RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

New Suggestive Genetic Loci and Biological Pathways for Attention Function in Adult Attention-Deficit/Hyperactivity Disorder

Silvia Alemany,[1,2,3]* Marta Ribasés,[4,5,6] Natàlia Vilor-Tejedor,[1,2,3] Mariona Bustamante,[1,2,3,7] Cristina Sánchez-Mora,[4,5,6] Rosa Bosch,[4,5] Vanesa Richarte,[4,5,6,8] Bru Cormand,[9,10,11] Miguel Casas,[4,5,6,8] Josep A. Ramos-Quiroga,[4,5,6,8] and Jordi Sunyer[1,2,3,12]**

[1]Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain

[2]Universitat Pompeu Fabra (UPF), Barcelona, Spain

[3]CIBER Epidemiology and Public Health (CIBERESP), Madrid, Spain

[4]Department of Psychiatry, Hospital Universitari Vall d'Hebron, Barcelona, Spain

[5]Biomedical Network Research Centre on Mental Health (CIBERSAM), Barcelona, Spain

[6]Psychiatric Genetics Unit, Vall d'Hebron Research Institute (VHIR), Universitat Autònoma de Barcelona, Barcelona, Spain

[7]Center for Genomic Regulation (CRG), Barcelona, Spain

[8]Department of Psychiatry and Legal Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

[9]Facultat de Biologia, Departament de Genètica, Universitat de Barcelona, Catalonia, Spain

[10]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain

[11]Institut de Biomedicina de la Universitat de Barcelona (IBUB), Catalonia, Spain

[12]IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain

Manuscript Received: 31 December 2014; Manuscript Accepted: 22 June 2015

Attention deficit is one of the core symptoms of the attention-deficit/hyperactivity disorder (ADHD). However, the specific genetic variants that may be associated with attention function in adult ADHD remain largely unknown. The present study aimed to identifying SNPs associated with attention function in adult ADHD and tested whether these associations were enriched for specific biological pathways. Commissions, hit-reaction time (HRT), the standard error of HRT (HRTSE), and intraindividual coefficient variability (ICV) of the Conners Continuous Performance Test (CPT-II) were assessed in 479 unmedicated adult ADHD individuals. A Genome-Wide Association Study (GWAS) was conducted for each outcome and,

Grant sponsor: Ministerio de Economía y Competitividad; Grant number: SAF2012-33484; Grant sponsor: AGAUR; Grant numbers: 2014SGR-0932, 2014SGR1357; Grant sponsor: NVT thanks for her PhD Grant FI-DGR 2015; Grant sponsor: Instituto de Salud Carlos III-FIS; Grant numbers: PI11/00571, PI11/01629, PI12/01139; Grant sponsor: SA thanks for her Postdoctoral contract Sara Borrell CD14/00214; Grant sponsor: MR is a a recipient of contract of a Miguel Servet CP09/00119; Grant sponsor: Plan Nacional Sobre Drogas; Grant number: PNSD#2011-0080; Grant sponsor: Departament de Salut, Government of Catalonia, Spain; 7th Framework Programme for Research, technological Development and Demonstration, European Commission (CSM is a recipient of the contract AGGRESSOTYPE_FP7HEALTH2013/602805); European College of Neuropsychopharmacology (Grant reference: ECNP Networks); Grant sponsor: Spanish 'Ministerio de Economía y Competitividad'; Grant number: SAF2012-33484; Grant sponsor: AGAUR; Grant numbers: 2014SGR-0932, 2014SGR1357; Grant sponsor: Instituto de Salud Carlos III-FIS; Grant numbers: PI11/00571, PI11/01629, PI12/01139; Grant sponsor: Plan Nacional Sobre Drogas; Grant number: PNSD#2011-0080; Grant sponsor: Departament de Salut, Government of Catalonia, Spain; Grant sponsor: Instituto de Salud Carlos III; Grant number: CD14/00214; Grant sponsor: Agència de Gestió d'Ajuts Universitaris i de Recerca; Grant number: AGAUR FI-DGR 2015.

*Correspondence to:
Silvia Alemany, Centre for Research in Environmental Epidemiology (CREAL), C. Doctor Aiguader 88, 08003 Barcelona, Spain.
E-mail: salemany@creal.cat
**Correspondence to:
Marta Ribasés, Psychiatric Genetics Unit, Group of Psychiatry, Mental Health and Addictions, Vall d'Hebron Research Institute, Passeig Vall d'Hebron 119-129, 08035 Barcelona, Spain.
E-mail: mribases@ir.vhebron.net

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 14 July 2015
DOI 10.1002/ajmg.b.32341

subsequently, gene set enrichment analyses were performed. Although no SNPs reached genome-wide significance ($P < 5$ E−08), 27 loci showed suggestive evidence of association with the CPT outcomes ($P <$ E−05). The most relevant associated SNP was located in the *SORCS2* gene ($P = 3.65$E−07), previously associated with bipolar disorder (BP), Alzheimer disease (AD), and brain structure in elderly individuals. We detected other genes suggested to be involved in synaptic plasticity, cognitive function, neurological and neuropsychiatric disorders, and smoking behavior such as *NUAK1*, *FGF20*, *NETO1*, *BTBD9*, *DLG2*, *TOP3B*, and *CHRNB4*. Also, several of the pathways nominally associated with the CPT outcomes are relevant for ADHD such as the ubiquitin proteasome, neurodegenerative disorders, axon guidance, and AD amyloid secretase pathways. To our knowledge, this is the first GWAS and pathway analysis of attention function in patients with persistent ADHD. Overall, our findings reinforce the conceptualization of attention function as a potential endophenotype for studying the molecular basis of adult ADHD.

Key words: Attention-deficit/hyperactivity disorder; Conners Continuous Performance Test; adults; GWAS; *SORCS2*

How to Cite this Article:
Alemany S, Ribasés M, Vilor-Tejedor N, Bustamante M, Sánchez-Mora C, Bosch R, Richarte V, Cormand B, Casas M, Ramos-Quiroga JA, Sunyer J. 2015. New Suggestive Genetic Loci and Biological Pathways for Attention Function in Adult Attention-Deficit/Hyperactivity Disorder.
Am J Med Genet Part B 168B:459–470.

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by inappropriate symptoms of inattention, impulsivity, and motor restlessness (hyperactivity). Epidemiological studies estimate the prevalence of adult ADHD between 2.5% and 4.9% [Simon et al., 2009]. The core symptoms of ADHD (inattention, impulsivity, and hyperactivity) are associated with problems in remaining focused on a task especially for prolonged periods, as well as difficulties in organizing activities, prioritizing tasks, and time management [Haavik et al., 2010].

Although heritability estimates do not directly inform about the genetic etiological mechanisms or the number or size of genes affecting a given phenotype, they constitute an indicator of how relevant the genetic component can be for the disease of interest. In this regard, despite the high heritability estimated for childhood ADHD, ranging from 70% to 80% [Faraone et al., 2005; Burt, 2009], no genome-wide significant associations have been documented so far [Neale et al., 2008, 2010a,b; Mick et al., 2010; Hinney et al., 2011; Stergiakouli et al., 2012; Yang et al., 2013]. The search for specific genetic variants accounting for ADHD seems even more challenging in the case of adult ADHD since heritability estimates for this disorder during adulthood are substantially lower (30–38%) [Boomsma et al., 2010; Chang et al., 2013; Larsson et al., 2013a] with one exception reporting an heritability of 72% for adult ADHD [Larsson et al., 2013b]. Yet, it is still uncertain whether the modest heritability estimates can be explained by rater effects and what they may actually mean for molecular genetic studies of adult ADHD [Franke et al., 2012; Chang et al., 2013; Larsson et al., 2013a]. Also, similarly to child ADHD, the few genome-wide association studies (GWAS) conducted in adult ADHD to date revealed promising results but did not reach genome-wide significance [Lesch et al., 2008; Sanchez-Mora et al., 2014]. It can be argued that the lack of genome-wide significant associations in both child and adult ADHD may be due to the fact that GWAS in ADHD are still underpowered, with no study exceeding 3,000 patients so far; however, strategies other than increasing sample size can be used to advance in the identification of common genetic variants associated with the disorder, such as focusing on candidate endophenotypes [Hawi et al., 2015].

The concept of endophenotype refers to heritable quantitative traits, either cognitive or physiological, expected to be more directly related to dysfunction in neural systems than diagnosis facilitating the identification of genetic variants associated with the disease [Castellanos and Tannock, 2002; Hawi et al., 2015]. In this context, some neurocognitive traits may be used as endophenotypes because inattention and hyperactivity, core symptoms of ADHD, are closely related to cognitive domains such as executive function, attention, memory, and intelligence [Franke et al., 2012]. Indeed, these neurocognitive measures present evidences to be considered endophenotypes for ADHD including the fact that they are heritable, associated with ADHD and unaffected relatives often exhibit intermediate performance between probands and healthy controls [Hawi et al., 2015]. Focusing on attention, clinical research indicates that adult ADHD patients present less symptoms of hyperactivity or impulsivity and more inattentive symptoms compared to children with ADHD [Haavik et al., 2010]. Also, studies examining attention function have shown that adults with ADHD are more variable, less accurate, and slower than expected in attention performance tasks [Oberlin et al., 2005].

Interestingly, self and informant rated attention problems has been reported as a highly heritable trait in young adults [Chang et al., 2013], albeit the outcome analyzed in this study as attention problems also included symptoms of hyperactivity and, impulsivity. Furthermore, there is evidence suggesting that attention performance and ADHD share familial influences, which include common genetic and environmental factors [Kuntsi et al., 2010]. However, which specific genetic variants underlie attention deficits in adult ADHD subjects remains unclear.

In the current study, we conducted a GWAS aimed to identify SNPs associated with attention function in adult ADHD. In addition, we test whether specific biological pathways were enriched for these associations using gene set enrichment analyses (GSEA).

MATERIALS AND METHODS

Participants

The sample comprised 479 adult ADHD patients (65.8% males; mean age 32.8 years, SD = 10.8; 59.5% combined, 36.5% inattentive, 2.9% hyperactive-impulsive, 0.6% not otherwise specified, 0.2% residual, and 0.2% in partial remission) recruited and evaluated at the Psychiatry Department of the Hospital Universitari Vall d'Hebron (Barcelona, Spain) according to DSM-IV TR criteria. ADHD diagnosis was based on the Spanish version of the Conners Adult ADHD Diagnostic Interview for DSM-IV (CAADID) and the Structured Clinical Interview for DSM-IV Axis I and II Disorders (SCID-I and SCID-II) [Ramos-Quiroga et al., 2012]. All patients were of Caucasian origin. Exclusion criteria for the ADHD patients cohorts were IQ < 80; pervasive developmental disorders; schizophrenia or other psychotic disorders; the presence of mood, anxiety, or personality disorders that might explain ADHD symptoms; birth weight ≤1.5 kg; and other neurological or systemic disorders that might explain ADHD symptoms. All the subjects included in this study were drug-naïve (stimulants or atomoxetine). None of the subjects was under pharmacological treatment when completing the neuropsychological test. The study was approved by the Ethics Committee of the relevant institution and informed consent was obtained from all subjects or parents in accordance with the Helsinki Declaration.

Measures

The Conners Continuous Performance Test-II (CPT-II; [Conners and Staff, 2000]) was used to evaluate attention. In this computerized test, subjects are required to press the spacebar when any letter, except the target letter (i.e., X), appears on screen. This test provides several measures. Initially, the current study focused on two error measures, omissions and commissions; and three variability measures, hit-reaction time (HRT), the standard error of the HRT (HRTSE), and intraindividual coefficient variability (ICV). Omissions result from the failure to respond to target letters. Commission errors are made when responses are given to non-targets. HRT is the average speed of correct responses for the entire test. HRTSE is a measure of response speed consistency. ICV (variability outcome in CPT-II) is a measure of response speed consistency within respondent and corresponds to the amount of variability the individual shows in 18 separate segments of the test in relation to his or her own overall standard error. This outcome was log-transformed to meet the assumptions of a Gaussian distribution. For all measures, higher scores indicate worse performance.

Notice that although we originally considered the five measures previously described, given the distribution of the omissions outcome, it was not possible to fit a non-skewed parametric distribution for this variable. For this reason, the omissions outcome was not finally assessed in the current study.

Thus, the final attention outcomes included commissions, HRT, HRTSE, and ICV.

Genotyping and Quality Control

Genomic DNA was isolated either from saliva using the Oragene DNA Self-Collection Kit (DNA Genotek, Inc., Ottawa, Ontario, Canada) or from peripheral blood lymphocytes by the salting-out procedure [Miller et al., 1988]. Genome-wide genotyping was performed with the Illumina HumanOmni1-Quad BeadChip platform. Quality control was implemented at the individual and SNP level using PLINK [Purcell et al., 2007] and included filtering subjects with low call rate (<98%) or gender discrepancy followed by filtering SNPs with minor allele frequency (MAF) <0.01, Hardy-Weinberg equilibrium test *P*-values PHWE < 1E−06 or call rate <0.99. The final number of genotyped SNPs included in the present study was 799.713 autosomal SNPs.

Statistical Analysis

Genome-wide association analyses were conducted using multiple linear regressions in SNP test [Marchini et al., 2007] for each of the four attention outcomes analyzed assuming additive genetic effects in autosomal chromosomes and including age and sex as covariates. Quantile–quantile (Q–Q) and Manhattan plots were computed with the qqman package of R to evaluate overall significance of autosomal SNP *P*-values. Genome-wide level of significance was set at $P < 5E{-}08$, and suggestive evidence of association was defined by $P < 1E{-}05$.

We used the adjusted coefficient of determination (R^2) to estimate effect sizes of susceptibility SNPs identified for each attention outcome.

Meta-Analysis Gene-set Enrichment of variaNT Associations (MAGENTA) software [Segre et al., 2010] was used to compute gene set enrichment analysis (GSEA) for each attention outcome based on Panther, KEGG, and Ingenuity data sources. MAGENTA calculated Gene set *P*-value in accordance with the single SNP lowest *P*-value within a 110 kb window upstream and 40 kb window downstream of the SNP. Gene *P*-values were adjusted for confounding factors, such as physical gene size, number of SNPs for each gene, and linkage disequilibrium between proximal markers. Adjusted *P*-values were ranked, and the gene set enrichment *P*-value for each biological pathway was calculated given significance threshold (95th percentile). This value was compared with that generated with randomly permuted pathways of identical size to test whether genes in the pathway were enriched more than would be expected by chance.

RESULTS

Table I shows age, sex ratio, ADHD subtypes, comorbidities, and scores for the four attention outcomes tested. Average scores for commissions, HRT, HRTSE, and ICV were 15.5 (SD = 7.5), 396.5 (SD = 65.8), 6.6 (SD = 2.6), and 10.0 (SD = 7.2), respectively (Table I). Additionally, in supplemental material (SM), the correlations between the attention outcomes analyzed (Supplemental Table SI) and scores for each outcome for the different ADHD diagnosis subtypes (Supplemental Table SII) can be found.

Genome-Wide Association Study

Q–Q plots of the observed versus expected *P*-values and Manhattan plots showing the distribution of negative log-transformed *P*-values for each attention outcome are shown in Figure 1. The

TABLE I. Descriptive Characteristics for the Variables of the Study Based on a Sample of 479 Patients With ADHD

Sex, males (%)	315 (65.8%)
Age	32.8 (10.8) (17/70)
ADHD subtypes, N (%)	
Combined type	285 (59.5%)
Inattentive type	175 (36.5%)
Hyperactive/impulsive type	14 (2.9%)
Not otherwise specified	3 (0.6%)
Inattentive type residual)	1 (0.2%)
Partial remission	1 (0.2%)
Comorbid disorders	
Drug use	193 (40.4%)
Borderline personality disorder	8 (1.7%)
Mood disorder	90 (19.5%)
Anxiety	149 (32.3%)
Personality disorder	345 (74.4%)
Oppositional defiant disorder	81 (21.3%)
Commissions (value; T-score)	15.5 (7.5) (0/35); 53.91 (11.05) (33.37/88.79)
HRT (value; T-score)	396.5 (65.8) (44.8/670.6); 51.05 (10.98) (18.57/93.77)
HRTSE (value; T-score)	6.6 (2.6) (2.5 /21.6); 56.00 (12.66) (24.83/97.67)
IIV (value; T-score)	10.0 (7.2) (0.2/61.7); 55.56(11.78) (30.34/101.20)

Percentage is indicated for categorical variables. Mean, SD, and maximum and minim are indicated for continuous variables.
HRT, hit-reaction time; HRTSE, standard error of HRT; IIV, intraindividual variability.

Q–Q plots showed no departure from the expected *P*-values distribution. Genomic control inflation factor (λ) is included in each Q–Q plot.

Although no SNPs reached genome-wide significance ($P < 5E{-}08$), 45 SNPs within 27 loci showed suggestive evidence of association with the different attention outcomes analyzed (Table II). Several of these SNPs were located within or close to genes of interest for attention function in the context of ADHD.

Three loci were associated with HRT: the intergenic rs1521365 ($\beta = -41.30$, $P = 3.54E{-}06$), which nearest gene is the fibroblast growth factor 20 (*FGF20)* gene, located 133 Kb proximal; the rs2000810 ($\beta = -87.84$, $P = 9.78E{-}06$), located in the neuropilin (NRP) and tolloid (TLL)-like 1 (*NETO1*) gene; and finally the rs4689642 ($\beta = 21.74$, $P = 9.58E{-}06$), within the sortilin-related VPS10 domain containing receptor 2 (*SORCS2*) gene.

Sixteen loci were associated with HRTSE. Interestingly, the previous loci associated with HRT, rs4689642, was also associated with HRTSE ($\beta = 1.01$, $P = 3.65E{-}07$) showing the second strongest association with any attention outcome in the study (Table II). Other loci of interest associated with HRTSE included rs6539247 ($\beta = -0.82$, $P = 3.11E{-}06$) and rs2569973 ($\beta = 0.78$, $P = 6.90E{-}06$). They are both located in the novel (nua) kinase family 1 (*NUAK1*) gene. Of note, rs6539247 is located at the 3′-UTR of this gene. Also associated with HRTSE were the SNP6-38440872 ($\beta = 3.20$, $P = 4.26E{-}06$), located in the BTB (POZ) domain containing 9 (*BTBD9*) gene; rs17147674 ($\beta = -2.20$, $P = 5.21E{-}06$), located in the discs large homolog 2 (*DLG2*) gene and rs16982689 ($\beta = 2.67$, $P = 6.35E{-}06$), located in topoisomerase (DNA) III beta (*TOP3B*) gene (Table II).

Seven loci were associated with ICV, two of them were also associated with HRTSE; the rs2341917 located in acyl-CoA oxidase-like (*ACOXL*) gene that showed the strongest association with any attention outcome in the study ($\beta = 0.29$, $P = 2.50E{-}07$), and the rs6539247 ($\beta = -0.20$, $P = 5.71E{-}07$) located in *NUAK1* gene. Another loci which may be of interest is the rs12914008 ($\beta = 0.60$, $P = 6.08E{-}06$) located in the cholinergic receptor nicotinic beta 4 (*CHRNB4)*.

Four intergenic loci were associated with commissions. None of these loci were within or near to genes of interest for ADHD.

Regarding the effect size, 4 loci accounted for 14.4% of the genetic variance of commissions, 3 loci accounted for 11.5% of the genetic variance of HRT, 16 loci accounted for 37.1% of the genetic variance of HRTSE, and 7 loci accounted for 14.8% of the genetic variance of ICV (Supplemental Table SIII). The effect size for each SNP ranged from 0.04 to 0.05 for commissions, around 0.04 for HRT, from 0.04 to 0.05 for HRTSE, and from 0.001 to 0.05 for ICV (Supplemental Table SIII).

Gene Set Enrichment Analysis Results

Gene set enrichment analysis identified 39 functional pathways significantly associated with the attention outcomes considering a 95th percentile cut-off (Table III). However, none of the pathways remained significant after correcting for multiple testing at 5% FDR level.

Several pathways were nominally associated with more than one attention outcome. The gap junction pathway (KEGG) was associated with commissions ($P = 0.012$) and HRT ($P = 0.029$). Axon guidance (KEGG) and a similar pathway, axon guidance mediated by semaphorins (Panther), were associated with commissions ($P = 0.050$) and ICV ($P = 0.040$), respectively. The ubiquitin proteasome pathway (Panther) was associated with

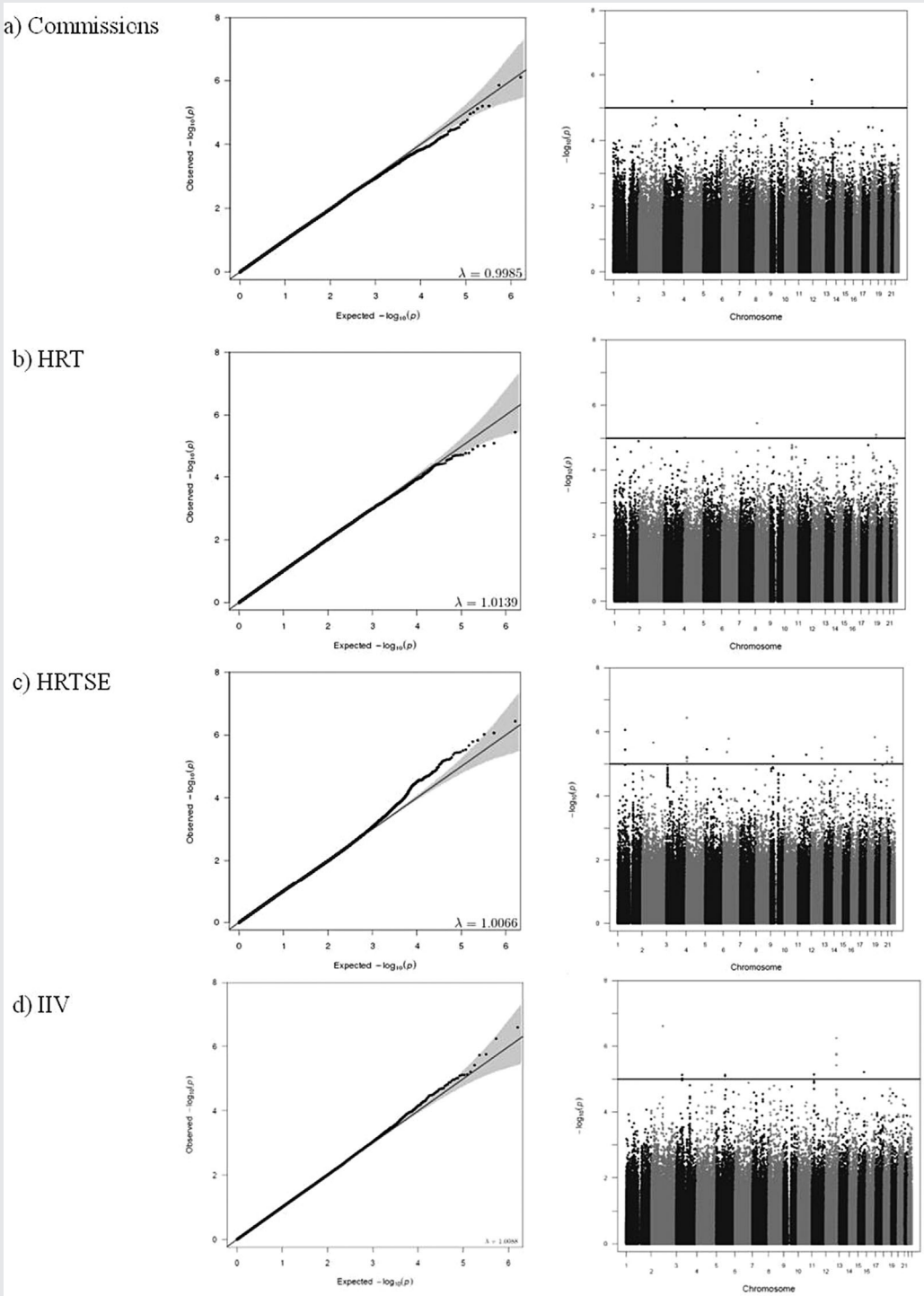

FIG. 1. Quantile–quantile plots (upper part) Manhattan plots (bottom part) of Genome-Wide Association analyses for (a) commissions, (b) HRT, (c) HRTSE, and (d) IIV. Genomic inflation factor (λ) is included in each Q–Q plot. The horizontal gray line in the Manhattan plots indicates the suggestive level of statistical significance ($P < E-05$).

both commissions ($P = 0.015$) and HRTSE ($P = 0.045$). Also, the PDGF signaling pathway (Panther) was associated with HRT ($P = 0.039$) and HRTSE ($P = 0.042$). Other pathways of interest include neurodegenerative disorders (KEGG) ($P = 0.027$) associated with commissions; the Alzheimer disease amyloid secretase pathway (Panther), associated with HRT ($P = 0.028$); and the Huntington's disease pathway (KEGG), associated with ICV ($P = 0.007$).

TABLE II. SNPs Associated With Attention Function Outcomes at $P < E-05$ (Sorted by Significance)

Attention outcome	SNP	CHR	Position[a]	Allele[b]	MAF	N	β	SE	*P*-value	Gene	Nearest gene
	rs13328379	8	20679030	T/C	0.140	478	−3.41	0.69	7.89E−07	Intergenic	*LOC286114*
Commissions	rs7936591	11	126802664	T/C	0.499	478	−2.08	0.46	7.61E−06	Intergenic	*BC030092*
	rs7642644	3	85003058	T/C	0.144	478	−3.15	0.70	6.34E−06	Intergenic	*LOC440970*
	rs2000651	18	21665508	T/C	0.328	478	2.31	0.52	9.89E−06	Intergenic	*SS18*
	rs1521365	8	16760719	T/C	0.06	477	−41.30	8.91	3.54E−06	Intergenic	*FGF20*
HRT	rs2000810	18	68652073	T/G	0.01	478	−87.84	19.66	9.78E−06	*NET01*	
	rs4689642	4	7266223	G/A	0.254	478	21.74	4.91	9.58E−06	*SORCS2*	
	rs4689642	4	7266223	G/A	0.254	478	1.01	0.20	3.65E−07	*SORCS2*	
	rs12095069	1	76235660	T/C	0.107	477	−1.36	0.28	8.64E−07	Intergenic	*ASB17*
	rs17058466	18	71734333	G/A	0.013	478	−3.73	0.77	1.46E−06	Intergenic	*BC037384*
	rs34892827	6	56450095	T/C	0.015	478	3.45	0.72	1.63E−06	*DST*	
	rs2341917	2	111427560	G/A	0.145	478	1.17	0.25	2.16E−06	*ACOXL*	
	rs259991	20	57161743	G/A	0.050	478	−1.78	0.38	2.94E−06	Intergenic	ZNF831
HRTSE	rs6539247	12	104984032	T/C	0.429	477	−0.82	0.17	3.11E−06	*NUAK1*	
	rs6893207	5	16420638	G/A	0.021	478	−2.88	0.62	3.49E−06	Intergenic	*ZNF622*
	rs1144333	1	76047688	G/A	0.096	478	−1.32	0.29	3.63E−06	*MSH4*	
	SNP6-38440872	6	38440872	C/A	0.016	476	3.20	0.70	4.26E−06	*BTBD9*	
	rs17147674	11	84267570	T/C	0.032	478	−2.20	0.48	5.21E−06	*DLG2*	
	rs10511779	9	26715702	T/C	0.144	478	1.10	0.24	5.80E−06	Intergenic	*CAAP1*
	rs16982689	22	20766319	T/C	0.022	478	2.67	0.59	6.35E−06	*TOP3B*	
	rs16878196	4	10724963	T/G	0.052	478	−1.71	0.38	6.51E−06	Intergenic	*MIR572*
	rs2569973	12	105015886	G/A	0.460	478	0.78	0.17	6.90E−06	*NUAK1*	
	rs28498503	18	74763943	T/C	0.158	477	1.03	0.23	7.37E−06	Intergenic	*SALL3*
	rs2341917	2	111427560	G/A	0.145	478	0.29	0.06	2.50E−07	*ACOXL*	
	rs6539247	12	104984032	T/C	0.451	477	−0.20	0.04	5.71E−07	*NUAK1*	
	rs12914008	15	76710560	G/A	0.302	478	0.60	0.13	6.08E−06	*CHRNB4*	
IIV	rs7122488	11	21830829	T/C	0.238	478	−0.20	0.05	7.32E−06	Intergenic	*NELL1*
	rs4681767	3	56734732	T/C	0.429	478	−0.18	0.04	7.50E−06	Intergenic	*ARHGEF3*
	rs1422110	5	85501896	G/A	0.460	478	−0.19	0.04	7.53E−06	Intergenic	*NBPF22P*
	rs7637449	3	56603071	G/A	0.350	478	−0.18	0.04	9.69E−06	Intergenic	*CCDC66*

HRT, hit-reaction time; HRTSE, standard error of HRT; IIV, intraindividual variability; SNP, single nucleotide polymorphism; CHR, chromosome; MAF, minor allele frequency; β, regression coefficient; SE, standard error.
LOC286114, uncharacterized LOC286114; *BC030092,* uncharacterized LOC101929497; *LOC440970,* long intergenic non-protein coding RNA 971; *SS18,* synovial sarcoma translocation; *FGF20,* fibroblast growth factor 20; *NET01,* neuropilin (NRP) and tolloid (TLL)-like 1; *SORCS2,* sortilin-related VPS10 domain containing receptor 2; *ASB17,* ankyrin repeat and SOCS box containing 17; *BC037384,* uncharacterized LOC100505853; *DST,* dystonin; *ACOXL,* acyl-CoA oxidase-like; *ZNF831,* zinc finger protein 831; *NUAK1,* NUAK family, SNF1-like kinase, 1; *ZNF622,* zinc finger protein 622; *MSH4,* mutS homolog 4; *BTBD9,* BTB (POZ) domain containing 9; *DLG2,* discs large homolog 2; *CAAP1,* caspase activity and apoptosis inhibitor 1; *TOP3B,* topoisomerase (DNA) III beta; *MIR572,* microRNA 572; *SALL3,* spalt-like transcription factor 3; *CHRNB4,* cholinergic receptor, nicotinic, beta 4; *NELL1,* NEL-like 1; *ARHGEF3,* rho guanine nucleotide exchange factor 3; *NBPF22P,* neuroblastoma breakpoint family; *CCDC66,* coiled-coil domain containing 66.
[a]Genome build NCBI37 (hg19).
[b]Effect allele/other allele.

TABLE III. Gene Set Enrichment Analysis (GSEA)

Outcome	Data source	Gene set	Size (N° of genes)	Nominal 95th Percentile			
				Expected	Observed	*P*-value	FDR
	Panther	Integrin signaling pathway	118	6	13	0.007	0.909
	KEGG	Keratan sulfate biosynthesis	15	1	4	0.007	0.200
	KEGG	Gap junction	89	4	10	0.012	0.353
	Panther	Ubiquitin proteasome pathway	51	3	7	0.015	0.621
	Ingenuity	NFKB Signaling	41	2	6	0.015	0.384
	KEGG	N-Glycan biosynthesis	42	2	6	0.017	0.459
	KEGG	Protein export	12	1	3	0.017	0.332
Commissions	Ingenuity	SAPK/JNK signaling	32	2	5	0.019	0.315
	Ingenuity	Interferon signaling	22	1	4	0.022	0.707
	KEGG	Focal adhesion	198	10	17	0.023	0.470
	KEGG	Neurodegenerative disorders	34	2	5	0.027	0.313
	KEGG	Neuroactive ligand–receptor interaction	278	14	21	0.035	0.443
	Ingenuity	Mitochondrial dysfunction	76	4	8	0.038	0.520
	KEGG	TGF-β signaling pathway	79	4	8	0.046	0.563
	KEGG	Axon guidance	126	6	11	0.050	0.490
	KEGG	B-cell receptor signaling pathway	67	3	7	0.050	0.485
	KEGG	Allograft rejection	16	1	4	0.007	0.288
	KEGG	Ether lipid metabolism	29	1	5	0.011	0.478
	KEGG	Biosynthesis of unsaturated fatty acids	22	1	4	0.022	0.460
	KEGG	Amino sugar and nucleotide sugar metabolism	44	2	6	0.022	0.582
HRT	Panther	Alzheimer disease amyloid secretase pathway	24	1	4	0.028	1
	KEGG	Gap junction	88	4	9	0.029	0.548
	KEGG	Autoimmune thyroid disease	25	1	4	0.032	0.528
	Panther	PDGF signaling pathway	50	3	6	0.039	1
	KEGG	Galactose metabolism	26	1	4	0.042	0.558
	KEGG	Graft-versus-host disease	16	1	3	0.043	0.488
	KEGG	Melanogenesis	96	5	9	0.046	0.567
	KEGG	GnRH signaling pathway	95	5	9	0.049	0.594
	KEGG	Vibrio cholerae infection	53	3	7	0.017	1
	KEGG	Riboflavin metabolism	16	1	3	0.041	1
HRTSE	Panther	PDGF signaling pathway	51	3	6	0.042	1
	Panther	Oxytocin receptor mediated signaling pathway	16	1	3	0.043	1
	Panther	Ubiquitin proteasome pathway	51	3	6	0.045	1
	Ingenuity	VDR/RXR activation	61	3	9	0.004	0.268
	KEGG	Huntington's disease	165	8	16	0.007	1.000
IIV	Panther	Angiotensin II-stimulated signaling through G proteins and β-arrestin	5	0	2	0.022	0.364
	KEGG	Glycosphingolipid biosynthesis—ganglio series	14	1	3	0.029	0.776
	Panther	Nicotinic acetylcholine receptor signaling pathway	35	2	5	0.029	1.000
	Panther	Axon guidance mediated by semaphorins	26	1	4	0.040	0.786

Pathways nominally associated with attention outcomes.
HRT, hit-reaction time; HRTSE, standard error of HRT; and IIV, intraindividual variability.

DISCUSSION

To our knowledge, this is the first GWAS and pathway (i.e., gene set enrichment) analysis of attention function in adult patients with ADHD. Despite no genome-wide significant results were detected ($P < 5E{-}08$), 27 loci fell below the suggestive threshold for significance ($P < E{-}05$). Some of these SNPs were located within or close to potential interesting genes for ADHD such as *SORCS2*, *NUAK1*, *FGF20*, *NETO1*, *BTBD9*, *DLG2*, *TOP3B*, and *CHRNB4*. Also, at the pathway level, several biological pathways were significantly associated with the different attention outcomes, although none remained significant after correcting for multiple testing. Nevertheless, some of these pathways are relevant in the context of ADHD, such as ubiquitin proteasome, axon guidance, neurode-

generative disorders, Alzheimer disease amyloid secretase, and Huntington's disease pathways.

The top hit, the rs2341917, was associated with HRTSE and ICV. This SNP is located in the *ACOXL* gene. The function of this gene is largely unknown. In GWAS, loci within this gene have been associated with susceptibility to alopecia areata [Betz et al., 2015] and chronic lymphocytic leukemia [Berndt et al., 2013; Speedy et al., 2014] among other diseases which, to our knowledge, are not related with attention or ADHD. Thus, the potential role of this gene in attention function in ADHD is unclear.

The second hit, the rs4689642, was associated with both HRT and HRTSE outcomes and it was located in the first intron of *SORCS2*. This gene encodes one family member of vacuolar protein sorting 10 (VPS10) domain-containing receptor proteins. Another SNP in the *SORCS2* gene, located approximately 200 kb apart from rs4689642, was associated with bipolar disorder in a GWAS [Ollila et al., 2009]. It is noteworthy that up to 20% of adults with ADHD also meet criteria for bipolar disorder [Kessler et al., 2006] and it has been suggested that these two psychiatric disorders may share etiological pathophysiological pathways [Landaas et al., 2012].

A recent study identified SORCS2 as a proneurotrophin receptor, mediating both trophic and apoptotic signals in conjunction with p75 (NTR) [Glerup et al., 2014]. Interestingly, a marked reduction in dopamine levels of frontal cortex was observed in *SorCS2* and *p75NTR* deficient animals [Glerup et al., 2014]. Also, recent findings suggest that *SORCS2* may be associated with risk for Alzheimer disease (AD), altered amyloid precursor protein (APP) processing [Reitz et al., 2013], and brain structure (temporal lobe) in elder individuals within the spectrum of Alzheimer disease [Kohannim et al., 2012]. In this context, rs6539247, located in the in the 3'UTR of the *NUAK1* gene, was associated with HRTSE. It has been suggested that this gene is associated with cortical amyloid-β, which plays a pivotal role in Alzheimer disease [Ramanan et al., 2014].

These findings are in line with the enrichment analyses results suggesting the implication of neurodegenerative disorders and AD amyloid secretase pathways in attention function in ADHD. The neurodegenerative disorders pathway includes biological pathways linked to neurodegenerative disorders such as AD, Parkinson Disease, or Huntington disease. The AD amyloid secretase pathway refers to the role of the amyloid precursor protein (APP) in the formation of amyloid plaques in AD. However, APP is not only linked to this pathologic process, as it has been suggested to be involved in neurite outgrowth and synaptogenesis, neuronal protein trafficking along the axon, transmembrane signal transduction, cell adhesion, and calcium metabolism [Zheng and Koo, 2006]. Taking all together, our findings suggest that genetic variants and biological pathways previously involved in AD may be also involved in attention performance in persistent ADHD. Of note, the AD amyloid pathway has been significantly associated with attention function in a GWAS conducted in a population-based children sample [Alemany et al., unpublished data].

Regarding the ubiquitin proteasome pathway, ubiquitination machinery has been suggested as a new disease mechanisms for adult ADHD in a previous GWAS conducted in the same clinical sample considered in the present study [Sanchez-Mora et al., 2014]. The present findings are in line with these findings supporting the role of ubiquitination processes specifically in attention function in these patients.

Other findings at the SNP-level are also interesting for ADHD research. First, the rs1521365, located close to *FGF20,* was associated with HRT. As a neurotrophin, *FGF20* plays critical roles not only in the growth and survival of neurons in early development but also in the biology of adult neurons [Lemaitre et al., 2010]. *FGF20* appears to be specifically expressed within the brain and particularly within neurons of the substantia nigra [Ohmachi et al., 2000] and cerebellum [Jeffers et al., 2001]. Of note, substantia nigra is a dopaminergic midbrain nuclei that has been shown to present an echogenic increased size in children ADHD patients compared with controls [Krauel et al., 2010; Romanos et al., 2010]. Also, neurochemical alterations and reduced gray matter volume in cerebellum have been reported in adult ADHD patients [Perlov et al., 2010; Seidman et al., 2011]. Besides this, *FGF20* has been associated with increased risk for Parkinson disease, specifically, the rs12720208 SNP [van der Walt et al., 2004], although several studies failed to replicate this association [de Mena et al., 2010].

Second, the *NETO1* gene was also associated with HRT. This gene encodes a predicted transmembrane protein containing two extracellular CUB domains followed by a low-density lipoprotein class A (LDLa) domain. *NETO1* gene regulates *N*-methyl-D-aspartate (NMDAR) function [Stohr et al., 2002], which is a major mediator of synaptic plasticity [Stephan et al., 2006]. It has been shown that corticolimbic NMDAR hypofunction is one of the core molecular mechanisms relevant for phenotypes observed in animal models of schizophrenia [Belforte et al., 2010]. Consistently, deletion of *Neto1* leads to deficits in synaptic plasticity in mice [Ng et al., 2009].

Third, a SNP within *BTBD9* was associated with HRTSE. This gene encodes a BTB/POZ domain-containing protein that has been associated with susceptibility to Restless Legs Syndrome (RLS) [Stefansson et al., 2007; Yang et al., 2011]. In this regard, ADHD is frequently coincident with sleep disorders such as the RLS [Zak et al., 2009; Hvolby, 2014] and both disorders, ADHD and RLS, are associated with a common dopaminergic dysfunction [Tilma et al., 2014] and iron deficiency [Cortese et al., 2008]. The genetic link between ADHD and RLS syndrome has been explored concluding that the role of *BTBD9* needs further study since it has been related to iron storage [Schimmelmann et al., 2009]. Our findings support the role of this gene in attention function in ADHD.

Fourth, the rs17147674 located in *DLG2* gene was also associated with HRTSE. This gene encodes the postsynaptic density protein known as chapsyn-110 [Egger et al., 2014]. It has been reported that individuals with mutations in *DLG2* made significantly more errors in tests of visual discrimination acquisition/cognitive flexibility compared to controls [Nithianantharajah et al., 2013]. Also, a recent study suggested the implication of a gain in copy number variants in *DLG2* gene in autism [Egger et al., 2014]. Despite the SNP detected in the present study is not in the region of this CNV, our results support the involvement of the *DLG2* gene in ADHD, which has been suggested to share genetic risk factors with autism [Reiersen et al., 2008; Ronald et al., 2008; Polderman et al., 2014].

Fifth, the rs16982689 located in *TOP3B* gene was associated with HRTSE. This gene encodes a DNA topoisomerase, an enzyme that

controls and alters the topologic states of DNA during transcription. A phenotypic effect of a deletion in the 22q11.22 region, including the *TOP3B* gene, has been associated with neurodevelopmental disorders such as the fragile X mental retardation syndrome [Stoll et al., 2013].

Finally, the rs12914008 located in *CHRNB4* gene was associated with ICV. The 15q25 region, which includes the *CHRNA5–CHRNA3–CHRNB4* gene cluster that encodes the nicotinic acetylcholine receptor a5, a3, and b4 subunits, respectively, has been implicated in susceptibility to smoking behavior [Saccone et al., 2009; Liu et al., 2010]. Interestingly, the prevalence of smoking is higher in patients with ADHD compared to the general population [Bukstein, 2012]. Nicotine consumption has been hypothesized to increase dopaminergic transmission which may enhance cognitive function in ADHD patients [Bukstein, 2012]. In this regard, a recent study found an association between tobacco smoking and the *CHRNA3* gene of this cluster in patients with ADHD but not in controls [Polina et al., 2014]. Whether the *CHRNB4* gene plays a specific role in attention function in adult ADHD related or not to smoking behavior requires further research.

Of note, the possible functionality of the genetic variants discussed above is currently unknown. To our knowledge, none of the loci were in linkage disequilibrium with any potential functional coding SNP with the exception of the missense SNP rs12914008 (*CHRNB4)*.

Since inattention is a core symptom of ADHD, it might be expected from a GWAS on attention function in patients with ADHD to detect associations with SNPs or genes previously associated with ADHD itself such as those related to dopamine transmission (e.g., *SLC6A3* or *DRD4*) or the *CDH13* gene identified in several studies [Hawi et al., 2015]. This was not the case for the present study. While it is likely that common susceptibility variants may exert pleiotopic effects on both attention function and ADHD, the current study may not have enough power to detect such variants. Of note, our findings in relation to the effect sizes suggest that the genetic variance of the attention outcomes analyzed is accounted by multiple loci of small effects with individual contributions below 6% for all outcomes (R^2 ranging from 0.001 to 0.05). These results are similar to the proposed polygenic hypothesis where multiple risk genes of minor/modest effects would contribute to the etiology of ADHD [Hawi et al., 2015]. However, the overall effect size accounted by all the loci showing suggestive evidence of association for each outcome (ranging from 14.4% to 37.1%, Supplemental Table SIII) was much higher than the previously reported 3.3% of variance accounted for ADHD by all loci combined [Kuntsi et al., 2006]. Although this may encourage further genetic research using candidate endophenotypes, it is difficult to compare these effect sizes since the phenotypes and the study designs, case–control in most of the GWAS in ADHD performed so far, are different. Furthermore, it is important to consider the limitations of our study and interpret the effect sizes with caution.

In this regard, it is worth mentioning that the most relevant findings were found in the HRT, HRTSE, and ICV attention outcomes. Reaction time (RT) variability is one of the most replicated deficits in ADHD [Castellanos and Tannock, 2002] and there is evidence reporting a strong link between RT variability and ADHD at phenotypic and genetic levels [Kuntsi and Klein, 2012] and previous research highlights RT as a promising cognitive target for molecular genetics investigation [Kuntsi et al., 2010]. Our findings are largely in agreement with these evidences. Also, we would like to point out two results: First, *SORCS2* was associated to both HRT and HRTSE, and *ACOXL* and *NUAK1* were associated to both HRTSE and ICV, which suggests certain genetic overlap that can be explained by the fact that these outcomes are phenotypically significantly correlated (Supplemental Table SII). Second, HRTSE was the outcome accumulating a higher number of genetic loci showing suggestive evidence of association, which may explain the fact that both global effect size and individual genetic contributions were larger for HRTSE compared to commissions, HRT and ICV. Furthermore, the overall effect size was larger for HRTSE (37.1%) than for commissions (14.4%) (Supplemental Table SIII). This is in keeping with recent results indicating that reaction time variability was genetically correlated with both inattention and hyperactivity ADHD symptoms in a twin sample from the general population whereas commission errors presented lower and non-significant genetic correlations with these symptoms [Kuntsi et al., 2014]. Overall, these findings suggest that HRTSE may be a particularly suitable outcome for genetic association studies in ADHD.

As a potential endophenotype, HRTSE is a quantitative neurocognitive measure that can be objectively assessed. Furthermore, even in a modest sample size such as the one used in this study, it was possible to identify several genetic variants potentially interesting for the etiology of ADHD. However, more research is needed specifically addressing the heritability and performance in unaffected relatives for this outcome in adult ADHD.

Our results should be interpreted in the context of some limitations. First, the sample size was modest for a GWAS approach even considering a candidate endophenotype as an outcome of interest. Also, we examine multiple phenotypes which may inflate type 1 error. Thus, replication in larger samples is required to confirm our results. This issue also limited the power of the study to analyse potential differences among ADHD subtypes. Second, because we used a sample with a wide age range, we were more likely to find genetic effects with an enduring influence throughout life span than identifying or replicating previous findings present in specific developmental periods [Kohannim et al., 2012].

Despite these limitations, the present study was able to identify a number of genetic variants susceptible to play a role in the molecular basis of attention function in adult ADHD. The SNPs pointed out above are located within or close to genes which are involved in neuronal processes, neurodegenerative diseases, or other neuropsychiatric or neurological conditions which may be comorbid with ADHD (i.e., bipolar disorder, restless legs syndrome, autism spectrum disorders, and smoking behavior). These evidences add neurobiological and clinical plausibility to the implication of these SNPs in the etiology of ADHD. Furthermore, our findings reinforce the conceptualization of attention function as a potential endophenotype for studying the molecular basis of adult ADHD. Thus, our promising results may suggest that future GWAS focusing of attention function in larger samples of ADHD patients are likely to detect major genetic effects at more restrictive significance thresholds.

CONFLICT OF INTEREST

None of the authors have conflict of interests or relevant financial interests or personal affiliations in connection with the content of this manuscript.

ACKNOWLEDGMENTS

We are grateful to all the patients and their families for their kind participation. We thank all the clinical collaborators that contributed to the diagnosis of the probands. We would like to thank M. Monfort for technical support at the Spanish National Genotyping Center (CEGEN) in Barcelona and M. Bayes from the National Center for Genomic Analysis (CNAG), Barcelona. Financial support was received from the Spanish 'Ministerio de Economía y Competitividad' (SAF2012-33484), AGAUR (2014SGR-0932, 2014SGR1357), "Instituto de Salud Carlos III-FIS" (PI11/00571, PI11/01629, PI12/01139), "Plan Nacional Sobre Drogas" (PNSD#2011-0080), and "Departament de Salut," Government of Catalonia, Spain. Silvia Alemany thanks the "Instituto de Salud Carlos III" for her Sara Borrell postdoctoral grant (CD14/00214). Marta Ribasés is a recipient of a Miguel de Servet contract from the "Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación", Spain (CP09/00119). Natàlia Vilor-Tejedor is funded by a predoctoral grant from the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR FI-DGR 2015) Generalitat de Catalunya. Cristina Sánchez-Mora is a recipient of a contract from the 7th Framework Programme for Research, technological Development and Demonstration, European Commission (AGGRESSOTYPE_FP7HEALTH2013/602805).

REFERENCES

Alemany S, Vilor-Tejedor N, Bustamante M, Llop S, Rebagliato M, Alvarez-Pedrerol M, Forns J, Júlvez J, Sunyer J. (unpublished data). A Genome-wide association study of attention function in a population-based sample of children.

Belforte JE, Zsiros V, Sklar ER, Jiang Z, Yu G, Li Y, Quinlan EM, Nakazawa K. 2010. Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. Nat Neurosci 13(1):76–83.

Berndt SI, et al. 2013. Genome-wide association study identifies multiple risk loci for chronic lymphocytic leukemia. Nat Genet 45(8):868–876.

Betz RC, et al. 2015. Genome-wide meta-analysis in alopecia areata resolves HLA associations and reveals two new susceptibility loci. Nat Commun 6:5966.

Boomsma DI, et al. 2010. Genetic epidemiology of attention deficit hyperactivity disorder (ADHD index) in adults. PLoS ONE 5(5):e10621.

Bukstein OG. 2012. Attention deficit hyperactivity disorder and substance use disorders. Curr Top Behav Neurosci 9:145–172.

Burt SA. 2009. Rethinking environmental contributions to child and adolescent psychopathology: A meta-analysis of shared environmental influences. Psychol Bull 135(4):608–637.

Castellanos FX, Tannock R. 2002. Neuroscience of attention-deficit/hyperactivity disorder: The search for endophenotypes. Nat Rev Neurosci 3(8):617–628.

Conners C, Staff. M. 2000. Conners' Continuous Performance Test (CPT II) computer programs for Windows technical guide and software manual. North Tonawanda Multi-Health Systems.

Cortese S, Lecendreux M, Bernardina BD, Mouren MC, Sbarbati A, Konofal E. 2008. Attention-deficit/hyperactivity disorder, Tourette's syndrome, and restless legs syndrome: The iron hypothesis. Med Hypotheses 70(6):1128–1132.

Chang Z, Lichtenstein P, Asherson PJ, Larsson H. 2013. Developmental twin study of attention problems: High heritabilities throughout development. JAMA Psychiatry 70(3):311–318.

de Mena L, et al. 2010. FGF20 rs12720208 SNP and microRNA-433 variation: No association with Parkinson's disease in Spanish patients. Neurosci Lett 479(1):22–25.

Egger G, et al. 2014. Identification of risk genes for autism spectrum disorder through copy number variation analysis in Austrian families. Neurogenetics 15(2):117–127.

Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, Sklar P. 2005. Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry 57(11):1313–1323.

Franke B, et al. 2012. The genetics of attention deficit/hyperactivity disorder in adults, a review. Mol Psychiatry 17(10):960–987.

Glerup S, et al. 2014. SorCS2 regulates dopaminergic wiring and is processed into an apoptotic two-chain receptor in peripheral glia. Neuron 82(5):1074–1087.

Haavik J, Halmoy A, Lundervold AJ, Fasmer OB. 2010. Clinical assessment and diagnosis of adults with attention-deficit/hyperactivity disorder. Expert Rev Neurother 10(10):1569–1580.

Hawi Z, Cummins TD, Tong J, Johnson B, Lau R, Samarrai W, Bellgrove MA. 2015. The molecular genetic architecture of attention deficit hyperactivity disorder. Mol Psychiatry 20(3):289–297.

Hinney A, et al. 2011. Genome-wide association study in German patients with attention deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 156B(8):888–897.

Hvolby A. 2014. Associations of sleep disturbance with ADHD: Implications for treatment. Atten Defic Hyperact Disord 7(1):1–18.

Jeffers M, Shimkets R, Prayaga S, Boldog F, Yang M, Burgess C, Fernandes E, Rittman B, Shimkets J, LaRochelle WJ, Lichenstein HS. 2001. Identification of a novel human fibroblast growth factor and characterization of its role in oncogenesis. Cancer Res 61(7):3131–3138.

Kessler RC, et al. 2006. The prevalence and correlates of adult ADHD in the United States: Results from the National Comorbidity Survey Replication. Am J Psychiatry 163(4):716–723.

Kohannim O, et al. 2012. Discovery and replication of gene influences on brain structure using LASSO regression. Front Neurosci 6:115.

Krauel K, Feldhaus HC, Simon A, Rehe C, Glaser M, Flechtner HH, Heinze HJ, Niehaus L. 2010. Increased echogenicity of the substantia nigra in children and adolescents with attention-deficit/hyperactivity disorder. Biol Psychiatry 68(4):352–358.

Kuntsi J, Klein C. 2012. Intraindividual variability in ADHD and its implications for research of causal links. In: Stanford C, Tannock R, editors. Behavioral neuroscience of attention deficit hyperactivity disorder and its treatment. Verlag Berlin Heidelberg: Springer. pp 67–91.

Kuntsi J, Neale BM, Chen W, Faraone SV, Asherson P. 2006. The IMAGE project: Methodological issues for the molecular genetic analysis of ADHD. Behav Brain Funct 2:27.

Kuntsi J, Pinto R, Price TS, van der Meere JJ, Frazier-Wood AC, Asherson P. 2014. The separation of ADHD inattention and hyperactivity-impulsivity symptoms: Pathways from genetic effects to cognitive impairments and symptoms. J Abnorm Child Psychol 42(1):127–136.

Kuntsi J, et al. 2010. Separation of cognitive impairments in attention-deficit/hyperactivity disorder into 2 familial factors. Arch Gen Psychiatry 67(11):1159–1167.

Landaas ET, Halmoy A, Oedegaard KJ, Fasmer OB, Haavik J. 2012. The impact of cyclothymic temperament in adult ADHD. J Affect Disord 142(1–3):241–247.

Larsson H, Asherson P, Chang Z, Ljung T, Friedrichs B, Larsson JO, Lichtenstein P. 2013a. Genetic and environmental influences on adult attention deficit hyperactivity disorder symptoms: A large Swedish population-based study of twins. Psychol Med 43(1):197–207.

Larsson H, Chang Z, D'Onofrio BM, Lichtenstein P. 2013b. The heritability of clinically diagnosed attention deficit hyperactivity disorder across the lifespan. Psychol Med 10:1–7.

Lemaitre H, et al. 2010. Genetic variation in FGF20 modulates hippocampal biology. J Neurosci 30(17):5992–5997.

Lesch KP, et al. 2008. Molecular genetics of adult ADHD: Converging evidence from genome-wide association and extended pedigree linkage studies. J Neural Transm 115(11):1573–1585.

Liu JZ, et al. 2010. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. Nat Genet 42(5):436–440.

Marchini J, Howie B, Myers S, McVean G, Donnelly P. 2007. A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet 39(7):906–913.

Mick E, et al. 2010. Family-based genome-wide association scan of attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 49(9):898–905 e3.

Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16(3): 1215.

Neale BM, et al. 2008. Genome-wide association scan of attention deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 147B(8):1337–1344.

Neale BM, et al. 2010a. Case-control genome-wide association study of attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 49(9):906–920.

Neale BM, et al. 2010b. Meta-analysis of genome-wide association studies of attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 49(9):884–897.

Ng D, et al. 2009. Neto1 is a novel CUB-domain NMDA receptor-interacting protein required for synaptic plasticity and learning. PLoS Biol 7(2):e41.

Nithianantharajah J, et al. 2013. Synaptic scaffold evolution generated components of vertebrate cognitive complexity. Nat Neurosci 16(1):16–24.

Oberlin BG, Alford JL, Marrocco RT. 2005. Normal attention orienting but abnormal stimulus alerting and conflict effect in combined subtype of ADHD. Behav Brain Res 165(1):1–11.

Ohmachi S, Watanabe Y, Mikami T, Kusu N, Ibi T, Akaike A, Itoh N. 2000. FGF-20, a novel neurotrophic factor, preferentially expressed in the substantia nigra pars compacta of rat brain. Biochem Biophys Res Commun 277:355–360.

Ollila HM, Soronen P, Silander K, Palo OM, Kieseppa T, Kaunisto MA, Lonnqvist J, Peltonen L, Partonen T, Paunio T. 2009. Findings from bipolar disorder genome-wide association studies replicate in a Finnish bipolar family-cohort. Mol Psychiatry 14(4):351–353.

Perlov E, Tebarzt van Elst L, Buechert M, Maier S, Matthies S, Ebert D, Hesslinger B, Philipsen A. 2010. H(1)-MR-spectroscopy of cerebellum in adult attention deficit/hyperactivity disorder. J Psychiatr Res 44(14): 938–943.

Polderman TJ, Hoekstra RA, Posthuma D, Larsson H. 2014. The co-occurrence of autistic and ADHD dimensions in adults: An etiological study in 17,770 twins. Transl Psychiatry 4:e435.

Polina ER, et al. 2014. ADHD diagnosis may influence the association between polymorphisms in nicotinic acetylcholine receptor genes and tobacco smoking. Neuromolecular Med 16(2):389–397.

Purcell S, et al. 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3):559–575.

Ramanan VK, et al. 2014. APOE and BCHE as modulators of cerebral amyloid deposition: A florbetapir PET genome-wide association study. Mol Psychiatry 19(3):351–357.

Ramos-Quiroga JA, et al. 2012. Criterion and concurrent validity of Conners Adult ADHD Diagnostic Interview for DSM-IV (CAADID) Spanish version. Rev Psiquiatr Salud Ment 5(4):229–235.

Reiersen AM, Constantino JN, Grimmer M, Martin NG, Todd RD. 2008. Evidence for shared genetic influences on self-reported ADHD and autistic symptoms in young adult Australian twins. Twin Res Hum Genet 11(6):579–585.

Reitz C, et al. 2013. Independent and epistatic effects of variants in VPS10-d receptors on Alzheimer disease risk and processing of the amyloid precursor protein (APP). Transl Psychiatry 3:e256.

Romanos M, Weise D, Schliesser M, Schecklmann M, Loffler J, Warnke A, Gerlach M, Classen J, Mehler-Wex C. 2010. Structural abnormality of the substantia nigra in children with attention-deficit hyperactivity disorder. J Psychiatry Neurosci 35(1):55–58.

Ronald A, Simonoff E, Kuntsi J, Asherson P, Plomin R. 2008. Evidence for overlapping genetic influences on autistic and ADHD behaviours in a community twin sample. J Child Psychol Psychiatry 49(5):535–542.

Saccone NL, et al. 2009. The CHRNA5-CHRNA3-CHRNB4 nicotinic receptor subunit gene cluster affects risk for nicotine dependence in African-Americans and in European-Americans. Cancer Res 69(17): 6848–6856.

Sanchez-Mora C, et al. 2014. Case-control genome-wide association study of persistent attention-deficit hyperactivity disorder identifies FBXO33 as a novel susceptibility gene for the disorder. Neuropsychopharmacology 40:915–926.

Schimmelmann BG, et al. 2009. Exploring the genetic link between RLS and ADHD. J Psychiatr Res 43(10):941–945.

Segre AV, Groop L, Mootha VK, Daly MJ, Altshuler D. 2010. Common inherited variation in mitochondrial genes is not enriched for associations with type 2 diabetes or related glycemic traits. PLoS Genet 6(8): e1001058.

Seidman LJ, Biederman J, Liang L, Valera EM, Monuteaux MC, Brown A, Kaiser J, Spencer T, Faraone SV, Makris N. 2011. Gray matter alterations in adults with attention-deficit/hyperactivity disorder identified by voxel based morphometry. Biol Psychiatry 69(9):857–866.

Simon V, Czobor P, Balint S, Meszaros A, Bitter I. 2009. Prevalence and correlates of adult attention-deficit hyperactivity disorder: Meta-analysis. Br J Psychiatry 194(3):204–211.

Speedy HE, et al. 2014. A genome-wide association study identifies multiple susceptibility loci for chronic lymphocytic leukemia. Nat Genet 46(1):56–60.

Stefansson H, et al. 2007. A genetic risk factor for periodic limb movements in sleep. N Engl J Med 357(7):639–647.

Stephan KE, Baldeweg T, Friston KJ. 2006. Synaptic plasticity and dysconnection in schizophrenia. Biol Psychiatry 59(10):929–939.

Stergiakouli E, et al. 2012. Investigating the contribution of common genetic variants to the risk and pathogenesis of ADHD. Am J Psychiatry 169(2):186–194.

Stohr H, Berger C, Frohlich S, Weber BH. 2002. A novel gene encoding a putative transmembrane protein with two extracellular CUB domains

and a low-density lipoprotein class A module: Isolation of alternatively spliced isoforms in retina and brain. Gene 286(2):223–231.

Stoll G, et al. 2013. Deletion of TOP3beta, a component of FMRP-containing mRNPs, contributes to neurodevelopmental disorders. Nat Neurosci 16(9):1228–1237.

Tilma J, Thomsen PH, Ostergaard JR. 2014. A possible coexistence between restless legs syndrom and attention deficit hyperactivity disorder. Ugeskr Laeger 176(4):pii: V11120672.

van der Walt JM, et al. 2004. Fibroblast growth factor 20 polymorphisms and haplotypes strongly influence risk of Parkinson disease. Am J Hum Genet 74(6):1121–1127.

Yang L, et al. 2013. Polygenic transmission and complex neuro developmental network for attention deficit hyperactivity disorder: Genome-wide association study of both common and rare variants. Am J Med Genet B Neuropsychiatr Genet 162B(5):419–430.

Yang Q, Li L, Chen Q, Foldvary-Schaefer N, Ondo WG, Wang QK. 2011. Association studies of variants in MEIS1, BTBD9, and MAP2K5/SKOR1 with restless legs syndrome in a US population. Sleep Med 12(8):800–804.

Zak R, Fisher B, Couvadelli BV, Moss NM, Walters AS. 2009. Preliminary study of the prevalence of restless legs syndrome in adults with attention deficit hyperactivity disorder. Percept Mot Skills 108(3):759–763.

Zheng H, Koo EH. 2006. The amyloid precursor protein: Beyond amyloid. Mol Neurodegener 1:5.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the pulisher's web-site.