Case-Control Study of Six Genes Asymmetrically Expressed in the Two Cerebral Hemispheres: Association of *BAIAP2* with Attention-Deficit/Hyperactivity Disorder

Marta Ribasés, Rosa Bosch, Amaia Hervás, Josep Antoni Ramos-Quiroga, Cristina Sánchez-Mora, Anna Bielsa, Xavier Gastaminza, Sílvia Guijarro-Domingo, Mariana Nogueira, Núria Gómez-Barros, Susanne Kreiker, Silke Groß-Lesch, Christian P. Jacob, Klaus-Peter Lesch, Andreas Reif, Stefan Johansson, Kerstin J. Plessen, Per M. Knappskog, Jan Haavik, Xavier Estivill, Miguel Casas, Mònica Bayés, and Bru Cormand

Background: Attention-deficit/hyperactivity disorder (ADHD) is a childhood-onset neuropsychiatric disease that persists into adulthood in at least 30% of patients. There is evidence suggesting that abnormal left-right brain asymmetries in ADHD patients may be involved in a variety of ADHD-related cognitive processes, including sustained attention, working memory, response inhibition and planning. Although mechanisms underlying cerebral lateralization are unknown, left-right cortical asymmetry has been associated with transcriptional asymmetry at embryonic stages and several genes differentially expressed between hemispheres have been identified.

Methods: We selected six functional candidate genes showing at least 1.9-fold differential expression between hemispheres (*BAIAP2*, *DAPPER1*, *LMO4*, *NEUROD6*, *ATP2B3,* and *ID2*) and performed a case-control association study in an initial Spanish sample of 587 ADHD patients (270 adults and 317 children) and 587 control subjects.

Results: The single- and multiple-marker analysis provided evidence for a contribution of *BAIAP2* to adulthood ADHD ($p = .0026$ and $p = .0016$, respectively). We thus tested *BAIAP2* for replication in two independent adult samples from Germany (639 ADHD patients and 612 control subjects) and Norway (417 ADHD cases and 469 control subjects). While no significant results were observed in the Norwegian sample, we replicated the initial association between *BAIAP2* and adulthood ADHD in the German population ($p = .0062$).

Conclusions: Our results support the participation of *BAIAP2* in the continuity of ADHD across life span, at least in some of the populations analyzed, and suggest that genetic factors potentially influencing abnormal cerebral lateralization may be involved in this disorder.

Key Words: ADHD, attention-deficit hyperactivity disorder, *BAIAP2*, brain asymmetry, case-control association study

Attention-deficit/hyperactivity disorder (ADHD) is a common childhood-onset disorder characterized by impaired attention, hyperactivity, and impulsivity that affects 8% to 12% of children and 3% to 5% of adults (1–4). At least 30% of ADHD patients diagnosed in childhood continue to suffer from the disorder into adulthood and several evidences suggest a stronger genetic component in persistent than in remitting ADHD (5–9). Although its pathophysiology is unknown, brain imaging and neuropsychological studies support that impairment of various brain regions may account for ADHD symptoms (10–14) and growing evidence points toward underlying disrupted anatomical (15–22) and functional (23–31) hemispheric brain asymmetries. In this regard, ADHD has been associated with a right hemisphere dysfunction, mainly based on abnormal right-sided fronto-striatal-pallidal activity (15,18,23,25,32–38). This deviation from the common pattern of cerebral lateralization may be involved in a variety of impairments in ADHD individuals, including their core symptoms of attention and impulsivity, as well as executive functions.

Although the exact mechanisms underlying brain laterality are unknown, cerebral asymmetry is considered a complex and highly heritable phenotype (39–41). Interestingly, the gene expression pattern in the central nervous system displays asymmetries that overlap with those in the brain's functional organization, suggesting that multiple genes are likely to interact to

From the Department of Psychiatry (MR, RB, JAR-Q, CS-M, AB, XG, MN, NG-B, MC), Hospital Universitari Vall d'Hebron; Child and Adolescent Mental Health Unit (AH, SG-D), Hospital Mútua de Terrassa; and Department of Psychiatry and Legal Medicine (JAR-Q, MC), Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain; Departments of Psychiatry, Psychosomatics and Psychotherapy (SK, SG-L, CPJ, K-PL, AR) and Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy (SK), University of Würzburg, Würzburg, Germany; Department of BioMedicine (SJ, JH), University of Bergen; Center of Medical Genetics and Molecular Medicine (SJ, PMK) and Division of Psychiatry (KJP, JH), Haukeland University Hospital; and Department of Biological and Medical Psychology (KJP) and Medical Genetics and Molecular Medicine (PMK), Department of Clinical Medicine, University of Bergen, Bergen, Norway; Genes and Disease Program (XE, MB), Center for Genomic Regulation (CRG-UPF); CIBER Epidemiología y Salud Pública (XE, MB); Centro Nacional de Genotipado (CeGen) (XE, MB); Departament de Genètica (BC), Facultat de Biologia, Universitat de Barcelona; CIBER Enfermedades Raras (BC); and Institut de Biomedicina de la Universitat de Barcelona (IBUB) (BC), Barcelona, Catalonia, Spain.

Authors MR and RB contributed equally to this article.

Address correspondence to Bru Cormand, Ph.D., Universitat de Barcelona, Departament de Genètica, Facultat de Biologia, Av. Diagonal 645, 08028 Barcelona, Barcelona, Spain; E-mail: bcormand@ub.edu.

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determine adult cerebral structure (41). In addition, left-right cortical asymmetry in humans has been associated with transcriptional asymmetry between hemispheres at early embryonic stages (42). In this regard, Sun *et al.* (42) identified 27 differentially expressed genes in left and right human embryonic 12-weeks cortex and suggested that their asymmetric expression is related to asymmetric cortical development (42).

Since the asymmetry of human cerebral hemispheres appears to have a molecular basis, we reasoned that altered gene expression may be involved in the abnormal brain lateralization observed in ADHD subjects and, consequently, may contribute to the genetic predisposition to this neurodevelopmental disorder. We selected six functional candidate genes from those described by Sun *et al.* (42) that showed 1.9- to 8-fold differential expression between hemispheres (brain-specific angiogenesis inhibitor 1-associated protein 2 [*BAIAP2*]; Dapper antagonist of beta-catenin homolog 1 [*DAPPER1*]; LIM domain only 4 [*LMO4*]; Neurogenic differentiation 6 [*NEUROD6*]; ATPase, Ca++ transporting plasma membrane 3 [*ATP2B3*]; and inhibitor of DNA binding 2 [*ID2*]) (42) and conducted an initial case-control study in 587 ADHD patients (270 adults and 317 children) and 587 sex-matched unrelated control subjects from Spain. The observed results were then tested for replication in two additional independent case-control samples from Germany and Norway (639 and 417 adult ADHD patients and 612 and 469 control subjects, respectively).

Methods and Materials

Subjects

Table S1 in Supplement 1 shows the clinical description of the 1643 ADHD Caucasoid patients included in the study.

Spain. Five hundred eighty-seven patients with ADHD were recruited: 270 adults (65.9% combined, 29.7% inattentive, and 4.4% hyperactive-impulsive) and 317 children (73.5% combined, 21.8% inattentive, and 4.7% hyperactive-impulsive). Seventy-eight percent of patients were male. The control sample consisted of 531 unrelated Caucasoid blood donors matched for sex with the ADHD group in which DSM-IV ADHD symptomatology was excluded under the following criteria: 1) not having previously been diagnosed with ADHD and 2) answering negatively to the life-time presence of the following DSM-IV ADHD symptoms: 1) often has trouble keeping attention on tasks, 2) often loses things needed for tasks, 3) often fidgets with hands or feet or squirms in seat, and 4) often gets up from seat when remaining in seat is expected. The average age at assessment was 30.2 years (SD = 12.1) for adult patients, 9.3 years (SD = 2.6) for child patients, and 39.9 years (SD = 17.0) for control subjects.

Replication Population 1: Germany. The German sample consisted of 639 adult ADHD patients (67.1% combined, 25.4% inattentive, and 7.5% hyperactive-impulsive) and 612 sex-matched unrelated control subjects. Three hundred thirty-two of those were extensively interviewed for absence of ADHD and did not fulfill DSM-IV ADHD criteria (43,44), while the remaining control subjects consisted of unscreened blood donors and University staff not explicitly screened for absence of psychiatric disorders, although the scope of the study was explained to these individuals. Fifty percent of patients were male (n = 321). The average age at assessment was 34.3 years (SD = 10.4) for patients and 31.2 years (SD = 10.3) for control subjects.

Replication Population 2: Norway. The clinical sample consisted of 417 adult ADHD subjects (75.5% combined, 10.8% inattentive, 3.4% hyperactive-impulsive, and 10.3% subthreshold) with an age at assessment of 35.4 years (SD = 11.5). Fifty-two percent of patients were male (n = 218). The control sample included 469 sex-matched unrelated subjects. Two hundred sixty-nine of them, with an average age at assessment of 29.1 years (SD = 6.5), had no previous ADHD diagnosis and were recruited using a random selection of persons born in Norway from 1967 to 1989, whereas the rest were healthy blood donors for whom no information about ADHD symptoms was available.

Clinical Assessment

Diagnosis was blind to genotype. The study was approved by the ethics committee of each institution and informed consent was obtained from all subjects. A more detailed description of the different diagnostic instruments used was published previously (45).

Spanish Population: Adult ADHD. The ADHD diagnosis was based on the Structured Clinical Interview for DSM-IV Axis I and Axis II Disorders (SCID-I and SCID-II) and the Conners' Adult ADHD Diagnostic Interview for DSM-IV (CAADID). Severity of ADHD symptoms was evaluated using the long version of the Conners' ADHD Rating Scale (self-report [CAARS-S:L] and observer [CAARS-O:L]), the ADHD Rating Scale (ADHD-RS), the ADHD Screening Checklist, and the Wender Utah Rating Scale (WURS) for retrospective symptoms. The level of impairment was measured by the Clinical Global Impression (CGI) included in the CAADID Part II and the Sheehan Disability Inventory (46).

Spanish Population: Childhood ADHD. Patients were evaluated with the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL) reported by parents. Attention-deficit/hyperactivity disorder symptoms were assessed using the Conners' Parent Rating Scale and the Conners' Teacher Rating Scale. Exclusion criteria for the adult and childhood Spanish populations were IQ <70; pervasive developmental disorders; schizophrenia or other psychotic disorders; the presence of mood, anxiety, dissociative, or personality disorders that might explain ADHD symptoms; adoption; sexual or physical abuse; birth weight <1.5 kg; and other neurological or systemic disorders that might explain ADHD symptoms. For additional information see Ribasés *et al.* (9).

German Population. Patients were extensively examined using an open interview by an experienced psychiatrist (C.P.J.), as well as SCID-I and SCID-II (47). Personality assessment was done using the Revised NEO Personality Inventory (NEO PI-R) and Tridimensional Personality Questionnaire (TPQ) (48,49); severity of ADHD was measured with the WURS interview (50). When available, chart reviews were performed and information from relatives and school reports was considered. Eligibility criteria for the study were ADHD according to DSM-IV, onset before the age of 7 years via retrospective diagnosis, lifelong persistence, current diagnosis and age at recruitment between 18 and 65 years. Exclusion criteria for patients were the restricted appearance of ADHD-like symptoms (lack of concentration, hyperactivity, or impulsivity) during episodes of Axis I disorders, as well as a current diagnosis of not withdrawn drug/alcohol abuse/dependence; a lifetime diagnosis of bipolar I disorder, schizophrenia, or any other psychotic disorder; and mental retardation (IQ level <80; multiple-choice word test [MWT-B] <13 points).

Norwegian Population. Patients were recruited using a National Registry of adults diagnosed with ADHD in Norway during 1997 to 2005. Diagnosis was made according to the ICD-10 research criteria (51), with two modifications: allowing

for the inattentive subtype in DSM-IV to be sufficient for the diagnosis and allowing for the presence of comorbid psychiatric disorders, as long as the criteria for ADHD were present before the appearance of the comorbid disorder. This diagnostic strategy was chosen as a compromise between the fact that ICD-10 is the official diagnostic system in Norway and the need to have an assessment comparable with the DSM-IV criteria. Until May 2005, this diagnostic assessment was mandatory for adult patients in Norway who were to be considered for treatment with stimulant drugs. All patients were formally diagnosed with ADHD before inclusion, but subtype data were not systematically available at the time of the primary diagnosis. Thus, the ADHD clinical subtype was assessed using the Adult ADHD Self-Report Scale (ASRS) (52) with a cutoff of 17 or more on each subscale (10.3% were diagnosed as subthreshold). Severity of past and current ADHD symptoms in patients and control subjects was evaluated using the WURS (53) and the ASRS.

DNA Isolation

DNA samples were isolated either from saliva using the Oragene DNA Self-Collection Kit (DNA Genotek, Kanata, Ontario, Canada) or from blood by the salting-out procedure or using magnetic bead technology with the Chemagic Magnetic Separation Module I and the Chemagic DNA Kit (Chemagen, Baesweiler, Germany). DNA concentrations were determined using the PicoGreen dsDNA Quantitation Kit (Molecular Probes, Eugene, Oregon).

Genes and Single Nucleotide Polymorphisms

From the 27 differentially expressed genes previously described (42), we considered their involvement in brain functions, the degree of asymmetry in their expression (from 1.9- to 8-fold), as well as SNPlex (Applied Biosystems, Foster City, California) design constraints, and finally genotyped six of them: *LMO4*, *BAIAP2*, *DAPPER*, *NEUROD6*, *ATP2B3,* and *ID2* (Table S2 in Supplement 1). For single nucleotide polymorphism (SNP) selection, we used information on the Centre d'Etude du Polymorphisme Humain (CEPH) panel from the HapMap database (Release 20; http://www.hapmap.org). We evaluated, with the linkage disequilibrium (LD) select software (ldSelect, http://droog.gs.washington.edu/ldSelect.html), the LD pattern of each candidate gene plus 3- to 5-kilobase (kb) flanking sequences. Tagging single nucleotide polymorphisms (tagSNPs) were selected at an r^2 threshold of .85 from all SNPs with minor allele frequency (MAF) >.15 for genes with fewer than 15 tagSNPs (*DAPPER1, LMO4, NEUROD6*, *ATP2B3,* and *ID2*) and MAF >.25 for *BAIAP2*, which had more than 15 tagSNPs. Thirty-one tagSNPs were chosen with these criteria. An additional nonsynonymous SNP, rs17832998, located within exon 4 of *DAPPER1*, was included in the analysis. To detect population admixture, 48 unlinked anonymous SNPs located at least 100 kb distant from known genes were also genotyped (54).

Genotyping

Spanish and German Populations. We assessed the 32 selected SNPs with an automated assay design pipeline (http://ms.appliedbiosystems.com/snplex/snplexStart.jsp). A proper design could not be achieved for two SNPs (Table S2 in Supplement 1). All SNPs were genotyped using the SNPlex platform. Two HapMap samples (NA11992 and NA11993) were included in all assays and a concordance rate of 100% was obtained.

Norwegian Population. Genotyping was carried out by the multiplex MassARRAY *iPLEX* System (SEQUENOM, San Diego, California). A total of 12 *BAIAP2* SNPs passed the assay design requirements and 11 SNPs were successfully genotyped with a final genotyping call rate of 98.6%. Genotype concordance rate was 100% for internal control individuals (n = 253 genotypes) and duplicates (n = 88 calls).

Statistical Analyses

We performed a two-stage association study. We first carried out a case-control association analysis in a Spanish sample that included adult and child ADHD patients and control subjects. Genes showing positive signals were then tested for replication in two independent case-control samples from Germany and Norway.

The analysis of minimal statistical power was performed post hoc using the Genetic Power Calculator software (http://pngu.mgh.harvard.edu/~purcell/gpc) (55), assuming an odds ratio (OR) of 1.5, prevalence of .05, significance level of .05, and the lowest MAF of .16. We tested genetic stratification in the Spanish and German samples by analyzing the SNPs in Hardy-Weinberg equilibrium (HWE) (10) from the 48 anonymous SNPs set with two different approaches: 1) the F-statistics (Fst) coefficient calculated by the Weir and Cockerham approach with the FSTAT software (http://www2.unil.ch/popgen/softwares/fstat.htm); and 2) the method of Pritchard and Rosenberg (9). These data were not available for the Norwegian sample.

Single-Marker Analysis. The analysis of HWE ($p < .01$) and the comparison of genotype and allele frequencies were performed using the SNPassoc R package (http://www.cran.r-project.org/web/packages/SNPassoc) (56). Dominant and recessive models were considered for SNPs displaying nominal association when either genotypes under a codominant model or alleles were taken into account. Genotype frequencies of SNPs within chromosome X were examined in female subjects, whereas in the comparison of allele frequencies, both male and female subjects were analyzed. Bonferroni correction in the initial association study, considering 30 SNPs, two age groups, and the comparison of genotype and allele frequencies, corresponds to a significance threshold of $p < 4.2\text{e-}04$, whereas for the replication study, where 13 SNPs and genotype and allele frequencies were considered, significance was set at $p < .0019$.

Multiple-Marker Analysis. To minimize multiple testing and type I errors (α), we decided a priori to restrict the haplotype-based association study to genes nominally associated with ADHD in the single-marker analyses. The best two-marker haplotype from all possible combinations was identified. Likewise, additional markers (up to four) were added in a stepwise manner to the initial two-SNP haplotype and subsequently assigned specific estimated haplotypes to individuals with the PHASE software (http://www.stat.washington.edu/stephens/software.html) (57). Significance was estimated using 10,000 permutations with the UNPHASED software (http://www.mrc-bsu.cam.ac.uk/personal/frank) (58). Since the expectation-maximization algorithm does not accurately estimate low haplotype frequencies (59), haplotypes with frequencies <.1 were excluded. To avoid bias and ensure accurate haplotype estimations, we confirmed results with the PLINK program (http://pngu.mgh.harvard.edu/purcell/plink) (60). We also tested those allelic combinations showing positive association in the overall ADHD sample in the two diagnostic groups of combined and inattentive ADHD. The hyperactive-impulsive group was not considered due to its small sample size.

Table 1. Association Study in 270 Adult ADHD Patients (178 Combined ADHD, 80 Inattentive ADHD, and 10 Hyperactive-Impulsive ADHD Patients) and 270 Sex-Matched Unrelated Control Subjects from Spain and 639 Adult ADHD Patients (429 Combined ADHD, 162 Inattentive ADHD, and 48 Hyperactive-Impulsive ADHD Patients) and 612 Sex-Matched Unrelated Control Subjects from Germany

		Genotypes								Alleles	
		Genotypes				Genotypes 11 Versus 12 + 22		Genotypes 11 + 12 Versus 22		Allele 2 Versus Allele 1	
Gene	SNPs	11	12	22	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Spanish Population											
BAIAP2	rs8079781	177 (66.0)	83 (31.0)	8 (3.0)	.011	1.69 (1.20–2.44)	.0026	—	.49	1.49 (1.12–2.00)	.0061
		144 (53.3)	115 (42.6)	11 (4.1)							
	rs4969385	171 (63.4)	90 (33.3)	9 (3.3)	.020	1.64 (1.15–2.27)	.0053	—	.65	1.43 (1.07–1.89)	.014
		139 (51.5)	120 (44.4)	11 (4.1)							
German Population											
BAIAP2	rs8079626	311 (49.2)	250 (39.6)	71 (11.2)	.048	1.32 (1.05–1.67)	.015	—	.62	1.19 (1.01–1.41)	.039
		258 (42.3)	278 (45.6)	74 (12.1)							

ADHD, attention-deficit/hyperactivity disorder; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

Results

We considered tagSNPs in six functional candidate genes differentially expressed in the right and left human embryonic cortex (*BAIAP2*, *DAPPER1*, *LMO4*, *NEUROD6*, *ATP2B3,* and *ID2*) (42) in a Spanish sample of 587 ADHD cases (270 adults and 317 children) and 587 control subjects. Of the 32 SNPs initially selected, two were discarded because they did not pass through the SNPlex design pipeline. Thus, a total of 30 SNPs with an average genotype call rate of 99.5% (SD = .48) were finally used (Table S2 in Supplement 1). The minimal statistical power was 59.5% and 66.4% when adult or childhood samples were considered, respectively.

Once we excluded evidence for population stratification (Fst coefficient: Theta = .000, 95% confidence interval [CI] = .000–.001; Pritchard and Rosenberg: $p = .32$ for adults and $p = .57$ for children), we compared genotype and allele frequencies between adult or childhood Spanish ADHD patients and their sex-matched unrelated control subjects. The single-marker analysis identified two SNPs in *BAIAP2* displaying nominal association with ADHD in the adult dataset: rs8079781 ($p = .0026$; OR = 1.69 [1.20–2.44]) and rs4969385 ($p = .0053$; OR = 1.64 [1.15–2.27]; Table 1 and Table S3 in Supplement 1), differences that did not remain statistically significant after Bonferroni correction. No association, however, was observed in the childhood ADHD sample.

We further considered *BAIAP2* for a haplotype-based analysis only in the adult dataset. All the associations described below remained significant once adjusted for multiplicity. The study of the 13 *BAIAP2* SNPs revealed a four-marker haplotype (rs8079626/rs11657991/rs7503597/rs7210438) associated with adult ADHD (global *p* value = .0052; Figure 1, Table 2). The analysis of the contribution of individual haplotypes to the phenotype showed overrepresentation of the A-G-G-C allelic combination ($p = .0016$; OR = 1.64 [1.20–2.22]) and a trend toward underrepresentation of the A-C-G-C haplotype in the adult sample ($p = .014$; OR = 1.59 [1.09–2.32]; Table 3). We then considered the frequency of the A-G-G-C risk haplotype carriers and confirmed the association between *BAIAP2* and adult ADHD ($p = .0092$, OR = 1.60 [1.13–2.25]). Interestingly, these differences were specific to the combined ADHD subgroup (global *p* value = 6.6e-04; Table 2) with overrepresentation of the same risk haplotype ($p = 4.2e-04$, OR = 1.87 [1.33–2.64]; Table 3) and an increased frequency of carriers of this allelic combination in this clinical dataset ($p = .0035$; OR = 1.77 [1.21–2.60]). No evidence of association between *BAIAP2* and the inattentive clinical subset was observed.

Replication Studies

The 13 SNPs within the *BAIAP2* gene were selected for follow-up in replication adult cohorts from Germany and Norway. Taking the *BAIAP2* SNP with the lowest MAF (.23), the minimal statistical power was 92.7% and 82.0% in the German or Norwegian populations, respectively.

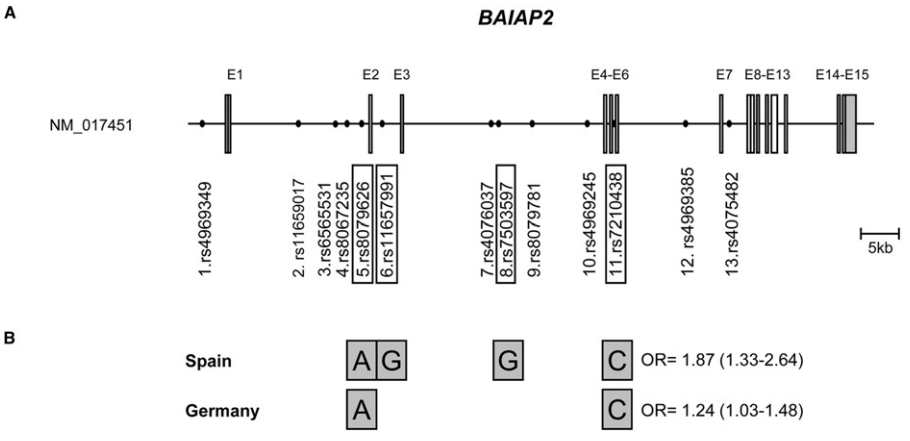

Figure 1. Haplotype analysis of the *BAIAP2* gene in adult ADHD. **(A)** Diagram of the *BAIAP2* gene with all the tagSNPs included in the present study. Boxes correspond to exons. Coding and noncoding exonic regions are indicated in white and gray, respectively. In bold and boxed, SNPs that conform the risk haplotype associated with adult ADHD in the Spanish dataset. **(B)** Allelic combinations associated with adult ADHD in the Spanish and German samples. ADHD, attention-deficit/hyperactivity disorder; SNP, single nucleotide polymorphism; tagSNPs, tagging single nucleotide polymorphisms.

Table 2. Haplotype Analysis of 13 *BAIAP2* SNPs in a Clinical Sample of 270 Adult ADHD Patients, 178 Combined ADHD Adult Subjects, and 270 Control Subjects from Spain Using the UNPHASED Software

	ADHD			Combined ADHD		
Marker[a] Haplotype	Global *p* Value	Best Haplotype *p* Value (Adjusted *p* Value)	Risk Haplotype-OR (95% CI)	Global *p* Value	Best Haplotype *p* Value (Adjusted *p* Value)	Risk Haplotype-OR (95% CI)
5 6	.0046	.0011 (.0051)	1.55 (1.19–2.01)	—	—	—
5 6 8	.0032	.0015 (.0096)	1.40 (1.09–1.79)	—	—	—
5 6 8 11	**.0052**	**.0016 (.0079)**	**1.64 (1.20–2.22)**	**6.6e-04**	**4.2e-04 (.0018)**	**1.87 (1.33–2.64)**

In bold the best allelic combination (highest OR); 5-rs8079626, 6-rs11657991, 8-rs7503597, and 11-rs7210438.
ADHD, attention-deficit/hyperactivity disorder; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

The single-marker analysis was first performed in the German sample. We found no evidence of population substructure (Fst coefficient: Theta = .000, 95% CI = .000–.001; Pritchard and Rosenberg: $p = .32$) and detected a nominal association between rs8079626 and ADHD (Table 1 and Table S4 in Supplement 1), one of the SNPs identified in the multiple-marker analysis of the Spanish sample. The multiple-marker approach showed evidence of association between adult ADHD and a two-marker haplotype (rs8079626/rs7210438) that contains two of the four SNPs present in the *BAIAP2* risk haplotype of the Spanish sample (global p value = .019; Figure 1, Table 4). Consistently with the results in the Spanish cohort, we observed an overrepresentation of the A-C allelic combination in the German ADHD sample ($p = .030$; OR = 1.21 [1.03–1.42]), whereas the G-C haplotype was downrepresented ($p = .0062$; OR = 1.28 [1.07–1.53]; Figure 1, Table 5). As in the Spanish series, once we subdivided patients according to the ADHD subtypes, the association between *BAIAP2* and adult ADHD remained significant only in the combined ADHD sample (global p value = .0031; Table 4).

No evidence of association between adult ADHD and the *BAIAP2* gene was detected in the Norwegian cohort in the analysis of single or multiple markers (Table S4 in Supplement 1).

Discussion

The purpose of this study was to analyze the involvement of brain asymmetry-related genes in the susceptibility to ADHD through a population-based association study. There are consistent data that support the existence of functional asymmetry in the brain (39,40,42). This segregation of human brain functions between hemispheres is associated with asymmetries in anatomical structures (42,61). In addition to environmental effects, growing evidence supports that genes play an essential role in the development of the human brain (39–41). Although little is known about genetic factors underlying brain lateralization, several genes are differentially expressed in the two hemispheres, some of which could be involved in the development of right-left asymmetries (42). As patients with ADHD show deviations from the typical pattern of cerebral asymmetry that may account for a large number of ADHD-related symptoms (12, 62,63), we suggest a relationship between genes differentially expressed in brain hemispheres and the vulnerability to this neurobehavioral disorder.

We performed the first comprehensive screen of common variants in six functional candidate genes showing at least 1.9-fold differential expression between hemispheres (42), conducted an initial case-control study in a Spanish sample, and provided preliminary evidence for the contribution of *BAIAP2* to adult ADHD. Subsequently, SNPs within this ADHD-associated gene were tested for replication in two additional independent adult samples from Germany and Norway. Despite the well-characterized and large case-control populations, the *BAIAP2* gene showed evidence for replication in the German but not in the Norwegian cohort. These results point to reconsider the robustness of this finding and raise several considerations:

1. Discrepancy could be attributed to clinical heterogeneity either within or across European populations. However, all patients fulfilled DSM-IV criteria for ADHD, and the Spanish and German ADHD samples were evaluated using a common set of diagnostic instruments.
2. Different frequencies of clinical subtypes or comorbid disorders that co-occur with ADHD could also explain the lack of association in one of the three populations considered (Table S5 in Supplement 1).
3. The study design ensured a high level of genetic coverage in *BAIAP2* (86.7%) and the replication cohorts were well-powered (>80%) to detect a nominal effect of the magni-

Table 3. Haplotype Distributions of rs8079626, rs11657991, rs7503597, and rs7210438 *BAIAP2* SNPs in 270 Adult ADHD Patients, 178 Combined Adult ADHD Subjects, and 270 Control Subjects from Spain

	ADHD			Combined ADHD		
Marker[a] Haplotype	Cases ($n = 270$)	Control Subjects ($n = 270$)	Haplotype-Specific *p* Value; OR (95% CI)	Cases ($n = 178$)	Control Subjects ($n = 270$)	Haplotype-Specific *p* Value; OR (95% CI)
5 6 8 11						
G G G C	99 (27.2)	124 (31.5)	—	64 (27.4)	124 (31.5)	—
A G G C	139 (38.2)	108 (27.4)	.0016; 1.64 (1.20–2.22)	97 (41.4)	108 (27.4)	4.2e-04; 1.87 (1.33–2.64)
A C G C	53 (14.6)	84 (21.3)	.014; 1.59 (1.09–2.32)[b]	28 (12.0)	84 (21.3)	.0017; 1.99 (1.25–3.17)[b]
A C T T	73 (20.0)	78 (19.8)	—	45 (19.2)	78 (19.8)	—
$\chi^2 = 12.8$; $df = 3$; $p = .0052$				$\chi^2 = 17.1$; $df = 3$; $p = 6.6e\text{-}04$		

ADHD, attention-deficit/hyperactivity disorder; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.
[a]5-rs8079626, 6-rs11657991, 8-rs7503597, and 11-rs7210438.
[b]When odds ratio < 1, the inverted score is shown.

Table 4. Haplotype Analysis of 13 *BAIAP2* SNPs in 639 Adult ADHD Patients, 429 Combined ADHD Adult Patients, and 612 Control Subjects from Germany Using the UNPHASED Software

Marker[b] Haplotype	ADHD			Combined ADHD		
	Global *p* Value	Best Haplotype *p* Value (Adjusted *p* Value)	Risk Haplotype-OR (95% CI)	Global *p* Value	Best Haplotype *p* Value (Adjusted *p* Value)	Risk Haplotype-OR (95% CI)
5 11	**.019**	**.0062 (.018)**	**1.21 (1.03–1.42)**	**.031**	**.014 (.038)**	**1.24 (1.03–1.48)**
4 5 11	.035	.0074 (.029)	1.21 (1.02–1.46)	—	—	—
4 5 8 11	.071	.033 (ns)	—	—	—	—

In bold the best allelic combination (highest OR); 4-rs8067235, 5-rs8079626, 8-rs7503597, and 11-rs7210438.
ADHD, attention-deficit/hyperactivity disorder; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

tude estimated in the Spanish adult ADHD population. However, population-specific effects such as different LD patterns cannot be discarded.

4. Furthermore, it is possible that the true effect is less than the point estimate (OR = 1.50, 95% CI = 1.12–2.0) in the initial finding due to the winner's curse effect (64). This could mean that the real risk in Northern European populations is closer to an OR of 1.2 (95% CI = 1.00–1.45), as estimated in the German sample, implying that larger samples are required to detect significant association.
5. The absence of association in one of the replication populations might also indicate that common *BAIAP2* SNPs of moderate effect may not play a major role in ADHD. Further genetic analyses in other large datasets are required to confirm these results and disclose the functional variants involved.
6. Finally, we have previously conducted a pooled genome-wide association analysis using an Affymetrix 500K SNP chip in 343 adult ADHD cases and 250 control subjects from Germany, >90% of which have also been examined in the present study (44). When reanalyzing these data, it became evident that two SNPs in *BAIAP2* were nominally significant: rs8080815 (p = .024) and rs8066330 (p = .002). These SNPs are located 950 and 1902 base pair (bp) proximal from and in strong LD (D′ = 1) with rs7210438, one of the variations contained in both the German and Spanish risk haplotypes. Furthermore, several SNPs in a putative isoform of *BAIAP2*, *BAIAP2L1* (*BAIAP2-like 1*), were also nominally significant: rs13232181 (p = .004), rs7812180 (p = .0006), and rs6465675 (p = .028).

BAIAP2 is expressed at higher levels in the left human cerebral cortex (42) and participates in neuronal proliferation, survival, and maturation (65–68). It encodes the insulin receptor tyrosine kinase substrate protein of 53 kDa (IRSp53) (69), a member of a group of downstream signaling molecules that participate in the signal transduction pathways of insulin and insulin-like growth factor 1 (IGF-1). Interestingly, IRSp53 selectively localizes at synapses (70) and although its specific function is unknown, it may participate in the insulin and/or IGF-1 dependent signaling pathways at the postsynaptic apparatus of excitatory synapses (71) and might be involved in insulin receptor-dependent learning and cognitive behavior in adult rats (72). Interestingly, several groups have suggested the involvement of abnormal cerebral glucose metabolism in ADHD, although results are controversial (73–75). *BAIAP2* expression in rat cerebral cortices is enhanced by treatment with methamphetamine, a drug that has successfully been used to treat ADHD (76). A potential role for the insulin receptor signaling pathway in the pathogenesis of ADHD is also suggested by our previously conducted genome-wide association study (GWAS), where not only *BAIAP2* and its isoform *BAIAP2L1* were nominally significant, but also *GRB10*, which encodes a protein known to bind to and regulate the insulin receptor (44).

The fact that the association between ADHD and *BAIAP2* was only observed in the adult group is in agreement with previous results and suggests a distinct genetic load between persistent and remitting ADHD (5,6,8,9). In this regard, alterations in this gene might contribute to the maintenance of ADHD symptoms in the subgroup of children in whom the disorder will persist throughout the life span. Alternatively, confounding factors, such as environmental influences, comorbidities, or IQ, could also contribute to these differences and should be considered in further analyses. Follow-up studies of child ADHD patients may allow us to discern between individuals with and without

Table 5. Haplotype Distributions of rs8079626 and rs7210438 *BAIAP2* SNPs in 639 Adult ADHD Patients, 429 Combined ADHD Adult Patients, and 612 Control Subjects from Germany

Marker[a] Haplotype	ADHD			Combined ADHD		
	Cases (n = 639)	Control Subjects (n = 612)	Haplotype-Specific *p* Value; OR (95% CI)	Cases (n = 429)	Control Subjects (n = 612)	Haplotype-Specific *p* Value; OR (95% CI)
5 11						
A C	590 (51.2)	537 (46.4)	.030; 1.21 (1.03–1.42)	406 (51.8)	537 (46.4)	.030; 1.24 (1.03–1.48)
G C	315 (27.4)	376 (32.6)	.0062; 1.28 (1.07–1.53)[b]	214 (27.3)	376 (32.6)	.014; 1.28 (1.05–1.57)[b]
A T	247 (21.4)	243 (21.0)	—	164 (20.9)	243 (21.0)	—
χ^2 = 7.9; df = 2; p = .019				χ^2 = 7.0; df = 2; p = .031		

ADHD, attention-deficit/hyperactivity disorder; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.
[a]5-rs8079626 and 11-rs7210438.
[b]When odds ratio < 1, the inverted score is shown.

symptomatic remission and their genotype data could be analyzed back to determine the genetic influence on the stability of ADHD symptoms.

Methodological Limitations

In the present study, we carried out a quite conservative two-stage statistical approach to address multiple testing. We first performed an association study considering single markers. However, as this strategy may neglect information about the joint contribution of different SNPs, for the single gene nominally associated with ADHD, we further moved on to a multiple-marker analysis. Although this approach allowed us to limit the number of tests, it may also increase the probability of false negative results for those genes in which the multiple-marker approach was not performed (77) and we do not know a priori whether individual SNPs and/or a combination of markers confer susceptibility to ADHD. However, we cannot overlook the fact that, although haplotype differences were significant after adjustment for multiplicity, the *BAIAP2* individual SNPs identified under the single-marker approach did not remain significant after the Bonferroni correction. As this multiple correction is often overconservative, particularly when the dependence between statistical tests is high (78), further studies are required to gain more insight into the involvement of individual *BAIAP2* SNPs, as well as specific haplotypes, in ADHD.

Because SNPs were selected to ensure genetic coverage according to LD criteria and all four *BAIAP2* SNPs that make up the ADHD risk haplotype are intronic and located far from any splice site or branch point, the *BAIAP2* sequence variants associated with ADHD may not have functional implications by themselves but rather be in LD with the causative variants. This idea is also supported by the fact that the Spanish and German risk haplotypes overlap (Figure 1). Further sequencing of *BAIAP2* may allow the identification of genetic variants directly involved in the predisposition to this complex phenotype.

Our findings provide tentative evidence for the contribution of *BAIAP2* to adult ADHD, support its participation in the persistence of the disorder, and suggest that genetic factors influencing abnormal cerebral lateralization may be involved in the predisposition to this neurodevelopmental disorder. To our knowledge, this is the first association study showing that a gene potentially involved in cerebral asymmetry may be considered a good candidate for ADHD. However, further investigation is required to replicate our results and to establish the participation of brain asymmetry-related genes in the adult outcome of the disorder.

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Supplementary material cited in this article is available online.

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