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REVIEW

Regulation of neural circuit formation by protocadherins

Stacey L. Peek[1,2] · Kar Men Mah[2] · Joshua A. Weiner[2,3]

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Abstract The protocadherins (Pcdhs), which make up the most diverse group within the cadherin superfamily, were first discovered in the early 1990s. Data implicating the Pcdhs, including ~60 proteins encoded by the tandem *Pcdha*, *Pcdhb*, and *Pcdhg* gene clusters and another ~10 non-clustered Pcdhs, in the regulation of neural development have continually accumulated, with a significant expansion of the field over the past decade. Here, we review the many roles played by clustered and non-clustered Pcdhs in multiple steps important for the formation and function of neural circuits, including dendrite arborization, axon outgrowth and targeting, synaptogenesis, and synapse elimination. We further discuss studies implicating mutation or epigenetic dysregulation of *Pcdh* genes in a variety of human neurodevelopmental and neurological disorders. With recent structural modeling of Pcdh proteins, the prospects for uncovering molecular mechanisms of Pcdh extracellular and intracellular interactions, and their role in normal and disrupted neural circuit formation, are bright.

Keywords Dendrites · Dendritic arborization · Axon outgrowth · Axonal targeting · Synaptogenesis · Synapse elimination · Cell adhesion molecules · Disease

Abbreviations

Pcdhs	Protocadherins
EC	Extracellular cadherin
PP1a	Protein phosphatase 1a
P	Postnatal day
WIRS	WRC interacting receptor sequence
WRC	WAVE regulatory complex
FAK	Focal adhesion kinase
PKC	Protein kinase C
SACs	Starburst amacrine cells
LTP	Long-term potentiation
Mdm2	Murine double minute 2
EFMR	Epilepsy and mental retardation limited to females
FIRES	Febrile infection-related epilepsy syndromes
ASDs	Autistic spectrum disorders
RTT	Rett syndrome
MECP2	*Methyl-CpG-binding protein 2*
ADHD	Attention deficit hyperactivity disorder
DS	Down syndrome
FASDs	Fetal alcohol spectrum disorders
HD	Huntington's disease
mHtt	Mutant *huntingtin* gene
GWAS	Genome-wide association studies
SCZ	Schizophrenia
BPD	Bipolar disorder
MDD	Major depressive disorder

Stacey Peek and Kar Men Mah contributed equally to this review.

✉ Joshua A. Weiner
joshua-weiner@uiowa.edu

Stacey L. Peek
stacey-peek@uiowa.edu

Kar Men Mah
karmen-mah@uiowa.edu

[1] Interdisciplinary Graduate Program in Neuroscience, The University of Iowa, Iowa City, IA, USA

[2] Department of Biology, The University of Iowa, Iowa City, IA, USA

[3] Department of Psychiatry, The University of Iowa, 143 Biology Building, Iowa City, IA 52242, USA

Introduction

The formation of proper neural circuitry requires intricate orchestration of cell–cell interactions at many stages of neurodevelopment, including neuronal differentiation and migration, axon outgrowth, dendrite arborization, and synaptogenesis. These processes are believed to involve specific molecular recognition between cell surface proteins on apposed neurons, or between those on neurons and glial cells such as astrocytes. Binding between appropriate cell adhesion molecules, and between other surface-bound ligands and receptors, can initiate cascades of intracellular signaling that allow neurons to develop and to function properly within the nervous system. For the development of a functional nervous system, each neuron must "choose" the partner neurons on which it will make, potentially, thousands of synapses. Since Sperry formulated the classic "Chemoaffinity Hypothesis", which posits that specific chemical or molecular cues guide the formation of circuitry during development, identifying such cues has been a major goal of developmental neuroscience [1]. Many studies have focused on diverse families of adhesion molecules, including the immunoglobulin and cadherin superfamilies, as likely candidates to provide a large repertoire of cell surface recognition molecules capable of producing a barcode-like identity for each cell. Additionally, molecular diversity can be generated by alternative splicing within individual genes: examples include the vertebrate neurexin genes, which can generate thousands of distinct proteins [2], and the *Drosophila* Dscam1 gene, a member of the immunoglobulin superfamily which can generate 19,008 unique extracellular regions, each one facilitating strictly homophilic recognition [3, 4]. In the case of *Dscam1*, such molecular recognition in most cases results in repulsion rather than adhesion, allowing each neuron to distinguish self from non-self to avoid fasciculation between its own dendrites and axons [5–8].

In vertebrates, top candidates for providing molecular diversity in cell–cell interactions, both adhesive and repulsive, are the protocadherins (Pcdhs), including those encoded by ~60 genes organized into three gene clusters (the clustered Pcdhs: α-, β-, and γ-Pcdhs) and another ten encoded by non-clustered (δ-Pcdh) genes. Protocadherins, discovered initially by Sano et al. [9] in a screen for cadherin-related genes, represent the largest group within the cadherin superfamily, membership in which is characterized by the presence of repeated extracellular cadherin (EC) motifs of approximately 100 amino acids. The "classical" cadherins have five EC repeats, while Pcdhs have six (clustered Pcdhs, δ2-Pcdhs) or seven (δ1-Pcdhs). While the classical cadherins signal to the actin cytoskeleton via catenin binding sites in their intracellular domains, the Pcdhs lack such sites and thus integrate into distinct signaling pathways. All of the Pcdh families have been implicated in multiple steps in neural circuit formation, and genetic studies are increasingly associating Pcdh gene mutations, copy number variations, and epigenetic dysregulation in neurodevelopmental disorders. Here, we review the data demonstrating roles for the clustered and non-clustered Pcdhs in axon outgrowth and targeting, dendrite arborization, and synaptogenesis, and highlight the evidence indicating their potential involvement in human neurodevelopmental and neurological disorders. Note that we focus here exclusively on the clustered Pcdhs and δ-Pcdhs, and on functions related to the establishment of neural circuitry; several other reviews can be consulted for other Pcdh roles [10–16] and for discussion of other cadherin superfamily molecules with roles in neural development [17–19].

The clustered protocadherins

In 1998, Kohmura et al. [20] discovered a number of cadherin-related genes, termed *Cadherin-Related Neuronal Receptors* (CNRs), using a yeast two-hybrid screen for interactors of the Fyn tyrosine kinase. Subsequent database searches using these sequences uncovered a larger family of cadherin-related genes that are now referred to as the clustered Protocadherins (Pcdhs). The clustered Pcdh genes are found in three tandem arrays (termed *Pcdha*, *Pcdhb*, and *Pcdhg*) spanning about 1 MB at human chromosome 5q31 and mouse chromosome 18 [21–23] (Fig. 1). Similar Pcdh gene clusters are found in other vertebrates, such as zebrafish and *Xenopus*, but with varying numbers of genes and distinct arrangements of clusters [24–27]. Each large "variable" exon (14 in *Pcdha,* 22 in both *Pcdhb* and *Pcdhg*) encodes six EC domains, a transmembrane domain, and a unique cytoplasmic domain of about 90 amino acids. Each variable exon is expressed from its own promoter through a "promoter choice" mechanism [28–32]. For *Pcdha* and *Pcdhg,* transcription continues through the 3′ end of the cluster, and the 5′-proximal variable exon is spliced to three small "constant" exons that encode a shared ~125 amino acid C-terminal domain (Fig. 1). No such constant exons exist within the *Pcdhb* gene cluster, and thus each variable exon produces a complete transmembrane protein [32, 33]. Further complexity is added to α-Pcdh isoforms by alternative splicing within the *Pcdha* constant exon 3, generating longer "A" type or shorter "B" type C-termini [21]. Immunohistochemistry and protein biochemistry indicates that α- and γ-Pcdhs are localized to axons and dendrites, and are found at some, but not all, synapses [20, 33–40, 47]. While fewer studies on the β-Pcdhs have

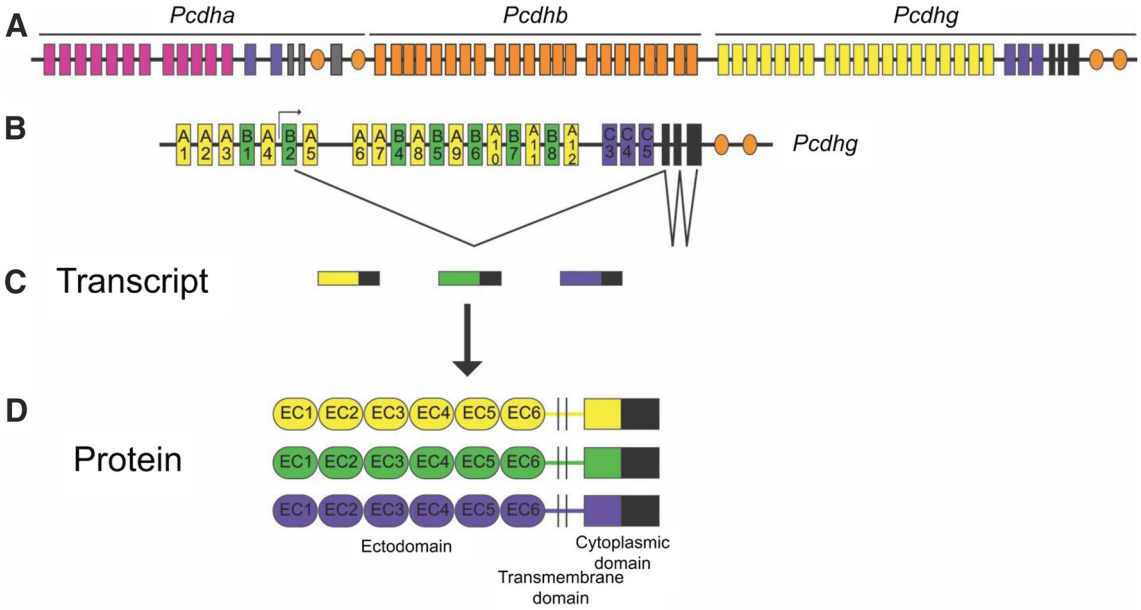

Fig. 1 The protocadherin gene clusters. **a** Schematic of the murine *Pcdha, Pcdhb,* and *Pcdhg* gene clusters on chromosome 18. A very similar structure is observed for the human clusters at chromosome 5q31. **b** The exon structure of the *Pcdhg* cluster is expanded below, with an example of the transcription initiation and splicing pattern (for B2, in this instance). **c** Schematic of the *Pcdhg* spliced transcripts generated by the cluster; each mature transcript consists of one large variable exon (*yellow*, *green*, or *purple*) and the three small constant exons (*black*). **d** Protein structure of the γ-Pcdhs (α-Pcdhs are identical in structure; β-Pcdhs lack any constant domain). Six extracellular cadherin (EC) repeats, a transmembrane domain, and a variable cytoplasmic domain are encoded by each variable exon; the constant exons encode a 125 amino acid C-terminal domain. Ovals indicate the sites of "cluster control regions", enhancers required for normal expression patterns of the Pcdh clusters

been reported, it has been shown that two members of this cluster, β16 and β22, are similarly preferentially localized to dendritic and post-synaptic density compartments in retinal and cerebellar neurons [41, 42, 43].

The *Pcdhg* cluster encodes γ-Pcdh proteins that can be grouped into three subfamilies on the basis of sequence similarity, termed γ-Pcdh-A, -B, and -C; however, γ-Pcdh-C3, -C4, and -C5 are more similar to α-Pcdh-C1 and -C2, found within the *Pcdha* cluster, than they are to any other γ-Pcdhs [22] (Fig. 1). All five C-type isoforms are expressed bi-allelically and ubiquitously in neurons, while the other *Pcdha, Pcdhb* and *Pcdhg* isoforms are expressed in an apparently sparser, monoallelic, and stochastic fashion [44–46]. Mice lacking the entire *Pcdhg* cluster die shortly after birth with massive neuronal apoptosis in the spinal cord and brainstem [47]; in contrast, mice lacking the *Pcdha* and/or the *Pcdhb* clusters are viable and fertile [48]. Mice in which only the ubiquitously expressed C3, C4, and C5 *Pcdhg* exons have been deleted phenocopy the null in many respects, while those lacking only the sparsely expressed A1, A2, and A3 *Pcdhg* exons are viable and fertile [49]. This is consistent with the existence of distinct, non-redundant roles for one or more C-type γ-Pcdhs, a possibility borne out by the demonstration that γ-Pcdh-C3, uniquely among γ-Pcdhs, can interact with Axin1 to inhibit canonical Wnt signaling [50].

Schreiner and Weiner [51] first showed, using a K562 cell aggregation assay and protein biochemistry, that individual γ-Pcdh proteins interact in a strictly homophilic manner in *trans*, while engaging in promiscuous *cis*-interactions with each other through their extracellular domains. This finding subsequently was confirmed and extended by Maniatis and colleagues, who showed that all of the 58 clustered Pcdhs are capable of homophilic *trans*-interaction [52]. Interestingly, while the α-Pcdhs can mediate homophilic interaction, most require *cis*-interaction with β- or γ-Pcdhs to reach the cell surface to do so [52]; this finding ascribes a functional role to an earlier report that γ-Pcdhs regulate the surface expression of α-Pcdhs [53]. Schreiner and Weiner [51] also found that while the EC1 motifs of γ-Pcdhs are required for *trans*-interactions, the homophilic specificity of these interactions are, rather, facilitated by matches between apposing EC2 and EC3 domains. Consistent with this, sequence analysis across α-Pcdh and γ-Pcdh family members reveals more divergence in EC2/3 than in other EC domains [51, 52]. While homophilic interaction of γ-Pcdhs can induce stable adhesive cell–cell contacts and cell aggregation [51, 52, 54, 55], some phenotypes (see below) in *Pcdhg* and *Pcdha* mutant mice are consistent with the possibility that homophilic interaction leads to repulsive signaling [56–59].

Recent crystallographic data and protein structure modeling, coupled with biochemical assays, represent a major leap forward in understanding the domains and amino acid residues that mediate *cis*- and *trans*-interactions among the clustered Pcdhs. In 2015 and 2016, the crystal structures of the extracellular domains of at least two isoforms each of α-Pcdhs, β-Pcdhs, and the γ-Pcdh-A, γ-Pcdh-B, and γ-Pcdh-C subfamilies were reported by two independent laboratories [60–64]. These reveal a highly specific anti-parallel *trans*-interaction interface facilitated by head-to-tail contacts involving EC1–EC4. Consistent with earlier results indicating the importance of EC2/3 for homophilic interaction [51, 52], antiparallel contact between these domains is a common feature of all the determined structures and models. Structural data also confirm biochemical experiments, demonstrating a critical role for EC6 in mediating *cis*-interactions between dimers of clustered Pcdhs as well as in the regulation of surface delivery of these proteins [52, 61, 64] (Fig. 2). Interestingly, biophysical measurements of several γ-Pcdh-A and γ-Pcdh-B subfamily isoforms suggested that members of the γ-Pcdh-B subfamily are capable of homophilic interactions in *cis,* but γ-Pcdh-A subfamily members are not, suggesting *cis*-multimerization is not completely promiscuous and adding another level of complexity [61]. Together with earlier cell aggregation studies, these new crystal structure and biophysical data suggest that the unit of clustered Pcdh adhesion is either a discrete *trans*-dimer of *cis*-dimers, or a zipper-like lattice whose extent may be regulated by the degree of isoform matching and mismatching between two cells [61, 64].

The non-clustered protocadherins

The ten non-clustered Pcdhs, also referred to as δ-Pcdhs, can be grouped into three subfamilies based on homology: δ0 (Pcdh20), δ1 (Pcdh1, Pcdh7, Pcdh9, and Pcdh11X/Y), and δ2 (Pcdh8/PAPC, Pcdh10, Pcdh17, Pcdh18, Pcdh19). The δ1 subfamily proteins have seven EC repeats, while δ0 and δ2 Pcdhs have six EC repeats [65] (Fig. 2). As for the clustered Pcdhs, in most δ-Pcdh genes a single large first exon encodes the amino-terminal signal peptide, multiple EC repeats, a single transmembrane domain, and a small part of the intracellular domain; the remainder of the intracellular domain is encoded by the remaining exons. *Pcdh1* and *Pcdh11* differ, in that two exons encode the EC domains [66]. Non-clustered Pcdhs exhibit a form of

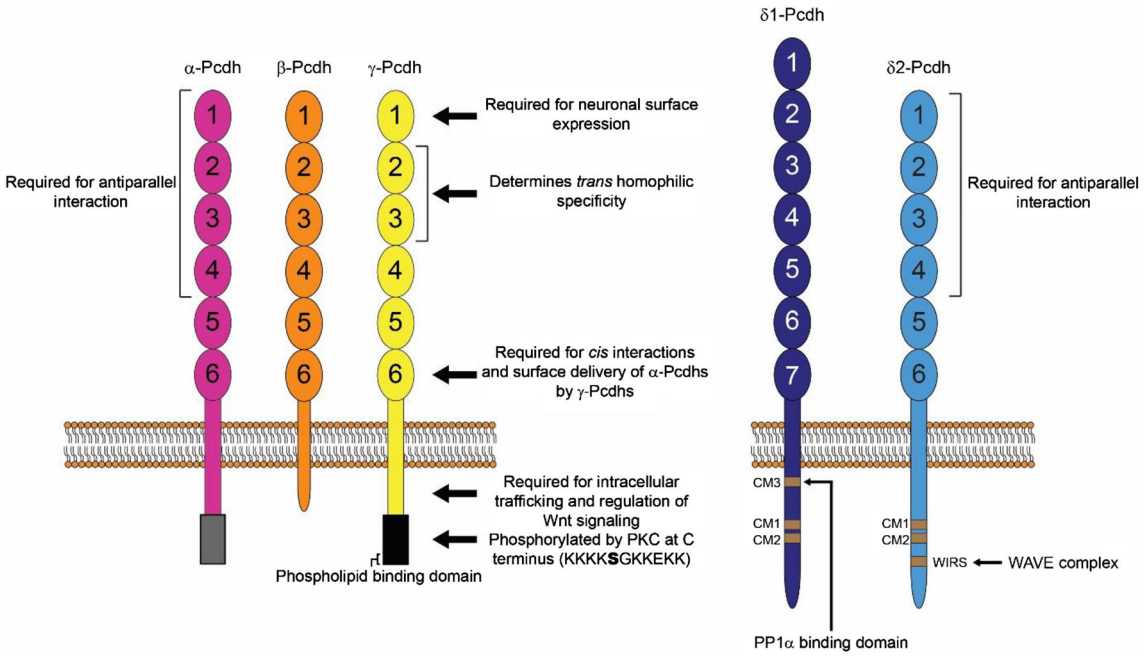

Fig. 2 Domain structures of clustered and non-clustered protocadherins. Schematic of the protein domains found in α-, β-, and γ-Pcdhs, with known roles of each domain indicated. EC1 is required for efficient surface expression (and therefore homophilic interaction) of clustered Pcdhs. EC2 and EC3 determine the specificity of antiparallel homophilic binding, which also involves contacts between EC1 and EC4. This antiparallel *trans*-interaction of opposed EC1–4 domains has also been shown to occur for the δ2-Pcdhs. EC6 of the γ-Pcdhs is required for interaction with α-Pcdhs for their efficient delivery to the cell surface. The variable cytoplasmic domain is involved in γ-Pcdh intracellular trafficking and their regulation of Wnt signaling, while a serine at the lipid-binding C-terminus of the constant domain is a target for PKC phosphorylation. The cytoplasmic domains of δ1- and δ2-Pcdhs harbor conserved domains (CM1–3) that may bind several intracellular signaling proteins, including PP1α. The δ2-Pcdhs (and the δ1-Pcdh Pcdh9) also harbor a wave interacting receptor sequence (WIRS) motif that mediates interaction with the WAVE complex

alternative splicing of downstream exons, producing isoforms with slight variations in the intracellular domain. Alternative use of small exons that encode the 5′ untranslated region or the variable cytoplasmic domain results in a large number of splice variants for *Pcdh11* and *Pcdh19*, both of which are located on the X-chromosome in human and mouse [66, 67]. Additionally, there are conserved motifs in the cytoplasmic domains of the δ-Pcdhs (CM1 and CM2); δ1-Pcdhs also have a CM3 that is absent in δ2-Pcdhs, and which has been found to bind protein phosphatase 1α (PP1α) [66] (Fig. 2). Non-clustered Pcdhs are found throughout the central nervous system; as for the clustered Pcdhs, the proteins are localized to axons and dendrites and are found perisynaptically and at some synapses [68–72].

In contrast to the recent studies determining crystal structures of many clustered Pcdh domains, few structural data have yet been reported for non-clustered Pcdhs. Using sequence coevolution analysis and comparison to γ-Pcdh structures, it was suggested that non-clustered Pcdhs likely interact via antiparallel EC1–4 contacts, in a manner similar to the clustered Pcdhs [63]. Recently, Cooper et al. [73] reported structural data for EC1–4 of zebrafish Pcdh19, a δ2-Pcdh family member, which indicate a similar architecture to that of clustered Pcdhs. This study also presented evidence that Pcdh19 binds homophilically using an antiparallel dimer interface. Mutations in Pcdh19 lead to a female-limited form of epilepsy (see "Epilepsy" section below); a large proportion of these are missense mutations in the EC domain. These mutations lead to protein misfolding and structural instability, which when replicated in vitro, were found to inhibit Pcdh19's adhesive ability in bead aggregation assays [73].

Several groups have sought to identify roles for non-clustered Pcdhs in homophilic cell adhesion. Though multiple δ-Pcdhs have been reported to exhibit homophilic adhesion, including Pcdh10 [72], Pcdh8 [74], Pcdh17 [68], and Pcdh19 [75], as for the clustered Pcdhs this seems to be modest in comparison to the classical cadherins [76]. Interestingly, several δ-Pcdhs have been shown to interact in *cis* with classical cadherins to regulate adhesion: Pcdh8 (also known as paraxial protocadherin in *Xenopus* and Arcadlin in mammals) [70, 74] antagonizes adhesion by C-cadherin [77] and induces the internalization of N-cadherin at synapses in response to increased electrical activity [70]. Pcdh19 can mediate homophilic adhesion only when in a *cis*-complex with N-cadherin, and this interaction precludes homophilic adhesion through N-cadherin itself [78]. Thus, it is possible that the non-clustered Pcdhs can either mediate or disrupt cell adhesion, depending presumably on the presence or absence of a variety of co-receptors or intracellular cofactors.

Roles in axon outgrowth and targeting

Clustered protocadherins

Several studies by the Yagi laboratory have established that the α-Pcdhs are important for the targeting of several axon types (see Table 1 for a summary of roles). Hasegawa et al. [79, 80] investigated a role for the α-Pcdhs in the sorting of olfactory sensory axons. Olfactory sensory neurons expressing a particular odorant receptor typically project axons to specific glomeruli in the main olfactory bulb. In *Pcdha* mutant mice lacking the constant exons (resulting in an apparent null allele), however, olfactory axons projected to multiple small extraneous glomeruli, instead of coalescing into a single glomerulus. While multiple glomeruli targeted by axons expressing a given odorant receptor can be found to some extent in younger wild type mice, in *Pcdha* mutants these multiple smaller glomeruli persisted over time [79]. Thus, while early stages of olfactory sensory neuron axon outgrowth do not require the α-Pcdhs, they are required in the final stages of axonal coalescence on individual glomeruli [79]. Subsequently, by using a transgenic mouse line that produces truncated α-Pcdh isoforms, the same group found that the α-Pcdh cytoplasmic region is essential for this process. It was further shown that the disruption of axon coalescence could be rescued by constitutive expression of even a single α-Pcdh isoform [80]. Recently, it was reported that the β- and γ-Pcdhs are also important for the development of olfactory axon projections: mice lacking the *Pcdhb* or *Pcdhg* cluster exhibited disrupted glomerular structure and altered axon projections, with the greatest defects observed when multiple clusters were deleted [48].

Serotonergic neurons also require the longer A-type cytoplasmic tail of α-Pcdh proteins for their proper axon projection and penetration into several brain target regions [81]. In *Pcdha* mutant animals lacking either the entire constant region, or just the A-type cytoplasmic tail, serotonergic axons approached their normal targets, but failed to form extensive axonal arbors within them, in contrast to wild-type animals [81]. This phenotype did not involve loss of any serotonergic neurons, as the number and distribution of *SERT*-expressing neurons within the raphe nuclei was similar between *Pcdha* mutants and controls [81]. These observations suggest a potential clinically relevant role for α-Pcdhs, as the serotonergic system is known to be involved in a wide variety of mood and anxiety disorders.

More recently, Meguro et al. [58] found that the terminals of retinal ganglion cells projecting to the dorsal lateral geniculate nucleus formed large aggregates in *Pcdha* mutant animals. These retinogeniculate terminals seemed to project appropriately early on in development,

Table 1 Selected roles for protocadherins in axon outgrowth and targeting

Gene symbol	Protein name	Results	Selected references
Pcdha	α-Protocadherins	Defect in the projection of olfactory axons in *Pcdha* mutant mice. This phenotype can be rescued by the constitutive expression of a single α-Pcdh isoform	Hasegawa et al. [79, 80]
		Serotonergic axons of *Pcdha* mutant animals lacking either the entire constant region or the A-type cytoplasmic tail approach their targets, but do not form extensive axonal arbors within them	Katori et al. [81]
		Terminals of retinal ganglion cells projecting to dorsal lateral geniculate nucleus in *Pcdha* knockout mice form large aggregates, resulting in reduced visual acuity	Meguro et al. [58]
Pcdhg	γ-Protocadherins	Spinal proprioceptive Ia afferent terminals are aberrantly expanded and aggregated in the ventral horn of *Pcdhg* mutant animals. These animals also exhibit fewer collateral projections of Ia afferent axons onto ventral interneurons. A similar phenotype is observed in animals lacking only the *Pcdhg* C3, C4 and C5 variable exons	Prasad and Weiner [59], Chen et al. [49]
Pcdha, Pcdhb, and *Pcdhg*	All clustered protocadherins	Mice lacking any and all combinations of clustered Pcdhs exhibited disrupted glomerular structure and altered axonal projections in olfactory neurons	Hasegawa et al. [48]
Pcdh7	Protocadherin 7, NF-protocadherin (*Xenopus*)	Expression of dominant-negative Pcdh7 lacking the extracellular domains resulted in truncated or absent retinal ganglion cell axons	Piper et al. [85]
		Morpholino knockdown of Pcdh7 caused retinal ganglion cell axons to be insensitive to the guidance molecule netrin-1 and resulted in axons that do not exit the optic nerve head	Leung et al. [86]
		Homophilic interactions between axonal Pcdh7 and surrounding neuroepithelial Pcdh7 are required for axonal extension in the mid-optic tract; furthermore these appear to be facilitated by Sema3A-induced local translation of Pcdh7 in the growth cone, which requires the Sema3A receptor neuropilin-1	Leung et al. [87]
Pcdh10	Protocadherin 10, OL-protocadherin	Striatal neurons lacking Pcdh10 exhibit impaired axon outgrowth. Deletion of Pcdh10 in mice led to the absence of a corticospinal tract and stalled or misrouted corticofugal and thalamocortical projections	Uemura et al. [88]
		Pcdh10 expression is regulated by activity in the olfactory epithelium. Misexpression of Pcdh10 in transgenic mice led to aberrant coalescence of some olfactory axons and ectopic positioning of some glomeruli	Williams et al. [69]
Pcdh17	Protocadherin 17	Amygdala axons in mice lacking Pcdh17 fail to extend proper axons, while ectopic expression of Pcdh17 via in utero electroporation results in misrouted axons	Hayashi et al. [92]
Pcdh18b	Protocadherin 18b	Morpholino-mediated knockdown of Pcdh18b resulted in shorter motor axons with fewer branches, a phenotype that could be rescued by co-injection of a BAC containing the Pcdh18b gene. Pcdh18b or Nap1 knockdown resulted in growth cones with fewer filopodia	Biswas et al. [93]

only forming aberrant aggregates between postnatal day (P)10 and P14, just before eye opening and refinement of retinogeniculate projections. *Pcdha* mutant mice exhibited reduced visual acuity, but the orientation and direction selectivity of neurons in the primary visual cortex were apparently normal [58, 82]. Together, these studies from the Yagi group strongly support a crucial role for α-Pcdhs in the proper targeting and refinement of long axonal projections in the CNS.

Though axon outgrowth, targeting, and tract formation appear to be grossly normal in *Pcdhg* mutant neurons [83, 84], γ-Pcdhs have been implicated in the proper patterning of axon terminals. Prasad and Weiner [59] demonstrated disrupted distribution of spinal proprioceptive Ia afferent terminal arbors in the ventral horn of *Pcdhg* mutant animals. Mutant Ia afferent terminals were aberrantly expanded and aggregated around their primary motor neuron targets, a phenotype reminiscent of that reported for *Pcdha* mutant retinogeniculate projections [58]. There were also fewer collateral projections of Ia afferent axons onto ventral interneurons in *Pcdhg* mutant animals, even when apoptosis of these ventral interneurons was blocked by the introduction of a *Bax* null alelle [59]. Subsequent studies showed that this phenotype was recapitulated in mice lacking only the C3, C4, and C5, but not the A1, A2, and A3, variable exons of the *Pcdhg* cluster

[49]. Using conditional *Pcdhg* mutant mice and multiple Cre transgenic lines to restrict the loss of γ-Pcdhs to dorsal root ganglion neurons, spinal interneurons, or motor neurons, Prasad and Weiner presented evidence that γ-Pcdhs provide a homophilic cue between Ia afferents and their target ventral interneurons; in the absence of this cue, Ia afferents fail to project collaterals and concentrate their terminals onto motor neurons [59]. The observed aggregation of Ia afferent terminals in *Pcdhg* mutants, and of retinogeniculate terminals in *Pcdha* mutants, might also represent a disruption of self-avoidance analogous to that demonstrated (see below) by Lefebvre et al. [57] in the dendrites of certain *Pcdhg* mutant neurons. This possibility should be addressed in these systems in future experiments by the reintroduction of an individual α- or γ-Pcdh isoform: if self-avoidance is disrupted in the mutants, this single-isoform expression should rescue the observed phenotypes.

Non-clustered protocadherins

Numerous studies have revealed roles for multiple non-clustered Pcdhs in the regulation of axonogenesis, axon outgrowth, and axon targeting (see Table 1 for a summary of roles). Expression of dominant-negative Pcdh7 (also known as NF-protocadherin) lacking the extracellular domain in *Xenopus* embryos indicated a role for this Pcdh in axonogenesis [85]. This manipulation resulted in retinal ganglion cells with truncated or completely absent axons, with few reaching the optic tectum. Consistent with this, expression of a truncated, dominant-negative TAF-1, a known cytoplasmic cofactor that interacts with the intracellular domain of Pcdh7, results in a similar phenotype [85]. Subsequent work showed that Pcdh7 modulates responses to axon guidance molecules Sema3a and netrin-1 to regulate optic nerve axons at several discrete points. First, morpholino knockdown of *Pcdh7* renders retinal axons insensitive to netrin-1, a critical signal that attracts axons toward the optic nerve head. Netrin-1 induces the degradation of Pcdh7 protein, which may be required for proper axon exit from the eye [86]. Subsequently, after axons cross the optic chiasm and continue toward the optic tectum, Pcdh7 plays a second role in guidance [87]. Upon reaching the mid-optic tract, an area where surrounding expression of Sema3A is high, axons must turn caudally. Homophilic interactions between axonal Pcdh7 and surrounding neuroepithelial Pcdh7 are required for axonal extension in this area; interestingly, these appear to be facilitated by Sema3A-induced local translation of Pcdh7 in the growth cone, which requires the Sema3A receptor neuropilin-1 [87]. In this manner, Pcdh7 is dynamically regulated by axon guidance cues to facilitate multiple steps in retinotectal axon guidance [86, 87].

Deletion of *Pcdh10* (also known as *OL-Pcdh*) in mice led to abnormal projections of a number of axon tracts that extend through the ventral telencephalon, an area of high Pcdh10 expression [88]. *Pcdh10* knockout mice completely lack a corticospinal tract, and corticofugal and thalamocortical projections are stalled or misrouted. Putative Nkx2.1+ and Islet-1+ guidepost cells in the ventral telencephalon are absent in *Pcdh10* knockouts, implying that a loss of cellular cues leads to the disruption of axon pathfinding [88]. Striatal neurons lacking Pcdh10 also exhibit impaired axon outgrowth both in vivo and in vitro [88]. An additional role for Pcdh10 in the targeting of olfactory axons to glomeruli was suggested by Williams et al., [69], who show that *Pcdh10* expression is regulated by activity in the olfactory epithelium. Misexpression of Pcdh10 in transgenic mice led to aberrant coalescence of some olfactory axons and ectopic positioning of some glomeruli [69]; this is particularly intriguing given the prominent role for the *Pcdha* cluster in olfactory axons, and suggests that multiple clustered and non-clustered Pcdh pathways are important for the development of olfactory circuitry.

A number of studies have established non-clustered Pcdhs as regulators of cytoskeletal signaling pathways critical for axon extension and pathfinding. GST-pulldown experiments identified Nap1, a molecule essential for neuronal differentiation and process outgrowth [89] that forms a complex with WAVE1 and Abi-1 to regulate actin rearrangement [90], as interactors of Pcdh10 (OL-Pcdh) [91]. The binding site for Nap1 is conserved between Pcdh10 [91], Pcdh19 [75], Pcdh17 [92], and Pcdh18b [93], suggesting a cytoskeletal signaling mechanism common to multiple non-clustered Pcdhs. This is bolstered by the discovery of the WRC interacting receptor sequence (WIRS), a conserved motif, present in the cytoplasmic tails of ~120 transmembrane molecules, that binds the WAVE regulatory complex (WRC) [94]. Many Pcdhs, including the α-Pcdhs and several members of the δ1- and δ2-Pcdh families harbor WIRS motifs, and a subset was confirmed to bind the WRC in GST-pulldown assays [94] (Fig. 2). Transfection of *Pcdh10* into U251 cells, a motile human astrocytoma line with movement similar to growth cones, results in colocalization with Nap1 at cell–cell contact sites and increases movement, but only when Pcdh10+ cells contact each other [91]. Parallel results were found when U251 cells were transfected with Pcdh17, and cell–cell contact sites with Pcdh17–Nap1 colocalization formed protruding edges [92]. Deletion of the Nap1 binding site from these δ-Pcdhs abolishes Nap1 recruitment to homophilic cell–cell junctions between cultured cells and between amygdala axons in explant cultures [91, 92].

Knockout of *Pcdh17* in mice prevented the normal extension and pathfinding of amygdala axons, while

ectopic expression of *Pcdh17* by in utero electroporation disrupted the pattern of amygdala axon outgrowth [92]. Interestingly, *Pcdh17* constructs lacking either the Nap1-binding domain or the entire cytoplasmic domain also disrupted axon patterning when overexpressed; however, only the mutant constructs (not wildtype *Pcdh17*) resulted in aberrant axon clumping, indicating distinct roles for intracellular signaling downstream of Pcdh17 homophilic adhesion [92]. In the zebrafish model, Biswas et al. [93] also presented evidence that Pcdh18b promotes axon outgrowth and branching via Nap1. Morpholino-mediated knockdown of *Pcdh18b* resulted in shorter motor axons with fewer branches, a phenotype that could be rescued by co-injection of a BAC containing the *Pcdh18b* gene. Live imaging of zebrafish embryos in which either Pcdh18b or Nap1 had been knocked down by morpholinos revealed that caudal primary motor neuron growth cones exhibited a lower density of filopodia; nevertheless, filopodia dynamics remained unaffected, suggesting that Pcdh18b and Nap1 are important primarily for forming new filopodia [93]. Together, these studies represent convergent evidence that non-clustered Pcdhs regulate several steps in axonogenesis, axon outgrowth, and axon pathfinding via signaling partners that control actin rearrangement.

Roles in dendrite arborization

Clustered protocadherins

Much progress has been made recently in identifying roles for the clustered Pcdhs, primarily the γ-Pcdhs, in the elaboration of dendritic arbors (see Table 2 for a summary of roles). Using *Emx1*-Cre transgenics to restrict mutation of a conditional *Pcdhg* allele to the cerebral cortex and circumvent the neonatal lethality found in constitutive mutants [47], Garrett et al. [83] found that γ-Pcdhs are essential for the proper formation of dendritic arbors in pyramidal neurons. Cortical neurogenesis, neuronal migration, and formation of major axonal tracts progressed normally in the *Pcdhg* mutant cortex, but the cortex appeared thinner due to loss of the cell-sparse, apical dendrite tuft-rich layer I [83]. Quantification of dendrite arbor complexity by Sholl analyses of layer V and layer II/III pyramidal neurons revealed a significant reduction in the absence of γ-Pcdhs. *In vivo* and in vitro experiments analyzing cell signaling revealed that this mutant phenotype was associated with aberrant activation of focal adhesion kinase (FAK), consistent with the important earlier finding that autophosphorylation (at Y397) and activation of FAK (and its homologue PYK2) is prevented by its interaction with the γ-Pcdh constant domain [95]. In *Pcdhg* mutant cortical neurons, protein kinase C (PKC) was also hyperactive and its target protein MARCKS was hyperphosphorylated, consistent with prior evidence that PKC negatively, and unphosphorylated MARCKS positively, regulate dendrite arborization [83]. Pharmocological inhibition of FAK or PKC, as well as overexpression of nonphosphorylatable MARCKS, rescued dendrite complexity in cortical neurons, confirming the involvement of a FAK/PKC/MARCKS signaling pathway downstream of γ-Pcdhs [83].

Concurrent work from Wu and colleagues offers complementary observations on the importance of both α- and γ-Pcdhs in dendrite arborization [96]. These authors found that *Pcdha* mutant hippocampal neurons both in vivo and in vitro had simplified dendritic arbors as well as a reduction in dendritic spine density, though this phenotype was not quite as severe as that observed following *Pcdhg* cluster knockdown by RNAi in vitro [96]. Consistent with Chen et al. [95] and Garrett et al. [83], both PYK2 and FAK were found to be hyperactivated in *Pcdha* mutant neurons, while activity of the Rac1 Rho GTPase was reduced [96]. Constitutively active Rac1 could rescue the dendritic phenotype of both *Pcdha* mutant, and *Pcdhg* knockdown, hippocampal neurons [96]. Together, Garrett et al. [83] and Suo et al. [96] provide convergent evidence that the clustered Pcdhs promote dendrite arborization in forebrain neurons via signaling mediated by adhesion kinases and Rho family GTPases.

Interestingly, given their apparent role in negatively regulating PKC activity in cortical neurons, the γ-Pcdhs' ability to inhibit FAK is, itself, negatively regulated by PKC. Keeler et al. [97] identified a serine residue within the γ-Pcdh constant domain that is phosphorylated by PKC in vitro and in vivo; this phosphorylation reduces γ-Pcdh-mediated FAK inhibition, providing a potential feedback mechanism. Overexpression of a non-phosphorylatable (S/A mutant) γ-Pcdh isoform in cortical neurons can increase dendrite arborization cell-autonomously, while overexpression of a phosphomimetic (S/D mutant) isoform, or treatment with the PKC activator PMA, can reduce dendrite arborization [97]. This raises the intriguing possibility that homophilic *trans*-interaction might lead to conformational changes preventing phosphorylation (or encouraging dephosphorylation) of the γ-Pcdh constant domain, though this remains to be shown experimentally. In further support of a role for γ-Pcdhs in dendrites, reduced dendrite arborization and dendritic spine density were observed in olfactory bulb neurons following lentiviral-Cre-driven *Pcdhg* mutation in their progenitors [98].

Although numerous in vitro cell aggregation and cell culture assays indicate that clustered Pcdhs can mediate homophilic cell adhesion [9, 51, 52, 54, 99–102], analysis of γ-Pcdh function in the retina indicates a role in repulsive signaling leading to self-avoidance. Lefebvre et al. [57]

Table 2 Selected roles for protocadherins in dendrite arborization

Gene symbol	Protein name	Results	Selected references
Pcdha	α-Protocadherins	*Pcdha* knockout resulted in decreased dendrite arborization in hippocampal neurons, in vivo and in vitro. This arborization defect was dependent on a PYK2/Rac pathway	Suo et al. [96]
Pcdhg	γ-Protocadherins	*Pcdhg* knockout reduced dendrite arborization of cortical layer V pyramidal neurons. γ-Pcdh inhibition of a FAK/PKC/MARCKS pathway is required for normal arborization	Garrett et al. [83]
		Pcdhg knockdown of hippocampal neurons in vitro resulted in reduced dendritic arborization as well as activation of PYK2 and FAK and increased Rac1 inhibition	Suo et al. [96]
		Pcdhg knockout in retinal starburst amacrine cells (SACs) and cerebellar Purkinje cells led to aberrant self-crossing of dendrites isoneuronally. Single γ-Pcdh isoform expression rescued this self-avoidance defect, but led to aberrant heteroneuronal avoidance of SAC dendrites	Lefebvre et al. [57]
		Pcdhg knockout in individual subventricular zone progenitor cells caused a reduction in dendritic arborization in olfactory bulb granule cells derived from these progenitors	Ledderose et al. [98]
		A serine residue within the γ-Pcdh constant domain is phosphorylated by PKC in vitro and in vivo, which reduced γ-Pcdh-mediated FAK inhibition. The overexpression of a non-phosphorylatable γ-Pcdh isoform increased dendrite arborization cell-autonomously, while the overexpression of a phosphomimetic reduced dendrite arborization	Keeler et al. [97]
		In the cerebral cortex, homophilic γ-Pcdh *trans*-interactions between neurons, and between neurons and astrocytes, promoted dendrite growth and arbor complexity	Molumby et al. [55]
Pcdha, Pcdhb, and *Pcdhg*	All clustered protocadherins (and/or possibly other genes affected by manipulation)	CTCF knockout cortex exhibited decreased *Pcdha*, *Pcdhb*, and *Pcdhg* expression and a reduction of dendrite arborization. Cultured hippocampal neurons also had reduced dendritic arbors. Note that phenotype may be due in part to disruption of other CTCF targets	Hirayama et al. [105]
		Dnmt3b methylation of *Pcdha* and selected *Pcdhb* and *Pcdhg* promoters determines expression frequency of Pcdhs within Purkinje neurons. Cell-specific knockout of *Dnmt3b* led to self-avoidance defects in Purkinje cell dendritic arbors. Note that phenotype may be due in part to disruption of other Dnmt3b targets	Toyoda et al. [106]
Pcdh7	Protocadherin 7, NF-protocadherin (*Xenopus*)	Dominant-negative Pcdh7 expression disrupted retinal ganglion cell dendrite arborization	Piper et al. [85]
Pcdh11X	Protocadherin 11X, protocadherin-X	shRNA knockdown of Pcdh11x in cultured cortical neurons increased the number of primary and secondary branches and the number of branch points, while the overexpression of Pcdh11X led to the opposite outcome; the PI3K-Akt pathway is implicated in the regulation of this process	Wu et al. [107]

revealed a distinct dendritic phenotype in *Pcdhg* mutant retinal starburst amacrine cells (SACs), the dendrites of which normally avoid crossing and fasciculating with each other, allowing for proper dendritic field spread. In contrast to wild-type SACs, *Pcdhg* mutant SACs are defective in self-avoidance, and their dendrites aberrantly cross and fasciculate with each other [57]. A similar increase in dendrite self-crossing was observed in *Pcdhg* mutant cerebellar Purkinje cells, a cell type which, like SACs, elaborates a self-avoiding, planar dendritic arbor. Remarkably, the SAC self-crossing phenotype was rescued cell-autonomously when only a single γ-Pcdh isoform was re-expressed by using a Cre-inducible transgene. When all SACs expressed the same γ-Pcdh isoform, however, they no longer properly intermingled with their neighbors, suggesting that the diversity of γ-Pcdh isoforms normally allows intra-neuronal self-avoidance while permitting inter-neuronal overlap for proper receptive field coverage [57]. As of yet, there are no assays directly demonstrating cell repulsion mediated by clustered Pcdh *trans*-

interactions, though as noted above, some axonal phenotypes are consistent with such a self-avoidance mechanism [58, 59]. It is possible that a switch from adhesion to repulsion involves distinct, cell type-specific, cytoplasmic signaling partners downstream of the clustered Pcdhs themselves; alternatively, repulsion could be mediated through other transmembrane molecules (e.g., DSCAM) that might interact in *cis* with the Pcdhs in some cell types. Though the signaling pathway through which γ-Pcdhs might promote repulsive self-avoidance remains unknown, Gibson et al. [103] have shown that γ-Pcdh-mediated self-avoidance in Purkinje cell dendrites is genetically independent of a similar self-avoidance role played by Slit/Robo signaling.

Recently, Molumby et al. [55] presented evidence that, at least in the cerebral cortex, homophilic γ-Pcdh *trans*-interactions between neurons, and between neurons and astrocytes, can positively promote dendrite growth and arbor complexity. Using the same Cre-inducible, single-isoform transgenic mouse lines reported by Lefebvre et al. [57], Molumby et al. [55] generated mice in which labeled cortical pyramidal neurons encountered increased or decreased γ-Pcdh homophilic matching with surrounding cells in vivo. Neurons surrounded entirely by other neurons and astrocytes expressing the same single γ-Pcdh isoform exhibited significantly increased dendrite growth and complexity; in contrast, isolated neurons expressing a single-isoform that few surrounding cells expressed exhibited severely deficient dendrite arbor complexity. The promotion of dendrite arborization via homophilic γ-Pcdh interactions appears to be a local phenomenon, rather than involving arbor-wide signaling, as only those branches encountering mis-matching cells showed decreased complexity [55]. Additionally, astrocytes, which highly express the γ-Pcdhs [35], promoted the arborization of pyramidal neuron dendrites cell non-autonomously through homophilic γ-Pcdh interactions [55]. In in vitro assays, Molumby et al. [55] found no evidence that neurons and astrocytes expressing the same γ-Pcdh isoform avoid each other; on the contrary, neuronal dendrites growing on astrocytes expressing the same single γ-Pcdh isoform concentrated the protein along contact sites, a result reminiscent of earlier observations in cultured hippocampal neurons [99]. As noted above, both neuronal types currently known to exhibit self-avoidance defects in the absence of γ-Pcdhs [56, 57] elaborate planar dendritic arbors, in contrast to the bushy arbors that cortical neurons develop. It may be that γ-Pcdh interactions can lead to distinct downstream effects in different cell types, in a way that depends on the ultimate structure and purpose of that neuron's dendritic arbor.

Finally, the phenotypes observed in mice in which clustered Pcdh gene expression is globally disrupted bolster the conclusion that these molecules are critical to the normal elaboration of dendritic arbors. The zinc finger transcription factor/insulator protein CTCF binds at several sites within the *Pcdh* gene clusters and was found to positively regulate the expression of *Pcdh* genes in numerous cell lines [28–30, 104]. In a forebrain-restricted conditional CTCF knockout mouse, clustered Pcdh genes (among many others) are severely downregulated, and dendrite arborization of both cortical and hippocampal neurons is significantly reduced, consistent with Garrett et al. [83] and Suo et al. [96]; barrel formation in the somatosensory cortex is also disrupted [105]. A separate study on the methylation of Pcdh gene promoters by the DNA methyltransferase Dnmt3b showed that loss of Dnmt3b led to constitutive expression of many Pcdh genes that are normally stochastically expressed [106]. Dnmt3b mutant Purkinje cells displayed increased self-crossing in Purkinje cells (similar to that observed by Lefebvre et al. [57]), but also reduced overall dendrite arborization and total length, which was not reported previously [106]. While these results support the importance of clustered Pcdhs in the regulation of dendrite arborization in many neuronal populations, it should be noted that the loss of CTCF or Dnmt3b also leads to dysregulation of several hundreds of non-Pcdh genes that could contribute to the phenotypes observed.

Non-clustered protocadherins

As noted below, several non-clustered Pcdhs play important roles in the regulation of dendritic spines. Nevertheless, in contrast to the extensive evidence that clustered Pcdhs regulate dendrite arborization, as of yet there are few reports indicating a similar role for the non-clustered Pcdhs in arborization per se. While the most severe retinal ganglion cell phenotypes following expression of dominant-negative Pcdh7 or TAF-1 in *Xenopus* embryos were found in axons, reduced dendrite arbors were also observed in some neurons [85]. In cultured cortical neurons, shRNA knockdown of *Pcdh11X* increased, and overexpression of *Pcdh11X* decreased, the number of primary and secondary branches as well as branch points [107]. Biochemical and pharmacological evidence indicates that Pcdh11X may inhibit dendrite arborization by upregulating a PI3K-Akt pathway [107].

Roles at synapses

Clustered protocadherins

Early subcellular fractionation studies found α- and γ-Pcdh proteins in synaptosome preparations and postsynaptic density protein fractions, and immunohistochemistry and

immunoelectron microscopy showed them at some, but far from all, synapses [20, 35, 36, 39, 40, 47]. While less is known about the β-Pcdh proteins, at least two (β16 and β22) are enriched in synaptic layers and can localize to subsets of retinal synapses [41, 42, 43]. While immunostaining for pre- and post-synaptic markers was significantly lower in the constitutive *Pcdhg* null mice studied initially [47], increased neuronal apoptosis clouded the interpretation of this observation. By crossing these constitutive null mice with those harboring a null allele of the proapoptotic gene *Bax* to genetically block neuronal apoptosis, Weiner et al. [84] were able to confirm a primary function for the γ-Pcdhs in regulating synaptic density in the embryonic spinal cord (see Table 3 for a summary of roles). *Pcdhg* hypomorphic spinal cord neurons cultured from a distinct mutant mouse line differentiated and survived in vitro, but exhibited fewer and weaker synapses, as analyzed by both immunohistochemistry and electrophysiology [84]. In contrast, Lefebvre et al. [36] analyzed mice in which *Pcdhg* loss was confined to the retina, and found that any reduction in synaptic density was entirely attributable to increased neuronal apoptosis; when cell death was blocked by the addition of a *Bax* null allele, synaptic density was normal, and electrophysiology revealed functional visual circuits. In addition to these manipulations of the entire *Pcdhg* locus, Li et al. [108] presented evidence that the γ-Pcdh-C5 isoform interacts with the γ2 subunit of the $GABA_A$ receptor, promotes surface clustering of this receptor, and regulates the number of GABAergic synapses in cultured hippocampal neurons.

While γ-Pcdhs are concentrated in synaptic protein fractions [39, 40, 47], immunostaining indicates that much of the protein does not localize to synapses, but is rather found perisynaptically, or in other compartments of dendrites and axons [35, 40]. Garrett and Weiner [35] showed that γ-Pcdhs are highly expressed by astrocytes, which localize γ-Pcdhs to their perisynaptic processes. By restricting *Pcdhg* disruption to astrocytes using *Gfap-Cre* transgenics, it was confirmed that astrocytic γ-Pcdhs account for much of the signal observed in immunostaining experiments [35]. In vivo, excitatory and inhibitory synaptogenesis was significantly delayed in the spinal cord of embryos with *Pcdhg* mutant astrocytes (but wild-type neurons). This was confirmed and extended by analyzing neuron/astrocyte co-cultures in which wild-type or *Pcdhg* mutant cells were combined; when wild-type neurons were surrounded by mutant astrocytes, synaptogenesis was delayed, though it could eventually reach wild-type levels [35]. When mutant neurons were surrounded by wild-type astrocytes, however, synapse density never reached normal levels, consistent with an earlier report from the same group [84]. The effect of γ-Pcdhs on synaptogenesis was contact dependent, which is consistent with the localization of γ-Pcdhs to astrocyte-neuron contacts [35]. Combined with the demonstration that homophilic γ-Pcdh interaction between astrocytes and neurons can regulate cortical dendrite arborization [55], these data identify a molecular mechanism through which glia control the formation of neuronal circuitry during development.

Several studies have implicated the α- and γ-Pcdhs in the regulation of dendritic spines, the postsynaptic sites of excitatory transmission, in cortical and hippocampal neurons. In addition to identifying dendritic arborization defects in *Pcdha* null mutant hippocampal neurons, Suo et al. [96] also observed a reduction in overall spine density. Spine density was also decreased in cultured hippocampal neurons in which the γ-Pcdhs had been knocked down using shRNA. These spine deficiencies could be rescued by knockdown of Pyk2, which as noted above can inhibit Rac1; consistent with this, constitutively active Rac1 can rescue spine density [96]. Additionally, Ledderose et al. [98] reported that *Pcdhg* knockout olfactory granule cells exhibit a near-complete lack of spines; it should be noted, however, that the spines in these axonless neurons are somewhat different in structure and function to the typical spines found in cortical or hippocampal neurons. In contrast to the shRNA knockdown results of Suo et al. [96], Molumby et al. [109] examined *Pcdhg* null cortical neurons in vivo and found an increase in overall spine density, primarily due to more thin spines. Consistent with this, overexpression of a single γ-Pcdh isoform (γ-Pcdh-A1) in the cortex in vivo using a Cre-activated transgenic mouse line resulted in significantly decreased overall spine density, due to reductions in mushroom and thin spines accompanied by a smaller increase in stubby spines [109]. Furthermore, co-expression of a γ-Pcdh isoform can inhibit the increase in spine density seen in cultured hippocampal neurons following overexpression of neuroligin-1 [109]. Thus, in the cortex in vivo the γ-Pcdhs may actually inhibit spine formation and/or stabilization, perhaps in favor of promoting dendrite arborization.

Finally, an interesting report by Tarusawa et al. [110] presents evidence that the clustered Pcdhs are important for the preferential synaptic connections made by "sister neurons" deriving from the same cortical progenitor and found in a clonal column. These authors generated chimeric mice using GFP+ induced pluripotent stem (iPS) cells derived from mice lacking either the DNA methyltransferase Dnmt3b, or all three Pcdh clusters (*Pcdha*, *Pcdhb*, and *Pcdhg*), whose genes are known to be regulated by Dnmt3b [106]. Sister neuron reciprocal connections are altered in iPS-derived neurons in either case: the increase with development of reciprocal connectivity is lost in Dnmt3b KO neurons, while in triple cluster knockout neurons, reciprocal connectivity is aberrantly high early in development, and aberrantly low later in development.

Table 3 Selected roles for protocadherins at synapses

Gene symbol	Protein name	Results	Selected references
Pcdha	α-Protocadherins	Overall dendritic spine density was reduced in *Pcdha* mutant animals and cultured neurons	Suo et al. [96]
Pcdhg	γ-Protocadherins	Loss of γ-Pcdhs in vivo led to decreased spinal cord synapses; neurons cultured from *Pcdhg* hypomorphic allele mice exhibited fewer and physiologically weaker synapses	Weiner et al. [85]
		γ-Pcdhs are highly expressed by astrocytes, in perisynaptic processes. γ-Pcdh wild-type neurons that are surrounded by mutant astrocytes exhibit delayed synaptogenesis, in vivo and in vitro	Garrett and Weiner [34]
		Cultured hippocampal neurons in which γ-Pcdhs are knocked down using shRNA exhibited fewer spines	Suo et al. [96]
		Olfactory granule cells of *Pcdhg* knockout mice have a near-complete loss of spines	Ledderose et al. [98]
		Loss of *Pcdhg* in cortical neurons led to an increase in overall spine density while the overexpression of a single γ-Pcdh isoform in vivo resulted in decreased overall spine density. Disruptions in spine morphologies were also observed. The increase in spine density due to neuroligin-1 overexpression in vitro can be blocked by the co-expression of a single γ-Pcdh isoform	Molumby et al. [109]
Pcdha, Pcdhb, and *Pcdhg*	All clustered Protocadherins (and/or possibly other genes affected by manipulation)	Dendritic spines were reduced in pyramidal and hippocampal neurons of CTCF knockout animals. These neurons also exhibited reduced mEPSCs. Note that phenotype may be due in part to disruption of other CTCF targets. Neurons lacking all 3 clusters, or the methyltransferase Dnmt3b, also exhibit disrupted reciprocal connectivity within cortical columns derived from the same progenitor cell	Hirayama et al. [105], Tarusawa et al. [110]
Pcdh8	Protocadherin 8, Arcadlin (mammals), paraxial protocadherin (*Xenopus*)	Antibodies blocking Pcdh8 homophilic interactions led to a reduction in EPSP amplitudes and disruption of long-term potentiation in hippocampal slices in vitro	Yamagata et al. [74]
		Pcdh8 causes a decrease in spine number by activation of a MAPK pathway resulting in Pcdh8/N-cadherin endocytosis. Consequently, neurons cultured from Pcdh8 knockout mice have increased dendritic spines, but only in the presence of N-cadherin	Yasuda et al. [70]
Pcdh10	Protocadherin 10, OL-protocadherin	Pcdh10 knockdown prevented MEF2-induced synapse elimination. Pcdh10 is necessary for ubiquitination and proteasomal degradation of PSD-95, which leads to spine pruning	Tsai et al. [120]
Pcdh17	Protocadherin 17	Loss of Pcdh17 in mice increased the number of synaptic vesicles at presynaptic terminals, though there is no change in synapse density. Overexpression of Pcdh17 in cortical neurons in vitro increased mobility of synaptic vesicles along axons	Hoshina et al. [68]

Though an integrated interpretation of this complex pattern of results is not yet formulated, it would seem that proper repertoires of clustered Pcdhs within cortical neurons are important for the formation of specific synapses between them.

Non-clustered protocadherins

While the expression patterns of individual clustered Pcdhs appear to be stochastic or, for the five C-type isoforms, ubiquitous, expression of several non-clustered Pcdhs has been shown to mark particular neuronal populations and circuits. Spatiotemporal expression patterns of the non-clustered Pcdhs divide the brain into only partially overlapping regions, suggesting that neurons in a given functional network express the same repertoire of Pcdhs [66, 68, 72, 76, 88, 111–114]. For example, Hoshina et al. [68] show that Pcdh17 has a topographic expression pattern in the cortex, basal ganglia, and thalamus that is opposite to that of Pcdh10, such that the two complement each other and rarely overlap. Pcdh10 expression is a feature of visual and limbic system networks [72, 111, 112]. Demonstrating region-specific expression of non-clustered Pcdhs in the cortex, Kim et al. [112] also found that the same Pcdh was

expressed in the corresponding relay nucleus of the thalamus for each region. Interestingly, given the results of Tarusawa et al. [110] described above, it was recently shown that Pcdh19 expression marks discrete columns of neurons derived from Pcdh19+ progenitor cells in the zebrafish optic tectum, and that loss of Pcdh19 disrupts this columnar organization and leads to defective vision-driven behaviors [115]. These findings are consistent with the possibility that differential expression of non-clustered Pcdhs regulates the formation of specific neuronal networks during development.

Using two super-resolution microscopy techniques, Hoshina et al. [68] showed that Pcdh17 is juxtaposed to both VGLUT1/PSD95-labeled excitatory synapses and VGAT/Gephyrin-labeled inhibitory synapses in the basal ganglia. Immuno-electron microscopy using a Pcdh17 antibody also found synaptic and perisynaptic labeling of membranes. *Pcdh17* knockout in mice leads to an increase in the number of synaptic vesicles clustered at the presynaptic terminal, which correlates with an increase in paired-pulse ratios and decreased synaptic depression following prolonged stimulation. Thus, Pcdh17 seems to play an important role in regulating presynaptic physiology in corticostriatal circuits, as knockouts exhibit enhanced synaptic efficacy in the absence of any overall change in synapse density [68] (see Table 3 for a summary of roles).

As discussed above, Pcdh17 (along with other δ-Pcdhs) interacts with Nap1, a component of the WAVE complex [92] that regulates actin dynamics. Neurons generated from *Nap1* mutant neuroepithelial cells fail to differentiate and extend dendrites and axons due to disrupted actin reorganization [89]. Synaptic vesicles are surrounded by networks of actin filaments, suggesting that actin dynamics can affect their movement. Consistent with this, overexpression of *Pcdh17* in cortical neurons in vitro increased the mobility of synaptic vesicles along axons [68]. Furthermore, Pcdh17, like Pcdh19, can form a complex with N-cadherin [78]; N-cadherin and β-catenin in turn regulate actin dynamics proposed to increase the pool of synaptic vesicles available for release at the presynaptic terminal [116–118]. Given this, it is not hard to imagine a scenario in which Pcdh17 knockout results in increased N-cadherin/β-catenin signaling, increasing the population of synaptic vesicles primed for release, as indicated by Hoshina et al. [68]. These results are consistent with work in invertebrates, where mutations of WAVE complex components or interactors such as MIG-10 [119] or Cyfip [120] have been shown to disrupt synaptic vesicle size, clustering, or endocytosis.

There is also substantial evidence that non-clustered Pcdhs regulate synaptic plasticity. In an effort to identify mRNAs upregulated during synaptic activity, Yamagata et al. [74] identified a gene they named *arcadlin*, the rat orthologue of human *Pcdh8* and *Xenopus PAPC*, in a seizure-induced hippocampal cDNA library (for consistency we will refer to the encoded protein as Pcdh8). Disruption of Pcdh8 homophilic interaction with function-blocking antibodies reduced excitatory postsynaptic potential (EPSP) amplitudes and abolished long-term potentiation (LTP) in hippocampal slices in vitro [74]. Subsequent morphological analysis of dendrites in Pcdh8 knockout hippocampal cultures demonstrated increased dendritic spine density, indicating that Pcdh8 normally acts to limit spine density [70]. Further experiments revealed that Pcdh8 interacts with N-cadherin in *cis* via their transmembrane domains, just as Pcdh19 and Pcdh17 can [78]. Homophilic interaction of Pcdh8 results in activation of the kinase TAO2β, which binds to the Pcdh8 cytoplasmic domain. This leads to the activation of p38MAPK, which in turn initiates a feedback loop by phosphorylating TAO2β, resulting in Pcdh8-mediated N-cadherin endocytosis and decreased spine density. Consistent with such a mechanism, re-expression of Pcdh8 in knockout hippocampal cultures decreased levels of N-cadherin on the cell surface, and restored spine density to wild type levels; similarly, siRNA knockdown of N-cadherin or expression of dominant-negative N-cadherin also reversed the increase in spine density observed in *Pcdh8* knockout neurons [70]. Collectively, these studies define a molecular mechanism through which Pcdh8/arcadlin controls activity-induced synaptic plasticity.

These results are congruent with subsequent work on *Pcdh10*, a known target gene of the transcription factor MEF2 downstream of synaptic activity, which was found to be important for synapse elimination in hippocampal neurons [121]. Pcdh10 expression is regulated by both MEF2 and by binding of its RNA to FMRP, an RNA binding protein mutated in Fragile X syndrome. Activation of MEF2 by depolarization increases ubiquitination of PSD95 by the E3 ubiquitin ligase, murine double minute 2 (Mdm2); Pcdh10 binds ubiquitinated PSD95 and helps target it for proteasomal degradation, which results in decreased dendritic spine density. Interfering with Pcdh10's interaction with the proteasome prevents MEF2-dependent degradation of PSD95 and subsequent synapse elimination [121]. Together, these studies provide evidence for activity-induced expression of non-clustered Pcdhs that participate in complex molecular cascades regulating structural rearrangements that underlie synaptic plasticity.

Roles in behavior and neurological or neurodevelopmental diseases

Epilepsy

Epilepsy and mental retardation limited to females (EFMR) is a rare, X-linked dominant disorder with early seizure

onset. Genomic sequencing of six probands from families with EFMR revealed a mutation in *PCDH19*, located on the X-chromosome, in all six families [122]. Since this finding, other studies have implicated *PCDH19* mutations in a spectrum of epileptic disorders including Dravet syndrome [123, 124], and febrile infection-related epilepsy syndromes (FIRES) [125]. Mutations have been reported in all exons of the *PCDH19* gene except for exon 2, with a large majority occurring in exon 1, coding for the extracellular domain (see Depienne [126] for a thorough review of *PCDH19* mutations). Leonardi et al. [127] compared sequences from *PCDH19* containing four de novo mutations to make predictions about protein structure. Three of these mutations were predicted to affect homophilic interaction, calcium binding, and/or protein folding. The fourth mutation resulted in a truncated protein lacking the entire intracellular domain [127]. These findings parallel the finding of Emond et al. [78] that a duplication near EC2–EC3 disrupts calcium-dependent adhesion of the Pcdh19–N-cadherin complex.

Although the symptoms and severity of these epileptic disorders vary widely, patients with *PCDH19* mutations all have early seizure onset initiated by febrile sickness, followed by decreased frequency with age, and mutations are often associated with intellectual disabilities, developmental delay, and behavioral disturbances [128–140]. Analyzing behavioral problems described in all cases of *PCDH19* mutations, Camacho et al. [129] found the comorbidity of *PCDH19* mutations and intellectual disability to be 75.4%, with 46.9% considered severe. Furthermore, behavioral disturbances, half of which involved autism spectrum disorder, were observed in 55.4% of cases [129].

Unlike most X-linked disorders in which the most severe phenotypes are seen in males, males carrying *PCDH19* mutations are unaffected [141]: Nearly all patients reported with epileptic disorders resulting from *PCDH19* mutations have been female. What might underlie this unusual sex bias? One hypothesis is that sexually dimorphic genes compensate for the loss of functional Pcdh19 in males. For example, two sexually dimorphic genes, *AKRIC2* and *AKRIC3*, are dysregulated in *PCDH19* mutation-positive females [142]. These genes are regulated by steroid receptors and encode metabolizing enzymes for allopregnanolone, a neurosteroid that associates with and modulates $GABA_A$ receptors, causing prolonged hyperpolarization and preventing overexcitability. *AKRIC2* and *AKRIC3* mRNA, AKRIC3 protein, and allopregnanolone blood levels are all decreased in patients with *PCDH19* mutations [142]. Another hypothesis proposes cellular interference as a mechanism for male sparing in *PCDH19* mutation disorders. The term cellular interference has been borrowed from studies of craniofrontonasal syndrome, which has an inheritance pattern similar to that of Pcdh19-associated epilepsies [143]. Due to X-chromosome silencing, random inactivation leads to heterozygous females expressing the *PCDH19* mutation in a mosaic pattern, whereas hemizygous males express the mutation ubiquitously. Cellular interference suggests that mosaic expression results in neighboring cells with different forms of Pcdh19: either the mutated or wild-type form, or no Pcdh19. As a result, cellular interfaces with mismatched Pcdhs may not engage in proper cell adhesion [124, 126]. This hypothesis gained support when Depienne et al. [124] found a *PCDH19* deletion in a rare male patient diagnosed with Dravet Syndrome. Fluorescent in situ hybridization with probes for *PCDH19* labeled only 53% of fibroblasts cultured from this patient, indicating mosaic inheritance. Thus far, mouse studies have failed to provide firm support for cellular interference. A *Pcdh19* homozygous null mouse does not present with spontaneous seizures or exhibit any gross brain morphological defects [71]. Interestingly, even in female *Pcdh19* heterozygous mice, in which Pcdh19 expression is mosaic in the brain due to X-inactivation, no spontaneous seizures or other behavioral abnormalities were reported [71]. Mouse models in which mutated forms of Pcdh19 are introduced may be of interest in the future.

Autism spectrum disorders (ASDs)

Autism spectrum disorders are characterized by social deficits, difficulties in communicating both verbally and non-verbally, repetitive behaviors, and exceedingly focused interests. Intellectual disabilities manifest at an early age for >50% of patients with these disorders [144]. Deficits arising during the period of synapse formation [145], mutations and copy number variants found in genes encoding synaptic proteins [146–148], and perturbed dendritic spines observed in both post-mortem brains of ASD patients and in ASD mouse models [149–151] all point to ASD as a disorder of synapses. Multiplex families and twin studies have shown that ASDs have heritability as high as 90% [145, 152, 153]. This has led to a large number of genomic studies searching for the molecular etiology of ASD, which together have proposed 695 candidate genes [154] among them *PCDH8, PCDHGA1, PCDHB15* [152], *PCDH9* [148, 155], *PCDH10* [155, 156], *PCDH15* [157], *PCDHB4* [158], and the *PCDHA* gene cluster [159].

One member of the δ2-Pcdh family, Pcdh10, has been linked to ASD particularly strongly. Copy number variation at the *PCDH10* locus has been found in ASD patients [155, 156]. Functional imaging of the amygdala points to abnormal amygdala activity in ASD patients; in particular, the basal lateral amygdala, which is strongly implicated in social and communication deficits [160]. *PCDH10* is

highly expressed in the limbic system including the basolateral amygdala [72, 111, 161], and *Pcdh10* haploinsufficiency in an ASD mouse model leads to decreased social behavior, disrupted electrophysiological responses in the amygdala, and increased density of immature, filopodia-like dendritic spines [161]. As noted above, this is consistent with the demonstration that Pcdh10 is implicated in a synapse and dendritic spine elimination pathway that requires two other genes implicated in ASD, *Mef2* and *Fmr1* [121].

Both Pcdh7 (a δ1-Pcdh) and Pcdhβ1 have a potential link to Rett syndrome (RTT), a neurological disorder on the ASD spectrum found almost exclusively in females and caused by a mutation in *Methyl-CpG-Binding protein 2 (MECP2)*, a gene that encodes a methylated DNA-binding protein that regulates transcription [162]. Miyake et al. [163] show that MeCP2 binds to, and can suppress the activity of, the promoter regions of the *PCDHB1* and *PCDH7* genes in neuroblastoma cells. Consistent with this, mRNA levels of both *PcdhB1* and *Pcdh7* are increased in MeCP2 null mice compared to controls; the former, but not the latter, is also abnormally expressed in samples from the frontal cortex of RTT patients [163].

Finally, Pcdhs have been implicated in neuropsychiatric disorders including ASD, intellectual disabilities, and attention deficit hyperactivity disorder (ADHD) resulting from a duplication of the 16p13.11 locus, which contains seven genes [164]. Fujitani et al. [164] present evidence that a microRNA, miR-484, is the gene responsible for the behavioral phenotypes modeled in mice. Manipulation of miR-484 expression in cortical progenitors in vitro and in vivo indicated that it is required for the differentiation of radial glia into neurogenic basal progenitors. In silico analysis identified the *Pcdh19* 3′UTR as a target for miR-484, and luciferase assays confirmed that miR-484 downregulates *Pcdh19*. Knockdown of *Pcdh19* by shRNA delivered via in utero electroporation led to increased differentiation of radial glia into basal progenitors, consistent with *Pcdh19* downregulation being the mechanism by which miR-484 acts [164]. Though it is not yet clear the extent to which Pcdh19 contributes to the various defects in 16p13.11 microduplication syndrome, its implication in this disorder further strengthens its link to neurological disease.

Down syndrome and fetal alcohol spectrum disorders

Given the important functions the γ-Pcdhs play in proper dendrite formation [18, 35, 55–57, 83, 96–98, 103, 105, 106], it is perhaps not surprising that they have been implicated in Down syndrome (DS) and Fetal alcohol spectrum disorders (FASDs). Both of these neurodevelopmental disorders are characterized by defective dendritic arborization, abnormal synaptic formation, and disrupted dendritic spine morphology [165–167]. Microarray studies have uncovered hypermethylation of *Pcdhg* genes and promoters at multiple developmental stages in DS tissues including several brain regions [168, 169]. Bisulfite pyrosequencing confirmed that multiple *Pcdhg* A and B subfamily gene promoters were hypermethylated in DS samples, and targeted RNA sequencing showed that this hypermethylation was, indeed, correlated with lower *Pcdhg* gene expression [169]. Though it remains to be demonstrated that reduced γ-Pcdh protein levels are causal for any of the dendritic or synaptic defects seen in DS, the phenotypes observed in *Pcdhg* null cortex (as discussed above, decreased dendrite arborization and altered dendritic spine number and morphology) are certainly consistent with such a possibility [55, 83]. Studies exploring methylation patterns in FASD similarly discovered increased methylation of the clustered Pcdhs both in the brains of FASD mouse models and in buccal cells of human FASD patients [170, 171].

Neurodegenerative diseases

Several studies have provided hints that Pcdhs may be relevant to neurodegenerative diseases, particularly Huntington's disease (HD), which is caused by increased length of CAG repeat sequences in exon 1 of the *huntingtin* gene [172]. The disease is characterized by motor disturbances, cognitive decline, and psychiatric symptoms [173] with an underlying pathology of degeneration of medium spiny neurons of the striatum, spreading later to cortical pyramidal neurons [174, 175]. To better understand the molecular changes caused by the increased CAG repeat in the mutant *huntingtin* (*mHtt*) gene, Langfelder et al. [175] profiled mRNA from brain tissues of an allelic series of knock-in mice harboring a range of CAG repeat severity. Using RNA-seq, 13 gene modules were identified whose expression co-varied with CAG length, 4 of which were highly enriched in Pcdh genes, including 37 of the 58 clustered Pcdhs and several δ-Pcdhs including Pcdh20 and Pcdh11X/Y [175]. Interestingly, a previous study found that *Pcdh20* mRNA levels are increased in the striatum of the YAC128 transgenic HD mouse model, while the mRNA levels are decreased in the caudate of humans with HD; though this is corroborating evidence that Pcdh20 levels may be associated with HD, the disparity between mouse and human results needs to be resolved [176]. Carrasquillo et al. [177] reported a SNP in *Pcdh11X* associated with increased susceptibility to late-onset Alzheimer's disease. Subsequent genome-wide association studies (GWAS), however, found no association between this polymorphism and Alzheimer's patients [178, 179].

Schizophrenia and mood disorders

Several studies have found evidence for differential expression of the Pcdh genes in patients with schizophrenia (SCZ) and bipolar disorder (BPD). Real time PCR of postmortem tissue from dorsal lateral prefrontal cortex, an area of the brain strongly implicated in SCZ, of affected patients found a decrease of *Pcdh17* mRNA compared to non-affected subjects [180]. Studies of the mechanism of effective treatment of the antipsychotic drug olanzapine point to methylation of gene promoters in disease-causing pathways. Methylated DNA immunoprecipitation in rats treated with therapeutic levels of olanzapine reveal differential methylation of the promoters of *Pcdh7, Pcdh8, Pcdh9, Pcdha11, Pcdha9, Pcdha5*, and *Pcdh19* in the cerebellum and *Pcdhga8* and *Pcdh7* in the hippocampus [181]. Considering the epigenetic control of clustered Pcdh isoform expression and its functional consequences on dendritic arborization [106, 182] discussed above, olanzapine-induced methylation of some clustered (as well as non-clustered) Pcdh genes is worth noting. In a significant study by Nakazawa et al. [183], induced pluripotent stem cells (iPSCs) were derived from monozygotic twins diagnosed with treatment-resistant schizophrenia. Each twin was differentially responsive to clozapine, a drug that is effective in 60–70% of treatment-resistant patients, but which is used only when other options have proven ineffective due to its rare but life-threatening side effects. Neurons were differentiated from each twin's iPSCs and exposed to clozapine. RNA-Seq analysis revealed that many clustered *Pcdh* genes were differentially regulated by clozapine in neurons derived from the clozapine-responding twin compared to those derived from the non-responding twin [183]. By comparing monozygotic twins with identical genomes, but distinct responses to clozapine, this study implicates Pcdhs in the molecular mechanisms through which neuropsychiatric drugs may act.

Looking for genetic loci associated with susceptibility to SCZ and BPD, several studies have identified chromosomal locus 5q31, which contains the three *Pcdh* gene clusters [184–190]. Pedrosa et al. [191] discovered an SNP in an enhancer (HS5-1) that can affect expression of the *Pcdha* cluster, noting a significant increase in homozygosity of one allele in BPD patients. Although the effect of this SNP has not been tested, it occurs in predicted transcription factor binding sites, so it could conceivably alter expression levels of *Pcdha* genes [191].

As discussed above, α-Pcdhs play important roles in elaboration of serotonergic projections [81], a branch of the nervous system implicated in mood disorders such as BPD and major depressive disorder (MDD) [192–194]. Additionally, increased hippocampal expression of *Pcdhga11* was observed in a congenital learned helplessness rat model of depression [195]. Some of the strongest evidence linking Pcdhs to mood disorders, however, comes from the study of PCDH17. In mood disorders, structural abnormalities and dysfunction of areas associated with emotional processing such as the prefrontal cortex, hippocampus, and the amygdala are observed; PCDH17 is strongly expressed in these areas [68, 92, 196]. Recently, a large-scale meta-analysis of ~30,000 MDD and BPD cases and controls identified a number of SNPs within the *PCDH17* gene locus that were linked to increased susceptibility to mood disorders [196]. One particular SNP (rs9537793) in the 3′ region downstream of *PCDH17* held up as significantly associated across several analyses, and was studied further. This risk allele also associated with decreased hippocampus and amygdala volume in patients, and with increased activity of the amygdala in response to negative emotional stimuli, a response often observed in patients with mood disorders. RNA sequencing analysis of *PCDH17* expression levels in iPSCs from patients diagnosed with BPD, neurons derived from these iPSCs, and postmortem tissue from the frontal cortex of patients diagnosed with BPD (but not those with MDD), showed a significant increase [196]. Additionally, overexpression of *Pcdh17* in mouse primary cortical neurons in vitro led to decreased overall density of dendritic spines, with a particular loss of "mature" mushroom spines accompanied by a relative increase in the proportion of small, stubby spines [196]. This is consistent with the fact that post-mortem brain tissues from patients diagnosed with mood disorders often show disruptions in dendritic spine density and morphology [197, 198]. The report of Chang et al. [196] is particularly satisfying not only because it combines many levels of analysis and includes thousands of case/control comparisons, but also because its results are consistent with prior work, discussed above, by Hoshina et al. [68], who found that *Pcdh17* knockout mice exhibit decreased measures of depression. Together, these studies strongly implicate Pcdh17 expression levels in the neuropathology underlying mood disorders.

Negative early life experiences such as maltreatment cause brain and behavior changes that affect mental health into adulthood and increase the risk of developing psychological illnesses [199–202]. These changes are thought to be mediated in part by epigenetic control of numerous genes [203] including Pcdhs. Using microarray analysis of ~7 million bp regions of human chromosome 5 (containing the *Pcdh* clusters and glucocorticoid receptor gene *NR3C1*) and the syntenic region of rat chromosome 18, Suderman et al. [204] and McGowan et al. [205] find that clustered Pcdh promoters are enriched in hypermethylation sites in the hippocampi of, respectively, adult victims of child abuse [204] and rats that received poor maternal care as pups [205]. Furthermore, qPCR of rat hippocampal

tissue revealed that good maternal care corresponded with increased transcription of 20 of the 33 *Pcdh* genes examined [205]. Given that chronic stress can result in disrupted dendrite arborization and dendritic spine formation [206, 207], these results are consistent with the roles identified for clustered Pcdhs in the regulation of proper dendrite development and the regulation of spine and synapse numbers.

Concluding remarks

The pace of data defining Pcdh functions and the molecular mechanisms underlying them has accelerated greatly over the past decade. Both clustered and non-clustered Pcdhs have now been implicated in every step of neural circuit formation, and the genes that encode them are increasingly found to be mutated or dysregulated in a wide variety of neurological and neurodevelopmental disorders, demonstrating their clinical relevance. Though it is still unclear how disease etiology or progression is regulated by Pcdhs, the growing information about their many roles will form the basic science foundation that will be required for any future therapeutic approaches that seek to modulate or restore Pcdh function. While it is perhaps too early to make broad generalizations about unique functions of distinct Pcdh families, it is gratifying that a few patterns are beginning to emerge.

Thus far, the clustered Pcdhs have been implicated primarily in the late stages of axon terminal formation, while the non-clustered Pcdhs seem more important in initial axon outgrowth and early pathfinding events. In mice with disrupted *Pcdha* or *Pcdhg* genes, axons of olfactory neurons, retinal ganglion cells, or Ia proprioceptive neurons project properly to their final targets, but once there, fail to form properly distributed terminal arbors [48, 49, 58, 59, 80–82] (Table 1). In contrast, disruption of *Pcdh7*, *Pcdh17*, or *Pcdh18b* results in non-existent or shorter axons in cortical neurons, retinal ganglion cells, motor neurons, or amygdala neurons [77, 85–88, 92] (Table 1). Some of this distinction may be due to several non-clustered Pcdhs interacting with Nap1 and the WAVE complex, though some Pcdha proteins also have a predicted WIRS site [94]. Different roles in axons may also reflect distinct timing of Pcdh action, due either to developmental patterns of gene expression, or to mechanisms that regulate Pcdh protein concentration in axons, including both trafficking and local translation, as was shown for Pcdh7 in retinal ganglion cell axons [88].

Another emerging theme is that many Pcdhs, both clustered and non-clustered, seem able to negatively regulate dendritic spine and synapse density, either by inhibiting synaptogenesis or by promoting synapse elimination. In cortical neurons lacking *Pcdhg* genes, spine density is increased in vivo; an opposite effect is seen when a single *Pcdhg* isoform is overexpressed in the cortex [109]. This may be due to the ability of γ-Pcdhs to bind to and inhibit the functionality of neuroligin-1 [109]. Similarly, a number of non-clustered Pcdhs can interact with other synaptic molecules to promote endocytosis and dendritic spine loss: Pcdh8 does this through interaction with N-cadherin [70], while Pcdh10 induces synapse elimination by interacting with ubiquitinated PSD-95 [120]. Thus, it seems clear that, in addition to mediating *trans* homophilic adhesion themselves, Pcdhs can act in *cis* to regulate the stability or turnover of other proteins with synaptic functions. At the same time, the clustered Pcdhs also appear to be important for reciprocal connectivity between cortical "sister neurons" derived from the same progenitor [110], so it is possible that Pcdhs help stabilize only the "proper" synaptic connections during development.

Of course, these apparent themes may simply reflect our still-incomplete understanding of the panoply of functional roles each Pcdh plays during neural circuit formation. One thing that remains puzzling is why some Pcdhs appear to play differing, even opposite, roles in particular neuronal subsets. For example, knockout and knockdown studies have suggested that the γ-Pcdhs can promote synaptogenesis in embryonic spinal cord neurons [34, 85] and increase spine density in hippocampal [96] or olfactory [98] neurons, but negatively regulate synapse and spine density in cortical neurons [109]. Similarly, the γ-Pcdhs can mediate homophilic adhesion in cellular assays [51], and homophilic matching appears to encourage cell contact and promote dendrite arbor growth in cortical or hippocampal neurons [55, 96, 99]. Nevertheless, knockout phenotypes in other neurons such as retinal starburst amacrine cells and Purkinje cells indicate that homophilic γ-Pcdh interactions lead to repulsive signaling required for proper dendrite self-avoidance [56, 57]. Presumably, this reflects the ability of Pcdhs to interact with a wide range of cell surface and cytoplasmic signaling partners, which may differ in each cell type, at different times in development, and/or in distinct subcellular compartments. A major effort in future research should be focused on trying to understand the cell type and context-dependent signaling mechanisms through which Pcdhs act to regulate neural circuit formation and function.

Overall, the research described in this review has firmly established the diverse Pcdhs as major players in the formation and function of neural circuits, and their links to disease indicate that they should be fruitful future targets of therapeutic approaches aimed at their amelioration. Given the current rapid pace of discovery, it seems certain that

many new roles and molecular mechanisms will be identified in the near future.

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