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Genetic variants in SLC9A9 are associated with measures of Attention-deficit/hyperactivity disorder symptoms in families

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Abstract

Objective—A family was previously identified that cosegregates a pericentric inversion, inv(3)(p14 : q21), with an early-onset developmental condition, characterized by impulsive behavior and intellectual deficit. The inversion breakpoints lie within DOCK3 and SLC9A9 at the p-arm and q-arm, respectively. Based on this report, these genes were selected to be evaluated in a family-based attention-deficit/hyperactivity disorder (AD/HD) association study.

Methods—Conners' Parent (CPRS) and Teacher (CTRS) Rating Scales of AD/HD symptoms and Conners' Continuous Performance Test (CPT) measures were collected and a minimal number of tagging singlenucleotide polymorphisms (SNPs) in each gene were selected for analysis. Analyses were performed on families who met research criteria for AD/HD. Using the program, QTDT, each tagging SNP was tested for association with *T*-scores from the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) subscales according to the CTRS and CPRS, and five CPT measures.

Results—After adjusting for multiple testing, a SNP in the 3′ UTR of SLC9A9, rs1046706, remained significantly associated (false discovery rate, q value < 0.05) with scores on the DSM-IV hyperactive-impulsive and total symptom subscales according to the CTRS and errors of commission on the CPT. In addition, an intronic SLC9A9 SNP, rs2360867, remained significantly associated with errors of commission.

Conclusion—Our results suggest that SLC9A9 may be related to hyperactive-impulsive symptoms in AD/HD and the disruption of SLC9A9 may be responsible for the behavioral phenotype observed in the inversion family. The association with SLC9A9 is particularly interesting as it was recently implicated in a genome-wide association study for AD/HD. Further investigation of the role of SLC9A9 in AD/HD and other behavioral disorders is warranted.

Keywords

attention-deficit/hyperactivity disorder; Conners' Continuous Performance Test; Conners' Parent Rating Scale; Conners' Teacher Rating Scale; genetics; psychiatry; single-nucleotide polymorphism

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Introduction

Attention-deficit/hyperactivity disorder (AD/HD) is a common psychiatric disorder, characterized by inattention and/or hyperactivity and impulsivity, which are considered extreme for an individual's developmental level. According to the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) diagnostic criteria for AD/HD, there are three main subtypes: combined type, predominately inattentive type, and predominately hyperactive-impulsive type. The estimated prevalence of AD/HD for school-aged children is 3–7%, with males more commonly affected than females (Albayrak *et al.*, 2008; Wallis *et al.*, 2008).

AD/HD has a complex etiology, influenced by both genetic and environmental factors. Evidence for a strong genetic component is characterized by familial clustering, heritability estimated at 0.8, and support from twin and adoption studies (Albayrak *et al.*, 2008). There have been numerous candidate gene association studies [reviewed in Ref. (Bobb *et al.*, 2005)], a handful of genome-wide linkage studies (Fisher *et al.*, 2002; Bakker *et al.*, 2003; Arcos-Burgos *et al.*, 2004; Hebebrand *et al.*, 2006; Ogdie *et al.*, 2006; Asherson *et al.*, 2008; Romanos *et al.*, 2008), and multiple whole-genome association studies published to date for AD/HD [reviewed in Ref. (Franke *et al.*, 2009)]. Positive association signals in several candidate genes, including DAT1, DRD2, DRD4, DRD5, 5-HTT, 5-HT2A, and SNAP25, have been replicated in multiple studies, but none seem to have a large effect size (Wallis *et al.*, 2008). There has also been limited success in identifying linkage regions that replicate across studies (Wallis *et al.*, 2008). Thus, while much progress has been made in the field of AD/HD genetics it is clear that much remains to be investigated.

Importantly, much of the genetic analysis for AD/HD defined the phenotype from a qualitative perspective; the presence or absence of an AD/HD diagnosis. A more recent trend in the study of AD/HD genetics has been to focus on endophenotypes or intermediate phenotypes of AD/HD. As proposed originally by Gottesman and Gould (2003). endophenotypes for neuropsychiatric conditions like AD/HD are appealing because the qualitative phenotypes are often fraught with phenotypic heterogeneity resulting in a loss of power to identify genetic associations. There are several benefits from using an endophenotype: (i) the trait is quantitative which should provide more power over a dichotomous trait, (ii) both unaffected and affected individuals can be included in the analysis which increases the sample size, and (iii) it is an intermediate phenotype which should be closer to the primary cause(s) of the disorder and thus may help to address phenotypic heterogeneity. Endophenotypes for neuropsychiatric conditions can take a variety of forms, including rating scales, biomarkers, and neuroanatomical features. Our group has previously used and discussed the rationale for selecting Conners' Continuous Performance test (CPT) measures as AD/HD endophenotypes (Kollins *et al.*, 2008). The CPT is administered on the computer where a series of target and nontarget letters flash on the screen and the individual is instructed to press the spacebar as quickly as possible after he/she views a target letter. A series of measurements can then be obtained from these test results (Table 1). We, and others, have shown that some of these CPT-type measures are correlated with AD/HD symptoms or affection status (Epstein *et al.*, 2003; Kollins *et al.*, 2008) and are heritable (Groot *et al.*, 2004; Kuntsi *et al.*, 2006; Kollins *et al.*, 2008). In our data set, heritabilities for these measures ranged from 28 to 57% (Kollins *et al.*, 2008). Both these features, the correlation with affection status and having a heritable component, are important for selecting an appropriate endophenotype for genetic analysis (Castellanos and Tannock, 2002). Another approach to define endophenotypes is to focus on symptom counts, either in raw form, or based on *T*-scores from questionnaires. In this study, we focused on endophenotypes derived from both the CPT and questionnaire data.

Historically, most candidate genes for AD/HD were selected based on their involvement in neurotransmission. In this analysis, we elected to perform a candidate gene study, but chose an alternative approach for defining AD/HD candidate genes, focusing on genes located at cytogenetic breakpoints that have been associated with AD/HD-like phenotypes. Our candidate gene selection was based on a report of a family that cosegregates a pericentric inversion with an early onset developmental condition, characterized by impulsive behavior and intellectual deficit (De Silva *et al.*, 2003; Efron *et al.*, 2003). A total of eight children, four with and four without the inversion, were clinically evaluated. Children with the inversion exhibited more signs of conduct problems, hyperactive-impulsive tendencies, and learning problems according to the Conners' Parent Rating Scale (CPRS) (Efron *et al.*, 2003). In addition, the mean intelligence quotient (IQ) was significantly lower (P=0.03) among individuals with the inversion compared with individuals without the inversion (Efron *et al.*, 2003). At the time the De Silva *et al.* (2003) and Efron *et al.* (2003) manuscripts were published, the inversion breakpoints were described as being located in the 3p14 and 3q21 bands as determined by cytogenetic analysis using G-banding. The group later mapped the precise location of the inversion breakpoints and found that they occurred within intron 19 of the dedicator of cytokinesis 3 (DOCK3) and intron 13 of the solute carrier family 9 (sodium/hydrogen exchanger) isoform 9 (SLC9A9) at the p-arm and q-arm, respectively (De Silva *et al.*, 2003). The true cytogenetic locations of DOCK3 and SLC9A9 are 3p21 and 3q24, respectively, and we believe the low resolution of the G-banding technique led to the discrepant cytogenetic positions in the original report. In addition to mapping the inversion breakpoints, De Silva *et al.* (2003) assessed the spatial expression patterns for both DOCK3 and SLC9A9 in the brain. They found that DOCK3 was expressed at the highest levels in the frontal, temporal, and occipital lobes, whereas SLC9A9 was expressed highest in the medulla and spinal cord (De Silva *et al.*, 2003). Given these data, it is possible that the disruption of one or both of these genes contribute to the behavioral phenotype segregating in this family (De Silva *et al.*, 2003). There are several mechanisms by which this inversion may produce a phenotypic effect, including disruption of a regulatory region, haploinsufficiency of one or both genes, or the creation of a novel or abnormal protein with altered function (De Silva *et al.*, 2003).

DOCK3, a presenilin-binding protein, belongs to the DOCK180 family of proteins and is highly expressed in the neurons (Kashiwa *et al.*, 2000; Cote and Vuori, 2002; Namekata *et al.*, 2002). There is additional supporting evidence for the 3p21 region and DOCK3 as AD/HD candidates. A genome-wide association study for bipolar disorder (BPD) identified two SNPs within DOCK3 that were significantly associated with BPD in two independent populations (Baum *et al.*, 2008). This is of interest because BPD and AD/HD share some overlapping characteristics and sometimes co-occur. In addition, a previous study described an individual with a 3p21.31 duplication who exhibited many AD/HD-like characteristics, such as hyperactive-impulsive behaviors, attention deficits, and poor school performance (Stuart *et al.*, 2007).

SLC9A9 is a Na+/H+ exchanger protein that is localized to the golgi and postgolgi endocytic compartments (Nakamura *et al.*, 2005). Additional studies also support SLC9A9 as an AD/HD candidate. A family-based association study found several SNPs in different regions of SLC9A9 that were nominally associated (most significant result: P=0.01, odds ratio=1.61) with the DSM-IV combined type AD/HD (Brookes *et al.*, 2006). In addition, a genome-wide linkage analysis for AD/HD identified a marker located in SLC9A9, D3S1569, that showed weak linkage (single-point logarithm of the odds: 1.37) with AD/HD (Fisher *et al.*, 2002). Among the candidate genes examined in a recent genome-wide association study, SLC9A9 showed the strongest evidence for association with AD/HD measures (Lasky-Su *et al.*, 2008).

Based on the inversion, inv(3)(p21 : q24), that cosegregates with an AD/HD-like phenotype in a family and the other supportive studies described above, we selected DOCK3 and SLC9A9 as candidates to be evaluated in a family-based AD/HD genetic association study.

Methods

Study population

A total of 167 families were ascertained for the study if at least one child between the ages of 5 and 12 years met enrollment criteria. A detailed description of the study population is provided in Table 2. Families were enrolled if the proband: (i) met DSM-IV criteria for AD/HD, (ii) was not home-schooled (so that parent and teacher informants were independent), (iii) had a full scale IQ estimate greater than 70 as determined by the Wechsler Intelligence Scale for Children, Fourth Edition intelligence test vocabulary and block design subtest scores (individuals who scored between 70 and 80 had to score greater than 70 on the Vineland Adaptive Behavior Scale Adaptive Behavior Composite Score), and (iv) had Clinical Global Impressions Scale Severity Scores ≥3. Families were excluded from the study if the child: (i) was not the biological child of the primary caregiver, (ii) was diagnosed with a pervasive developmental disorder, (iii) displayed significant signs of developmental delay, or (iv) had a coexisting medical, neurological, or genetic disorder. Families were ascertained from two collection sites: Duke University Medical Center, North Carolina and University of North Carolina, Greensboro, North Carolina. All participating family members provided written informed consent that had been approved by both institutional review boards.

Conners' Parent and Teacher Rating Scales

The revised, long versions of the CPRS and CTRS of AD/HD symptoms were collected on affected and unaffected siblings aged 5–18 years (Conners, 1997). We focused on the Conners' DSM-IV subscales: inattentive, hyperactive-impulsive, and total symptom count, and used *T*-scores, which are adjusted for age and sex, for all analyses.

T-scores are scores that are relative to a normative group, or the population average. For example, a *T*-score of 50 means that the participant obtained a score that is considered average for individuals from the normative group of the same sex and age. The normative sample for these measures include 2482 and 1973 children and adolescents for the CPRS and CTRS, respectively (Conners, 1997). Individuals aged 3–17 years were recruited from over 200 schools in over 10 provinces and 45 states in Canada and the US (Conners, 1997). The composition of ethnic groups was similar to our own study population, although it is suggested that the standard norms for the revised Conners' rating scales are robust to differences in ethnicity (Conners, 1997).

Conners' Continuous Performance Test

All willing family members were administered the CPT. The CPT and associated measurements have been previously described in detail (Conners and MHS Staff, 2000). Participants completed the CPT on a desktop computer in a room with minimal distractions. Three hundred and sixty letters appeared on the screen for roughly 14 min and participants were instructed to press the spacebar whenever a target letter (non-'X') appeared on the screen. Five measurements from the CPT were used for our analysis (Table 1). Descriptions on how to interpret these measurements are also provided in Table 1.

T-scores, which are adjusted for age and sex, were calculated for all measurements. The normative data consist of 1920 individuals ascertained through two different studies from 17 states and three provinces in the US and Canada (Conners and MHS Staff, 2000). The

general population norms seem to be applicable to minority groups (African–Americans and Asians) (Conners and MHS Staff, 2000).

Genotyping and quality control

A subset of our parents and children (*n*=452 individuals) were genotyped using the Illumina Infinium Human-Hap300 duo chip (Illumina, Inc., San Diego, California, USA). As described in detail below, individuals (*n*=204) who were ascertained after the initial genome screen were genotyped using TaqMan 'Assays-on-Demand' or 'Assays-by Design' products (Applied Biosystems, Foster City, California, USA).

Quality of the Illumina data was assessed using PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell *et al.*, 2007). SNPs (*n*=315 980) were submitted for quality checks. Call rates exceeded 98% for all 452 individuals, one individual was excluded because of a sex discrepancy, and two individuals were excluded because of per-family Mendelian errors in excess of 1%. Out of the 315 980 SNPs submitted, 6109 SNPs were excluded because of a minor allele frequency (MAF) less than 0.05, 13 SNPs were excluded because of Mendelian errors in more than 4 families, and 629 SNPs were excluded because of deviations from Hardy–Weinberg equilibrium (HWE) ($P<0.000001$). In total, three individuals and 6751 SNPs did not pass our quality control checks.

To address the multiple testing problem, a minimal number of haplotype-tagging SNPs present on the Illumina chip were selected for analysis using LDSelect (DOCK3: *n*=10, $r^2>0.8$; SLC9A9: *n*=79, $r^2>0.64$) (Carlson *et al.*, 2004). A subset of these tagging SNPs were also selected as TaqMan assays (Applied Biosystems) to type in the more recently ascertained study participants to increase sample size, and ultimately power in the data set. All DOCK3 SNPs (*n*=10) and a subset of the SLC9A9 SNPs (*n*=15) were included in the expanded screen. SLC9A9 SNPs were selected if they tagged at least one additional SNP or were positioned close to an intron/exon border. Quality control for TaqMan genotype data was as follows: (i) two Centre d'Etude du Polymorphism Humain controls and blinded duplicates were used for every 94 samples and required to match 100%, and (ii) pedigree inconsistencies were identified using PEDCHECK (O'Connell and Weeks, 1998); families were excluded from subsequent analyses for markers if the inconsistencies could not be resolved.

As stated above, the total number of individuals genotyped for each SNP varied because of the costs associated with genotyping individuals ascertained after the initial genome screen. Six hundred and twenty-six individuals were genotyped for all 10 DOCK3 SNPs, whereas the number of individuals genotyped for the 79 SLC9A9 SNPs varied (minimum=422 individuals and maximum=626 individuals). SAS 9.1 (SAS Institute, Cary, North Carolina, USA) was used to calculate the MAF using all individuals and, because of our limited sample size, a MAF less than 10% was used as a further exclusion criterion for SNPs selected for analysis. All SNPs were tested for significant deviations from HWE using exact tests by the Genetic Data Analysis Program (Zaykin *et al.*, 1995). HWE was calculated separately for affected and unaffected individuals. Specifically, the first affected and first unaffected individual from each family were used for the analysis.

Statistical analysis

Heritability was estimated for all traits using Sequential Oligogenic Linkage Analysis Routines (Almasy and Blangero, 1998). The polygenic command was used in Sequential Oligogenic Linkage Analysis Routines, which provides an estimate of the total additive genetic heritability. The software program, QTDT (http://www.sph.umich.edu/csg/abecasis/QTDT/index.html), was used to test for association

in our families. Specifically, we used the orthogonal association model (-ao) while modeling environmental, polygenic, and additive major locus effects (-wega) (Abecasis *et al.*, 2000a). Empirical *P* values were estimated from 1000 Monte Carlo permutations, which makes this test robust to small sample sizes and non-normal data (Abecasis *et al.*, 2000b). To adjust for multiple testing, we used the Benjamini–Hochberg false discovery rate (FDR) procedure to correct for the number of SNPs evaluated across both genes (n=89) and used an FDR-adjusted *P* value (*q* value) threshold of 0.05 to determine significance (Benjamini and Hochberg, 1995). SAS 9.1 was used to calculate the FDR *q* values. We did not adjust for the number of phenotypes tested because there is not an ideal way to properly account for the fact that many of the traits are highly correlated. To assist in the interpretation of our results, we calculated the pairwise linkage disequilibrium [LD (D′) and r^2] between all selected tagging SNPs using GOLD (Abecasis and Cookson, 2000). The teacher-generated hyperactive-impulsive *T*-scores and the CPT measurement, variability, were log-transformed for all analyses to achieve a more normally distributed phenotype.

Results

Heritability of quantitative traits

Heritabilities of all quantitative traits were estimated using our study population (Table 3). None of the DSM-IV subscales from the CPRS were significantly heritable, whereas scores on the hyperactive-impulsive and total symptom subscales according to the CTRS and all CPT scores, except variability, were significantly heritable.

Association analyses

Using QTDT, each tagging SNP was tested for association with parent and teacher DSM-IV subscales and five CPT measurements. We identified two SNPs in SLC9A9 that remained significant after applying an FDR *q* value threshold of 0.05 to correct for testing 89 SNPs (Table 4 and Fig. 1). None of the 10 DOCK3 tagging SNPs remained significantly associated with any of the 11 quantitative traits examined. The first significant SNP, rs1046706, in the 3′UTR of SLC9A9 was associated with *T*-scores on the DSM-IV hyperactive-impulsive and total symptom subscales according to the CTRS and the CPT measure, errors of commission (Table 4). The major allele (T) was associated with higher hyperactive-impulsive symptom counts [55.23±10.48 (GG), 61.21±13.68 (TG), 63.09± 13.79 (TT) (mean ± standard deviation)], total symptom counts [56.32±9.99 (GG), 65.38±12.69 (TG), 66.00±12.46 (TT)], and errors of commission [47.56±9.51 (GG), 50.91±10.94 (TG), 52.75±11.25 (TT)]. It is worth noting that rs1046706 was also nominally associated with hyperactive-impulsive (*P*= 0.001), inattentive (*P*=0.021), and total symptom scores (*P*=0.003) according to the CPRS, inattentive scores (*P*=0.007) according to the CTRS and the CPT measure, hit reaction time (*P*=0.002) (Table 4). In addition, it does not appear that rs1046706 is in high linkage disequilibrium (LD) with other typed SNPs in our population or any known SNPs in the HapMap Caucasian population (Collins *et al.*, 1998). The second significant SLC9A9 SNP, rs2360867, is intronic and remained associated (FDR *q* value <0.05) with the CPT measure, errors of commission [48.15±12.53 (TT), 50.56±10.65 (CT), 52.52±10.48 (CC)]. This intronic SNP is in high LD with other known SNPs in the HapMap Caucasian population. Of note, the 3′UTR SNP, rs1046706, and the intronic SNP, rs2360867, are in minimal LD (r^2=0.001) in our study population.

Discussion

We selected two genes, DOCK3 and SLC9A9, to be evaluated in an AD/HD family-based association study. After adjusting for multiple testing, we identified a 3′UTR SNP (rs1046706) in SLC9A9 that was significantly associated with scores on the DSM-IV

hyperactive-impulsive and total symptom subscales according to the CTRS, and the CPT measure, errors of commission. Although the following findings did not withstand correction for multiple testing, the 3′UTR SNP was also nominally associated with five additional AD/HD measures suggesting that our association finding is consistent across multiple measures of impulsivity and, to a lesser extent, inattentiveness. We do not find it particularly surprising that the 3′UTR SNP remained significantly associated with the Conners' scores according to teacher ratings, but not parent ratings given the lower heritability estimates for those measures in our study population and the fact that multiple studies have shown that informant ratings are often discordant [reviewed in Ref. (De Los and Kazdin, 2005)]. Nonetheless, we are encouraged to find that rs1046706 showed nominal associations with hyperactive-impulsive, inattentive, and total symptom subscales according to the CPRS. Interestingly, this 3′UTR SNP does not seem to be tagging additional known SNPs and may itself be functionally relevant. However, verification of this would require experimental work that was not carried out in the present study. The 3′UTR sequence contains a variety of regulatory elements that may impact gene expression levels. MicroRNA binding sites are often located in the 3′UTR of genes and polymorphisms located within or near a microRNA-binding site are particularly good functional candidates for affecting gene expression. For example, a dihydrofolate reductase 3′UTR SNP that was located 14 bp away from a miR-24 binding site caused dihydrofolate reductase overexpression by interfering with the normal function of miR-24 (Mishra *et al.*, 2007). In addition, the rate of mRNA decay is an important post-transcriptional mechanism, and can be influenced by both cis-elements found primarily in the 3′UTR and various RNA-binding proteins (Bolognani and Perrone-Bizzozero, 2008). Interestingly, these interactions which affect mRNA stability have recently been described in neurons (Bolognani and Perrone-Bizzozero, 2008).

We also identified a SNP, rs2360867, located in intron 5 of SLC9A9, which remained significantly associated with errors of commission after adjustment for multiple testing. Increased errors of commission are thought to reflect impulsive behavior, although we do not see any evidence of association with hyperactive-impulsive symptoms according to the teacher or parent rating scales. Interestingly, intron 5 of SLC9A9 has been previously associated with combined type AD/HD (Brookes *et al.*, 2006). Brookes *et al.* (2006) found weak evidence of association with rs2360867 and AD/HD (WHAP: *P* value=0.036, UNPHASED: *P* value=0.216).

SLC9A9 is ubiquitously expressed and may play a role in basic cellular functioning (Nakamura *et al.*, 2005). There is evidence suggesting that SLC9A9 interacts with RACK1, which is a scaffolding protein found in the brain (Ohgaki *et al.*, 2008). In addition, a recent study showed that SLC9A9 was significantly upregulated as a result of the RNAi knock-down of NPAS4, which is a transcription factor that is activated in response to neuronal membrane depolarization (Morrow *et al.*, 2008). Although SLC9A9 expression was unaltered in response to neuronal membrane depolarization alone (Morrow *et al.*, 2008), it seems to be either directly or indirectly regulated by a transcription factor induced by neuronal activity. Furthermore, several functional studies showed that SLC9A1 (member of the SLC9A family) null mice have increased hippocampal neuronal excitability, signs of ataxia, and seizures (Cox *et al.*, 1997; Bell *et al.*, 1999; Gu *et al.*, 2001). Interestingly, seizure-prone rat strains exhibit common characteristics of AD/HD and autism spectrum disorders (Gilby, 2008).

Given our relatively small sample size, the heritability estimates for the subscales according to the CPRS and CTRS, and the CPT measures should be interpreted cautiously. Although it appears at first glance that the CTRSs, but not the CPRSs are significantly heritable in our population, the overall trends are consistent with one another (hyperactive-impulsive

h^2>total symptom count h^2>inattentive h^2) and both the hyperactive-impulsive and the total symptom count estimated heritabilities according to the parent ratings are bordering on significance. Several studies have reported higher heritability estimates of AD/HD subscales according to parent ratings than what we have estimated in our study population (Kuntsi and Stevenson, 2001; Price *et al.*, 2001). Possible explanations for the observed discrepancy include differences in the power of the study, study population characteristics, informant characteristics, rating scale, and the analysis method used.

Although we are encouraged by our findings, we recognize that there were several limitations to our study. We had a relatively small population size and therefore did not have sufficient power to detect disease variants occurring at a low frequency or that have a small effect size. We also did not have access to an independent dataset for replication of our finding. In addition, we may not have sufficiently covered all the variation in DOCK3 and SLC9A9 as a result of selecting only SNPs that were present on the Illumina chip. Finally, we were unable to adequately address all aspects of the phenotype that cosegregated with the 3p21 : 3q24 inversion as we excluded individuals in our study with extreme developmental delay (IQ<70). Thus, one area for future study would be to focus on individuals with a lower IQ to determine whether the association with SLC9A9 extends to other IQ levels.

We found significant associations with SLC9A9 and AD/HD measures in our families. Our data suggest that disruption of this gene contributes to aspects of the AD/HD-like phenotype found in the inversion family. Our association finding of SLC9A9 with AD/HD is of particular interest as this gene has been identified as an AD/HD candidate in several studies. These include association findings in our own population and another independent population (Brookes *et al.*, 2006; Lasky-Su *et al.*, 2008), suggestive evidence for linkage in one study (Fisher *et al.*, 2002), and an inversion that directly disrupts SLC9A9 cosegregates with an AD/HD-like phenotype in a family (De Silva *et al.*, 2003; Efron *et al.*, 2003). Our findings, combined with the findings of earlier studies, warrant further investigation of SLC9A9 in relation to AD/HD and other behavioral disorders.

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Fig. 1.
SLC9A9
SLC9A9 gene diagram with significant SNPs shown (Microsoft Office Visio, Microsoft, Washington DC, USA).

Table 1

Description of CPT measures[a,b]

CPT measurement	Description	Interpretation
Errors of commission	Number of times a nontarget ('X') was responded to	Response inhibition/impulsivity; higher scores reflect impulsive or fast response sets
Hit reaction time	Mean response time to all targets	Fast reaction times with high commission errors indicate impulsivity
Hit reaction time SE	Variability of hit reaction times	Refers to erratic nature of responding and may represent attentional lapses
Detectability	Derived from signal detection theory, this index is the distance between the signal and noise distributions in standard score units; scores index the participant's ability to discriminate between targets and non-targets	Sustained attention; higher scores indicate better discrimination
Variability	Variability (standard deviation) of hit reaction time SE values	'Within respondent' variability; variability in the consistency of an individual's response times

CPT, Continuous Performance Test; SE, standard error.

[a] Table adapted from Kollins *et al.* (2008).

[b] Conners and MHS Staff (2000).

Table 2

Study population characteristics

	Proband generation		Parental generation		Grandparental generation	
	N, mean	%, SD	*N*, mean	%, SD	*N*, mean	%, SD
Individuals	283		378		106	
AD/HD affection status						
Affected	186	65.72	74	19.58	7	6.60
Combined	92	32.51	22	5.82	1	0.94
Hyperactive	21	7.42	13	3.44	2	1.89
Inattentive	73	25.80	39	10.32	4	3.77
Questionable	15	5.30	71	18.78	13	12.26
Unaffected	62	21.91	206	54.50	76	71.70
Not available	20	7.07	27	7.14	10	9.43
Race						
Non-Hispanic White	230	81.27	284	75.13	91	85.85
African–American	50	17.67	51	13.49	1	0.94
Asian–American	3	1.06	4	1.06	2	1.89
American–Indian	0	0	0	0	1	0.94
Not available	0	0	39	10.32	11	10.38
Sex						
Male	175	61.84	195	51.59	54	50.94
Female	108	38.16	183	48.41	52	49.06
Phenotyping						
FSIQ	252	89.05	114	30.16	9	8.49
Estimate	106.16	15.03	110.47	14.17	108.11	18.55
Age (years) at examination	9.43	2.77	39.71	6.28	64.05	6.35
CPRS	260	91.87	NA		NA	
Age (years) at examination	8.81	2.78	NA	NA	NA	NA
CTRS	201	71.02	NA		NA	
Age (years) at examination	8.82	2.53	NA	NA	NA	NA
CPT	241	85.16	130	34.39	10	9.43
Age (years) at examination	9.2	2.76	38.93	6.42	64.6	7.86
Genotyping[a]						
Included in initial screen	193	68.20	185	48.94	44	41.51
Additional typing for select SNPs	88	31.10	87	23.02	29	27.36

AD/HD, attention-deficit/hyperactivity disorder; CPRS, Conners' Parent Rating Scale; CPT, Continuous Performance Test; CTRS, Conners' Teacher Rating Scale; FSIQ, Full Scale Intelligence Quotient; NA, not available; SD, standard deviation, SNP, single-nucleotide polymorphism.

[a]Please refer to the methods section for more details.

Table 3

Heritabilities of phenotypes

Traits	*N*	h^2	h^2 SE	*P* value
CPRS: DSM-IV subscales				
Inattentive	260	0.02	0.15	0.443
Hyperactive-impulsive	260	0.25	0.16	0.055
Total symptom count	260	0.19	0.16	0.106
CTRS: DSM-IV subscales				
Inattentive	201	0.16	0.27	0.277
Log-hyperactive-impulsive	201	0.76	0.23	0.002*
Total symptom count	201	0.46	0.23	0.026*
CPT				
Errors of commission[a]	381	0.40	0.12	4.0×10^{-4}*
Hit reaction time	381	0.55	0.12	4.4×10^{-6}*
Hit reaction time SE	381	0.27	0.11	0.008*
Detectability[a]	380	0.27	0.12	0.010*
Log-variability	381	0.14	0.11	0.102

CPRS, Conners' Parent Rating Scales; CPT, Continuous performance test; CTRS, Conners' teacher rating scales; DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition; h^2, heritability; *N*, number of individuals; SE, standard error.

[a]Residual kurtosis is above 0.8. Errors of Commission: 1.12; detectability: 0.98.

*$P<0.05$

Table 4

QTDT results for significant SNPs[a]

				CTRS			CPRS			CPT				
Gene	**SNP**	**Basepair location**	**MAF**	**Inattentive *P* value**	**log-HI *P* value**	**TSC *P* value**	**Inattentive *P* value**	**HI *P* value**	**TSC *P* value**	**Comm *P* value**	**Hit RT *P* value**	**Hit RT SE *P* value**	**log-variability *P* value**	**Detectability *P* value**
SLC9A9	rs1046706	144467790	0.44	0.007	0.00E + 00[b,c]	0.00E + 00[b,c]	0.021	0.001[d]	0.003	0.001[c]	0.002	0.852	0.487	0.062
SLC9A9	rs2360867	144888353	0.43	0.153	0.961	0.399	0.851	0.471	0.646	0.00E + 00[b,c]	0.128	0.821	0.548	0.022

Comm, errors of commission; CPRS, Conners' Parent Rating Scale; CPT, Continuous Performance Test; CTRS, Conners' Teacher Rating Scale; FDR, false discovery rate; HI, hyperactive-impulsive; Hit RT, hit reaction time; Hit RT SE, hit reaction time standard error; MAF, minor allele frequency; TSC, total symptom count.

[a] Only SNPs that remained significant after adjustment for multiple testing are shown.

[b] Treated as 1×10^{-5} for FDR calculation.

[c] FDR *q* value <0.05.

[d] FDR *q* value: 0.10 >q > 0.05.