehan K, Lowe N, Kirley A, Shields D, Fitzgerald M, Gallagher L, Gill M. Preferential Transmission of Paternal Alleles at Risk Genes in Attention-Deficit/Hyperactivity Disorder. American Journal of Human Genetic. 2005; **77**:958–965.
20 Heils A, Mössner R, Lesch KP. The human serotonin transporter gene polymorphism--basic research and clinical implications. J Neural Transm. 1997; **104**(10): 1005–14.
21 Hirvonen M, Laakso A, Nagren K, Rinne JO, Pohjalainen T, Hietala J. C957T polymorphism of the dopamine D2 receptor (DRD2) gene affects striatal DRD2 availability in vivo. Mol Psychiatry. 2004; **9**(12): 1060–1. No abstract available. Erratum in: Mol Psychiatry. 2005;**10**(9): 889.
22 Jensen PS, Martin BA, Cantwell DP. Comorbidity in ADHD: Implications for research, practice and DSM-IV. Journal of the American Academy of Child and Adolescent Psychiatry. 1997; **36**:1065–1079.
23 Kim SJ, Badner J, Cheon KA, Kim BN, Yoo HJ, Kim SJ, Cook E, Leventhal BL, Kim YS. Family-Based Association Study of the Serotonin Transporters Gene Polymorphisms in Korean ADHD Trios. American Journal of Medical Genetics Pard B. 2006; **139B**:14–18.
24 Kobayashi K, Kurosawa Y, Fujita K, Nagatsu T: Human dopamine beta-hydroxylase gene: two mRNA types having different 3'-terminal regions are produced through alternative polyadenylation. Nucleic Acids Res. 1989; **17**(3):1089–102.
25 Kopeckova M, Paclt I, Goetz P. Polymorphisms and low plasma activity of dopamine-beta-hydroxylase in ADHD children. Neuro Endocrinol Lett. 2006 Dec; **27**(6):748–54. Review.
26 Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science. 1996; **274**(5292): 1527–31.
27 McGough JJ. Attention-Deficit/Hyperactivity Disorder Pharmacogenomics. Biological Psychiatry. 2005; **57**:1367–1373.
28 Michelhaugh SK, Fiskerstrand C, Lovejoy E, Bannon MJ, Quinn JP. The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. J Neurochem. 2001 Dec;**79**(5): 1033–8.
29 Mill J, Asherson P, Browes C, D'Souza U, Craig I. Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: evidence from brain and lymphocytes using quantitative RT-PCR. Am J Med Genet. 2002; 114B: 975–979.
30 Mill J, Xu X, Ronald A, Curran S, Price T, Knight J, Craig I, Sham P, Plomin R, Asherson P. Quantitative trait locus analysis of candidate gene alleles associated with attention deficit hyperactivity disorder (ADHD) in five genes: DRD4, DAT1, DRD5, SNAP-25, and 5HT1B. Am J Med Genet B Neuropsychiatr Genet. 2005; **133**(1): 68–73
31 Paclt I, Florian J: Psychofarmakoterapie dětského a dorostového věku. Grada. 1998; p 405.
32 Paclt I, Koudelová J. Dopamin-beta-hydroxylase in plasma of psychiatric patients. Activ. nerv. super. 1990; **32**(1): 67.
33 Paclt I, Koudelová J. The activity of the dopamine-beta-hydroxylase (DBH) in an experiment in animals in norm and in humans suffering with special developmental dopaminergic disturbance. Proceeding of the IBC International Conference on Dopaminergic Disorders. April 28–29. 1997; Boston, MA, USA.
34 Paclt I, Koudelová J, Křepelová A, Uhlíková P, Gazdíková M, Bauer P. Biochemical markers and genetic research of ADHD. Neuro Endocrinol Lett. 2005; **26**(4):423–30. Review.
35 Purper-Ouakil D, Wohl M, Mouren MC, Verpillat P, Ades J, Gorwood P. Meta-analysis of family-based association studies between the dopamine transporter gene and attention deficit hyperactivity disorder. Psychiatr Genet. 2005 Mar; **15**(1): 53–9.
36 Šerý O, Theiner P, Hladilová R, Šteif R, Balaštíková B, Drtílková I. Genes for Il-6 and DRD2 correlate to hyperkinetic disorder. (in Czech) Česká a Slovenská psychiatrie. 2003; **99**(8): 404–109.
37 Stevenson J, Asherson P, Hay D, Levy F, Swanson **J**, Thapar A, Willcutt E. Characterizing the ADHD phenotype for genetic studies. Developmental science. 2005; **8**(2): 115–121.
38 Tannock R. Attention deficit hyperactivity disorder: advances in cognitive, neurobiological, and genetic research. J Child Psychol Psychiatry. 1998; **39**(1): 65–99.
39 Thapar A, O'Donovan M, Owen MJ. The genetics of attention deficit hyperactivity disorder. Hum Mol Genet. 2005 Oct 15; **14**(Spec No. 2): R275–82.
40 Todd RD, Huang H, Smalley SL, Nelson SF, Willcutt EG, Pennington BF, Smith SD, Faraone SV, Neuman RJ. Collaborative analysis of DRD4 and DAT genotypes in population-defined ADHD subtypes. J Child Psychol Psychiatry. 2005 Oct; **46**(10): 1067–73.
41 VanNess SH, Owens MJ, Kilts CD. The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. BMC Genet. 2005; **6**: 55.
42 Volkow ND, Ding Y, Fowler JS, Wang GJ, Logan J, Gatley SJ, Hitzemann R, Smith G, Fields SD, Gur R. Dopamine Transporters Decrease with Age. The Journal of Nuclear Medicine. 1996; **37**(4): 554–559.
43 Wohl M, Purper-Ouakil D, Mouren MC, Ades J, Gorwood P. Meta-analyse des genes candidats dans le trouble deficit attentionnel avec hyperactivite (TDAH). [Meta-analysis of candidate genes in attention-deficit hyperactivity disorder]. Encephale. 2005 ; **31**(4 Pt 1): 437–47.
44 Xu X, Mill J, Chen CK, Brookes K, Taylor E, Asherson P. Family-based association study of serotonin transporter gene polymorphisms in attention deficit hyperactivity disorder: no evidence for association in UK and Taiwanese samples. Am J Med Genet B Neuropsychiatr Genet. 2005 Nov 5; **139**(1): 11–13.
45 Zabetian CP, Anderson GM, Buxbaum SG, Elston RC, Ichinose H, Nagatsu T, et al. A quantitative-trait analysis of human plasma-dopamine beta-hydroxylase activity: evidence for a major functional polymorphism at the DBH locus. Am J Hum Genet. 2001; **68**(2):515–22.