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Long term pharmacotherapy by methylfenidate or atomoxetine DAT 1 10/10 ADHD children in correlation with results of the imaging methods

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Abstract

OBJECTIVES: ADHD is one of the most significant diagnostic units in child and adolescent psychiatry. The occurrence in children is 5–6% and 50–80% continued to adult age. The presence of individual genes (polymorphism) on particular symptoms and processes in ADHD are not known. It is estimated that ADHD symptoms are up to 80% to genetic. The higher density of resultant DAT 1 protein was observed in ADHD patients in comparison with controls. The question was if DAT 1 10/10 predicted bad prognoses in long term therapy.
METHODS: We compared 30 ADHD DAT 1 10/10 adolescents treated for 5–6 years. Patients with 30 control adolescents. They were the same age of probands and controls. All these subjects were examined by child psychiatry scales (Conners, Achenbach…). Biological changes were tested by MRI specific CNS volumometry.
RESULTS: We didn't confirm bad prognoses in long term therapy with methylphenidate or atomoxetine in ADHD children DAT 1 10/10 in long term therapy. In MRI specific CNS volumometry were not identify any differences in controls and ADHD probands. Gray matter thickness was significantly higher in prefrontal and occipital areas in patients compared to control in prefrontal and occipital areas with cluster-wise p-value<0.05. By this method were not identify any cerebrum damage in long term therapy by methylphenidate and atomoxetine.

INTRODUCTION

ADHD (Attention-deficit hyperactivity disorder) nowadays represents one of the most significant diagnostic units in the psychiatry of children and adolescents and today it is also a significant unit in the psychiatry of adults (Mick & Faraone *et al.* 2008). The occurrence in children is estimated to be 5–6% (Polanczyk & Jensen 2008), 50%–80% (Frodl *et al.* 2012) of patients continue to display these symptoms, particularly attention disorder into adulthood and they can be aggressive, have behaviour disorders and dis-sociality (Asherson 2009).

ORIGINAL ARTICLE

A lot of psychiatric disorders including ADHD have a genetic basis (polygenic inheritance), however the specific influence of the presence of individual genes on particular symptoms and process of ADHD are not known. It is possible to relate up to 80% of phenotypic characteristics to the genetic factors at this disorder (Faraone *et al.* 2005).

Polymorphisms DAT1 and DRD4 are most frequently indicated as high-risks for ADHD. The studies of DAT1 genes were focused mainly on polymorphism 40-bp VNTR located in 3'UTR, 10 repeat. The issue is that there is a variable number of repetitive units in the length of the 40 pair's basis. In connection with ADHD, allele with 10 repeats (10R) was identified as a high-risk. This variant is connected with a higher expression of dopamine transporter (Mill *et al.* 2002), in striatum. The higher density of resultant DAT1 protein was observed at ADHD patients in comparison with the controls (Krause *et al.* 2003). Polymorphism at gene DAT1 determines the sensitivity to methylphenidate, which operates through dopamine transporters (Krause 2008). Homozygotes for 10R allele (10/10) are less sensitive to methylphenidate, an assumption was pronounced on the basis of this reason that it is suitable to indicate higher doses of methylphenidate or a different type of medication (Szobot *et al.* 2011; Winzberg & Comings 1999). Question is if these results are obvious (Šerý *et al.* 2015)?

Tab. 1. Probands (n=30).

Patients	DBH1021 (C-1021T)	DBH444 (G444A)	DBH910 (Ala/Ser)	DBH1603 (C1603T)	DBH1912 (C1912T)	DBHintron5 (TaqI)	DRD2	DRD3	DBH (19 bp ins/del)	DAT1	5-HTT
ADHD 1	2	1	1	1	2	2	1	2	3	3	3
ADHD 2	2	2	1	1	1	2	1	3	1	3	2
ADHD 3	1	2	1	1	1	3	3	2	3	3	2
ADHA4	1	1	1	1	1	2	3	2	3	3	3
ADHD 5	3	2	1	1	2	2	2	3	3	3	2
ADHD 6	2	2	1	1	2	3	1	3	1	3	2
ADHD 7	3	3	1	1	2	1	1	2	3	3	1
ADHD8	1	2	1	1	1	3	2	3	3	3	3
ADHD 9	1	2	1	1	1	1	1	2	2	3	2
ADHD 10	2	2	1	1	1	2	1	2	3	3	2
ADHD 11	2	2	1	1	1	2	1	2	3	3	2
ADHD 12	1	1	1	1	1	2	1	3	1	3	2
ADHD 13	2	3	1	1	1	3	1	1	1	3	2
ADHD 14	1	1	1	1	1	1	2	3	2	3	2
ADHD 15	2	3	1	1	1	1	2	3	3	3	1
ADHD 16	3	2	1	1	1	2	1	3	2	2	3
ADHD 17	2	2	1	1	1	2	2	3	1	3	1
ADHD 18	2	2	1	1	2	2	2	2	2	3	2
ADHD 19	2	2	1	1	1	1	2	2	1	3	1
ADHD 20	2	3	2	2	1	2	1	2	2	3	2
ADHD 21	1	1	2	1	1	2	1	3	2	3	2
ADHD 22	1	2		1	1	3	2	3	1	3	2
ADHD 23	2	1	1	1	1	3	1	3	2	3	2
ADHD 24	1	1	1	1		2	2	3	2	3	3
ADHD 25	1	3	1	1	1	3	2	1	3	3	3
ADHD 26	3	3	1	1	1	1	2	3	3	3	3
ADHD 27	1	1	1	1	1	2	3	1	2	3	1
ADHD 28	3	3	1	1	2	1	2	3	1	3	2
ADHD 29	3	2	1	1	1	2	1	3	2	3	3
ADHD 30	1	2	1	1	1	3	1	3	1	3	3

MATERIAL AND METHODS

On the basis of our previous results of family study of polymorphisms DAT (Paclt *et al.* 2013) – from the total amount of 357 probands (ADHD children and members of their families) there were homozygotes 9/9 29 individuals, heterozygotes 9/10 142 individuals and homozygotes 10/10 186 individuals 49 patients from this cohort were 9–17 years old. We have gained the informed consent of patients or parents at 30 cases.

30 ADHD patients from DAT1 (10/10 homozygotes) mean age 15.6±2.5 y/o (3 girls) with the history of at least 5 years treatment by methylphenidate or atomoxetine underwent clinical and MRI examination at least 3 weeks after pharmacotherapy termination. 30 healthy controls (mean age 16.5±1.8 y/o, 6 girls). Age of patients and controls don't different in statistic point.

Psychiatric examination and scaling

All the subjects, namely ADHD patients (combined type or hyperactivity/impulsive type) without comorbidity and comparable age check, were examined by two independent child psychiatrists and by testing battery. Pharmacotherapy: methylfenidate (Ritalin, Concerta) 10–60 mg/day (90%), atomoxetine (Strattera) 1–1.2 mg/kg (10%). Testing, questionnaire examinations served for the assessment of comorbidity symptoms and the assessment of ADHD symptoms

Tab. 2. Controls (n=30).

Controls	DBH1021 (C-1021T)	DBH444 (G444A)	DBH910 (Ala/Ser)	DBH1603 (C1603T)	DBH1912 (C1912T)	DBHintronS (TaqI)	DRD2	DRD3	DBH (19 bp ins/del)	DAT1	5-HTT
1	1	2	1	1	2	2	1	2	2	2	3
2	1	3	1	1	2	1	2	3	2	3	3
3		3	1	1	1	1	2	3	2	3	2
4	1	2	1	1	2	2	2	2	3	2	2
5	1	2	1	1	1	2	1	3	2	3	2
6	1	1		1	1	3	1	3	3	3	1
7	1	3	1	1		1	1	3	1	3	1
8	1	2	1	1	1	2	1	2	2	2	3
9	1	2	1	1	1	2		1	2	3	2
10	1	1	1	1	1	3		1	3	2	2
11		3	1	1	2	1	1	3	2	3	1
12	1	2	2	1	1	1	1	2	2	3	2
13	1	1	1	1		2	1	3	1	3	2
14	1	1	1	1		2		2	2	3	3
15	1	1	1		1	3	1	2	2	3	1
16	1	3	1	1	1	1	1	2	1	2	3
17	1	2	1		1	2	1	3	3	3	3
18	2	1	1	1	1	1	2	3	2	3	2
19	2	2	2	1	1	2	1	3	2	2	2
20	2	2	1		1	2	1	3	2	3	3
21	2	2		1	1	2		2	2	2	3
22	1	1	1	1	1	3	1	2	3	2	2
23	1	1	1		1	3	1	3	3	3	1
24	1	1	1	1	1	3	1	1	2	2	2
25	1	2	1	1	1	2	1	1	3	3	3
26	1	1	1	1	1	3	3	3	2	2	2
27	1	2	1	1	1	2	2	3	2	2	2
28	1	1	1	1	1	3	1	3	3	2	1
29	This sample was devalued.										
30	This sample was devalued.										

– Conner's CPQ (1985) a questionnaire for parents, Czech version Paclt 1998.

Genetic examination

The detection of polymorphisms (Table 3) at both groups was made by SnapShot. At the first phase, particular DNA segments containing studied polymorphisms were amplified by multiplex PCR in thermo cycler Veriti (Applied Biosystems, USA). Consequently, particular polymorphisms were detected using oligonucleotides designed according to requirements of SnapShot method in combination with SnapShot PCR Mix (Applied Biosystems, USA). The products of SnapShot reaction will be analysed on Genetic Analyzer 3130 appliance and will be assessed in GeneMapper 3.2 programme (Applied Biosystems, USA).

Tab. 3. Characteristics of polymorphisms.

DBH1021 (C-1021T)	rs1611115	C/C = 1
proximální promotor		C/T = 2
		T/T = 3
DBH444 (G444A)	rs1108580	G/G = 1
p.Glu148Glu Exon2		G/A = 2
		A/A = 3
DBH910 (Ala/Ser)	rs4531	G/G = 1
p.Ala304Ser Exon5		G/T = 2
		T/T = 3
DBH1603 (C1603T)	rs6271	G/G = 1
p.Arg535Cys Exon11		G/A = 2
		A/A = 3
DBH1912 (C1912T)	rs129882	C/C = 1
p.Arg638Cys Exon12		C/T = 2
		T/T = 3
DBHintron5 (TaqI)	rs2519152	A/A = 1
c.1024+192T>C		A/G = 2
		G/G = 3
DRD2	rs1800497	G/G = 1
9,5 kb downstream from 3´		G/A = 2
		A/A = 3
DRD3	rs6280	C/C = 1
c.456C>T (p.Ser9Gly) Exon1		C/T = 2
		T/T = 3
DBH (19 bp ins/del)	rs?	S/S = 1
-4784-4803 promoter		S/L = 2
S = short; L = long		L/L = 3
DAT1	rs?	9/9 = 1
3´UTR repetice délka 40 bp		9/10 = 2
10 opakování / 9 opakování		10/10 = 3
5-HTT	rs?	14/14 = 1
5´- UTR 20-23 bp VNTR		14/16 = 2
14 repetic		16/16 = 3
16 repetic		

MRI Methods

Subjects were examined by the same clinical and MR protocols at 3 T whole-body MR system equipped with Tx/Rx head coil. All subjects were informed about the examination protocol and signed informed consent approved by the local ethical committee. PRESS sequences with TR/TE = 5000/30 and 135 ms were used in the left and right putamen and surrounding area in the basal ganglia with volume of interest of 11.2 ml. For the spectra quantification, segmentation to white and grey matter and CSF content was done using T1-weighted MPRage imaging sequence (TR/TE = 2300/4.43 ms, 1 9 1 9 1 mm voxel size). Metabolite concentrations were calculated using LCModel technique. Data evaluation was done using standard statistical tests. Grey and white matter surfaces were reconstructed from T1-weighted images using standard FreeSurfer image processing stream (Dezortová *et al.* 2015) and registered to mean surface template. Vertex-wise statistical evaluation of gray matter thickness with the fixed effect 'diagnosis' and the continuous factor 'age' using general linear model with cluster-wise correction for multiple comparison was performed.

RESULTS

Evaluation scale CPQ

The most striking difference between control subjects and patients with ADHD group are under headings hyperactivity and learning disorders $p<0.000$ and hyperactivity/ impulsivity $p<0.000$. Significant differences are also items for behavioral disorders and anxiety $p<0.009$ and $p<0.012$. Though long-term treatment of patients with ADHD was significantly improved, nevertheless some symptoms persisted. The above items are in the group of patients compared with standards showed statistically significant difference. But the results confirm clinical improvement in therapy for a long time (Table 4).

Grey and white matter surfaces were reconstructed from T1-weighted images using standard FreeSurfer image processing stream (Dezortová *et al.* 2015) and registered to mean surface template. Vertex-wise statistical evaluation of gray matter thickness with the fixed effect 'diagnosis' and the continuous factor 'age' using general linear model with cluster-wise correction for multiple comparison was performed.

Tab. 4. Statistical analysis of scale CPQ.

		N *	Mean	Std. Deviation	t-test	*p*-value	Mann-Whitney U	*p*-value	Cohen's d
CPQ_conduct dissorder	controls	25	1.40	1.555	-2.72	0.009	203.000	0.019	-0.77
	patients	26	3.19	2.912					
CPQ_anxiety	controls	25	1.28	2.031	-2.595	0.012	180.500	0.005	-0.73
	patients	26	3.19	3.099					
CPQ_imp/hyp	controls	25	1.04	1.306	-4.970	0.000	80.500	0.000	-0.74
	patients	27	5.22	4.013					
CPQ_learning disorders	controls	25	0.92	1.320	-4.925	0.000	99.000	0.000	-1.41
	patients	27	3.44	2.225					
CPQ_psych_pot	controls	25	1.32	1.282	0.581	0.564	257.000	0.177	
	patients	26	1.08	1.671					
CPQ_asoc_chov	controls	25	0.00	0.000	-1.934	0.059	287.500	0.028	-0.54
	patients	28	0.25	0.645					
CPQ_puntickar	controls	25	1.08	1.382	-0.944	0.350	321.500	0.749	
	patients	27	1.63	2.589					
CPQ_sval_nap	controls	25	0.12	0.332	-1.770	0.083	286.000	0.169	
	patients	27	0.56	1.188					

*Some mistakes in 30 protocols.

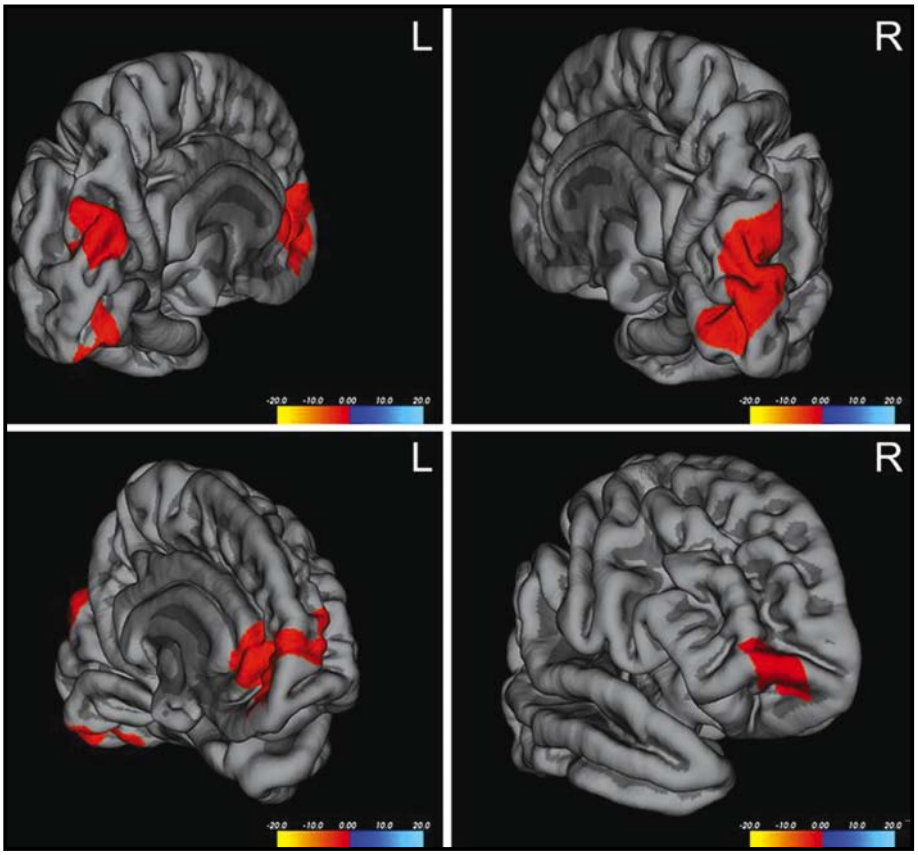

Fig. 1. Brain average surface revealing clusters with significantly higher grey matter thickness in ADHD patients.

Grey matter thickness was found to be significantly higher in patients in prefrontal and occipital areas with cluster-wise p-value<0.05.

DISCUSSION

Results of MR spectroscopy show that although the patients were treated in the long term by psychostimulants in a small member in atomoxetine. Their MRI profile in prefrontal and the occipital gray matter is not affected. Treated ADHD children have significantly thicker gray matter in the prefrontal and occipital area. We hypothesize that this finding can be a consequence of medication by mthylfenidate and atomoxetine. We didn't confirm therapy complications and the bad results in ADHD DAT 1 10/10 patients.

These are some problems of interpretation in data from non-longitudinal study.

The majority of structural MRI studies have (out of necessity) adopted a naturalistic, cross-sectional deign. This does not allow for any conclusions on cause and effect or a more informative within-subject evaluation of stimulant treatment effects.

It is necessary to monitor the number of probands, the type of long-term medication and meet the requirement of complex method of examination by imaging methods.

In our publication, we presented only some of the data from a broader research, including detailed assessments of individual parameters (for further assessment, see other scales, detailed analysis, used medication, characteristics of basal ganglia and spectroscopy focusing on the caudate nucleus and the nucleus lentiformis) compared to patients with ADHD without pharmacotherapy. Those results will be presented in some of the same authors' other papers.

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