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Association of the glutamate receptor subunit gene *GRIN2B* with attention-deficit/hyperactivity disorder

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Abstract

The glutamatergic signaling pathway represents an ideal candidate susceptibility system for attention-deficit/ hyperactivity disorder (ADHD). Disruption of specific *N*-methyl-D-aspartate-type glutamate receptor subunit genes (*GRIN1*, *2A–D*) in mice leads to significant alterations in cognitive and/or locomotor behavior including impairments in latent learning, spatial memory tasks and hyperactivity. Here, we tested for association of *GRIN2B* variants with ADHD, by genotyping nine single nucleotide polymorphisms (SNPs) in 205 nuclear families identified through probands with ADHD. Transmission of alleles from heterozygous parents to affected offspring was examined using the transmission/disequilibrium test. Quantitative trait analyses for the ADHD symptom dimensions [inattentive (IA) and hyperactive/impulsive (HI)] and cognitive measures of verbal working memory and verbal short-term memory were performed using the FBAT program. Three SNPs showed significantly biased transmission ($P < 0.05$), with the strongest evidence of association found for rs2284411 ($\chi^2 = 7.903$, 1 degree of freedom, $P = 0.005$). Quantitative trait analyses showed associations of these markers with both the IA and the HI symptom dimensions of ADHD but not with the cognitive measures of verbal short-term memory or verbal working memory. Our data suggest an association between variations in the *GRIN2B* subunit gene and ADHD as measured categorically or as a quantitatively distributed trait.

Keywords

association; attention-deficit/hyperactivity disorder; genetic; GRIN2B; NMDA

Attention-deficit/hyperactivity disorder (ADHD) is an impairing childhood condition characterized by age-inappropriate regulation of attention, motor activity and impulsivity (Shastry 2004; Wilens & Dodson 2004). Several studies implicate deficits in executive control as contributing to the cognitive defects associated with ADHD (Barkley 1997;

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Oosterlaan *et al.* 1998; Pennington & Ozonoff 1996; Schachar & Logan 1990). Executive control encompasses a complex set of cognitive processes, including reasoning, attention, planning and problem-solving skills (Pennington & Ozonoff 1996), that direct goal-oriented responses in a context-dependent manner (Hughes & Graham 2002). Working memory, an example of executive control, refers to the temporary maintenance of task-related information 'online' for future access and manipulation (Baddeley 1992). Impairments in both verbal (Karatekin & Asarnow 1998) and spatial (Barnett *et al.* 2001; Karatekin & Asarnow 1998) working memory have been shown in children with ADHD. Recent meta-analyses of working memory in ADHD indicate impairments in children (Martinussen *et al.* 2005) and adults (Hervey *et al.* 2004) in verbal working memory.

Imaging, lesion and animals studies have suggested a role for the prefrontal cortex (PFC) in mediating executive control (Fernandez-Duque & Posner 2001; Goldman-Rakic 1987; Levy & Goldman-Rakic 2000; Pennington & Ozonoff 1996). In divided attention tasks, patients with ADHD used the left inferior aspect of the basal ganglia to a lesser extent compared with controls (Shafritz *et al.* 2004). Therefore, genes encoding components that support PFC and striatal circuitry necessary for cognitive processes would be candidate targets for molecular studies of ADHD.

Although the exact etiology of ADHD is unknown, it is thought that the dysregulation of neurotransmitter systems underlies the pathogenesis and associated cognitive and locomotor deficits of this disorder. Based on pharmacological and/ or animal knockout studies, the dopaminergic and serotonergic systems are strongly implicated in ADHD (Gainetdinov *et al.* 1999; Giros *et al.* 1996). In addition, similar types of studies support involvement of the glutamatergic system in behavioral models related to ADHD (Adler *et al.* 1998; Carlsson & Carlsson 1989; Corbett *et al.* 1995; Newcomer *et al.* 1999; Raber *et al.* 1997; Sakimura *et al.* 1995; Tang *et al.* 1999; Zhang *et al.* 2002). Further evidence indicates that interactions between dopamine, serotonin and glutamate systems may influence the cognitive and/or behavioral dimensions thought to be affected in ADHD (Gainetdinov *et al.* 2001; Miyamoto *et al.* 2001; Mohn *et al.* 1999). Disruption of *GRIN2A*, a glutamate receptor subunit gene, in mice causes increased metabolism of dopamine and serotonin in the frontal cortex and striatum, and impaired spatial learning (Miyamoto *et al.* 2001). In addition, these mice exhibited additional increases in locomotor activity that was attenuated by dopamine or serotonin receptor antagonists (Miyamoto *et al.* 2001). In the dopamine transporter knockout mouse, blockage of glutamate receptor activity by antagonists augmented hyperactivity, whereas drugs that enhanced glutamatergic neurotransmission inhibited hyperactivity (Gainetdinov *et al.* 2001). The PFC, an area intimately linked to executive control and likely to be involved in ADHD, receives inputs from both dopaminergic and glutamatergic neurons. Genetic studies have found positive associations between components of the glutamate (Turic *et al.* 2004, 2005), dopamine (Barr *et al.* 2001; Lowe *et al.* 2004; Misener *et al.* 2004; Swanson *et al.* 1998) and serotonin (Hawi *et al.* 2002; Manor *et al.* 2001; Quist *et al.* 2000, 2003) pathways and ADHD. Interestingly, a genome-wide scan of affected sib pairs yielded a peak LOD score of 2.6 for multipoint quantitative trait analysis in 12p13, the region of the *GRIN2B* glutamate receptor subunit gene (Fisher *et al.* 2002).

Glutamate receptors are responsible for the majority of excitatory synaptic transmission and plasticity in the central nervous system (reviewed in Ozawa *et al.* 1998). Because of this central role in neuronal communication and synaptogenesis, glutamate receptors control several cellular and cognitive processes (Riedel *et al.* 2003). Ionotropic glutamate receptors are subdivided into three categories based on their respective agonists, namely α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, kainate or *N*-methyl-D-aspartate (NMDA). The classic learning and memory receptors, NMDA receptors (NMDARs), are composed of heteromeric complexes containing an obligatory NR1 (GRIN1) subunit, plus an additional NR2 (GRIN2A–D) or NR3 (GRIN3A,B) subunit (Riedel *et al.* 2003). The exact NMDAR subunit composition delineates receptor function including neurotransmitter affinity, Ca^{2+} permeability and susceptibility to Mg^{2+} (Cull-Candy *et al.* 2001).

The NMDAR subunit *GRIN2B* plays an essential role in memory and learning by regulating key aspects of synaptic plasticity (Kutsuwada *et al.* 1996; Tang *et al.* 1999). Over-expression of *GRIN2B* in the forebrain of mice enhanced hippocampal long-term potentiation, spatial learning and memory and improved learning processes involved in fear extinction (Tang *et al.* 1999). Knockout studies of *GRIN2B* indicate that this subunit is essential for development, neuronal patterning and synaptic transmission in the CA1 region of hippocampal slices (Kutsuwada *et al.* 1996). GRIN2B protein is highly expressed throughout the embryonic brain in rodents, but after birth, the protein becomes restricted to the forebrain (Watanabe *et al.* 1992). High levels of GRIN2B protein are detected in the hippocampus (Monyer *et al.* 1994) and layer II neurons of the PFC (Rudolf *et al.* 1996), brain areas that are critical for spatial learning and memory tasks, and executive function, respectively. Similarly, in adult human brain slices, GRIN2B protein is highly expressed in the pyramidal cells of the frontal and parietotemporal cortex, with lower expression in hippocampal and basal ganglia structures (Schito *et al.* 1997). Given its spatially restricted expression in frontostriatal brain structures and its critical role in learning and memory, it is possible that *GRIN2B* contributes to the cognitive deficits associated with ADHD.

We hypothesized that *GRIN2B* represents a candidate gene for ADHD. We genotyped nine polymorphisms within the *GRIN2B* gene in a large clinically ascertained sample of probands with ADHD, affected siblings and their parents. We tested for association between these *GRIN2B* alleles and ADHD using the transmission/disequilibrium test (TDT), which tests for the biased transmission of alleles from heterozygous parents to their affected offspring. For polymorphisms that showed significant evidence of association, we employed a quantitative trait TDT approach to test for association with the different ADHD symptom dimensions (IA and HI). We also examined whether a relationship existed between these alleles and the quantitative traits of verbal short-term memory and verbal working memory.

Materials and methods

Study sample

The study sample included 205 small nuclear families from the Toronto area, with 47 affected siblings. A total of 252 affected children between the ages of 7 and 16 (204 boys and 48 girls) were used in this study. The ethnic background of the sample was largely of European Caucasian descent, with 10% comprising other or mixed ethnic backgrounds

including African, Chinese, Indian and Native American. Genotyping of both parents was achieved in 150 families, and in 55 families, only one parent was genotyped. Division of the affected children among the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) ADHD subtypes was 27% predominantly IA, 14% predominantly HI and 59% of the combined subtype. The mean IQ of the sample as measured by the Wechsler Intelligence Scale for Children III (WISC-III) full-scale performance was 102.9 ± 12.8. The means and distribution for the ADHD symptom dimensions were as follows: IA parent-reported mean = 5.8 ± 2.0 (n = 229), HI parent-reported mean = 5.6 ± 2.3 (n = 229), IA teacher-reported mean = 5.3 ± 2.2 (n = 219) and HI teacher-reported mean = 4.3 ± 2.8 (n = 219).

Diagnostic assessment of ADHD

Probands and affected siblings meeting the criteria for ADHD as outlined in the DSM-IV (American Psychiatric Association, 1994) were recruited from the Child Development and Neuropsychiatry Clinics at the Hospital for Sick Children (Toronto, Canada). The criteria for assessment and diagnosis of subjects used for this study have been previously described (Adams *et al.* 2004; Barr *et al.* 1999). In brief, information about ADHD symptoms was collected from semistructured interviews conducted with parents (Parent Interview for Child Symptoms (PICS)-IV) (Ickowicz *et al.* 2006) and teachers (Teacher Telephone Interview (TTI)-IV). Additional information was obtained from supplementary assessments and questionnaires: Children's Depression Inventory (Kovacs 1995), Children's Manifest Anxiety Scale (Reynolds & Richmond 1985), Clinical Evaluation of Language Fundamentals 3rd Edition (Semel *et al.* 1995), Conners Parent and Teacher Rating Scales-Revised (Conners 1997) and Ontario Child Health Survey Scales-Revised (Boyle *et al.* 1993). Exclusion criteria included scoring below 80 on both the performance and the verbal scales of the WISC-III (R. T., The Hospital for Sick Children, unpublished results; Wechsler 1991), evidence of neurological or pervasive developmental disorder, bipolar affective disorder, psychosis, Tourette syndrome or chronic multiple tics and/or a first-degree relative diagnosed with bipolar affective disorder or schizophrenia. Children were medication free for a minimum of 24 h prior to assessment and cognitive testing. Approval for this protocol was obtained from the Hospital for Sick Children Research Ethics Board. Informed written consent and assent were obtained for all subjects.

Cognitive phenotype measures

Measures of verbal short-term and working memory were used in this genetic study. Verbal working and short-term memory was evaluated using the digit span subtest of the WISC-III (Wechsler 1991). This assessment provides an overall memory score that is divided into two parts, digits forward (DF) and digits backward (DB). The DF measures verbal short-term memory, whereby the experimenter reads aloud a sequence of digits (1/second) and the subject is asked to repeat them in the order in which they were presented. The DB measures verbal working memory, whereby the experimenter reads a series of numbers aloud and the subject is required to repeat the numbers in reverse order. Both components were corrected for age using population-based norms (Kaplan *et al.* 1999). The means and distribution for digit span performance were as follows: digit span forward mean = 9.1 ± 2.8 (n = 166) and digit span backward mean = 8.8 ± 3.1 (n = 166).

Genotyping

Single nucleotide polymorphisms (SNPs) were genotyped for this study using an ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the TaqMan 5′ nuclease assay for allelic discrimination. SNPs used in the analysis were selected from confirmed polymorphisms in public databases with probes and primers available commercially (Assay on Demand or Assay by Design) from Applied Biosystems. Polymerase chain reaction (PCR) mixture (5 μl) contained 30 ng genomic DNA, 2 μl TaqMan Universal PCR Master Mix (Applied Biosystems) and 0.1 μl allelic discrimination mix (20× mix, Applied Biosystems) containing 36 μm of each primer and 8 μm of each probe. Thermal cycling conditions were 95°C for 1 min, followed by 40–50 cycles of 95°C for 15 seconds, 58–60°C for 1 min. Results were obtained from the allelic discrimination end-point analysis mode of the ABI 7900HT software package version 2.0.

Statistical analysis

For the categorical analysis of ADHD, we used the extended TDT (ETDT) program (Sham & Curtis 1995) to test for the biased transmission of individual alleles. Transmission of haplotypes was analyzed using the TRANSMIT program (Clayton 1999). Haplotypes with a frequency of less than 10% were not included in the analysis. To calculate the coefficients of linkage disequilibrium (LD), D' and r^2, between marker alleles in the parental chromosomes, we used HAPLOVIEW v2.03 (Barrett *et al.* 2005). Permutation-based analysis was performed using the UNPHASED program (Dudbridge 2003). The FBAT program (Horvath *et al.* 2001) was used to analyze *GRIN2B* variants in relation to ADHD symptom dimensions of IA and HI, and the cognitive phenotypes of verbal short-term memory and verbal working memory. As is recommended in the application of the FBAT statistic, an offset was used in the quantitative TDT analyses. The choice of offset varied by trait and was based on mean scores derived from the population.

Results

For this study, we investigated genetic variation in the NMDAR 2B subunit gene (*GRIN2B*) in relation to ADHD by genotyping nine *GRIN2B* SNPs in 205 nuclear families identified through a proband with ADHD. The organization of the *GRIN2B* gene is shown in Fig. 1, and the genomic location of the markers (SNPs) is illustrated in Fig. 1 and Table 1.

We tested for biased transmission of the alleles for each variant using the TDT statistic, obtained using the ETDT program (Sham & Curtis 1995) (Table 1). Significant TDT results ($P < 0.05$) or a trend toward significance was observed for four SNPs located in intron 3, rs2268115, rs2300256, rs2284411 and rs2284407. Following permutation-based analysis to correct for multiple comparisons, the global significance level for rs2284411 was 0.03. We did not observe biased transmission of any of the remaining SNPs. The intron 3 SNPs are clustered upstream of exon 4, and as such, we sequenced this area in a subset of probands with ADHD ($n = 41$). In this sample, we did not find any variations within the exon 4 sequence or within or around splice sites.

The degree of LD varied among the nine SNPs examined (Table 2). This most likely is a factor of the wide distribution of the SNPs, the large genomic size of *GRIN2B* and its complex LD structure. HapMap genotyping in the Centre d'Etude de Polymorphisme Utah (CEU) population revealed over 40 haplotype blocks with more than 120 common haplotypes at frequencies greater than 10%. Five of the nine selected SNPs fall within separate haplotype blocks.

Using the TRANSMIT program (Clayton 1999), we performed haplotype analysis on the four positive SNPs (Table 3). The global transmit results were significant for haplotypes with a frequency greater than 10% [$\chi^2 = 13.835$, 4 degrees of freedom (df), $P = 0.008$] and for all haplotypes ($\chi^2 = 25.126$, 9 df, $P = 0.003$).

We next performed quantitative trait TDT analysis using the FBAT program (Horvath *et al.* 2001) to evaluate the relationship between the four positive SNPs and the ADHD symptom dimensions of IA and HI. Scores for these quantitative traits were ascertained through interviews with both parents and teachers. The results of the FBAT analyses are shown in Table 4. Symptom scores showed a significant association of markers rs2300256 and rs2284411 with IA (parent reported and/or teacher reported) and a trend for rs2268115 and rs2284407 (parent reported). For HI, a significant association was observed for rs2284407 and a trend toward significance for rs2268115 (parent reported). Therefore, *GRIN2B* markers were associated with ADHD as observed using both a categorical definition (Table 1) and ADHD symptoms using quantitative trait analysis (Table 4).

Last, we investigated whether there was an association between the four positive SNPs in intron 3 and verbal short-term and working memory. Scores for these quantitative traits were obtained using the digit span test. FBAT results are shown in Table 4. We did not observe a significant association between the positive intron 3 SNPs and short-term or working memory.

Discussion

We have shown an association of variants in the *GRIN2B* gene and ADHD. In our sample, four of the nine SNPs we genotyped showed biased transmission. Quantitative trait analysis of these four markers indicated an association with the IA and HI symptom dimensions of ADHD. In contrast, there was no significant association of *GRIN2B* with verbal short-term or working memory.

GRIN2B is a moderately large gene comprising 13 exons and spanning a genomic region of ~400 kb located at chromosome 12p13.1. The four positive *GRIN2B* markers are all located within intron 3 and therefore do not alter the amino acid sequence of the protein. However, these changes may have an effect on the regulatory aspects of *GRIN2B* expression such as transcription, messenger RNA processing, nuclear export or alteration of secondary structure. Alternatively, rather than having a direct functional effect, the positive variants identified in this study are more likely to be in LD with a functional variant. Screening of the *GRIN2B* gene in patients with schizophrenia failed to identify SNPs that alter the coding sequence (Nishiguchi *et al.* 2000; Ohtsuki *et al.* 2001; Williams *et al.* 2002). Similarly,

sequencing of exon 4 in 41 probands with ADHD in our sample did not show any functional changes. These results may reflect the high selective pressure imposed on the *GRIN2B* sequence. Indeed, the human *GRIN2B* gene has 98% overall amino acid sequence identity with mouse and rat sequences (Ishii *et al.* 1993; Kutsuwada *et al.* 1992; Schito *et al.* 1997).

Our haplotype analysis of the four most significant markers reached global significance; however, no single haplotype was more informative than single markers because the biased transmissions were split across several haplotypes. Haplotype analysis of the remaining markers was not performed. Determining the best method for the analysis of haplotypes when data from multiple markers are available is currently a subject of investigation by a number of groups, with yet no clear consensus. In some cases, the use of EHAP software has proven fruitful (Seltman *et al.* 2001, 2003). EHAP software allows one to use the evolutionary relationship that exists among haplotypes to focus on specific groups, thus increasing the power of the analysis and maximizing the information gained from the transmissions of the alleles (Seltman *et al.* 2001). However, this method is recommended for regions of high LD and, for this reason, is not suitable for examining the markers selected in this study.

Quantitative TDT analysis showed a moderate association between preferentially transmitted alleles and the ADHD symptom dimensions of IA and HI. This suggests that variants in *GRIN2B* contribute to both symptom dimensions. Twin studies indicate that there will be genes common to both dimensions as well as genes unique to each dimension (Hudziak *et al.* 1998; Sherman *et al.* 1997; Willcutt *et al.* 2000). Our data indicate that *GRIN2B* falls in the former category.

We did not observe a relationship between *GRIN2B* and verbal working memory or verbal short-term memory as measured using the digit span test. This is in contrast to the critical role *GRIN2B* plays in memory acquisition and storage (Loftis & Janowsky 2003). We cannot exclude the possibility that *GRIN2B* may contribute to alternate cognitive processes such as spatial working memory. For example, spatial memory is enhanced in animal models when *GRIN2B* is overexpressed in the PFC (Tang *et al.* 1999) and impaired when NMDARs are blocked in nonhuman primates (Taffe *et al.* 2002). A recent meta-analysis on childhood ADHD studies determined a larger overall effect size for spatial working memory vs. verbal working memory, suggesting that spatial working memory may comprise a larger component of the cognitive findings in ADHD (Martinussen *et al.* 2005). However, a recent meta-analysis did not support deficits in visuospatial working memory in studies of adults with ADHD (Hervey *et al.* 2004). Future work could investigate spatial memory tasks to determine if *GRIN2B* plays a role in possible memory deficits in populations with ADHD.

Collectively, our data suggest the involvement of variations in the *GRIN2B* subunit gene and ADHD. Replication of these results in independent ADHD samples would further support a role for the *GRIN2B* gene in contributing to this disorder. Further investigation with the aim of identifying the functional variants for *GRIN2B* that underlie ADHD is warranted and will aid in the clarification of the molecular mechanisms underlying this disorder.

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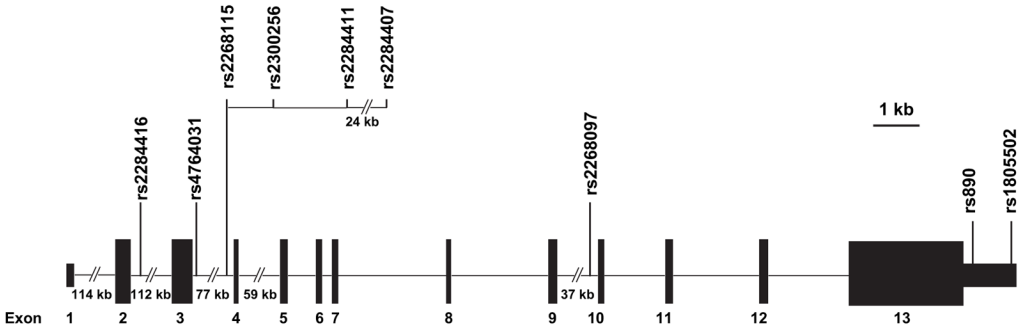

Figure 1. Schematic representation of the genomic organization of the human *GRIN2B* gene (NT_009714.16)

The location of nine polymorphic sites (SNPs) are indicated. 5′- and 3′-untranslated sequences are represented by small black boxes and exonic sequences are represented by large black boxes.

Table 1

Transmission/disequilibrium test analysis and allele frequencies of the *GRIN2B* markers in ADHD

SNP	Location	Chromosome location*	Allele	Frequency†	Transmissions	Nontransmissions	χ^2	*P* value‡
rs2284416	Intron 2	13 810 481	T	0.489	97	83	1.089	0.297
			G	0.511	83	97		
rs4764031	Intron 3	13 780 840	A	0.621	82	76	0.228	0.695
			C	0.379	76	82		
rs2268115	Intron 3	13 760 992	T	0.563	100	70	5.294	0.021
			G	0.437	70	100		
rs2300256	Intron 3	13 759 677	G	0.484	102	78	3.200	0.074
			A	0.516	78	102		
rs2284411	Intron 3	13 757 439	T	0.354	95	60	7.903	0.005
			C	0.646	60	95		
rs2284407	Intron 3	13 733 473	G	0.615	93	66	4.585	0.032
			T	0.385	66	93		
rs2268097	Intron 9	13 644 099	A	0.632	74	71	0.062	0.803
			G	0.368	71	74		
rs890	3′ UTR	13 606 575	A	0.505	81	71	0.658	0.417
			C	0.495	71	81		
rs1805502	3′ UTR	13 605 448	A	0.814	55	52	0.084	0.772
			G	0.186	52	55		

*University of California, Santa Cruz (UCSC) May 2004 National Center for Biotechnology Information (NCBI) Build 35.

†Allele frequencies are reported for the parental chromosomes in this sample.

‡1 df, two sided.

UTR, untranslated region.

Table 2

Linkage disequilibrium between *GRIN2B* markers in this sample

	SNP	1	2	3	4	5	6	7	8	9
1	rs2284416		0.20	0.16	0.17	0.21	0.06	0.29	0.13	0.04
2	rs4764031	0.03		0.45	0.56	0.38	0.41	0.19	0.13	0.46
3	rs2268115	0.02	0.15		0.99	1.00	0.55	0.13	0.06	0.03
4	rs2300256	0.03	0.17	0.72		0.63	0.65	0.17	0.04	0.00
5	rs2284411	0.02	0.05	0.42	0.24		0.47	0.04	0.17	0.18
6	rs2284407	0.00	0.16	0.24	0.25	0.07		0.47	0.10	0.44
7	rs2268097	0.05	0.04	0.01	0.02	0.00	0.21		0.39	0.79
8	rs890	0.02	0.01	0.00	0.00	0.02	0.01	0.09		0.92
9	rs1805502	0.00	0.03	0.00	0.00	0.00	0.03	0.08	0.19	

D' values are shown in the upper half of the table, r^2 values in the lower half of the table.

Table 3

Haplotype analysis of the *GRIN2B* markers in ADHD

	Polymorphism									
Haplotype	**rs2268115**	**rs2300256**	**rs2284411**	**rs2284407**	**Haplotype frequency***	**Observed†**	**Expected‡**	**Var(*O* – *E*)§**	**χ^2 (1 df)¶**	***P* value#**
G1	T	G	T	G	0.244	96.134	82.719	27.354	6.579	0.013
G2	T	A	T	G	0.170	61.024	53.668	18.115	2.987	0.084
G3	T	A	C	T	0.162	47.386	57.828	18.497	5.894	0.014
G4	G	A	C	T	0.277	89.114	95.427	29.876	1.334	0.856

Global chi-squared test on 4 df for haplotypes with frequencies greater than 10% = 13.835, $P = 0.008$.

*Only haplotypes with a frequency greater than 10% were used in the analysis.

†Test statistic representing the observed number of transmissions.

‡Expected values of the test statistic under the null hypothesis of no linkage or association.

§Variance of (observed – expected) transmissions.

¶(Observed – expected)^2/ Var($O - E$).

#1 df, two sided.

Table 4

FBAT analysis of *GRIN2B* allele transmission in relation to verbal short-term and working memory and the ADHD symptom dimensions of IA and HI

		n^*	$S^\dagger$	E(S)‡	Var(S)	Z	$P^\S$	n^*	$S^\dagger$	E(S)‡	Var(S)	Z	$P^\S$
Marker	**Allele**	**PICS IA symptoms**						**TTI IA symptoms**					
rs2268115	T	92	549	493	830	1.941	0.052	91	536	494	850	1.431	0.153
	G		440	496					439	480			
rs2300256	G	110	607	537	959	2.267	0.023	109	587	538	986	1.565	0.118
	A		558	628					568	617			
rs2284411	T	98	466	397	785	2.451	0.014	96	450	387	772	2.281	0.023
	C		561	630					538	601			
rs2284407	G	91	631	580	796	1.813	0.070	90	610	566	739	1.630	0.103
	T		372	423					347	391			
		PICS HI symptoms						TTI HI symptoms					
rs2268115	T	92	618	560	957	1.902	0.057	90	426	393	709	1.235	0.217
	G		450	509					372	405			
rs2300256	G	110	613	563	1027	1.569	0.116	108	441	421	905	0.649	0.517
	A		587	638					530	550			
rs2284411	T	98	473	437	871	1.226	0.220	96	325	291	656	1.327	0.184
	C		642	678					454	488			
rs2284407	G	92	689	629	923	2.003	0.045	88	487	451	689	1.376	0.169
	T		385	446					288	324			
		Digit span forward						Digit span backward					
rs2268115	T	68	−135	−114	242	−1.320	0.187	68	−107	−102	285	−0.240	0.811
	G		−95	−115					−109	−113			
rs2300256	G	84	−129	−120	290	−0.549	0.583	83	−102	−100	314	−0.104	0.917
	A		−139	−149					−166	−168			
rs2284411	T	74	−89	−66	248	−1.473	0.141	74	−47	−72	271	1.549	0.121
	C		−123	−146					−179	−154			
rs2284407	G	67	−151	−142	260	−0.571	0.568	67	−80	−78	274	−0.103	0.918
	T		−66	−75					−77	−78			

*Number of informative families.

[†]Test statistic.

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

[§]1 df, two sided.

PICS, Parent Interview for Child Symptoms; TTI, Teacher Telephone Interview.