SNPs in Dopamine D2 Receptor Gene (DRD2) and Norepinephrine Transporter Gene (NET) Are Associated with Continuous Performance Task (CPT) Phenotypes in ADHD children and their families.

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Abstract:

Haplotype-tagging SNP analyses were conducted to identify molecular genetic substrates of quantitative phenotypes derived from performance on a Continuous Performance Task (CPT). Three hundred sixty-four individuals were sampled from 152 families ascertained on the basis of at least one child having ADHD. Probands, their affected and unaffected siblings, and parents were administered a CPT. Four different components of performance were analyzed and tested for association with SNPs from 10 candidate genes involved in monoaminergic function. After correcting for multiple comparisons and controlling for multiple individuals from the same family, significant associations were identified between commission errors and SNPs in the DRD2 gene (rs2075654, rs1079596), and between reaction time variability and a SNP in the NET gene (rs3785155). These findings suggest that commission errors and reaction time variability are excellent candidates as ADHD endophenotypes based on previously published criteria. Results also shed light on the molecular genetic basis of specific processes that may underlie the disorder.

Keywords: ADHD | endophenotype | DRD2 | norepinephrine transporter gene | continuous performance task phenotypes | child psychology | medical genetics

Article:

INTRODUCTION

Considerable effort has been devoted to the identification of the molecular genetic basis of Attention Deficit Hyperactivity Disorder (ADHD), which is one of the most commonly diagnosed psychiatric disorders in children and adults [Goldman et al., 1998; Faraone et al., 2005; Waldman and Gizer, 2006]. The majority of molecular genetic studies to date, however,

have only demonstrated small main effects for a range of candidate genes, most of which are associated with neurotransmission in monoamine systems (e.g., dopamine, norepinephrine, serotonin) [Faraone et al., 2005; Waldman and Gizer, 2006]. It has been argued that the variability observed across molecular genetic studies of ADHD is based, in part, on the phenotypic heterogeneity that is part of the clinical condition [Buitelaar, 2005; Thapar et al., 2006].

More recently, emphasis has been placed on the identification of sub-phenotypes or endophenotypes of ADHD that may help address the problem of phenotypic heterogeneity [Hudziak, 2001; Castellanos and Tannock, 2002; Doyle et al., 2005a,b]. In a seminal article, Castellanos and Tannock 2002 delineated the essential features of an ADHD endophenotype that would increase the likelihood of identifying stronger associations between genetic factors and endophenotypic expression. Specifically, they noted that an endophenotype should be continuously quantifiable, should predict the disorder probabilistically, should be closer to the site of primary causative agent than to diagnostic categories, and should be anchored in neuroscience.

An emerging body of research suggests that neuropsychological, neuroanatomical, and neurofunctional deficits among ADHD patients may be suitable candidates for endophenotypes based on the criteria described by Castellanos and Tannock. Continuous performance tests (CPT) are among the most widely used neuropsychological tests in patients with ADHD. These tasks usually assess both sustained attention and the ability to inhibit responding under some conditions. In a typical task, a respondent is instructed to attend to a series of target stimuli and to make a response (a mouse click or key press, for example) as quickly as possible following target stimuli. On some proportion of trials however, a non-target stimulus is presented and the respondent is instructed to withhold responding. Performance on CPTs therefore yields a number of different measures that are believed to represent different aspects of executive functioning. Errors of omission, or not responding to a target stimulus, are believed to index sustained attention. Errors of commission, or responding to a non-target stimulus, index response inhibition. Other aspects of performance include reaction time and reaction time variability, which are believed to index attentional regulation [Castellanos et al., 2005; Sonuga-Barke and Castellanos, 2007].

CPTs have been widely used to differentiate patients with ADHD from those without. Several meta-analyses report moderate to large effect sizes for different aspects of CPT performance across ADHD and non-ADHD groups [Frazier et al., 2004; Hervey et al., 2004]. CPT performance is therefore both distributed continuously (i.e., quantitatively across clinical and non-clinical groups) and predicts ADHD probabilistically, meeting several of the criteria for a promising endophenotype described by Castellanos and Tannock 2002.

The genetic basis of processes underlying CPT performance, such as inhibitory control and reaction time variability, among ADHD individuals and their families has also been examined,

with both family-based and twin studies supporting the heritability of these processes [Nigg et al., 2004; Kuntsi et al., 2005]. The molecular genetic basis of CPT performance as it relates to ADHD has also been studied in several studies over the past several years, with work focusing primarily on variants of several different dopaminergic genes.

Errors of commission on CPT tasks have been associated with variation in several different genes, including the dopamine D4 receptor DRD4 gene [Manor et al., 2002a; Kieling et al., 2006], the dopamine transporter gene (DAT1; [Loo et al., 2003]), the dopamine D5 receptor DRD5 gene [Manor et al., 2004] and the monoamine oxidase A gene (MAOA, [Manor et al., 2002b]). Similarly, variants of the same genes have also been associated with response variability on CPT performance [Manor et al., 2002a, 2004; Loo et al., 2003; Bellgrove et al., 2005a,b].

It is noteworthy that the findings from these molecular genetic studies of CPT performance in ADHD samples have been achieved using relatively small samples from ethnically diverse backgrounds (e.g., Irish, American, Israeli, British), suggesting that the effects are robust.

Since the search for valid neuropsychological endophenotypes of ADHD is still nascent, there are potential limitations to the above studies that may help guide the refinement of subsequent empirical work. Most notably, the majority of previous molecular genetic studies of CPT performance have focused primarily on a comparatively limited number of variable-number-of-tandem-repeat (VNTR) polymorphisms in a handful of genes. One exception to this paradigm was a study that found overall CPT performance and response variability influenced by a single nucleotide polymorphism (SNP) in the DRD4 gene [Bellgrove et al., 2005b]. Genetic analyses using more contemporary haplotype-tagging SNP approaches allow for a more complete examination of how variation in specific genes may be associated with specific phenotypes. Rather than focusing on a single, repetitive polymorphism within a gene, one examines multiple, independent SNPs across the gene.

The purpose of the present study, therefore, was to examine the molecular genetic associations between specific candidate genes and CPT performance using a haplotype-tagging SNP approach. We also expanded our examination of candidate genes to study other genes involved in monoamine neurotransmission. To this end, we concentrated on the following 10 genes: dopamine D1 receptor (DRD1), dopamine D2 receptor (DRD2), dopamine D3 receptor DRD3, DRD4, DAT1, serotonin 1B (HTR1B), serotonin transporter (SLC6A4), norepinephrine transporter (NET), dopamine beta hydroxylase (DBH), and synaptosomal associated protein (SNAP-25).

METHODS

Subjects

Statistical analyses were performed on a total of 364 individuals from 152 families ascertained on the basis of at least one child between the ages of 5 and 12 years who met research criteria for ADHD. Sixty-nine families contained a single affected child with no other known family history of ADHD. The remaining 83 families were comprised of an affected child with additional confirmed or suspected family history of ADHD. Mean age of participating children was 9.27 years (SD = 2.78 years) and mean age of participating parents was 41.11 years (SD = 9.32 years). Further details regarding demographics and baseline characteristics of the analyzed sample are presented in Table I. Data were collected at two academic sites (Duke University Medical Center and University at North Carolina Greensboro), which are both located in central North Carolina. Affected individuals and family members provided written informed consent, per study protocols approved by the respective institutional review boards (IRBs).

Table I. Sample Characteristics

		N/mean	%/SD
Probands/affected Sibs (**includes possibly affected)		180	49.5%
ADHD subtype			
Combined		84	46.7%
Hyperactive		20	11.1%
Inattentive		67	37.2%
Possibly affected		9	5.0%
CPRS ADHD index	N = 178	74.8	10.3%
CTRS ADHD index	N = 167	65.3	12.4%
Race			
Non-Hispanic White		143	79.4%
Non-Hispanic African-American		29	16.1%
Asian-American		3	1.7%
Hispanic White		4	2.2%
Hispanic Black		1	0.6%
Gender			
Male		125	69.4%
Female		55	30.6%
FSIQ estimate	N = 179	106.7	15.2%
Affected parent		41	11.3%
ADHD subtype			
Combined		15	36.6%
Hyperactive		8	19.5%
Inattentive		18	43.9%
NOS		0	0.0%
CPRS ADHD index		N/A	
CTRS ADHD index		N/A	
Race			
Non-Hispanic White		32	78.1%
Non-Hispanic African-American		9	22.0%
Asian-American		0	0.0%
Gender			
Male		14	34.2%
Female		27	65.9%
Unaffected/uncertain child		50	13.7%

		N/mean	%/SD
Affection status			
Unaffected		47	94.0%
Uncertain		3	6.0%
CPRS ADHD index	N = 46	52.7	10.4%
CTRS ADHD index	N = 14	54.2	14.3%
Race			
Non-Hispanic White		44	88.0%
Non-Hispanic African-American		3	6.0%
Asian-American		0	0.0%
Hispanic White		2	4.0%
Hispanic Black		1	2.0%
Gender			
Male		25	50.0%
Female		25	50.0%
FSIQ estimate	N = 49	108.7	13.6%
Unaffected/uncertain parent		93	25.6%
Affection status			
Unaffected		88	94.6%
Uncertain		5	5.4%
CPRS ADHD index		N/A	
CTRS ADHD index		N/A	
Race			
Non-Hispanic White		76	81.7%
Non-Hispanic African-American		15	16.1%
Asian-American		0	0.0%
Hispanic White		2	2.2%
American-Indian		0	0.0%
Gender			
Male		33	35.5%
Female		60	64.5%

All children in the family were given a standard assessment battery which included parent diagnostic interviews [Shaffer et al., 2000], parent and teacher versions of the Conners' Rating Scales to assess cross-situational symptom presentation and developmental deviance of ADHD symptoms [Conners, 1997], performance tasks (continuous performance task [CPT; see below]) and a brief cognitive screening (a short form of the Wechsler Intelligence Scale to estimate Full Scale IQ [FSIQ]). Adults also completed the Conners' Adult ADHD Rating Scale and the CPT. Data from all cases, including diagnostic interview results, rating scale results, IQ and CPT performance, were reviewed by an expert panel to determine final diagnoses, based on DSM-IV criteria. Based on the expert panel review, individuals were classified into one of two categories: “affected/possibly affected” and “unaffected/uncertain.” Affected/Possibly Affected individuals either met all research criteria for ADHD diagnosis (based on DSM-IV criteria) or met nearly all research criteria and the expert panel strongly suspected ADHD given the preponderance of evidence from the assessment. Individuals classified as unaffected/uncertain either did not meet research criteria for ADHD or had insufficient data to determine affection status. It is relevant

that this approach to categorizing affection status has been used successfully with other psychiatric phenotypes in genetic studies [Yonan et al., 2003]. In addition, CPT data from all participants, regardless of affection status, was used for analysis (see below). Comorbidity with other psychiatric conditions was not reason for exclusion unless ADHD was not the primary diagnosis. Blood was obtained from the affected child, all available siblings and parents under IRB-approved procedures. DNA was extracted from whole blood using the Gentra Puregene methodology as supplied by Qiagen Inc. (Valencia, California).

Primary Outcome Measure

Conners Continuous Performance Test (CPT; [Conners, 2000a]). The Conners continuous performance test was completed on an IBM-compatible desktop computer in a quiet setting with minimal distractions. Three hundred sixty total letters appeared on the computer screen, one at a time, each for approximately 250 msec. The 360 trials were presented in 18 blocks of 20 trials each. The blocks differed only in the interstimulus intervals (ISI) between letter presentations, which lasted 1-, 2-, or 4-sec.

Participants were instructed to press the spacebar when any letter except the letter “X” appeared on the screen. The percentage of trials when letters other than “X” appeared was 90% across all ISI blocks. Given the low rate of “no-go” trials, the task as administered emphasized inhibitory control. Reaction time was measured from the point at which any letter other than “X” appeared on the screen until the spacebar was pressed. Only successful non-“X” trials, or trials where the participant correctly pressed the spacebar when presented with a target stimulus was included for reaction time (RT) data analysis. The total Conners' CPT task takes approximately 14 min to complete. Six primary outcome variables were derived from CPT performance for the present analyses and are described in Table II. For each of the outcome variables, T-scores were used since they adjust for age and sex, thus allowing comparability of data from children of all ages and adults. The version of the CPT that was used provides normative data for children as young as 6 years. A small proportion of our sample (5%) was 5 years of age at the time of testing. For these individuals, the 6-year-old norms were used.

Table II. Description of Measures Analyzed From CPT [From Conners, 2000b]

Dependent measure	Description	Interpretation measured
Errors of omission	Number of targets (non-X stimuli) that were presented but not responded to	Sustained attention; higher scores indicate lack of orienting to stimuli, or slow, sluggish response style
Errors of commission	Number of non-targets (X-stimuli) that were 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; slow reaction times with high omission errors indicate inattentionpulsivity
Hit reaction time standard error	Variability of hit reaction times	Refers to erratic nature of responding and may represent attentional lapses (see text for more details)

Dependent measure	Description	Interpretation measured
Detectability (d′)	Derived from signal detection theory, this index is the distance between the signal and noise distributions in standard score units; scores index the subject's ability to discriminate between targets and non-targets	Sustained attention; higher scores indicate better discrimination
Response style	Evaluates speed/accuracy tradeoff	High scores indicate cautious response style; low scores indicate more impulsive response style

Genotyping

SNP genotyping was performed using the Illumina Infinium HumanHap300 duo (Illumina, Inc., San Diego, CA). For all genotype assays, quality control measures included genotyping two Centre d'Etude du Polymorphism Humain (CEPH) controls for every 94 unique samples. Each unique sample was included in the subsequent statistical analysis only if the sample efficiency for the entire screen was over 98%. Further, individual markers were excluded if they did not achieve over 98% efficiency across all the unique samples. PEDCHECK was used to identify and eliminate markers by families due to pedigree inconsistencies [O'Connell and Weeks, 1998]. LD Select [Carlson et al., 2004] was used to identify haplotyped-tagging SNPs within our 10 candidate genes (DRD1, DRD2, DRD3, DRD4, DAT1, HTR1B, SLC6A4, NET, DBH, and SNAP-25). Table III lists the location and minor allele frequency for each of the SNPs that were assessed.

Table III. Listing of SNPs Used for Analysis

Chromosome	Gene	Probe	Base pair location	Minor allele frequency
11	DRD4	HCV1611535	615085	0.33
11	DRD4	rs35134589	615695	0.25
11	DRD4	rs3758653	626399	0.18
11	DRD4	rs936461	626496	0.40
11	DRD4	7-repeat	629818–630292	0.20
11	DRD4	rs11246226	631191	0.43
5	SLC6A3	rs27072	1447522	0.19
5	SLC6A3	rs40184	1448077	0.49
5	SLC6A3	rs11133767	1454580	0.34
5	SLC6A3	rs6869645	1457548	0.09
5	SLC6A3	rs6347	1464412	0.31
5	SLC6A3	rs27048	1465645	0.43
5	SLC6A3	rs37022	1468629	0.21

Chromosome	Gene	Probe	Base pair location	Minor allele frequency
5	SLC6A3	rs2042449	1469646	0.18
5	SLC6A3	rs37020	1471374	0.48
5	SLC6A3	rs464049	1476905	0.48
5	SLC6A3	rs460700	1482969	0.26
5	SLC6A3	rs11737901	1483616	0.31
5	SLC6A3	rs409588	1483834	0.27
5	SLC6A3	rs460000	1485825	0.26
5	SLC6A3	rs403636	1491354	0.15
5	SLC6A3	rs2617605	1495521	0.35
5	SLC6A3	rs6346	1496163	0.02
5	SLC6A3	rs6350	1496199	0.07
5		rs3756450	1501148	0.17
9		rs1076153	135487964	0.20
9		rs1076150	135488582	0.49
9	DBH	rs2797849	135491762	0.33
9	DBH	rs3025388	135493077	0.18
9	DBH	rs2007153	135493640	0.39
9	DBH	rs2519155	135494419	0.32
9	DBH	rs1108580	135494935	0.46
9	DBH	rs1108581	135495062	0.23
9	DBH	rs2873804	135495465	0.47
9	DBH	rs5320	135497294	0.07
9	DBH	rs5324	135498479	0.02
9	DBH	rs1611123	135498904	0.45
9	DBH	rs1611125	135499133	0.47
9	DBH	rs1541333	135501206	0.46
9	DBH	rs1541332	135501337	0.48
9	DBH	rs2519154	135502096	0.42
9	DBH	rs2797853	135502336	0.28
9	DBH	rs2283123	135505118	0.11
9	DBH	rs2283124	135505151	0.12
9	DBH	rs77905	135507918	0.47
9	DBH	rs2097628	135509523	0.38

Chromosome	Gene	Probe	Base pair location	Minor allele frequency
9	DBH	rs2073833	135510103	0.43
9	DBH	rs2073837	135512749	0.31
9	DBH	rs129882	135513490	0.20
17	SLC6A4	rs1042173	25549137	0.41
17	SLC6A4	rs4325622	25550601	0.39
17	SLC6A4	rs3794808	25555919	0.42
17	SLC6A4	rs140701	25562658	0.40
17	SLC6A4	rs140700	25567515	0.07
17	SLC6A4	rs6354	25574024	0.20
17	SLC6A4	rs2066713	25575791	0.35
17	SLC6A4	rs8071667	25576899	0.19
20		rs6104567	10143433	0.24
20		rs1889189	10145086	0.31
20	SNAP25	rs6039769	10146954	0.29
20	SNAP25	rs6032826	10151817	0.27
20	SNAP25	rs3787303	10156748	0.21
20	SNAP25	rs2423486	10160212	0.28
20	SNAP25	rs2423487	10161095	0.10
20	SNAP25	rs363032	10166644	0.10
20	SNAP25	rs363039	10168496	0.35
20	SNAP25	rs363043	10174146	0.31
20	SNAP25	rs363016	10179174	0.40
20	SNAP25	rs363050	10182257	0.37
20	SNAP25	rs6074113	10190011	0.48
20	SNAP25	rs362563	10191251	0.07
20	SNAP25	rs362569	10194733	0.39
20	SNAP25	rs362584	10202475	0.29
20	SNAP25	rs6039806	10206654	0.45
20	SNAP25	rs6077718	10209142	0.12
20	SNAP25	rs6039807	10211576	0.50
20	SNAP25	rs2284297	10213897	0.05
20	SNAP25	rs362549	10217890	0.45
20	SNAP25	rs6108463	10228505	0.24

Chromosome	Gene	Probe	Base pair location	Minor allele frequency
20	SNAP25	rs362988	10229370	0.47
20	SNAP25	rs4813925	10234313	0.36
20	SNAP25	rs1051312	10235088	0.45
20	SNAP25	rs362552	10244217	0.37
11	DRD2	rs2242592	112784640	0.40
11	DRD2	rs6279	112786283	0.38
11	DRD2	rs6277	112788669	0.47
11	DRD2	rs2075654	112794276	0.13
11	DRD2	rs2587548	112797422	0.46
11	DRD2	rs1076563	112801119	0.47
11	DRD2	rs1079596	112801829	0.14
11	DRD2	rs2471857	112803549	0.14
11	DRD2	rs4586205	112812339	0.34
11	DRD2	rs4620755	112814829	0.14
11	DRD2	rs7125415	112815891	0.11
11	DRD2	rs4648318	112818599	0.32
11	DRD2	rs17601612	112822955	0.32
11	DRD2	rs4274224	112824662	0.49
11	DRD2	rs4581480	112829684	0.15
11	DRD2	rs7131056	112834984	0.49
11	DRD2	rs4350392	112840927	0.14
11	DRD2	rs4938019	112846601	0.14
11	DRD2	rs12364283	112852165	0.06
5	DRD1	rs4867798	174800505	0.29
5	DRD1	rs4532	174802756	0.34
5	DRD1	rs5326	174802802	0.13
6	HTR1B	rs13212041	78227843	0.24
6	HTR1B	rs6298	78229711	0.27
3	DRD3	rs2134655	115340891	0.25
3	DRD3	rs963468	115345577	0.38
3	DRD3	rs3773678	115352768	0.19
3	DRD3	rs2630351	115357749	0.06
3	DRD3	rs167771	115358965	0.23

Chromosome	Gene	Probe	Base pair location	Minor allele frequency
3	DRD3	rs167770	115362252	0.30
3	DRD3	rs226082	115363703	0.30
3	DRD3	rs324029	115364313	0.30
3	DRD3	rs10934256	115368342	0.19
3	DRD3	rs1486009	115371222	0.04
3	DRD3	rs6280	115373505	0.35
3		rs9825563	115382910	0.33
16	SLC6A2	rs2242446	54247926	0.25
16	SLC6A2	rs36030	54250791	0.16
16	SLC6A2	rs17307096	54251784	0.34
16	SLC6A2	rs3785143	54252607	0.07
16	SLC6A2	rs192303	54257725	0.34
16	SLC6A2	rs41154	54260207	0.39
16	SLC6A2	rs36024	54263892	0.42
16	SLC6A2	rs187714	54264000	0.38
16	SLC6A2	rs36023	54264755	0.42
16	SLC6A2	rs36021	54269451	0.43
16	SLC6A2	rs3785152	54274051	0.08
16	SLC6A2	rs40147	54274341	0.29
16	SLC6A2	rs1814269	54274529	0.42
16	SLC6A2	rs36016	54277535	0.47
16	SLC6A2	rs3785155	54279891	0.15
16	SLC6A2	rs880711	54280882	0.18
16	SLC6A2	rs11862589	54281443	0.49
16	SLC6A2	rs879519	54281912	0.50
16	SLC6A2	rs5568	54287625	0.34
16	SLC6A2	rs1566652	54289076	0.36
16	SLC6A2	rs36010	54289169	0.04
16	SLC6A2	rs5569	54289336	0.27
16	SLC6A2	rs36009	54290121	0.08
16	SLC6A2	rs42460	54295157	0.08
16		rs10521330	54297655	0.20

Statistical Analysis

Hardy–Weinberg equilibrium was assessed using exact tests implemented in the Genetic Data Analysis program [Zaykin et al., 1995]. Pairwise linkage disequilibrium (D′ and r2) between markers within each gene was calculated using the software package GOLD [Abecasis and Cookson, 2000]. All analyses were conducted using programs specifically designed for family data, to account for the dependency among relatives. As described above, several quantitative phenotypes from the CPT were examined for both genetic association and linkage with our SNP data. One of the requirements for these analyses was that the trait under investigation be normally distributed. Several transformations were tested for CPT omission errors and response style, but none produced a normal distribution of these phenotypes, largely because of distributional skewness. These variables were subsequently removed from analysis. The remaining CPT phenotypes (commission errors, hit reaction time, hit reaction time standard error, and detectability) did not significantly deviate from a normal distribution and were used without transformation in all analyses.

Pearson correlations were calculated for each pairwise combination of the CPT variables. The association between CPT variables and affection status was evaluated using Generalized Estimating Equations (GEE) with the PROC GENMOD procedure in SAS version 9.1. The GEE approach controls for familial correlation among individuals from the same family. Further, we examined the association between the SNPs and affection status using the association in the presence of linkage (APL) test. APL provides a novel test for association in the presence of linkage that also correctly infers missing parental genotypes by estimating identity-by-descent parameters (IBD) [Chung et al., 2006].

The heritabilities of CPT phenotypes were assessed in our data set using Sequential Oligogenic Linkage Analysis Routines (SOLAR) [Almasy and Blangero, 1998]. The “polygenic” command in SOLAR was used to obtain heritabilities. This analysis uses familial data to estimate how much of the variance in a quantitative phenotype is due to heritable factors. The QTDT [Abecasis et al., 2000a,b] and SOLAR were used to test for the presence of genetic association and genetic linkage, respectively.

For the QTDT, we present the Monks–Kaplan exact test because it is a conservative TDT-like method for families with multiple siblings with or without parents. The exact test version of Monks–Kaplan was used because of the small sample size of our data set. We accounted for possible false-positive associations due to multiple testing by adjusting the nominal P-values using the false discovery rate (FDR) procedure developed by Benjamini and Hochberg 1995 and chose a threshold of 0.10 for declaring significance. The concept of the FDR was proposed to relax the stringent property of Bonferroni correction. As originally proposed, the FDR first ranks all P-values from high to low ($P(N) > P(N-1) > \ldots > P(1)$). Each P-value is then compared to $(i \times 0.05)/N$, where i is the rank of the observed P-value and N is the total number of SNPs. When $P(j) < 0.05/j$ is significant, SNPs ranked below P(j) are also declared to be significant.

With the threshold set at 0.10, on average, 10% of associations identified by this procedure as significant will be false-positive discoveries. We calculated the FDR q-values using PROC MULTTEST in SAS version 9.1.

RESULTS

The Pearson correlations amongst the four CPT phenotypes generally were high and statistically significant, with the exception of the correlations between commission errors and hit reaction time standard error ($r = 0.01$; $P = 0.91$), and between hit reaction time standard error, and detectability ($r = -0.02$, $P = 0.64$), suggesting that these performance parameters measured independent processes.

The heritabilities of the CPT phenotypes are shown in Table IV. Age was tested as a covariate in all four estimates of heritability, but was not significantly associated with any of the phenotypes. Thus, reported heritability estimates are without including age as a covariate. It is important to note that since age-adjusted T-scores were used for analyses, the lack of age effects was not unexpected. Estimates of heritability for the four significant phenotypes fell between 28% and 57%, which is generally considered to be reasonably heritable for genetic analysis of a quantitative trait.

Table IV. Heritability Estimates for CPT Parameters

Outcome	Heritability estimate	*P*-value
Commission errors	0.4273	0.0002
Hit Reaction time	0.5682	0.0000
Hit Reaction time Standard error	0.2827	0.0068
Detectability	0.3154	0.0043

Descriptive data for CPT and results from the analysis predicting affection status from these variables are included in Table V. Commission errors were significantly associated with affection status ($P = 0.04$), but the other CPT parameters failed to reach statistical significance although there were several trends in the expected direction (P-values for hit reaction time, reaction time standard error, and variability, were all at or below $P = 0.10$). A number of SNPs from SNAP-25, DBH, NET, SLC6A4, DRD3, and DRD4 were nominally associated with affection status in this sample (data not shown, P's = 0.01–0.04), but none withstood multiple testing corrections.

Table V. Means and SDs for CPT Parameters in Affected and Unaffected Individuals

Outcome	Mean (SD)	*P*-value for parameter predicting affection status[a]
Commission errors		0.04
Affected	50.86 (11.84)	

Unaffected	48.27 (9.46)	
Hit reaction time		0.10
Affected	52.38 (11.93)	
Unaffected	50.27 (11.62)	
Hit reaction time standard error		0.10
Affected	53.70 (10.61)	
Unaffected	51.77 (11.32)	
Variability		0.07
Affected	52.72 (11.33)	
Unaffected	50.61 (10.60)	
Detectability		0.24
Affected	50.77 (11.33)	
Unaffected	49.42 (10.25)	

a Based on APL analysis (see text).

Significant results of the QTDT analysis for genetic association with the CPT phenotypes are shown in Table VI. Commission errors were significantly associated with SNPs in two of the genes (DRD3 and DRD2), although only the associations with DRD2 SNPs (rs2075654 and rs1079596) remained significant after applying the FDR correction.

Table VI. Results From QTDT Analyses

Gene	SNP	CPT outcome	Uncorrected *P*-value	FDR q-value
SLC6A3 (DAT1)	rs37020	HRTSE	0.008	0.352
	rs464049	HRTSE	0.031	0.8184
	rs409588	HRTSE	0.041	0.891
	rs2042449	Detectability	0.049	0.98
DBH	rs1611125	Detectability	0.049	0.98
	rs77905	Detectability	0.047	0.98
SNAP-25	rs6104567	HRT	0.007	0.924
	rs363016	HRT	0.041	0.99
	rs6104567	HRTSE	0.015	0.49
DRD2	**rs2075654**	**Commission**	**<0.0001**	**0.0132**
	rs1079596	**Commission**	**0.001**	**0.066**
	rs2471857	Commission	0.003	0.132
	rs7131056	Commission	0.025	0.825
	rs7125415	Detectability	0.006	0.792
DRD3	rs3773678	Commission	0.043	0.97

Gene	SNP	CPT outcome	Uncorrected *P*-value	FDR q-value
	rs3773678	HRT	0.042	0.99
	rs3773678	Detectability	0.045	0.98
SLC6A2 (NET)	**rs3785155**	**HRTSE**	**<0.0001**	**0.0132**
	rs880711	HRTSE	0.004	0.264

Hit reaction time standard error showed significant associations with SNPs in the DAT1, SNAP-25, and NET genes. Only one SNP in the NET gene (rs3785155) remained significant after correcting for FDR. Detectability was significantly associated with SNPs in the DAT1, DBH, DRD2, and DRD3 genes, although the magnitude of these associations was generally lower that those observed for commission errors and hit reaction time standard error, and none of the associations remained significant at the FDR threshold ($q < 0.10$). Similarly, hit reaction time was associated with SNPs in the SNAP-25 and DRD3 genes, but these associations were less robust and did not withstand corrections for multiple comparisons.

DISCUSSION

The present study found nominally significant associations between parameters of CPT performance and SNPs in six different genes associated with monoamine function: DRD2, DRD3, DAT1, DBH, NET, and SNAP-25. After correcting for multiple comparisons, however, only SNPs in the DRD2 and NET genes were significantly associated with commission errors and hit reaction time standard error, respectively.

Two of the genes we examined in this study, DRD4 and DAT1 have been associated with aspects of CPT performance in previous studies [Manor et al., 2002a; Loo et al., 2003; Bellgrove et al., 2005b; Kieling et al., 2006]. We found no associations with any SNPs in the DRD4 gene and although several associations between hit reaction time standard error were nominally associated with DAT1 SNPs, these failed to withstand corrections for multiple testing. We specifically examined the DRD4 7-repeat allele given its strong previous association with CPT phenotypes [Manor et al., 2002a; Langley et al., 2004; Kieling et al., 2006]. However, this marker was not associated with any of the quantitative phenotypes that we examined.

One possible reason that we did not replicate these previous findings was that the composition of our sample differed. Without exception, previous studies that have investigated the molecular genetics of performance-based phenotypes using the CPT and other similar tasks have used only clinical samples that were usually stratified on the basis of single risk alleles (e.g., the 7-repeat allele of the DRD4, or the 10-repeat of the DAT1). Our approach was different in that we analyzed data from both ADHD probands and their affected and unaffected siblings, as well as parents who also varied with respect to their ADHD presentation.

Compared to most previous studies that have examined the molecular genetic basis of CPT performance, our sample was considerably larger, affording more statistical power to identify meaningful genotype-phenotype relationships. Our findings are consistent with a number of studies that have shown both reaction time variability (indexed in this study by hit reaction time standard error) and commission errors as being among the most sensitive cognitive measures to discriminate ADHD from non-ADHD samples [Frazier et al., 2004; Hervey et al., 2004]. Moreover, reaction time variability has also been shown to be heritable in family and twin-based studies [Kuntsi et al., 2006].

Based on previous recommendations for evaluating candidate endophenotypes [Castellanos and Tannock, 2002], the present findings lend strong support for the use of commission errors and reaction time variability in future molecular genetic studies of ADHD. As noted these phenotypes predict the disorder probabilistically and are continuously quantifiable. We have also demonstrated that these phenotypes are heritable. The remaining criteria described by Castellanos and Tannock are that the endophenotype should be more proximal to the causative agent (i.e., the genotype) than the diagnostic category with which it is associated, and it should also be anchored in neuroscience. Regarding the latter, both inhibitory control and intra-subject response variability have been shown to have distinct and dissociable neural bases [Aron et al., 2007; Clare Kelly et al., 2008]. Since specific genes that we have found to be associated with inhibitory control and response time variability (DRD2 and NET) are more strongly associated with the candidate endophenotypes than the disorder itself, the former criterion regarding proximity to causal agents is also met.

The specific associations observed in the present study are also consistent with other work linking the endophenotypes, candidate genes, and monoaminergic dysfunction. Both norepinephrine and dopamine activity have previously been hypothesized to be associated with unique aspects of cognitive dysfunction characteristic of ADHD [Viggiano et al., 2004; Pliszka, 2005]. Variation in the DRD2 gene has been consistently associated with a range of substance use disorders, including alcohol abuse/dependence and nicotine dependence [Noble, 1998, 2000; Munafo et al., 2004], both of which are more common in individuals diagnosed with ADHD [Wilens, 2007]. Moreover, deficits in inhibitory control are thought to be central to the development of many substance use disorders [Ivanov et al., 2008]. A recent study also demonstrated that individuals with alcohol dependence who carried the TaqIA polymorphism of the DRD2 gene exhibited poorer inhibitory control on a CPT [Rodriguez-Jimenez et al., 2006]. Our group also reported that DRD2 genotype interacts with self-reported symptoms in a population based sample of young adults to predict lifetime risk of regular smoking [McClernon et al., 2008]. Taken together these findings suggest that DRD2 modulated effects on inhibitory control may represent a plausible mechanism for risk of subsequent substance use problems in individuals with ADHD. The present findings point to specific regions of the DRD2 gene that should be investigated further to evaluate this hypothesis.

Previous work has also shown that attention and attentional lapses, like those believed to be indexed by reaction time variability are largely mediated through noradrenergic pathways, suggesting a critical role of the NET receptor in these kinds of processes [Smith and Nutt, 1996]. As such, our finding of strong associations between reaction time variability and SNPs on the NET receptor gene is consistent with previous neurobiological work.

At least two limitations to the present study are endemic to the haplotype tagging SNP analysis of any complex trait. First, in order to adequately survey the genetic variation across any given gene, a large number of SNPs are required. This in turn reduces power to detect effects of small size when appropriate statistical corrections are applied. As such, some of our nominally significant findings may actually be meaningful, but our FDR correction renders conclusions about these associations tenuous. Second, there are some genes for which reasonable SNP coverage across the gene was not available. For example, for the DRD4 and HTR1B genes, only five and two tagging SNPs were identified, respectively. The consequence of this limitation is that rare variations in these genes may be associated with CPT phenotypes, but we did not examine SNPs with frequencies less than 0.02 (see Table III).

Two additional related limitations are worth noting. First, only commission errors from the CPT significantly predicted affection status in our data set, although hit reaction time standard error trended in the expected direction. Second, we failed to find associations between the SNPs of interest (i.e., those associated with commission errors and hit reaction time standard error) and affection status. Together, these findings warrant caution in the interpretation of the potential for these endpoints as viable endophenotypes for the diagnosis of ADHD.

In spite of these limitations, our findings are the first to show strong associations that withstand FDR corrections between hapolotype tagging SNPs and quantitative CPT endophenotypes. Of particular note is that these findings suggest distinct genetic substrates for traits that are associated with two of the core features of ADHD: errors of commission (impulsivity) and reaction time variability (attention/attentional lapses). Of course, this interpretation is likely to be somewhat oversimplified, but the data represent an important incremental next step in linking specific genetic variation to quantitative phenotypes that may improve our understanding of ADHD.

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