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Protocadherin-19 Expression and Function in Vertebrate Brain Review

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Introduction:

Cell-adhesion molecules, such as cadherins, play a pivotal rule in the development and formation of a functional central nervous system (CNS) and maintenance of the system in vertebrates. Cadherins constitute a large superfamily of calcium-dependent cell adhesion molecules, with each family having distinct domain structures (Patel et al., 2006). The protocadherin family is the largest of the cadherin superfamily. The overall domain structure of protocadherins (pcdhs) is similar to that of most other cadherins in that it consists of a large extracellular domain, a short transmembrane domain and a cytoplasmic domain. Pcdhs differ from other cadherins mainly in their sequences in the extracellular domain, and in the cytoplasmic tail. The extracellular domain of pcdhs contains five or more ectodomains (EC), which is encoded by a single large exon (Basu, 2015). **Figure 1** illustrates the differences in the domain structure between classical cadherins and pcdhs. Within this pcdh family, there are more than 80 different members identified thus far and are all primarily found in the CNS (Hirayama & Yagi., 2006). The pcdh subgroups have diverse functions, ranging from being responsible for cell movement during epiboly and involvement in convergent extension and somitogenesis during embryogenesis to formation of neural circuits in CNS (Emond et al., 2009).

Classical Cadherin:

Protocadherin:

Figure 1 shows the differences in domain structure between classical cadherins and pcdhs. Classical cadherins have five ectodomains, with EC3 and EC5 being specific characteristics shown by the diagonal lines. Pcdhs have more than five ectodomains, represented by ECn. (TMD – transmembrane domain, C-terminal domain – cytoplasmic domain)

The development of an accurate and complex vertebrate CNS with its networks of neurons requires activities of numerous cell-adhesion molecules. Various studies have been carried out on examining expression and functions of different pcdhs, including protocadherin-19 (pcdh19), in vertebrate CNS. Pcdh19 is the 82nd member of the pcdh family (Tai et al., 2010). A recent study by Hynes et. al (2010) described how mutations in PCDH19 result in neurological diseases in human females, suggesting that PCDH19/pcdh19 plays an important role in the proper functionality of the vertebrate brain.

Based on genomic organization, domain structures and sequences, pcdhs can be divided into two separate categories; clustered and non-clustered (Rubinstein, 2015). Clustered refers to having a specific genomic localization organized into gene clusters. Clustered pcdhs consist of pcdh α, β, and γ (Morishita, & Yagi, 2007). Non-clustered refers to the pchds that do not have a specific genomic cluster organization and are scattered throughout the genome. The non-clustered pcdhs consist of Pcdh δ and solitary pcdhs (Morishita & Yagi, 2007). The subgroup pcdh δ can be further divided into two categories based on the presence or absence of a

phosphatase-1α (PP1α) binding domain and the number of ectodomain (EC) repeats. Group pcdh δ1 contains this binding domain and has seven EC repeats, while pcdh δ2 does not, and contains six EC repeats (Kim et al., 2011). Based on the characteristics presented, pcdh19 belongs to the pcdh δ2 group (Morishita & Yagi, 2007).

Pcdh19 expression and function have been analyzed in several model organisms including zebrafish, mouse, rat and humans, using various techniques including PCR, in situ hybridization, immunocytochemistry, morpholino antisense oligonucleotide technique, and/or mutation studies. Using in situ hybridization and systematic resequencing, the PCDH19/pcdh19 gene was first isolated in humans (Dibbens et al., 2008) after a long process of trying to understand a disease known as epilepsy and mental retardation limited to females (EFMR, Juberg & Hellman, 1971). Studies done on the human PCDH19 reveal that the gene has a signal sequence consisting of 23 amino acid residues, six conserved EC repeats, a transmembrane domain, and a conserved c-terminal domain (Kolc et al., 2017). After discovering this gene in humans, other model organisms were researched to better understand pcdh19 expression and/or function. Polymerase chain reaction (PCR), a technique used to amplify specific segments of DNA, allowed the domain organization of pcdh19 to be characterized. The PCR study showed that zebrafish pcdh19 also contains an extracellular domain, a single-pass transmembrane domain, and an intracellular domain. The extracellular domain is encoded by a single large exon, containing six EC repeats, as well as four conserved cysteine bases. Like other cadherins, it has a short transmembrane domain and a cytoplasmic C-terminal domain (Emond et al., 2009). Reverse-transcription PCR also led to the finding that pcdh19 contains two splice variants, which are both expressed during development (Emond et al., 2009). A similar domain structure was found in the mouse pcdh19 gene (Schaarschuch & Hertel, 2017). In all the organisms studied, the

C-terminal domain of the pcdh19 contains conserved CM1 and CM2 motifs. (Kolc et al., 2017). A simplified domain structure of the pcdh19 discussed is shown in **figure 2**.

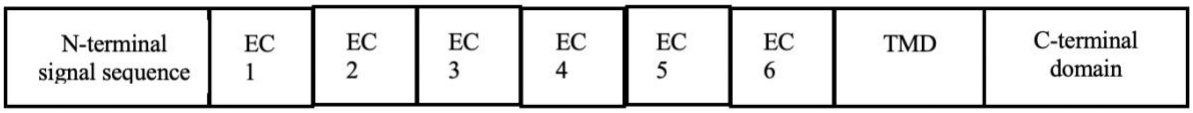

Figure 2 displays a simplified version of the pcdh19 domain structure. (TMD represents the transmembrane domain).

Although the domain structure is similar across the organisms, there are differences present in the amino acid sequences between these organisms. The pcdh19 gene consists of six exons, which is the region of the gene that is coded for protein. The first exon is responsible for coding all of the extracellular domains and transmembrane domains. Exons 2-6 code for the C-terminal domain, which is where splice variants are found (Kolc et al., 2017). **Figure 3** shows amino acid sequences comparison between human PCDH19, zebrafish pcdh19 and mouse pcdh19.

ZPcdh19	YTVEEELRAGTKIANVTADAKVAGFALGNRQP*YLRVISNSEPRWVNLSP*AGLLITKQKIDRDAVC
HPCDH19	YSVEEEQRAGTVIANVAKDAREAGFALDPRQASAFRVVSNSAPHLVDINPSSGLLVTKQKIDRDLLC
MPcdh19	YSVEEEQRAGTVIANVAKDAREAGFALDPRQASAFRVVSNSAPHLVDINPSSGLLVTKQKIDRDLLC
ZPcdh19	RQTPKCFISLEVMSN***SMEICVIKIEIIDVNDNAPRRFPTNHIDIEISENAAPGTRFPLEGASDPDSGS
HPCDH19	RQSPKCIISLEVMSS***SMEICVIKVEIKDLNDNAPSFPAAQIELEISEAASPGTRIPLDSAYDPDSGS
MPcdh19	RQSPKCIISLEVMSS***SMEICVIKVEIKDLNDNAPSFPAAQIELEISEAASPGTRIPLDSAYDPDSGS
ZPcdh19	NGIQTYTITPNDIFGLEIKTRGDGSKIAELVVEKTLDRETQSRYTFELTAEDGGDPPKSGTVQLNIKV
HPCDH19	FGVQTYELTPNELFGLEIKTRGDGSRFAELVVEKSLDRETQSHYSFRITALDGGDPPRLGTVGLSIKV
MPcdh19	FGVQTYELTPNELFGLEIKTRGDGSRFAELVVEKSLDRETQSHYSFRITALDGGDPPHMGTVGLSIKV
ZPcdh19	IDSNDNNPVFDEPVYTVNVLENSPINTLVIDLNATDPDEGTNGEVVYSFINFVSNLTKQMFKIDPKTG
HPCDH19	TDSNDNNPVFSESTYAVSVPENSPPNTPVIRLNASDPDEGTNGQVVYSFYGYVNDRTRELFQIDPHSG
MPcdh19	TDSNDNNPVFGESTYSVSVPENSPPNTPVIRLNASDPDEGTNGQVVYSFYGYVNDRTRELFQIDPHSG
ZPcdh19	VITVNGVLDHEELHIHEIDVQAKDLGPNSIPAHCKVIVNVIDINDNAP**EIKLLSE*NSEMVEVSENA
HPCDH19	LVTVTGALDYEEGHVYELDVQAKDLGPNSIPAHCKVTVSVLDTNDNPP**VINLLSV*NSELVEVSESA
MPcdh19	LVTVTGALDYEEGHVYELDVQAKDLGPNSIPAHCKVTVSVLDTNDNPP**IINLLSV*NSELVEVSESA
ZPcdh19	PLGYVIALVRVSDNDSGANGKVQCRLQGNVPFRLN*EFESFSTLILVDGRLDREQRDMYNLTILAEDSG
HPCDH19	PPGYVIALVRVSDRDSGLNGRVQCRLLGNVPFRLQ*EYESFSTILVDGRLDREQHDQYNLTIQARDGG
MPcdh19	PPGYVIALVRVSDRDSGLNGRVQCRLLGNVPFRLQ*EYESFSTILVDGRLDREQHDQYNLTIQARDSG

ZPcdh19	YPPLRSSKSFAVKVTDENDNPPYFTKPHYQAMVLENNVPGAFLLAVSARDPDLGMNGTVSYEIIKSEVRGMS
HPCDH19	VPMLQSAKSFTVLITDENDNHPHFSKPYYQVIVQENNTPGAYLLSVSARDPDLGLNGSVSYQIVPSQVRDMP
MPcdh19	VPMLQSAKSFTVRITDENDNHPHFSKPYYQVIVQENNTPGAYLLSVSARDPDMGLNGSVSYQIVPSQVRDMP
ZPcdh19	VESYVTVNSN*GEIYGVRAFNHEDTRTFEFKVSAKDGGDP*PLTSNATVRIVVLDVNDNTPVMTTPPLVNGTAEV
HPCDH19	VFTYVSINPNSGDIYALRSFNHEQTKAFEFKVLAKDGGLP*SLQSNATVRRVILDVNDNTPVITAPPLINGTAEV
MPcdh19	VFTYVSINPNSGDIYALRSFNHEQTKAFEFKVLAKDGGLP*SLQSNATVRRVILDVNDNTPVITAPPLINGTAEV
ZPcdh19	SIPKNAGVGYLVTQIKADDYDEGENGRLTYSISEG*DMAYFEIDQINGEVRTTKTFGENAKPSYQITVVAHDHG
HPCHD19	YIPRNSGIGYLVTVVKAEDYDEGENGRVTYDMTEG*DRGFFEIDQVNGEVRTTRTFGESSKSSYELIVVAHDHG
MPcdh19	YIPRNSGIGYLVTVVKADDYDEGENGRVTYDMTEG*DRGFFEIDQVNGEVRTTRTFNENSKPSYELIVVAHDHG
ZPcdh19	QTSLSASAYIVIYLSPDLNAQEQ*********IGPVNLSLIFIIALGSIAVILFVTMIFVAVKCKRDNKEIRTYNCRVAEY
HPCHD19	KTSLSASALVLIYLSPALDAQES*********MGSVNLSLIFIIALGSIAGILFVTMIFVAIKCKRDNKEIRTYNCRIAEY
MPcdh19	KTSLSASALVLIYLSPALDAQES*********MGSVNLSLIFIIALGSIAGILFVTMIFVAIKCKRDNKEIRTYNCRIAEY
ZPcdh19	SYGNQKKSSKKKKLSKNDIRLVPRDVEETDKMNVVSCSSLTSSLNYFDYHQQTLPLGCRRSESTFLNVENQN
HPCHD19	SYGHQKKSSKKKKISKNDIRLVPRDVEETDKMNVVSCSSLTSSLNYFDYHQQTLPLGCRRSESTFLNVENQN
MPcdh19	SYGHQKKSSKKKKISKNDIRLVPRDVEETDKMNVVSCSSLTSSLNYFDYHQQTLPLGCRRSESTFLNVENQN
ZPcdh19	SRNAAPNHGYHHTFTGQGPQQPDLIINGMPLPETENYSIDSSYVNSRAHLIKSTSTFKDMEGNSLKDSGHEE
HPCHD19	TRNTSANHIYHHSFNSQGPQQPDLIINGVPLPETENYSFDSNYVNSRAHLIKS-STFKDLEGNSLKDSGHEE
MPcdh19	TRNTTASHIYHHSFNSQGPQQPDLIINGVPLPETENYSFDSNYVNSRAHLIKS-STFKDLEGNSLKDSGHEE
ZPcdh19	SDQTDSEHDVQRGHYADTAVNDVLNMTVPSNNSQIPDQDQSEGFHCQDECRILGHSDRCWMP
HPCHD19	SDQTDSEHDVQRSLYCDTAVNDVLNTSVTSMGSQMPDHDQNEGFHCREECRILGHSDRCWMP
MPcdh19	SDQTDSEHDVQRSLYCDTAVNDVLNTSVTSMGSQMPDHDQNEGFHCREECRILGHSDRCWMP

Figure 3. Amino acid sequence comparison between zebrafish pcdh19, human PCDH19, and the mouse pcdh19. Identical amino acid residues are highlighted by the yellow shading. This figure is modified from Liu et al., 2009.

Using ClustalW2, which is a sequence alignment program, comparisons between zebrafish pcdh19, human PCDH19, and mouse pcdh19 were performed (Liu et al., 2009). The similarities range from roughly 70-95% and are shown in **table 1**. On the variant of the gene that was sequenced, the human PCDH19 is most similar to the mouse pcdh19, while is about 71% identical to the zebrafish pcdh19 (Liu et al., 2009).

Comparisons	Percent Similar
HPCDH19 / Zpcdh19	71.28
HPCDH19 / Mpcdh19	96.46
Zpcdh19 / Mpcdh19	71.76

Table 1 shows the similarities in the amino acid sequences between the three organisms. (Note: HPCHDH19 – human PCDH19, Zpcdh19 – zebrafish pcdh19, and Mpcdh19 – mouse pcdh19)

This review focuses on the expression and function of pcdh19 in the zebrafish, mouse, rat and human brains.

Pcdh19 in the telencephalon

The most anterior part of the vertebrate CNS is known as the telencephalon. It is the largest brain region in birds and mammals. The telencephalon contains a pair of olfactory bulbs and a pair of cerebral hemispheres. In mammals, the cerebral hemispheres consist of cerebral cortex as well as subcortical structures including the hippocampus and basal ganglia. This defined region has pivotal roles in cognition, motor function, and emotion (Visel &Taher, 2013). With said functions, the telencephalon is quite a sophisticated region. Therefore, anything we learn about its molecular expression and function may likely help us understand more about this brain region. It is therefore important to study pcdh19 expression and function in the telencephalon.

Pcdh19 expression is detected in the telencephalon in both embryonic zebrafish (Liu et al., 2009) and adult zebrafish (Liu et al., 2015). Pcdh19 mRNA (*pcdh19*) is observed in the forebrain of zebrafish embryos 12 hours post fertilization (hpf). As development proceeds, the anterodorsal portion of the forebrain becomes the telencephalon while the ventroposterior portion develops into the diencephalon (Kimmel et al., 1995). *Pcdh19* is expressed in the telencephalon of 18-24 hpf zebrafish, and its expression becomes restricted to the lateral and dorsal regions of telencephalon in 36 hpf zebrafish. *Pcdh19* expression in the telencephalon of 48 and 72 hpf zebrafish is more apparent in the dorsal half of the region. This dorsal region (called pallium) is homologous to the cortex of mammals (Wullimann et al., 1996). In adult zebrafish, *pcdh19* is expressed by both the olfactory bulb and cerebral hemispheres (Liu et al.,

2015). As in the embryonic zebrafish, *pcdh19* expression levels are higher in the anterior and dorsolateral telencephalon. Strong *pcdh19* expression is also detected in the ventricular region along the midline. Interestingly, the dorsolateral zebrafish telencephalon is topologically corresponding to the mammal hippocampus, while the ventromedial zebrafish telencephalon, is topologically corresponding to the mammal amygdala (Muller et al., 2011)

Studies conducted on the developing mouse embryo also found high levels of *pcdh19* in the dorsal cortex of the telencephalon, as well as the lateral ganglionic eminence (Gaitan & Bouchard, 2006). Mice are found to have a high level of expression of *pcdh19* in the hippocampus in developing and adult mice (Pederick et al., 2016). The adult mice show expression of *pcdh19* in the dorsal and ventral portions of the amygdala (Schaarschuch & Hertel, 2017). In developing rat (post-natal day 3, P3), *pcdh19* expressing cells are found in the olfactory bulb, throughout the cerebral cortex and basal ganglia, hippocampus and amygdala (Kim et al., 2007).

High levels of *PCDH19* is also found in the human embryonic CNS, with the highest levels being in the cortex. This expression is highest 8-16 weeks post-conception (Pederick et al., 2017). Although it was shown that *PCDH19* has high expression in neural progenitor cells in the adult human, there is no information on spatial distribution of PCDH19 in the adult human brain (Homan et al., 2018). **Figures 4, 5** and **6** provide visual representations of where *pcdh19* is found in the telencephalon of embryonic and adult zebrafish.

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Pcdh19 in the diencephalon

The diencephalon serves as a major relay station for sensory information and houses the thalamus, , hypothalamus, and epithalamus. This region is involved in the relay and processing

of sensory and motor information, as well as control of autonomic functions (Lim & Golden, 2007). With the diencephalon serving such important and diverse functions, it is of interest to neurobiologists to learn about pcdh19 in the development, maintenance and normal function of the diencephalon.

Pcdh19 expression is detected in the diencephalon in both embryonic zebrafish (Liu et al., 2009) and adult zebrafish (Liu et al., 2015). *Pcdh19* is observed in the ventroposterior portion of the forebrain, the precursor of the diencephalon at 12 hpf. *Pcdh19* is expressed in the diencephalon of 18-24 hpf zebrafish, with stronger expression in the dorsal thalamus, ventroanterial to the optic tectum. *Pcdh19* expression becomes more restricted in the dorsal and central thalamus in 36-72 hpf zebrafish (Liu et al., 2009). In adult zebrafish, high levels of *pcdh19* expression are observed in the suprachiasmatic nucleus and periventricular regions of the hypothalamus. *Pcdh19* is also expressed in the habenula and dorsal saccus of the epithalamus. As in the embryonic zebrafish, *pcdh19* is also expressed in the anterior, dorsal and ventral thalamus of the adult zebrafish (Liu et al., 2015).

In the mouse embryo, *pcdh19* expression is found in the diencephalon and pituitary gland, which is a structure located at the base of the hypothalamus (Gaitan & Bouchard, 2006). No detailed information about the spatial distribution of *pcdh19* in the diencephalon of the developing mice is available. In adult mice *pcdh19* is expressed in in the anterior hypothalamus and anterior thalamus (Schaarschuch & Hertel, 2017). In the P3 rat brain, *pcdh19* expression is detected in most regions of the thalamus and hypothalamus (Kim et al., 2007). Moreover, the lateral habenula nucleus of the epithalamus also contains *pcdh19* expressing cells.

PCDH19 expression was noted in various regions of the embryonic human CNS, but no information about spatial distribution of this gene in the embryonic human brain was provided

(Pederick et al., 2017). Although it was shown that *PCDH19* has high expression in neural progenitor cells in the adult human, information about specific locations of these cells was not reported (Homan et al., 2018).

Expression of *pcdh19* in developing and adult zebrafish brains is summarized in **Figures 4, 5,** and **6**

Pcdh19 in the midbrain

The vertebrate midbrain is located between the diencephalon and hindbrain, consisting of the tectum and the tegmentum (Saladin, 2018). The tectum is the dorsal part of the midbrain, and contains primarily the optic tectum in nonmammals, and superior and inferior colliculi in mammals. The remaining part of the midbrain is the tegmentum containing numerous structures including periaqueductal grey area, red nucleus and reticular formation. The optic tectum is the major visual center in nonmammals. The superior colliculi play a role in orienting head and eyes toward visual stimuli, while the inferior colliculi is a major center for processing auditory information (Kinkhabwala et al., 2011; Saladin, 2018). The tegmentum participates in diverse brain functions including regulating breathing, cardiovascular activities, arousal, consciousness, and coordination of certain movements. Information about pcdh19 expression and function in the midbrain can help us learn more about the molecular nature of this brain region.

Pcdh19 expression is detected in the midbrain in both embryonic zebrafish (Liu et al., 2009) and adult zebrafish (Liu et al., 2015). No obvious *pcdh19* is found in the midbrain of zebrafish embryos at 12 hpf. However, at 18 and 24 hpf, the *pcdh19* is expressed in both the optic tectum and tegmentum. At 36 hpf, *pcdh19* is confined to regions in the optic tectum and dorsal tegmentum. This expression pattern continues at 50 and 72 hpf (Liu et al., 2009). In adult

zebrafish, *pcdh19* is strongly expressed in the cellular layers of the optic tectum. *Pcdh19* expression in the tegmentum of adult zebrafish is mainly found in the dorsal and lateral regions (Liu et al., 2015).

Pcdh19 was expressed in the midbrain of embryonic mouse, although its detailed spatial distribution of this gene in the midbrain is not reported (Gaitan & Bouchard, 2006). There is no published report, to the best of my knowledge, on *pcdh19* expression in the midbrain of adult mice. *PCDH19* expression is detected in various regions of the embryonic human CNS, but there is no information on its spatial distribution in either the embryonic or adult human brain (Pederick et al., 2017). In the 3P rat brain, *pcdh19* expression is detected in both the superior and inferior colliculi (Kim et a., 2007).

Expression of *pcdh19* in developing and adult zebrafish brains is summarized in **Figures 4, 5,** and **6**

Pcdh19 in the hindbrain

The vertebrate hindbrain is an important region in controlling and coordinating movement and houses the cerebellum and medulla oblongata, and pons. The hindbrain coordinates functions that are fundamental to survival (Kinkhabwala et al., 2011; Saladin 2018). Major functions of the hindbrain include control of movements, balancing, respiration, cardiovascular activities, and conveying sensory information to the brain. With the said functions, the hindbrain is quite an important region of the vertebrate brain. Therefore, anything we learn about its molecular expression and function may likely help us understand more about this brain region. It is therefore important to study pcdh19 expression and function in the hindbrain.

Pcdh19 expression is detected in the hindbrain in both the embryonic zebrafish (Liu et al., 2009) and adult zebrafish (Liu et al., 2015). *Pcdh19* is observed in the hindbrain of zebrafish embryos 12 hpf, as two thick vertical bands, likely corresponding to rhombomeres one and four. *Pcdh19* expression is detected in both the cerebellum and medulla of embryos at 18 and 24 hpf. Expression of this gene in the medulla is stronger in the dorsal half of this region. In older embryos of 36-72 hpf, a stronger *pcdh19* expression is seen in the dorsolateral regions of the both the cerebellum and medulla, while its expression in the ventromedial hindbrain is further reduced (Liu et al., 2009). In the adult zebrafish, *pcdh19* is found to be expressed in in the granular regions of the cerebellum where most cell bodies, including Purkinje cells reside (Liu et al., 2015). *Pcdh19* expression was also noted in the medulla, where the expression is detected in nuclei of cranial nerves (trigeminal to vagal). In addition, *pcdh19*-expressing cells are also found within the reticular formation (Liu et al., 2015).

Expression of *pcdh19* is also present in the hindbrain of the mouse embryo (Gaitan & Bouchard, 2006) and cerebellum of adult mice. Similar to zebrafish, Purkinje cells in adult mice express *pcdh19* (Pederick et al., 2017). *PCDH19* expression is detected in various regions of the embryonic human CNS, and in neural progenitor cells in adult humans, Pederick et al., 2017). Again, no information about their spatial distribution is reported. There is no published report, to the best of my knowledge, on *pcdh19* expression in the hindbrain of developing rat.

Expression of *pcdh19* in developing and adult zebrafish brains is summarized in **Figures 4, 5,** and **6**

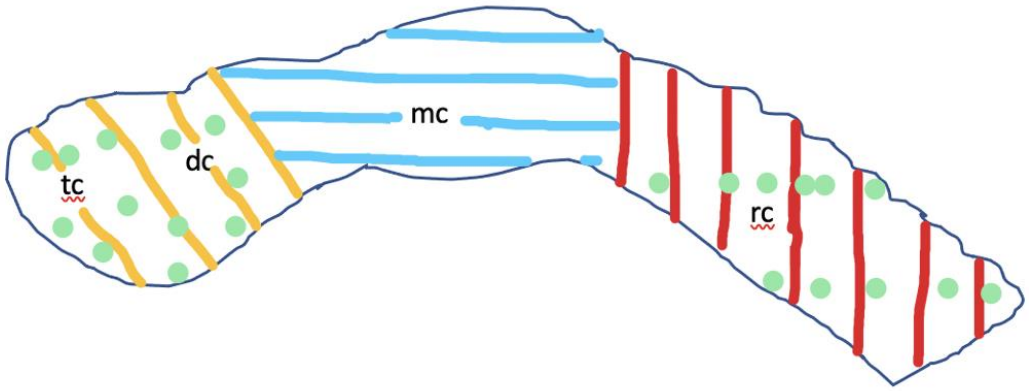

Figure 4 shows a schematic, sagittal representation of a 12 hpf embryonic zebrafish brain, highlighting some of the major areas where *pcdh19* is found. (yellow – forebrain/telencephalon, diencephalon; blue – midbrain, red – hindbrain/rhombencephalon) Green dots represent regions with high level of *pcdh19* expression.

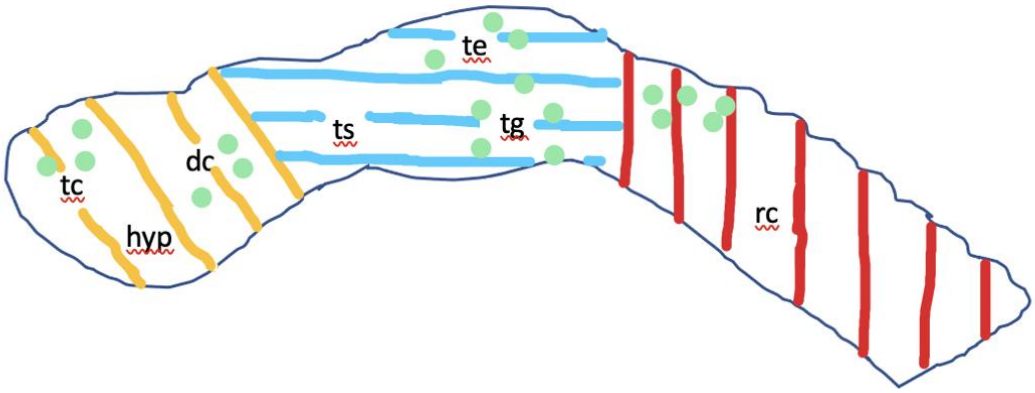

Figure 5 shows a schematic, sagittal representation of a 24hpf embryonic zebrafish brain, highlighting some of the major areas where pcdh19 is found. (yellow – forebrain/telencephalon, diencephalon; blue – midbrain; te-tectum; tg-tegmentum, red – hindbrain/rhombencephalon) Green dots represent regions with high levels of *pcdh19* expression.

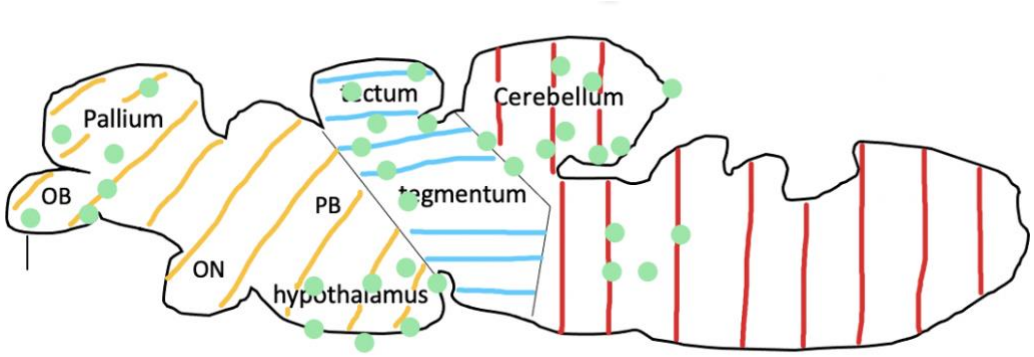

Figure 6 shows a schematic, sagittal representation of the adult zebrafish brain, highlighting some of the major areas where pcdh19 is found. (yellow – forebrain/telencephalon, diencephalon; blue – midbrain, red – hindbrain; OB – olfactory bulb, PB – pineal body, ON-optice nerve) Green dots represent regions with high levels of *pcdh19* expression.

In summary, *pcdh19* is expressed in all major brain regions in both developing and adult zebrafish, mice, rat and humans. There is much more information about pcdh19 expression in the brains of zebrafish and rat than in mice and humans. Importantly, *pcdh19* is expressed by similar or homologous regions in the telencephalon, diencephalon and/or cerebellum in all four species surveyed.

Pcdh19 Function in Zebrafish

Antisense morpholino oligonucleotides technique was initially used to study pcdh19 function in zebrafish brain development (Emond et al., 2009). Zebrafish embryos with reduced pcdh19 function show defects in neural plate convergence, suggesting that pcdh19 plays a role in

brain morphogenesis (Emond et al., 2009). In zebrafish larvae with pcdh19 knockout, functional neural connectivity is drastically altered, suggesting that there is a delay in synaptogenesis (Light & Jontes, 2019). If synaptogenesis is delayed, there is a possibility that the neuronal connections needed for proper development may not form.

To further test pcdh19's function in organizing the vertebrate CNS, zebrafish pcdh19 knockout mutants were tested for positive phototaxis, which is a behavior in which a zebrafish swims towards a light. Compared to wildtype zebrafish, significantly fewer mutant zebrafish reached the light, and those that did took longer time (Cooper et al., 2015). Structural study of the optic tectum, visual center of nonmammals, of pcdh19 mutant zebrafish revealed that assembly columns of neurons in the optic tectum were severely disrupted in the mutant fish. Furthermore, neural cell migration was also disrupted in the mutant pcdh19 zebrafish (Light & Jontes, 2019).

Pcdh19 Function in Mice

Various studies have been performed on mice to better comprehend cellular and molecular mechanisms underlying pcdh19 function in the CNS. In pcdh19 null mice, an increase in migrating neurons is observed, but this does not lead to a significant change in the positioning of neurons in the developing and adult mice brains, and no obvious gross morphological defects are detected in the mutant mice (Pederick et al., 2016). This is in contrast to the zebrafish study using morpholino antisense oligonucleotides (see above), in which brain morphogenesis is affected and gross brain defects are observed. This apparent difference is likely due to a lack of compensatory mechanisms in the morphant zebrafish (Pederick et al., 2016).

Despite the finding showing no difference in neuronal positions and gross brain morphology, pcdh19 knockout and heterozygous female mice show significantly higher seizure incident rates compared to wild type mice (Rakotomamonjy et al., 2020), These seizures are observed in the frontal lobe of the cerebral hemisphere. In contrast, heterozygous males do no display increased rates of seizures. These findings suggest that pcdh19 pathogenesis has a sex-related characteristic, namely, a unique pattern of X-linked inheritance. Further analysis using both in vitro and in vivo methods showed that pcdh19 plays an important role in sorting of neural progenitor cells in developing mice cortex (Pederick et al., 2017). In female pcdh19 heterozygous mice, pcdh19-expressing cells (i.e. from the wildtype X-chromosome) sort out from pcdh19 negative cells (from the mutated X-chromosome), likely contributing to abnormal brain circuits formation responsible for the increased epilepsy. Heterozygous male mice have no functional pcdh19, and no pcdh19-dependent cell sorting. Normal pcdh19 function in these heterozygous (called hemizygous, because it is X-linked) male is likely compensated by activities of other similar pcdhs, such as pcdh17, that have similar expression patterns in the brain as pcdh19, resulting in no detectable change in seizure rates. Indeed, expression studies in zebrafish and rats clearly show that pcdh17 and pcdh19 expression domains overlap extensively (Kim et al., 2007; Liu et al., 2015). In summary, the molecular mechanism underlying X-linked pcdh19 function depends more on the nature of X-chromosome inactivation (random) than the actual function of the gene. However, more studies are required to explain why increased rates of seizures are also detected in homozygous female mice.

Additionally, it was found that heterozygous knockout female mice, in addition to showing increased rates of seizures, also displayed autism-like behaviors when placed with other mice and objects. Surprisingly, hemizygous pcdh19 knockout male mice (without any pcdh19

function) also display autism-like behaviors. These results demonstrate that pcdh19 function is required for normal mouse brain development and function (Lim et al., 2019).

Pcdh19 Function in Humans

Understanding the functioning of PCDH19 in humans is important in better understanding the health and disease of human beings. Much of the research done on humans has been from mutation studies. More than 100 different mutations in PCDH19 have been identified, including nonsense, missense, small nucleotide changes, alterations to splice sites, and others (Niazi et al., 2018). Interestingly, there have been no mutations found in exon 2 (Depienne & LeGuern, 2012).

The mutations that occur in exon 1 become pathological due to the fact that exon 1 is the largest exon, containing the extracellular domains responsible for cell-cell interactions (Niazi et al., 2018). Also interesting is that amino acid substitutions mutations found in the cytoplasmic domain, or exons 3 to 6, appear to have little or no deleterious effects on the protein function. In some patients, the whole PCDH19 gene was deleted (Depienne et al., 2011), which supports the idea that the mutation leads to a loss of function.

Similar to mice, hemizygous humans show differences between males and females (Dibbens et al., 2008). Males hemizygous for the PCDH19 gene show no abnormal cognitive function and no seizures, whereas hemizygous females show early severe seizures and mental retardation (Dibbens et al., 2008). This finding suggests impairment in the frontal and temporal lobes. Since the absence of the gene led to no overall effect on the males, it is probable that PCDH19 may not be an essential protein for humans, and the absence in males is compensated by another protein (e.g. PCDH17) or proteins (see above). Like the mice, random X inactivation

in the hemizygous females, lead to sorting of PCDH19 positive cells from PCDH19 negative cells, causing brain tissue mosaicism and abnormal formation of brain circuits (Depienne et al., 2009; Koc et al., 2019). This explains why mutations in the X-linked PCDH19 gene can cause epilepsy and mental retardation limited to females (EFMR). These detrimental effects are not seen in males due to the fact that males have only one X chromosome, which allows their cells to be homogenous in regard to PCDH19 expression and function (Kolc et al., 2019).

Conclusion

Much has already been learned about pcdh19/PCDH19 expression and function in the vertebrate CNS. This protein plays an important role in cell-cell adhesion throughout the CNS, which also regulates communication between cells. Pcdh19/PCDH19 is expressed in equivalent regions throughout the zebrafish, mouse, rat, and human brain, where these regions all play pivotal rules in the proper functioning of the CNS. The most important region of this gene is exon 1, which is the region that encodes for protein the domain that is responsible for cell to cell adhesion. Mutations in the gene cause a disrupt in the interaction between pcdh19+/PCDH19+ and pcdh19-/PCDH19- cells, which can result in disorganization and dysfunction of the CNS. In humans, mutations in this region can lead to early onset of seizures in female infants, which evolve to become more severe with affective symptoms. With our knowledge of pcdh19/PCDH10 expression and function in the vertebrate CNS, researchers and clinicians may develop potential strategies for treating patients with loss of PCHH19 function.

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