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Functional Genomics of Attention-Deficit/ Hyperactivity Disorder (ADHD) Risk Alleles on Dopamine Transporter Binding in ADHD and Healthy Control Subjects

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Abstract

Background—The main aim of this study was to examine the relationship between dopamine transporter (DAT) binding in the striatum in individuals with and without attention-deficit/hyperactivity disorder (ADHD), attending to the 3′-untranslated region of the gene (3′-UTR) and intron8 variable number of tandem repeats (VNTR) polymorphisms of the DAT (*SLC6A3*) gene.

Methods—Subjects consisted of 68 psychotropic (including stimulant)-naïve and smoking-naïve volunteers between 18 and 55 years of age (ADHD $n = 34$; control subjects $n = 34$). Striatal DAT binding was measured with positron emission tomography with 11C altropine. Genotyping of the two DAT (*SLC6A3*) 3′-UTR and intron8 VNTRs used standard protocols.

Results—The gene frequencies of each of the gene polymorphisms assessed did not differ between the ADHD and control groups. The ADHD status ($t = 2.99$; $p < .004$) and 3′-UTR of *SLC6A3* 9 repeat carrier status ($t = 2.74$; $p < .008$) were independently and additively associated with increased DAT binding in the caudate. The ADHD status was associated with increased striatal (caudate) DAT binding regardless of 3′-UTR genotype, and 3′-UTR genotype was associated with increased striatal (caudate) DAT binding regardless of ADHD status. In contrast, there were no significant associations between polymorphisms of DAT intron8 or the 3′-UTR-intron8 haplotype with DAT binding.

Conclusions—The 3′-UTR but not intron8 VNTR genotypes were associated with increased DAT binding in both ADHD patients and healthy control subjects. Both ADHD status and the 3′-UTR polymorphism status had an additive effect on DAT binding. Our findings suggest that an

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AJF, AAB, HB, and AC report no biomedical financial interests or potential conflicts of interest.

BKM has the following financial interests: patent holder on 11C-131I-altropine, and other DAT imaging agents; Alseres licensed Altropine from Harvard University; Navidea Biopharmaceuticals, a radiopharmaceutical developer, is evaluating an option to license Altropine from Alseres; consultant for Prexa Pharmaceuticals; and patent holder on numerous dopamine transporter inhibitors.

ClinicalTrials.gov: An Open Label Phase I/II Study of the Safety and Dopamine Transporter Binding Properties of C-11 Altropine in Normal Human Subjects and in Subjects With ADHD; <http://clinicaltrials.gov/ct2/show/NCT00302380?term=NCT00302380&rank=1>; NCT00302380.

ADHD risk polymorphism (3'-UTR) of *SLC6A3* has functional consequences on central nervous system DAT binding in humans.

Keywords

ADHD; altropane; dopamine; dopamine transporter; genetics; PET imaging

Attention-deficit/hyperactivity disorder (ADHD) has long been hypothesized to be associated with impaired dopaminergic neurotransmission (1). One potential source of dopaminergic dysregulation has been thought to be impaired dopamine transporter (DAT) functioning, because the DAT is a key regulator of the dopamine (DA) system (2). We (3,4) and others (5–12) have documented (on average) over-activity (13) of the DAT in humans with positron emission tomography (PET) with the highly selective DAT ligand C11-altropane.

Although the etiology of alterations in DAT activity is not entirely clear, mutations of the DAT gene (*SLC6A3*) could affect DAT expression and lead to dysregulated DAT and impaired dopaminergic neurotransmission. In animal models, mutations of the DAT gene (*SLC6A3*) have been associated with increased striatal DAT activity (14–16), and abnormal striatal DAT function has been associated with decreased dopaminergic tone, hyperactivity, and deficits in inhibitory behavior (17–19).

In humans, two variable number of tandem repeat (VNTR) *SLC6A3* polymorphisms have been studied. One of these *SLC6A3* VNTR polymorphisms is a 40-base pair (bp) VNTR located in the 3'-untranslated region of the gene (3'-UTR), which produces two common alleles with 9- and 10-repeats (9R and 10R). An intron8 VNTR polymorphism of *SLC6A3* produces two common alleles comprising 5- and 6-repeats of 30 base pairs. These two VNTR polymorphisms have been studied individually and in tandem as a 3'-UTR-intron8 haplotype. Although in humans these 3'-UTR and intron8 VNTR polymorphisms as well as the 3'-UTR-intron8 haplotype of *SLC6A3* have been associated with ADHD (20), the precise effects of these polymorphisms remain unknown. In vitro, the 3'-UTR of sequence-defined DAT alleles differentially affected the level of reporter gene expression in HEK-293 cells (transfected with two different luciferase expression vectors or reporters). The human 9 tandem repeat sequence of the DAT 3'-UTR yielded higher luciferase activity than the 10R sequence, irrespective of the promoter. These findings provided robust evidence that the 9R length in the 3'-UTR of the DAT gene conceivably is associated with increased DAT protein in human brain compared with the 10R sequence and that variability in the length or the sequence of the 3'-UTR of the DAT gene influences DAT protein expression in the brain (21). The findings further showed that not only length but single nucleotide variations in DAT 3'-UTR segments modify reporter gene expression and might contribute to the diversity of DAT expression between individuals.

Advances in PET technology and the availability of a highly selective ligand for the DAT, C11-altropane, now allow for the imaging of the DAT in vivo in humans, facilitating the direct in vivo examination of the product of the DAT gene and its polymorphisms in humans.

A number of studies have examined the relationship of the *SLC6A3* gene and striatal DAT activity in humans (22). Two recent studies were conducted in healthy volunteers and reported significant relationships between the 3'-UTR and the intron8 VNTR polymorphisms as well as the combined 3'-UTR-intron8 haplotype of *SLC6A3* and altered DAT availability in the striatum (23,24). Two other studies in subjects with ADHD (one pediatric [25], and one adult [26]) reported inconsistent results with the 3'-UTR

polymorphism but did not examine the 3'-UTR-intron8 haplotype. There is a need to further investigate this polymorphism in PET studies of subjects with ADHD, considering that this 3'-UTR-intron8 haplotype of *SLC6A3* has been suggested as particularly relevant for the pathophysiology of ADHD (27,28).

To this end, the main aim of this study was to use PET neuroimaging of DAT to examine the relationship between central DAT activity in the striatum in individuals with ADHD attending to the DAT1 3'-UTR and intron8 polymorphisms as well as the 3'-UTR-intron8 haplotype. We hypothesized, on the basis of previous work and theoretical considerations, that there would be a selective and significant association between DAT1 3'-UTR and intron8 polymorphisms as well as the 3'-UTR-intron8 haplotype and DAT activity in the striatum in individuals with ADHD. Because other genetic polymorphisms associated with dopaminergic function have also been linked to ADHD, we also examined their role on affecting DAT functioning. To the best of our knowledge, this is the most comprehensive evaluation of the effects of *SLC6A3* gene polymorphisms on striatal DAT binding in an ADHD sample.

Methods and Materials

Subjects

Subjects consisted of 68 volunteers between 18 and 55 years of age with and without ADHD. The subjects with ADHD satisfied full diagnostic criteria for DSM-IV ADHD (see details in the following text). Control subjects could not have more than two mild symptoms of ADHD and no first degree relative with ADHD. We excluded subjects who were smokers or had other clinically significant chronic medical or current psychiatric conditions, histories of seizure disorder or head injury, abnormal baseline laboratory values, IQ <80, drug or alcohol abuse or dependence, or prior or current treatment with psychotropic medications (including stimulants). All subjects were right-handed. All subjects had an electrocardiogram, full blood count, blood chemistries, and a urinalysis (including drug screen and, in women, a pregnancy test). This study was approved by the institutional review board, and all subjects signed a written informed consent before inclusion in the study.

Assessment

To assess inclusion and exclusion criteria, subjects underwent a comprehensive clinical assessment that included a psychiatric evaluation by a board certified psychiatrist with expertise in adult ADHD, a structured diagnostic interview, medical history, and vital signs. The structured diagnostic interview used was the Structured Clinical Interview for DSM-IV (29), supplemented for childhood disorders by modules (DSM-IV ADHD and conduct disorder) from the Kiddie Schedule for Affective Disorder and Schizophrenia for School-Age Children (Epidemiologic Version) (30). To have been given a full diagnosis of adult ADHD, the subject must have: 1) met full DSM-IV-R criteria (at least 6 of 9 symptoms) for inattentive and/or hyperactive/impulsive domains by the age of 7 years as well as within the past month; 2) described a chronic course of ADHD symptomatology from childhood to adulthood; and 3) endorsed currently at least moderate level of impairment attributed to the ADHD symptoms.

Procedures

PET Imaging—Images were acquired with a HR+ (CTI, Knoxville, Tennessee) PET camera. The primary imaging parameters of the HR+ camera are in-plane and axial resolution of 4.5 mm full-width-at-half-maximal, 63 contiguous slices of 2.42 mm separation. Images were acquired in three-dimensional mode and reconstructed with the

filtered back projection (FBP) algorithm with a ramp filter of 4.00 mm. Photon attenuation measurements were made with rotating pin sources containing ^{68}Ge and used to correct the emission data.

C-11 altropane is a highly suitable ligand for DAT imaging, because it has relatively high DAT affinity (K_m : 12 nmol/L), high DAT selectivity, and low nonspecific binding (31). For each scan, approximately 5 mCi of C-11 altropane was injected intravenously over 30 sec, and serial PET images were acquired. Dynamic image collection started at the same time as the infusion, and images were acquired in 15-sec frames for the first 2.0 min, in 1-min frames for the next 4.0 min, and in 2-min frames for the last 27 frames, 60 min in all. All projection data were corrected for nonuniformity of detector response, dead time, random coincidences, and scattered radiation. Regions of interest representing the striatum (left, right caudate nucleus and left, right putamen) and cerebellum were drawn manually on the PET images. This procedure was repeated for all slices in which the structures were visualized at full intensity (away from edge slices). For each frame, regions of interest of like structures were averaged to yield average striatal and cerebellar time activity curves. Time activity curves from the regions with DAT bindings were fitted to a simplified reference region model (32) to calculate binding potential for each region.

Genotyping

Genotyping of the *SLC6A4* (serotonin [5-HTT]) intron and promoter (5HTTLPR) polymorphisms and *DRD4* VNTRs was performed with the following protocol. Genomic DNA (5 ng) was amplified in a 7- μL reaction with KlenTaq DNA Polymerase (.2 U), the proprietary KlenTaq Buffer (1 $\times$), deoxyribonucleotide triphosphates (200 $\mu\text{mol/L}$ each), glycerol (5% for *SLC6A4*, and 10% for *DRD4*), Betaine (1 mol/L), and the marker specific primers (.2 $\mu\text{mol/L}$). The *SLC6A4* intron and *DRD4* VNTR primers were as follows: *SLC6A4*_IN02-F VIC-GTCAGTATCACAGGCTGCGAG, *SLC6A4*_IN02-R TGTTCCTAGTCTTACGCCAGTG, *DRD4*-EX03B-F VIC-GACCGCGACTACGTGGTCTACTC, *DRD4*-EX03B-R CTCAGGA-CAGGAACCCACCGAC. The *SLC6A4*_IN02-R and *DRD4*-EX03B-R primer also contains a proprietary tail that helps stabilize the amplified product. The *SLC6A4* promoter VNTR primers were as follows: *SLC6A4*_PRO-F 6FAM-ATGCCAGCACCTAACCCTAA TGT, *SLC6A4*_PRO-R GGACCGCAAGGTGGGCGGGA. Amplification was performed with the following protocol: 13 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec beginning at 61.5°C for the *SLC6A4* markers and 69.5°C for the *DRD4* marker, and dropped .5°C every cycle and primer extension at 72°C for 30 sec; 37 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec at 55°C for the *SLC6A4* markers and 63°C for the *DRD4* marker, and primer extension at 72°C for 30 sec; 72°C for 1 hour.

Genotyping of the two *DAT1* VNTRs, referred to as DAT1 (*DAT1* 3'-UTR) and DAT30 (*DAT1* intron8), used the following protocol. Genomic DNA (5 ng) was amplified in a 7- μL reaction with HotStarTaq DNA Polymerase (.2 U), the proprietary HotStar-Taq Buffer (1 $\times$), deoxyribonucleotide triphosphates (200 $\mu\text{mol/L}$ each), and the marker specific primers (.2 $\mu\text{mol/L}$). The DAT30 reaction also contained an additional 1 mmol/L of magnesium. Primers were as follows: DAT1-F 6FAM- TGTGGTGTAGGGAACGG CCTGAG, DAT1-R CCTCCTGGAGGTCACGGCTCAAGG, DAT30-F 6FAM-GCTTGGGAAGGAAGGG, DAT30-R TGTGTGCGTGCATGTGG. The DAT1-R and DAT30-R primers also contain the proprietary tail. Amplification for DAT1 was performed as follows. Samples were heated at 92°C for 9 min to activate the HotStarTaq Polymerase. This is followed by 12 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec beginning at 64.5°C and dropped .5°C every cycle and primer extension at 72°C for 30 sec; 37 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec at 58°C and primer

extension at 72°C for 30; 72°C for 1 hour. The DAT30 conditions were the same with the exception that the denaturation temperatures were 4°C higher.

Amplified products were pooled and combined with size standards (LIZ-250) before being analyzed on an ABI-3730 (Applied Biosystems, Foster City, California). GeneMapper v3.5 (Applied Biosystems) was used to analyze the raw results from the ABI3730; however, a genotype was not considered final until two laboratory personnel had independently checked (and corrected) the GeneMapper results and both individuals were in agreement.

Statistical Analysis

The DAT binding was compared in subjects with (i.e., at least one copy) and without (i.e., zero copies) putative risk alleles (DRD4 7-repeat allele, DAT1 3'-UTR 9R allele, DAT1 intron8 6-repeat allele, STin2 12-repeat allele, and 5-HTTLPR long allele). The risk alleles (i.e., number of VNTR repeats) were selected on the basis of the results of prior meta-analyses (20).

Categorical data were analyzed by χ^2 analysis; continuous parametric data were analyzed by analysis of variance with post hoc pairwise comparisons (Scheffe) or unpaired *t* test; and the rank sum test was used for nonparametric data. Associations between continuous variables were evaluated with Pearson correlations. We chose a significance level of .05. All tests were two-tailed.

Results

Sociodemographic Characteristics of the Sample

As shown in Table 1, ADHD participants had a higher percentage of men and were somewhat older than control subjects. In our sample DAT binding decreased 7%/decade. Thus, we controlled for age and sex in all subsequent analyses. Of the ADHD subjects, 62% ($n = 21$ of 34) were combined type, 35% ($n = 12$ of 34) were inattentive subtype, and 3% ($n = 1$ of 34) were hyperactive subtype.

Genotype and ADHD Status

As shown in Table 2, the gene frequencies of each of the gene polymorphisms assessed did not differ between the ADHD and control groups. In addition, gene frequencies were in Hardy Weinberg equilibrium.

ADHD, Genes, and DAT Binding

As depicted in Table 3, both ADHD status and 3'-UTR (9R) carrier status of *SLC6A3* (but not intron8 or the 3'-UTR-intron8 haplotype) were independently and additively associated with increased striatal binding in the caudate. In other words, ADHD status was associated with increased striatal (caudate) DAT binding (effect size .58) regardless of 3'-UTR polymorphism, and 3'-UTR (9R) carrier status was associated with increased striatal (caudate) DAT binding (effect size .63) regardless of ADHD status. In contrast, there were no significant associations between polymorphisms of the *DRD4* and *5-HTT* genes with DAT binding. The associations of ADHD subtype and number of inattentive and hyperactive symptoms to DAT binding in the caudate were not statistically significant.

Discussion

We examined the relationship of DAT1 3'-UTR and intron8 polymorphisms and the 3'-UTR-intron8 haplotype of the DAT gene (*SLC6A3*) to DAT expression as measured by PET with 11C altropane binding in adults with ADHD and healthy control subjects. We did not

find, in contrast to the study hypothesis, an association between the haplotype of 3'-UTR-intron8 haplotype and DAT binding. Instead, we found that only the 3'-UTR polymorphism was associated with DAT binding levels in both ADHD and healthy control subjects. The findings parallel the discovery that polymorphisms of variable length in the DAT 3'-UTR affected levels of reporter gene expression in vitro. With either an HSV-TK or SV40 promoter, vectors containing the 9R sequence yielded higher levels of luciferase gene expression than vectors containing the 10R sequence (21). It is possible that the direction and the magnitude of luciferase levels observed in the immortalized cell line HEK-293 cells conceivably are influenced by cell-specific proteins interacting with the 3'-UTR sequences and not reflect physiological regulation of the DAT gene in brain dopamine neurons. Nonetheless, the parallel observations made with in vivo imaging and in vitro analyses on reporter gene expression suggest that polymorphisms of length in the 3'-UTR of the DAT gene might contribute to the dynamic processes that regulate DAT density in the brain. A number of possible mechanisms, including modulation of the promoter region or RNA stability, might account for these observations. Collectively, these data also portend that a DAT 3'-UTR of a particular sequence might function differently, depending on the sequence of the DAT promoter or other regions of the DAT gene in any given allele.

We also found that both ADHD and the 3'-UTR polymorphism had an additive effect on DAT binding. In contrast, there were no significant associations of polymorphisms of the *DRD4* and *5-HTT* genes with DAT binding. These findings suggest that the 3'-UTR polymorphism of the *SLC6A3* gene is a key mutation affecting expression of the DAT gene in humans, suggesting that its presence might influence clinical expression.

The finding that gene frequencies of all the gene polymorphisms assessed (Table 1) were not different between the ADHD and control groups is not surprising, considering that the gene effects reported in previous studies with ADHD subjects were very small, and positive results have been largely limited to meta-analyses (20).

Our finding showing that a significant association between *SLC6A3* polymorphisms and DAT bindings was limited to the 3'-UTR polymorphism and to its 9R carrier allele is consistent with results from a recent PET study that reported that 9R carrier alleles were significantly associated with higher striatal DAT binding in healthy control subjects (23,24). However, our results are partially inconsistent with those of a recent meta-analysis that failed to show statistically significant associations between striatal DAT binding (as determined by single photon emission computed tomography) and VNTR alleles of the 3'-UTR of *SLC6A3* despite an association (effect size [Hedges' *g*] = .66) in the same direction (22).

Our results showing an association between the 3'-UTR polymorphism of the DAT gene and ADHD is consistent with recent studies in adults that suggested that the 9R (9/9 and 9/10) carrier status is the risk polymorphism of the DAT (*SLC6A3*) gene in adults with persistent ADHD (28,33). Although the 10/10 genotype of the 3'-UTR VNTR had been thought to be a risk factor for ADHD in children, in a meta-analysis of over 3000 adults, Franke *et al.* (28) reported that the 9R genotype was associated with persistent ADHD. The authors concluded that a differential association of DAT1 with ADHD in children and adults might explain the inconsistencies observed in earlier association studies. Taken together, these results suggest that the 9R alleles of the 3'-UTR polymorphism might account for increased DAT binding in adults with persistent ADHD.

Our failure to find a significant relationship between the intron8 (*SLC6A3*) VNTR or the intron8-3'-UTR haplotype with striatal DAT binding is inconsistent with a small literature that reported a significant such association in healthy control subjects (23,24). Although the

reasons for these discrepant results are not entirely clear, our subsample with intron8 data was small, and we might not have had sufficient statistical power to detect a relationship. More work is needed to clarify this issue.

Our finding of an association between ADHD status and DAT binding independently of the 3'-UTR polymorphism is consistent with a recent meta-analysis of published results (13) (including an earlier, smaller version of this sample [4]) that documented increased striatal binding of 14% in ADHD ($p < .05$) across studies. Although the authors postulated that their findings of increased binding were due to previous stimulant exposure, this interpretation is not supported by the data, because of misclassifications of stimulant use status in participants in some of the studies included in the meta-analysis (34). Despite the suggestion that therapeutic stimulant use might affect DAT binding, two long-term (12- and 18-month) stimulant treatment studies of nonhuman primates reported no effect on DAT or D2/ D3 receptors as well as DA tone (35,36). Because our sample was entirely stimulant-naïve, it cannot address the issue of whether therapeutic doses of stimulants affect DAT binding.

To the best of our knowledge, there are only two previous studies that assessed the effects of *SLC6A3* genotypes on DAT binding (one pediatric [25] and one adult study [26]) in subjects with ADHD. Similar to the current study, both groups had previously reported increased striatal DAT binding in subjects with ADHD compared with normal control subjects (9,11,12). However, the gene findings in these studies of ADHD alone were different from each other and (somewhat) from our study. Krause *et al.* (26) reported no difference in striatal DAT binding in relationship to the 9R carrier alleles of the 3'-UTR of *SLC6A3* in 29 adults with ADHD. However, they noted a pronounced effect of smoking to lower DAT binding. Although not statistically significant, when they restricted the analysis to the 19 adults with ADHD who were not smokers they found increased DAT binding in the 9R carriers, similar to our study. In contrast, Cheon *et al.* (25) reported decreased DAT binding associated with the 9R carrier alleles of the 3'-UTR of *SLC6A3* in children with ADHD. The later result might be partially explained by the Franke *et al.* (28) and Barkley *et al.* (33) findings (preceding text) suggesting a different influence of the 3'-UTR of *SLC6A3* between children and adults. Clearly more work is needed to reconcile these discrepant findings.

The finding that ADHD was associated with dysregulation of the DAT independently of the 3'-UTR polymorphism suggests that in some individuals with ADHD, dysregulation of DAT is due to other yet unidentified risk factors, including other polymorphisms of the DAT gene (*SLC6A3*) and the promoter region of the gene. The grouping together of multiple alleles solely by the number of repeat sequences (length) of the 3'-UTR might confound the association of the DAT gene with ADHD and mask the relevance of other sequence variations that might be involved in DAT gene regulation (21). Additionally, influences of other genes or acquired nongenetic factors might affect DAT binding. More work is needed to identify these other risk factors.

Our results should be viewed in light of some methodological limitations. Although the sample size was substantial, it might not have been large enough for all the analyses. Although the 3'-UTR of *SLC6A3* seems to be a functional polymorphism in humans, it is a small part of *SLC6A3*. Fuller coverage of the gene is needed to examine other areas of *SLC6A3* and their relationship to DAT binding. Because the sample was largely Caucasian, our findings might not generalize to other ethnic groups. Further research should examine other regions of *SLC6A3* in a larger, more diverse sample.

Despite these considerations, our findings suggest that the ADHD-associated risk polymorphism (3'-UTR) of *SLC6A3* has functional consequences on central nervous system

DAT binding in humans. Furthermore, our results imply that some of the DAT (and therefore dopamine) dysregulation in ADHD could be due to an over-representation of the 9R allele of the 3'-UTR risk polymorphisms of SLC6A3. These results advance our understanding of ADHD risk genes and DAT expression in humans.

There are a number of clinical implications of our work. Our work and the work of others continue to document dysregulation of dopamine and the dopamine transporter in ADHD that is not accounted for by stimulant use. Some of this DAT dysregulation is due to a polymorphism of the DAT gene (9R carriers [VNTR] of 3'-UTR *SLC6A3*). However, ADHD makes an independent contribution to DAT binding, suggesting an as yet unidentified genetic or nongenetic mechanism for this phenomenon. Moreover, other studies have reported that the relationship of this DAT gene polymorphism is different in persistent and nonpersistent ADHD.

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In 2012, JB received an honorarium from the MGH Psychiatry Academy and The Children's Hospital of Southwest Florida/Lee Memorial Health System for tuition-funded Continuing Medical Education courses.

In 2011, JB gave a single unpaid talk for Juste Pharmaceutical Spain, received honoraria from the MGH Psychiatry Academy for a tuition-funded CME course, and received honoraria for presenting at an international scientific conference on ADHD. He also received an honorarium from Cambridge University Press for a chapter publication. He received departmental royalties from a copyrighted rating scale used for ADHD diagnoses, paid by Eli Lilly, Shire, and AstraZeneca; these royalties are paid to the Department of Psychiatry at MGH.

In 2010, JB received a speaker's fee from a single talk given at Fundación Dr. Manuel Camelo A.C. in Monterrey Mexico. He provided single consultations for Shionogi Pharma and Cipher Pharmaceuticals; the honoraria for these consultations were paid to the Department of Psychiatry at the MGH. He received honoraria from the MGH Psychiatry Academy for a tuition-funded CME course.

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Table 1

Demographic Data

	ADHD (<i>n</i> = 34)		Control (<i>n</i> = 34)		Statistic	
	mean	SD	mean	SD	<i>t</i>	<i>p</i>
Age (yrs)	32.8	9.1	27.7	6.8	2.64	.01
SES	2.3	1.1	2.4	1.3	.27	NS
IQ	113	11.3	113	13.4	.02	NS
	<i>n</i>	%	<i>n</i>	%	χ^2	<i>p</i>
Sex (male)	23	68%	12	35%	7.12	.01

ADHD, attention-deficit/hyperactivity disorder; NS, not significant; SES, socioeconomic status.

Table 2

Gene Frequencies

Gene	ADHD (n = 34)		Control (n = 36)		Statistic	
	n	%	n	%	χ^2	p
<i>SLC6A3_Utr3'</i>	18	53%	15	44%	.53	NS
<i>SLC6A3_In8'</i>	7 ^a	54%	9 ^b	47%	.13	NS
<i>DRD4</i>	10 ^c	30%	10 ^d	32%	.03	NS
<i>SLC6A4_In</i>	8 ^e	35%	11 ^f	39%	.11	NS
<i>SLC6A4_Pr</i>	8 ^g	35%	11 ^h	41%	.19	NS

ADHD, attention-deficit/hyperactivity disorder; NS, not significant.

^aDenominator = 13.

^bDenominator = 19.

^cDenominator = 33.

^dDenominator = 31.

^eDenominator = 23.

^fDenominator = 28.

^gDenominator = 23.

^hDenominator = 27.

Table 3
Relationship of DAT Binding (Caudate) to ADHD Status and Genetic Polymorphism

Gene	n	ADHD vs. Control		Gene		Interaction	
		t	p	t	p	t	p
<i>SLC6A3_Utr3'</i>	68	2.99	.004	2.74	.008	.21	NS
<i>SLC6A3_In8'</i>	32	2.51	.018	.37	NS	.61	NS
<i>DRD4</i>	64	3.08	.003	1.53	NS	.75	NS
<i>SLC6A4_In</i>	51	2.69	.010	.09	NS	.72	NS
<i>SLC6A4_Pr</i>	50	2.95	.005	-1.04	NS	.26	NS

ADHD, attention-deficit/hyperactivity disorder; DAT, dopamine transporter; NS, not significant