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Understanding CELSRs - Cadherin EGF LAG seven-pass G-type receptors

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Abstract

The cadherin EGF LAG seven-pass G-type receptors (CELSRs) are a special subgroup of adhesion G protein-coupled receptors (GPCRs), which are pivotal regulators of many biological processes such as neuronal/endocrine cell differentiation, vessel valve formation and the control of planar cell polarity during embryonic development. All three members of the CELSR family (CELSR1-3) have large ecto-domains that form homophilic interactions and encompass more than 2,000 amino acids. Mutations in the ecto-domain or other gene locations of CELSRs are associated with neural tube defects (NTDs) and other diseases in humans. *Celsr* knockout (KO) animals have many developmental defects. Therefore, specific agonists or antagonists of CELSR members may have therapeutic potential. Although significant progress has been made regarding the functions and biochemical properties of CELSRs, our knowledge of these receptors is still lacking, especially considering that they are broadly distributed but have few characterized functions in a limited number of tissues. The dynamic activation and inactivation of CELSRs and

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the presence of endogenous ligands beyond homophilic interactions remain elusive, as do the regulatory mechanisms and downstream signaling of these receptors. Given this motivation, future studies with more advanced cell biology or biochemical tools, such as conditional KO mice, may provide further insights into the mechanisms underlying CELSR function, laying the foundation for the design of new CELSR-targeted therapeutic reagents.

Keywords

G Protein-Coupled Receptor (GPCR); Adhesion; CELSR; Development; Planar Cell Polarity (PCP)

Introduction

G protein-coupled receptors (GPCRs) govern the information exchange between the extracellular environment and intracellular activity and, as a group, comprise the largest transmembrane protein superfamily (Chai *et al*. 2014, Venkatakrishnan *et al*. 2013). All GPCRs have seven conserved transmembrane helices, enabling the receptor to undergo a conformational change upon receiving extracellular stimuli, which in turn couples to downstream effectors and ultimately generates cellular responses. (Rosenbaum *et al*. 2009, Hanson & Stevens 2009, Shukla *et al*. 2008, Kahsai *et al*. 2011). The adhesion GPCRs form a unique subfamily within the GPCR superfamily. Each of these special GPCRs has a very large N-terminal ecto-domain that encompasses potential adhesion motifs/repeats and the conserved GPCR proteolytic site (GPS), which is part of the GPCR autoproteolysis-inducing (GAIN) domain (Arac *et al*. 2012, Paavola & Hall 2012, Sun *et al*. 2013) (Singer *et al*. 2013, Fredriksson *et al*. 2003, Bjarnadottir *et al*. 2007, Hu *et al*. 2014, Paavola & Hall 2012) (Fig. 1A and 1B and Fig. S3D). Many adhesion GPCRs are processed into two subunits after cleavage at their GPS (Lin *et al*. 2004, Hsiao *et al*. 2011, Hu *et al*. 2014, Arac *et al*. 2012). In recent years, cellular, physiological and genetic studies have revealed that many adhesion GPCR members, such as CELSRs, VLGR1/GPR98 and HE/GPR64, have important functions for neuronal system development, male fertility and other physiological processes. (Paavola *et al*. 2011, Bae *et al*. 2014, Li *et al*. 2008, Jin *et al*. 2007, Boutin *et al*. 2012, Curtin *et al*. 2003, McGee *et al*. 2006, Davies *et al*. 2004, Piao *et al*. 2004).

The CELSR family, whose name is derived from cadherin EGF LAG seven-pass G-type receptor, is a special subgroup of adhesion GPCRs because proteins of this family have cadherin repeats at their far N-termini. These proteins are involved in important functions during development, and genetic mutations within this receptor family are related to human diseases. Evolutionarily, CELSRs emerged early compared with most other adhesion GPCRs, and the discovery of a *Drosophila* counterpart, *Flamingo (fmi)*, has facilitated their genetic study (Usui et al. 1999, Langenhan et al. 2013). The initial characterization of the functions of *fmi* revealed important roles for CELSRs in cell adhesion and cell-cell contacts (Usui *et al*. 1999, Nishimura *et al*. 2012, Formstone 2010). Accordingly, recent studies have demonstrated essential roles for all three members of the human *CELSR* family (*CELSR1*, *CELSR2* and *CELSR3*) in vertebrate planar cell polarity (PCP) during embryonic neuronal development and in cell rearrangement during vessel valve formation (Formstone & Mason

2005, Nishimura et al. 2012, Tatin *et al*. 2013, Curtin et al. 2003, Doudney & Stanier 2005, Devenport & Fuchs 2008, Ravni *et al*. 2009, Yates *et al*. 2010b, Wu *et al*. 2011, Feng *et al*. 2012a, Boutin et al. 2012, Tissir *et al*. 2010, Qu *et al*. 2010, Zhou *et al*. 2009, Wada *et al*. 2006, Lewis *et al*. 2011, Feng *et al*. 2012b). Human genetic analysis has also shown that mutations in *CELSR1* and *CELSR2* are associated with neural tube defects (NTDs) and cardiovascular disease (CVD), respectively (Doudney & Stanier 2005, Allache *et al*. 2012, Juriloff & Harris 2012, Yates *et al*. 2010a, Robinson *et al*. 2012, Adams *et al*. 2014, Talmud *et al*. 2009, McVey *et al*. 2014, Arvind *et al*. 2014, Samani *et al*. 2008, Waterworth *et al*. 2010, Saade *et al*. 2011, Braun *et al*. 2012). Despite these progresses in CELSR research, our knowledge of these receptors is limited; indeed, CELSRs have known functions in only several physiological processes, despite their broad distribution, and the roles of CELSRs at the molecular level and their regulatory mechanisms remain elusive. In this mini-review, we discuss the current understanding of CELSRs, addressing the gene and protein structures of CELSRs, the tissue distribution of CELSRs, the known functions of CELSRs in neuronal, pancreas and intraluminal valve formations, the genetic association of *CELSRs* with diseases and potential CELSR signaling properties, all of which may inform future CELSR research.

1. The Cloning and Gene Structure of CELSRs

Celsr1 was first identified in mice as an orphan seven transmembrane domain receptor (Hadjantonakis *et al*. 1997). Because of its large size and low abundance, only partial cDNAs of *Celsr1* containing transmembrane domain 2 to the C-terminus and an additional 878 bp in the 3′ untranslated region were initially cloned from a mouse embryonic cDNA library at 8.5 days post-coitum. The cloned partial mouse *Celsr1* (mm*Celsr1*) cDNA (ME2 clone) was used to map its gene location on mouse chromosome 15 (Fig. S1B). The mouse *Celsr1* ME2 clone was then used to screen and isolate human *CELSR1* cosmid clones. The human *CELSR1 (hsCELSR1)* locus was mapped to chromosome 22q13.3 by fluorescence in situ hybridization (FISH) using these human *CELSR1* cosmid clones (Fig. S1A). The human *CELSR1* gene spans 177 kb and is organized into 35 exons; the mouse *Celsr1* gene spans 135 kb and has 37 exons (Fig. S1C and S1D). Two years after the identification of Celsr1 in mice, a *Celsr* ortholog from *Drosophila* was independently cloned and functionally characterized by two research labs, who named this ortholog *Flamingo (fmi)* and *Starry night (stan)* (Usui *et al*. 1999, Chae *et al*. 1999). The reported translational product of *fmi* encodes 3,575 amino acids, and *stan* mRNA is more than 12 kb in length and encodes 3,579 putative amino acids.

When *Celsr1* was identified, a search for proteins containing EGF-like domains resulted in the cloning of partial cDNAs from two other *Celsr* family members, *Celsr2* and *Celsr3*, which were named MEGF2 and MEGF3, respectively (Nakayama *et al*. 1998). Human *CELSR2* was later mapped to human chromosome 1p21-.13 (3.05cR from CHLC.GCT8C07), which spans 26 kb and is organized into 34 exons, whereas human *CELSR3* is located on chromosome 3p21 (1.61cR from WI-7862, lod > 3), which encompasses 27 kb and has 35 exons (Fig. S1A and S1C) (Formstone *et al*. 2000). Mouse *Celsr2* is located on chromosome 3 F3, which spans 25 kb and is organized into 35 exons, and mouse *Celsr3* is located on chromosome 9 F2, which spans 27 kb and is organized into 37 exons (Fig. S1B and S1D).

2. Deduced CELSR Protein Structures

All CELSRs contain a hepta-helical transmembrane region and a large extracellular domain and belong to the adhesion GPCR subfamilies. Human full-length CELSR1 has an N-terminal extracellular domain of 2,465 amino acids and a seven transmembrane domain at its C terminus. As a cadherin EGF LAG seven-pass G-type receptor, the N-terminus of CELSR1 contains nine atypical cadherin repeats, six epidermal growth factor (EGF)-like domains, two laminin G (LAG) domains, and one hormone receptor motif (HormR) close to the GPS (Fig. 1A, 1B).

The N-terminal end of CELSRs contains nine cadherin repeats (110 residues, also called extracellular cadherin (EC) domains), which have signature DXD, DRE, or DXNDNAPXF motifs and are responsible for extracellular calcium binding (Fig. 1A, 1B) (Takeichi 1990, Shapiro & Weis 2009). The crystal structures of several classical cadherin ecto-domains and fragments have been previously solved (Caddy *et al*. 2010, Allache *et al*. 2012). In these structures, the cadherin repeat monomer is composed of seven beta strands and is similar to immunoglobulin constant domains (Overduin *et al*. 1996, Overduin *et al*. 1995) (Fig. S3A). Connections between successive cadherin repeats require calcium coordination (Georgieva *et al*. 2003, Tissir *et al*. 2002a). Early experiments showed that calcium promotes the tissue-like clump aggregation of cadherin-expressing cells, and recent FRET results have shown trans-dimer formation (between cells) of two individual cadherin pairs (Shima *et al*. 2002, Takeichi *et al*. 1981, Zhang *et al*. 2009). By contrast, cadherins may form weak cis-dimers (interactions between molecules within the same cell) without calcium. Therefore, cadherin repeat structures provide a likely structural mechanism for calcium-regulated cadherin interaction. However, despite potential trans-cadherin interaction-mediating CELSR2/3 signaling, the structures of CELSRs cadherin repeats have not been resolved, and calcium-regulated cis/trans-dimerization has not yet been investigated in detail (Shima *et al*. 2007).

After the nine cadherin repeats in CELSRs, there are five calcium-binding EGF domains (EGF-CA) separated by two laminin G domains (Fig. 1A, 1B and S2). Each EGF-CA domain has approximately 40 amino acids, and the laminin G domain has a jelly roll fold structure (Hohenester *et al*. 1999) (Fig. S3B). Both of these domains are also potential calcium receivers. One EGF-Lam domain in CELSR1/2 and two EGF-Lam domains in CELSR3 follow the EGF-CA and laminin G domains. The EGF-Lam domain is approximately 60 amino acids in length and has eight conserved cysteines. There is a hormone receptor motif (HormR) at the C-terminus of these repeats (Arac *et al*. 2012) (Fig. 1A and S3C). The HormR has two conserved tryptophans and several conserved cysteines. In addition to the N-terminal adhesion domains, HormR is often observed in the adhesion GPCR family and is occasionally regarded as a potential ligand binding domain (Paavola & Hall 2012, Arac *et al*. 2012). HormR deletion in the *Drosophila* CELSR homolog *fmi* results in a loss of receptor function (Tissir *et al*. 2002b). However, all adhesion GPCR HormRs lack an N-terminal α-helix, which is an essential element for peptide and hormone interactions with B1/Secretin-like GPCRs, such as the GLP-1 receptor (Underwood *et al*. 2010, Runge *et al*. 2008). Whether the HormR of CELSRs participates in ligand/hormone interaction is unknown and requires further investigation.

The GAIN domains of CELSRs follow their HormR regions (Fig. 1A and 1B and S3D). In vitro, several adhesion GPCRs are processed into two fragments at their GPS via a cis-auto hydrolysis mechanism, in which a conserved T/S/C functions as a nucleophile and a conserved H serves as a general base during proteolysis (Lin *et al*. 2004, Hsiao *et al*. 2011, Hu *et al*. 2014, Arac *et al*. 2012). Interestingly, only human CELSR2 has the general base H2355 and nucleophile T2357, whereas the conserved nucleophile is replaced by A in hsCELSR1 and G in hsCELSR3, and the general base H is replaced by R in hsCELSR3 (Fig. 2A). Therefore, whether hsCELSRs are separated into different subunits through auto-proteolysis or regulated by proteases in physiological contexts requires additional experimentation.

After the GPS of CELSRs, there is a seven transmembrane helical region (Fig. 1A, 1B and 2B). A conserved Cys pair from the first extracellular loop and second extracellular loop (C2531 and C2603 from hsCELSR1; C2439 and C2511 from hsCELSR2; and C2602 and C2674 from hsCELSR3) can form a disulfide bond to stabilize the extracellular structure of CELSRs for efficient ligand binding or conformational change (Venkatakrishnan *et al*. 2013) (Fig. 2B). Disulfide bonds broken by C-A mutations have been reported to decrease ligand binding and inactivate GPCRs, such as the gonadotropin-releasing hormone receptor (Gross *et al*. 2001)

After the extracellular region, the seven transmembrane helix is the hub in the key lock model of CELSR, in which the extracellular signal is transduced to the cytoplasmic region of the CELSR by conformational change. Several residues, including the conserved P2618 in TM5 and W2668 in the TM6 of hsCELSR1 (P2526 and W2576 in hsCELSR2; P2689 and W2738 in hsCELSR3), corresponding to $P211^{5.50}$ and the toggle switch $W286^{6.48}$ of the β2-adrenergic receptor, may have important functions during such conformational changes (Lefkowitz *et al*. 2008, Cherezov *et al*. 2007, Rosenbaum *et al*. 2007, Rasmussen *et al*. 2007) (Fig. 2B). Next to the trans-membrane region, the intracellular part of CELSRs serves as a docking point for downstream effectors in the cytoplasm and includes intracellular loops 1-3 and the C-tail. The third intracellular loop of CELSRs contains approximately 20 amino acids, which provide structural flexibility for the receptor (Fig. 2B). After the seventh helix, CELSRs have different cytoplasmic tail lengths. Whereas hsCELSR1 and hsCELSRS2 have 304 and 305 amino acids, respectively, in their C-termini, hsCELSR3 has a longer C-tail containing 532 amino acids. All CELSR C-termini are enriched with Ser, Thr, Pro and acidic residues, such as Glu and Asp. The cytoplasmic region of CELSR1 contains multiple potential phosphorylation sites and SH3 binding motifs because of its enriched Ser and Pro. Moreover, each CELSR has several predicted PEST regions that may regulate receptor turnover in the cell (Meyer *et al*. 2011). Cadherins reportedly undergo degradation once the PEST region is exposed following dissociation from its binding partners (Huber *et al*. 2001, Singh *et al*. 2006). Whether a similar regulatory mechanism occurs with CELSRs requires further investigation.

3. Tissue Distribution

The original characterization of the *Celsr Drosophila* counterpart *fmi* identified its expression in the epithelium and nervous systems of both embryos and imaginal tissues

(Usui *et al*. 1999). In mammals, *Celsr*1, *Celsr*2 and *Celsr3* are ubiquitously expressed in many tissues (Table 1 and Fig. S4). During development, *Celsr1-3* expression is regulated spatially and temporally, suggesting that they have distinct functions. The complementary pattern of *Celsr1* and *Celsr3* expression is an interesting feature in different developmental systems.(Tissir *et al*. 2002, Formstone & Little 2001, Shima *et al*. 2002).

Celsr1-3 is broadly expressed in the nervous system from embryonic and early post-natal development until the adult stage (Formstone *et al*. 2000, Hadjantonakis *et al*. 1997, Tissir *et al*. 2002, Devenport & Fuchs 2008, Tissir & Goffinet 2006). All three members are expressed in the brain and spinal cord (Tissir *et al*. 2002b), cochlea (Shima *et al*. 2002, Curtin *et al*. 2003, Montcouquiol *et al*. 2008), and pigment cells of the eye (Shima *et al*. 2002) (Fig. S4). During development, *Celsr* members exhibit different expression patterns in the brain. The mRNA expression of *Celsr1* is primarily confined to areas of neural stem cell (NSC) proliferation, including the ventricular zones during brain development, telencephalic ependymal zones and the subgranular layer of dentate gyrus in the adult brain (Boutin *et al*. 2012). By contrast, *Celsr3* is primarily expressed in post-mitotic neural cells but not in neural stem cells (Boutin *et al*. 2012). The pattern of *Celsr2* expression overlaps with that of *Celsr1* and *Celsr3*, which has been observed in both NSC and post-mitotic neural cells (Boutin *et al*. 2012). The expression of *Celsr1* is reduced by decreasing the number of NSCs during early postnatal development. Although *Celsr3* expression is downregulated postnatally, it is still present in the cerebellar granular layer, the hilus of the dentate gyrus, the rostral migratory stream and the central regions of the olfactory bulb. Recent studies have also shown that *Celsr3* is expressed during normal development of the ON visual pathway circuitry in the inner retina (Lewis *et al*. 2011). In contrast with the dynamic change in *Celsr1/3* expression, *Celsr2* expression remains stable throughout life

Celsr1-3 expression is also observed in other non-neural systems, such as the skin, lungs, heart, kidney, and spleen, as well as the digestive and reproductive systems (Fig. S4B-S4D) (Formstone *et al*. 2000, Shima *et al*. 2002, Yates *et al*. 2010, Zhang *et al*. 2011). In the rodent testis, *Celsr3* is expressed exclusively in post-mitotic germ cells, whereas *Celsr1* and *Celsr2* are expressed in Sertoli cells, with persistent post-natal down-regulation of *Celsr1* and *Celsr2* in adults (Fig. S4C) (Beall *et al*. 2005, Johnson *et al*. 2000). The loss of *Celsr2* and *Celsr3* function in the developing pancreas reduces insulin-producing β-cell differentiation from endocrine progenitors (Fig. S4C). (Cortijo *et al*. 2012). CELSR1 participates in the development of skin hair by interacting with other PCP proteins (Fig. S4D) (Ravni *et al*. 2009,Devenport & Fuchs 2008). CELSR1 and the PCP protein VANGL2 were also important in lymphatic vessels in regulating adherens junctions and directing cell rearrangements during intraluminal valve formation (Fig. S4D) (Tatin *et al*. 2013). Both *Celsr2* and *Celsr1* are expressed in whiskers; however, *Celsr3* transcripts are not detected in whiskers (Fig. S4A)

At the protein level, only CELSR1 has been characterized because of a lack of specific CELSR2/3 antibodies. CELSR1 protein expression has been demonstrated in hair germ cells and the epidermal cells of embryonic skin (Formstone & Little 2001), as well as the floor and roof plates of the hindbrain and spinal cord (Hadjantonakis *et al*. 1998). Interestingly, both full-length CELSR1 (p400) and a cleaved CELSR1 fragment (p85) have been detected

by the CELSR1-specific antibody. This CELSR1 fragment is not produced by auto-cleavage at the GPS but may act through an uncharacterized proteolytic process (Hadjantonakis *et al*. 1998).

4. The Physiological Actions of CELSRs

(1) The physiological actions of CELSRs in the nervous system—CELSRs have been characterized as critical regulators of neuronal migration, dendrite growth and axon guidance, in addition to vertebrate PCP and neuronal tube closure. The importance of CELSRs in facial branchiomotor (FBM) migration was first revealed by an ENU (N-ethyl-N-nitrosourea (chemical formula: C3H7N3O2)) mutagenesis screen for alterations in FBM migration in zebrafish, which led to the identification of four mutations in the *celsr2/off road* locus (Wada *et al*. 2006). Compared with their wild-type counterparts, the FBM neurons in c*elsr2* mutants are unable to migrate caudally to r6. The dysfunction in FBM migration of *celsr2* zebrafish mutants is phenocopied by *Celsr2*$^{Dgen/Dgen}$ mutant mice. The conditional inactivation of *Celsr2* in FBM neurons under *Isl1-Cre* causes a phenotype similar to that of *Celsr2*$^{Dgen/Dgen}$ mutant mice, indicating that specific CELSR2 signaling inside the FBM cells directly regulates FBM neuron migration. By contrast, although the conditional inactivation of *Celsr1* with *Isl1-Cre* does not cause apparent migrational dysfunction, the specific inactivation of *Celsr1* in progenitors by *Nk6.2-Cre* causes abnormal rostral migration (Qu *et al*. 2010). Although *Celsr3* deficiency does not impair the ability of FBM neurons to migrate, *Celsr3* is an important player in the tangential movement of caltetinin-positive interneurons and the radial movement of calbindin-positive interneurons (Formstone & Mason 2005). Moreover, *Celsr3*$^{ko/ko}$ and *Celsr2*$^{Dgen/Dgen}$ double-mutant mice exhibit an exacerbation of the phenotype of *Celsr2*$^{Dgen/Dgen}$ mutant mice, which are phenocopies of *Fzd3* mutant mice (Qu et al. 2010, Qu *et al*. 2014). These results suggest that *Celsr1-3* are coordinated to regulate FBM neuron migration during development. Whereas *Celsr1* has an important function in guiding the direction of FBM neuron migration, *Celsr2* and *-3* may operate together to control the ability of FBM cells to migrate (Qu *et al*. 2010).

In addition to their key functions in FBM cell migration, *Celsr2* and *Celsr3* also play important roles in dendrite development and axon guidance (Boutin et al. 2012). Flamingo mutant flies exhibit impaired reciprocal inhibition of dendritic growth from homologous neuroblasts of the opposite hemisegment (Gao et al. 2000, Boutin et al. 2012). In mammals, RNAi-mediated *Celsr2* knockdown results in simplified dendritic trees of Purkinje neurons and a decreased cortical pyramidal neuron length (Shima et al. 2004). By contrast, *Celsr3* regulates neurite growth in an opposing manner (Shima et al. 2007). *Celsr3* knockdown with RNAi increases rather than decreases dendrite length. In addition to its inhibitory role in dendrite growth, *Celsr3* is essential for axon guidance. *Celsr3* mutant mice exhibit multi-faceted defects in the development of axonal tracts and anterior-posterior guidance defects in the brainstem (Tissir et al. 2005, Fenstermaker et al. 2010). Regional inactivation of *Celsr3* further demonstrates the requirement for functional *Celsr3* in axonal projections to the anterior commissure and sub-cerebral targets (Zhou et al. 2008). In the peripheral nervous system, *Celsr3*-deficient axons fail to respond to ehrinA-EphA forward signaling (Chai et al. 2014). Taken together, the results demonstrate that *Celsr2* is important in the

dendrite length in pyramidal neurons and the complexity of the Purkinje neurons and that *Celsr3* is essential in many steps of axon guidance and plays important roles in brain wiring.

Accumulating evidence has also demonstrated that *Celsr1* is a key regulator of vertebrate PCP and neuronal tube closure. A chick *Celsr1/fmi-1* ortholog, *c-fmi1*, was shown to play a conserved role in neural tube closure and additional roles in asymmetry and somitogenesis (Formstone & Mason 2005). By using ENU mutagenesis experiments to identify mice that exhibit head-shaking behavior, belly curling and spinning during tail suspension, researchers have identified two heterozygous *celsr1* mutants (*celsr1*^{crsh} and *celsr1*$^{Sc\gamma}$) that have defects in the orientation of the sensory hair cells of the cochlea, which is a typical phenotype of PCP (Curtin *et al*. 2003). Both homozygous *celsr1*^{crsh} and *celsr1*$^{Sc\gamma}$ mice displayed several defects in neural tube closure that lead to perinatal lethality. Six *Celsr1* mutations were then identified in human fetuses that have the severe neural tube defects called craniorachischisis (CRN) (Robinson *et al*. 2012). All of these *Celsr1* mutations have been shown to affect CELSR1 protein trafficking to the plasma membrane in vitro. Two novel *Celsr1* mutations were also recently associated with spina bifida (Lei *et al*. 2014).

A detailed mechanistic study suggested that CELSR1 regulates the polarized bending of the neural plate by coordinating with Dishevelled, DAAM1 and PDZ-RhoGEF to increase Rho kinase activity (Fig. 3) (Nishimura *et al*. 2012). Up-regulated Rho kinase activity is essential for actomyosin-dependent contraction in a planar-polarized manner and promotes the simultaneous apical constriction and midline convergence of neuroepithelial cells. Moreover, recently published data have suggested that two distinct PCP signals operate in brain ciliated cells. The first signal, which is coordinated by *Celsr 2/3, Fzd3* and *Vangl2*, organizes the single-cell polarity of individual cells; the second signal, which is governed by *Celsr1, Fzd3*, and *Vangl2*, regulates tissue polarity in both radial progenitors and ependymal cells (Boutin *et al*. 2014). Using conditional *Celsr2* and *-3* mutants, another recent study suggested that *Celsr2* and *Celsr3* function redundantly to regulate axonal guidance in the prosencephalon using mechanisms other than classical epithelial PCP (Qu et al. 2014)

(2). The essential roles of *Celsr2* and *Celsr3* in pancreas β cell differentiation and glucose homeostasis—During pancreas development, mRNA transcripts of *Celsr1* and *Celsr3* are detected in the pancreatic bud at E11.5, and all three *CELSRs* were broadly expressed in the progenitors, exocrine cells and endocrine cells of the pancreatic epithelium at E14.5 using in situ hybridization (Cortijo *et al*. 2012). A constitutive loss of *Celsr3* in *celsr3* KO mice led to reduced insulin-containing β cells after E14.5. The essential role of *Celsr3* in pancreatic β cell differentiation from progenitor cells is not due to its function in the nervous system but rather its function in the pancreas because specifically inhibiting *Celsr3* expression in epithelial pancreas progenitor cells by crossing *Celsr3* $^{flox/flox}$ mice with *Pdx1-cre* mice reproduced the phenotype of *Celsr3* KO mice. The capacity to clear glucose was decreased in *Celsr3* $^{flox/flox}$*/Pdx1-cre* mice and compared with *Celsr3*$^{flox/flox}$ mice, was accompanied by decreased numbers of adult islet β cells (Cortijo *et al*. 2012). Therefore, *Celsr3* is an essential gene in pancreatic β cell development and appropriate glycemic control.

Celsr2 mRNA is significantly increased in the pancreatic buds of Celsr3 KO mice at E14.5. In Celsr2/Celsr3 DKO mice, a dramatic decrease in cells that produce glucagon (α cells), insulin (β cells), ghrelin, polypeptide (pp cells) and somatostatin (δ cells) is observed after E14.5 (Cortijo et al. 2012). The loss of both Celsr2 and -3 results in reduced JNK substrate c-JUN phosphorylation, and the JNK agonist anisomycin increases pancreatic β-cell differentiation in Celsr3 KO mice. These results suggest that Celsr2 and Celsr3 might have redundant functions in pancreas development and promote endocrine cell differentiation by regulating JNK pathways.

(3). *Celsr1* function in the intraluminal valve formation of lymphatic vessels— In developing lymphatic valves, endothelial cells profoundly change their shape and polarity and undergo rearrangement to form intraluminal leaflets that require precise regulation at multiple levels. Along with valve initiation, the expression of PCP protein CELSR1 is induced around $Prox1^{high}$ cell clusters at E16.5 and then in endothelial cells intersecting with the flow directions at E17.5 and valve leaflets at E18.5 (Tatin *et al*. 2013). During cell rearrangement, both CELSR1 and its interacting protein VANGL2 are recruited from the filopodia to discrete plasma membrane micro-domains at cell-cell contacts. Both $Celsr1^{Crsh}$ mutant mice and $Celsr1^{flox/flox}$:*Prox1-*Cre^{ERT2} embryos exhibit abnormal organization in their lymphatic valves. By contrast, $Celsr2^{-/-}$ and $Celsr3^{flox/flox}$:*Prox1-*Cre^{ERT2} embryos have normal valves. Further mechanistic studies have suggested that *Celsr1* plays a pivotal role during lymphatic endothelial cell rearrangement by regulating the stabilization of VE-cadherin and the maturation of the adherens junctions (Tatin *et al*. 2013). These results demonstrate the essential role of *Celsr1* in directing cell rearrangements during intraluminal valve formation in lymphatic vessels.

5. Pathological Action in Relation to Diseases

In humans, mutations in the *CELSR1-3* genes cause many diseases, such as neural tube defects in the nervous system, coronary heart disease and tumors (Table 2). As the key regulator of vertebrate PCP, CELSR1 has been characterized as an essential gene in the process of neural tube closure by studies using different animal models (Curtin *et al*. 2003). Consistently, multiple *CELSR1* mutations have been identified as rare mutations in the neural tube defects (NTDs), one of the most severe central nervous system diseases at birth (Allache *et al*. 2012, Robinson *et al*. 2012, Juriloff & Harris 2012, Doudney & Stanier 2005). Among the identified mutations of *CELSR1* associated with NTDs, 30 mutations occur in the extracellular domain, including R541W, V551M, Q757R, A773V, Q834X, R836C, G934R and V1008L in the protocadherin repeats; V1244I after the protocadherin repeats; D1365N, R1886H, D1889N, and P1976T in the EGF-like domain; D1401G, T1443P, R1456Q, R1526W, G1628R, S1726G, and R1835C in the laminin G-like domain; A2075V, R2121C, S2190L, A2228V, N2230S, R2312P, A2339T, P2352S, and R2359C between the last laminin EGF-like domain and the GPS; and R2438Q close to the GPS (Table 2 and Fig. 4) (Allache *et al*. 2012, Robinson *et al*. 2012). Five *CELSR1* mutations (N2739T, S2964L, P2937L, R2949Q and S2963-T2966 del) have been observed in the C-terminal cytoplasmic tails. The S2963-T2966 del mutation has a deleted SSR motif, which is a potential PKC phosphorylation site of CELSR1, indicating that kinase-regulated signals are potentially impaired in this special mutant.

Non-coding genetic mutations of *CELSR2* are associated with cardiovascular disease (CVD) (Qi *et al*. 2011, Waterworth *et al*. 2010, Jeemon *et al*. 2011, Samani *et al*. 2008, Samani *et al*. 2007, Kathiresan *et al*. 2008, Nakayama *et al*. 2009) (Table2). A genetic mutation at the 3' untranslated regions of *CELSR2* (rs660240) is associated with circulating LDL levels and coronary artery disease (CAD) (Waterworth *et al*. 2010). Other non-coding genetic variants in an intergenic region between the *CELSR2* and *PSRC1* genes (rs646776 and rs599839 in the CELSR2-PSRC1-SORT1 locus) are associated with reduced LDL-c levels, CAD and MI risk in Hispanic individuals and cardiovascular disease in type 2 diabetes mellitus (T2DM) (Virtanen *et al*. 2012, Chow *et al*. 2012, Samani *et al*. 2007, Sandhu *et al*. 2008, Arvind *et al*. 2014, Qi *et al*. 2011, Kathiresan *et al*. 2008, Nakayama *et al*. 2009). However, the functional relevance of *CELSR2* in lipid metabolism and cardiovascular disease has not been well described and awaits further investigation.

The PCP pathway also plays vital roles in carcinogenesis. As the core components of PCP, CELSRs have also been observed in different tumors (Table 2). Aberrant DNA methylation of the *CELSR1* gene CpG loci has been identified in hepatocellular carcinomas (Ammerpohl *et al*. 2012), and the downregulation of *CELSR1* has been identified in cases of non-nodal mantle cell lymphoma (Del Giudice *et al*. 2012). In contrast, both *CELSR1* and several other PCP pathway components, such as *VANGL2*, *PRICKLE1*, *FRIZZLED3/7* and *DISHEVELLED2/3*, are up-regulated in B lymphocytes of chronic lymphocytic leukemia patients (Kaucka *et al*. 2013). Considering the specific temporal and spatial regulatory roles of different *CELSR* members during embryonic development, *CELSRs* may be correlated with the progression of specific tumor types at particular stages. Further evaluation of *CELSR* members in different tumor types and tumor stages is required.

6. Potential Signaling

In the fruit fly, the human *CELSR* orthologs *fmi* and *stan* have been found to functionally interact with key PCP proteins, including the seven transmembrane receptors *Frizzled*, tetraspanin Van Gogh (vang), Dishevelled (dsh), Prickle (pk), and Diego (dgo), and probably other, less clearly identified, members (Boutin et al. 2012). In mammals, *Celsr* works together with Frizzled3 and -6, Dishevelled1 and -2 and Vangl1 and -2 (Fig. 3) (Boutin *et al*. 2012). Downstream of CELSRs, CELSR1 may regulate Rho kinase activity through Disheveled, DAAM1 and PDZ-RhoGEF during the polarized bending of the neural plate (Fig. 3) (Nishimura *et al*. 2012), whereas *Celsr*2 and -3 may regulate the JNK pathway and the phosphorylation status of c-Jun during pancreatic development (Cortijo *et al*. 2012). During neurite growth, the activation of *Celsr2/3* increases intracellular calcium through a PLC activity-dependent pathway (Shima *et al*. 2007). However, the signaling events triggered by *CELSRs* activation or inactivation have not been studied in detail. One potential cause is the lack of efficient in vitro research tools, such as selective compounds that specifically activate or inactivate *Celsr* members.

Future directions

The three *CELSRs* are broadly expressed in neuronal, digestive and reproductive systems and in the skin, lungs, heart, and other tissues (Table 1 and Fig. S4). The functional roles of

CELSRs have been extensively studied in neuronal systems by using specific KO models. For example, homozygotes of *Celsr3* KO mice perish just after birth and present severe axonal defects (Zhou *et al*. 2008, Tissir *et al*. 2005). The homozygotes of $Celsr^{Crsh}$ and $Celsr^{Sc\gamma}$ mutants and 20% of the homozygotes of *Celsr1* KO mice are embryonic lethal with defects in neural tube closure (Devenport & Fuchs 2008, Curtin *et al*. 2003, Yates *et al*. 2010b, Ravni *et al*. 2009). *Celsr2* KO mice also exhibited abnormal FBM neuron migration (Qu *et al*. 2010, Tissir *et al*. 2010). The elucidation of specific roles for each *Celsr* member in tissues other than the neuronal system is blocked by the severe defects of *CELSRs* KO mice because most functions and tissue development are controlled by neuronal activity or interact extensively with the nervous system. By using specific *Celsr* conditional KO mice, the functional roles of *Celsr2/3* in pancreatic development and *Celsr1* in intraluminal valve formation have been recently characterized (Cortijo *et al*. 2012, Tatin *et al*. 2013). In the future, specific *Celsr* roles in different tissues/organs may be elucidated by combining these $Celsr^{flox/flox}$ mice with tissue-specific Cre mice.

Although the functional importance of *CELSRs* during tissue-level development has been appreciated following these recent animal studies, the explicit roles of *CELSRs* at the molecular and cellular levels remain elusive. CELSRs belong to the seven transmembrane receptor (GPCR) superfamily, which consists of the largest group of transmembrane proteins that sense extracellular information and transduce the signals inside the cells to initiate intracellular responses (Chai et al. 2014, Zhang & Xie 2012, Wang *et al*. 2014). CELSRs have large ecto-domains that provide a platform for multiple ligands or protein interactions; however, no explicit small ligands have been clearly demonstrated as either natural or artificial ligands for CELSRs. Previous studies have suggested that CELSR2 and CELSR3 elicit downstream signaling through selective homophilic binding but exhibit the opposite functions during neurite growth (Shima *et al*. 2007). Therefore, the ecto-domain of a CELSR from one cell may be the ligand for the CELSR from a neighboring cell. In that case, the homophilic CELSR ecto-domain interaction region should fold and remain intact to form specific trans-interactions, excluding non-specific cis-homophilic interactions that could induce unwanted signaling cascades. Direct evidence and mapping of the specific interface are required to confirm that one ecto-domain of the CELSR is the ligand for other CELSRs. The homophilic interaction of the large ecto-domain of CELSRs does not exclude the possibility that CELSRs interact with other adhesion molecules, most of which also contain multiple EGF or cadherin repeats. A detailed biochemical examination of CELSR homophilic interactions and potential CELSR interactions with other adhesion molecules should be undertaken to define the natural ligands of CELSRs.

CELSRs belong to the adhesion GPCR subfamily (or LNB7TM GPCR subfamily), which all contain a GPS in their GAIN domain (Singer *et al*. 2013, Arac *et al*. 2012, Sun *et al*. 2013). Several GPCRs, including GPR56, BAI1 and VLGR1, have been shown to enhance their constitutive activity after removing their N-terminal regions following GPS cleavage (Paavola & Hall 2012, Paavola *et al*. 2011, Hu *et al*. 2014). In recent studies, CELSR2 and CELSR3 were uncleaved after their exogenous transfection in cells (Shima et al. 2007, Lin et al. 2004, Sun et al. 2013). However, this finding does not exclude the possibility that

CELSRs could be separated into different fragments in certain physiological or pathological contexts.

Previous studies have revealed that CELSR1 may regulate Rho kinase activity inside the cells and that CELSR2 and CELSR3 may interfere with the JNK pathway and control the phosphorylation status of c-Jun. However, it is not clear whether CELSRs elicit signaling through G proteins, such as other adhesion GPCRs, including GPR56 and LEC1, or through non-classical G protein signaling pathway, such as Smoothened or Frizzled (Paavola & Hall 2012). In a study on the functional role of *Celsr2/3* in neurite growth, applying CELSR recombinant cadherin repeats (CELSR2-CR and CELSR3-CR) significantly increased the concentration of intracellular calcium, which is specifically blocked by the PLC inhibitor U73122 (Shima *et al*. 2007). These results indicated that CELSR 2/3 may signal through Gq coupling. However, the CELSR2-CR-/CELSR3-CR-induced intracellular calcium increase is much slower than with typical Gq-coupled GPCRs. Therefore, further studies are required to characterize the specific G protein coupling and explicit downstream signaling cascades of CELSRs.

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References

Adams JN, Raffield LM, Freedman BI, Langefeld CD, Ng MC, Carr JJ, Cox AJ, Bowden DW. Analysis of common and coding variants with cardiovascular disease in the Diabetes Heart Study. Cardiovasc Diabetol. 2014; 13:77. [PubMed: 24725463]

Allache R, De Marco P, Merello E, Capra V, Kibar Z. Role of the planar cell polarity gene CELSR1 in neural tube defects and caudal agenesis. Birth Defects Res A Clin Mol Teratol. 2012; 94:176–181. [PubMed: 22371354]

Ammerpohl O, Pratschke J, Schafmayer C, et al. Distinct DNA methylation patterns in cirrhotic liver and hepatocellular carcinoma. Int J Cancer. 2012; 130:1319–1328. [PubMed: 21500188]

Arac D, Boucard AA, Bolliger MF, Nguyen J, Soltis SM, Sudhof TC, Brunger AT. A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. EMBO J. 2012; 31:1364–1378. [PubMed: 22333914]

Arvind P, Nair J, Jambunathan S, Kakkar VV, Shanker J. CELSR2-PSRC1-SORT1 gene expression and association with coronary artery disease and plasma lipid levels in an Asian Indian cohort. J Cardiol. 2014

Bae BI, Tietjen I, Atabay KD, et al. Evolutionarily dynamic alternative splicing of GPR56 regulates regional cerebral cortical patterning. Science. 2014; 343:764–768. [PubMed: 24531968]

Beall SA, Boekelheide K, Johnson KJ. Hybrid GPCR/cadherin (Celsr) proteins in rat testis are expressed with cell type specificity and exhibit differential Sertoli cell-germ cell adhesion activity. J Androl. 2005; 26:529–538. [PubMed: 15955893]

Bjarnadottir TK, Fredriksson R, Schioth HB. The adhesion GPCRs: a unique family of G protein-coupled receptors with important roles in both central and peripheral tissues. Cell Mol Life Sci. 2007; 64:2104–2119. [PubMed: 17502995]

Boutin C, Goffinet AM, Tissir F. Celsr1-3 cadherins in PCP and brain development. Curr Top Dev Biol. 2012; 101:161–183. [PubMed: 23140629]

Boutin C, Labedan P, Dimidschstein J, et al. A dual role for planar cell polarity genes in ciliated cells. Proc Natl Acad Sci U S A. 2014; 111:E3129–3138. [PubMed: 25024228]

Braun TR, Been LF, Singhal A, et al. A replication study of GWAS-derived lipid genes in Asian Indians: the chromosomal region 11q23.3 harbors loci contributing to triglycerides. PLoS One. 2012; 7:e37056. [PubMed: 22623978]

Caddy J, Wilanowski T, Darido C, et al. Epidermal wound repair is regulated by the planar cell polarity signaling pathway. Dev Cell. 2010; 19:138–147. [PubMed: 20643356]

Chae J, Kim MJ, Goo JH, Collier S, Gubb D, Charlton J, Adler PN, Park WJ. The Drosophila tissue polarity gene starry night encodes a member of the protocadherin family. Development. 1999; 126:5421–5429. [PubMed: 10556066]

Chai G, Zhou L, Manto M, Helmbacher F, Clotman F, Goffinet AM, Tissir F. Celsr3 is required in motor neurons to steer their axons in the hindlimb. Nat Neurosci. 2014; 17:1171–1179. [PubMed: 25108913]

Cherezov V, Rosenbaum DM, Hanson MA, et al. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science. 2007; 318:1258–1265. [PubMed: 17962520]

Chow KB, Sun J, Chu KM, Tai Cheung W, Cheng CH, Wise H. The truncated ghrelin receptor polypeptide (GHS-R1b) is localized in the endoplasmic reticulum where it forms heterodimers with ghrelin receptors (GHS-R1a) to attenuate their cell surface expression. Mol Cell Endocrinol. 2012; 348:247–254. [PubMed: 21903149]

Cortijo C, Gouzi M, Tissir F, Grapin-Botton A. Planar cell polarity controls pancreatic beta cell differentiation and glucose homeostasis. Cell Rep. 2012; 2:1593–1606. [PubMed: 23177622]

Curtin JA, Quint E, Tsipouri V, et al. Mutation of Celsr1 disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. Curr Biol. 2003; 13:1129–1133. [PubMed: 12842012]

Davies B, Baumann C, Kirchhoff C, Ivell R, Nubbemeyer R, Habenicht UF, Theuring F, Gottwald U. Targeted deletion of the epididymal receptor HE6 results in fluid dysregulation and male infertility. Mol Cell Biol. 2004; 24:8642–8648. [PubMed: 15367682]

Del Giudice I, Messina M, Chiaretti S, et al. Behind the scenes of non-nodal MCL: downmodulation of genes involved in actin cytoskeleton organization, cell projection, cell adhesion, tumour invasion, TP53 pathway and mutated status of immunoglobulin heavy chain genes. Br J Haematol. 2012; 156:601–611. [PubMed: 22150124]

Devenport D, Fuchs E. Planar polarization in embryonic epidermis orchestrates global asymmetric morphogenesis of hair follicles. Nat Cell Biol. 2008; 10:1257–1268. [PubMed: 18849982]

Doudney K, Stanier P. Epithelial cell polarity genes are required for neural tube closure. Am J Med Genet C Semin Med Genet. 2005; 135C:42–47. [PubMed: 15800847]

Feng J, Han Q, Zhou L. Planar cell polarity genes, Celsr1-3, in neural development. Neurosci Bull. 2012a; 28:309–315. [PubMed: 22622831]

Feng J, Xu Y, Wang M, Ruan Y, So KF, Tissir F, Goffinet A, Zhou L. A role for atypical cadherin Celsr3 in hippocampal maturation and connectivity. J Neurosci. 2012b; 32:13729–13743. [PubMed: 23035085]

Fenstermaker AG, Prasad AA, Bechara A, Adolfs Y, Tissir F, Goffinet A, Zou Y, Pasterkamp RJ. Wnt/planar cell polarity signaling controls the anterior-posterior organization of monoaminergic axons in the brainstem. J Neurosci. 2010; 30:16053–16064. [PubMed: 21106844]

Formstone CJ. 7TM-Cadherins: developmental roles and future challenges. Adv Exp Med Biol. 2010; 706:14–36. [PubMed: 21618823]

Formstone CJ, Barclay J, Rees M, Little PF. Chromosomal localization of Celsr2 and Celsr3 in the mouse; Celsr3 is a candidate for the tippy (tip) lethal mutant on chromosome 9. Mamm Genome. 2000; 11:392–394. [PubMed: 10790539]

Formstone CJ, Little PF. The flamingo-related mouse Celsr family (Celsr1-3) genes exhibit distinct patterns of expression during embryonic development. Mech Dev. 2001; 109:91–94. [PubMed: 11677057]

Formstone CJ, Mason I. Expression of the Celsr/flamingo homologue, c-fmi1, in the early avian embryo indicates a conserved role in neural tube closure and additional roles in asymmetry and somitogenesis. Dev Dyn. 2005; 232:408–413. [PubMed: 15614764]

Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol. 2003; 63:1256–1272. [PubMed: 12761335]

Gao FB, Kohwi M, Brenman JE, Jan LY, Jan YN. Control of dendritic field formation in Drosophila: the roles of flamingo and competition between homologous neurons. Neuron. 2000; 28:91–101. [PubMed: 11086986]

Georgieva L, Nikolov I, Poriazova N, Jones G, Toncheva D, Kirov G, Owen MJ. Genetic variation in the seven-pass transmembrane cadherin CELSR1: lack of association with schizophrenia. Psychiatr Genet. 2003; 13:103–106. [PubMed: 12782967]

Gross J, Grimm O, Ortega G, Teuber I, Lesch KP, Meyer J. Mutational analysis of the neuronal cadherin gene CELSR1 and exclusion as a candidate for catatonic schizophrenia in a large family. Psychiatr Genet. 2001; 11:197–200. [PubMed: 11807409]

Hadjantonakis AK, Formstone CJ, Little PF. mCelsr1 is an evolutionarily conserved seven-pass transmembrane receptor and is expressed during mouse embryonic development. Mech Dev. 1998; 78:91–95. [PubMed: 9858697]

Hadjantonakis AK, Sheward WJ, Harmar AJ, de Galan L, Hoovers JM, Little PF. Celsr1, a neural-specific gene encoding an unusual seven-pass transmembrane receptor, maps to mouse chromosome 15 and human chromosome 22qter. Genomics. 1997; 45:97–104. [PubMed: 9339365]

Hanson MA, Stevens RC. Discovery of new GPCR biology: one receptor structure at a time. Structure. 2009; 17:8–14. [PubMed: 19141277]

Hohenester E, Tisi D, Talts JF, Timpl R. The crystal structure of a laminin G-like module reveals the molecular basis of alpha-dystroglycan binding to laminins, perlecan, and agrin. Mol Cell. 1999; 4:783–792. [PubMed: 10619025]

Hsiao CC, Chen HY, Chang GW, Lin HH. GPS autoproteolysis is required for CD97 to up-regulate the expression of N-cadherin that promotes homotypic cell-cell aggregation. FEBS Lett. 2011; 585:313–318. [PubMed: 21156175]

Hu QX, Dong JH, Du HB, et al. Constitutive Galphai Coupling Activity of VLGR1 and its Regulation by PDZD7. J Biol Chem. 2014

Huber AH, Stewart DB, Laurents DV, Nelson WJ, Weis WI. The cadherin cytoplasmic domain is unstructured in the absence of beta-catenin. A possible mechanism for regulating cadherin turnover. J Biol Chem. 2001; 276:12301–12309. [PubMed: 11121423]

Jeemon P, Pettigrew K, Sainsbury C, Prabhakaran D, Padmanabhan S. Implications of discoveries from genome-wide association studies in current cardiovascular practice. World J Cardiol. 2011; 3:230–247. [PubMed: 21860704]

Jin Z, Tietjen I, Bu L, Liu-Yesucevitz L, Gaur SK, Walsh CA, Piao X. Disease-associated mutations affect GPR56 protein trafficking and cell surface expression. Hum Mol Genet. 2007; 16:1972–1985. [PubMed: 17576745]

Juriloff DM, Harris MJ. A consideration of the evidence that genetic defects in planar cell polarity contribute to the etiology of human neural tube defects. Birth Defects Res A Clin Mol Teratol. 2012; 94:824–840. [PubMed: 23024041]

Kahsai AW, Xiao K, Rajagopal S, Ahn S, Shukla AK, Sun J, Oas TG, Lefkowitz RJ. Multiple ligand-specific conformations of the beta2-adrenergic receptor. Nat Chem Biol. 2011; 7:692–700. [PubMed: 21857662]

Kathiresan S, Melander O, Guiducci C, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet. 2008; 40:189–197. [PubMed: 18193044]

Kaucka M, Plevova K, Pavlova S, et al. The planar cell polarity pathway drives pathogenesis of chronic lymphocytic leukemia by the regulation of B-lymphocyte migration. Cancer Res. 2013; 73:1491–1501. [PubMed: 23338609]

Langenhan T, Aust G, Hamann J. Sticky signaling--adhesion class G protein-coupled receptors take the stage. Sci Signal. 2013; 6:re3. [PubMed: 23695165]

Lefkowitz RJ, Sun JP, Shukla AK. A crystal clear view of the beta2-adrenergic receptor. Nat Biotechnol. 2008; 26:189–191. [PubMed: 18259173]

Lei Y, Zhu H, Yang W, Ross ME, Shaw GM, Finnell RH. Identification of novel CELSR1 mutations in spina bifida. PLoS One. 2014; 9:e92207. [PubMed: 24632739]

Lewis A, Wilson N, Stearns G, Johnson N, Nelson R, Brockerhoff SE. Celsr3 is required for normal development of GABA circuits in the inner retina. PLoS Genet. 2011; 7:e1002239. [PubMed: 21852962]

Li S, Jin Z, Koirala S, Bu L, Xu L, Hynes RO, Walsh CA, Corfas G, Piao X. GPR56 regulates pial basement membrane integrity and cortical lamination. J Neurosci. 2008; 28:5817–5826. [PubMed: 18509043]

Lin HH, Chang GW, Davies JQ, Stacey M, Harris J, Gordon S. Autocatalytic cleavage of the EMR2 receptor occurs at a conserved G protein-coupled receptor proteolytic site motif. J Biol Chem. 2004; 279:31823–31832. [PubMed: 15150276]

McGee J, Goodyear RJ, McMillan DR, et al. The very large G-protein-coupled receptor VLGR1: a component of the ankle link complex required for the normal development of auditory hair bundles. J Neurosci. 2006; 26:6543–6553. [PubMed: 16775142]

McVey D, Samani N, Webb T. 214 Generation of Isogenic Cell Lines to Study a Single Disease Associated Variant at the 1P13 Cad Risk Locus. Heart. 2014; 100(Suppl 3):A117.

Meyer RD, Srinivasan S, Singh AJ, Mahoney JE, Gharahassanlou KR, Rahimi N. PEST motif serine and tyrosine phosphorylation controls vascular endothelial growth factor receptor 2 stability and downregulation. Mol Cell Biol. 2011; 31:2010–2025. [PubMed: 21402774]

Montcouquiol M, Jones JM, Sans N. Detection of planar polarity proteins in mammalian cochlea. Methods Mol Biol. 2008; 468:207–219. [PubMed: 19099257]

Nakayama K, Bayasgalan T, Yamanaka K, et al. Large scale replication analysis of loci associated with lipid concentrations in a Japanese population. J Med Genet. 2009; 46:370–374. [PubMed: 19487539]

Nakayama M, Nakajima D, Nagase T, Nomura N, Seki N, Ohara O. Identification of high-molecular-weight proteins with multiple EGF-like motifs by motif-trap screening. Genomics. 1998; 51:27–34. [PubMed: 9693030]

Nishimura T, Honda H, Takeichi M. Planar cell polarity links axes of spatial dynamics in neural-tube closure. Cell. 2012; 149:1084–1097. [PubMed: 22632972]

Overduin M, Harvey TS, Bagby S, Tong KI, Yau P, Takeichi M, Ikura M. Solution structure of the epithelial cadherin domain responsible for selective cell adhesion. Science. 1995; 267:386–389. [PubMed: 7824937]

Overduin M, Tong KI, Kay CM, Ikura M. 1H, 15N and 13C resonance assignments and monomeric structure of the amino-terminal extracellular domain of epithelial cadherin. J Biomol NMR. 1996; 7:173–189. [PubMed: 8785495]

Paavola KJ, Hall RA. Adhesion G protein-coupled receptors: signaling, pharmacology, and mechanisms of activation. Mol Pharmacol. 2012; 82:777–783. [PubMed: 22821233]

Paavola KJ, Stephenson JR, Ritter SL, Alter SP, Hall RA. The N terminus of the adhesion G protein-coupled receptor GPR56 controls receptor signaling activity. J Biol Chem. 2011; 286:28914–28921. [PubMed: 21708946]

Piao X, Hill RS, Bodell A, et al. G protein-coupled receptor-dependent development of human frontal cortex. Science. 2004; 303:2033–2036. [PubMed: 15044805]

Qi L, Ma J, Qi Q, Hartiala J, Allayee H, Campos H. Genetic risk score and risk of myocardial infarction in Hispanics. Circulation. 2011; 123:374–380. [PubMed: 21242481]

Qu Y, Glasco DM, Zhou L, et al. Atypical cadherins Celsr1-3 differentially regulate migration of facial branchiomotor neurons in mice. J Neurosci. 2010; 30:9392–9401. [PubMed: 20631168]

Qu Y, Huang Y, Feng J, Alvarez-Bolado G, Grove EA, Yang Y, Tissir F, Zhou L, Goffinet AM. Genetic evidence that Celsr3 and Celsr2, together with Fzd3, regulate forebrain wiring in a Vangl-independent manner. Proc Natl Acad Sci U S A. 2014; 111:E2996–3004. [PubMed: 25002511]

Rasmussen SG, Choi HJ, Rosenbaum DM, et al. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature. 2007; 450:383–387. [PubMed: 17952055]

Ravni A, Qu Y, Goffinet AM, Tissir F. Planar cell polarity cadherin Celsr1 regulates skin hair patterning in the mouse. J Invest Dermatol. 2009; 129:2507–2509. [PubMed: 19357712]

Robinson A, Escuin S, Doudney K, Vekemans M, Stevenson RE, Greene ND, Copp AJ, Stanier P. Mutations in the planar cell polarity genes CELSR1 and SCRIB are associated with the severe neural tube defect craniorachischisis. Hum Mutat. 2012; 33:440–447. [PubMed: 22095531]

Rosenbaum DM, Cherezov V, Hanson MA, et al. GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. Science. 2007; 318:1266–1273. [PubMed: 17962519]

Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. Nature. 2009; 459:356–363. [PubMed: 19458711]

Runge S, Thogersen H, Madsen K, Lau J, Rudolph R. Crystal structure of the ligand-bound glucagon-like peptide-1 receptor extracellular domain. J Biol Chem. 2008; 283:11340–11347. [PubMed: 18287102]

Saade S, Cazier JB, Ghassibe-Sabbagh M, et al. Large scale association analysis identifies three susceptibility loci for coronary artery disease. PLoS One. 2011; 6:e29427. [PubMed: 22216278]

Samani NJ, Braund PS, Erdmann J, et al. The novel genetic variant predisposing to coronary artery disease in the region of the PSRC1 and CELSR2 genes on chromosome 1 associates with serum cholesterol. J Mol Med (Berl). 2008; 86:1233–1241. [PubMed: 18649068]

Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. N Engl J Med. 2007; 357:443–453. [PubMed: 17634449]

Sandhu MS, Waterworth DM, Debenham SL, et al. LDL-cholesterol concentrations: a genome-wide association study. Lancet. 2008; 371:483–491. [PubMed: 18262040]

Shapiro L, Weis WI. Structure and biochemistry of cadherins and catenins. Cold Spring Harb Perspect Biol. 2009; 1:a003053. [PubMed: 20066110]

Shima Y, Copeland NG, Gilbert DJ, Jenkins NA, Chisaka O, Takeichi M, Uemura T. Differential expression of the seven-pass transmembrane cadherin genes Celsr1-3 and distribution of the Celsr2 protein during mouse development. Dev Dyn. 2002; 223:321–332. [PubMed: 11891983]

Shima Y, Kawaguchi SY, Kosaka K, Nakayama M, Hoshino M, Nabeshima Y, Hirano T, Uemura T. Opposing roles in neurite growth control by two seven-pass transmembrane cadherins. Nat Neurosci. 2007; 10:963–969. [PubMed: 17618280]

Shima Y, Kengaku M, Hirano T, Takeichi M, Uemura T. Regulation of dendritic maintenance and growth by a mammalian 7-pass transmembrane cadherin. Dev Cell. 2004; 7:205–216. [PubMed: 15296717]

Shukla AK, Sun JP, Lefkowitz RJ. Crystallizing thinking about the beta2-adrenergic receptor. Mol Pharmacol. 2008; 73:1333–1338. [PubMed: 18239031]

Singer K, Luo R, Jeong SJ, Piao X. GPR56 and the developing cerebral cortex: cells, matrix, and neuronal migration. Mol Neurobiol. 2013; 47:186–196. [PubMed: 23001883]

Singh GP, Ganapathi M, Sandhu KS, Dash D. Intrinsic unstructuredness and abundance of PEST motifs in eukaryotic proteomes. Proteins. 2006; 62:309–315. [PubMed: 16299712]

Sun JP, Li R, Ren HZ, Xu AT, Yu X, Xu ZG. The very large G protein coupled receptor (Vlgr1) in hair cells. J Mol Neurosci. 2013; 50:204–214. [PubMed: 23180093]

Takeichi M. Cadherins: a molecular family important in selective cell-cell adhesion. Annu Rev Biochem. 1990; 59:237–252. [PubMed: 2197976]

Takeichi M, Atsumi T, Yoshida C, Uno K, Okada TS. Selective adhesion of embryonal carcinoma cells and differentiated cells by Ca2+-dependent sites. Dev Biol. 1981; 87:340–350. [PubMed: 6793433]

Talmud PJ, Drenos F, Shah S, et al. Gene-centric association signals for lipids and apolipoproteins identified via the HumanCVD BeadChip. Am J Hum Genet. 2009; 85:628–642. [PubMed: 19913121]

Tatin F, Taddei A, Weston A, Fuchs E, Devenport D, Tissir F, Makinen T. Planar cell polarity protein Celsr1 regulates endothelial adherens junctions and directed cell rearrangements during valve morphogenesis. Dev Cell. 2013; 26:31–44. [PubMed: 23792146]

Tissir F, Bar I, Goffinet AM, Lambert De Rouvroit C. Expression of the ankyrin repeat domain 6 gene (Ankrd6) during mouse brain development. Dev Dyn. 2002a; 224:465–469. [PubMed: 12203740]

Tissir F, Bar I, Jossin Y, De Backer O, Goffinet AM. Protocadherin Celsr3 is crucial in axonal tract development. Nat Neurosci. 2005; 8:451–457. [PubMed: 15778712]

Tissir F, De-Backer O, Goffinet AM, Lambert de Rouvroit C. Developmental expression profiles of Celsr (Flamingo) genes in the mouse. Mech Dev. 2002b; 112:157–160. [PubMed: 11850187]

Tissir F, Qu Y, Montcouquiol M, et al. Lack of cadherins Celsr2 and Celsr3 impairs ependymal ciliogenesis, leading to fatal hydrocephalus. Nat Neurosci. 2010; 13:700–707. [PubMed: 20473291]

Underwood CR, Garibay P, Knudsen LB, Hastrup S, Peters GH, Rudolph R, Reedtz-Runge S. Crystal structure of glucagon-like peptide-1 in complex with the extracellular domain of the glucagon-like peptide-1 receptor. J Biol Chem. 2010; 285:723–730. [PubMed: 19861722]

Usui T, Shima Y, Shimada Y, Hirano S, Burgess RW, Schwarz TL, Takeichi M, Uemura T. Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. Cell. 1999; 98:585–595. [PubMed: 10490098]

Venkatakrishnan AJ, Deupi X, Lebon G, Tate CG, Schertler GF, Babu MM. Molecular signatures of G-protein-coupled receptors. Nature. 2013; 494:185–194. [PubMed: 23407534]

Virtanen KJ, Remes V, Pajarinen J, Savolainen V, Bjorkenheim JM, Paavola M. Sling compared with plate osteosynthesis for treatment of displaced midshaft clavicular fractures: a randomized clinical trial. J Bone Joint Surg Am. 2012; 94:1546–1553. [PubMed: 22832887]

Wada H, Tanaka H, Nakayama S, Iwasaki M, Okamoto H. Frizzled3a and Celsr2 function in the neuroepithelium to regulate migration of facial motor neurons in the developing zebrafish hindbrain. Development. 2006; 133:4749–4759. [PubMed: 17079269]

Wang HM, Dong JH, Li Q, et al. A stress response pathway in mice upregulates somatostatin level and transcription in pancreatic delta cells through Gs and beta-arrestin 1. Diabetologia. 2014

Waterworth DM, Ricketts SL, Song K, et al. Genetic variants influencing circulating lipid levels and risk of coronary artery disease. Arterioscler Thromb Vasc Biol. 2010; 30:2264–2276. [PubMed: 20864672]

Wu G, Huang X, Hua Y, Mu D. Roles of planar cell polarity pathways in the development of neural [correction of neutral] tube defects. J Biomed Sci. 2011; 18:66. [PubMed: 21864354]

Yates LL, Papakrivopoulou J, Long DA, Goggolidou P, Connolly JO, Woolf AS, Dean CH. The planar cell polarity gene Vangl2 is required for mammalian kidney-branching morphogenesis and glomerular maturation. Hum Mol Genet. 2010a; 19:4663–4676. [PubMed: 20843830]

Yates LL, Schnatwinkel C, Murdoch JN, Bogani D, Formstone CJ, Townsend S, Greenfield A, Niswander LA, Dean CH. The PCP genes Celsr1 and Vangl2 are required for normal lung branching morphogenesis. Hum Mol Genet. 2010b; 19:2251–2267. [PubMed: 20223754]

Zhang B, Tran U, Wessely O. Expression of Wnt signaling components during Xenopus pronephros development. PLoS One. 2011; 6:e26533. [PubMed: 22028899]

Zhang R, Xie X. Tools for GPCR drug discovery. Acta Pharmacol Sin. 2012; 33:372–384. [PubMed: 22266728]

Zhang Y, Sivasankar S, Nelson WJ, Chu S. Resolving cadherin interactions and binding cooperativity at the single-molecule level. Proc Natl Acad Sci U S A. 2009; 106:109–114. [PubMed: 19114658]

Zhou L, Bar I, Achouri Y, et al. Early forebrain wiring: genetic dissection using conditional Celsr3 mutant mice. Science. 2008; 320:946–949. [PubMed: 18487195]

Zhou L, Qu Y, Tissir F, Goffinet AM. Role of the atypical cadherin Celsr3 during development of the internal capsule. Cereb Cortex. 2009; 19(Suppl 1):i114–119. [PubMed: 19349379]

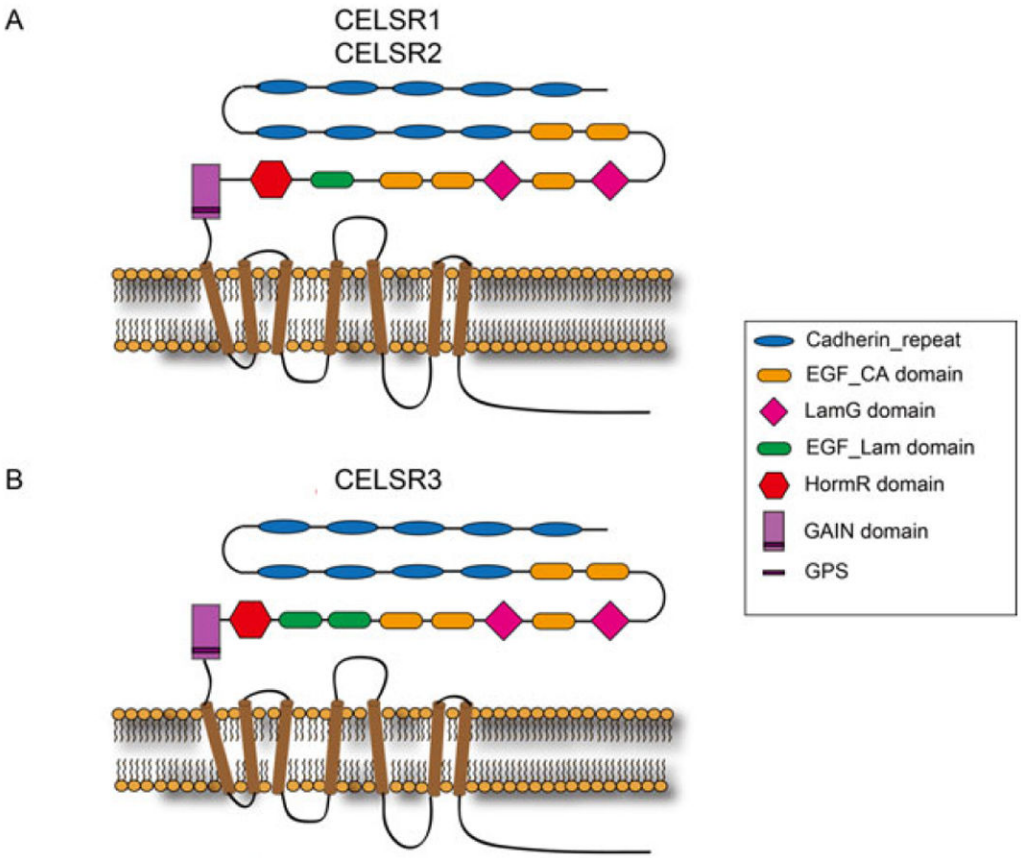

Figure 1. Domain architecture of the CELSR protein

(A/B) Snake-like domain architecture of CELSR1-3 proteins. Domains are indicated by shaded boxes with different colors, with the exception of trans-membrane regions, which are highlighted by brown bars with helices; purple, GAIN domain; red, HormR domain; green, EGF-Lam domain; orange, EGF-CA domain; magenta, LamG domain; and blue, cadherin repeat.

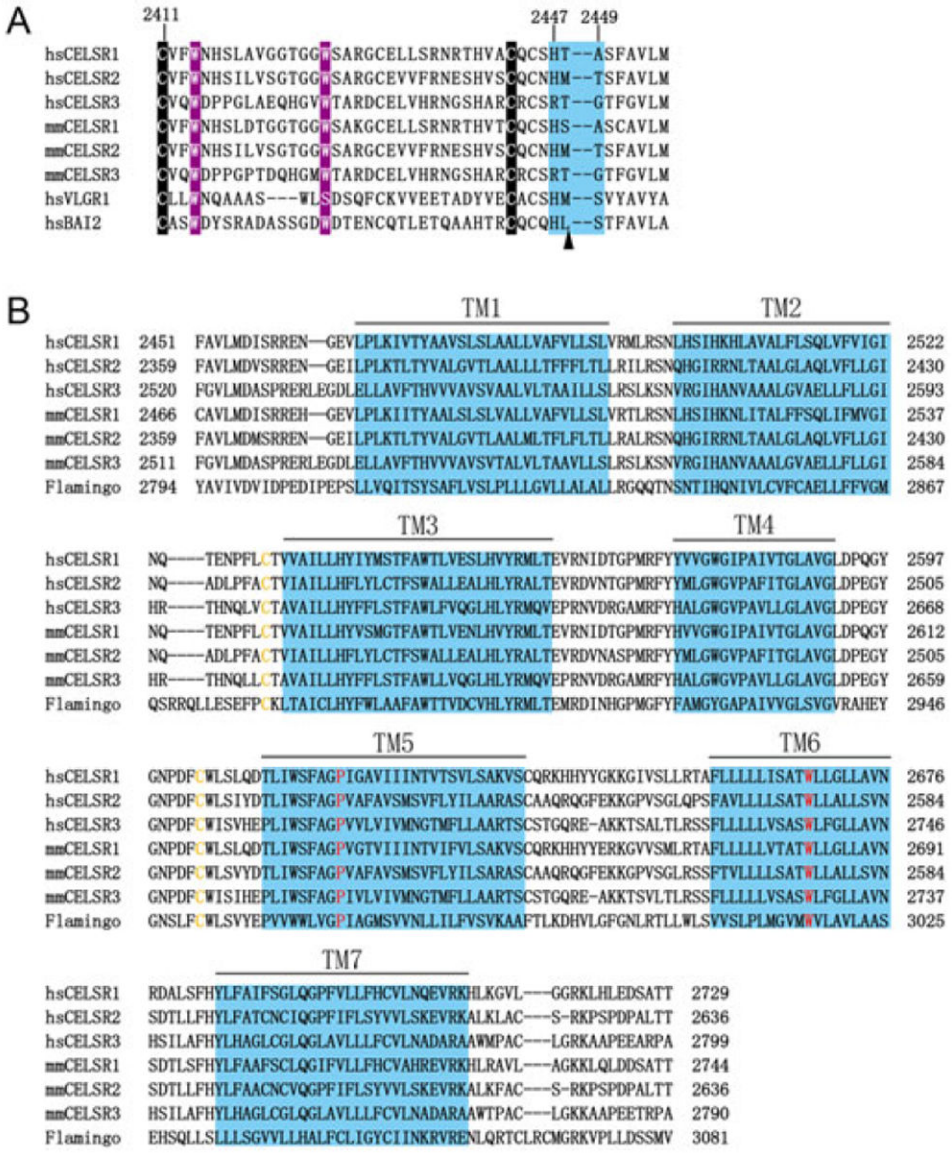

Figure 2. Sequence alignments of the GPS and transmembrane region of the CELSR1-3

(A) Sequence alignment of the GPS for CELSR1-3 proteins and several other adhesion GPCRs.

(B) Sequence alignment of the transmembrane regions of the CELSR proteins from humans, mice and *Drosophila*.

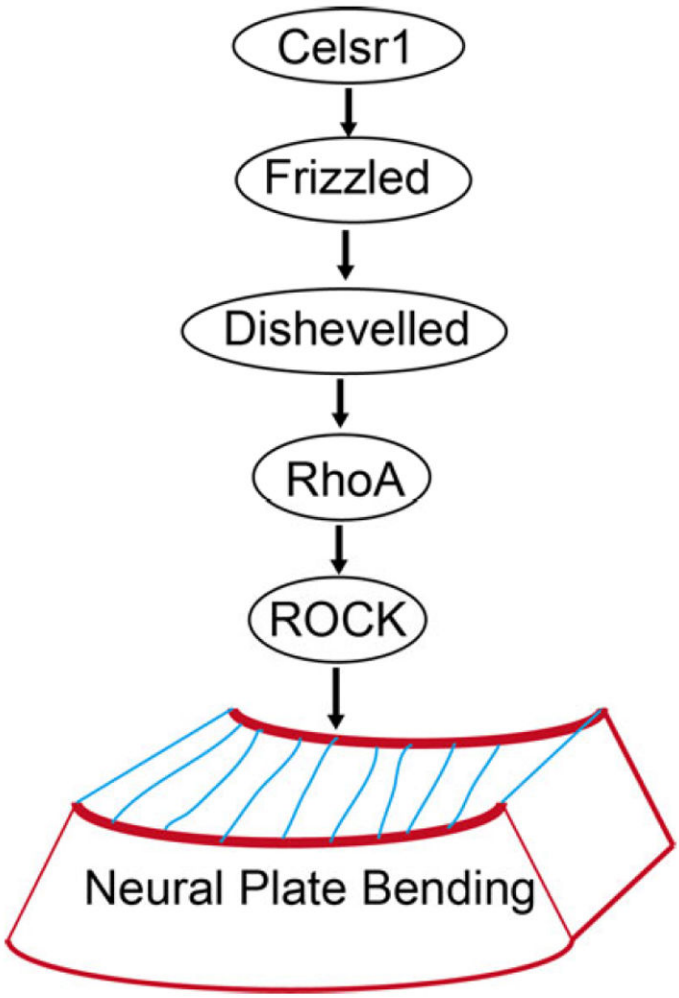

Figure 3. Schematics for the signaling cascade downstream of CELSR1

The signaling cascade downstream of CELSR1 during the regulation of neural tube closure.

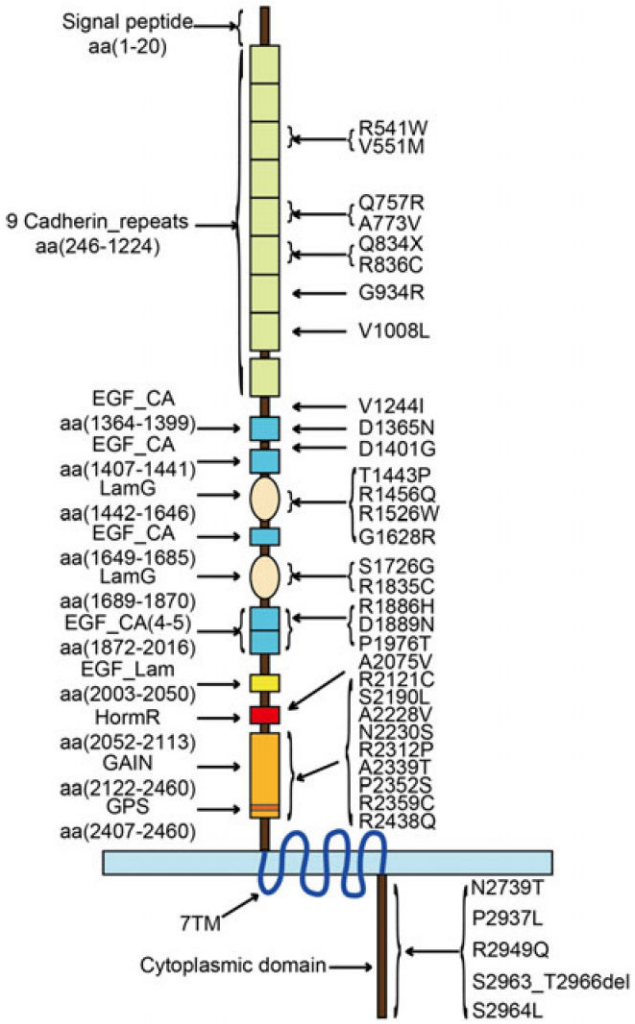

Figure 4. Novel CELSR1 mutations in patients with neural tube defects (NTDs)

A schematic representation of the predicted protein structure of CELSR1 with structural modules (left) and the approximate positions of different NTDs mutations (right) indicated by arrows. Abbreviations: aa, amino acids; EGF, epidermal growth factor; GAIN, GPCR Autoproteolysis Inducing (GAIN) domain. GPS, G protein-coupled receptor proteolytic site; and TM, transmembrane.

Table 1

Distribution of *Celsr1-3* in tissues

Classification	Tissue	Reference
Celsr1	Brain, spinal cord, eye	(Hadjantonakis *et al*. 1997)
		(Formstone *et al*. 2000)
	Cochlea	(Montcouquiol *et al*. 2008)
		(Shima *et al*. 2002)
		(Curtin *et al*. 2003)
	Esophagus and the stomach	(Shima *et al*. 2002)
	Kidney	(Shima *et al*. 2002)
		(Zhang *et al*. 2011)
	Lung	(Formstone *et al*. 2000)
		(Yates *et al*. 2010b)
	Spleen	(Formstone *et al*. 2000)
	Skin hair	(Ravni *et al*. 2009)
		(Devenport & Fuchs 2008)
	Lymphatic vessels	(Tatin *et al*. 2013)
Celsr2	Brain, spinal cord, eye	(Hadjantonakis *et al*. 1997)
		(Formstone *et al*. 2000)
	Cochlea	(Shima *et al*. 2002)
	Esophagus and the stomach	(Shima *et al*. 2002)
	Whiskers	(Shima *et al*. 2002)
	Hair follicles and serous glands	(Shima *et al*. 2002)
	Lung	(Formstone *et al*. 2000)
	Spleen	(Formstone *et al*. 2000)
	Heart	(Formstone *et al*. 2000)
	Testis	(Beall *et al*. 2005)
	Pancreas	(Cortijo *et al*. 2012)
Celsr3	Brain, spinal cord, eye	(Hadjantonakis *et al*. 1997)
		(Formstone *et al*. 2000)
	Cochlea	(Shima *et al*. 2002)
	Testis	(Beall *et al*. 2005)
	Inner retina	(Lewis *et al*. 2011)
	Pancreas	(Cortijo *et al*. 2012)

Table 2

***CELSR1-3* and different diseases**

Classification	Diseases	Reference
CELSR1	Neural tube defects	(Allache *et al*. 2012)
		(Robinson *et al*. 2012)
		(Doudney & Stanier 2005)
		(Juriloff & Harris 2012)
		(Nishimura *et al*. 2012)
	Hepatocellular carcinoma	(Ammerpohl *et al*., 2012)
	Mantle cell lymphoma	(Del Giudice *et al*. 2012)
	Chronic lymphocytic leukemia	(Kaucka *et al*. 2013)
CELSR2	Cardivasocular disease	(Qi *et al*. 2011)
		(Waterworth *et al*. 2010)
		(Jeemon *et al*. 2011)
		(Virtanen *et al*. 2012)
		(Chow *et al*. 2012)
		(Samani *et al*. 2007)
		(Kathiresan *et al*. 2008)
		(Nakayama *et al*. 2009)