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Genome-Wide Analysis Reveals Four Novel Loci for Attention-Deficit Hyperactivity Disorder in Korean Youths

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Objectives: The molecular mechanisms underlying attention-deficit hyperactivity disorder (ADHD) remain unclear. Therefore, this study aimed to identify the genetic susceptibility loci for ADHD in Korean children with ADHD. We performed a case-control and a family-based genome-wide association study (GWAS), as well as genome-wide quantitative trait locus (QTL) analyses, for two symptom traits.

Methods: A total of 135 subjects (71 cases and 64 controls), for the case-control analysis, and 54 subjects (27 probands and 27 unaffected siblings), for the family-based analysis, were included.

Results: The genome-wide QTL analysis identified four single nucleotide polymorphisms (SNPs) (rs7684645 near *APELA*, rs12538843 near *YAE1D1* and *POU6F2*, rs11074258 near *MCTP2*, and rs34396552 near *CIDEA*) that were significantly associated with the number of inattention symptoms in ADHD. These SNPs showed possible association with ADHD in the family-based GWAS, and with hyperactivity-impulsivity in genome-wide QTL analyses. Moreover, association signals in the family-based QTL analysis for the number of inattention symptoms were clustered near genes *IL10*, *IL19*, *SCL5A9*, and *SKINTL*.

Conclusion: We have identified four QTLs with genome-wide significance and several promising candidates that could potentially be associated with ADHD (*CXCR4*, *UPF1*, *SETD5*, *NALCN-AS1*, *ERC1*, *SOX2-OT*, *FGFR2*, *ANO4*, and *TBL1XR1*). Further replication studies with larger sample sizes are needed.

Key Words: Attention-deficit hyperactivity disorder; Genome-wide association study; Asian population; Case-control study; Family-based study.

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INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, and affects 5−8% of children worldwide.[1)] ADHD is associated with academic under-achievement and dysfunctional relationships with family members and peers.[2)] It is a heterogeneous and complex disorder, and its pathophysiology remains largely unknown.

Previous twin and adoption studies have suggested a strong genetic contribution to ADHD, and a meta-analysis of twin studies has reported an average heritability of 76%.[3)] Several candidate gene association studies have investigated ADHD risk genes, including dopamine-related genes (*DRD4*, *DRD5*, and *SLC6AC*), serotonin-related genes (*HTR1B* and *SLC6A4*), and synaptic vesicle fusion-related gene *SNAP-25*.[4)] However, efforts to replicate these results have been inconsistent.[5)] Furthermore, many common gene variants with small effects are considered to contribute to ADHD.[3)]

Genome-wide association studies (GWAS) are powerful tools for detecting, at several hundred thousand positions in the genome, common genetic polymorphisms that influence disease susceptibility and quantitative traits. Numerous GWAS have been conducted to identify ADHD risk loci using either case-control or family-based designs,[4)] and a recent meta-

analysis of GWAS has revealed 12 genome-wide significant loci for ADHD.[6] Moreover, a family-based quantitative trait loci (QTL) analysis has previously identified significant associations with cell-cell adhesion gene *CDH13*.[7] The single nucleotide polymorphisms (SNPs) implicated in previous GWAS of ADHD are located at the sodium/proton exchanger *SLC9A9*,[8] glutamate receptor *GRM5*,[9] and cholinergic receptor *CHRNA7*.[10] However, these results have not been sufficiently replicated, and the effect of these genes on ADHD pathogenesis has been subject to considerable controversy. In addition, most GWAS for ADHD have been performed in European and American populations and, to our knowledge, few studies have been performed in Asian cohorts.[11] To explore the risk variants related to ADHD predisposition in a Korean population, we assessed the genetic susceptibility loci for ADHD by conducting a case-control and a family-based GWAS in Korean children with ADHD.

METHODS

Participants

Subjects with ADHD and their unaffected siblings were recruited from November 2012 to April 2015 at the children's outpatient psychiatric clinic of Asan Medical Center, Seoul, Korea. Typically developing children were recruited as controls through the Internet bulletin board of Asan Medical Center. All participants were 6–12 years old and were of Korean ancestry. All subjects were genetically unrelated. Subjects were excluded from this study if they satisfied one or more of the following criteria: 1) suspected mental retardation or an IQ score of less than 80; 2) history of ADHD medication (stimulants or atomoxetine) in the past three months; 3) history of low birth weight of less than 2.5 kg; 4) presence of congenital genetic disorders, acquired brain injury (e.g., cerebral palsy), seizure, or other neurological disorders; and 5) past and/or current history of bipolar disorder, schizophrenia, other childhood psychotic disorders, organic mental disorder, or pervasive developmental disorder. Cases with comorbid disorders, such as tic or anxiety disorders, that did not require pharmacological treatment were included.

This study was approved by the Institutional Review Board at Asan Medical Center (2012-0767). Written informed consent was obtained from the parents and written assent was obtained from the subjects.

Measures

All subjects and their parents underwent clinical evaluation by child psychiatrists. A diagnosis of ADHD and comorbid psychiatric disorders was confirmed according to the diagnostic criteria in Diagnostic and Statistical Manual of Mental Disorders-IV-Text Revision (DSM-IV-TR)[12] and Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL).[13] All subjects also completed the Korean Wechsler Intelligence Scale for Children-Fourth Edition (K-WISC-IV).[14] Two quantitative traits for QTL analysis were derived from the K-SADS-PL ADHD sections: the total number of 1) inattention and 2) hyperactivity-impulsivity symptoms as per the DSM-IV-TR criteria.

Genotyping and quality control

Genomic DNA was extracted from whole blood. The samples were genotyped using an Affymetrix Axiom™ KORV1.0-96 Array (Affymetrix, Santa Clara, CA, USA). Genotyping was performed according to the standard Affymetrix protocol at DNA Link (Seoul, Korea). The detailed protocol is described in Supplementary Material (in the online-only Data Supplement). SNPs that did not pass the Hardy-Weinberg equilibrium test ($p<1.00E-07$), those with low minor allele frequency (case ≤ 0.01 and control ≤ 0.01), and those with low marker call rate (case ≤ 0.95 or control ≤ 0.95) were excluded. Markers with $p<0.001$ were inspected using cluster plots. Owing to the small sample size, only autosomal SNPs were included in the family-based analysis.

Statistical analyses

Statistical procedures were performed using PLINK (http://zzz.bwh.harvard.edu/plink/)[15] and SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA). First, the case-control and the family-based GWAS were performed. For the case-control analysis, parametric tests were performed, including chi-square test for dominant and recessive alleles and Cochran-Armitage trend test for co-dominant alleles. We also conducted a non-parametric test, Jonckheere-Terpstra test, for dominant, recessive, and co-dominant alleles. For the family-based analysis, we conducted a sibling-transmission disequilibrium test.[16] Second, genome-wide QTL analyses, with either a case-control or a family-based design, were conducted to test the association with the two quantitative traits of ADHD. For QTL analyses, regression analyses with an additive model were performed using PLINK. To control for multiple comparisons, we considered p-values lower than 5.0E-08 to be statistically significant genome-wide.[17]

RESULTS

After quality control procedures were completed, 135 individuals (71 cases and 64 controls) and 525356 SNPs (63.1%), for the case-control analysis, and 27 sibling pairs (27 probands and 27 unaffected siblings) and 432921 SNPs (52.4%), for the family-based analysis, were included. Table 1 presents

the demographic and clinical characteristics of the study subjects. Between subjects with ADHD and typically developing children in the case-control analysis, a significant difference was found in age (p=0.002), gender (p=0.030), IQ (p=0.002), and comorbid diagnosis of oppositional defiant and conduct disorder (p=0.014). When ADHD subjects were compared with their unaffected siblings, a significant difference in gender was found (p=0.021).

In the case-control and the family-based GWAS, none of the variants reached genome-wide significance (p<5.00E-08). Table 2 lists the top SNPs (p<1.00E-05) of the case-control GWAS, which include rs34442475, adjacent to *CXCR4*, and rs2238652, adjacent to *UPF1*. These SNPs also showed possible association with the number of inattention symptoms (p=2.74E-02 and 1.59E-03) and hyperactivity-impulsivity symptoms (p=1.70E-04 and 1.80E-04) in the case-control QTL analysis. In the family-based GWAS, none of the SNPs had a p-value lower than 1.00E-05. Supplementary Fig. 1 (in the online-only Data Supplement) and Fig. 1 show the Manhattan plots and quantile-quantile (Q-Q) plots of the case-control and family-based GWAS, respectively.

Table 3 presents the list of SNPs with p-values lower than 1.00E-05 in the case-control QTL analysis. Two SNPs showed possible association with the number of inattention symptoms and five SNPs exhibited a possible association with the number of hyperactivity-impulsivity symptoms. These seven SNPs showed a trend towards association with ADHD in the case-control GWAS (p<0.05).

Table 1. Demographic and clinical characteristics of the study subjects

	Case-control analysis				Family-based analysis			
	ADHD (n=71)	Control (n=64)	t or χ^2	p-value	ADHD (n=27)	Unaffected sibling (n=27)	t or χ^2	p-value
Age, mean (SD)	7.9 (1.8)	8.9 (2.0)	−3.165	0.002	8.4 (1.8)	9.1 (2.2)	−1.144	0.258
Gender (boys), n (%)	53 (74.6)	36 (56.3)	5.072	0.030	22 (81.5)	13 (48.1)	6.577	0.021
IQ	99.3 (15.9)	107.4 (14.1)	−3.093	0.002	101.8 (18.7)	110.9 (16.4)	−1.903	0.063
ADHD subtype, n (%)								
Inattentive	27 (38.0)				15 (55.6)			
Hyperactive-impulsive	8 (11.3)				1 (3.7)			
Combined	29 (40.8)				8 (14.8)			
NOS	7 (9.9)				3 (5.6)			
Comorbid diagnosis, n (%)								
ODD/CD	7 (5.2)	0 (0)	6.655	0.010	3 (11.1)	0 (0)	3.176	0.236
Anxiety disorder	2 (2.8)	4 (6.3)	0.937	0.420	1 (3.7)	0 (0)	1.019	1
Tic disorder	2 (2.8)	2 (3.1)	0.011	1	2 (7.4)	0 (0)	2.077	0.491
Mood disorder	1 (1.4)	0 (0)	0.908	1	0 (0)	0 (0)		
Symptom count								
Inattention	6.4 (1.7)	1.3 (1.6)	18.165	<0.001	6.7 (1.9)	1.8 (1.6)	10.373	<0.001
Hyperactivity-impulsivity	4.7 (2.4)	0.5 (0.9)	13.642	<0.001	4.2 (2.5)	0.6 (0.8)	6.916	<0.001

ADHD: attention-deficit hyperactivity disorder, CD: conduct disorder, NOS: attention-deficit hyperactivity disorder not otherwise specified, ODD: oppositional defiant disorder, SD: standard deviation

Table 2. List of SNPs with p values<1.00E-05 in the case-control GWAS

								GWAS		Genome-wide QTL analysis			
rs number	Chr	Position	Minor allele	Closest gene	MAF	OR	95% CI	Case-control p value	Family-based p value	Case-control inattention p value	Case-control hyperactivity-impulsivity p value	Family-based inattention p value	Family-based hyperactivity-impulsivity p value
rs34442475	2	137064385	C	*CXCR4*	0.412	2.30	1.41–3.78	1.60E-06	5.64E-01	2.74E-02	1.70E-04	3.24E-01	3.13E-01
rs2238652	19	18942559	T	*UPF1*	0.289	4.79	2.28–10.0	3.12E-06	3.17E-01	1.59E-03	1.80E-04	4.89E-01	4.14E-01

Chr: chromosome, CI: confidential interval, *CXCR4*: chemokine (C-X-C motif) receptor 4, GWAS: genome-wide association study, MAF: minor allele frequency, OR: odds ratio, QTL: quantitative trait locus, SNP: single nucleotide polymorphism, *UPF1*: up-frameshift suppressor 1

In the family-based genome-wide QTL analysis, four SNPs, including rs7684645 adjacent to apelin receptor early endogenous ligand (*APELA*), rs12538843 adjacent to Yae1 domain containing 1 (*YAE1D1*) and POU class 6 homeobox 2 (*POU6F2*), rs11074258 adjacent to multiple C2 domains, transmembrane 2 (*MCTP2*), and rs34396552 adjacent to cell death-inducing DFFA-like effector A (*CIDEA*), showed a genome-wide significant association with the number of inattention symptoms. Table 4 describes the SNPs with p-values lower than 1.00E-06. Most of these SNPs also showed possible association with ADHD in the family-based GWAS, and/or with the number of hyperactivity-impulsivity symptoms in the family-based genome-wide QTL analysis ($p<0.05$). In fact, in the family-based QTL analysis, 153 SNPs and 18 SNPs had p-values lower than 1.00E-05 for the number of inattention symptoms and for the number of hy-

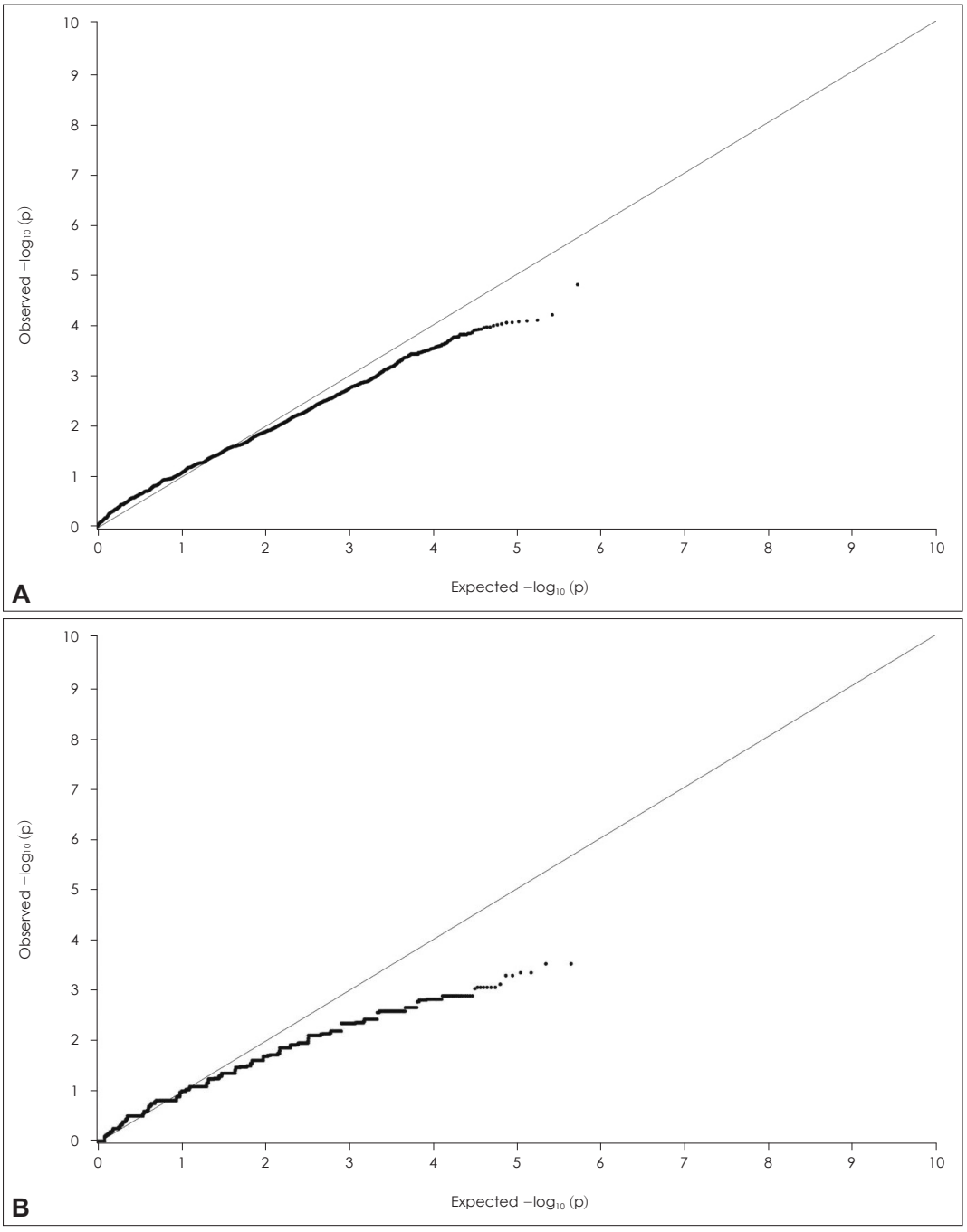

Fig. 1. Q-Q plot of association results. A: Q-Q plot of case-control GWAS, B: Q-Q plot of family-based GWAS, GWAS: genome-wide association study, Q-Q: quantile-quantile.

peractivity-impulsivity symptoms, respectively. The regional association plots (Fig. 2), established using genotype data from the family-based QTL analysis for the number of inattention symptoms, indicated that moderately associated SNPs (p<1.00E-04) were tightly linked to rs11119570, located near *IL10* and *IL19*; and rs214220, located near *SLC5A9* and *SKINTL*. Supplementary Fig. 1 (in the online-only Data Supplement) and Fig. 1 describe the Manhattan-plots and Q-Q plots, respectively, of the genome-wide QTL analyses.

DISCUSSION

In this study, we conducted GWAS of ADHD and genome-wide QTL analyses of ADHD symptoms in Korean children with ADHD. In the case-controlled and the family-based GWAS, we did not identify any significant genome-wide SNPs.

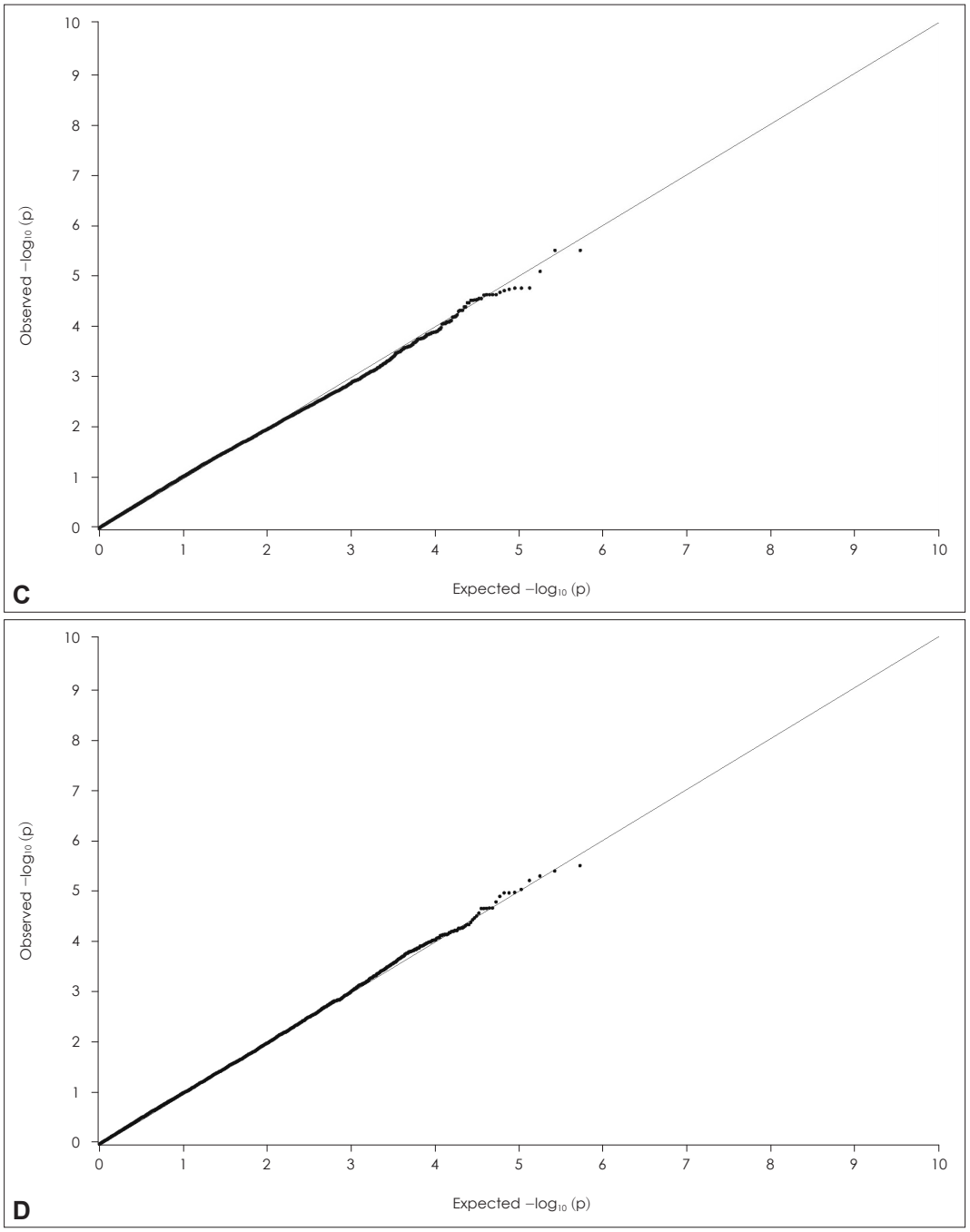

Fig. 1. Q-Q plot of association results. C: Q-Q plot of case-control genome-wide QTL analysis for inattention symptom count, D: Q-Q plot of case-control genome-wide QTL analysis for hyperactivity-impulsivity symptom count. Q-Q: quantile-quantile, QTL: quantitative trait locus.

However, in the genome-wide QTL analysis, we identified four SNPs (rs7684645 near *APELA*, rs12538843 near *YAE1D1* and *POU6F2*, rs11074258 near *MCTP2*, and rs34396552 near *CIDEA*) that were significantly associated with the number of inattention symptoms of ADHD. These SNPs showed possible association with ADHD in the family-based GWAS, and with hyperactivity-impulsivity in the genome-wide QTL analysis.

Among genes adjacent to the four aforementioned SNPs, rs7684645 is located in the intergenic region adjacent to the *APELA* gene, located at 4q32.3. *APELA* plays a key role in cardiac development as a motogen, by promoting endoderm and mesendoderm cell migration during gastrulation.[18] Boso et al.[19] reported that the plasma level of apelin is reduced in patients with autism spectrum disorder, thus suggesting a possible association with neurodevelopmental disorders.

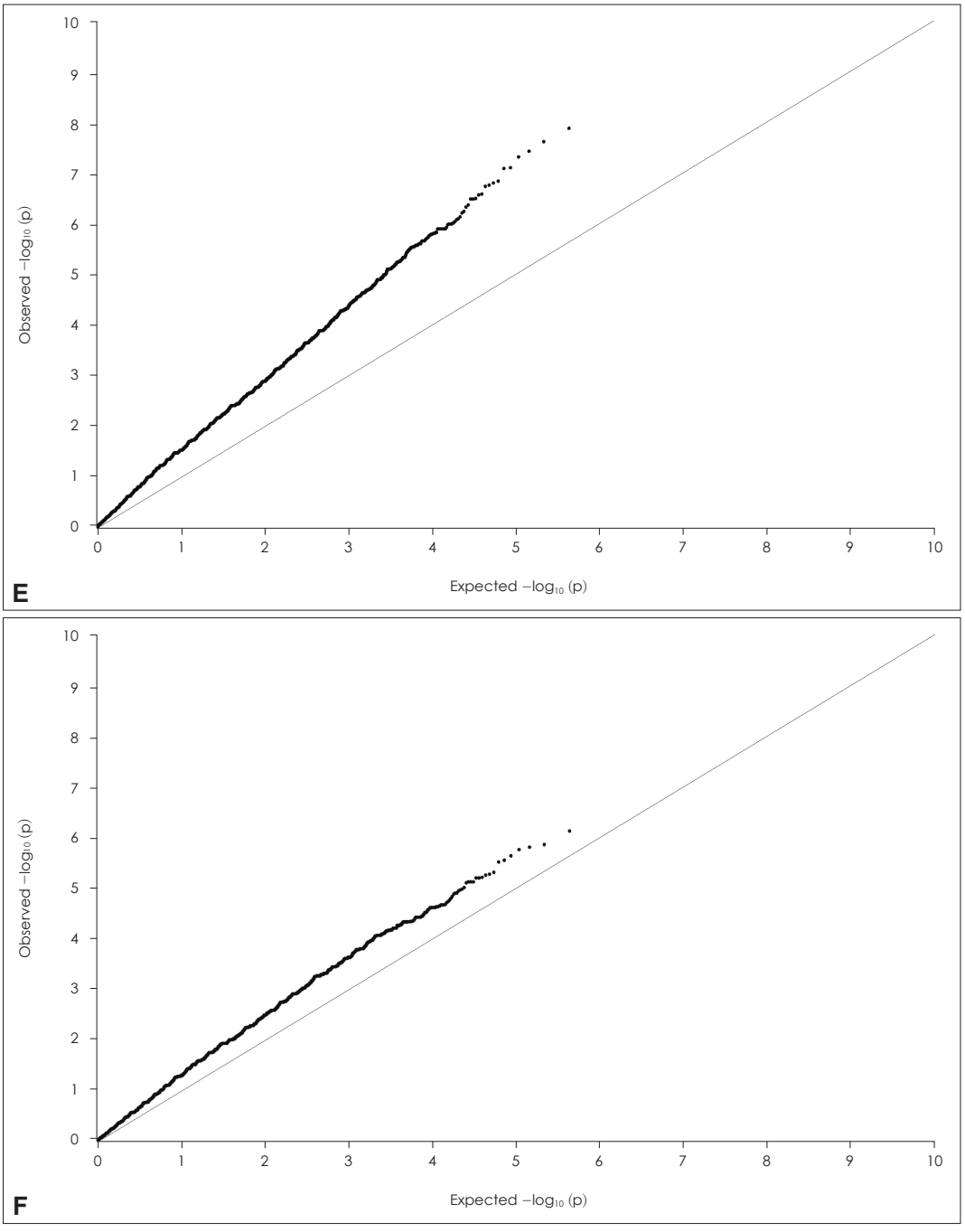

Fig. 1. Q-Q plot of association results. E: Q-Q plot of family-based genome-wide QTL analysis for inattention symptom count, F: Q-Q plot of family-based genome-wide QTL analysis for hyperactivity-impulsivity symptom count. Q-Q: quantile-quantile, QTL: quantitative trait locus.

Table 3. List of SNPs with p values < 1.00E-05 in the genome-wide QTL analysis (case-control analysis)

rs_number	Chr	Position	Gene	Region	Minor allele	MAF	GWAS		Genome-wide QTL analysis			
							Case-control p value	Family-based p value	Case-control inattention p value	Case-control hyperactivity-impulsivity p value	Family-based inattention p value	Family-based hyperactivity-impulsivity p value
rs117354149	3	9506285	*SETD5*	Missense, cds, exon, UTR-3	C	0.085	5.26E-04	5.64E-01	3.15E-06	1.42E-03	7.90E-01	1
rs9513794	13	101402224	*NALCN-AS1*	Intron	T	0.326	2.80E-03	1	8.22E-06	3.09E-03	9.25E-01	8.24E-01
rs61913097	12	1449199	*ERC1*	Intron	C	0.182	4.52E-03	(–)	1.27E-02	3.15E-06	(–)	(–)
rs11917999	3	181256247	*SOX2-OT*	Intron	G	0.222	3.33E-04	2.51E-01	7.89E-04	4.03E-06	8.65E-02	4.90E-02
rs117059665	10	123330437	*FGFR2*	Intron	T	0.048	2.56E-03	(–)	4.54E-04	5.05E-06	(–)	(–)
rs609728	12	101375646	*ANO4*	Intron	T	0.164	9.46E-04	8.33E-02	5.44E-04	6.18E-06	5.83E-02	1.12E-01
rs74490514	3	176641395	*TBL1XR1*, *LINC01209*	Upstream, downstream	T	0.038	1.41E-02	(–)	5.94E-02	9.29E-06	(–)	(–)

ANO4: anoctamin 4, Chr: chromosome, *ERC1*: ELKS/RAB6-interacting/CAST family member 1, *FGFR2*: fibroblast growth factor receptor 2, GWAS: genome-wide association study, *LINC01209*: long intergenic non-protein coding RNA 1209, *MAF*: minor allele frequency, *NALCN-AS1*: NALCN antisense RNA 1, QTL: quantitative trait locus, *SETD5*: set domain containing 5, SNP: single nucleotide polymorphism, *SOX2-OT*: SOX2 overlapping transcript, *TBL1XR1*: transducin (beta)-like 1 X-linked receptor 1

Moreover, rs12538843 was located between genes *POU6F2* and *YAE1D1*. The *POU6F2* gene is expressed within the central nervous system, kidney, adrenal gland, heart, stomach, muscle, and eye.[20] It might be involved in the early steps of differentiation of amacrine and ganglion cells. Anney et al.[21] reported a possible association between *POU6F2* and autism spectrum disorder, and suggested that *POU6F2* may be associated with neurodevelopmental disorders. The function of *YAE1D1*, on the other hand, remains unknown. Further assessments of the function of *POU6F2* and *YAE1D1*, and of the role of rs12538843 in the pathogenesis of ADHD are needed.

Rs11074258 is located upstream of the *MCTP2* gene. This gene is involved in intercellular signal transduction and synapse function via its calcium-ion binding activity. Previous studies have supported the association between *MCTP2* and ADHD. Mick et al.[8] suggested a possible association between gene *MCTP1*, a paralog of *MCTP2*, and ADHD in a family-based GWAS (p=1.59E-05). Furthermore, using the Biological Network Gene Ontology tool, Poelmans et al.[22] found that the gene ontology process "calcium ion binding," which plays an important role in neurite migration, was significantly enriched in the 14 ADHD-associated genes.

Rs34396552 is located in the intergenic region, near the *CIDEA* gene. *CIDEA* is homologous to a murine protein known to activate apoptosis in mice. Its human homolog is known to regulate lipolysis in human adipocytes, and is also related to obesity.[23] Several studies have reported an association between ADHD and obesity, and have suggested that this comorbidity may be due to a shared genetic component.[24]

Besides the four SNPs with genome-wide significance, association signals of the family-based QTL analysis for the number of inattention symptoms were also clustered near the *IL10*, *IL19*, *SCL5A9*, and *SKINTL* genes. *IL19* and *IL10* encode cytokines that belong to the *IL10* cytokine subfamily, and play a key role in immune regulation and inflammation; furthermore, *SKINTL* is a newly identified immunoglobulin superfamily gene.[25] In addition, the immune and inflammatory system has been implicated in the pathogenesis of ADHD[26] and other psychiatric disorders such as autism.[27] Polymorphisms in *IL10* may be involved in increased risk for major depressive disorder.[28] On the other hand, *SLC5A9* is a sodium-dependent transport channel of D-mannose, D-glucose, and D-fructose; its role in the pathogenesis of ADHD remains unclear.

In this study, only the family-based QTL analysis identified genome-wide significant associations. Previous reports have suggested that case-control studies may be more powerful than family-based studies when investigating complex human traits, including qualitative and quantitative traits.[29]

Table 4. List of SNPs with p values<1.00E-06 in the family-based genome-wide QTL analysis

rs_number	Chr	Position	Gene	Region	Minor allele	MAF	GWAS		Genome-wide QTL analysis			
							Case-control p value	Family-based p value	Case-control inattention p value	Case-control hyperactivity-impulsivity p value	Family-based inattention p value	Family-based hyperactivity-impulsivity p value
rs7684645	4	165796146	*APELA*	Upstream,	A	0.387	6.51E-01	1.34E-03	4.31E-01	7.83E-01	1.29E-08	1.50E-04
rs12538843	7	39600097	*POU6F2, YAE1D1*	Intron, downstream, upstream	A	0.287	1.85E-01	2.70E-03	3.99E-01	2.67E-01	2.37E-08	1.54E-02
rs11074258	15	94770799	*MCTP2*	Upstream	C	0.170	6.57E-02	1.57E-03	4.79E-02	7.24E-02	3.67E-08	2.39E-03
rs34396552	18	12286113	*CIDEA*	Downstream	G	0.359	2.93E-01	2.70E-03	4.25E-01	7.69E-02	4.76E-08	1.41E-03
rs35493881	18	68153563	*GTSCR1*	Upstream	–	0.359	1.33E-02	3.89E-03	4.54E-02	2.32E-01	7.79E-08	7.73E-03
rs1239704	13	51152611	*DLEU1, DLEU7*	Intron, downstream	G	0.324	2.52E-01	4.51E-03	5.30E-01	7.91E-02	8.03E-08	1.74E-02
rs11119570	1	206968235	*IL19, IL10*	Upstream	A	0.396	1.98E-01	4.51E-03	2.67E-01	5.66E-01	1.43E-07	4.76E-04
rs6015071	20	56314639	*PMEPA1, MIR4532*	Upstream	A	0.274	6.38E-01	3.48E-02	7.98E-01	4.87E-01	1.56E-07	2.94E-02
rs3116816	6	29149442	*OR2J2, OR14J1*	Exon, downstream, upstream	A	0.398	1.38E-01	2.70E-03	9.09E-01	3.92E-01	1.72E-07	3.44E-03
rs6954881	7	56369816	*NUPR1L*	Downstream, upstream	G	0.472	2.42E-01	2.28E-03	1.02E-01	5.39E-01	1.82E-07	2.80E-03
rs1239682	13	51165494	*DLEU1, DLEU7*	Intron, downstream	C	0.333	3.00E-01	7.53E-03	3.45E-01	8.47E-01	2.59E-07	1.36E-02
rs6073330	20	42737494	*JPH2, TOX2*	Downstream	A	0.481	3.57E-03	4.68E-03	1.45E-02	9.42E-02	2.69E-07	3.23E-02
rs6796	7	6502367	*KDELR2, DAGLB*	UTR-3, intron, exon	C	0.491	8.41E-02	2.70E-03	7.82E-01	4.34E-01	3.16E-07	3.33E-02
rs74120710	10	12937634	*CCDC3, CAMK1D*	Downstream	T	0.157	5.46E-02	8.15E-03	3.38E-01	5.12E-01	3.23E-07	3.91E-03
rs62214554	20	52807262	*CYP24A1, PFDN4*	Upstream	G	0.250	3.06E-01	8.15E-03	5.62E-01	2.43E-01	3.23E-07	1.81E-02
rs10800919	1	203336808	*PRELP, FMOD*	Upstream, downstream	C	0.371	2.74E-01	1.24E-02	2.14E-01	2.55E-01	4.25E-07	4.76E-03
rs1523609	7	6535517	*GRID2IP, KDELR2*	Downstream, upstream	G	0.482	3.34E-01	4.68E-03	8.20E-01	6.31E-01	4.68E-07	3.95E-02
rs2817619	1	11603063	*PTCHD2*	Downstream	T	0.442	1.04E-01	2.70E-03	3.82E-02	8.80E-01	5.69E-07	4.69E-04
rs16823921	2	145376116	*TEX41*	Upstream	G	0.333	3.07E-01	1.57E-03	8.32E-01	3.56E-01	6.12E-07	6.81E-05
rs10868138	9	86917301	*SLC28A3*	Missense, exon	C	0.093	2.47E-01	1.43E-02	3.83E-01	1.23E-01	7.29E-07	2.60E-03
rs9316596	13	22469240	*LINC00424, LINC00540*	Upstream, downstream	A	0.343	8.41E-02	1.26E-02	2.29E-01	3.93E-01	7.95E-07	3.78E-02
rs6699651	1	7652387	*CAMTA1*	Intron	T	0.106	–	8.15E-03	–	–	8.34E-07	1.33E-04
rs4945333	11	78920819	*TENM4*	Intron	G	0.245	5.57E-02	6.66E-03	4.98E-01	5.83E-01	9.03E-07	4.52E-02
rs214220	1	48622182	*SKINTL, SLC5A9*	Intron, upstream	C	0.500	1.35E-01	7.53E-03	3.85E-02	8.37E-01	9.63E-07	1.42E-02
rs9464011	6	53866266	*MLIP*	Intron	C	0.102	2.92E-01	8.15E-03	6.87E-01	8.87E-01	3.05E-04	7.38E-07

APELA: apelin receptor early endogenous ligand, *CAMK1D*: calcium/calmodulin-dependent protein kinase ID, *CAMTA1*: calmodulin binding transcription activator 1, *CCDC3*: coiled-coil domain containing 3, Chr: chromosome, *CIDEA*: cell death-inducing DFFA-like effector A, *CYP24A1*: cytochrome P450, family 24, subfamily A, polypeptide 1, *DAGLB*: diacylglycerol lipase, beta, *DLEU1*: deleted in lymphocytic leukemia 1, *DLEU7*: deleted in lymphocytic leukemia, 7, *FMOD*: fibromodulin, *GRID2IP*: glutamate receptor, ionotropic, delta 2 (Grid2) interacting protein, *GTSCR1*: Gilles de la Tourette syndrome chromosome region, candidate 1, GWAS: genome-wide association study, *IL10*: interleukin 10, *IL19*: interleukin 19, *JPH2*: junctophilin 2, *KDELR2*: *KDEL* (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2, *LINC00424*: long intergenic non-protein coding RNA 424, *LINC00540*: long intergenic non-protein coding RNA 540, MAF: minor allele frequency, *MCTP2*: multiple C2 domains, transmembrane 2, *MIR4532*: microRNA 4532, *MLIP*: muscular LMNA-interacting protein, *NUPR1L*: nuclear protein, transcriptional regulator, 1-like, *OR14J1*: olfactory receptor, family 14, subfamily J, member 1, *OR2J2*: olfactory receptor, family 2, subfamily J, member 2, *PFDN4*: prefoldin subunit 4, *PMEPA1*: prostate transmembrane protein, androgen induced 1, *POU6F2*: POU class 6 homeobox 2, *PRELP*: proline/arginine-rich end leucine-rich repeat protein, *PTCHD2*: patched domain containing 2, QTL: quantitative trait locus, *SKINTL*: skint-like, pseudogene, *SLC28A3*: solute carrier family 28, member 3, *SLC5A9*: solute carrier family 5, member 9, SNP: single nucleotide polymorphism, *TENM4*: teneurin transmembrane protein 4, *TEX41*: testis expressed 41, *TOX2*: TOX high mobility group box family member 2, *YAE1D1*: Yae1 domain containing 1.

However, other reports have concluded that family-based designs can be more powerful than case-control designs when evaluating the genetic risk for common complex diseases, as the case-control design is more susceptible to bias due to population stratification or phenotype misclassification.[30]

Some limitations of our study should be considered when interpreting its results. First, our sample size was small. Similar to previous ADHD GWAS, our current analysis did not yield any significant genome-wide associations, except for the number of inattention symptoms in the family-based QTL

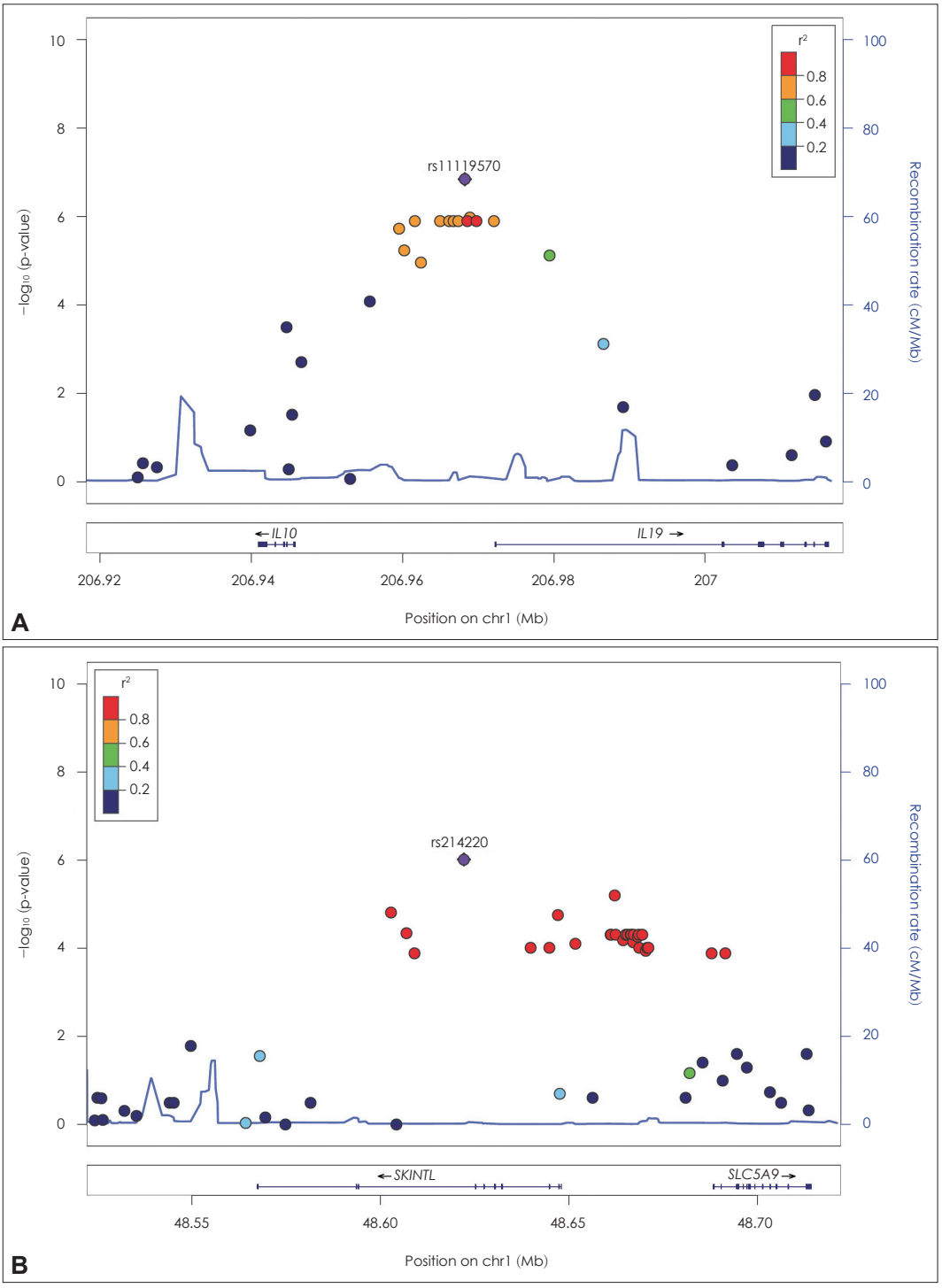

Fig. 2. Regional association plots. A: Regional association plot near *IL10* and *IL19*, B: Regional association plot near *SKINTL* and *SLC5A9*. Chr: chromosome, *IL10*: interleukin 10, *IL19*: interleukin 19, *SLC5A9*: solute carrier family 5, member 9, *SKINTL*: skint-like, pseudogene.

analysis. A large-scale, nationwide, or international consortium analysis or meta-analysis could overcome this issue. Second, significant differences in age, gender, IQ, and comorbid diagnosis of oppositional defiant and conduct disorder were found between ADHD subjects and controls. Moreover, when comparing ADHD subjects and their unaffected siblings, significant differences in gender were noted. We cannot disregard the possibility that such differences in gender, age, IQ, and comorbid diagnosis could have masked some true associations. Third, in QTL analysis, only symptoms of inattention and hyperactivity were used and intermediate phenotypes of ADHD, such as neuropsychological test results, were not included. Fourth, we excluded subjects with a history of recent ADHD medication that could affect quantitative traits of inattention and hyperactivity-impulsivity. However, this may have caused a selection bias by excluding children with such severe symptoms of ADHD that medication was required. Fifth, it must be noted that in the Q-Q plot of the family-based genome-wide QTL analysis, the observed p values of a large number of variants are inflated rather than matched to a uniform distribution. It is possible that the sample size was not large enough, and that some outliers influenced our results. To address this issue, further replication studies with larger sample sizes are needed.

CONCLUSION

We have identified four QTLs (rs7684645, rs12538843, rs11074258, and rs34396552) with genome-wide significant associations to ADHD and several promising candidates. Further investigation of these valuable candidates, using independent samples and related functional studies, are warranted. Moreover, analyses using larger ADHD sample sizes are likely to reveal additional common genetic risk loci for this complex disorder.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.5765/jkacap.2018.29.2.62.

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Conflicts of Interest

The authors have no financial conflicts of interest.

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