Review

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Latrophilins updated

Abstract: Latrophilins (LPHN) are part of a yet unexplored family of receptors comprising three isoforms, LPHN1-3, and belonging to a unique branch of G protein-coupled receptors (GPCR) named adhesion GPCR (aGPCR). LPHN are considered to be prototypical models for the study of aGPCR as they are one of the most evolutionary conserved members. Previously described as the target for a potent neurotoxin from the black widow spider venom, LPHN are now being studied under a whole new perspective. Indeed, recent advances have provided a better understanding of different aspects of this prototypical family of receptors: 1) elucidation of LPHN ectodomain organization by crystallography has unveiled a new functional domain with great repercussion on all the other members of the aGPCR family, 2) proteomic approaches have opened the gate to unsuspected functional characteristics of LPHN cellular role, and 3) genetic approaches have provided hints into the physiological functions of LPHN in specific systems and organisms. Moreover, genomic linkage studies screening human patients from diverse genetic backgrounds have involved LPHN gene defects in human disorders such as attention-deficit hyperactivity disorder and cancer. In this review, we will provide a historical perspective addressing experimental research on these receptors while highlighting the new advances and discoveries concerning LPHN functions. As GPCR still represent the most studied targets for the development of pharmacological approaches aiming at alleviating human disorders, the relevance of studying LPHN retains a high pertinence to better understand these receptors for the treatment of human diseases.

Keywords: attention-deficit hyperactivity disorder; G protein-coupled receptor; intercellular adhesion; latrotoxin; synapse formation.

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Introduction

Have you ever been bitten by a black widow spider? The ones that have experienced this unpleasant encounter display symptoms ranging from acute pain, paralysis, and even death (1). Black widow spider venom is meant to induce paralysis and death for many of the spider's prey, which are for the most part insects but can also affect crustacean and vertebrates (2, 3). Paralysis in vertebrates is caused by the action of one component of the black widow spider venom on the peripheral nervous system, a toxin named α-latrotoxin, which was purified as part of a complex mix of other proteinaceous entities constituting the harmful concoction (4). Experimental studies conducted with this compound proved to be very useful in elucidating neurotransmitter release mechanisms, as α-latrotoxin can be active on every type of vertebrate synapse, no matter which neurotransmitter is involved: acetylcholine, noradrenaline, dopamine, glutamate, and encephalin (5–10). Interestingly, the toxin's action can be separated into two modes: a) a Ca^{2+}-dependent and b) a Ca^{2+}-independent effect (11, 12). However, it is noteworthy that the two modes of action elicited by α-latrotoxin only coexist in interneuronal synapses as only one mode is present in the neuromuscular junction (Ca^{2+}-dependent mode only). The specific effect of α-latrotoxin toward neurotransmitter release suggested early on that specific receptors might be responsible for the toxin's action on neuronal membranes (13). Later on, high-affinity binding of the toxin was observed in a preparation of dog cerebral cortex synaptosomes, a finding that elicited a heightened interest toward the discovery of α-latrotoxin receptors (14). α-Latrotoxin modes of action suggested that the receptors would be a) highly expressed in nervous system (if not exclusively expressed in brain) and b) Ca^{2+} sensitive as to their binding for α-latrotoxin. The first receptor to be isolated and identified from bovine brain solubilizates using affinity chromatography to α-latrotoxin was

part of a new family of cell adhesion molecules named neurexins (NRXN) (15). These polymorphic proteins qualified as α-latrotoxin receptors as they were found enriched in brain tissues and dependent on Ca^{2+} for α-latrotoxin binding (16, 17). The endogenous role of NRXN would later be revealed as extremely important in events determining synaptic function (18, 19) and as will be discussed below. Although more protein fragments were also isolated in bovine brain solubilizates, a technical mishap delayed the identification of yet another α-latrotoxin receptor. The protein band corresponding to 120 kDa was disregarded because of its similarity to the size of immobilized α-latrotoxin that might elute from the column. Three independent groups were able to overcome this hurdle and published the identification of a neuronal GPCR-like molecule as a second receptor for α-latrotoxin. This receptor fulfilled the characteristics previously described for α-latrotoxin action because it could bind the toxin in the absence of Ca^{2+} with high affinity and possessed a high expression in the brain (20–22).

The objective of this review is not to summarize the extensive literature on α-latrotoxin actions but will focus on the GPCR-like molecule and the recent advances regarding molecular and genetic aspects linking this receptor to physiological and pathophysiological functions. We will refer to this receptor by the name latrophilin in order to keep a consistent nomenclature system throughout.

Nomenclature of latrophilins: past, present and future

Past protein nomenclature

At the time of its discovery as a toxin receptor, the research groups involved gave a different name to this seven-transmembrane receptor. *Latrophilin* (*LPHN*) was adopted by the group of Ushkaryov et al., whereas the acronym *CIRL*, standing for Calcium Independent Receptor for α-Latrotoxin, was put forward by the group of Petrenko et al. (20, 21). On the other hand, the group of Südhof et al. combined both CIRL and latrophilin to reach the abbreviation *CL* (22).

Past gene nomenclature

As genome sequencing became more successful, many names were given to *LPHN* genes. Indeed, during the same time period, a gene described as human LPHN1 gene and termed *lphh1* was found to be involved in breast cancer. It was later found that this gene encoded for the human ortholog of LPHN2 (23). Moreover, the sequencing of the human genome allowed the identification of three related genes named *lec1*, *lec2*, and *lec3*, as they encoded for lectomedins, which are proteins characterized by their lectin and olfactomedin domains (24). However, further sequence comparisons demonstrated that *lec1*, *lec 2*, and *lec 3* were the same as *lphn2*, *lphn1*, and *lphn3*, respectively. The sequencing of arthropod genome revealed the presence of only one LPHN gene ortholog named *dCIRL* (25). Finally, supporting the evolutionary conservation of LPHN genes, *lat-1* and *lat-2* were assigned to the *Caenorhabditis elegans* genome and found to encode proteins with 25–28% similarity to mammalian LPHN (26, 27).

Present and future nomenclature

In an effort to simplify the growing nomenclature of LPHN and other receptors of the adhesion-GPCR family, a naming system was tentatively discussed by the IUPHAR. This system uses a combination of four common letters (ADGR), ascribing membership to the adhesion receptor class, followed by the first letter of the most used name, and ending with the number given to the gene isoform. Therefore, because 'latrophilin' is the most widely used name, the new nomenclature assigns the term "ADGRL" to LPHN ranging from ADGRL1-3 corresponding to LPHN1-3, respectively (28).

Tissue distribution and developmental expression of latrophilins

Despite the high sequence homology between LPHN isoforms, each protein has a differential expression pattern. This difference in expression pattern is observed both spatially and developmentally.

Distribution of latrophilins in rodent tissues

Northern blotting experiments performed on rat tissues showed that in contrast to other brain-confined molecules, like NRXN for example, LPHN were not found exclusively in the brain but also in other non-neuronal tissues, although in variable amounts. In fact, the mRNA expression of LPHN1 and LPHN3 was predominantly observed in

brain, LPHN1 mRNA being the most abundant. A very low amount of LPHN3 mRNA was found in the heart, placenta, pancreas, kidneys, and testes, whereas LPHN1 mRNA was also found in the kidneys, lungs, and spleen (29–31). In contrast, LPHN2 mRNA revealed a weak presence in the brain but had a more ubiquitous distribution: its expression was observed mainly in the liver and lungs and also in the placenta, heart, kidneys, pancreas, spleen, and ovaries. At the protein level, LPHN2 was weakly detected in the brain, heart, lung, kidney, and spleen tissues, whereas LPHN1 protein could mostly be detected in the brain (29). Subsequent studies using rat and mouse tissues, and analyzing the expression profile of adhesion GPCR family members by quantitative real-time PCR, showed that mRNAs for LPHN1 and LPHN3 are primarily enriched in mouse brain and almost absent in other tissues, whereas LPHN2 was found in most mouse tissues (32), in agreement with previous studies. However, unlike results from previous studies using rat tissues, the levels of LPHN1 and LPHN3 mRNAs, in liver and lung, respectively, were comparable to the quantities detected in the brain (32).

Distribution of latrophilins in human tissues

Regarding human tissues, the expression pattern for LPHN2 mRNA was the same as the one observed in rodents, the mRNA being ubiquitously detected with little variation in levels between tissues tested. However, there were some differences in relation with LPHN1 and LPHN3 mRNA expression profiles. Similarly to their rodent orthologs, human LPHN1 and LPHN3 mRNA were found enriched in the brain (22). It is noteworthy that LPHN mRNA levels vary between different regions of the human brain. Northern blot analysis for LPHN3 and corroboration at the protein level using an anti-LPHN3 antibody indicated that high-mRNA expression was observed in amygdala, caudate nucleus, cerebellum, and prefrontal cortex. Lower levels of LPHN3 mRNA were also detected in corpus callosum, hippocampus, occipital pole, frontal lobe, temporal lobe, and putamen, whereas no expression was detected in the thalamus, medulla, or spinal cord (33). Besides, LPHN1 expression in the brain is cell type specific, strictly expressed in neurons but not glial cells (34, 35). However, human LPHN1 mRNA was found in significant quantities outside of the brain, a result that contrasts with its rodent ortholog (22). Recent human studies employing microarray analysis and confirmation by real-time PCR indicated that both LPHN2 and LPHN3 are highly expressed in adult and fetal adrenal glands (36). Therefore, these data suggest that LPHN, especially LPHN2, could have a widespread physiological function.

Developmental regulation of LPHN expression

As would be expected from molecules regulating tissue development, the expression of all three LPHN isoforms is highly controlled throughout tissue maturation. The expression pattern of LPHN2 and LPHN3, evaluated on human adrenal glands, revealed that both isoforms were highly expressed and that mRNA levels were significantly higher in fetal vs. adult tissue (36). Likewise, ulterior studies analyzing the expression levels of LPHN mRNA by *in situ* hybridization in rat and mouse brain during postnatal development showed that LPHN2 mRNA appeared very early, reaching its highest level a few days after birth and then decreasing to become hardly detectable in adult animals. In contrast, LPHN1 mRNA levels increased as the brain underwent maturation. As for LPHN3 mRNA brain expression, its peak was observed immediately after birth, followed by a decrease, similar to LPHN2 mRNA decay pattern. In contrast, real-time quantitative PCR done with mouse brain tissues demonstrated that the expression profile of LPHN3 mRNA mimicked LPHN1 pattern, while LPHN2 behaved differently: both LPHN1 and LPHN3 mRNA levels increased after birth reaching a plateau during the period describing intense synaptogenesis, while LPHN2 mRNA levels underwent a constant decrease (34, 37). As the function of LPHN seems tightly linked to the development of neuronal tissue, these data suggest that each LPHN isoform may contribute differently in shaping neuronal networks.

Latrophilins: synaptic localization

The effect of α-latrotoxin in inducing massive neurotransmitter release has been attributed to its interaction with synaptic targets. Latrotoxin-binding studies in primary cultures of cerebellar neurons revealed that these targets appear to be located both pre- and postsynaptically (38). The current view suggests that NRXN, the Ca^{2+}-dependent receptor for α-latrotoxin, is mostly presynaptic, but the consensus on LPHN localization has been hard to build. Their localization has been attributed both to pre- and postsynaptic compartments (39–41). The reason for this discrepancy is still unclear but might come from differences in the methods used. The evidence for presynaptic localization of LPHN comes from antibody staining studies analyzed by electron microscopy and cell fractionation data separating synaptosomes in different portions

(40). Additionally, LPHN and its endogenous ligand teneurin (TEN) are both found enriched in growth cones, a structure that is crucial for synaptogenesis events (42). This enrichment, which suggests at least a presynaptic localization, seems to describe a different role for these molecules than the one played in established networks because in this system, a *cis*-interaction mechanism of action would prevail over a *trans*-interaction mechanism. On the other hand, the evidence to support postsynaptic localization of LPHN comes from high-throughput proteome analysis of the postsynaptic density and from cell fractionation data (39, 41). Moreover, the strong binding of LPHN to proteins constituting molecular scaffolds of the postsynaptic density also reinforces their postsynaptic positioning (34, 41).

Latrophilins: structural domains and related functions

LPHN are characterized by the presence of a number of adhesion motifs, N-terminally located, and of a heptahelical structure, C-terminally located. These characteristics bring LPHN into an unexplored family of GPCR-like molecules named adhesion-GPCR (aGPCR). Studies on the structure of LPHN paved the way for understanding crucial aspects regarding aGPCR function. The first consideration is that the modular arrangement of LPHN adhesion domains suggests that protein-protein interaction can occur in a modular fashion. In other words, every adhesion motif may constitute an independent binding unit, which would be self-sufficient. The second consideration concerns the discovery of a new signature domain that solidifies and supports its belonging not only to the aGPCR family but also to an evolutionary related family of proteins called polycystic kidney disease proteins. These different aspects will be reviewed according to this point of view for each of the LPHN adhesion domains.

The LPHN domain structure is described in Figure 1 and comprises the following motifs/domain in the N-terminal adhesion patch: 1) the lectin-like domain immediately followed by, 2) the olfactomedin-like domain, 3) the serine/threonine-rich sequence, 4) the hormone-binding domain, and finally, 5) the signature GAIN domain encompassing the GPS site. The C-terminal domains comprise (1) the seven-transmembrane region interspaced and connected by intra as well as extracellular loops and (2) the cytoplasmic tail bearing a PDZ-binding motif.

The NTF

The lectin-like domain

This domain was first described structurally after crystallographic studies performed on the mouse and *C. elegans* rhamnose-binding lectin domain (RBL domain) (43). This domain initially identified in sea urchin eggs is relatively rare and do not possess any amino acid sequence similarity to known lectin classes. Classic RBL or lectin-like domain proteins function by binding rhamnose, a sugar that has no known biosynthetic pathway in animals and therefore is found only rarely (44). They exert their binding function by clustering and/or oligomerizing, possibly to increase their avidity to carbohydrates (45, 46). However, the LPHN lectin-like domain was suspected to mediate protein-protein interactions instead of having canonical sugar-binding activities. This conclusion was reached since the lectin-like domain had very low affinity for rhamnose. This suspicion turned out to be supported by interaction of the lectin-like domain with the newly identified TEN (37, 47). These ligands will be discussed in the “Latrophilin ligands and associated functions” section. This interaction of TEN with the isolated lectin-like domain was of lower affinity than with the full-length LPHN extracellular domain although still retaining a nanomolar-binding constant. This suggested that the lectin-like domain was not fully recapitulating the binding pocket, but that it was sufficient for LPHN/TEN interaction. In mouse neuronal cultures, this interaction regulates the maintenance or formation of both excitatory and inhibitory synapses (37). Evolutionary speaking, the lectin-like domain is present in all vertebrate and invertebrate LPHN, thus, seems to have a conserved function in these organisms (27). In parallel, TEN, which are known ligands for LPHN lectin-like domain, are also evolutionary conserved in vertebrates and invertebrates. More intriguing is the presence of both proteins in the same non-neuronal tissues. However, the function of lectin-like domain-dependent interaction described by LPHN/TEN complex in other tissues than the brain is not well substantiated at this time.

The extracellular alternatively splice insert A

Adhesion molecules are known to modulate their ligand-ligand interactions by the generation of multiple isoforms able to differentially direct homophilic or heterophilic interactions. All three *lphn* genes contain a mini-exon between the lectin and olfactomedin domains flanked by canonical

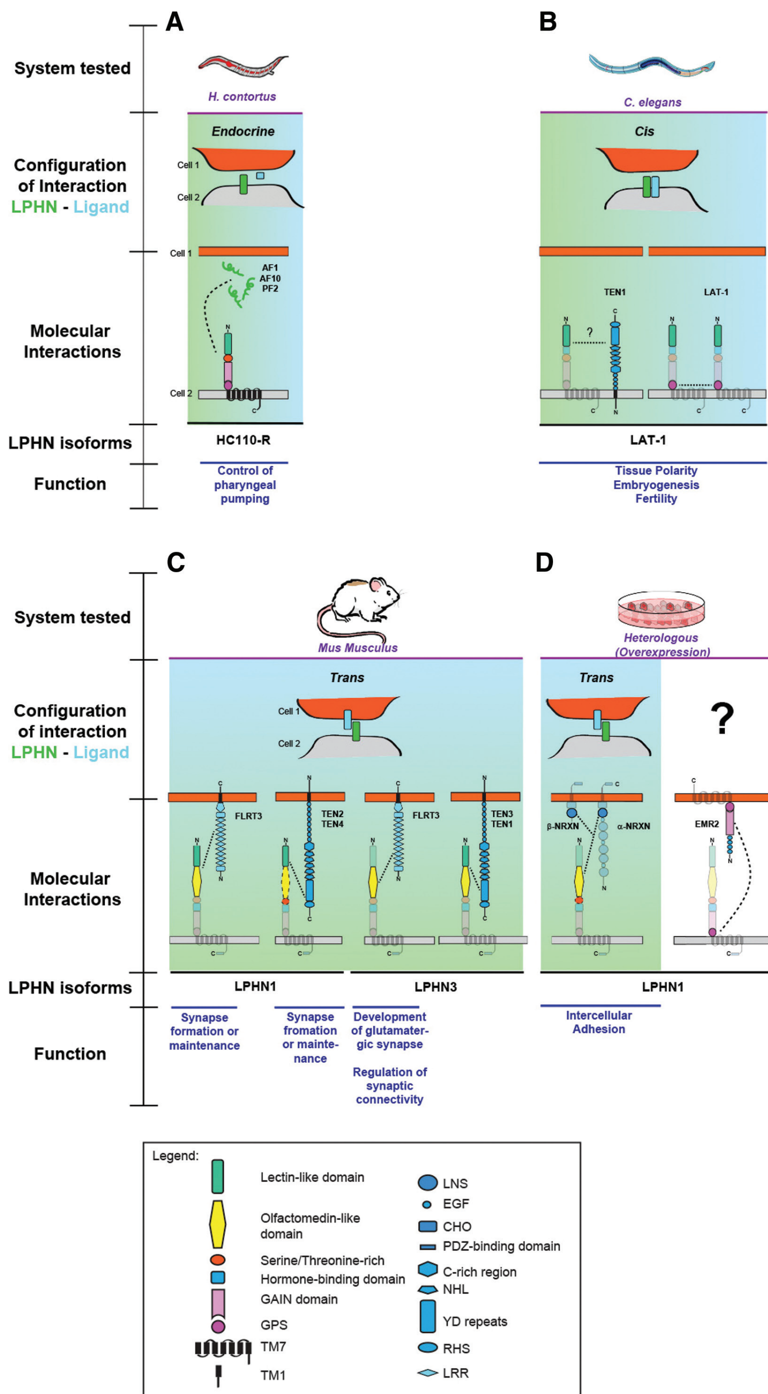

Figure 1 Latrophilins and their endogenous ligands.
Represented are molecular interactions involving LPHN and grouped first by systems studied, then binding configuration [Endocrine in (A), *cis* in (B), *trans* in (C) and (D), or unknown [?] in (D)], followed by proteins or molecules binding LPHN and finally known functions elicited through LPHN isoforms. Systems studied comprise *Haemonchus contortus*, *C. elegans*, *Mus musculus*, and heterologous cells. Ligands represented are neuropeptides AF1, AF10, PF2 in (A); *cis*-interacting adhesion molecules TEN1 (teneurin1) and LAT-1 in (B); *trans*-interacting adhesion molecules FLRT (fibronectin-like domain-containing leucine-rich transmembrane protein), TEN (teneurin), and NRXN (neurexin) in (C) and (D); as well as EMR2 (EGF-like module containing mucin-like hormone receptor-like 2) in (D). Molecular interactions are described for each LPHN domains for the given ligand when known: interacting domains are highlighted in bright color while the remaining of the protein is colored with a transparency level. A dashed line between extracellular domains involved indicates protein-protein interactions. Legends to molecule schematics are shown in detail below. Note that LPHN is represented at the cell-surface of ‘Cell 2’ for clarity purposes in all panels; in no way do we refer to cell specializations like synapses for instance.

donor/acceptor sites and, therefore, susceptible to alternative splicing. The same sequence is translated for *lphn1* and *lphn3* genes, whereas *lphn2* gene contains an additional splice insert sequence resulting in two differently translated inserts. Splicing will therefore generate different isoforms for LPHN with possibly different functions. Indeed, splice site A insert affects LPHN1 affinity for TEN but does not significantly change its affinity for either NRXN or FLRT3, two endogenous ligands of LPHN (37). This mechanism is susceptible to affect ligand selection in a neuronal network where LPHN establish a specific interaction pattern with its different ligands. For example, by changing its affinity for TEN, LPHN1 might then preferentially interact with FLRT3. Hence, alternative splicing in LPHN confers different ligand specificities. It is noteworthy that the splicing pattern of the ubiquitously expressed *lphn2* gene drastically differs from heart to brain, two tissues that account for most of its expression in mice: only one splice variant is exclusively present in the heart, while two are equally present in the brain (37). This suggests that alternative splicing of LPHN is a physiological process that is under stringent tissue-specific regulation, susceptible to generate various LPHN-ligand complexes.

The olfactomedin-like domain

The olfactomedin-like domain is of newer occurrence in the evolutionary three linking vertebrates LPHN. Its presence has been detected only in mammals and not in insects (27). This domain remains incompletely characterized, as it is the only adhesion motif of LPHN that has not yet been characterized structurally. Its sequence similarity to olfactomedin-1, olfactomedin-related and mucin proteins suggested, just like the lectin-like domain, that it may mediate protein-protein interactions. In fact, this domain was found to be involved in the binding of NRXN, a family of adhesion molecules influencing synapse formation and function, as will be discussed below. Intriguingly, this binding was only detected for LPHN1, as LPHN2 and LPHN3 did not bind NRXN (37, 47). Additionally, another class of LPHN endogenous ligands named FLRT3 (fibronectin-like domain containing leucine-rich transmembrane protein 3) was found to be interacting through the olfactomedin domain as well (37, 47). This domain seems to participate also in the formation of the binding pocket for TEN. Indeed, while the lectin domain is sufficient to bind TEN, only when it is coupled to the olfactomedin-like domain does it fully recapitulate the high-affinity interaction observed with the full-length LPHN extracellular domain. This denotes that it either provides some structural determinants to the binding pocket by stabilizing the lectin domain-dependent binding pocket or that it provides complementary direct binding determinants locking the ligand into place. In summary, the olfactomedin domain has a protein-binding activity distinct from the lectin domain and independent from other adhesion motifs of LPHN.

The serine-threonine rich region

The molecular structure as well as the function of this domain remains unknown to this day. Using bioinformatics analysis, it was suggested that the domain might contain O-glycosylation sites, which would act as a stack region between the olfactomedin domain and the hormone-binding domain. This assumption has recently been tested using electron microscopy analysis of a soluble protein containing LPHN3 extracellular domain (47). Although this precise domain could not be located reliably, mutational analysis suggested that O-glycosylation could be abrogated by deleting the Ser/Thr domain, according to subsequent mass spectrometry analysis. The role of this domain seems to be structurally important for separating two globular domains on LPHN3 extracellular region. It is the extracellular region that is the most unconserved among the LPHN (22).

The hormone-binding domain

This domain was named as such because of its similarity to a region of the corticotrophin-releasing factor receptor (CRF receptor), which possesses a hormone-binding activity (48). Its structure has been determined and contains a unique fold (49). It is tentatively proposed that this domain may be able to bind a polypeptide or small molecule in order to activate LPHN for transducing an intracellular cascade. However, the presumed hormone-binding site is blocked by the presence of the GAIN domain and would require a conformational change in order to engage in a mechanism of hormone activation involving the transmembrane helices. Whether this domain is involved in any molecular interactions remains to be determined.

The GAIN domain

It is probably the best-known domain as it represents a signature for the aGPCR family. First described as a Bai-homology domain, because of its resemblance to brain-angiogenesis inhibitor receptor then discovered around

the same time period, it was renamed after the structure was determined by the groups of Axel Brunger and Thomas Südhof using protein crystallography (22, 49). The authors used both LPHN1 and Bai3 extracellular domains to describe a completely new fold that was conserved among all members of the aGPCR family. Upon analysis of the crystallographic data, a break in the polypeptide chain was clearly identifiable in LPHN1 crystal structure, which led to the name of GPCR Autoproteolysis Inducing domain (GAIN domain). The complex fold was revealed for the first time and able to bring crucial information on the structural biology of aGPCR. Indeed, the GAIN domain, comprising 320 residues, can be divided in two subdomains, A and B, each forming a separate entity contributing to the overall folding of the domain. The N-terminally located subdomain A contains six α-helices, while subdomain B comprises 13 β-strands and two α-helices. Located at a sharply kinked loop between the last two β-strands of the GAIN domain is the presence of a scissile bond that composes the G-protein-coupled receptor proteolysis site (GPS). This site encompasses a region where LPHN and other aGPCR are found to be cleaved, therefore, generating a molecule containing a N-terminal fragment (NTF) and a C-terminal fragment (CTF).

LPHN cleavage: autoproteolysis hypothesis

The partition leading to the generation of NTF and CTF fragments of LPHN is suspected to be the consequence of autoproteolysis (49). Indeed, the relay of charges contributed by the subdomain A and B generates a stable intermediate leading to the cleavage of a conserved bond at the GPS site. This bond is made accessible by the creation of a kink, which is stabilized by the presence of conserved disulfide bonds. Moreover, the production of the cleavage reaction intermediates is favored by keeping the newly exposed N-terminal hydrophobic residue embedded in a network of hydrophobic charges provided by the rest of the GAIN domain. Another charge network between conserved residues at this site assures that the cleaved β-strand stays well in place. The same charges are also responsible for maintaining the rest of the GAIN domain in place so that it is still noncovalently attached to the CTF through hydrophobic interactions (49).

LPHN cleavage: enzymatic hypothesis

Although evidences from LPHN GAIN domain crystal structure support the catalysis of autoproteolytic cleavage, it is also possible that other types of activities might be involved in disconnecting the NTF from the CTF fragment. Indeed, there are few evidences that some enzymatic activity might intersect in the process of creating these fragments. Supposing that the cleavage at the GPS site is an enzymatic process, the potential enzyme(s) would have to be colocalized with the receptor in intracellular compartments because this cleavage occurs early on during the receptor biosynthesis as the presence of cleaved LPHN can be tracked down from the endoplasmic reticulum (ER) (50).

BACE1

Also known as β-site amyloid precursor protein cleaving enzyme 1 or β-secretase 1, this enzyme is responsible for the extracellular cleavage of amyloid peptide protein (APP) [reviewed in (51)]. This cleavage generates a soluble form of APP and leaves behind a form of membrane-bound APP that will then be cleaved by γ-secretase. This process leads to the generation of the pathological form of APP according to the amyloid-β hypothesis. BACE1 is produced as an immature form, proBACE1, which possesses robust catalytic activity from the moment it is translated into the ER, coinciding with LPHN cleavage at the GPS (52, 53). Growing body of evidences suggested that BACE1 function might involve the shedding of non-APP substrates (54). A recent attempt at an unbiased screen for endogenous substrates of BACE1 in neurons used pharmacological inhibition followed by affinity purification of biotinylated sugars to detect shedded membrane proteins (55). These assays used a membrane-permeant inhibitor of BACE1 that may also inhibit the activity of its zymogen in the endosomes and secretory pathways such as ER and Golgi compartments. Purification of soluble proteins revealed that all LPHN were differentially shedded after BACE1 inhibition, therefore, generating altered levels of NTF in the extracellular medium: soluble NTF levels for LPHN1, LPHN2, and LPHN3 were all found to be reduced after BACE1 inhibition. Importantly, the fact that these NTF fragments were found to be soluble in the extracellular medium indicates that at least a fraction of the generated NTF can be released from the CTF. Whether BACE1 participates in GPS cleavage is unclear, but it is noteworthy that its canonical recognition sequence is adequately described by the GPS sequence. Indeed, BACE1 preferred cleavage sites seem to require a Leucine at *P1* position and a polar residue at position *P2* and *P1′*, a consensus site that is reminiscent of the one displayed at the GPS of LPHN isoforms, where the site of cleavage is located between the leucine residue and the polar threonine residue [$C^{P4}(S/N)^{P3}H^{P2}L^{P1}{\star}T^{P1'}N^{P2'}$; *star:*

cleaved bond] (49, 56). Moreover, the soluble peptides identified for LPHN1, for example, comprised exclusively the N-terminal adhesion region sequences, suggesting that the enzymatic activity is a) membrane proximal and b) targeted to that region alone, leaving other extracellular sequences untouched (55). It is noteworthy that cleavage modulation of LPHN1 ligand, NRXN, is also observed after BACE1 inhibition. These data hint to an unsuspected role of BACE1 in LPHN shedding as a non-APP substrate.

Second site cleavage in the GAIN domain

An alternative cleavage site has been identified between the GPS site and the first transmembrane domain of LPHN1 (57). Cleavage activity at the second proteolysis site might explain the recovery of LPHN NTF fragments as extracellular soluble forms observed in BACE1 inhibition studies (discussed above). It is unclear which enzyme is responsible for this catalysis as interfering with the GPS site cleavage leads to a more efficient cleavage at the second site, suggesting a homeostatic mechanism between the two sites. Also, treatments enhancing protein kinase C activity were shown to inhibit the extent of the second site cleavage, suggesting that the activity of the molecular component involved in this cleavage can be regulated by modulating cellular levels of phosphorylation (58).

GAIN domain function vs. GPS site contribution

The physiological role of the GAIN domain is still unclear but points to a very important structural determinant separating the adhesion function from the activation function of aGPCR, in general, and of LPHN, in particular. As for one role, it contributes the crucial charges involved in the autoproteolytic cleavage of LPHN (49). Although the GAIN domain does not fit into the classic mold of adhesion domains, its high conservation throughout evolution coupled to its membrane proximity suggest more than just a structural contribution. Identification of ligands for the GAIN domain remains an important challenge, as it is likely that intermolecular interactions might modulate its function. As a potential correlate, LPHN exogenous ligand α-latrotoxin binds LPHN via the GAIN domain and is involved in physically re-associating LPHN NTF to their CTF (49, 59). Discovery of endogenous ligands for the GAIN domain would constitute a major advance in the field as this protein fold is found in all members of the adhesion GPCR family. Quite intriguingly, most of the mutations involving LPHN in diseases like cancers, are found in the GAIN domain (60). This would suggest that the GAIN domain may modulate the activity of the receptor or influence its biological function.

The role of GPS cleavage is still elusive. It was first suggested that GPS cleavage was important for surface exposure of the LPHN as earlier studies showed that GPS site nonconserved mutations led to reticulum endoplasmic retention of the generated mutants (50). However, more recent studies described GPS mutations that eliminate receptor cleavage but that still allow the proper membrane localization of LPHN (49). A consequence of GPS cleavage is to separate the NTF from the CTF; therefore, it could act to physically segregate two different functions of the receptor: the adhesion function on the one hand, and the signaling function on the other hand. This is well exemplified by studies targeted at rescuing the two different phenotypes observed for LPHN1 null mutants in *C. elegans*, a fertilization defect and a developmental defect, by overexpressing different LPHN constructs (61). Indeed, while the presence of NTF is required to rescue both phenotypes, the CTF is absolutely required to rescue the developmental defect but not the fertilization defect.

The CTF

The seven transmembrane domains and intertransmembrane loops

As heptahelical domains are characteristic of GPCR, these domains constitute a second important hallmark of adhesion GPCR after the GAIN domain. LPHN seven-transmembrane domains bear a high sequence homology between the different isoforms. This high level of homology suggests a common function between LPHN1, 2, and 3 transmembrane domains, which might not only serve to tether them to the membrane but may also show a similar pattern of rearrangement leading to activation of intracellular cascades. However, the transmembrane domains have been shown to be unnecessary for mediating certain LPHN-dependent functions, suggesting that LPHN work as bimodular proteins where the NTF and the CTF engage in different functions (61).

The cytoplasmic C-terminal tail

Like for many GPCR, the cytoplasmic loops allow for the receptor intracellular interface to interact with

components from inside the cell. This mode of action might lead to the translation of extracellular binding events into the generation of intracellular signaling cascades. Because LPHN are adhesion GPCR, it would be expected that they adopt similar cellular pathways as for classical GPCR like G-protein coupling and desensitization comprising phosphorylation by G protein-coupled receptor kinases (GRKs) and internalization following binding by β-arrestin, for example. Emerging evidence suggests that the LPHN intracellular modifications and interactions might well account for a significant portion of the function of these receptors. However, how these receptors initiate an intracellular signaling cascade is an open question that remains to be answered in the field.

G-protein coupling

Although LPHN have not been shown to functionally couple to G-proteins, they can physically associate with G-protein complexes. Among them, the G-proteins $G\alpha_{q/11}$ and $G\alpha_o$ (but not $G\alpha_s$) can be pulled down along with brain LPHN that has been affinity purified on a α-latrotoxin column (21, 62). Because GPCR are in constant equilibrium with their G-proteins, overexpression paradigms are often used to shift the equilibrium toward activation of the coupled G-protein in question. However, overexpression of LPHN in heterologous cells led to confusing results including the absence of cAMP pathway activation, which is consistent with LPHN not being physically linked to $G\alpha_s$ but also the absence of IP_3 production, which would come from activating $G\alpha_{q/11}$, a protein shown to interact with the receptor (21).

Phosphorylation

As for desensitization mechanisms, they are largely unknown for LPHN. Given that GPCR are subject to phosphorylation to achieve desensitization by GRK proteins and then recruitment of β-arrestin leading to internalization, it will be very interesting to see if LPHN follow a similar pattern of desensitization. It is noteworthy that the presence of phosphorylation was observed for all three LPHN in synaptosomes from mouse brain (Figure 2). The phosphorylation sites map to multiple serine/threonine/tyrosine residues in the cytoplasmic tail (63, 64), which would suggest that phosphorylation events might regulate LPHN function including perhaps its activation state or its internalization.

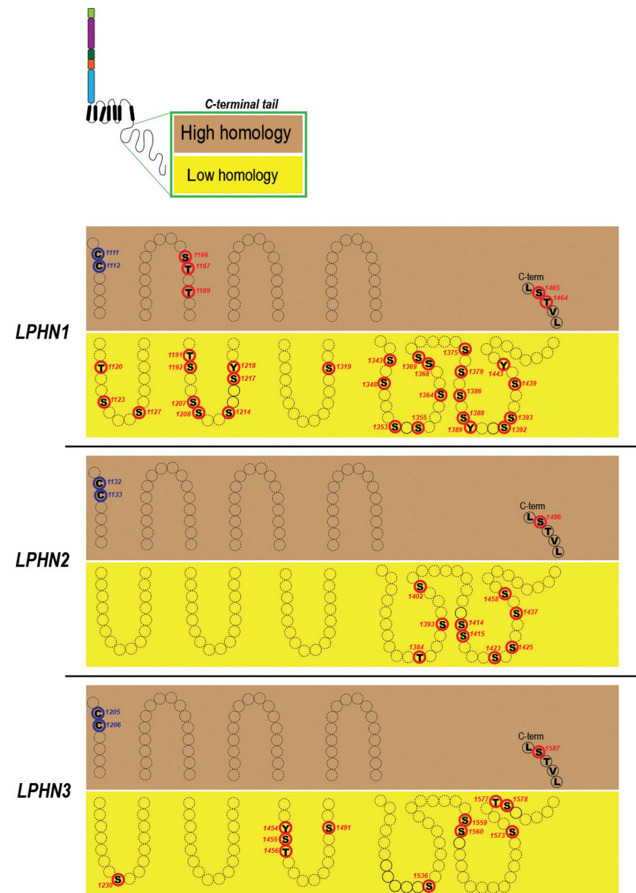

Figure 2 Latrophilin isoforms and identified intracellular phosphorylation sites.
Schematic representation of LPHN C-terminal tail residues parted in two regions of either high homology or low homology. These were deducted from protein alignment showing regions of high residue identity vs. low residue identity (high and low homology regions, respectively). Serine (S), threonine (T), and tyrosine (Y) residues are identified by their positioning number from the immature LPHN proteins and are displayed in open red circles (63, 64). Note that a) most of the phosphorylation sites identified so far reside in the C-terminal tail and do not involve the intracellular loops, and b) there is a high concentration of phosphorylation events in regions that are of low homology between the LPHN. In all three LPHN, the PDZ-binding motif, comprising residues LVTSL, undergoes phosphorylation either on the conserved Ser or both Ser and Thr residues. Potential palmytoylation sites at conserved cysteine (C) residues are also identified in open blue circles. Dashed open circles represent gaps between residues.

Recruitment of intracellular proteins: PDZ-binding motif and C-tail

Another characteristic of LPHN cytoplasmic tail is the presence of PDZ-binding motifs. These motifs are known to anchor various molecules like protein scaffolds located at

the synapse. LPHN PDZ-binding motifs have been shown to interact with scaffold proteins from the SHANK family (34, 41). SHANK proteins are structurally associated to membrane-associated guanylate kinases or MAGUKs and have been described as proteins primarily located at post-synaptic compartments of excitatory synapses. Although it is tempting to conclude that LPHN might act exclusively postsynaptically in excitatory synapses, reports show that immunoreactivity to the cytosolic tail can be detected in the presynaptic compartment (40). Moreover, the function of LPHN is not restricted to excitatory synapses as interfering with its ligand-binding function affects both inhibitory and excitatory synapses (37). Also of recent interest is the involvement of LPHN in intracellular complexes comprising TRIP8b, an adaptor protein involved in protein trafficking and able to recruit other molecules important for exocytosis (65, 66). However, it is unclear whether or not this interaction is dependent on the PDZ-binding motif.

Latrophilin ligands and associated functions

The biological function of LPHN is still unclear to this date. One key element that has been slowing down the progress in that sense was the lack of endogenous ligands. Until recently, LPHN were described as orphan receptors. The first attempts at discovering endogenous ligands for LPHN were performed in the parasitic nematode *Haemonchus contortus*, which expresses a LPHN-like receptor termed HC110-R, a target for a new class of cyclic depsipeptides used as anthelmintic drugs (67). This search identified three neuropeptides of the FMRFamide-like family (AF1, AF10, and PF2), exhibiting a low-affinity interaction with the N-terminal fragment of HC110-R (Figure 1A). These ligands were then proposed to be involved in the control of pharyngeal pumping for this nematode worm (68). However, more recent efforts led to the discovery of the first high-affinity endogenous ligands for LPHN, which describe families of adhesion motif-containing transmembrane proteins (TEN, NRXN, and FLRT), supporting their putative role in cell-cell interactions and signaling (37, 40, 69, 70).

Heterophilic interactions

Teneurins

Recent biochemical screens from brain tissues identified a family of neuronally expressed type II transmembrane glycoproteins termed teneurins (TEN) as specific endogenous ligands for LPHN (37, 40). TEN are composed of a relatively short N-terminal cytoplasmic sequence, a single transmembrane region, and a long extracellular sequence containing multiple EGF-like repeats, a region of conserved cysteines and unique YD repeats (71). TEN EGF domains are closely related to the ones from another class of extracellular matrix proteins called tenascin from which they get their name as tenascin-like major proteins (Ten-m). Vertebrates possess four teneurin genes (*Ten-m1*, *2*, *3*, and *4*) expressing four TEN proteins (TEN1, 2, 3 and 4), whereas invertebrates have usually one or two related genes. TEN can form homodimers and can also undergo homophilic binding to each other in invertebrates and vertebrates (37, 71–74). Complementary but nonoverlapping expression of TEN in the *Drosophila* olfactory system and the mice cerebellum, hippocampus, and visual pathway (retina, dorsal lateral geniculate nucleus, superior culiculus, and visual cortex) suggests that their homophilic interactions could instruct interneuronal connections (75, 76). Indeed, this role has been substantiated by studies done on the *Drosophila* olfactory system and the mouse visual system, in which they are suspected to direct axon targeting events (72, 73, 77, 78). The function of TEN in the nervous system also involves the promotion of neurite outgrowth, cell adhesion, dendritic morphology, and synapse formation. Although the molecular dissection of most of TEN function is still unclear, their role in cell adhesion and the formation of synapses *in vitro* seems to rely on their heterophilic interactions with LPHN (37, 40). Although initial studies suggested that LPHN could only interact with TEN2, other reports revealed that a more promiscuous molecular network exists between the two protein families (37, 40, 47, 69). Because both proteins LPHN1 and TEN2 are enriched in synapses of cultured hippocampal neurons and that LPHN1 is localized in the presynaptic membranes, while TEN2 is mostly postsynaptic, it was thus suggested that they can form *trans*-synaptic complexes to regulate the formation or maintenance of synapses (40). Indeed, as a correlate of *trans*-interaction, heterologously expressed LPHN1 in HEK293 cells was able to induce the recruitment of hemisynapses containing the postsynaptic marker PSD95, from co-cultured neurons (40). Alternatively, TEN are also thought to exert their function through *cis*-interaction with LPHN. In *C. elegans*, the individual and combined effects on embryonic morphogenesis and fertility of the orthologs *lat-1* and *ten-1* genes indicated that both are acting in *cis* (parallel) implying a synergistic rather than linear interaction between them. In this complex, they may function

as co-receptors for a common ligand with different signaling responses into the same cell (61).

This LPHN-TEN interaction network involves the molecular contribution of the lectin-like domain from LPHN but is also supported by determinants originating from the olfactomedin domain and the Ser/Thr-rich sequence (Figure 1C). The combination of the LEC-OLF-S/T domains exhibits a higher affinity for TEN2 and TEN4 than the LEC domain by itself (37). Interestingly, the LPHN-TEN interaction can be modulated by alternative splicing of LPHN Splice Site A located at the junction of the LEC and OLF domains (37).

As both signaling pathways associated with either TEN or LPHN are a matter of active investigation, the next step will be to elucidate if the LPHN-TEN interaction can generate intracellular signaling. Addition of soluble TEN2 ligand to hippocampal neurons causes an increase of intracellular Ca^{2+} concentration, an effect reminiscent of GPCR coupling to protein $G\alpha_q$ for the modulation of intracellular Ca^{2+} stores, as is currently suggested for the action of LPHN. Although this assumption is tempting, it remains to be demonstrated whether this neuronal signal is actually mediated by LPHN. Additionally, the anterograde signal elicited through TEN would be a subject of great interest as it has been shown that the activation of mitogenic pathways is a part of their cellular signaling profile (79, 80). Moreover, the ability of TEN to undergo proteolysis by a yet unknown mechanism and to release an intracellular domain acting on the transcriptional machinery after translocation to the nucleus interrogates if this can be modulated by their heterophilic interactions (81).

Neurexins

NRXN are presynaptic type-1 transmembrane proteins, that in mammals are encoded by three homologous genes (NRXN 1-3), each of which is controlled by two different promoters (an upstream 'α' and a downstream 'β' promoter) producing two principal isoforms, longer α-NRXN and shorter β-NRXN (82). They contain large extracellular domains (α-NRXN contain three EGF-like repeats and six LNS repeats, β-NRXN contain only one LNS domain), one transmembrane region, and a very short cytoplasmic tail. Similarly to LPHN, NRXN were discovered as neuronal receptors for α-latrotoxin, a component of Black Widow spider venom (15, 21, 83). NRXN are mainly expressed presynaptically in the brain, where they may perform different functions (18, 84). Their role as presynaptic cell adhesion molecules was supported after the discovery of their canonical endogenous ligands, the postsynaptic transmembrane proteins, neuroligins (85, 86).

NRXN was identified as a high-affinity LPHN1 ligand using *in vitro* assays, a finding that is surprising given that both adhesion molecules were known for years to be individual receptors for α-latrotoxin (70). This interaction was specific for LPHN1 as no binding to NRXN could be seen for either LPHN2 and 3 (47). However, assays using affinity chromatography on brain tissues to isolate NRXN ligands did not yield any LPHN peptides (87–89). The canonical NRXN ligand, neuroligin, directly competes against LPHN interaction on NRXN with a higher affinity, which might explain the results from brain affinity chromatography assays (70). Domains of interaction comprised the sixth LNS domain of NRXN (the only LNS domain present for β-NRXN) and the olfactomedin-like domain of LPHN1, along with a short sequence engulfing the Ser/Thr-rich region (Figure 1D). Interestingly, although LPHN are evolutionary conserved, the olfactomedin-like domain is absent from LPHN homologs expressed in *C. elegans* and *Drosophila melanogaster*, which implies that this interaction is not essential for basic synapse formation events.

A hallmark of NRXN is their propensity to be submitted to extensive alternative splicing, a process that can generate more than 3000 isoforms (82). α-NRXN contain five sites of alternative splicing (SS1 to SS5), while β-NRXN bear two sites (SS4 and SS5). As polymorphic adhesion molecules, NRXN are suspected to interact with many different ligands and to have many different functions as a result of alternative splicing. A unifying trend for the binding function of NRXN is the importance of SS4. Indeed, NRXN interaction with their numerous ligands depends on the splicing events at SS4 (87, 88, 90–92). Like for other NRXN ligands, alternative splicing at SS4 regulates the binding to LPHN1. Indeed, the presence of an insert in SS4 disrupts the interaction and completely abrogates the binding to LPHN1 (70).

The role of this interaction and how it differentiates the function of invertebrate LPHN vs. vertebrate LPHN remains an open question. Also, while the LPHN1-NRXN binding has been observed to stabilize cell-cell junctions in a *trans* configuration, it is possible that this binding additionally occurs in *cis* given the joint presence of LPHN and NRXN on the presynaptic membrane. Finally, because both molecules are α-latrotoxin receptors, the fact that they interact with each other arises the possibility that LPHN1 and NRXN might conduct jointly, rather than independently, the action of the toxin. Such a cooperative effect between NRXN1 and LPHN1 has been observed for the effect of α-latrotoxin on mice engineered

with a genetic deletion of both adhesion molecules, but this needs further clarification at this point (93).

FLRT3

Another LPHN endogenous adhesion ligand candidate identified is FLRT3, fibronectin-like domain-containing leucine-rich transmembrane protein 3 (37, 69). FLRT are glycosylated membrane proteins composed of 10 leucine-rich repeats surrounded by C-terminal and N-terminal cysteine flanking regions, a fibronectin-like domain, a transmembrane domain, and a short intracellular tail (94). In vertebrates, there are three FLRT isoforms (FLRT1-3) encoded by different genes, *Flrt1-3*; they are expressed in the brain with additional expression for FLRT1 in the kidney, FLRT2 in the heart, skeletal muscle, and pancreas, and FLRT3 in many other tissues (95). In neurons, FLRT are involved in many different processes, but it was only recently that FLRT3 has been shown to participate in synapse formation through its interaction with LPHN1 and LPHN3 (37, 47, 69). Of interest, FLRT3 extracellular domain can interact with the olfactomedin-like domain of LPHN similarly to its other ligand NRXN (Figure 1C) (47). Like for TEN, the interaction LPHN-FLRT3 is of high affinity, but unlike TEN, FLRT3 binding to LPHN is not regulated by alternative splicing of the latter, confirming a distinct binding mechanism between LPHN and these two unrelated ligands (37, 47).

The fact that FLRT3 was detected postsynaptically suggests that LPHN and FLRT3 may form a *trans*-synaptic complex. Moreover, the pair LPHN3-FLRT3 has been shown to play an important role in glutamatergic synapse development, regulating excitatory synapse number *in vitro* and *in vivo* (69).

EMR2

Studies dissecting the cleavage of aGPCR revealed that LPHN could interact with other members of this receptor family, specifically with EMR2 standing for EGF-like module containing mucin-like hormone receptor-like 2 (Figure 1D). Although belonging to the same family, EMR2 and LPHN ectodomains have no sequence similarity (96). Also, unlike LPHN distribution, EMR2 is expressed on neutrophils, monocytes, macrophages, and dendritic cells. EMR2 ligation potentiates the activation and recruitment of human neutrophils and acts as a regulator of PMN function. It binds chondroitin sulfate with a potential role during cell adhesion and migration (97). LPHN1-EMR2 interaction has been demonstrated using overexpression paradigms. Indeed, experiments with chimeras of LPHN1 and EMR2 suggested that they could cross-interact by exchanging their NTF (96). This result is interesting in the view of previous reports suggesting that LPHN NTF and CTF fragments can behave independently. According to this study, when the NTF is separated from its complementary fragment, its ligand binding characteristics are modified. Following ligand binding, there would be a dynamic re-association with its own complementary fragment or with the CTF of another aGPCR subunit. Intriguingly, the authors detected cross-interactions between LPHN1 NTF and EMR2 CTF, although the complex did not bind α-latrotoxin. These data could unveil a new mechanism for aGPCR function in general, and for LPHN in particular, insinuating that LPHN can exchange their adhesion domains with the transmembrane domains of other aGPCR, therefore, creating a receptor unit with unique binding and signaling properties. Further studies are necessary to dissect this mechanism.

Homophilic interactions

Some members of the aGPCR family, to which LPHN belongs, have been reported to form homodimers. This kind of structure plays a regulatory role in modulating the expression and function of these receptors (98). In fact, very little is known about the extent and basis of homointeraction for aGPCR (99).

LPHN1 was shown to undergo receptor clustering after the addition of the exogenous ligand. LPHN homointeraction was observed between the NTF and the CTF fragments rather than between ectodomains only. This would form a complex with subsequent signal transduction properties, but no evidence of a direct receptor interaction was demonstrated (59). Subsequently, a model for the mode of action of the heterologous receptor LAT-1 was proposed in which the homodimerization of the LAT-1 NTF could be ligand induced through the lectin domain, leading to the cross-activation of the 7TM domain via the GPS of the partner molecule. The homologous pairing of two receptor molecules through GPS interactions with the transmembrane domains provided biochemical evidence of LAT-1 homodimers, while sedimentation assays provided biophysical evidence of the homodimers (61). However, if LPHN are capable of homophilic binding, it must be in a *cis* configuration because LPHN-expressing cells were not capable of mediating cell-cell adhesion when presented

on two different cell populations, suggesting that they are not homophilic cell adhesion molecules (37).

Association of latrophilins with human health disorders

Given LPHN widespread expression in mammals, they are expected to influence multiple events in development and maturation of tissues, highlighting that LPHN roles might be that are common in their adhesive properties but divergent in their cellular functions. For example, LPHN2 presence in the heart might affect cardiovascular health, but its presence in the brain might affect neuronal synapse function, both functions mediated by its adhesion properties to known and unknown extracellular ligands. The high homology of LPHN isoforms residing in their transmembrane domain and adhesion motifs would suggest a common function for adhesion properties and membrane localization, while the low homology residing in its intracellular region would suggest heterogeneity of signal transduction mechanisms. Therefore, genetic defects in each LPHN may generate a distinct phenotype. We are reviewing, here, human disorders that have been associated with LPHN genetic defects and will also discuss possible overlap with comorbid or coexisting disorders in relation to newly discovered ligand interactions.

Attention-deficit hyperactivity disorder

Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that affects a number of children by hindering their ability to participate in normal social behaviors. It is one of the most commonly diagnosed psychiatric disorders in children. ADHD is characterized by coexistence of attentional problems and hyperactivity (100). The persistence of ADHD throughout adolescence and adulthood brings about comorbidity phenotypes such as depression, anxiety, and addiction (101). Owing to the highly inheritable nature of this disorder, genetic defects are suspected to play an important role in the development of this neuropathology. Dopaminergic and serotonergic genes were among the first to be associated with the development of ADHD and have been studied extensively (100, 102). Indeed, defects in dopaminergic and serotonergic pathways influence mobility and anxiety, two conditions found in the etiology of ADHD. Moreover, ADHD symptoms can be alleviated by treating patients with pharmacological agents acting on the dopamine transporter-DAT-, thereby, supporting the dopaminergic contribution to the disease. However, recent advances have identified genetic variants of the LPHN3 gene as susceptibility factors for the development of ADHD in children and also for the persistence of ADHD in adulthood (103) and reviewed in (104). A minimal critical region of approximately 325 kb identified as a susceptibility locus associated with ADHD in linkage studies, mapped to the LPHN3 gene encompassing exons 4–19 coding for most of the translated protein. This link between LPHN3 and ADHD was extended to populations from USA, Columbia, Germany, Spain, and Norway, hinting to a crucial contribution of this receptor in human neurodevelopmental diseases. LPHN3 genetic variants associated with ADHD can be divided in three mutational groups: 1) missense, 2) synonymous, and 3) intronic changes (105). Interestingly, although multiple polymorphisms were observed, very few of the mutations identified were predicted to affect coding regions or canonical splice sites in the LPHN3 gene (Figure 3). However, five mutations representing missense changes target important functional domains. These mutations target nonconserved residues between LPHN3 and its cognate isoforms LPHN1 and LPHN2. While this suggests a lower impact on protein function, these polymorphisms should not be neglected as LPHN3 might have a different function than LPHN1 or LPHN2 in brain and maybe in other tissues as well. As for intronic or synonymous changes, they were highly prevalent, but their function is unclear at present. It is, therefore, possible that noncoding variants might quantitatively or qualitatively affect LPHN3 expression. The expression pattern of brain-enriched LPHN3 is consistent with a role in adult brain development as its mRNA expression continues throughout adulthood and is highest during that time period in mice (37). Also, LPHN3 prevalence in ADHD-related brain regions such as amygdala, caudate nucleus, cerebellum, and cerebral cortex supports a role in disease-affected areas (22, 33). Furthermore, its newly identified endogenous ligand, FLRT3, has also been linked to ADHD. These evidences unveil a central role for LPHN3 and its ligands in the etiology of ADHD.

How does this adhesion GPCR influence brain development? Loss of function mutants, targeting the zebrafish LPHN homolog LPHN3.1, displayed impulsive motor phenotype and a reduction, as well as misplacement, of dopamine-positive neurons, pointing to a neurodevelopmental role of LPHN3 in affecting motility behaviors (106). Additionally, LPHN3 null mice studies revealed changes in mRNA-encoding transporters involved in the dopaminergic and serotonergic pathways in newborns and in the striatal levels of dopamine and serotonin in adulthood

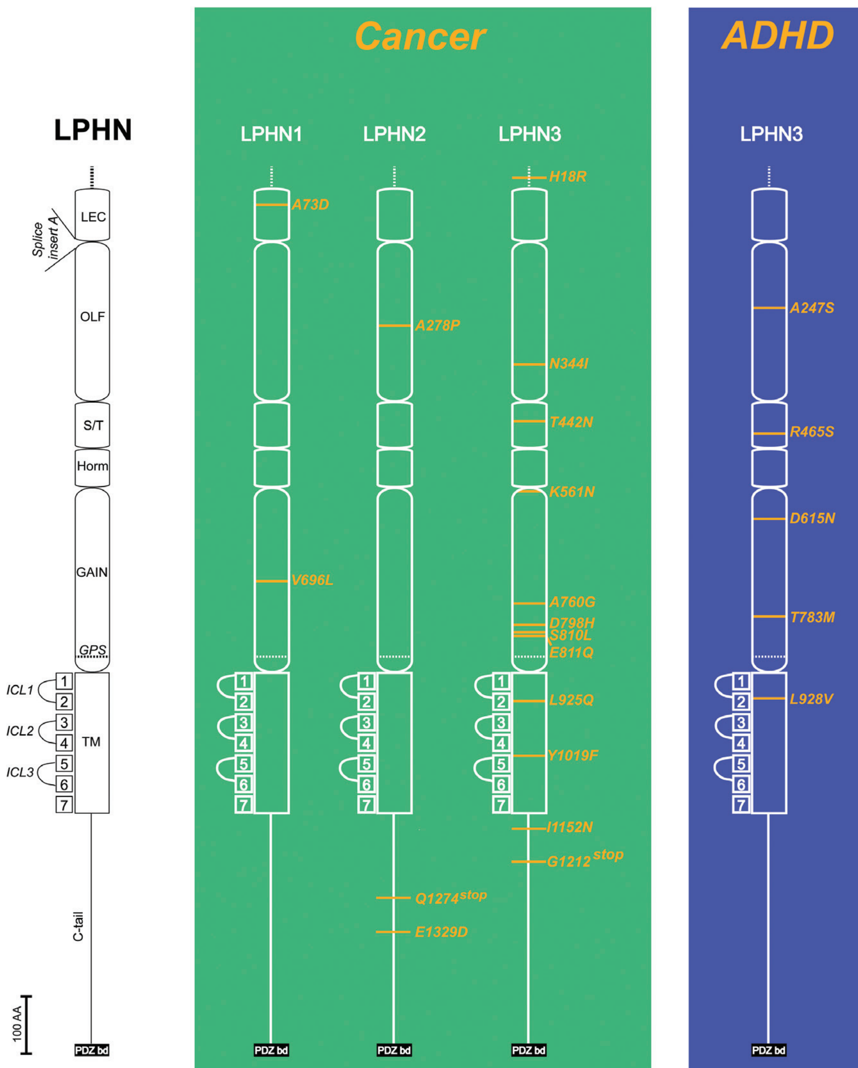

Figure 3 Missense mutations identified for latrophilins in human cancer samples and patients diagnosed with ADHD.
Schematic representation of LPHN and their structural domains: LEC, lectin-like domain; OLF, olfactomedin-like domain; Horm, hormone-binding domain; S/T, serine/threonine-rich domain; GAIN, GPCR autoproteolysis inducing domain; GPS, GPCR proteolysis site; TM, seven-transmembrane domains region (1–7); C-tail, carboxy-terminal tail; PDZ bd, PDZ-binding domain. Missense mutations are shown for each LPHN (LPHN1, 2, and 3) and identified by a positioning number, which is adjacent to the affected structural domain in the immature proteins (containing the signal peptide, which is represented by a dashed line). Note that all three LPHN have mutations linking them to cancer, but only LPHN3 has been identified as a molecular target for mutations affecting the development or susceptibility to ADHD. The majority of identified missense mutations in all LPHN are clustered in their extracellular domains, the GAIN domain being the most targeted for LPHN3 in either cancer or ADHD samples.

(107). These mice displayed a hyperactive phenotype in behavioral tests and were more sensitive to the locomotor stimulant effects of cocaine, which is consistent with reward pathway-associated defects seen in ADHD patients. Such corroborating phenotypes for LPHN3 genetic defects unveil a possible overlap between the function of this class of aGPCR and molecular components of monoamine signaling such as dopamine and serotonin receptors/transporters.

Cancer

Dysregulation in the cell cycle constitutes a hallmark of the function of oncogenes and proto-oncogenes, affecting cell division and leading to development of cancerous phenotype. In contrast, tumor-suppressor genes act as protectors of the cell, therefore, allowing normal cell function to go on, under biological stress. *Lphn2* gene (or *Lphh1*) was initially isolated and characterized as

part of a study aiming at identifying genes that are comprised in a chromosomal region involved in human breast cancer (23). While the function of LPHN in cell cycle is still unclear, its implication in asymmetric cell division has been highlighted in *C. elegans* embryogenesis (27). Indeed, LPHN mutant-specific cell lineages fail to properly align mitotic spindles in time during specific stages of the blastomere division, therefore, leading to a loss of tissue polarity. This phenotype depends on the coupling of adhesion function to receptor signaling through LPHN as it can only be rescued when both functional NTF and CTF domains are expressed. Interfering with LPHN function in *Tribolium castaneum* (TC0001872/CIRL) leads to a similar phenotype resulting in the arrest of larval growth and development (108). Therefore, LPHN intracellular signaling following cell-cell adhesion might be a function that certain cells require in order to remain in synchronicity with other cells for normal mitotic divisions, a process that is repressed in the induction of cancer. A change in the adhesion properties of LPHN-deficient cells may alter intercellular communication leading to unorthodox cell divisions. Although this is a speculation at this point, the continuous involvement of adhesion motif-related mutations for LPHN proteins in human cancers provide some support for this hypothesis. Additional linkage studies have revealed numerous LPHN loss-of-function mutations and involved LPHN presumably as tumor-suppressor genes in different types of cancers such as lung adenocarcinoma, ovarian cancer, and urothelial carcinoma of the bladder (Figure 3) (60, 109).

Psychiatric comorbidity with ADHD: potential common molecular pathways

LPHN operates partly through adhesion mechanisms. All the ligands identified so far for LPHN are associated with disorders affecting neurological properties. It is, therefore, possible that the LPHN-induced pathway might participate in pathophysiological processes through these ligands. Comorbid diseases are common for patients suffering from ADHD, which has been associated with LPHN genetic defects.

LPHN and NRXN: molecular links between ADHD and autism?

NRXN genetic defects as well as defects affecting their canonical postsynaptic partners, neuroligins, have been associated with autism spectrum disorders (ASDs) in genome-wide association studies performed on different populations diagnosed with ASDs (110–118). The core features of ASDs are characterized by social skills and communication deficits, stereotypies, and ritualistic behaviors. Other common symptoms that have been characteristic of individuals with an ASD include problems with impulsivity, hyperactivity, and attentional deficits, which are clinical presentations that also constitute hallmarks of ADHD (119). A significant proportion of patients suffering from autism display symptoms associated with ADHD, a condition described as psychiatric comorbidity. It is estimated that as much as 20–70% of the autistic population displays features of ADHD (120). This overlap often complicates diagnosis to the point where autistic children commonly receive a diagnosis of ADHD first (121). Both disorders being neurodevelopmental in nature, they might, therefore, find commonalities in their etiology. Some researchers have reported common structural brain abnormalities that are shared in those with ASD and ADHD, and given the higher rate of ASD symptoms in children and their siblings diagnosed with ADHD, a common genetic etiology is beginning to emerge in the scientific community (122–124). While it is becoming increasingly clear that the NRXN-neuroligin axis and their related signaling molecules contribute to the pathophysiology of ASDs, the intriguing evidence that LPHN can interact with NRXN to form adhesion complexes *in vitro* brings another level of complexity for this genetically diverse psychiatric disorder (70). It is not clear to this date if LPHN-NRXN pathway is involved in this comorbidity phenotype in humans, but this will be worthwhile exploring in the future with further studies.

LPHN and TEN: molecular links between ADHD and bipolar disorder?

Another psychiatric disorder that could involve LPHN function includes the development of bipolar disorder (BD). BD is an episodic recurrent pathological mood disturbance that ranges from extreme elation or mania to severe depression, usually accompanied by disturbances in thinking and behavior, and often by psychotic features like delusions and hallucinations. Like for ADHD, the development of BD suggests the contribution of substantial inheritable genetic factors evidenced by a family history of mood or psychotic illness in patients with established disease. Indeed, genome-wide association studies conducted on vast samples of patients diagnosed with BD revealed the complexity and diversity of genetic contributions to the development of this disorder. More than five

chromosomal regions were identified as part of risk loci for BD development in humans, one of them circumscribing the gene for TEN4 (125–128). Moreover, studies have shown that there is comorbidity between ADHD (which is LPHN related) or BD (which is TEN related) and disorders involving reward behaviors possibly implicating the dopaminergic pathway such as addiction and alcoholism, for example (101, 129). Because TEN possesses signaling capabilities through its interaction with LPHN, it is conceivable that an alteration to TEN levels might also affect cellular pathways implicating LPHN (40). Moreover, given that LPHN genetic deficiencies affect molecular components of the dopaminergic system that is important for reward behaviors, there is a potential for convergence of cellular function between LPHN and TEN signaling pathways that might be responsible for the existence of BD comorbidity disorders like addiction and anxiety, for example.

Expert opinion

Since its discovery in 1998 as a neuronal receptor for α-latrotoxin, research on the physiological functions of LPHN has been incremental. Substantial advances in elucidating LPHN functions came from the identification of its endogenous ligands, more than a decade following its discovery. Not only will this tell us more about LPHN functions but will also enlighten our knowledge on the function of their interacting partners. Convergence of biochemical and genetic approaches are now paving the way to unveil the importance of LPHN in participating in cellular processes that will show to be very diverse as they are expressed in various tissues.

Outlook

We foresee that the short-term future will hold the following discoveries and challenges:

1. Other sets of LPHN ligands will be discovered and characterized, which will shed more light on the function of these receptors and of the interacting ligands.
2. Genetic dysfunctions of LPHN associated to neurological disorders will be linked to specific behaviors in vertebrates.
3. LPHN somatic mutations found in cancer will be used to help elucidate the cellular biology of LPHN.
4. Non-neuronal functions will be discovered for the different LPHN isoforms in vertebrates.
5. Intracellular signaling initiated by endogenous ligand binding to LPHN will be dissected.

Highlights

LPHN regulate cell-cell adhesion events that have linked them to synaptogenesis in mammals and as important regulators of tissue polarity and fertility in invertebrates. Their high degree of evolutionary conservation puts emphasis on their essential role for governing intercellular processes through adhesion. Adhesion events mediated by LPHN involve a series of modular domains, each establishing their own sets of interactions to participate in heterophilic adhesion with other cell adhesion molecules such as TEN, FLRT, and NRXN. Known previously to mediate the deleterious effects of α-latrotoxin, their physiological function mediated by recently identified endogenous ligands still remains elusive. LPHN genes are targeted by multiple mutations in association with cancer and the development of ADHD.

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List of abbreviations

EGF	epidermal growth factor
LNS	laminin α-neurexin-sex hormone-binding globulin
PDZ	postsynaptic density protein, *Drosophila* disc large tumor suppressor and zona occludens-1 protein
NHL	NCL-1, HT2A and Lin-41
YD repeats	tyrosine-aspartate repeats
RHS	retrotransposon hot spot
CHO	O-linked glycosylation

SHANK SH3 and multiple ankyrin repeat domains protein
cAMP cyclic adenosine monophosphate
IP_3 inositol triphosphate
TRIP8b tetratricopeptide repeat-containing Rab8b-interacting protein
PMN polymorphonuclear leukocytes.

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