Contribution of LPHN3 to the genetic susceptibility to ADHD in adulthood: a replication study

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Attention-deficit/hyperactivity disorder (ADHD) is a common developmental disorder characterized by a persistent pattern of inattention and/or hyperactivity-impulsivity. Using families from a genetic isolate, the Paisa population from Colombia, and five independent datasets from four different populations (United States, Germany, Norway and Spain), a highly consistent association was recently reported between ADHD and the latrophilin 3 (LPHN3) gene, a brain-specific member of the LPHN subfamily of G-protein-coupled receptors that is expressed in ADHD-related regions, such as amygdala, caudate nucleus, cerebellum and cerebral cortex. To replicate the association between LPHN3 and ADHD in adults, we undertook a case–control association study in 334 adult patients with ADHD and 334 controls with 43 single nucleotide polymorphisms (SNPs) covering the LPHN3 gene. Single- and multiple-marker analyses showed additional evidence of association between LPHN3 and combined type ADHD in adulthood [P = 0.0019; df = 1; odds ratio (OR) = 1.82 (1.25–2.70) and P = 5.1e-05; df = 1; OR = 2.25 (1.52–3.34), respectively]. These results further support the LPHN3 contribution to combined type ADHD, and specifically to the persistent form of the disorder, and point at this new neuronal pathway as an essential susceptibility factor for ADHD throughout the lifespan.

Keywords: Adult ADHD, attention-deficit/hyperactivity disorder, case–control association study, LPHN3

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Attention-deficit/hyperactivity disorder (ADHD) is a common developmental disorder characterized by a persistent pattern of inattention and/or hyperactivity-impulsivity (Biederman & Faraone 2005; Swanson et al. 1998). Data from twin, family and adoption studies show that genetic factors play an essential role in the etiology of the disorder (Albayrak et al. 2008; Faraone et al. 2005; Maher et al. 1999). The worldwide pooled prevalence of ADHD is around 5.3% in school-aged children (Polanczyk et al. 2007). Attention-deficit/hyperactivity disorder can persist into adolescence and adulthood with an estimated prevalence rate of 4.4% (Kessler et al. 2006) and higher familial aggregation, suggesting that genes may play a stronger role in the etiology of persistent than in remitting ADHD (Faraone et al. 2000a).

Although dopaminergic and serotonergic candidate genes have been extensively examined in ADHD (Faraone et al. 2005; Mick & Faraone 2008; Oades 2008; Ribases et al. 2009b; Swanson et al. 2007; Thapar et al. 2005), Arcos-Burgos et al. recently reported a consistent association between ADHD and common genetic variants within the latrophilin 3 (LPHN3) gene, a brain-specific member of the LPHN subfamily of G-protein-coupled receptors that is expressed in ADHD-related regions, such as amygdala, caudate nucleus, cerebellum and cerebral cortex (Arcos-Burgos et al. in press; Krain & Castellanos 2006; Sugita et al. 1998). This association was first observed in a genetic isolate (Paisa population in Colombia) and was confirmed in a total sample comprising 2627 cases, 2531 controls and 1202 relatives from five different populations (Paisas, United States, Germany, Norway and Spain), supporting the implication of a new neuronal pathway in the predisposition to ADHD as well as in the response to treatment with stimulant medication (Arcos-Burgos et al. in press). Interestingly, although the specific role of LPHN3 in ADHD remains unknown, different lines of investigation suggest the involvement of this family of G-protein-linked receptors in synaptic neurotransmitter release as well as in neurodegeneration in response to ischemia and hypoxia (Bin Sun et al. 2002; Ichtchenko et al. 1998; Krasnoperov et al. 1997; Sugita et al. 1998). LPHN1 and LPHN2 mediate the Ca2+-independent neurotransmitter release from presynaptic nerve terminals induced by α-latrotoxin, a potent excitatory neurotoxin that stimulates synaptic vesicle exocytosis (Ichtchenko et al. 1998; Krasnoperov et al. 1997; Sugita et al. 1998). In addition,
the expression of LPHNs is highly regulated during postnatal brain development, with LPHN3 exhibiting its highest expression levels immediately after birth (Kreienkamp et al. 2000). Although follow-up studies have shown that ADHD symptoms persist into adulthood in most children and adolescents with ADHD (Biederman et al. 1996; Faraone et al. 2002; Kuntsi et al. 2005; Polanczyk & Rohde 2007), genetic studies have mainly focused on pediatric samples. Thus, little is known about common susceptibility factors involved in the etiology of ADHD addressing the diagnostic continuity of the disorder throughout the lifespan (Franke et al. 2010; Ribases et al. 2008, 2009b). In this regard, six of the seven independent ADHD samples studied by Arcos-Burgos et al. consisted primarily of children and adolescents, and only the Norwegian clinical sample consisted exclusively of adult patients with ADHD (Arcos-Burgos et al. in press). To replicate the strong association between LPHN3 and ADHD in an additional sample of adults with ADHD, we performed a case–control association study in 334 adults with ADHD and 334 controls with 43 single nucleotide polymorphisms (SNPs) covering, in terms of linkage disequilibrium (LD), the LPHN3 gene.

Materials and methods

Subjects

We recruited 334 adult patients with ADHD at the Department of Psychiatry of the Hospital Universitari Vall d’Hebron of Barcelona (Spain) between 2004 and 2008 (65.3% combined type, 31.1% inattentive type and 3.6% hyperactive-impulsive type). All subjects met Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria for ADHD and 71% were males (n = 237). Diagnosis was blind to genotype and was based on the Structured Clinical Interview for DSM-IV Axis I and II Disorders (SCID-I and SCID-II) and the Conners’ Adult ADHD Diagnostic Interview for DSM-IV (CAADID Part I and II). A more detailed description of the different diagnostic instruments used was published previously (Ribases et al. 2009b). Exclusion criteria were IQ < 70, pervasive developmental disorders, schizophrenia or other psychotic disorders, ADHD symptoms due to mood, anxiety, dissociative or personality disorders, adoption, sexual or physical abuse, birth weight < 1.5 kg and other neurological or systemic disorders that might explain ADHD symptoms. The control sample, consisting of 334 unrelated adult subjects from the same geographic area in whom DSM-IV ADHD symptoms were excluded, was matched for sex with the ADHD subjects from the same geographic area in whom DSM-IV ADHD symptoms. The control sample, consisting of 334 unrelated adult patients and 42.3 years (SD 14.2) for controls. The study was approved by the ethics committee of Hospital Universitari Vall d’Hebron and informed consent was obtained from all subjects.

DNA isolation and quantification

Genomic DNA was isolated either from peripheral blood lymphocytes by the salting-out procedure or using magnetic bead technology with the Chemagic DNA kit (Chemagen AG, Baesweiler, Germany) or from saliva using the Oragene™ DNA Self-Collection kit (DNA Genotek Inc, Ottawa, Ontario, Canada).

SNP selection, plex design, genotyping and quality control

Information on the Centre d’Etude du Polymorphisme Humain (CEPH) panel from the HapMap database (release 22, March 2007) was used for SNP selection. To minimize redundancy and to ensure full genetic coverage, we evaluated the LD pattern of the LPHN3 gene with LD-SELECT software version 1.0 (bioinfo.bsd.uchicago.edu/HapMap-LDSelect-Processor.html; Carlson et al. 2004). TagSNPs were selected at an $r^2$ threshold of 0.80 from all SNPs with minor allele frequency (MAF) > 0.2. Forty-six tagSNPs were chosen with these criteria (31 in multiloci bins and 15 singletons). Two additional SNPs with MAF < 0.2, rs1947275 and rs1397547, were also included in the analysis because they showed positive association with ADHD in a previous study by Arcos-Burgos et al. (in press). We evaluated the 48 selected SNPs with the automated assay design pipeline at ms.apppliedbiosystems.com/snpplex/snpplexStart.jsp. A proper design could not be achieved for one SNP, which translates into a design rate of 97.9% (Table S1). All SNPs were genotyped using the SNPlex™ platform (Applied Biosystems, Foster City, CA, USA). Two CEPH samples (NA10860 and NA10861) were included in all genotyping assays and a concordance rate of 100% with HapMap data was obtained (n = 90 genotypes).

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the 43 SNPs that were finally considered in the study is shown in Fig. 1. The minimal statistical power of the χ² test for the combined, inattentive and all ADHD patient subgroups was 15.2%, 10.9% and 18.1% considering a codominant model, 19.7%, 13.7% and 23.4% under a dominant model and 5.6%, 5.4% and 5.7% for the recessive model. Population stratification in this dataset was previously excluded by analyzing 48 unlinked anonymous SNPs located at least 100 kb distant from known genes (Ribases et al. 2008, 2009a,b).

The single-marker analysis identified one SNP in LPHN3 displaying nominal association with ADHD: rs2122643 [SNP20; P = 0.018; df = 1; OR = 1.45 (1.07–1.97); Table 1; Fig. 1]. Once we subdivided patients according to clinical subtype, three SNPs, rs2122643 (SNP20), rs1868790 (SNP21) and rs6858066 (SNP25), were associated with combined type ADHD and two SNPs, rs4860106 (SNP35) and rs13115125 (SNP37), with inattentive type ADHD. However, after correcting for multiple testing, only rs6858066 (SNP25) remained associated with combined ADHD [P = 0.0019; df = 1; OR = 1.82 (1.25–2.70); Table 1 and Fig. 1].

We then performed a haplotype-based analysis within the combined type ADHD subgroup and all the associations described below remained significant when adjusted for multiple comparisons by a permutation test. The study of the 43 LPHN3 SNPs showed a three-marker haplotype (rs1868790/rs6813183/rs12503398; SNP21/SNP28/SNP29) strongly associated with combined type ADHD (global P-value = 8.3e-04; df = 3; Table 2; Fig. 2). The analysis of the contribution of individual allelic combinations to this clinical subtype showed over-representation of the T-C-A haplotype [P = 7.5e-05; df = 1; OR = 2.06 (1.46–2.90)] and a trend toward under-representation of the A-G-G combination in this group of patients [P = 0.018; df = 1; OR = 1.38 (0.96–2.00); Table 2]. When we considered the frequency of individuals carrying the T-C-A risk haplotype, the association between LPHN3 and combined type ADHD in adults was confirmed [P = 5.1e-05; df = 1; OR = 2.25 (1.52–3.34); data not shown]. Although these differences were not detected in the inattentive type subgroup, significant association was also observed when all ADHD patients were considered (global P-value = 0.0028; df = 3; Table 2), with over-representation of the same T-C-A risk haplotype [P = 1.9e-04; df = 1; OR = 1.80 (1.31–2.48); Table 2] and an increased frequency of carriers of this allelic combination in this clinical dataset [P = 1.65e-04; df = 1; OR = 2.01 (1.40–2.88)]. The PAR, estimating the proportion of combined type ADHD in the present study that is attributable to the LPHN3 risk haplotype, was calculated as 12.35%.

Discussion

In this study, we replicated the recently described association between LPHN3 and ADHD, supporting the involvement of this gene in the susceptibility to this psychiatric disorder in adults. As the majority of ADHD samples investigated by Arcos-Burgos et al. were pediatric (82.9%) (Arcos-Burgos et al. in press), our data provide further evidence of common susceptibility factors for ADHD extending from childhood to adulthood (Faraone et al. 2000a,b; Kuntsi et al. 2005; Polanczyk & Rohde 2007; Ribases et al. 2008, 2009b; Spencer et al. 2007).

The original study describing the association between LPHN3 and ADHD identified a significantly associated area delimited by SNPs rs1901223 and rs1355368, located in the central part of the gene between introns 6 and 9. In this regard, the combined single- and multiple-marker family-based and case–control association studies performed with patients of the Paisa isolate showed association between ADHD and the LPHN3 SNPs rs1901223, rs1376307, rs6813183 and rs1355368. A meta-analysis of seven independent samples from five populations confirmed the initial association. Although the associated SNPs were not the same (rs6551665, rs1947274 and rs2345039), they are located within the same region of the gene and have varying degrees of LD with the original SNPs (Fig. 2). Within the Arcos-Burgos et al. replication samples, only the Norwegian population included adult ADHD patients and its separate analysis showed evidence for significant associations between ADHD and rs1868790 and rs10446786, both in LD with SNPs also showing association in the Paisa population (Fig. 2) (Arcos-Burgos et al. in press).

The three SNPs of the risk haplotype identified in our Spanish adult ADHD sample, rs1868790 (SNP21), rs6813183 (SNP28) and rs12503398 (SNP29), are in common or in LD with SNPs identified in the Paisas, the Norwegians and/or the worldwide sample meta-analysis (Fig. 2) (Arcos-Burgos et al. in press). Thus, two of them, rs1868790 (SNP21) and rs6813183 (SNP28), were also associated with ADHD in the Norwegian or the Paisa samples, respectively, whereas the third, rs12503398 (SNP29), was in strong LD with two SNPs identified in the Colombian isolate (D′ > 0.87; r² > 0.68; Fig. 2); also, SNP rs6813183 (SNP28) was in moderate LD with rs2345039 (D′ = 1; r² = 0.25), one of the SNPs pinpointed in the previous meta-analysis by Arcos-Burgos et al. Although we have genotyped the other two markers highlighted in this meta-analysis, rs6551665 and rs1947274, they were not included in our final analysis because of abnormal HWE distribution of rs6551665 that was tagging rs1947274. However, these two SNPs are in strong LD with rs6858066 (SNP25), which is found associated in the single-marker analysis performed in our Spanish adult ADHD dataset (D′ > 0.95 and r² > 0.74).

Whether the different ADHD clinical subtypes share genetic risk factors remains unclear. The strong association between ADHD and LPHN3 detected in the present study was seen in the combined type (and to a lesser extent in the total ADHD sample) but not in the inattentive subgroup. Although the previous study by Arcos-Burgos et al. did not take into account diagnostic subgroups in the association of LPHN3 with ADHD, our results are in agreement with previous studies supporting the validity of the DSM-IV distinction between the combined type and predominantly inattentive type, suggesting that differential genetic factors may participate in distinct ADHD clinical subgroups (Larsson et al. 2006; Lowe et al. 2004; Rasmussen et al. 2004; Ribases et al. 2008, 2009a,b; Smoller et al. 2006; Sobanski et al. 2008; Todd et al. 2001, 2005; Woo & Rey 2005). Furthermore, the
Figure 1: LD plot showing $D'$ values of the 43 $LPHN3$ SNPs (from 5' to 3') in the control sample considered in the present study. Haploview v4.2 (http://www.broadinstitute.org/haploview) was implemented to determine the LD blocks within the gene using the confidence interval method. Asterisks and boxes indicate SNPs nominally associated with ADHD in the single- and multiple-marker analyses, respectively. Underlined, the SNP associated with ADHD in the single-marker analysis after correcting for multiple testing.
Table 1: Association study of 43 SNPs covering the LPHN3 gene in 334 adult patients with ADHD (218 with combined type ADHD, 104 with inattentive type ADHD and 12 with the hyperactive-impulsive type) and 334 sex-matched unrelated controls.

<table>
<thead>
<tr>
<th>SNP*</th>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases n (%)</td>
<td>Controls n (%)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>All ADHD</td>
<td>1165</td>
<td>143</td>
</tr>
<tr>
<td>Combined ADHD</td>
<td>70</td>
<td>101</td>
</tr>
<tr>
<td>rs2122643 (20)</td>
<td>(49.5)</td>
<td>(42.9)</td>
</tr>
<tr>
<td>Inattentive ADHD</td>
<td>75</td>
<td>96</td>
</tr>
<tr>
<td>rs4860106 (35)</td>
<td>(34.6)</td>
<td>(44.2)</td>
</tr>
<tr>
<td>rs3115125 (37)</td>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>

*SNP number according to Fig. 1 is shown in brackets.
†When odds ratio <1, the inverted score is shown.
‡Correction for multiple testing on the basis of the SpD of matrices of pairwise LD between SNPs of the LPHN3 gene (P < 0.002).
Table 2: Haplotype analysis of 43 LPHN3 SNPs in a clinical sample of 334 adult patients with ADHD and 334 controls using the UNPHASED software version 3.0.13; haplotype distributions in the two-marker analysis (rs1868790 and rs12503398); three-marker analysis (rs1868790, rs6813183 and rs12503398) and four-marker analysis (rs1868790, rs6813183, rs12503398 and rs734644).

<table>
<thead>
<tr>
<th>Marker haplotype</th>
<th>Combined ADHD</th>
<th>Inattentive ADHD</th>
<th>All ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Global P-value</td>
<td>Best haplotype P-value (adjusted P-value)</td>
<td>Risk haplotype</td>
</tr>
<tr>
<td>21 29</td>
<td>0.0038</td>
<td>5.1e-04 (0.0036)</td>
<td>1.86 (1.36–2.55)</td>
</tr>
<tr>
<td>21 28 29</td>
<td>8.3e-04</td>
<td>7.5e-05 (0.001)</td>
<td>2.06 (1.46–2.90)</td>
</tr>
<tr>
<td>21 28 29 31</td>
<td>7.6e-04</td>
<td>6.2e-05 (0.004)</td>
<td>2.00 (1.41–2.84)</td>
</tr>
</tbody>
</table>

In bold, the best allelic combination (highest OR).

*21-rs1868790; 28-rs6813183; 29-rs12503398; 31-rs734644.

†Under-represented in ADHD cases.
linkage of the LPHN3 locus with conduct disorder, nicotine dependence and alcohol abuse/dependence (Jain et al. 2007) is consistent with a differential association between LPHN3 and ‘antisocial ADHD’ (Christiansen et al. 2008; Faraone et al. 1998, 2000c). Alternatively, it is also possible that the limited sample size of the inattentive clinical sample may account for the absence of association observed when this ADHD group was considered and, thus, further studies in larger samples are required.

In conclusion, our findings replicate the association between LPHN3 and ADHD described by Arcos-Burgos et al. (in press) specifically with the persistent form of the disorder, and further highlight a new neuronal pathway as a common susceptibility factor for ADHD in childhood and adulthood. As all the associated variants identified are intronic, further functional studies and possibly deep sequencing of LPHN3 will be required to identify causal variants that will allow delineating the pathogenesis of this complex phenotype.

References


Arcos-Burgos, M., Jain, M., Acosta, M.T. et al. (in press) A common variant of the latrophilin 3 gene, LPHN3, confers susceptibility to...


Association of the \textit{LPHN3} gene with adult ADHD


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Supporting Information

Additional Supporting Information may be found in the online version of this article:

\textbf{Table S1:} Description of the 48 SNPs in the \textit{LPHN3} gene (NT_022778.15) initially selected for the SNPlex analysis.

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