

Association between polymorphisms of the *DBH* and *DAT1* genes and attention deficit hyperactivity disorder in children from Jordan

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Abstract. Attention deficit hyperactivity disorder (ADHD) is one of the most common neuropsychiatric disorders in children. In this study, the association between 10-repeats in the *DAT1* gene and the (GT)_n repeat in the *DBH* gene and ADHD was examined in children from Jordan. In addition, the levels of dopamine-β-hydroxylase enzyme activity in the plasma of ADHD children were evaluated. Fifty children with ADHD and 50 age- and gender-matched control subjects were recruited. The results showed significant differences between the ADHD group and controls with respect to the plasma levels of dopamine-β-hydroxylase enzyme activity (25.4 ± 2.3 vs. 84.7 ± 5.0 μmol/min; $p < 0.01$). Moreover, the 10-repeat *DAT1* gene and (GT)_n *DBH* gene polymorphisms were significantly associated with ADHD development ($p < 0.05$). In conclusion, the *DBH* and *DAT1* genes appear to play a role in the development of ADHD in the Jordanian population.

Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most common neuropsychiatric and behavioral childhood disorders (1), affecting approximately 12% of school-aged children, with a higher prevalence in boys than girls (2). The disorder is characterized by reduced attention and hyperactivity (3-5); ADHD children are disorganized and have trouble fulfilling plans and completing tasks (6). The symptoms have been attributed, mainly, to lower norepinephrine and dopamine activities. Research has indicated a malfunction in dopamine-β-hydroxylase (DBH), which is responsible for maintaining the balance between dopamine and norepinephrine concentrations (2,7-11).

Recent data suggest that various polymorphisms of the dopamine-related genes are related to ADHD. Studies involving children from the US, Czech Republic, Finland, Brazil and India demonstrated a correlation between *DBH* gene polymorphisms and increased susceptibility to ADHD (1,10-14). Moreover, studies from the US, Taiwan, UK and China revealed strong associations between ADHD and polymorphisms of the dopamine transporter gene (*DAT1*) (12,15,16). Despite accumulating evidence, no studies have examined these relationships in Arab/Middle Eastern populations. Therefore, in this study the plasma levels of DBH enzyme activity were evaluated in ADHD children from Jordan. In addition, the association between polymorphisms of the *DAT1* and *DBH* genes and ADHD was examined.

Materials and methods

Subjects. Fifty children with ADHD (2.5-14 years of age) were recruited from the King Abdullah University Hospital and the Princess Rahmah Teaching Hospital. The children were diagnosed with ADHD according to the Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) criteria by a pediatric neurologist. Fifty healthy children, matched for age and gender, were included in the study as controls. The control children attended the same hospitals for reasons other than neuropsychiatric disorders. Written informed consent was obtained from the participants' guardians according to the Jordan University of Science and Technology Institutional Review Board.

DNA extraction. DNA was extracted from EDTA blood samples obtained from the participants using the Wizard DNA Extraction kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The concentration of the extracted DNA was measured using SmartSpect™3000 (Bio-Rad, Hertfordshire, UK). DNA samples were stored at -20°C until use.

Genotyping of the *DAT1* polymorphism. The 40-bp variable number tandem repeat (VNTR) of the *DAT1* 3'-untranslated region was analyzed using polymerase chain reaction (PCR) as previously described (17). Detection of various repeats was

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Table I. Distribution of the genotypes of the *DAT1* polymorphism.

Genotype	Patients n (%)	Control n (%)
6/6	1 (2)	0 (0)
6/7	2 (4)	0 (0)
7/7	3 (6)	3 (6)
7/9	2 (4)	10 (20)
7/10	0 (0)	2 (4)
9/9	7 (14)	17 (34)
9/10	17 (34)	14 (28)
9/11	1 (2)	0 (0)
10/10	16 (32)	4 (8)
10/11	1 (2)	0 (0)

p<0.001, based on the Fisher's exact test.

performed using 12% polyacrylamide gel and proper DNA ladders.

Genotyping of the *DBH* polymorphism. The *DBH* dinucleotide repeat was performed using previously described primers and PCR conditions (18). The PCR products were digested with *HaeIII* at 37°C for 2 h. The digested PCR products were analyzed using 12% polyacrylamide gels, and the sizes of the dinucleotide repeat were measured using proper DNA ladders (19).

Determination of dopamine- β -hydroxylase activity. The activity of the *DBH* enzyme was measured using the patch adsorption method according to the protocol used by Nagatsu and Udenfriend (20). In brief, 50 μ l of serum was added to 350 μ l of distilled water, and the diluted serum was added to 600 μ l of a reagent mixture [200 μ l of sodium acetate buffer (1 mol/l, pH 5.0), 50 μ l of sodium fumarate (0.2 mol/l), 50 μ l of ascorbic acid (0.2 mol/l), 50 μ l of catalase (1 mg/ml), 50 μ l of tyramine (0.4 mol/l), 50 μ l of pargyline (20 mmol/l) and 150 μ l of N-ethylmaleimide (0.2 mol/l)]. As a blank, 50 μ l of serum was diluted to 400 μ l by distilled water, boiled at 95°C for 5 min and then added to 600 μ l of the reagent mixture described above. The reaction mixtures were incubated at 37°C for 60 min with continuous shaking. At the end of the incubation period, the reactions were stopped by adding 200 μ l of trichloroacetic acid (3 molar), and the mixtures were centrifuged at 2,000 x g for 10 min. The supernatant was transferred to a new tube containing 200 μ l of Dowex-50 (H⁺, 200-400 mesh), which was fully equilibrated with acetate buffer. The precipitants were then washed three times with distilled water coupled with centrifugation at 2,000 x g for 5 min each. After the last wash, 1 ml of NH₄OH (4 molar) was added to elute the adsorbed amines. The mixtures were then centrifuged at 2,000 x g for 5 min, and the supernatants were transferred to new tubes. Following this, 100 μ l of NaIO₄ (200 g/l) was added to the tube to convert the octopamine in the supernatant to para-hydroxybenzaldehyde. Finally, 100 μ l of Na₂S₂O₅ (10 g/l) was added to reduce the excess periodate. Absorbance was then measured at 330 nm using microcu-

Table II. Frequency of the *DAT1* alleles.

Alleles	Patients (n)	Controls (n)
DAT6	4	0
DAT7	10	18
DAT9	34	58
DAT10	50	24
DAT11	2	0

p<0.05, based on the Fisher's exact test.

Table III. Distribution of the genotypes of the (GT)_n *DBH* polymorphism in the studied population.

Genotype	Patients n (%)	Controls n (%)
A2/A3	29 (58)	9 (18)
A2/A4	1 (2)	28 (56)
A3/A4	0 (0)	6 (12)
A3/A6	0 (0)	1 (2)
A4/A6	20 (40)	6 (12)

p<0.05, based on the Fisher's exact test.

Table VI. Allelic frequencies of the (GT)_n *DBH* polymorphism in the studied population.

Genotype	Patients (n)	Controls (n)
A2	30	37
A3	29	16
A4	21	40
A6	20	7

p<0.001.

vets with a 1-cm light path. The activity of the enzyme was expressed in terms of μ mol/min; the level of *DBH* enzyme.

Statistical analysis. Statistical evaluation of the results was carried out by comparing allele, genotype and predicted phenotype distributions using the χ^2 test. If n<5, the Fisher's exact test was used. The SPSS 15.0 statistical software package (SPSS Inc., Chicago, IL, USA) was used for all statistical evaluations. Comparisons that involved two groups were carried out using the Student's t-test. A p-value of <0.05 was considered significant.

Results

The sample ratio of boys to girls was 4:1. The median age was 7.1 \pm 1.2 years for the ADHD subjects and 8 \pm 1.4 for the control group (p>0.05).

Table V. DBH enzyme activity in the blood levels of the study subjects.

Dopamine- β -hydroxylase	Patients (n=50)	Controls (n=50)	P-value
Activity (mean $\mu\text{mol/min}$)	25.0 \pm 2.3	84.6 \pm 5.0	<0.01
Description of activity			<0.01
High (>50 $\mu\text{mol/min}$)	4	43	
Low (<50 $\mu\text{mol/min}$)	46	7	

According to the distribution of the *DAT1* polymorphism genotypes shown in Table I, 9/9 and 9/10 were most common in the controls (34 and 28%, respectively). However, the most common genotypes in the ADHD group were 9/10 and 10/10 repeats (34 and 32%, respectively). Additionally, Table II shows that the 9-repeat allele (58%) was common in the controls, while the 10-repeat allele was common in the ADHD group (50%, $p<0.05$).

As shown in Table III, the frequency of (GT) n DBH polymorphism A2/A3 and A4/A6 genotypes was higher in the ADHD children (58 and 40%, respectively), while the frequency of the A2/A4 genotype was higher in the controls (56%, $p<0.05$). In agreement with the genotype distribution, Table IV shows that the A3 and A6 allelic frequencies were higher in the ADHD group, while the frequency of the A4 allele was higher in the control group ($p<0.001$).

The result also revealed a significant decrease ($p<0.01$) in the plasma DBH activity in the ADHD (25.412 \pm 2.32 $\mu\text{mol/min}$) compared to the control (84.689 \pm 5.01 $\mu\text{mol/min}$) children (Table V). Moreover, the percentage of children with abnormally low activity was higher ($p<0.01$, Table V) in the ADHD vs. the control group.

Discussion

The present study revealed that the frequency of the (GT) n repeat 5' in the *DBH* gene was higher in the ADHD group among Jordanian children. In addition, a significantly lower level of *DBH* activity was detected in ADHD children compared to the controls.

Previous studies have revealed that an imbalance between the dopaminergic and noradrenergic systems is implicated in the development of ADHD in children (10,16,21). The two systems are connected by the activity of the DBH enzyme, which is expressed in the central and peripheral nervous systems and catalyzes the conversion of dopamine to norepinephrine (22). Genetic variations in the *DBH* gene suggest that a lower enzyme activity increases the susceptibility of developing ADHD in children. Several studies have shown an increased *TaqI* A polymorphism in children with ADHD (12,14,23). In addition, several single-nucleotide polymorphisms in the *DBH* gene have been implicated in ADHD etiology (21). Moreover, the *DBH* (GT) n repeat polymorphism was found to contribute to the development of ADHD in children from the US (23). In the the present study, the *DBH* (GT) n polymorphism was associated with ADHD children, supporting the hypothesis that *DBH* plays a key role in the ADHD trait. Thus, similar to other populations, genetic varia-

tions in the *DBH* gene may play a role in the development of ADHD in Jordanian children.

Another gene that plays a key role in regulating the dopaminergic system is *DAT1*, which codes for a dopamine transporter expressed in pre-synaptic neurons (24). Previous studies have implicated excess dopamine clearance in the synapses of ADHD as part of the etiology of the disease (25). In addition, positron emission tomography imaging indicated dysregulation of *DAT1* in the striatum of subjects with ADHD (26,27). The present results indicate that VNTR in the 3' untranslated region of *DAT1* is associated with ADHD with a high frequency of the 10R allele in affected children. In agreement with this result, several studies (18,28) have found that homozygosity for the 10R allele of the *DAT1* gene was significantly greater in ADHD children from the US, Ireland and Czech Republic (12,29,30). In addition, a meta-analysis found a significant association between the 10R allele and ADHD development (31,32). Thus, similar to other populations, VNTR in the 3' untranslated region of *DAT1* may play a role in the etiology of ADHD in children from Jordan.

In support of the genetic analysis, DBH enzyme activity was significantly different in the ADHD group compared to the controls. The plasma DBH level can be used as a marker to reflect sympathetic noradrenergic activity, since the enzyme is released into the blood stream during synaptic transmission (33). Although plasma DBH enzyme activity varies among unrelated individuals (18), it is generally accepted that children with ADHD have decreased plasma and urine DBH activities (10,19,34).

As is the case in most association studies, reported negative and positive associations with a given genetic variant are common. In the examined polymorphisms, lack of association between ADHD and the *DBH* (GT) n repeat was reported in Indians (35). Similarly, the absence of an association between ADHD and VNTR of the *DAT1* 3'-untranslated region was observed in studies from Brazil and Norway (1,17,23,36-38). The discrepancy may be due to the complexity of ADHD, the differences in the genetic background of the studied populations, and the impact of environmental factors.

Previous studies have demonstrated that certain environmental factors, including maternal smoking or drinking habits during pregnancy, low birth weight, maternal age, stress and/or exposure to heavy metals such as zinc and mercury contribute to the development of ADHD (6,39). However, the contribution of environmental factors to the development of ADHD in Jordanian children was not examined in the present study; it will be investigated in future research.

Notably, DBH enzyme activity has been shown to change in individuals with certain diseases such as schizophrenia, Tourette syndrome, familial dysautonomia and orthostatic hypotension (40). Similarly, polymorphisms of *DAT1* are associated with bipolar disorder, Parkinson disease and drug abuse (41,42). Therefore, future studies should examine common variants of the *DBH* and *DAT1* genes in Jordanians.

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References

- Cheuk DK and Wong V: Attention-deficit hyperactivity disorder and blood mercury level: a case-control study in Chinese children. *Neuropediatrics* 37: 234-240, 2006.
- Sheehan K, Hawi Z, Gill M and Kent L: No association between TPH2 gene polymorphisms and ADHD in a UK sample. *Neurosci Lett* 412: 105-107, 2007.
- Gornick MC, Addington A, Shaw P, *et al*: Association of the dopamine receptor D4 (DRD4) gene 7-repeat allele with children with attention-deficit/hyperactivity disorder (ADHD): an update. *Am J Med Genet B Neuropsychiatr Genet* 144B: 379-382, 2007.
- Thapar A, O'Donovan M and Owen MJ: The genetics of attention deficit hyperactivity disorder. *Hum Mol Genet* 14: R275-R282, 2005.
- Tsai SJ: Attention-deficit hyperactivity disorder may be associated with decreased central brain-derived neurotrophic factor activity: clinical and therapeutic implications. *Med Hypotheses* 68: 896-899, 2007.
- Rowland AS, Lesesne CA and Abramowitz AJ: The epidemiology of attention-deficit/hyperactivity disorder (ADHD): a public health view. *Ment Retard Dev Disabil Res Rev* 8: 162-170, 2002.
- Gizer IR, Ficks C and Waldman ID: Candidate gene studies of ADHD: a meta-analytic review. *Hum Genet* 126: 51-90, 2009.
- Guan L, Wang B, Chen Y, *et al*: A high-density single-nucleotide polymorphism screen of 23 candidate genes in attention deficit hyperactivity disorder: suggesting multiple susceptibility genes among Chinese Han population. *Mol Psychiatry* 14: 546-554, 2009.
- Hesse S, Ballaschke O, Barthel H and Sabri O: Dopamine transporter imaging in adult patients with attention-deficit/hyperactivity disorder. *Psychiatry Res* 171: 120-128, 2009.
- Kopeckova M, Paclt I, Petrask J, Pacltova D, Malikova M and Zagatova V: Some ADHD polymorphisms (in genes *DAT1*, *DRD2*, *DRD3*, *DBH*, 5-HTT) in case-control study of 100 subjects 6-10 age. *Neuro Endocrinol Lett* 29: 246-251, 2008.
- Nyman ES, Ogdie MN, Loukola A, *et al*: ADHD candidate gene study in a population-based birth cohort: association with *DBH* and *DRD2*. *J Am Acad Child Adolesc Psychiatry* 46: 1614-1621, 2007.
- Barkley RA, Smith KM, Fischer M and 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. *Am J Med Genet B Neuropsychiatr Genet* 141B: 487-498, 2006.
- Kopeckova M, Paclt I and Goetz P: Polymorphisms and low plasma activity of dopamine-beta-hydroxylase in ADHD children. *Neuro Endocrinol Lett* 27: 748-754, 2006.
- Roman T, Schmitz M, Polanczyk GV, Eizirik M, Rohde LA and Hutz MH: Further evidence for the association between attention-deficit/hyperactivity disorder and the dopamine-beta-hydroxylase gene. *Am J Med Genet* 114: 154-158, 2002.
- Brookes KJ, Mill J, Guindalini C, *et al*: A common haplotype of the dopamine transporter gene associated with attention-deficit/hyperactivity disorder and interacting with maternal use of alcohol during pregnancy. *Arch Gen Psychiatry* 63: 74-81, 2006.
- Krause J: SPECT and PET of the dopamine transporter in attention-deficit/hyperactivity disorder. *Expert Rev Neurother* 8: 611-625, 2008.
- Qian Q, Wang Y, Zhou R, Yang L and Faraone SV: Family-based and case-control association studies of *DRD4* and *DAT1* polymorphisms in Chinese attention deficit hyperactivity disorder patients suggest long repeats contribute to genetic risk for the disorder. *Am J Med Genet B Neuropsychiatr Genet* 128B: 84-89, 2004.
- Wei J, Xu HM, Ramchand CN and Hemmings GP: Is the polymorphic microsatellite repeat of the dopamine beta-hydroxylase gene associated with biochemical variability of the catecholamine pathway in schizophrenia? *Biol Psychiatry* 41: 762-767, 1997.
- Galvin M, Ten Eyck R, Shekhar A, *et al*: Serum dopamine beta hydroxylase and maltreatment in psychiatrically hospitalized boys. *Child Abuse Negl* 19: 821-832, 1995.
- Nagatsu T and Udenfriend S: Photometric assay of dopamine - hydroxylase activity in human blood. *Clin Chem* 18: 980-983, 1972.
- Kopeckova M, Paclt I and Goetz P: Polymorphisms of dopamine-beta-hydroxylase in ADHD children. *Folia Biol* 52: 194-201, 2006.
- Hartman BK: Immunofluorescence of dopamine-hydroxylase. Application of improved methodology to the localization of the peripheral and central noradrenergic nervous system. *J Histochem Cytochem* 21: 312-332, 1973.
- Smith KM, Daly M, Fischer M, *et al*: Association of the dopamine beta hydroxylase gene with attention deficit hyperactivity disorder: genetic analysis of the Milwaukee longitudinal study. *Am J Med Genet B Neuropsychiatr Genet* 119B: 77-85, 2003.
- Parsian A and Zhang ZH: Human dopamine transporter gene polymorphism (VNTR) and alcoholism. *Am J Med Genet* 74: 480-482, 1997.
- Prince J: Catecholamine dysfunction in attention-deficit/hyperactivity disorder: an update. *J Clin Psychopharmacol* 28: S39-S45, 2008.
- Jucaite A, Fernell E, Halldin C, Forsberg H and Farde L: Reduced midbrain dopamine transporter binding in male adolescents with attention-deficit/hyperactivity disorder: association between striatal dopamine markers and motor hyperactivity. *Biol Psychiatry* 57: 229-238, 2005.
- Spencer TJ, Biederman J, Madras BK, *et al*: Further evidence of dopamine transporter dysregulation in ADHD: a controlled PET imaging study using altoprane. *Biol Psychiatry* 62: 1059-1061, 2007.
- Cook EH Jr, Stein MA, Krasowski MD, *et al*: Association of attention-deficit disorder and the dopamine transporter gene. *Am J Hum Genet* 56: 993-998, 1995.
- Drtilkova I, Sery O, Theiner P, *et al*: Clinical and molecular-genetic markers of ADHD in children. *Neuro Endocrinol Lett* 29: 320-327, 2008.
- Hawi Z, Segurado R, Conroy J, *et al*: Preferential transmission of paternal alleles at risk genes in attention-deficit/hyperactivity disorder. *Am J Hum Genet* 77: 958-965, 2005.
- Faraone SV and Khan SA: Candidate gene studies of attention-deficit/hyperactivity disorder. *J Clin Psychiatry* 67 (Suppl 8): 13-20, 2006.
- Yang XM, Wang SH, Yang YD, *et al*: A dopaminergic projection from the dorsal raphe nucleus to the inner ear. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 41: 857-860, 2006.
- O'Connor DT, Levine GL and Frigon RP: Homologous radio-immunoassay of human plasma dopamine-beta-hydroxylase: analysis of homospecific activity, circulating plasma pool and intergroup differences based on race, blood pressure and cardiac function. *J Hypertens* 1: 227-233, 1983.
- Galvin MR, Stilwell BM, Shekhar A, Kopta SM and Goldfarb SM: Maltreatment, conscience functioning and dopamine beta hydroxylase in emotionally disturbed boys. *Child Abuse Negl* 21: 83-92, 1997.
- Bhaduri N, Sarkar K, Sinha S, Chattopadhyay A and Mukhopadhyay K: Study on *DBH* genetic polymorphisms and plasma activity in attention deficit hyperactivity disorder patients from Eastern India. *Cell Mol Neurobiol* 30: 265-274, 2010.
- Genro JP, Polanczyk GV, Zeni C, *et al*: A common haplotype at the dopamine transporter gene 5' region is associated with attention-deficit/hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 147B: 1568-1575, 2008.
- Johansson S, Halleland H, Halmoy A, *et al*: Genetic analyses of dopamine related genes in adult ADHD patients suggest an association with the *DRD5*-microsatellite repeat, but not with *DRD4* or *SLC6A3* VNTRs. *Am J Med Genet B Neuropsychiatr Genet* 147B: 1470-1475, 2008.

38. Langley K, Turic D, Peirce TR, *et al*: No support for association between the dopamine transporter (DAT1) gene and ADHD. *Am J Med Genet B Neuropsychiatr Genet* 139B: 7-10, 2005.
39. Swanson JM, Kinsbourne M, Nigg J, *et al*: Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychol Rev* 17: 39-59, 2007.
40. Yoon DY, Rippel CA, Kobets AJ, *et al*: Dopaminergic polymorphisms in Tourette syndrome: association with the DAT gene (SLC6A3). *Am J Med Genet B Neuropsychiatr Genet* 144B: 605-610, 2007.
41. Bannon MJ: The dopamine transporter: role in neurotoxicity and human disease. *Toxicol Appl Pharmacol* 204: 355-360, 2005.
42. Kurian MA, Zhen J, Cheng SY, *et al*: Homozygous loss-of-function mutations in the gene encoding the dopamine transporter are associated with infantile parkinsonism-dystonia. *J Clin Invest* 119: 1595-1603, 2009.