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# Case-Control Genome-Wide Association Study of Attention- Deficit/Hyperactivity Disorder

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**Objective:** Although twin and family studies have shown attention-deficit/hyperactivity disorder (ADHD) to be highly heritable, genetic variants influencing the trait at a genomewide significant level have yet to be identified. Thus additional genomewide association studies (GWAS) are needed. **Method:** We used case-control analyses of 896 cases with *DSM-IV* ADHD genotyped using the Affymetrix 5.0 array and 2,455 repository controls screened for psychotic and bipolar symptoms genotyped using Affymetrix 6.0 arrays. A consensus SNP set was imputed using BEAGLE 3.0, resulting in an analysis dataset of 1,033,244 SNPs. Data were analyzed using a generalized linear model. **Results:** No genomewide significant associations were found. The most significant results implicated the following genes: *PRKG1*, *FLNC*, *TCERG1L*, *PPM1H*, *NXPH1*, *PPM1H*, *CDH13*, *HK1*, and *HKDC1*. **Conclusions:** The current analyses are a useful addition to the present literature and will make a valuable contribution to future meta-analyses. The candidate gene findings are consistent with a prior meta-analysis in suggesting that the effects of ADHD risk variants must, individually, be very small and/or include multiple rare alleles. *J. Am. Acad. Child Adolesc. Psychiatry*, 2010;49(9):906–920. **Key Words:** ADHD, genetics, genome-wide association, Imputation

Attention-deficit/hyperactivity disorder (ADHD) is among the most common childhood onset psychiatric disorders. The worldwide prevalence of ADHD in children is 8% to 12%<sup>1</sup> and the prevalence of ADHD in adults in the United States is approximately 4%.<sup>2,3</sup> Early studies found the risk of ADHD among parents of children with ADHD to be increased by between two- and eightfold, with similarly elevated risk among the siblings of ADHD subjects (for a review of this literature, see Faraone and Biederman <sup>4</sup>). Faraone et al.<sup>5</sup> extended these findings to families ascertained via adult probands meeting criteria for either full DSM-IV ADHD or late-onset ADHD.

Adoption and twin studies are necessary to disentangle genetic from environmental sources of transmission observed in family studies. Three studies found that biological relatives of ADHD <sup>6</sup> or hyperactive children <sup>7,8</sup> were more likely to have hyperactivity than adoptive relatives. A more direct method of examining the heritability of ADHD is to study twins: the extent to which monozygotic twins are more concordant for ADHD than dizygotic twins can be used to compute the degree to which variability in ADHD in the population can be accounted for by genes (i.e., heritability). Reviews of twin studies from the United States, Australia, Scandinavia and the European Union show heritability for ADHD to be approximately 75%, which places it among the most heritable of psychiatric disorders.<sup>9–11</sup>

Candidate gene association studies have focused heavily on catecholaminergic pathways,<sup>16–20</sup> the major target of most pharmacotherapies for ADHD.<sup>21</sup> However, genes within the serotonergic and neuro-developmental pathways have also been examined. A meta-analysis found nominally significant ( $p < .05$ ) associations at the following: SLC6A3/DAT1 (3'UTR VNTR and rs27072), DRD4 (exon 3 VNTR and rs1800955), DRD5 (148-bp allele), SLC6A4/5HTT (5HTTLPR), HTR1B (rs6296), and SNAP-25 (rs3746544)<sup>16</sup>; however, these effects, if present, are likely to be small and have not been unequivocally confirmed by prior genome-wide association scans of ADHD.<sup>22–25</sup>

The present work continues the search for ADHD susceptibility genes by completing a new, independent, multi-site case-control genome-wide association study (GWAS) of DSM-IV ADHD, using the Affymetrix 5.0 and 6.0 arrays.

In an attempt to find regions of chromosomes that might harbor genes for ADHD, several groups have conducted genome-wide linkage scans. This approach examines many DNA markers across the genome to determine whether any chromosomal regions are shared more often than expected among ADHD family members. These have produced mixed results, with some reporting evidence of linkage <sup>12,13</sup> and others not.<sup>14</sup> To determine whether there were any significant linkage signals among these studies, Zhou et al.<sup>15</sup> conducted a genome scan meta-analysis of these data. They reported genome-wide significant linkage ( $pSR = .00034$ ,  $pOR = .04$ ) for a region on chromosome 16 between 64 Mb and 83 Mb. Although this finding is intriguing and worthy of follow-up, the lack of significant findings for other loci suggests that many genes of moderately large effect are unlikely to exist, and that the method of association will be more fruitful in the search for ADHD susceptibility genes.

## Method

### Participants

The 1,150 cases used in the present analysis consist of (a) samples collected by a subset of the International Multicenter ADHD Genetics (IMAGE) Project sites but not included in the IMAGE GWAS [23](#); and (b) samples collected at additional sites (Frankfurt/Homburg, Trier, Wuerzburg, Germany, Scotland, and Cardiff, United Kingdom) that were assessed in a manner similar to IMAGE samples. Cases were identified mainly through outpatient clinics at the data collection sites. They were predominantly of European origin from the United Kingdom, Ireland, Germany, the Netherlands, and the United States. Of the cases, 81% met criteria for DSM-IV ADHD. Children had been referred for assessment of hyperactive, disruptive or disorganized behavior and had been clinically diagnosed as ADHD (or hyperkinetic disorder, the most closely equivalent category in the ICD-10 nomenclature used at some of the clinics). Clinical and demographic features of the case sample stratified by site are provided in [Table 1](#). All case data were collected with informed consent of parents and with the approval of the site's institutional review board or ethical committee.

At the IMAGE sites, parents of children were interviewed with the Parental Account of Childhood Symptom (PACS), a semi-structured, standardized, investigator-based interview developed as an instrument to provide an objective measure of child behavior. Both parents and teachers completed the respective versions of the Conners ADHD rating scales and the Strengths and Difficulties Questionnaire. Exclusion criteria were autism, epilepsy, IQ <70, brain disorder, and any genetic or medical disorder associated with externalizing behaviors that might mimic ADHD.

In Germany, families were recruited in order of clinical referral in the outpatient clinics in Wuerzburg, Homburg, and Trier. Families were of German white ancestry. All cases met DSM-IV criteria for ADHD. The index child was 6 years or more of age, and further affected siblings were included when at least 6 years of age. All children were assessed by full semistructured interview (Kiddie-Sads-PL-German Version or Kinder-DIPS) and parent and teacher ADHD DSM-IV–based rating scales to ensure pervasiveness of symptoms. Exclusion criteria were IQ < 80, comorbid autistic disorders or somatic disorders (e.g., hyperthyroidism, epilepsy, neurological diseases, severe head trauma), primary affective disorders, Tourette syndrome, psychotic disorders or other severe primary psychiatric disorders, and birth weight <2,000 g.

At the Cardiff site, children ages 6 to 16, of British, Caucasian ancestry, were assessed by interviewing parents with the Parent Child and Adolescent Psychiatric Assessment (CAPA)-a semi-structured research diagnostic interview and a telephone interview with the teacher using the Child ADHD Teacher Telephone Interview. All cases met diagnostic criteria for DSM-IV ADHD or ICD-10 hyperkinetic disorder or DSM-III-R ADHD and had IQ test scores above 70. Exclusion criteria were pervasive developmental disorder, Tourette

syndrome, psychosis or any neurological conditions.

**TABLE 1** Clinical and Demographic Characteristics of the Case Sample, Stratified by Site

Site	Sex (Male) n (%)	Age (y) Mean $\pm$ SD	ADHD Subtype			Comorbidity	
			IA n (%)	HI n (%)	C n (%)	ODD n (%)	CD n (%)
Cardiff	34 (97)	8.9 $\pm$ 2.0	3 (9)	6 (18)	25 (75)	22 (63)	4 (11)
Homburg	156 (85)	9.7 $\pm$ 1.8	45 (24)	17 (9)	121 (66)	64 (47) <sup>a</sup>	40 (23)
IMAGE I	40 (77)	11.5 $\pm$ 3.0	0 (0)	0 (0)	52 (100)	34 (65)	20 (39)
Ireland	123 (88)	10.9 $\pm$ 4.2	10 (8)	13 (11)	99 (81)	75 (55)	27 (20)
MGH	61 (69)	9.3 $\pm$ 2.5	26 (30)	10 (11)	52 (59)	53 (61)	13 (15)
Netherlands	66 (92)	11.2 $\pm$ 2.8	6 (11)	1 (2)	49 (88)	19 (33)	3 (5)
St. Andrews	47 (96)	10.5 $\pm$ 3.0	2 (4)	4 (8)	43 (88)	16 (34)	8 (17)
Wurzburg	203 (82)	11.0 $\pm$ 2.7	18 (7)	0 (0)	229 (93)	90 (36)	34 (14)
Total	730 (84)	10.5 $\pm$ 2.9	110 (13)	51 (6)	670 (81)	373 (47)	149 (18)

Note: Additional clinical information was missing for 29 subjects (MGH, n = 1; Ireland, n = 2; St. Andrews, n = 1; Wurzburg, n = 20; and Homburg, n = 5). ADHD = attention-deficit/hyperactivity disorder; C = combined type; HI = hyperactive-impulsive; IA = inattentive; IMAGE = International Multicenter ADHD Genetics Project; MGH = Massachusetts General Hospital.

<sup>a</sup>Oppositional defiant disorder (ODD) criteria were not assessed in subjects meeting criteria for conduct disorder (CD) at this site and were therefore not available in 40 subjects.

At the Scottish site, children ages 6 to 16 years, of British white ancestry, were assessed by interviewing parents with the Parent Child and Adolescent Psychiatric Assessment (CAPA), a semistructured research diagnostic interview. To confirm pervasiveness, teachers completed the Conners Teacher Rating Scale. All cases met diagnostic criteria for DSM-IV ADHD. Children with an IQ <70, autistic spectrum disorder, head injury, known chromosomal abnormality, encephalitis or significant medical conditions such as epilepsy were excluded.

The control sample (2,653 population controls of European ancestry) was collected for an institutional review board–approved GWAS of schizophrenia and have been described elsewhere.<sup>26</sup> Briefly, the control participants were drawn from a US nationally representative survey panel (of approximately 60,000 adult individuals at any one time, with constant turnover) ascertained via random digit dialing. Participants were screened for psychosis and bipolar disorder. Control participants were not screened for ADHD. A blood sample was collected via a US national phlebotomy service. Control participants gave written consent for their DNA to be used for medical research at the discretion of NIMH.

## Genotyping

Cases were genotyped using the Affymetrix 5.0 array at the State University of New York Upstate Medical University, Syracuse using the standard protocol issued by Affymetrix. The genotypes were called using both BRLMM-P and BIRDSUITE,<sup>27</sup> with any calling discrepancies coded as missing. Controls were genotyped using the Affymetrix 6.0 array, at the Broad Institute National Center for Genotyping and Analysis. Genotype calls were made with the BIRDSEED program, a module of the BIRDSUITE package.

The control genotype data initially quality controlled by the National Center for Biotechnology Information (NCBI). The quality control (QC) of the control data has been described in detail elsewhere.<sup>26</sup> Briefly, the 2,653 control samples used in the present analyses had call rates >97%, genders consistent with site reports, and 26% to 28.5% heterozygous genotypes, and were of European ancestry (as evaluated by EIGENSTRAT). The prior data-cleaning efforts for this set of genotypes include SNP call rate <95%, Hardy–Weinberg equilibrium,  $p$  value <10<sup>-6</sup>, MAF <1%, plate effects, and removal of SNPs showing more than two Mendelian errors (from a set of trios that are not included in these analyses) or discordant genotypes for duplicate samples.

## QC and Statistical Analyses

As the cases and controls were genotyped using different platforms, we undertook additional QC checks before conducting imputation. To ensure imputation quality, we applied more stringent QC exclusion thresholds and carefully examined differences between cases and controls. Our key criterion for QC consideration was call rate at the sample and SNP levels, as well as call rate differences between cases and controls. These sample and SNP exclusion criteria are found in [Table 2](#).

**TABLE 2** Summary of Case and Control Quality Control (QC) Filtering and Exclusion Criteria, in Order of Operation

QC metric	Cases: Affymetrix 5.0	Controls: Affymetrix 6.0
QC of individuals (n)		
Individuals before QC	1,150	2,653
Individuals call rate <98%	126	0
Related individuals removed	52	0
Ancestry outliers removed	76	198
Individuals after QC stage 2	896	2,455
QC of SNPs (n)		
SNPs on array	500,568	906,600
SNPs dropped because of genotyping failure/lack of annotation/previous NCBI QC	116,295	251,240
SNPs before QC stage 1	384,273	671,422
Merge SNPs	340,536	340,536
SNP call rate 98% (no. of SNPs)	9,349	611
SNP missing difference <0.5%	61,383	0
Allele frequency difference >15% c.f. HapMap3	12	0
Hardy–Weinberg disequilibrium ( $p < .000001$ )	4,822	0
1% MAF	768	0
Final SNP count for imputation		263,591
QC stage 3, after imputation (n)		
SNPs after imputation		1,253,831
Quality score >0.6		1,033,244

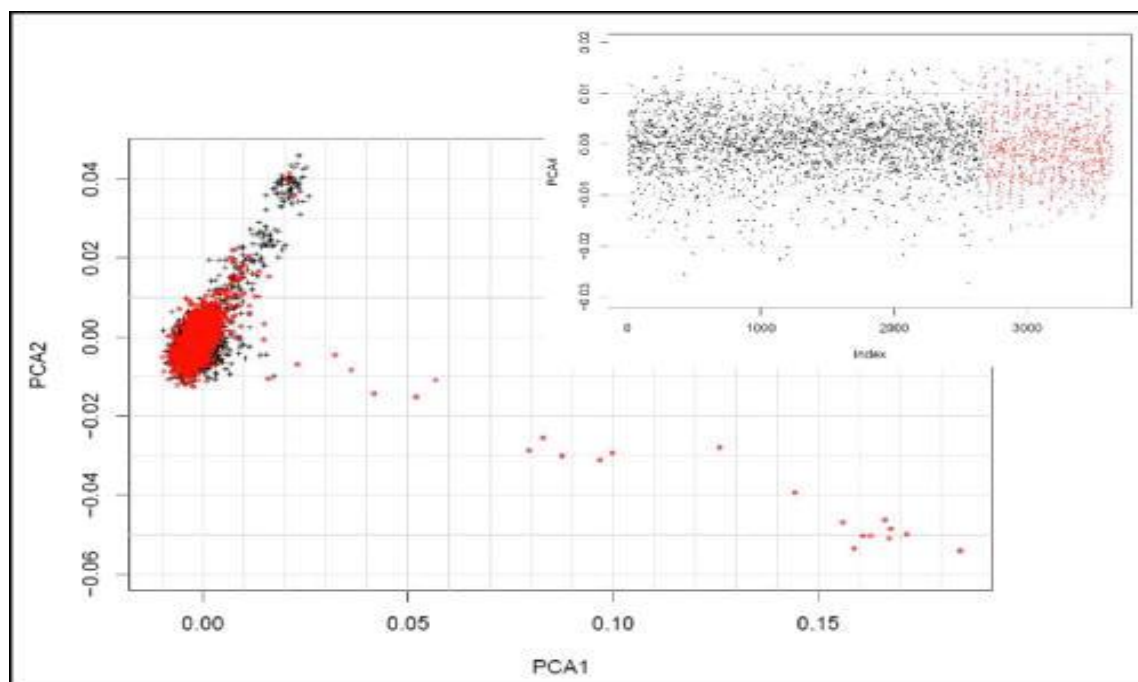
Note: MAF = minor allele frequency; QC = quality control; SNP = single-nucleotide polymorphism.  
<sup>a</sup>Control data were screened for individual call rates >98%, MAF >1% before National Center for Biotechnology Information (NCBI) release. Slight changes occurred when ancestral outliers were excluded.

In the first stage of QC, the case and control samples were screened for low call rate, low MAF, allele frequency differences relative to the European–American and Toscani Italian HapMap samples (CEU and TSI). The two datasets were then merged, resulting in a dataset of 896 cases and 2,455 controls, genotyped on 340,536 SNPs common to both samples. To define the set of SNPs which were included in the analysis, we conducted a first pass case/control analysis in PLINK [28](#) and examined the distribution of association test statistics, with a particular focus on the  $[\lambda]$  statistic (defined as the observed median  $[\chi^2]$  divided by the expected median  $[\chi^2]$ ). We observed a relatively high  $[\lambda]$  of 1.20. A strong correlation was also observed between significance values and call rate. This relationship was more pronounced for SNPs where call rate differences were observed between cases and controls.

Based on these results, we undertook a further round of QC excluding SNPs with a call rate <99%, call rate difference case-control >0.5%, MAF <1. This led to the exclusion of an additional 76,334 SNPs. We also increased the stringency of call rate at the individual level to 98%, which excluded an additional six cases. We chose these exclusion thresholds because SNPs in the excluded categories had a much higher rate of missingness than other SNPs, which could lead to spurious evidence for association. The resulting  $[\lambda]$  was 1.16 with only little evidence for technical inflation of test statistics, as indicated by further metadata such as call rate and Hardy–Weinberg equilibrium p value.

With the merged dataset, before QC, we estimated genome-wide identity by descent using PLINK, which derives this information from the pairwise identity-by-state patterns within the data. Based on these results, we excluded an additional 52 cases (seven avuncular/halfsibs, 35 siblings, 10 identical). To further examine population substructure within our sample, we applied the multidimensional scaling algorithm (MDS) in PLINK to a linkage disequilibrium pruned sample, after cleaning. This MDS algorithm is numerically equivalent to the principal component analysis implemented in Eigenstrat. These units represent the contribution of many hundreds to thousands of SNPs, which share covariance induced by technical artifacts or, more commonly, population stratification. The main plot in [Figure 1](#) shows the first two multidimensional scaling dimensions (PCA1 and PCA2). As shown in [Figure 1](#), the majority of subjects were tightly clustered, indicating that most of the subjects were of European ancestry. The following exclusion criteria were applied based on visual inspection of the MDS plots: MDS dimension 1 > 0.01; MDS dimension 2 > 0.01 and < -0.01; and MDS dimension 4 < -0.02.

**FIGURE 1** Population substructure within the case (red dots) and control (black dots) as assessed by multidimensional scaling. Note: PCA1 \_ first multidimensional scaling dimension.



Based on these thresholds, an additional 274 (198 controls, 76 cases) samples were excluded from the analysis. Thus the final sample consisted of 3,357 individuals (896 cases, 2,455 controls), genotyped on 226,110 SNPs. The [lambda] value arising from a PLINK case-control analysis was calculated as 1.08, suggesting that the methodological and technical confounds have mostly been accounted for. These data were then imputed using BEAGLE 3.0,<sup>29–31</sup> using the HapMap 3 phased CEU and TSI samples as a reference (410 haplotypes).<sup>32,33</sup> The imputation procedure was conducted jointly on cases and controls.

To restrict the analysis to well imputed SNPs, we applied a threshold of 0.6 to the quality score, resulting in an analysis dataset of 1,033,244 SNPs. The quality score was defined as the ratio of the variance of the dosages, as compared with the variance that is predicted from the allele frequency. This can be interpreted as the proportion of information generated by imputing versus genotyping the variant.

### Statistical Analyses

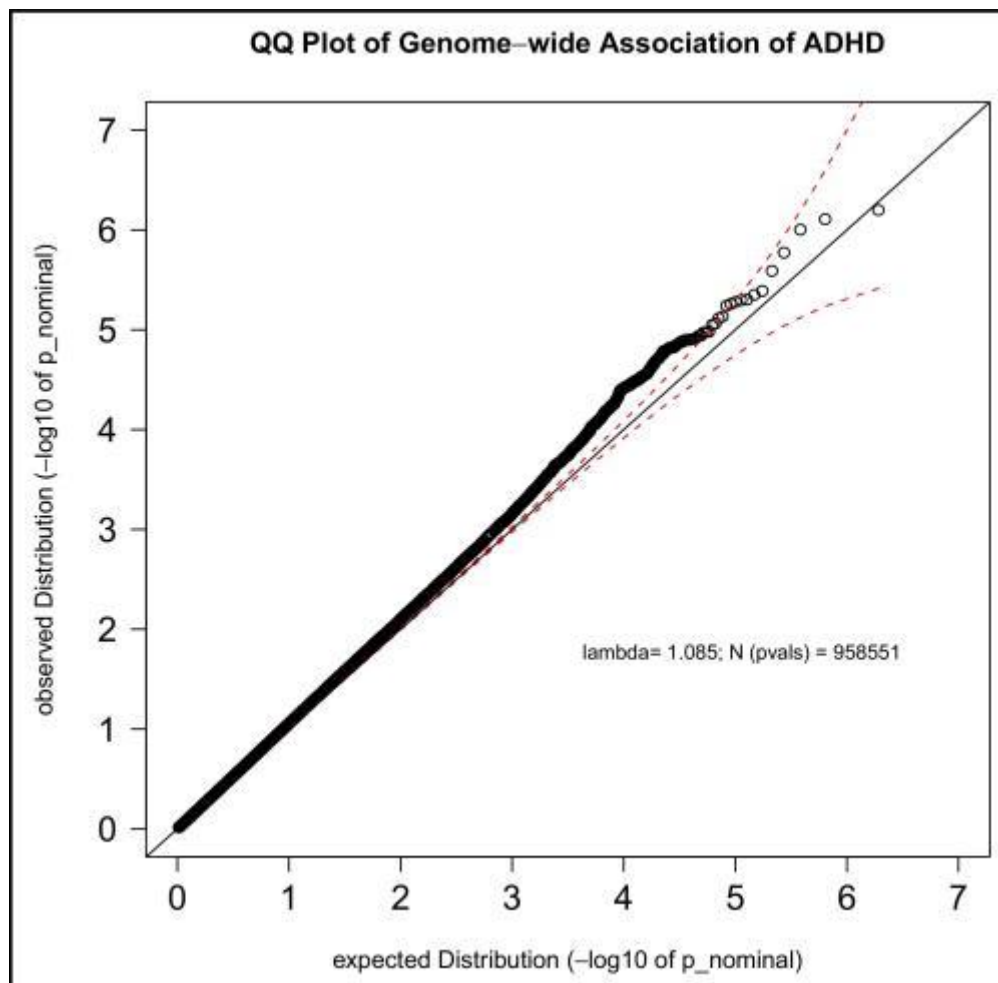
After the QC, we conducted association analysis using a generalized linear model (GLM) in R, using the 10 principal components from the MDS procedure as covariates. This model removes most of the effects of population stratification and any residual technical bias, which helps to control for false-positive results. The p value reported is the Wald statistic from the GLM, which is asymptotically distributed as a chi-square with 1 degree of freedom under the null hypothesis.



## Results

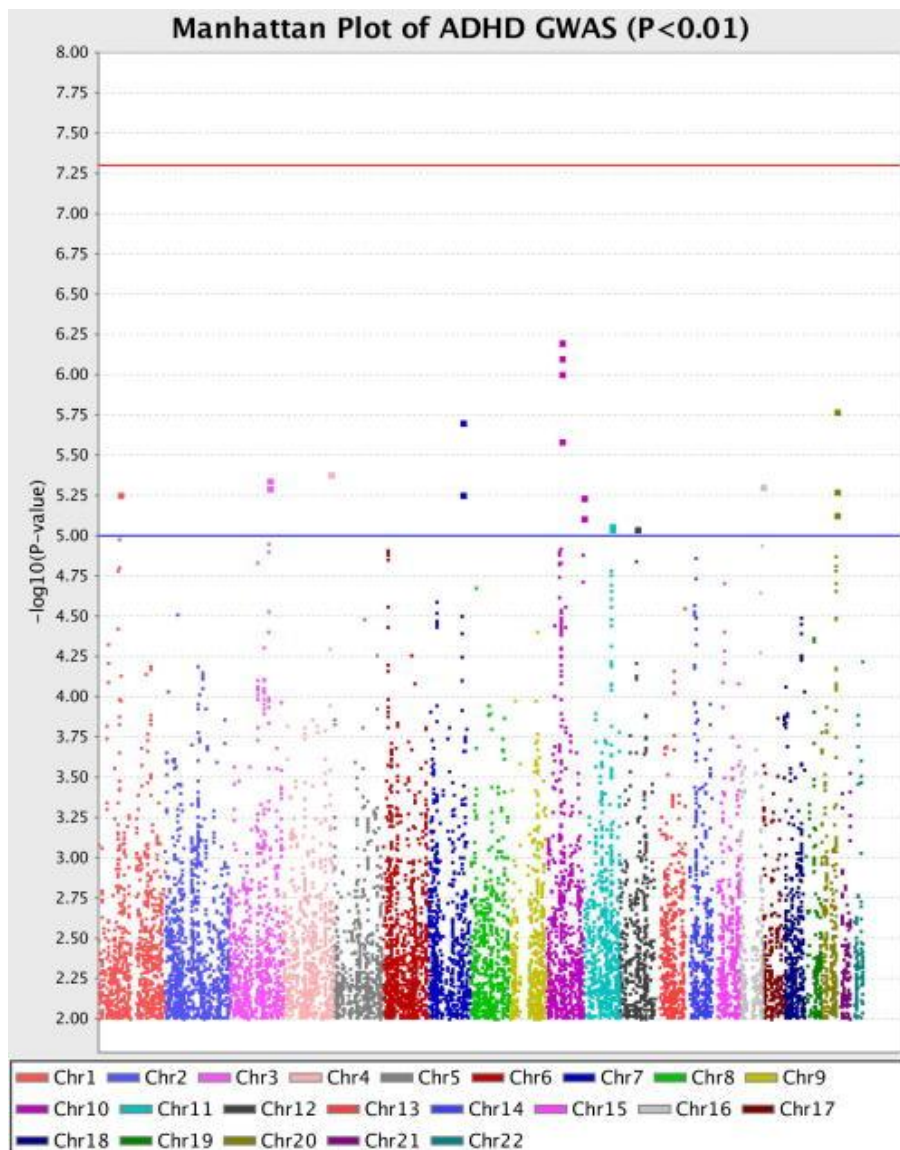
Because genotyping artifacts and ethnic differences between populations can confound GWAS analyses, it is essential to show the absence of artifacts. We do this with the quantile–quantile (QQ) plot in [Figure 2](#), which plots, for each SNP, the observed versus expected p value. In the presence of spurious associations, evidence for association would be seen across all SNPs, i.e., the plotted line would be above the diagonal line throughout the range plotted. We see some inflation above the diagonal, but this is small as measured by a lambda statistic ( $\lambda$ ) of 1.08, which is not much greater than the value of 1.0 expected when there is no inflation. Thus, the QQ plot suggests that our QC procedures removed most association signals that could be attributed to either technical artifacts or population stratification. We further examined the distribution of test statistics and  $\lambda$  by imputation status (genotyped vs. imputed) and by minor allele frequency (using 10 equally spaced bins). There was no obvious correlation of inflation of test statistics with either MAF or imputation status. Similarly there was no relationship between quality score and the distribution of the test statistic.

**FIGURE 2** Quartile– quartile plot of genome-wide association of attention-deficit/hyperactivity disorder.

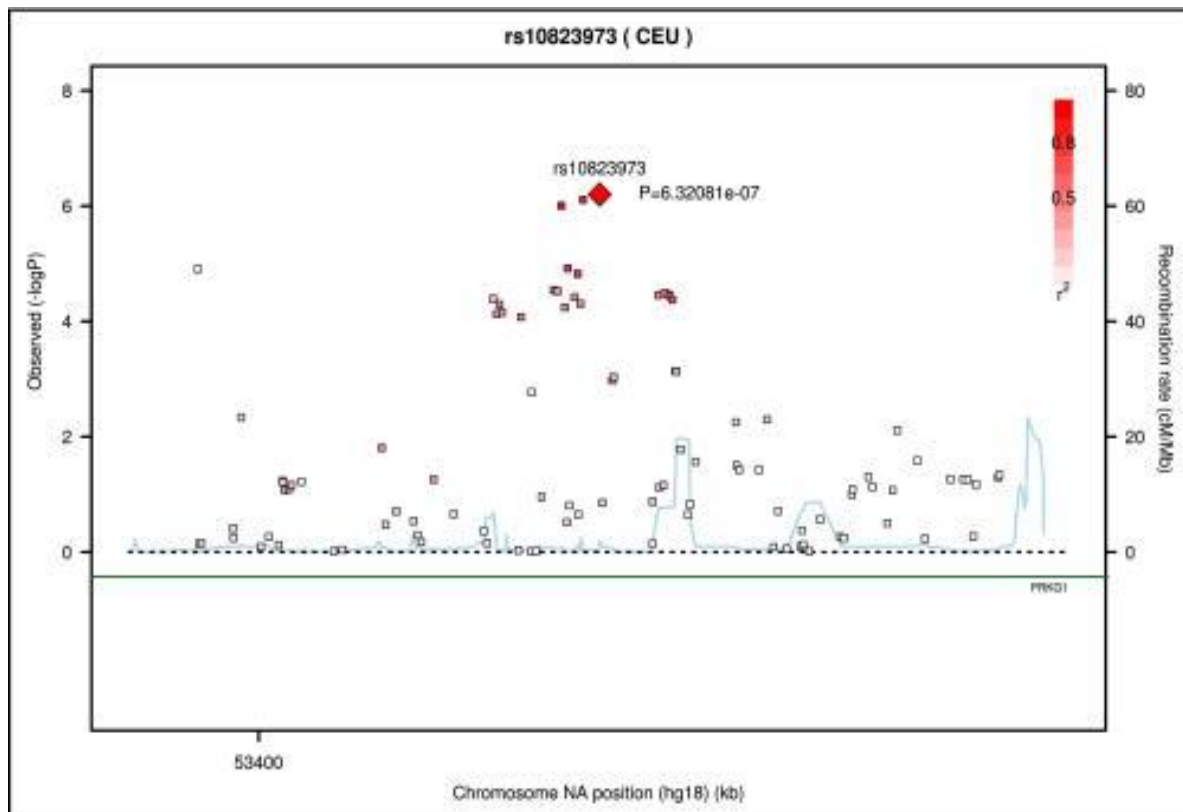


As shown in [Figure 3](#), the strongest signal was observed on chromosome 10 (10q21.1;  $p = 6.32 \times 10^{-7}$ ) driven by rs10823973 (C/G). The association signal from this SNP is supported by association from SNPs with which it is in strong LD. In this sample, the C allele was more common in cases than in controls, with a frequency of 57.8% in cases and 51.2% in controls. The SNP is located in PRKG1. As [Figure 4](#) shows, the association signal is also seen for nearby SNPs in the region, which suggests that the association is true rather than being caused by technical artifact or population stratification. This result lies in a well-established chromosome 10 inversion,<sup>34</sup> spanning 10q11.2 to 10q21.

**FIGURE 3** Manhattan plot from the genome-wide generalized linear model (GLM) analysis. Note: ADHD \_ attention-deficit/hyperactivity disorder; Chr \_ chromosome; GWAS \_ genome-wide association study.



**FIGURE 4** Regional association and linkage disequilibrium (LD) plot for the 10q21.1 region. Note: The most associated genotyped single-nucleotide polymorphism (SNP) is shown in the diamond in bright red, and the color of the remaining markers reflects the linkage disequilibrium ( $r^2$ ) with the top single-nucleotide polymorphism (SNP) in each panel (increasing red hue associated with increasing  $r^2$ ). The recombination rate (right-hand y axis) is plotted in light blue and is based on the European–American (CEU) HapMap Phase III population.



The next strongest signals were located on chromosomes 20q13.33 (rs17729098,  $p = 1.68 \times 10^{-6}$ ) and 7q31.1 (rs2291567,  $p = 1.97 \times 10^{-6}$ ). The association signal on chromosome 20 is likely artifactual, as there are no other SNPs in the region that show similar levels of association (Figure 5a), although this conclusion is limited by the poor coverage of SNPs in the region. The chromosome 7 finding, on the other hand, does show a broad region of association suggesting that this is not due to a technical artifact or population stratification (Figure 5b). We also examined the results for SNPs in the 18 genes considered in a recent meta-analysis.<sup>16</sup> However, none of these SNPs were significant at a level of  $p \leq .001$ .

**FIGURE 5** (A) Regional association and linkage disequilibrium (LD) plot for 20q13.33 region. (B) Regional association and LD plot for 7q32.1 region.

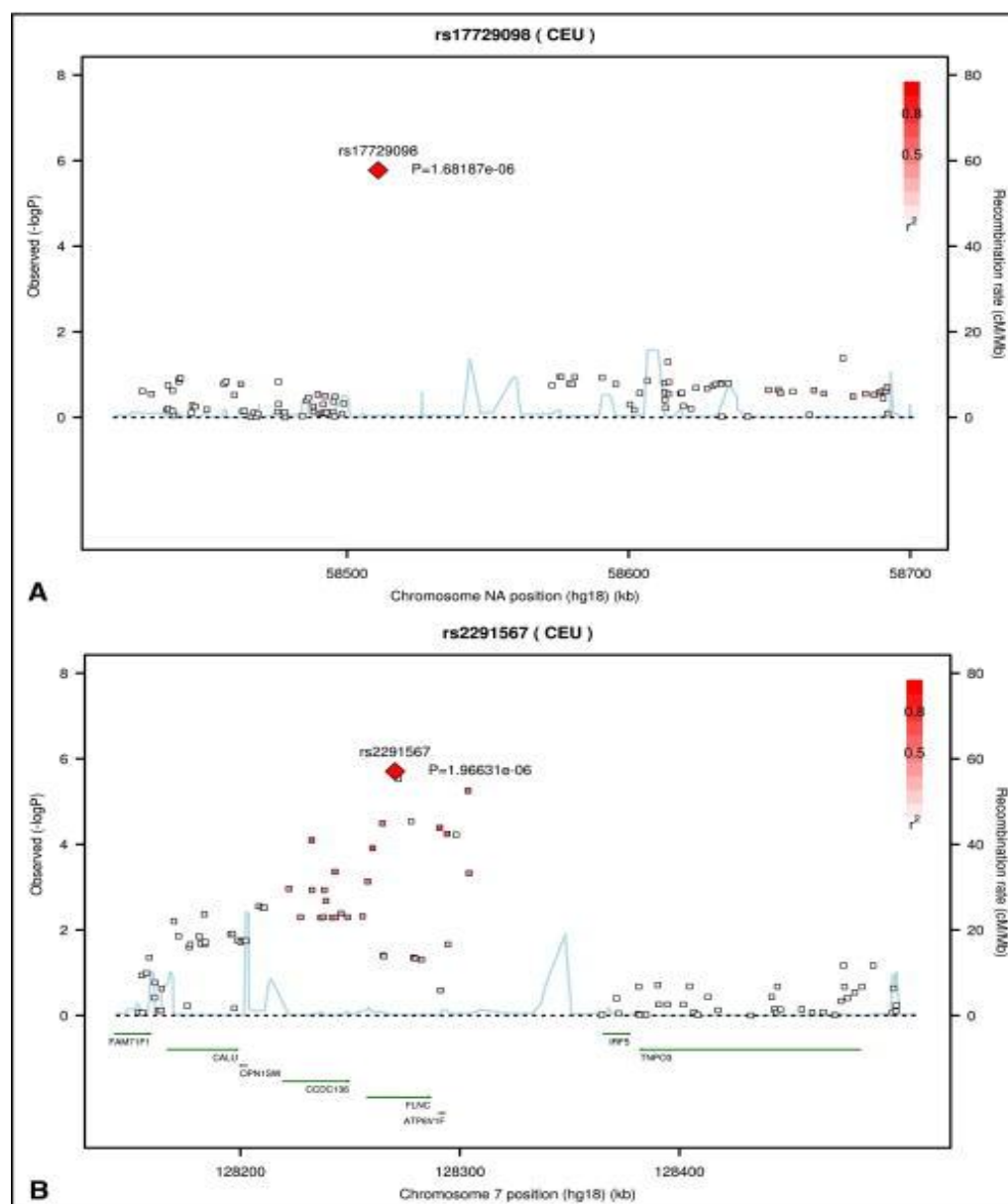


Table 3 presents the top 100 hits based on p value significance in our imputed dataset. The table indicates the gene name of the SNP fall within a gene. We and colleagues from three other GWAS Consortia have completed a meta-analysis that includes the data presented in this paper.<sup>35</sup> That meta-analysis comprised 2,064 trios, 896 cases, and 2,455 controls. In Table 3, we also give the p value for each SNP from the meta-analysis.

**TABLE 3** Top 100 Single-Nucleotide Polymorphisms (SNPs) from Imputed International Multicenter ADHD Genetics Project (IMAGE) II dataset

Chr	BP	SNP	A1	A2	IMAGE 2 p Value	Meta-Analysis p Value	Case Freq	Control Freq	GLM Estimate	Gene
10	53446292	rs1903977	A	T	1.50E-05	.1749	0.348	0.403	0.56072	PRKG1
10	53449486	rs10823973	C	G	6.32E-07	.3663	0.578	0.512	0.563132	PRKG1
10	53447049	rs10823964	T	G	7.84E-07	.2744	0.565	0.501	0.553325	PRKG1
10	53443918	rs10762524	T	G	9.89E-07	.3336	0.577	0.512	1.6774	PRKG1
20	58511043	rs17729098	T	C	1.68E-06	1.68E-06	0.978	0.951	0.883769	
7	128270463	rs2291567	T	C	1.97E-06	.04204	0.856	0.816	0.644948	FLNC
10	53377755	rs7092969	A	T	2.57E-06	.05565	0.792	0.739	0.870689	PRKG1
7	128272052	rs3734973	A	G	2.97E-06	.0661	0.861	0.822	-0.565169	FLNC
4	177360844	rs11731185	A	C	4.09E-06	.03243	0.602	0.662	0.894289	
3	145754148	rs10490827	T	C	4.48E-06	.01823	0.106	0.071	-0.53528	
16	86126636	rs4488454	C	G	4.97E-06	.003164	0.32	0.381	0.88796	
3	145768265	rs12633370	A	G	5.03E-06	.01686	0.104	0.069	-0.635448	
20	54266838	rs6014657	A	C	5.25E-06	.2581	0.191	0.243	-1.07922	
1	79972153	rs6672282	A	G	5.49E-06	.01273	0.929	0.957	0.906555	
7	128303706	rs13238831	T	C	5.57E-06	.0005903	0.87	0.835	-0.71242	
10	132876636	rs11593241	T	C	5.73E-06	.06239	0.833	0.876	-0.609587	TCERG1L
20	54282384	rs6014664	T	C	7.38E-06	.2728	0.211	0.263	0.700502	
10	132883393	rs2033791	A	G	7.66E-06	.1028	0.165	0.122	-0.506491	TCERG1L
11	102923368	rs716274	A	G	8.67E-06	.01452	0.378	0.437	-0.507027	
11	102923078	rs716273	T	C	8.98E-06	.01426	0.378	0.437	1.31019	
12	61326877	rs12317552	T	C	9.14E-06	.005663	0.065	0.044	0.693778	PPM1H
10	70699308	rs2394534	T	G	1.04E-05	.02563	0.804	0.761	-1.8826	
7	8595016	rs17151821	C	G	1.05E-05	.014	0.967	0.982	1.22331	NXPH1
1	79991162	rs7543390	A	C	1.06E-05	.1833	0.052	0.028	-0.663373	
10	132874346	rs1370868	A	G	1.07E-05	.2392	0.815	0.858	-0.862547	TCERG1L
3	145781004	rs12631326	T	C	1.14E-05	.01534	0.9	0.933	0.520709	
16	86128662	rs11117283	T	C	1.15E-05	.005478	0.668	0.61	2.28425	
7	8603077	rs2107281	T	C	1.19E-05	.05902	0.023	0.012	-0.508054	NXPH1
10	53444832	rs1903979	T	C	1.21E-05	.1637	0.346	0.401	0.591023	PRKG1
10	53391182	rs1903960	T	G	1.24E-05	.03183	0.784	0.735	1.34502	PRKG1
6	18588998	rs567621	T	C	1.25E-05	.3004	0.97	0.945	-0.49569	
10	44401400	rs1999774	A	T	1.25E-05	.1014	0.438	0.5	-1.35758	
6	18584639	rs532347	A	G	1.26E-05	.2679	0.031	0.055	-0.495269	
10	44393217	rs1021055	A	G	1.27E-05	.1103	0.438	0.5	0.857156	
3	145784349	rs16855659	T	C	1.28E-05	.01951	0.1	0.067	-0.495811	
10	44392825	rs899889	A	G	1.32E-05	.1055	0.44	0.501	1.33796	
6	18594466	rs490618	A	T	1.32E-05	.2535	0.97	0.945	0.688136	
10	132873811	rs2397752	T	C	1.33E-05	.4843	0.298	0.256	-0.62526	TCERG1L
20	54274113	rs1980818	C	G	1.37E-05	.2628	0.203	0.25	-0.579146	
14	43155518	rs8011986	T	C	1.40E-05	.2581	0.692	0.739	1.3277	
6	18594397	rs536700	C	G	1.42E-05	.2551	0.97	0.945	-1.25684	
12	61331522	rs10506445	A	G	1.44E-05	.0199	0.938	0.959	0.528822	PPM1H
3	107394229	rs7633167	A	C	1.49E-05	.04074	0.603	0.548	0.491204	
10	44393389	rs10900075	T	C	1.50E-05	.1204	0.561	0.5	-0.50273	
10	44424421	rs7069381	T	G	1.50E-05	.1185	0.439	0.5	-0.491285	
10	44398176	rs11239157	T	C	1.52E-05	.1195	0.439	0.5	-0.490879	
20	54277553	rs1326021	T	C	1.55E-05	.1755	0.736	0.682	0.545545	
1	79963454	rs6424669	C	G	1.60E-05	.05057	0.948	0.971	-1.20443	
11	102909121	rs7117196	T	C	1.64E-05	.3623	0.672	0.616	0.509248	
20	54277432	rs1326022	A	G	1.64E-05	.1636	0.266	0.32	-0.546283	
1	74879329	rs17095690	A	G	1.65E-05	.256	0.163	0.126	0.740204	C1orf173



Chr	BP	SNP	A1	A2	IMAGE 2 p Value	Meta-Analysis p Value	Case Freq	Control Freq	GLM Estimate	Gene
11	102899137	rs1481963	A	G	1.78E05	.8727	0.749	0.698	0.557108	TCERG1L
10	44391896	rs7083920	T	C	1.80E05	.1134	0.439	0.5	-0.486633	
14	43160678	rs7146388	A	G	1.85E05	.2889	0.694	0.74	-0.574504	
10	132874204	rs1583670	T	C	1.94E05	.1733	0.189	0.145	0.635465	
15	44499663	rs11853869	T	C	1.97E05	.34	0.975	0.988	-1.7472	
20	54279203	rs6064362	A	G	2.00E05	.1074	0.688	0.632	0.519797	
11	102909072	rs7105630	T	C	2.04E05	.9765	0.252	0.303	-0.548703	
8	20988666	rs6986709	T	C	2.12E05	.02959	0.104	0.071	0.795838	CDH13
20	54274415	rs6064361	T	C	2.20E05	.1339	0.311	0.366	-0.521177	
11	102863301	rs10895437	A	G	2.22E05	.712	0.256	0.307	-0.542526	
16	81813774	rs8045006	A	C	2.28E05	.000158	0.057	0.035	1.1611	
10	44409059	rs2061971	A	G	2.42E05	.06829	0.552	0.493	0.478701	
1	79931809	rs1524174	A	C	2.47E05	.009319	0.071	0.044	0.998981	
11	102899049	rs1481964	A	G	2.49E05	.1488	0.578	0.521	0.478905	HK1
7	34663070	rs1023556	T	C	2.61E05	.4215	0.271	0.225	0.564398	
14	33797982	rs11625217	A	G	2.71E05	.05322	0.53	0.474	0.472106	
10	70703703	rs2394538	T	C	2.77E05	.03182	0.627	0.676	-0.527049	
6	18606326	rs10498690	A	G	2.80E05	.3496	0.028	0.051	-1.34235	
11	102923294	rs10895458	A	C	2.81E05	.009655	0.449	0.506	-0.473362	
13	112568363	rs1278776	A	G	2.85E05	.0000285	0.048	0.027	1.19599	ATP11A
10	53442805	rs7893146	T	C	2.87E05	.02307	0.227	0.275	-0.549052	PRKG1
7	128277789	rs13227216	T	C	2.90E05	.1392	0.837	0.798	0.668785	FLNC
3	145751545	rs12631899	T	C	2.98E05	.03321	0.889	0.922	-0.782344	PRKG1
14	33801343	rs12432593	A	C	3.00E05	.07092	0.53	0.474	0.468952	
10	53443371	rs10823954	A	G	3.01E05	.02442	0.225	0.273	-0.551318	
7	34595629	rs2893482	A	G	3.01E05	.2577	0.722	0.768	-0.538738	
14	33814177	rs4624074	T	C	3.10E05	.04751	0.5	0.555	-0.471712	
2	50122120	rs17495366	T	C	3.12E05	.01472	0.762	0.712	0.53582	NRXN1
7	128264708	rs2291560	T	C	3.21E05	.002541	0.898	0.866	0.858583	FLNC
20	54285153	rs932792	A	C	3.22E05	.1638	0.678	0.623	0.502806	PRKG1
14	43080539	rs8005625	T	G	3.24E05	.6327	0.2	0.163	0.667015	
18	62468199	rs545245	T	C	3.24E05	.0188	0.606	0.661	-0.479264	
10	53458887	rs10762539	T	G	3.24E05	.1289	0.58	0.534	0.556207	
14	43175448	rs7140723	A	C	3.27E05	.7938	0.184	0.147	0.668814	
20	54269761	rs913000	T	C	3.34E05	.1422	0.269	0.321	-0.523468	
11	102900172	rs1481961	A	C	3.36E05	.1996	0.578	0.522	0.470946	PRKG1
5	113707103	rs36936	T	C	3.36E05	.0002923	0.234	0.19	0.566459	
7	34604539	rs6462569	T	C	3.40E05	.2659	0.278	0.232	0.535921	
10	53459394	rs7901487	T	C	3.42E05	.1268	0.419	0.464	-0.556738	
10	53457963	rs10823978	A	G	3.51E05	.02782	0.221	0.265	-0.593313	
3	127402754	rs4374483	A	G	3.57E05	.07212	0.475	0.415	0.471741	
7	34566246	rs10258145	A	G	3.58E05	.3281	0.28	0.234	0.53732	PRKG1
18	62467515	rs556372	T	C	3.59E05	.01625	0.606	0.66	-0.477123	
11	102904902	rs10895454	A	C	3.61E05	.1831	0.578	0.522	0.467765	
10	53459673	rs10762540	A	C	3.62E05	.1287	0.581	0.535	0.555974	
10	29605989	rs2796304	A	C	3.65E05	.09506	0.577	0.524	0.484793	
7	34572861	rs12690808	T	G	3.72E05	.3395	0.278	0.233	0.533947	
10	70696516	rs906219	A	C	3.73E05	.0368	0.377	0.33	0.525993	HKDC1
6	18614743	rs10456831	T	C	3.74E05	.5029	0.027	0.049	-1.34042	

Note: A = allele; BP = base pair; Chr = chromosome; Freq = frequency; GLM = generalized linear model.

## Discussion

We have reported an independent genome-wide association scan of ADHD. None of the SNPs achieved genome-wide significance ( $p < 5.0 \times 10^{-8}$ ), either in the sample reported here or in a meta-analysis or our results with other samples.<sup>35</sup> Given the extent to which ADHD is genetic, it is highly likely that within the set of SNPs with  $p$  value  $< 10^{-3}$  there are true associations for which we do not yet have sufficient power to unequivocally detect.

Although no finding achieved genome-wide significance, several of our top findings deserve further comment. The PRKG1 gene regulates neuronal migration, signal transduction, dendrite development, long-term potentiation, and forebrain development.<sup>36–39</sup> Thus, it is a reasonable candidate for a gene that might lead to brain abnormalities and ADHD.

One of our top findings was in the CDH13 gene ( $p = 2.28E-05$ ). CDH13 was implicated by a GWAS of 343 ADHD adults and 250 controls <sup>22</sup> and in the IMAGE GWAS of ADHD symptom counts.<sup>24,25</sup> This gene lies under a linkage peak implicated in a meta-analysis of ADHD linkage studies <sup>15</sup> and has been implicated in substance use disorders,<sup>40</sup> which co-occur with ADHD.<sup>41,42</sup>

Column 7 of [Table 3](#) helps with the interpretation of our findings in the context of other ADHD GWAS. This column gives the  $p$  value from a meta-analysis <sup>35</sup> that comprises the data in this paper and data from three other consortia: IMAGE,<sup>23</sup> PUWMA <sup>43</sup> and CHOP (unpublished data). As seen in [Table 3](#), most SNPs show a dramatically higher  $p$  value on the combined sample. No SNP increases in significance, and a few retain significance levels  $<.0001$ . This does not create confidence in the idea that many of our top 100 SNPs are true associations.

Our negative results indicating the existence of very small genetic effects when individual variants are considered alone is not surprising. GWAS findings are now emerging for other psychiatric disorders. There have been replicated copy number variation associations for schizophrenia <sup>44,45</sup> and for autism,<sup>46–48</sup> a genome-wide significant association for bipolar disorder,<sup>49</sup> and a significant association from a schizophrenia–bipolar dataset.<sup>50</sup> These early GWAS results suggest that, because of the many statistical comparisons required to scan the genome, large samples are needed to detect some genes, and extremely large samples are needed to detect many genes.

For example, the successful bipolar disorder GWAS, which detected two loci at genome-wide levels of significance, required 4,387 cases and 6,209 controls.<sup>49</sup> Studies of this size and larger implicated several genes for diabetes,<sup>51</sup> but a pooled sample of 60,000 subjects was required to definitively implicate a large set of genes.<sup>52</sup> For type 2 diabetes and Crohn's disease, mapping of one or a small number of disease-associated variants was successful in studies with sample sizes similar to that of the present study; however the vast majority of findings have emerged with the incorporation of multiple scans involving sample sizes many times larger than that presented here,<sup>52,53</sup> and in most cases consisted of genetic loci conferring odds ratios in the region of 1.1 to 1.4. The statistical requirement for large sample size for GWAS should not be interpreted as meaning that the effects of individual genetic variants are very much smaller than the effects of individual environmental variants; in fact, the latter are small as well.<sup>54</sup>

The general expectation from GWAS of complex disorders is for multiple genes of very small effect.<sup>55</sup> Backward

power calculations on some of the initial true results from these diseases indicate that many of the identified candidates were extremely unlikely to be detected from the initial study.<sup>55</sup> Thus these initial studies were either fortunate or many such effects (potentially 100 or more) with a similar effect size must exist. In this study we have not been fortunate, insofar as we did not identify a variant above genome-wide significance, which we define as  $5 \times 10^{-8}$ .<sup>56,57</sup> Concerning the existing candidate genes for ADHD, the genome-wide association data do not provide genome-wide significant support for any of the previously postulated candidates. That is not to say that these genes should be rejected from consideration, but rather that the effect sizes for each of these variants must be small if they are real effects, which is consistent with the meta-analyses of candidate gene studies.<sup>10,11,16</sup>

We have considered the pathophysiological and clinical implications of genetic effects so small that they cannot be detected with our current sample size. Such small effects can arise for several potential reasons. First, it may be correct that genetic risks for ADHD are due to numerous small additive effects of common risk variants; however, it is also possible that multiple rare variants of small to moderately large effect size could account for these findings.<sup>58</sup> Alternative explanations include sample heterogeneity, the possible interaction of genetic variants either within or between genes, and their interaction with environmental risk factors. Although the heritability of ADHD is high, this does not give an indication of the underlying genetic architecture, although it does imply that genetic influences are important for the etiology of the disorder. Recent modeling of complex behavioral and biological traits in the mouse suggests that as heritability increases the number of genetic variants involved increases, although effect sizes of individual variants remain small.<sup>59</sup> For ADHD, our expectation is that novel genes for ADHD will be identified from GWAS once sufficient whole-genome association data have been accumulated from the analysis of 5,000 to 10,000 cases.

Given the expense of GWAS, it is reasonable to ask whether genes of very small effect are worth discovering. Theoretical considerations suggest that the smallness of a gene effect should not be confused with the potential importance of its discovery. For example, should we someday discover a rare variant or a common variant of small effect that implicates a new biological pathway in ADHD, that pathway could then be searched for biological targets that might yield treatments which are more efficacious than standard therapies for the disorder. The discovery of such a variant would also focus research on the implicated gene and pathway, which could lead the discovery of similar variants.

The need to search for DNA variants that lead to ADHD cannot be understood without placing the disorder in the context of current knowledge. ADHD is a common disorder affecting up to 10% of children.<sup>1</sup> In the majority of cases, the disorder persists into adulthood <sup>60</sup> and is associated with serious impairments including traffic accidents,<sup>60</sup> increased health care use,<sup>60</sup> substance abuse,<sup>60</sup> unemployment,<sup>60</sup> divorce,<sup>60</sup> and risk behaviors for acquired immunodeficiency syndrome (AIDS).<sup>61</sup> Approximately 25% of ADHD patients do not respond well to currently available therapies.<sup>62,63</sup> Moreover, the currently preferred treatment for ADHD is stimulant medication. Although medications for ADHD are effective in controlling symptoms for many patients, they do not



“cure” the disorder. Even those patients who are receiving treatment are at risk for adverse outcomes.<sup>64</sup> Currently available treatments improve outcomes but leave patients with much residual disability and do not markedly improve the executive dysfunction seen in many ADHD patients. These treatments also have adverse effects, including delays in growth.<sup>65</sup>

The outcome of the present study may have been influenced by a number of limitations. Most notably is the issue of power. We had 80% power for an odds ratio of 1.65, assuming a multiplicative model and a 10% minor allele frequency. Thus we did not have sufficient power to detect smaller effects or the same effect at lower allele frequencies. Much larger samples or meta-analyses of the current samples will provide a stronger strategy for advancing knowledge regarding the molecular genetics of ADHD along with paradigms designed to search for rare genetic variants. Another limitation of the current study was the differences in genotyping platforms used to analyze case and control samples. This design limitation led to the exclusion of numerous SNPs through additional QC steps before imputation. Although it is possible that this might lead to artificial inflation of the test statistics, it is unlikely that this had much influence on the outcome of the study, given the relatively low genomic control value and the lack of significant results.

Imputation analysis, albeit extremely useful at generating estimates of association evidence at genetic loci that have not been typed, is not perfect. The uncertainty inherent in these analyses reduces the effective sample size, thus limiting power. Also, imputation, like genome-wide association, has limited capacity for the analysis of rarer variation. These analyses used population-based controls that were not screened for ADHD. Although this will have reduced power somewhat, given the prevalence of the disorder, we do not expect that this had much impact on the results. Finally, ADHD very likely is genetically heterogeneous, such that many different genetic architectures give rise to similar clinical presentations. This includes the possibility that rare variants account for part of the disorder's heritability. Such genetic heterogeneity and complexity reduces power to detect significant association.

In summary, although the current analyses have not identified any convincing results the sample is a useful addition to the present literature and has made a valuable contribution to the current meta-analysis of ADHD GWAS,<sup>35</sup> which combines data from four ADHD GWAS.

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