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From the Black Widow Spider to Human Behavior: Latrophilins, a Relatively Unknown Class of G Protein-Coupled Receptors, Are Implicated in Psychiatric Disorders

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Abstract

The findings of a recent study associate LPHN3, a member of the latrophilin family, with an increased risk of developing attention deficit/hyperactivity disorder (ADHD), the most common psychiatric disorder in childhood and adolescence. Latrophilins comprise a new family of G protein-coupled receptors of unknown native physiological function that mediate the neurotoxic effects of α -latrotoxin, a potent toxin found in black widow spider venom. This receptor–toxin interaction has helped to elucidate the mechanistic aspects of neurotransmitter and hormone release in vertebrates. Such unprecedented discovery points to a new direction in the assessment of ADHD and suggest that further study of this receptor family may provide novel insights into the etiology and treatment of ADHD and other related psychiatric conditions.

Keywords

ADHD; LPHN3; latrophilin; G protein-coupled receptor; α -latrotoxin

INTRODUCTION

Living cells are capable of detecting external stimuli by means of receptor molecules present in the cytoplasm, the nucleus, or embedded in the plasma membrane. The nature of these stimuli may be physical (e.g., variation in potential difference across the membrane or its mechanical deformation), but in most cases chemical ligands signal a change in the external environment. Chemical ligands range broadly from complex macromolecules to small solutes, and it is the fine-tuning of their interaction with highly specific receptors that ultimately determines cell function and fate.

There are several classes of receptors: the predominant and best characterized are the histidine kinase sensors (HK) in prokaryote cells [Szurmant et al., 2007; Yamada and Shiro, 2008], the receptor tyrosine kinases (RTK) in metazoans [Dengjel et al., 2009; Hausott et al., 2009] and the G-protein coupled receptors (GPCR) found in all eukaryotes [Pierce et al.,

2002; Cabrera-Vera et al., 2003]. These receptor classes have different membrane topologies that determine the mechanism of signal transduction. GPCRs have been the subject of intense research over the years; in fact, more than one-third of all current pharmaceuticals are directed at them. Such is their importance that 2009 was named “The Year in G-Protein Coupled Receptor Research” at the Endocrine Society's Annual Meeting ENDO 2009 [Millar and Newton, 2010], with 850 reviews and more than 8,000 original studies published.

In the last 10 years, latrophilins (LPHNs), a relatively new family of GPCRs, have received a great deal of attention as a potential target for drug discovery. LPHNs were discovered as receptors for α -latrotoxin (α -LTX), a potent neurotoxin isolated from black widow spider venom [Davletov et al., 1996; Lelianova et al., 1997]; this receptor family has since undergone extensive investigation involving regulated exocytosis of neurotransmitters and hormones [Davletov et al., 1998; Rahman et al., 1999; Silva et al., 2009b]. More recently, while studying the genetics of attention deficit/hyperactivity disorder (ADHD) our lab discovered linkage and association to one gene of the LPHN family. Common *LPHN3* alleles at the marker and haplotype level were found to confer a major risk of susceptibility to developing ADHD in a genetic isolate from Colombia, South America, results that were successfully replicated in European and U.S. populations [Arcos-Burgos et al., 2010a].

In this review, we will update the current knowledge on LPHNs and discuss recent findings that for the first implicate this receptor class in the etiology of psychiatry disorders.

MOLECULAR STRUCTURE OF LATROPHILINS

All GPCRs are confined to the plasma membrane and share a common topology of seven transmembrane segments (7TM) [Gether et al., 2002; Pierce et al., 2002; Schoneberg et al., 2002]. Activation of G proteins upon ligand binding to the receptor transmits the signal to various intracellular effectors that eventually affect gene expression and/or metabolic reactions [Landry and Gies, 2002; Cabrera-Vera et al., 2003].

LPHNs represent a putative adhesion-class GPCR family [Fredriksson et al., 2003; Bjarnadottir et al., 2007] with large extracellular and intracellular domains [Sugita et al., 1998]. Long N-terminus GPCRs contain several cell adhesion modules (e.g., cadherin, IgG, laminin A, thrombospondin type 1, galactose lectin, EGF) and transmembrane segments resembling those of group B GPCRs [Hayflick, 2000; Stacey et al., 2000; Krasnoperov et al., 2002], which suggests that these receptors, now termed long N-terminus group B (LNB) GPCRs, are naturally occurring chimeras of cell adhesion molecules and signaling receptors capable of transducing cell–cell interactions into intracellular signals [Hamann et al., 1996; Stacey et al., 2002]. A sequence comparison analysis of LPHN extracellular region reveals some interesting features: a rhamnose-binding lectin domain (RBL); a region homologous to olfactomedins; a long region of homology shared with a protein family of unknown function, referred to as BAI1–3 (brain-specific angiogenesis inhibitors); and a short, cysteine-rich sequence (Fig. 1).

The RBL domain is a relatively rare domain first characterized from sea urchin egg lectins (SUEL). So far, mouse LPHN1 is the only latrophilin for which the tridimensional structure

of the RBL domain and its rhamnose-binding properties have been determined [Vakonakis et al., 2008]. However, the fact that rhamnose is found only rarely in animals [Tymiak et al., 1993] makes it questionable as an endogenous ligand for LPHNs. Sequence analysis of the RBL domains of human LPHN3 and mouse LPHN1 (amino acids 35–124) using CrustalW algorithms (<http://services.uniprot.org/>) reveals a high degree of homology with other lectin-like proteins and the conservation of eight cysteine residues (Fig. 2), which is predictive of carbohydrate-binding properties of this domain in LPHNs. At least in mouse LPHN1, all eight cysteine residues are necessary for proper domain folding [Vakonakis et al., 2008].

Olfactomedins comprise a diverse family of secreted glycoproteins of unknown function (e.g., noelin, tiarin, and gliomedin) that are implicated in the development of the nervous system [Moreno and Bronner-Fraser, 2001; Tsuda et al., 2002; Eshed et al., 2005]. Secondary structure predictions confirm a more general helical structure of the N-terminal region in the olfactomedin-like sequences [Green and Klein, 2002], whereas LPHNs, on the other hand, are mostly strand-like, which suggests that the olfactomedin domain in LPHNs is likely the only domain related to olfactomedins.

The brain-specific angiogenesis inhibitors constitute another family of adhesion-class GPCRs, with three members identified so far, BAI1, 2 and 3. These genes are strongly expressed in brain [Shiratsuchi et al., 1997]. BAI1, a p53-inducible gene, has been implicated in the regulation of vascularization of malignancies including colorectal cancer, pulmonary adenocarcinoma, gastric cancer, and glioblastomas [Fukushima et al., 1998; Hatanaka et al., 2000; Lee et al., 2001; Kang et al., 2006]. Interestingly, a recent study reported an association between BAIAP2 (BAI associated protein 2, a left–right brain asymmetry-related gene) and ADHD in Spanish and German populations [Ribases et al., 2009].

The cysteine-rich sequence in LPHNs has a high homology with the sequences found in several GPCRs [Sugita et al., 1998; Krasnoperov et al., 2002]. LNB GPCRs have previously been found to undergo autocleavage at this cysteine-rich proteolysis site (GPS) that spans approximately 60 amino acids upstream of the first transmembrane segment [Gray et al., 1996; Krasnoperov et al., 1997; Nechiporuk et al., 2001] (Fig. 1). LPHNs are cleaved in the endoplasmic reticulum into an N-terminal fragment (p120) and a C-terminal fragment (p85), and this cleavage is necessary for efficient delivery to the plasma membrane and proper receptor function [Krasnoperov et al., 2002; Volynski et al., 2004; Krasnoperov et al., 2009; Silva et al., 2009a]. It was initially proposed that the GPS-dependent autoproteolysis of LPHNs was sufficient for receptor delivery to the cell surface, but recent evidence demonstrates that this sequence is also closely related to trafficking, possibly involving the interaction with auxiliary proteins along the secretory pathway [Deyev and Petrenko, 2010]. This proteolytic processing may be regarded as a mechanism of molecular compartmentalization of cell adhesion and G-protein activation functions.

The C-terminal cytoplasmic region of LPHNs does not show any particular domain structure, except that it contains multiple potential sites for palmitoylation and phosphorylation presumably implicated in the modulation of receptor activity, and several PEST sequences (rich in proline, glutamic acid, serine, and threonine) [Matsushita et al.,

1999]. PEST sequences confer susceptibility to quick degradation by proteolysis [Rogers et al., 1986; Roth et al., 1998; Sekhar and Freeman, 1998], so those proteins containing these motifs may be considerably short lived. There are three putative PEST sequences in LPHN1, two in LPHN2 and one in LPHN3 from rat or bovine origin, which suggests that LPHN1 may be more susceptible to rapid degradation compared to LPHN2 and LPHN3.

LATROPHILINS AS α -LTX RECEPTORS

Black widow spider venom contains at least 86 toxins [Duan et al., 2006], but α -LTX is the only one of these specifically affecting vertebrates [Rosenthal and Meldolesi, 1989]. This toxin is capable of inducing the secretion of neurotransmitters and hormones in its target cells by stimulating exocytosis [Rosenthal et al., 1990]. α -LTX has been found to stimulate exocytosis largely by creating ion channels and triggering a massive influx of Ca^{2+} in the presynaptic membrane [Ceccarelli et al., 1979; Fesce et al., 1986]. Cation currents, however, do not explain all of the toxin's effects. In fact, α -LTX can stimulate small vesicle exocytosis in the absence of extracellular Ca^{2+} [Igarashi et al., 2000; Verhage et al., 2000; Deak et al., 2009]. Both Ca^{2+} -dependent and -independent mechanisms rely on the toxin binding to specific surface receptors [Hlubek et al., 2000; Van Renterghem et al., 2000; Volynski et al., 2000]. To date, three receptors have been identified: neurexin, protein tyrosine phosphatase σ , and LPHNs [Sudhof, 2001; Ushkaryov et al., 2008]. LPHNs were identified simultaneously by two independent groups in bovine and rat brain extracts [Davletov et al., 1996; Krasnoperov et al., 1996; Lelianaova et al., 1997], and it was the high affinity co-purification with α -LTX that gave the receptor its name. Since LPHN-mediated toxic effects do not require Ca^{2+} influx, LPHNs were also designated as Ca^{2+} -independent receptors of α -LTX (CIRL). The precise mechanism of LPHN-mediated exocytosis in response to α -LTX is not well understood, but apparently the activation of intracellular G-protein signaling can be expendable. Transfection experiments in PC12 and chromaffin cells revealed that LPHN1 lacking its cytoplasmic tail still functions as an excellent α -LTX receptor [Sugita et al., 1998; Hlubek et al., 2000]. Likewise, α -LTX is capable of triggering massive exocytosis of β -hexosaminidase granules in LPHN1-transfected mast cells in the presence of extracellular Ca^{2+} , but when the cells were deprived of the divalent cation release of the enzyme was dramatically reduced [Hiramatsu et al., 2010]. These results demonstrate that LPHN1-dependent signal transduction is not required in the presence of extracellular Ca^{2+} and suggest that in normal physiological conditions LPHNs may exert their major effects by simply recruiting α -LTX for membrane insertion near presynaptic active zones, without actually transducing any intracellular signals.

LATROPHILIN-MEDIATED INTRACELLULAR SIGNALING

The effects of α -LTX have been studied thoroughly in endocrine cells and the presynaptic apparatus of neurons. As discussed above, this toxin is capable of inducing receptor-mediated vesicle exocytosis through both Ca^{2+} -dependent and -independent pathways. When LPHNs were identified as α -LTX receptors and further characterized as GPCRs, the moderate toxin's effects independent of extracellular Ca^{2+} made sense immediately. At that time, receptor-mediated activation of G proteins was a well-known mechanism implicated in

regulated exocytosis in secretory cells, so LPHN-mediated signaling might explain extracellular Ca^{2+} expendability.

Neurons and endocrine cells are very similar with respect to the composition of their characteristic small vesicles and dense-core granules, besides having a variety of molecular, biochemical, and functional similarities. Such similarities in the molecular machinery for secretion suggest that processes responsible for secretion of neurotransmitters and hormones are likely to have evolved from the trafficking machinery that controls secretion in more simple systems such as yeast cells [Bennett and Scheller, 1993]. The fact that small G proteins are highly enriched at synapses is more than a mere coincidence [de Maturana and Sanchez-Pernaute, 2010]. Lelianova et al. [1997] found that LPHN1 co-purified with $\text{G}\alpha_o$ in rat and bovine brain extracts, and that this interaction was involved in the induction by α -LTX of inositol-3-phosphate and cyclic AMP in transfected COS-7 cells [Lelianova et al., 1997]. Shortly after, another group confirmed these results with the new finding that $\text{G}\alpha_{q/11}$ was also linked to LPHN signaling [Rahman et al., 1999]. Coupling to $\text{G}\alpha_{q/11}$ was able to activate phospholipase C followed by mobilization of intracellular Ca^{2+} stores and, eventually, norepinephrine small vesicle exocytosis. Moreover, activation of protein kinase C by LPHN-mediated diacylglycerol release was able to increase the Ca^{2+} sensitivity of the molecular machinery of vesicle fusion to accelerate insulin secretion in pancreatic β cells [Hu et al., 2006]. Another study performed in *C. elegans* showed that UNC-13, a major presynaptic diacylglycerol receptor essential for vesicle-mediated neurotransmitter release in mammals as well, is involved in LPHN-dependent regulation of exocytosis [Brose et al., 2000; Willson et al., 2004]. As aforementioned, LPHN1-expressing mast cells are also capable of responding, although moderately, to stimulation by α -LTX in the absence of extracellular Ca^{2+} [Hiramatsu et al., 2010], mechanism that is possibly mediated by phosphorylation of the soluble *N*-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins SNAP-23, syntaxin-4, and VAMP-8. β -hexosaminidase release was decreased upon inhibition of protein kinase C and phospholipase C signaling.

LPHN C-terminal region has also been found to interact with synaptic multidomain scaffolding proteins of the ProSAP/SSTRIP/Shank family [Kreienkamp et al., 2000, 2002]. These proteins play an important role in regulating the architecture of the cytoskeleton at synapses; thus, their interaction with LPHNs might control the vesicular fusion/docking process by affecting the architecture of the plasma membrane at the active zones. More recently, Popova et al. [2007] demonstrated that LPHN1 interacts with TRIP8b, a Rab8b-interacting adapter protein involved in regulated exocytosis. Since Rab8 has been shown to localize at or near recycling endosomes [Hattula et al., 2006; Roland et al., 2007], it is possible that TRIP8b is involved in regulating LPHN concentration on the cell surface.

EXPRESSION OF LATROPHILINS

LPHN expression studies have been performed mainly in rats, probably because of the feasibility of laboratory breeding and the bigger brain size compared to mice. Three highly homologous LPHNs, LPHN1–3, have been identified so far. LPHN1 is found predominantly in the brain, but low levels of mRNA are also found in most tissues [Sugita et al., 1998], which is suggestive of a widespread function not directly relevant to neuronal activity.

LPHN2 is expressed primarily outside the brain (especially in liver and lung); although LPHN2 mRNA is detected in brain at low levels, the protein could not be detected by Western blot. LPHN3 expression is detectable mainly in brain [Sugita et al., 1998; Ichtchenko et al., 1999]. Matsushita et al. [1999] found by Northern blotting of RNA isolated from rat tissues that LPHN1 and 3 are almost exclusively brain-specific, although LPHN3 is much less abundant. LPHN1 mRNA was also found in very low amounts in the kidneys, lungs, and spleen.

Human studies reveal somewhat different findings from those described above. In a recent study, the GPCR expression pattern of human adult and fetal adrenal glands was compared using microarray analysis and confirmed by real-time PCR. The results showed that both LPHN2 and LPHN3 are highly expressed in this gland, but mRNA levels are significantly higher in fetal versus adult tissue [Xing et al., 2009]. Recently, we analyzed LPHN3 distribution in postmortem human brain tissue by Northern blot and revealed high-mRNA expression in amygdala, caudate nucleus, cerebellum, and prefrontal cortex. Lower expression was detected in corpus callosum, hippocampus, whole brain extract, occipital pole, frontal lobe, temporal lobe, and putamen. Expression was corroborated at the protein level using an anti-LPHN3 antibody. No expression was detected in thalamus, medulla, or spinal cord [Arcos-Burgos et al., 2010a].

LPHNs contain multiple splicing sites that result in different isoforms, with variations in both the extracellular and the intracellular domains. The positions of some of these sites seem to be conserved among LPHNs. A more detailed description of the splicing alternatives has been given [Matsushita et al., 1999]. Interestingly, the same LPHN can have a different number of isoforms across species (not all of them have been experimentally verified), which suggests an intricate physiological role for this receptor family. It is worth noting that human isoform 3 and mouse isoform 6 of LPHN3 might exist in soluble form. The amino acid sequences of these two isoforms correspond to only the extracellular portion of the proteins with no predicted transmembrane segments whatsoever. This phenomenon has been observed and well characterized for many other membrane receptors [Junttila et al., 2000; Jones et al., 2001; Suzuki et al., 2004; Tabata and Khurana Hershey, 2007; Secher, 2010]. Soluble counterparts of surface receptors may act as decoys that compete with the membrane-bound form for its specific ligand. The fact that LPHN1 and 3 possess highly conserved extracellular domains advocates for a more specialized function and may be indicative of selective pressure to maintain their ligand-binding specificity.

GENE-DRUG INTERACTIONS

Several studies focusing on a variety of genes have found interactions between LPHNs and several well-known drugs and chemicals (Table I). Most of these compounds mainly have an effect on LPHN mRNA expression, but for some others like 5-fluorouracil and methotrexate, which are cytostatics used in cancer treatment, LPHN proteins are capable of modifying the drugs' pharmacodynamic properties. Further interrogation of the brain-specific LPHN1 and LPHN3 might reveal important information about the pharmacodynamics of psychotropic drugs, with possible implications for the treatment of psychiatric disorders.

LATROPHILINS IN NON-NEURONAL PROCESSES

Despite the increased attention LPHNs have received in the last few years, the number of research studies is still limited. Most of the work has tried to decipher the molecular mechanisms of presynaptic exocytosis in vertebrates, and has mainly implicated the brain-specific sisters LPHN1 and LPHN3. For the more ubiquitously expressed LPHN2 we were able to identify two publications that address non-neuronal functions of LPHNs. One of the studies found that LPHN2 is present in the primitive streak and atrioventricular canal at the time of epithelial–mesenchymal transition (EMT) in the embryonic chicken heart [Doyle et al., 2006]. The loss of LPHN2 message led to inhibition of the EMT by altering gene expression, which suggests a role for LPHN2 in facilitating Ca^{2+} signaling within the cell, as its closely related partner, LPHN1, has been shown to do. In the other study, LPHN2 was implicated in the development of breast cancer in humans. The authors found that aberrant expression of LPHN2 (then named LPHH1) due to loss of heterozygosity in chromosome 1p31.1 participates in maintaining a malignant phenotype [White et al., 1998].

A very recent study has implicated LPHN1 in *C. elegans* embryogenesis [Langenhan et al., 2009]. This study provides strong evidence that LPHN1 plays an essential role in the alignment of cell division planes during the early embryonic stages. The authors succeeded in demonstrating that LPHN1 affects the anterior–posterior tissue polarity by interacting with a *wnt* spindle orientation pathway in the *C. elegans* embryo. Specifically, the RBL domain of LPHN1 was found to be indispensable to this interaction. LPHN1 expression was detected in the blastomeres in *C. elegans* early embryo and later in diverse tissues in the larval and adult stages, including the nervous system. The direct relevance of these results to vertebrate embryogenesis is remarkable, and it appears to support additional findings relating LPHNs to neuronal function in mammals.

LATROPHILINS IN NEURODEVELOPMENTAL PROCESSES

The brain-specific confinement of LPHN1 and LPHN3 and their function as GPCRs suggest possible roles in neuronal transmission and the regulation of neuronal viability. A previous study performed in rats showed that the differential expression of LPHNs in the brain affects susceptibility to ischemia-induced neuronal death. LPHN mRNAs were upregulated in CA1 neurons upon ischemic insult, whereas they were downregulated in CA3 neurons. Antisense oligonucleotides to LPHN1 and 3 mRNAs suppressed neuronal death associated with hypoxia in hippocampal and cortical cell cultures, suggesting a functional importance of these proteins in neurodegeneration [Bin Sun et al., 2002].

A striking behavioral finding was that mice lacking LPHN1 attend poorly to their offspring, which resulted in increased neonatal mortality [Tobaben et al., 2002]. In this study, homozygous mutant mice were indistinguishable in appearance from the wild type, and were fertile and viable for over 1 year, but the female knockout mice were less able than control mice to attend to their litters. As a consequence, when mouse pups were cared for by LPHN1-lacking females, most pups died within a week independently of the genotype. These data suggest that the LPHN1 deficiency does not significantly affect mouse brain

function, but results in some behavioral effects. Additional behavioral analyses will be required to determine the extent of these behavioral changes.

LATROPHILINS IN PSYCHIATRIC CONDITIONS

For the first time, we found an association between LPHNs and ADHD [Arcos-Burgos et al., 2010a]. ADHD is the most common behavioral disorder of childhood affecting 8–12% of children worldwide [Biederman and Faraone, 2005]. In our study we found a significant allelic association to ADHD of several markers located inside the *LPHN3* gene in chromosome 4q13.2. Iterative and expanded analyses of multiple generations of ADHD families provided a consistent and reliable presence of genetic association and linkage at the marker and haplotype level of *LPHN3* variants, a finding that was strongly replicated in American, German, Spaniard, and Norwegian samples. Pooling of all samples narrowed the area of association to critical extracellular and transmembrane domains, and a region responsible for LPHN3 mRNA splicing. We also found that the dosage of the *LPHN3* susceptibility haplotype varied inversely with the ratio of *N*-acetylaspartate/creatine (NAA/Cr), a measure of neuronal number known to be abnormal in ADHD.

The spatial and temporal expression of LPHN3 supports its role in the pathogenesis of ADHD. As mentioned above, LPHN3 is expressed in regions of the brain most affected in ADHD, that is, the amygdala, caudate nucleus, pontine nucleus, putamen, hippocampus, cerebral cortex, and cerebellar Purkinje cells [Krain and Castellanos, 2006; Arcos-Burgos et al., 2010a]. Most of these brain structures participate in three of the four main dopaminergic systems in the brain: the nigrostriatal, the mesolimbic, and the mesocortical systems. Also, LPHN3 appears to be expressed with increased intensity in more brain regions at earlier ages. For example, the 2-year-old individual we studied [Arcos-Burgos et al., 2010a] showed high levels of LPHN3 in the indusium griseum, a cerebral structure primarily involved in the embryological development of the limbic system. In addition, in situ hybridization experiments on rats have shown that the highest level of LPHN3 expression occurs in the forebrain immediately after birth and decreases during adult life [Ichtchenko et al., 1999]. Taken together, these data suggest that LPHN3 is strongly implicated in brain development at a time when ADHD is considered to arise, and provide new insights into the neurobiology and pathophysiology of human behavior.

Functional studies revealed that *LPHN3* variants are expressed in key brain regions related to attention and activity. Further, these variants affect metabolism in neural circuits implicated in ADHD and are associated with response to stimulant medication. Preliminary studies suggest that incidence of ADHD in the general population would be reduced by about 9% if the effect of the *LPHN3* variant that confers susceptibility to ADHD was controlled [Arcos-Burgos et al., 2010a].

Individuals with ADHD are at increased risk for substance use disorder (SUD) and disruptive behaviors [Molina et al., 1999; Palacio et al., 2004; Biederman et al., 2006; Szobot et al., 2007; Bukstein, 2008]. Children diagnosed with ADHD monitored during the transition into adolescence usually exhibit higher rates of alcohol, tobacco, and psychoactive drug use than children without ADHD [Biederman et al., 1995; Molina and Pelham, 2003].

The lifetime risk for SUD is approximately 50% in subjects with childhood ADHD persisting into adulthood [Biederman et al., 1995]. Similarly, a high ADHD prevalence is found in samples of adolescents with SUD [DeMilio, 1989; Horner and Scheibe, 1997; Kuperman et al., 2001]. In view of these findings, we decided to further interrogate markers within *LPHN3* for ADHD comorbidities in several independent samples. In each sample, markers were associated with SUD and disruptive behavior disorders (Arcos-Burgos et al., 2010b).

These results, together with the previous findings implicating *LPHN3* in the etiology of ADHD, strongly supports a broader role of *LPHN3* in the etiology of psychiatric disorders.

CONCLUSIONS

There is sufficient accumulated data to confidently link LPHNs to brain physiology. Although the precise functions of these receptors are still unknown, the growing evidence implicating them in neurotransmitter exocytosis, their expression profile, and their unique structural properties strongly advocate for a role in interneuron communication. Our new findings relating LPHNs to psychiatric disorders will certainly provide new insights into their function in neuronal processes, especially during developmental stages.

LPHN3 seems to be a promising gene in the study of ADHD and other neurodevelopmental disorders. Comparative sequence analysis of this gene between ADHD affected and control individuals might reveal important functional variations responsible for the ADHD phenotype. Given the complexity of neuronal networks, the relevance of any mutations identified may not be immediately clear, especially because LPHN endogenous ligand(s) have not been discovered yet. So far, α -LTX and more recently the veterinary antihelminthic drug emodepside [Willson et al., 2004; Harder et al., 2005], are the only known ligands for LPHNs, but the fact that these two agents do not occur naturally in vertebrates renders these interactions physiologically irrelevant. Finding the *LPHN3* endogenous ligand(s) will provide new insights into the possible role of LPHNs in the dopaminergic systems and will help us to understand, at least in part, the molecular basis of ADHD.

These results altogether open a new window into the evaluation of molecular substrates of ADHD and the development of new drugs targeted at specific brain genes and developmental pathways implicated in the disorder.

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FIG. 1.

General structure of latrophilins. The long extracellular region (approx. 850 aa) comprises four domains and at least eight potential *N*-glycosylation sites. From N- to C-terminus, a SUEL LECTIN domain (rhamnose-binding lectin or RBL), a region homologous to olfactomedins and myocilin, a homology region (HR) with BAI1-3, a cysteine-rich GPCR proteolysis site (gps), and a C-terminal region (LPHN).

FIG. 2.
A: Sequence comparison of RBL domains from lectins of different species. The open boxes show conserved cysteine residues across species. Green boxes, α -helices; yellow boxes, β -sheets; red letters: residues involved in carbohydrate binding in mouse LPHN1 RBL domain. B: Comparative modeling of human LPHN3 RBL domain based on mouse LPHN1 pdb file, using the SWISS-MODEL software (Peitsch, 1995; Guex and Peitsch, 1997; Schwede et al., 2003; Kopp and Schwede, 2004, 2006; Arnold et al., 2006). Sequence alignment reveals an 80% of identity. s, β -strands; h, α -helices.

TABLE 1

Drugs and Chemicals Known to Interact With Latrophilin Genes

Gene	Drug	Organism	Interaction	Refs.
LPHN1	Chlorine	<i>R. norvegicus</i>	Chlorine results in decreased expression of LPHN1 mRNA	Crosby et al. [2008]
	Ozone	<i>R. norvegicus</i>	[Ozone co-treated with chlorine] results in increased expression of LPHN1 mRNA	Crosby et al. [2008]
	Dietary fats	<i>M. musculus</i>	Dietary fats results in increased expression of LPHN1 mRNA	de Wilde et al. [2008]
	Doxorubicin	<i>H. sapiens</i>	LPHN1 protein affects the chemical susceptibility to doxorubicin	Gyorffy et al. [2006]
	Ethinyl estradiol	<i>R. norvegicus</i>	Ethinyl estradiol results in decreased expression of LPHN1 mRNA	Heneweer et al. [2007]
	Ozone	<i>R. norvegicus</i>	[Ozone co-treated with chlorine] results in increased expression of LPHN1 mRNA	Crosby et al. [2008]
	Palm oil	<i>M. musculus</i>	Palm oil results in increased expression of LPHN1 mRNA	de Wilde et al. [2008]
	Paraquat	<i>R. norvegicus</i>	Paraquat affects the expression of LPHN1 mRNA	Tomita et al. [2004]
	Valproic acid	<i>H. sapiens</i>	Valproic acid results in increased expression of LPHN1 mRNA	Plant et al. [2009]
	Vinclozolin	<i>R. norvegicus</i>	Vinclozolin affects the expression of LPHN1 mRNA	Skinner et al. [2008]
LPHN2	Acetaminophen	<i>M. musculus</i>	Acetaminophen affects the expression of LPHN2 mRNA	Beyer et al. [2007]
	Bisphenol A	<i>R. norvegicus</i>	Bisphenol A results in increased expression of LPHN2 mRNA	Naciff et al. [2002]
	Dietary fats	<i>M. musculus</i>	Dietary fats results in increased expression of LPHN2 mRNA	de Wilde et al. [2008]
	Ethanol	<i>R. norvegicus</i>	Ethanol results in increased expression of LPHN2 mRNA	Deaciuc et al. [2004]
	Fluorouracil	<i>H. sapiens</i>	LPHN2 protein affects the chemical susceptibility to fluorouracil	Gyorffy et al. [2006]
	Methotrexate	<i>H. sapiens</i>	LPHN2 protein affects the chemical susceptibility to methotrexate	Gyorffy et al. [2006]
	Mustard gas	<i>R. norvegicus</i>	Mustard gas affects the expression of LPHN2 mRNA	Dillman et al. [2005]
	Palm oil	<i>M. musculus</i>	Palm oil results in increased expression of LPHN2 mRNA	de Wilde et al. [2008]
LPHN3	Ethinyl estradiol	<i>R. norvegicus</i>	Ethinyl estradiol results in increased expression of LPHN3 mRNA	Naciff et al. [2005]
	Methylselenic acid	<i>H. sapiens</i>	Methylselenic acid results in increased expression of LPHN3 mRNA	Zhang et al. [2005]