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Association of Genetic Risk Severity With ADHD Clinical Characteristics

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This study sought to examine the association between the cumulative risk severity conferred by the total number of attention-deficit/hyperactivity disorder (ADHD) risk alleles of the *DAT1* 3′UTR variable number tandem repeat (VNTR), *DRD4* Exon 3 VNTR, and 5-*HTTLPR* with ADHD characteristics, clinical correlates, and functional outcomes in a pediatric sample. Participants were derived from case–control family studies of boys and girls diagnosed with ADHD, a genetic linkage study of families with children with ADHD, and a family genetic study of pediatric bipolar disorder. Caucasian children 18 and younger with and without ADHD and with available genetic data were included in this analysis (N = 591). The association of genetic risk severity with sociodemographic, clinical characteristics, neuropsychological, emotional, and behavioral correlates was examined in the entire sample, in the sample with ADHD, and in the sample without ADHD, respectively. Greater genetic risk severity was significantly associated with the presence of disruptive behavior disorders in the entire sample and oppositional defiant disorder in participants with ADHD. Greater genetic risk severity was also associated with the absence of anxiety disorders, specifically with the absence of agoraphobia in the context of ADHD. Additionally, one ADHD symptom was significantly associated with greater genetic risk severity. Genetic risk severity is significantly associated with ADHD clinical characteristics and co-morbid disorders, and the nature of these associations may vary on the type (externalizing vs. internalizing) of the disorder.

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Key words: genetic risk alleles; genetic risk severity; attention deficit disorder (ADD); attention-deficit/hyperactivity disorder (ADHD); DRD4, DAT1, SERT, 5-HTTLPR

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INTRODUCTION

Pediatric attention-deficit/hyperactivity disorder (ADHD) is among the most common childhood-onset psychiatric disorders with a prevalence of 5.5–9.3% in the United States [O'Connell et al., 2009] and 8–12% worldwide [Faraone et al., 2003]. Children and

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adolescents with this disorder experience educational difficulties [Loe and Feldman, 2007], problems with self-esteem [Barber et al., 2005], significantly impaired family and peer relationships [Hoza, 2007], and an overall decrease in quality of life [Coghill, 2010].

The extensive body of literature on the etiology of ADHD strongly suggests genes exert an influential role on the transmission, onset, and persistence of the disorder. Research places ADHD as one of the most heritable of psychiatric disorders with cross-sectional and longitudinal family, twin, and adoption studies demonstrating 70–80% heritability estimates, familial aggregation of symptoms, and familial clustering within and across generations [Faraone et al., 2005].

In the attempt to better understand the active role of genetics on the manifestation of ADHD, to identify chromosomal regions and susceptibility genes, and to chart the biological mechanisms of action, a number of approaches have been utilized, ranging from studies of candidate genes [Waldman et al., 1998; Comings et al., 2000a,b; Faraone et al., 2000b; Faraone and Khan, 2006], genetic linkage [Barr et al., 1999; Faraone et al., 1999; Ogdie et al., 2006; Lesch et al., 2008], and genome-wide association [Sklar, 2005; Hebebrand et al., 2006; Lasky-Su et al., 2008; Mick et al., 2008; Neale et al., 2008, 2010].

Despite such efforts, studies have yielded inconsistent results [Faraone et al., 2005; Waldman and Gizer, 2006]. For example, some of the first candidate gene studies of ADHD reported significant associations for the dopamine transporter gene (*DAT1*) [Cook et al., 1995], the dopamine D4 receptor (*DRD4*) [LaHoste et al., 1996; Licinio, 1996], and serotonin transporter gene (*5-HTT*) [Manor et al., 2001; Seeger et al., 2001]. Studies attempting to replicate the results yielded both positive and negative results for *DAT1* [Waldman et al., 1998; Swanson et al., 2000; Curran et al., 2001; Todd et al., 2001], *DRD4* [Castellanos et al., 1998; Rowe et al., 1998; Hawi et al., 2000; Faraone et al., 2001] and *5-HTT* [Kent et al., 2002; Langley et al., 2003; Xu et al., 2005; Wigg et al., 2006].

A number of hypotheses have been offered to explain these inconsistent results, the most investigated of which is the "common disorder"/"complex trait" hypothesis [Lander and Schork, 1994; Ioannidis et al., 2001; Lohmueller et al., 2003; Faraone, 2005]. "Complex trait" disorders are common disorders—psychiatric (e.g., major depression, bipolar disorder, ADHD, etc.), or medical (diabetes, asthma, osteoperosis, etc.)—insofar as their large prevalence in the population, and are characterized by phenotypic heterogeneity. That is, the disorders are indicated by a number of features, co-occurring in a variety of frequently non-overlapping patterns [Rice et al., 2001]. For example, the diagnosis of ADHD using the current DSM-IV-TR criteria may consist of either the Inattention Type or the Hyperactivity Type, with a possibility for a Combined (Inattention/Hyperactivity) Type. Each of the types consists of nine symptoms, only six of which are required to fulfill the diagnostic criteria [American Psychiatric Association, 2000]. "Complex trait" disorders, such as ADHD, are believed to result from the combined effects of variation within multiple interacting genes, each contributing a small magnitude of the overall risk, suggesting a polygenic etiology for the disorder [Lander and Schork, 1994; Rannala, 2001]. For example, many genes have been implicated in ADHD, such as the dopamine receptor D4 (*DRD4*) gene, the dopamine receptor D5 (*DRD5*) gene, the dopamine transporter (*DAT1*) gene, the dopamine beta hydroxylase (*DβH*) gene, the serotonin transporter (*5-HTT*) gene, the 5-hydroxytryptamine receptor 1B (*HTR1B*) gene, and the synaptosomal-associated protein 25 (*SNAP-25*) gene, all exerting small effects [Faraone, 2005; Mick and Faraone, 2008].

Because complex disorders exhibit polygenic etiology and do not follow readily predictable patterns of inheritance, the identification of risk genes for the purpose of an improved understanding of their influence on any disorder presents a number of challenges [Risch and Merikangas, 1996; Chakravarti, 1999]. Genomewide association studies suggest there is a polygenic risk component to ADHD [Cross-Disorder Group of the Psychiatric GWAS Consortium (in press)] and that this polygenic loading is associated with conduct disorder in ADHD patients [Hamshere et al., 2013].

Recently, a meta-analysis by Gizer et al. [2009], undertaken to determine which candidate genes of the 18 implicated in ADHD exhibit consistent evidence of association with childhood ADHD, found significant associations between *DAT1*, *DRD4*, *DRD5*, *5-HTT*, *HTR1B*, and *SNAP-25*. Given these promising findings and the paucity of knowledge on the polygenic effect on ADHD characteristics (e.g., age of onset, illness severity, and treatment), clinical correlates of ADHD (e.g., psychiatric disorder co-morbidity), and the resulting functional impairments (e.g., neuropsychological performance, behavioral and school characteristics), we hypothesized that the cumulative risk imparted by putative ADHD candidate gene risk alleles would predict the clinical features of the disorder. To test our hypothesis, we limited our selection of candidate risk alleles to those identified by Gizer et al. [2009] that had previously been genotyped in our sample. Of the risk alleles Gizer et al. [2009] identified in the meta-analysis, we had available data for the variable number tandem repeat (VNTR) in the 3′-untranslated region (UTR) of *DAT14*, the VNTR in Exon 3 of *DRD4*, and the serotonin-transporter-linked polymorphic region (*5-HTTLPR*) promoter in the *5-HTT* gene. The risk allele designated for *DAT1* is the 480-base pair (bp; or, 10-repeat) allele for the 3′-UTR, the risk allele designated for *DRD4* is the seven repeat of Exon 3, and the risk allele designated for *5-HTT* is the long allele of 5-HTTLPR. To the best of our knowledge, this is the first evaluation of the association between genetic risk severity compiled using the *5-HTT*, the *DAT1* and the *DRD4* total number of risk alleles and ADHD characteristics, clinical correlates, and functional outcomes in a pediatric sample.

MATERIALS AND METHODS

Subjects

Subjects were derived from identically designed longitudinal case–control family studies of boys [Biederman et al., 2006b] and girls [Biederman et al., 2006a] diagnosed with ADHD, a genetic linkage study of families with children with ADHD [Faraone et al., 2007], and a family genetic study of pediatric bipolar disorder [Wozniak et al., 2005]. Families were predominantly ascertained through the Clinical and Research Program in Pediatric Psychopharmacology within the Child Psychiatry Unit at the Massachusetts General Hospital. Remaining families were referred from local private child psychiatry and pediatric practices, self-referrals, newspaper and

support group advertisements, Internet postings, the outpatient psychiatry unit at Children's Hospital in Boston, and internal postings within the MGH system. For all studies, potential participants were excluded if they had been adopted, if their nuclear family was not available, if they had major sensorimotor handicaps (paralysis, deafness, and blindness), a history of psychosis or autism, inadequate command of the English language, or a Full Scale IQ <80.

After a complete description of the study, parents provided written informed consent for their children, and children and adolescents provided written assent. Adult offspring (those who were 18 years of age, N = 15) provided written informed consent to participate. Parents provided consent for offspring under the age of 18. Children and adolescents provided written assent to participate. Because we selected our candidate gene risk alleles based on the Gizer et al. [2009] meta-analysis findings, which focused on child and adolescent populations, we included ≤18-year-old participants with genetic data, only. A total of 591 participants were derived from the following studies: the boys study (N = 51), the girls study (N = 76), the ADHD linkage study (N = 267), and the family bipolar study (N = 197). All subjects were Caucasian. Of the 591 participants, 63% (N = 372) were male; 463 participants (M = 302; F = 161) met full criteria for ADHD and 128 (M = 70; F = 58) did not. The mean age of the sample was 11.1 years (SD = 3.4). The human research committee at Massachusetts General Hospital approved this study.

Assessment Procedures

All subjects were assessed with the same assessment battery. Detailed study methodologies for each study have been previously reported [Biederman et al., 2006a,b; Faraone et al., 2007]. Briefly, in all studies, psychiatric assessments relied on the Schedule for Affective Disorders and Schizophrenia for School-Age Children, Epidemiologic (K-SADS-E) [Orvaschel, 1985; Orvaschel and Puig-Antich, 1987] for subjects younger than 18 years of age and the Structured Clinical Interview for DSM-III or DSM-IV (SCID) [First et al., 1997] (supplemented with modules from the K-SADS-E to assess childhood diagnoses) for subjects 18 years of age. DSM-III criteria were used to diagnose participants of the boys and girls study (N = 127), whereas participants of the genetic linkage of ADHD and bipolar disorder family genetic studies (N = 464) were assessed using DSM-IV criteria.

Diagnoses were based on independent interviews with the mothers and direct interviews with subjects, except that children younger than 12 years of age were not directly interviewed. Combining data from direct and indirect interviews, we considered a diagnosis positive if it was endorsed in either interview. All interviews were blind to the subject's referral source or diagnostic status (ADHD or Control, or proband) and administered by highly trained and supervised psychometricians. The interviewers had undergraduate degrees in psychology and were extensively trained. First, they underwent several weeks of classroom style training, learning interview mechanics, diagnostic criteria, and coding algorithms. Then, they observed interviews by experienced raters and clinicians. They subsequently conducted at least six practice (non-study) interviews and at least three study interviews while being observed by senior interviewers. Trainees were not permitted to conduct interviews independently until they executed at least three interviews that achieved perfect diagnostic agreement with an observing senior interviewer. Based on 500 assessments from interviews of children and adults, the median kappa coefficient of agreement between a psychometrician and an experienced clinician was 0.98. Diagnoses were considered positive if DSM diagnostic criteria were unequivocally met (DSM-III-R for boys and girls

TABLE I. Association of Genetic Risk Severity With Sociodemographic Characteristics

	Mean ± SD	β	Adjusted R^2	R^2	F	t	*P*-value
Age							
All participants[a]	11.1 ± 3.4	−0.2	0.004	0.005	(1, 590)	−1.8	0.07
With ADHD[b]	11 ± 3.4	−0.3	0.01	0.01	(1, 462) = 0.04	−2.1	0.04*
Without ADHD[c]	11.6 ± 3.4	0.05	−0.01	0.000	(1, 127) = 0.04	0.2	0.8
SES							
All participants[a]	1.9 ± 1	0.02	−0.001	<0.000	(1, 590) = 0.4	0.7	0.5
With ADHD[b]	1.9 ± 0.9	0.01	−0.002	0.000	(1, 462) = 0.06	0.2	0.8
Without ADHD[c]	2 ± 1.1	0.08	−0.001	0.01	(1, 127) = 0.8	0.9	0.4
Sex							
All participants[a]						0.04	0.9
With ADHD[b]						0.02	0.9
Without ADHD[c]						0.1	0.9

*$P < 0.05$.
[a]N = 591.
[b]N = 463.
[c]N = 128.

study baseline; DSM-IV for the genetic linkage and bipolar study). Biederman et al. [1997] showed that 93% of children with a DSM-III-R diagnosis also received a DSM-IV diagnosis.

A committee of board-certified child and adult psychiatrists and experienced psychologists, who were blind to the subjects' ascertainment status and all other data, resolved diagnostic uncertainties. Diagnoses presented for review were considered positive only when the committee determined that diagnostic criteria were met to a clinically meaningful degree. We estimated the reliability of the diagnostic review process by computing kappa coefficients of agreement for clinician reviewers. For these diagnoses, the median reliability between diagnoses was 0.87.

Mothers provided information regarding their child's history of school problems (e.g., grade retention, special placements, and remedial assistance) and treatment history (i.e., counseling, medication, and hospitalization).

The Global Assessment of Functioning (GAF) rating scale was used to rate the overall functioning of participants. Participants were given a score of 0–90, with 90 being the highest possible score indicating the least impairment in their social, occupational, psychological, and physical functioning [American Psychiatric Association, 2000]. GAF scores were assigned to each participant based on the information obtained through the diagnostic interview and were also reviewed by the committee. Scores reported represent lowest lifetime score.

The Hollingshead Four-Factor Index was used to assess socioeconomic status on a scale of 1–5, 1 representing the most affluent social class and 5 the least affluent [Hollingshead, 1975].

The Child Behavior Checklist (CBCL) was used to measure behavioral and emotional problems. The CBCL is an affordable pencil and paper test completed by the child's caregiver, requiring no administration by a physician or rater. Use, scoring, and pricing

TABLE II. Association of Genetic Risk Severity and Clinical Characteristics

		Mean ± SD	β	Adjusted R^2	R^2	F	t	*P*-value
Past GAF score	All participants[a]	51 ± 9.3	−0.0009	−0.002	<0.000	(1, 590) = 0.03	−0.2	0.9
	With ADHD[b]	49 ± 8.2	−0.003	−0.002	0.000	(2, 461) = 0.2	−0.5	0.6
	Without ADHD[c]	56 ± 11	0.004	−0.01	0.002	(1, 127) = 0.3	0.5	0.6
Current GAF score	All participants[a]	56 ± 9	−0.004	−0.0007	0.001	(1, 590) = 0.6	−0.8	0.4
	With ADHD[b]	55 ± 8	−0.004	−0.001	0.001	(2, 461) = 0.4	−0.7	0.5
	Without ADHD[c]	62 ± 9	−0.2	−0.01	0.001	(1, 127) = 0.1	−0.3	0.8
Mean number of ADHD symptoms	All participants[a]	10.2 ± 3.5	0.01	−0.001	0.001	(1, 590) = 0.5	0.7	0.5
	With ADHD[b]	11.4 ± 2	0.03	<−0.000	0.003	(2, 461) = 0.9	0.9	0.3
	Without ADHD[c]	4 ± 3	0.01	−0.01	0.002	(1, 127) = 0.1	0.4	0.7
ADHD treatment and illness characteristics[d]								
Counseling					0.001	0.003	(2, 461) = 1.5	0.2
Hospitalization					−0.002	0.000	(2, 461) = 0.04	0.8
Medication					−0.002	0.000	(2, 461) = 0.3	0.6
Counseling + medication					−0.002	0.000	(2, 461) = 0.2	0.6
Illness impairment					−0.009	0.005	(2, 461) = 0.4	0.9
Age of onset		3.8 ± 2.3	−0.08	<−0.000	0.002	(2, 461) = 0.7	−0.8	0.4
School characteristics								
School tutoring	All participants[a]				−0.001	0.009	(1, 590) = 0.9	0.5
	With ADHD[b]				0.002	0.02	(2, 461) = 1.2	0.3
	Without ADHD[c]				−0.01	0.03	(1, 127) = 0.8	0.5
Placement in special class	All participants[a]				−0.005	0.006	(1, 590) = 0.6	0.8
	With ADHD[b]				−0.01	0.01	(2, 461) = 0.4	0.9
	Without ADHD[c]				−0.01	0.03	(1, 127) = 0.7	0.6
Repeated grade	All participants[a]				−0.007	0.003	(1, 590) = 0.3	0.9
	With ADHD[b]				−0.01	0.01	(2, 461) = 0.4	0.9
	Without ADHD[c]				−0.03	0.01	(1, 127) = 0.3	0.9

[a]N = 591.
[b]N = 463.
[c]N = 128.
[d]ADHD participants, only.

TABLE III. Association of Genetic Risk Severity With Individual Lifetime ADHD Symptoms

	With ADHD (N = 463)		Without ADHD (N = 128)		All participants (N = 591)	
ADHD symptom	**Test statistic**	***P*-value**	**Test statistic**	***P*-value**	**Test statistic**	***P*-value**
Difficulty remaining seated	$F(2, 461) = 0.1$	0.8	$F(1, 127) = 0.6$	0.4	$F(1, 590) = 0.01$	0.9
Fidgety	$F(2, 461) = 1.7$	0.2	$F(1, 127) = 1.7$	0.2	$F(1, 590) = 1.7$	0.2
Difficulty playing quietly	$F(2, 461) = 0.7$	0.4	$F(1, 127) = 0.03$	0.9	$F(1, 590) = 0.6$	0.4
Talks excessively	$F(2, 461) = 0.5$	0.5	$F(1, 127) = 0.04$	0.8	$F(1, 590) = 0.3$	0.6
Shifts activities	$F(2, 461) = 0.6$	0.4	$F(1, 127) = 0.7$	0.4	$F(1, 590) = 1.02$	0.3
Difficulty sustaining attention	$F(2, 461) = 0.1$	0.8	$F(1, 127) = 0.06$	0.8	$F(1, 590) = 0.01$	0.9
Difficulty following instructions	$F(2, 461) = 2.3$	0.1	$F(1, 127) = 0.00$	1.0	$F(1, 590) = 1.2$	0.3
Easily distracted	$F(2, 461) = 0.1$	0.8	$F(1, 127) = 0.3$	0.6	$F(1, 590) = 0.1$	0.7
Interrupts or intrudes	$F(2, 461) = 0.1$	0.8	$F(1, 127) = 0.04$	0.8	$F(1, 590) = 0.2$	0.7
Blurts out answers	$F(2, 461) = 0.3$	0.6	$F(1, 127) = 0.02$	0.9	$F(1, 590) = 0.2$	0.6
Difficulty waiting turn	$F(2, 461) = 7.7$	0.003**	$F(1, 127) = 0.1$	0.8	$F(1, 590) = 6.6$	0.01*
Acts before thinking	$F(2, 461) = 3$	0.1	$F(1, 127) = 0.01$	0.9	$F(1, 590) = 2.2$	0.1
Loses things	$F(2, 461) = 0.2$	0.6	$F(1, 127) = 0.2$	0.6	$F(1, 590) = 0.4$	0.5
Does not listen	$F(2, 461) = 0.4$	0.5	$F(1, 127) = 0.03$	0.9	$F(1, 590) = 0.4$	0.5

*$P < 0.05$.
**$P < 0.01$.

information is easily accessible at http://www.aseba.org/. The CBCL queries the parent about the child's behavior in the past 6 months and aggregates these data into behavioral problem T-scores. A computer program calculates the T-scores for each scale. Raw scores are converted to gender and age-standardized scores (T-scores having a mean = 50 and SD = 10). A minimum T-score of 50 is assigned to scores that fall at midpoint percentiles of $\leq$50 on the syndrome scales to permit comparison of standardized scores across scales. The parent (usually the mother) of each participant completed either the 1991 or the 2001 version of the CBCL. As previously reported [Spencer et al., 2011], we defined Deficient Emotional Self-Regulation (DESR) if a child had an aggregate cut-off score of $>$180 but $<$210 on the Anxiety/Depression, Aggression, and Attention scales of the CBCL (CBCL–DESR), and Severe Emotional Dysregulation if a child had an aggregate cut-off score of $\geq$210 on the same CBCL scales as above [Biederman et al., 2012].

Intellectual functioning was assessed through administration of the Vocabulary, Block Design, Digit Span, Digit Symbol, Digit Coding, and Arithmetic subtests of the WISC-R [Wechsler, 1974]. Using procedures suggested by Sattler [1988] we estimated Full Scale IQ from the Block Design and Vocabulary subtests of the WISC-R using age-corrected scaled scores. We computed the Freedom From Distractibility IQ from the Digit Span, Digit Coding, and Oral Arithmetic subscales of the WISC-R. We assessed reading and arithmetic achievement with subtests of the Wide Range Achievement Test—Revised [Jastak and Jastak, 1985].

Genotyping

Genotyping of the HTT polymorphism and DRD4 VNTRs was performed using the following protocol. Genomic DNA (5 ng) was amplified in a 7 μl reaction using KlenTaq DNA Polymerase (0.2 U; DNA Polymerase Technology, Inc., St. Louis, MO), the proprietary KlenTaq Buffer (1×), dNTPs (200 μM each), glycerol (5% for HTT and 10% for DRD4), Betaine (1 M), and the marker specific primers (0.2 μM). The DRD4 VNTR primers were as follows: DRD4-EX03B-F VIC-GACCGCGACTACGTGGTC-TACTC, DRD4-EX03B-R CTCAGGACAGGAACCCACCGAC. The DRD4-EX03B-R primer also contains a proprietary tail that helps stabilize the amplified product. The HTT promoter VNTR x primers were as follows: SLC6A4_PRO-F 6FAM-ATGCCAG-CACCTAACCCCTAATGT, SLC6A4_PRO-R GGACCG-CAAGGTGGGCGGGA. Amplification was performed with the following protocol: 13 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec beginning at 61.5°C for the HTT marker and 69.5°C for the DRD4 marker and dropped 0.5°C every cycle and primer extension at 72°C for 30 sec; 37 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec at 55°C for the HTT marker and 63°C for the DRD4 marker and primer extension at 72°C for 30 sec; 72°C for 1 hr.

Genotyping of DAT1 VNTRs used the following protocol. Genomic DNA (5 ng) was amplified in a of 7 μl reaction using HotStarTaq DNA Polymerase (0.2 U; Qiagen, Valencia, CA), the proprietary HotStarTaq Buffer (1×), dNTPs (200 μM each), and the marker-specific primers (0.2 μM). Primers were as follows: DAT1-F 6FAM–TGTGGTGTAGGGAACGGCCTGAG, DAT1-R CCTCCTGGAGGTCACGGCTCAAGG. The DAT1-R primers also contain the proprietary tail. Amplification for DAT1 was performed as follows. Samples were heated at 92°C for 9 min to activate the HotStarTaq Polymerase. This is followed by 12 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec beginning at 64.5°C and dropped 0.5°C every cycle and primer extension at 72°C for 30 sec; 37 cycles of denaturation for 30 sec at 93°C, annealing for 30 sec at 58°C and primer extension at 72°C for 30 sec; 72°C for 1 hr.

Amplified products were pooled and combined with size standard (LIZ-500, Applied Biosystems, Foster City, CA) before being

TABLE VI. Neuropsychological Profile Associated With Genetic Risk Severity

Test	Task	Sample	β	Adjusted R^2	R^2	F	t	*P*-value
WRAT-III	Reading SS	All participants[a]	0.002	−0.002	0.001	F(1, 590) = 0.3	0.5	0.6
		With ADHD[b]	0.004	−0.000	0.002	F(2, 461) = 0.8	0.9	0.4
		Without ADHD[c]	−0.005	−0.008	0.004	F(1, 127) = 0.3	−0.6	0.6
	Arithmetic SS	All participants[a]	−0.001	−0.001	0.001	F(1, 590) = 0.4	−0.7	0.5
		With ADHD[b]	0.002	<−0.002	<0.000	F(2, 461) = 0.3	0.5	0.6
		Without ADHD[c]	−0.001	−0.002	0.007	F(1, 127) = 0.8	−0.9	0.4
WISC-R	Full IQ	All participants[a]	0.007	0.3	0.5	F(1, 590) = 3.1	−1.6	0.2
		With ADHD[b]	0.008	−0.3	0.01	F(2, 461) = 1.2	−1.4	0.3
		Without ADHD[c]	−0.002	−0.01	0.001	F(1, 127) = 0.08	−0.3	0.5
	Block design	All participants[a]	−0.001	−0.008	<0.000	F(1, 590) = 0.00	−0.03	0.9
		With ADHD[b]	0.004	−0.008	<0.000	F(2, 461) = 0.02	0.1	0.9
		Without ADHD[c]	−0.31	0.4	0.5	F(1, 127) = 3.2	−1.8	0.2
	Vocabulary	All participants[a]	−0.03	−0.002	0.006	F(1, 590) = 0.7	−0.8	0.4
		With ADHD[b]	−0.02	−0.006	0.003	F(2, 461) = 0.4	−0.6	0.6
		Without ADHD[c]	−0.32	0.5	0.6	F(1, 127) = 5.1	−2.3	0.1
	Digit symbol	All participants[a]	−0.008	−0.004	0.004	F(1, 590) = 0.5	−0.7	0.8
		With ADHD[b]	−0.002	−0.008	<0.000	F(2, 461) = 0.00	−0.07	0.9
		Without ADHD[c]	0.08	−0.3	0.02	F(1, 127) = 0.05	0.2	0.8
	Arithmetic	All participants[a]	−0.03	−0.002	0.006	F(1, 590) = 0.7	−0.9	0.4
		With ADHD[b]	−0.03	−0.002	0.007	F(2, 461) = 0.8	−0.9	0.4
		Without ADHD[c]	0.03	−0.3	0.01	F(1, 127) = 0.03	0.2	0.9
	Digit span	All participants[a]	−0.02	−0.004	0.004	F(1, 590) = 0.5	−0.7	0.5
		With ADHD[b]	−0.02	−0.004	0.004	F(2, 461) = 0.5	−0.7	0.5
		Without ADHD[c]	−0.4	0.3	0.5	F(1, 127) = 3.1	−1.8	0.2
	Freedom from distractibility	All participants[a]	−0.006	−0.002	0.006	F(1, 590) = 0.8	−0.9	0.4
		With ADHD[b]	−0.005	−0.003	<0.006	F(2, 461) = 0.7	−0.8	0.4
		Without ADHD[c]	−0.006	−0.002	0.006	F(1, 127) = 0.09	−0.3	0.8
Stroop test	Word	All participants[a]	−0.002	−0.002	<0.000	F(1, 590) = 0.04	−0.2	0.8
		With ADHD[b]	−0.001	−0.003	<0.000	F(2, 461) = 0.3	−0.2	0.9
		Without ADHD[c]	−0.003	−0.01	<0.000	F(1, 127) = 0.04	−0.2	0.8
	Color	All participants[a]	0.003	−0.002	0.001	F(1, 590) = 0.2	0.4	0.7
		With ADHD[b]	0.007	−0.001	0.002	F(2, 461) = 0.7	0.8	0.4
		Without ADHD[c]	−0.009	−0.006	0.006	F(1, 127) = 0.5	−0.7	0.5
	Word/color	All participants[a]	0.002	−0.002	0.001	F(1, 590) = 0.1	0.4	0.7
		With ADHD[b]	0.003	−0.003	<0.000	F(2, 461) = 0.2	0.4	0.7
		Without ADHD[c]	−0.002	−0.01	<0.000	F(1, 127) = 0.03	−0.2	0.9
	Interference	All participants[a]	0.006	−0.001	0.001	F(1, 590) = 0.5	0.7	0.5
		With ADHD[b]	0.006	−0.002	0.001	F(2, 461) = 0.4	0.6	0.5
		Without ADHD[c]	0.007	−0.01	0.002	F(1, 127) = 0.14	0.4	0.7
Wisconsin card sorting test	Categories completed	All participants[a]	−0.02	−0.002	0.001	F(1, 590) = 0.3	−0.5	0.6
		With ADHD[b]	−0.03	−0.002	0.002	F(2, 461) = 0.5	−0.7	0.5
		Without ADHD[c]	0.03	−0.01	0.002	F(1, 127) = 0.1	0.3	0.7
	Perseverative errors	All participants[a]	−0.002	−0.002	0.0004	F(1, 590) = 0.2	−0.4	0.7
		With ADHD[b]	−0.001	−0.004	<0.000	F(2, 461) = 0.00	−0.02	0.9
		Without ADHD[c]	−0.007	−0.005	0.009	F(1, 127) = 0.7	−0.8	0.4
	Non-perseverative errors	All participants[a]	0.004	0.0001	0.003	F(1, 590) = 1.1	1	0.3
		With ADHD[b]	0.006	0.002	0.005	F(2, 461) = 1.4	1.2	0.2
		Without ADHD[c]	−0.003	−0.01	0.002	F(1, 127) = 0.12	−0.3	0.7
	Failure to maintain set	All participants[a]	−0.04	−0.001	0.002	F(1, 590) = 0.8	−0.9	0.4
		With ADHD[b]	−0.06	0.001	0.005	F(2, 461) = 1.3	−1.1	0.3
		Without ADHD[c]	0.04	−0.01	0.002	F(1, 127) = 0.13	0.4	0.7

[a]N = 591.
[b]N = 463.
[c]N = 128.

TABLE VII. Child Behavior Checklist (CBCL) Profile Associated With Genetic Risk Severity

	Sample	β	Adjusted R^2	R^2	F	t	*P*-value
CBCL scales							
Delinquent	All participants[a]	0.7	0.004	0.01	F(1, 590) = 1.7	1.3	0.2
	With ADHD[b]	0.8	0.006	0.01	F(2, 461) = 2	1.4	0.2
	Without ADHD[c]	−0.4	−0.06	0.003	F(1, 127) = 0.06	−0.2	0.8
Aggressive	All participants[a]	0.6	−0.001	0.004	F(1, 590) = 0.8	0.9	0.4
	With ADHD[b]	−1.1	−0.03	0.03	F(2, 126) = 0.5	−0.7	0.5
	Without ADHD[c]	0.7	<0.000	0.006	F(1, 127) = 1.07	1.03	0.3
Withdrawn	All participants[a]	0.9	0.01	0.02	F(1, 590) = 3.4	1.8	0.07
	With ADHD[b]	0.02	0.03	0.01	F(2, 461) = 2.9	2	0.06
	Without ADHD[c]	0.14	−0.06	<0.000	F(1, 127) = 0.00	0.06	0.9
Somatic	All participants[a]	−0.002	−0.005	<0.000	F(1, 590) = 0.03	−0.2	0.9
	With ADHD[b]	−0.3	−0.004	0.002	F(2, 461) = 0.3	−0.6	0.6
	Without ADHD[c]	2.8	0.06	0.11	F(1, 127) = 2.04	1.4	0.2
Anxious/depressed	All participants[a]	0.7	0.006	0.001	F(1, 590) = 1.1	1.1	0.3
	With ADHD[b]	0.5	−0.06	0.003	F(1, 127) = 0.04	0.2	0.8
	Without ADHD[c]	0.7	<0.000	0.01	F(2, 461) = 1.07	1.03	0.3
Social problems	All participants[a]	0.2	−0.005	0.007	F(1, 590) = 0.8	0.05	0.5
	With ADHD[b]	0.5	−0.002	0.004	F(2, 461) = 0.6	0.8	0.4
	Without ADHD[c]	0.1	−0.06	<0.000	F(1, 127) = 0.00	0.03	0.9
Thought problems	All participants[a]	−0.3	−0.004	0.001	F(1, 590) = 0.2	−0.5	0.6
	With ADHD[b]	−0.2	−0.005	<0.000	F(2, 461) = 0.07	−0.3	0.8
	Without ADHD[c]	−1.8	−0.02	0.04	F(1, 127) = 0.7	−0.9	0.4
Attention problems	All participants[a]	0.3	−0.003	0.009	F(1, 590) = 0.5	0.08	0.6
	With ADHD[b]	0.4	−0.004	0.002	F(2, 461) = 0.3	0.6	0.6
	Without ADHD[c]	−0.2	−0.06	<0.000	F(1, 127) = 0.01	−0.1	0.9
Composite scales							
Externalizing	All participants[a]	0.9	0.01	0.01	F(1, 590) = 1.8	1.1	0.2
	With ADHD[b]	1.1	0.007	0.01	F(2, 461) = 2.3	1.5	0.2
	Without ADHD[c]	0.09	−0.06	<0.000	F(1, 127) = 0.00	0.03	0.9
Internalizing	All participants[a]	0.7	−0.001	0.005	F(1, 590) = 0.9	0.9	0.3
	With ADHD[b]	0.5	−0.003	0.003	F(2, 461) = 0.5	0.7	0.5
	Without ADHD[c]	2.5	−0.01	0.05	F(1, 127) = 0.8	0.9	0.4
Total problems	All participants[a]	0.8	0.001	0.007	F(1, 590) = 0.8	0.7	0.2
	With ADHD[b]	0.8	0.002	0.008	F(2, 461) = 1.3	1.2	0.3
	Without ADHD[c]	0.7	−0.06	0.004	F(1, 127) = 0.06	0.3	0.8

[a]N = 591.
[b]N = 463.
[c]N = 128.

more robust than the evidence for its influence on internalizing disorders [DeYoung et al., 2006; Propper et al., 2007; Bakermans-Kranenburg et al., 2008]. Because two (*DRD4*, *DAT1*) of our three polymorphisms are part of the dopaminergic system, this may have influenced the results so that the association between genetic risk severity and disruptive behavioral disorders, but not anxiety disorders, may have been a result of the unequal and larger variance conferred by *DAT1* and *DRD4* versus *5-HTT* to disruptive

TABLE VIII. Emotion Dysregulation Profile Associated With Genetic Risk Severity

	With ADHD (N = 463)		Without ADHD (N = 128)		All participants (N = 591)	
	Test statistic	*P*-value	Test statistic	*P*-value	Test statistic	*P*-value
Severe emotion dysregulation (CBCL ≥ 210)	F(2, 461) = 1.8	0.2	F(1, 127) = 0.2	0.6	F(1, 590) = 1.3	0.3
CBCL-DESR	F(2, 461) = 0.5	0.5	F(1, 127) = 0.4	0.7	F(1, 590) = 0.4	0.5

behavior disorders and specifically, to ODD. More research is needed to investigate the relationship of genetic risk severity with internalizing versus externalizing disorders within the context of ADHD.

We also found that both in the entire sample and in the sample of children with ADHD-only, greater genetic risk severity was positively significantly associated with the ADHD symptom "has difficulty waiting turn." This is a novel finding that implicates one specific symptom out of the possible 14 as most strongly associated with a genetic etiology. More research is needed to understand the possible uniqueness of this symptom and its potential utility to rapidly screen for ADHD.

Additionally, although the question of whether genetic risk severity predicts ADHD diagnostic status was beyond the scope of this study, the observation that a sizeable portion of our sample not meeting criteria for ADHD shared a genetic risk severity similar to that of participants with ADHD bears elaboration. Other unmeasured sources of heterogeneity, both genetic and non-genetic (e.g., environmental effects) could be at play as potentially protective factors against ADHD in participants without ADHD with a similar genetic risk severity score as in participants with ADHD. This would be consistent with research on the role of the environment in ADHD, in particular, in its interaction with genetic factors [Milberger et al., 1997; Ficks and Waldman, 2009; Sonuga-Barke et al., 2009; Grizenko et al., 2012; Nikolas et al., 2012]. In fact, Gizer et al. [2009] found that 65% of the total variation in study effect sizes was due to sources of heterogeneity (e.g., study methodology, environmental effects) for *DAT1*, 54% for *DRD4* and 66% for *5-HTT*. Further research should examine the interaction of cumulative genetic risk severity with a number of environmental factors, including, but not limited to family environment [Thakur et al., 2012; van den Hoofdakker et al., 2012], perinatal factors [Milberger et al., 1997; Nijmeijer et al., 2010], and parenting styles [Sonuga-Barke et al., 2009; Kumsta et al., 2010; Lahey et al., 2011; Li and Lee, 2012]. Also, the finding that 22% of our sample not meeting criteria for ADHD shared a genetic risk severity similar to that of participants with ADHD suggests low penetrance for ADHD. More research is needed to better understand the role of accumulative genetic risk severity in ADHD.

Our results must be interpreted in the context of some methodological limitations. Although we did not exclude any ethnic group, all of our subjects were Caucasian. Thus, our results may not generalize to other racial and ethnic groups. Although our results are based on a large sample of subjects with risk allele data, the number of participants with 0, 1, 5, and 6 risk alleles was relatively small given the rare occurrence of such allelic permutations, which may limit statistical power to test our hypothesis and bias results. Additionally, 127 participants were assessed by DSM-III criteria and 464 participants were assessed by DSM-IV criteria and thus findings may have differed depending on the DSM version used. However, Biederman et al. [1997] showed that 93% of children with a DSM-III-R diagnosis also received a DSM-IV diagnosis, so any effect on these results would be minimal. Finally, our findings were of moderate effect size and statistical significance. Although these would not have been significant after correcting for multiple comparisons, our findings for disruptive behavior disorders remain compelling given that they conceptually replicate prior work [Hamshere et al., 2013]. The less-than-robust strength of these associations may be explained by Gizer et al.'s [2009] meta-analytic finding, which already indicated a significant but modest association between ADHD and the 10-repeat *DAT1* VNTR allele as well as between ADHD and the "long" allele of *5-HTTLPR*. The association between the 7-repeat *DRD4* VNTR indicated a moderate effect with substantial heterogeneity in effect size across studies included in the meta-analyses.

Despite these limitations, our study is the first to investigate the association of ADHD clinical characteristics and functional outcomes through the framework of an additive polygenic model comprising of the *DRD4*, *DAT1*, and *5-HTT* putative risk alleles for ADHD. Our findings document a significant association between genetic risk severity and co-morbid psychiatric disorders in the context of ADHD. They also document an association with the severity of genetic risk that is discrepant for the two clusters of disorders implicated (i.e., disruptive behavior disorders and anxiety disorders), suggesting that co-morbid internalizing versus externalizing disorders in ADHD may be influenced by independent genetic factors.

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