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Association study of the nicotinic acetylcholine receptor α4 subunit gene, *CHRNA4*, in attention-deficit hyperactivity disorder

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Abstract

Attention-deficit hyperactivity disorder (ADHD) is a common childhood-onset psychiatric condition with a strong genetic component. Evidence from pharmacological, clinical and animal studies has suggested that the nicotinic system could be involved in the disorder. Previous studies have implicated the nicotinic acetylcholine receptor α4 subunit gene, *CHRNA4*, in ADHD. Particularly, a polymorphism in the exon 2–intron 2 junction of *CHRNA4* has been associated with severe inattention defined by latent class analysis. In the current study, we used the transmission disequilibrium test (TDT) to investigate four polymorphisms encompassing this region of *CHRNA4* for association with ADHD in a sample of 264 nuclear families from Toronto. No significant evidence of biased transmission was observed for any of the marker alleles for ADHD defined as a categorical trait (all subtypes included), although one haplotype showed marginal evidence of under-transmission. No association was found with the ADHD predominantly inattentive subtype or with symptom dimension scores of inattention. On the contrary, nominally significant evidence of association of individual markers was obtained for the ADHD combined subtype and with teacher-rated hyper-activity–impulsivity scores, with the same haplotype being under-transmitted. Based on our results and others, *CHRNA4* may be involved in ADHD; however, its role in ADHD symptomatology remains to be clarified.

Keywords

Attention-deficit/hyperactivity disorder; *CHRNA4*; genetics; nicotinic receptor; transmission/disequilibrium test

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Attention-deficit hyperactivity disorder (ADHD) is a common childhood-onset psychiatric condition affecting 4–12% of children worldwide (Faraone *et al.* 2003) with a tendency to persist into adolescence and adulthood (Clarke *et al.* 2005). Family, twin and adoption studies have shown that this disorder is highly heritable (Biederman & Faraone 2005; Thapar *et al.* 1999) and multiple susceptibility genes are likely to be involved.

As currently recognized by the *Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition* (DSM-IV), the behavioral symptoms of ADHD load into two separate dimensions, one reflecting inattentive behavior and the other a combination of hyperactive and impulsive behavior. Twin studies have shown that the symptoms of inattention and hyperactivity/impulsivity are primarily explained by shared genetic influences; however, each symptom dimension of ADHD was also shown to be under unique genetic influence (Levy *et al.* 2001; Rasmussen *et al.* 2004; Sherman *et al.* 1997).

Catecholamine system dysfunction, particularly in the dopaminergic system, has been suggested in ADHD by pharmacological, imaging, molecular genetic and animal studies (Davids *et al.* 2003; Durston 2003; Seeman & Madras 1998; Thapar *et al.* 2005; Viggiano *et al.* 2003). Accumulating evidence indicate a potential role for the nicotinic system in modulating dopamine neurotransmission. Nicotinic acetylcholine receptors (nAChRs) are expressed in regions densely innervated by dopaminergic neurons (Arroyo-Jimenez *et al.* 1999; Gotti *et al.* 2006; Klink *et al.* 2001) and activation of presynaptic nAChRs is known to facilitate dopamine release in the nucleus accumbens and in the striatum (Grady *et al.* 2002; Picciotto *et al.* 1998). In addition, nAChRs signaling was shown to regulate the dopamine transporter gene transcription and function (Li *et al.* 2004; Parish *et al.* 2005), potentially affecting dopamine uptake.

Attention-deficit hyperactivity disorder is associated with an increased risk of early initiation of cigarette smoking (Milberger *et al.* 1997) and, consequently, a high prevalence of cigarette smoking is observed in children with ADHD as they reach adolescence and adulthood (Biederman *et al.* 2006; Lambert & Hartsough 1998). Lower cessation (stop smoking) ratios were reported for boys with ADHD compared with the general population (Pomerleau *et al.* 1995). In addition, maternal smoking during pregnancy was shown to be a significant risk factor for development of ADHD and ADHD symptoms for the offspring (Barman *et al.* 2004; Batstra *et al.* 2003; Kotimaa *et al.* 2003; Thapar *et al.* 2003). Clinical and animal studies have shown that nicotine receptor stimulation plays a role, either directly or by interactions with other neurotransmitters, in several executive function processes such as response inhibition, attention and working memory (Newhouse *et al.* 2004; Rezvani & Levin 2001). These processes are thought to underlie the cognitive and behavioral difficulties experienced by children with ADHD (Arnsten & Li 2005; Lijffijt *et al.* 2005; Luman *et al.* 2005; Martinussen *et al.* 2005; Willcutt *et al.* 2005). Specifically, nicotine or nicotinic agonists have been shown to improve attention in adult smokers and nonsmokers without attention deficits and adults with ADHD (Levin *et al.* 1998; Mancuso *et al.* 1999; Wilens *et al.* 1999, 2006), making nicotinic system genes attractive susceptibility genes for ADHD.

Neuronal nAChRs are ligand-gated ion channels composed of five subunits. Molecular analyses have identified nine alpha (α2–α10) and three beta (β2–β4) subunits in the central nervous system (Dani & Bertrand 2007), with the majority of high-affinity binding sites provided by receptors consisting of α4 and β2 subunits. Nicotinic agonists shown to improve ADHD symptoms in adults bind selectively to α4–β2 high-affinity complexes.

The gene coding for the nAChR α4 subunit, *CHRNA4*, contains six exons spanning ~17 kb on chromosome 20q13.2–13.3 (Steinlein *et al.* 1994, 1996). Genetic polymorphisms in the *CHRNA4* gene have been proposed to be associated with several psychiatric or behavioral disorders, including autosomal-dominant nocturnal frontal lobe epilepsy (Combi *et al.* 2004), febrile convulsions (Chou *et al.* 2003), Alzheimer's disease (Kawamata & Shimohama 2002), alcohol dependence (Kim *et al.* 2004) and vulnerability to nicotine addiction (Feng *et al.* 2004; Li *et al.* 2005).

Because of the strong indication for the involvement of the nicotinic acetylcholine system in ADHD, the *CHRNA4* gene has been tested as a candidate for ADHD in several genetic studies. Evidence of association was found with a dinucleotide repeat in intron 1 in an analysis of DSM-IV ADHD symptom scores among 326 individuals (271 cases with Tourette syndrome and 55 controls) (Comings *et al.* 2000), while Kent *et al.* (2001) found no significant evidence of association for a *Cfo*1 restriction site polymorphism in exon 5 in a study of 68 trios with DSM-IV-defined ADHD. Using families selected from a twin sample from Missouri (178 families), Todd *et al.* (2003) reported a relationship between a polymorphism in the exon 2–intron 2 junction, and severe inattention problems defined by a latent class analysis. Using the same markers, Bobb *et al.* (2005) did not find evidence for association with DSM-IV ADHD using a sample of 163 ADHD cases and 129 controls (also analyzed as families with 192 available parental DNAs). Finally, in a recent analysis of 51 genes including *CHRNA4* in 674 families with a child meeting the DSM-IV ADHD combined subtype criteria, Brookes *et al.* (2006) reported nominal evidence of association for one marker in the 5′ flanking region of *CHRNA4*. Although the results from these studies are conflicting, some of the studies were based on small sample sizes, and further the studies used different phenotypes for analysis and the samples had different clinical characteristics. In three of the studies, the majority or all of the samples was composed of the DSM-IV combined ADHD subtype (Bobb *et al.* 2005; Brookes *et al.* 2006; Kent *et al.* 2001), whereas over half of Todd *et al.*'s sample was composed of children with severe inattention problems.

In this study, we investigated the association between *CHRNA4* and ADHD in a sample of ADHD families collected in Toronto. Four markers in the *CHRNA4* promoter-intron 2 region and their haplotypes were tested for evidence of biased transmission in relation to ADHD or DSM-IV ADHD subtypes using the transmission disequilibrium test (TDT). Relationship between these variants and the symptom dimensions of inattention and hyperactivity/impulsivity, and with cognitive measures of verbal short-term and working memory were also assessed using quantitative analyses.

Materials and methods

Diagnostic assessment and subjects

Probands and affected siblings between 7 and 16 years old were referred to the Child Development and Neuropsychiatry Clinics at the Hospital for Sick Children, Toronto, and met DSM-IV criteria for ADHD. Diagnosis was based on information from semi-structured interviews of parents [Parent Interview for Child Symptoms (PICS-IV)] (Ickowicz *et al.* 2006) and teachers [Teacher Telephone Interview (TTI-IV)] (Tannock *et al.* 2002). Clinical information was also obtained from the following standardized questionnaires and assessments: Conners' Parent and Teacher Rating Scales – Revised (Conners 1997), Ontario Child Health Survey Scales – Revised (Boyle *et al.* 1993), Wide-Range Achievement Test – Revision 3 (Wilkinson 1993), Clinical Evaluation of Language Fundamentals, third edition (Semel *et al.* 1995), Children's Depression Inventory (Kovacs 1995) and Children's Manifest Anxiety Scale (Reynolds & Richmond 1985). Children who scored below 80 on both the Performance and Verbal Scales of the Weschler Intelligence Scale for Children, 3rd Edition (WISC-III) (Kaplan *et al.* 1999; Wechsler 1991) were excluded from the study, as were children who exhibited neurological or chronic medical illness, Tourette syndrome, chronic multipletics, bipolar affective disorder, psychotic symptoms or other anxiety, depressive or developmental disorders that might account for their behavior. All children were free of medication for 24 h before assessment. This protocol was approved by the Hospital for Sick Children's Research Ethics Board and informed written consent or assent (children) was obtained for all participants.

The study sample comprised 264 nuclear families recruited in the Toronto area, for a total of 313 affected children (81% boys). The majority of the families reported their ethnic background to be of European Caucasian descent, while 10% of families were of other or mixed background, including Chinese, African, Indian and Native Canadians. Both parents were genotyped in 192 families. The distribution of the affected children among the three DSM-IV ADHD subtypes was: 14% of the predominantly hyperactive/impulsive subtype, 24% of the predominantly inattentive subtype and 62% of the combined subtype.

For the quantitative analysis, we used the symptom scores obtained for each dimension from the PICS-IV and TTI-IV semi-structured interviews, as described previously (Laurin *et al.* 2005). These are clinician ratings of the symptoms based on behavioral description elicited from parents or teachers. Verbal short-term and working memory was assessed using the digit span subtest of the WISC-III. This test provides two subscale scores (digits forward, digits backward), which index the ability to store and manipulate auditory–verbal information, respectively.

Isolation of DNA and marker typing

DNA was extracted from blood leukocytes using a high salt method (Miller *et al.* 1988). We examined four markers in *CHRNA4*: rs755203 in the promoter and rs2273505, rs6090384 and rs3787141 in intron 2. rs2273505 and rs6090384 were genotyped by restriction enzyme digest. They were both amplified on the same fragment using the following primers: 5′-CCTGCACCTGAGCCACTG-3′ and 5′-ACGCTCT-GAATCAACCCTTG-3′. Polymerase

chain reaction (PCR) amplification (20 μl volume) was carried out with 60 ng of genomic DNA using the PCR Enhancer system (Invitrogen Tech-Line^{SM}; Invitrogen, Carlsbald, CA, USA), supplemented with 1.5 mM $MgCl_2$, for 35 cycles of 94°C, 40 s; 59°C, 40 s and 72°C, 40 s. PCR products were digested using the enzymes *Nla*III (New England Biolabs, Berverly, MA, USA) for rs2273505 and *Hin*P1I (New England Biolabs) for rs6090384. *Nla*III restriction fragments for rs2273505 (allele G: 334 and 48 bp; allele A: 212, 170 and 48 bp) and *Hin*P1I fragments for rs6090384 (allele G: 212 and 170 bp; allele A: 382 bp) were visualized by ethidium bromide staining on 2.5% agarose gels.

The markers rs755203 (C_8838223_10, Assay-on-Demand®; Applied Biosystems, Foster City, CA, USA) and rs3787141 (C__25800787_10, Assay-on-Demand®; Applied Biosystems) were genotyped with the ABI 7900-HT Sequence Detection Systems (Applied Biosystems) using the TaqMan 5′ nuclease assay for allelic discrimination. The PCR reactions (5 μl) contained 30 ng of genomic DNA, 2.5 μl of TaqMan Universal PCR Master Mix and 0.1 μl of allelic discrimination mix. The thermal cycling conditions were 95°C for 10 min and 50 cycles of 95°C, 15 s; and the annealing temperatures were 58 and 59°C, respectively, 1 min.

Statistical analysis

For categorically defined ADHD, we examined the allelic transmission of markers using the extended TDT program (Sham & Curtis 1995) and the haplotype transmission with TRANSMIT version 2.5, using the robust estimator of variance option (Clayton 1999). Quantitative trait TDT analyses, examining transmission of individual alleles or haplotypes in relation to dimensional symptom scores and short-term and working memory measures were carried out using the FBAT program version 1.5.5, with the additive model of inheritance (Horvath *et al.* 2001; Laird *et al.* 2000). We used population-based mean scores for the tests as an offset value to mean center the trait. *P*-values were not corrected for multiple tests. We did not observe significant departure from the Hardy–Weinberg equilibrium for the genotype frequencies.

Results

Based on a previous report showing evidence of association between severe inattention problems defined by latent class analyses, and markers at the exon 2–intron 2 junction of the *CHRNA4* gene (Todd *et al.* 2003), we performed a family-based association study using four markers encompassing this same region of *CHRNA4*. Two markers, rs2273505 and rs6090384, were investigated in the previous association report. Although rs2273506 was also assessed in the report of Todd *et al.* (2003), it was not included in the present study because of evidence of complete linkage disequilibrium (LD) with rs2273505. Instead, we selected rs755203 in the promoter and rs3787141 located in *CHRNA4* intron 2.

We first tested for biased transmission of marker alleles using the TDT statistic with categorically defined ADHD (all subtypes). As shown in Table 1, we did not observe significant evidence of biased transmission for any of the marker alleles. Similarly, no significant evidence for over-transmission was observed for any of the resulting four-marker

haplotypes, although one low-frequency haplotype (7.2%) was marginally under-transmitted ($P = 0.041$) (Table 2).

In light of results previously reported for a severe inattention latent class and trends for association of the DSM-IV inattention subtype, we conducted TDT analyses for DSM-IV predominantly inattentive subtype. No evidence of association between *CHRNA4* variants or haplotypes and this subtype was observed (Tables 1 and 2). However, the number of informative transmissions for this subgroup was small and is thus not conclusive. We next analyzed the marker alleles using a quantitative approach for ADHD inattentive symptom scores as reported by parents and teachers and found no evidence of relationship (Table 3).

In contrast, we obtained nominally significant over-transmission of the rs2273505-G ($P = 0.033$) and rs3787141-T ($P = 0.046$) alleles for families with children meeting the DSM-IV criteria for the ADHD combined subtype (Table 1). One haplotype, including the alternate alleles of the two associated markers above (A and C, respectively), was significantly under-transmitted (GAGC: $P = 0.004$) (Table 2). This is the same haplotype that showed evidence of under-transmission with categorical ADHD. However, these findings should be interpreted cautiously because ADHD subtype analysis leads to a lower number of informative transmissions for each marker and the frequency of the under-transmitted haplotype is low (7.2%). For this reason, we did not analyze the predominantly hyperactive/impulsive subtype because the number of informative transmission would be too small to be conclusive.

Of note is that we observed over-transmission of the rs2273505-G for the combined subtype in our sample, while the opposite allele, rs2273505-A, showed a strong trend toward over-transmission for the DSM-IV-defined inattentive subtype in the report of Todd *et al.* (uncorrected $P = 0.046$, corrected $P = 0.089$). We also found marginally significant evidence for association of the individual alleles rs2273505-G and rs3787141-T with the teacher-rated hyperactivity–impulsivity scores ($P = 0.026$ and $P = 0.044$, respectively) (Table 3). Haplotype analysis showed a significant negative relationship between the same under-transmitted haplotype G–A–G–C, and teacher-rated hyperactivity–impulsivity scores ($Z = -2.283$, $P = 0.022$) (Table 3).

Finally, as nicotine has been suggested to modulate short-term and working memory processes, we also tested the relationship between *CHRNA4* alleles and verbal short-term and working memory measures in this sample. No relationship between this gene and verbal short-term and working memory was observed for individual markers or any of the observed haplotypes (data not shown).

Discussion

In this study, we assessed the association between *CHRNA4* and ADHD in a clinically ascertained sample. We limited our study to four polymorphisms in the gene region previously implicated in ADHD, i.e. 5′ flanking region to intron 2 (Brookes *et al.* 2006; Comings *et al.* 2000; Todd *et al.* 2003). No association was observed between these variants and DSM-IV categorically defined ADHD or with inattentive symptoms. We found,

however, marginal evidence of association between *CHRNA4* variants and the DSM-IV ADHD combined subtype and with hyperactive/impulsive symptom scores. We also observed the under-transmission of a low-frequency haplotype for DSM-IV categorical ADHD and the DSM-IV ADHD combined subtype.

This report is the fourth showing evidence of association between ADHD and the *CHRNA4* gene, despite different ADHD phenotypes or markers/alleles being associated (see Fig. 1). Differences in the markers and alleles associated may reflect differences in the linkage disequilibrium with the true and as yet unidentified risk variant(s) and because of ethnic/population differences, divergent linkage disequilibrium patterns lead to the association of different alleles.

Differences in sample characteristics exist between the study samples. We used a clinically ascertained sample, while Todd *et al.* selected their sample from a birth record-based twin sample, which they first screened for the presence of three or more inattentive symptoms endorsed by a parent. The children then went through a clinical assessment and the ones that met DSM-IV criteria for ADHD were used to test for association with the DSM-IV-defined subtypes. The screening procedure based on inattention symptoms used by Todd and colleagues may have led to an over-representation of the children with inattentive problems, as illustrated by the high proportion of the primarily inattentive subtype for children meeting the DSM-IV criteria in Todd *et al.*'s sample (58% compared with 24% in our sample). This may have increased power for the analysis of the severe inattention latent class and the DSM-IV inattention subtype compared with other samples. Brookes *et al.*'s sample included families with only the ADHD combined subtype (93.5% male probands), while Kent *et al.*'s 68 trios (87% male probands) were also predominantly of the combined subtype (84%), with few of the inattentive subtype (7%, 5 probands) and Bobb *et al.*'s study of 163 probands (53% male probands) was composed of 94% of children with the combined subtype with 6% inattentive subtype. Finally, Comings *et al.*'s method of assessment was completely different based on ADHD symptom scores for individuals with Tourette syndrome.

In addition, the Missouri sample was highly enriched with female probands (45.6% vs. 19% in our sample). Important gender differences in symptomatology have been observed for ADHD. Attention-deficit hyperactivity disorder-affected girls exhibit greater intellectual impairment, lower levels of hyperactivity and lower rates of other externalizing behaviors compared with boys (Biederman *et al.* 2002; Gaub & Carlson 1997; Newcorn *et al.* 2001).

With regard to methodology, we did not perform analysis for latent-class-defined ADHD subtypes as reported by Todd *et al.* because examination of our sample showed that we would have a very small sample size for each group, especially for the severe inattentive class (less prevalent), owing to the predominance of the DSM-IV combined subtype in our clinical sample.

The severe inattention subtype as defined by latent class analysis is thought to represent a relatively pure primary inattention subset of those meeting DSM-IV criteria for predominantly inattentive subtype. The genetic factors involved in the severe inattention latent class might be different from the genetic factors involved in the inattention symptoms

that are also present in children with hyperactivity/impulsivity symptoms. Thus, our analysis of inattention symptoms defined quantitatively and previous analysis of the latent-class-defined inattention subtype may not be comparable. As Todd *et al.*'s results are stronger for latent-class-defined inattention subtype than for DSM-IV-defined inattentive subtype, we cannot exclude the possibility that the same variants are associated with pure inattention problems that could not be detected with the current sample. However, the most significant single marker finding from that study was for the marker rs6090384 with the latent class severe inattention group ($P = 0.007$, corrected $P = 0.015$). This marker was also significant for the analysis of all children with ADHD ($P = 0.028$) and the inattentive DSM-IV subtype ($P = 0.039$); however, these two analyses did not stand up to correction for multiple testing ($P = 0.055$ and $P = 0.076$, respectively). Thus, we would have expected a similar trend in our entire sample and our inattentive subtype, but this was not seen. Instead, we obtained positive results for the DSM-IV combined subtype and hyperactive/impulsive symptom scores suggesting that *CHRNA4* variants could be associated more with combined or hyperactive-impulsive problems in our sample.

Interestingly, although research on nicotine has focused primarily on attention processes and working memory, pre-natal exposure or acute administration of nicotine has been shown to stimulate locomotor activity levels in rodents (Benwell & Balfour 1992; Newman *et al.* 1999; Tizabi *et al.* 2000), and *CHRNA4*-deficient mice exhibit increases in several components of their ethogram, including locomotion, rearing and sniffing, over the course of habituation to a novel environment (Ross *et al.* 2000). Furthermore, maternal smoking has also been associated with symptoms of hyperactivity in children (Kotimaa *et al.* 2003).

In summary, using a family-based sample, we found nominal evidence of association between *CHRNA4* and ADHD, particularly with the DSM-IV ADHD combined subtype and with hyperactive/impulsive symptoms. We were unable to show an association between *CHRNA4* and inattentive symptoms albeit this may be the result of sample characteristics. Based on our results and the findings from previous studies, the involvement of *CHRNA4* in ADHD still remains unclear, although the 5′ region-intron 2 of the gene has repeatedly shown association with the disorder. Further investigation of *CHRNA4* 5′ region, including regulatory regions is thus warranted.

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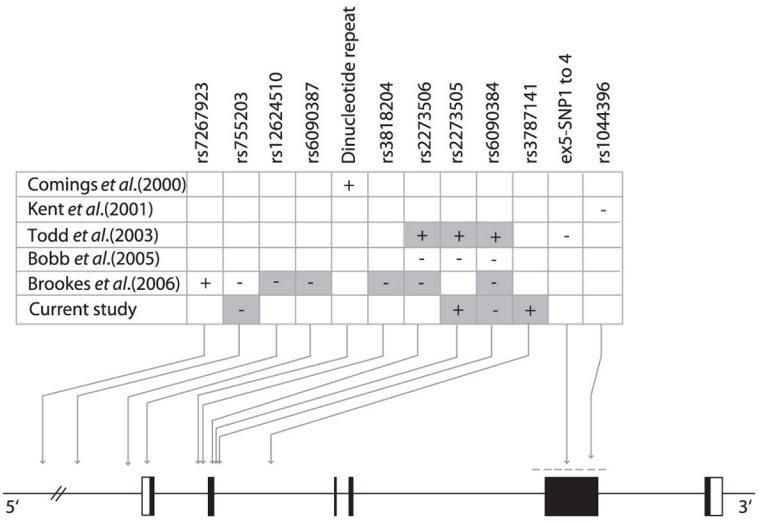

Figure 1. Schematic representation of the *CHRNA4* gene and association results reported for ADHD

Lower panel shows the structure of the *CHRNA4* gene. The six exons of the gene are represented by boxes, with black boxes for coding sequences and empty boxes for untranslated regions. Association findings are shown in the top panel with positive (+) and negative (−) results for the different studies. Of note, the Todd *et al.* findings presented here are not corrected for multiple tests and only rs6090384 remains significant after correction in that study. Markers involved in haplotypes showing evidence of association are shaded. Haplotypes from this study and Todd *et al.* were under-transmitted. The haplotype reported by Brookes *et al.* has a very low frequency (17 transmissions). Twelve other single nucleotide polymorphisms (SNPs), from intron 2 to 3′ untranslated region, have been tested by Brookes *et al.* (not shown here) and were all negative.

Table 1

Allele frequencies and TDT analysis of four SNPs in the *CHRNA4* gene

	rs755203		rs2273505		rs6090384		rs3787141	
Allele	**G**	**A**	**G**	**A**	**G**	**A**	**T**	**C**
Frequency	0.477	0.523	0.926	0.074	0.943	0.057	0.925	0.075
DSM-IV ADHD (all subtypes)								
Transmitted	108	105	40	26	25	18	37	26
Not transmitted	105	108	26	40	18	25	26	37
χ^2 (1 df)	0.042		2.970		0.581		1.921	
P-value	0.837		0.085		0.446		0.166	
DSM-IV predominantly inattentive subtype								
Transmitted	30	28	8	9	7	6	6	9
Not transmitted	28	30	9	8	6	7	9	6
χ^2 (1 df)	0.069		0.059		0.077		0.600	
P-value	0.793		0.808		0.782		0.439	
DSM-IV combined subtype								
Transmitted	55	61	25	12	9	10	24	12
Not transmitted	61	55	12	25	10	9	12	24
χ^2 (1 df)	0.310		4.568		0.053		4.000	
P-value	0.578		0.033		0.819		0.046	

Table 2

CHRNA4 haplotype frequencies and TRANSMIT analysis

		Transmission				
Haplotype*	**Frequency**	**Observed†**	**Expected‡**	**Var (O-E)**	**χ^2 (1 df)**	***P***
DSM-IV ADHD (all subtypes)§						
AGGT	0.492	290.92	288.51	66.61	0.087	0.768
GGGT	0.379	214.90	206.18	57.41	1.325	0.250
GAGC	0.072	34.97	43.58	17.82	4.161	0.041
AGAT	0.038	16.04	18.22	9.22	—	—
GGAT	0.021	11.12	11.86	4.09	—	—
DSM-IV ADHD predominantly inattentive subtype¶						
AGGT	0.492	69.98	70.21	20.97	0.003	0.960
GGGT	0.379	44.02	44.53	16.78	0.015	0.902
GAGC	0.072	11.98	10.91	4.67	0.244	0.622
AGAT	0.038	4.99	5.16	3.04	—	—
GGAT	0.021	3.01	3.14	1.02	—	—
DSM-IV ADHD combined subtype**						
AGGT	0.492	163.44	158.85	35.29	0.597	0.440
GGGT	0.379	118.49	116.44	30.47	0.138	0.711
GAGC	0.072	15.99	23.77	7.23	8.375	0.004
AGAT	0.038	9.05	8.84	4.09	—	—
GGAT	0.021	6.01	5.02	1.24	—	—

*Haplotypes with frequency >0.005 are listed.

†Test statistic representing the observed number of transmissions.

‡Expected value of the test statistic under the null hypothesis of no association.

§Global χ^2 on 3 df for haplotypes with frequencies >5% = 5.688, $P = 0.127$.

¶Global χ^2 on 3 df for haplotypes with frequencies >5% = 0.251, $P = 0.969$.

**Global χ^2 on 3 df for haplotypes with frequencies >5% = 8.430, $P = 0.038$.

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Table 3

FBAT analysis of *CHRNA4* allele and haplotype transmission in relation to ADHD symptom scores

		Inattentive symptoms					Hyperactive/impulsive symptoms				
Marker allele/haplotype	**No. of families**	**S***	**E(S)†**	**Var(S)**	***Z***	***P***	**S***	**E(S)†**	**Var(S)**	***Z***	***P***
Parent Interview for Child Symptoms											
rs755203 G	128	654.0	640.1	1072.6	0.424	0.671	622.3	619.6	1106.8	0.081	0.935
rs755203 A		680.1	694.0		−0.424		698.5	701.2		−0.081	
rs2273505 G	49	378.2	350.1	322.6	1.562	0.118	353.0	323.0	307.9	1.712	0.087
rs2273505 A		98.8	126.8		−1.562		85.6	115.7		−1.712	
rs6090384 G	36	261.2	256.7	213.9	0.311	0.775	254.9	244.5	232.5	0.682	0.495
rs6090384 A		81.0	85.6		−0.311		71.1	81.5		−0.682	
rs3787141 T	44	319.7	304.7	294.9	0.871	0.384	313.0	290.3	294.3	1.326	0.185
rs3787141 C		101.8	116.7		−0.871		86.6	109.4		−1.326	
AGGT	113	702.8	713.7	1027.2	−0.340	0.734	713.4	708.1	1031.5	0.167	0.868
GGGT	112	555.5	528.5	892.4	0.905	0.366	555.0	532.3	908.2	0.754	0.451
GAGC	42	100.7	120.7	279.3	−1.193	0.233	86.5	115.5	272.4	−1.756	0.079
AGAT	26	50.7	52.7	132.9	−0.176	0.860	46.6	56.3	158.2	−0.771	0.441
GGAT	10	33.9	32.6	74.1	0.148	0.882	36.3	33.1	73.9	0.372	0.710
Teacher Telephone Interview											
rs755203 G	126	617.1	639.6	1207.5	−0.646	0.518	499.8	509.9	970.4	−0.325	0.745
rs755203 A		697.5	675.1		0.646		508.8	498.6		0.325	
rs2273505 G	48	353.9	328.1	302.2	1.482	0.138	340.8	301.0	317.4	2.231	0.026
rs2273505 A		92.5	118.3		−1.482		66.3	106.0		−2.231	
rs6090384 G	37	225.6	233.7	187.6	−0.591	0.554	200.0	203.3	197.1	−0.231	0.817
rs6090384 A		86.0	77.9		0.591		71.0	67.8		0.231	
rs3787141 T	43	313.9	296.4	280.2	1.044	0.297	320.3	284.5	316.2	2.010	0.044
rs3787141 C		93.5	111.0		−1.044		68.3	104.0		−2.010	
AGGT	110	695.1	692.5	1015.5	0.082	0.935	514.3	516.9	854.8	−0.090	0.928
GGGT	108	512.6	510.2	922.0	0.080	0.936	488.0	456.2	824.0	1.110	0.267
GAGC	41	93.6	115.3	305.1	−1.242	0.214	67.0	108.7	334.1	−2.283	0.022
AGAT	26	57.6	49.3	116.9	0.765	0.444	44.5	36.9	103.1	0.751	0.453

		Inattentive symptoms					Hyperactive/impulsive symptoms				
Marker allele/haplotype	**No. of families**	**S***	**E(S)†**	**Var(S)**	***Z***	***P***	**S***	**E(S)†**	**Var(S)**	***Z***	***P***
GGAT	10	33.3	28.8	60.5	0.576	0.565	29.8	30.6	84.0	−0.096	0.924

*Test statistic.

†Expected value of the test statistic under the null hypothesis of no association.