Clustered Protocadherins Emerge as Novel Susceptibility Loci for Mental Disorders

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The clustered protocadherins (cPcdhs) are a subfamily of type I single-pass transmembrane cell adhesion molecules predominantly expressed in the brain. Their stochastic and combinatorial expression patterns encode highly diverse neural identity codes which are central for neuronal self-avoidance and non-self discrimination in brain circuit formation. In this review, we first briefly outline mechanisms for generating a tremendous diversity of cPcdh cell-surface assemblies. We then summarize the biological functions of cPcdhs in a wide variety of neurodevelopmental processes, such as neuronal migration and survival, dendritic arborization and self-avoidance, axonal tiling and even spacing, and synaptogenesis. We focus on genetic, epigenetic, and 3D genomic dysregulations of cPcdhs that are associated with various neuropsychiatric and neurodevelopmental diseases. A deeper understanding of regulatory mechanisms and physiological functions of cPcdhs should provide significant insights into the pathogenesis of mental disorders and facilitate development of novel diagnostic and therapeutic strategies.

Keywords: axonal tiling and myelination, neuronal self-recognition and dendrite self-avoidance, cell adhesion, neuropsychiatric diseases, neuronal connectivity, gene dysregulation, 3D genome architecture, clustered protocadherins

INTRODUCTION

The assembly of complex neural circuits, which are essential for accurate processing of sensory and cognitive information, requires individual neurons to discriminate self from non-self. This neuronal discrimination is thought to be accomplished by cell identity codes – neural recognition addresses determined by specific cell-surface receptors (Zipursky and Grueber, 2013). Given that there are billions of neurons each forming up to twenty thousand synapses in the human brain (Pakkenberg and Gundersen, 1997; DeFelipe et al., 1999), it's fascinating how neurons use limited number of genes to generate such seemingly unlimited diversities. Failure in generating diversified neuronal codes causes deficits in neural circuit formation and may be related to pathogenesis of brain disorders.

Vertebrates adopt several mechanisms to achieve molecular diversity in different systems. For example, in the immune system, individual B and T cells express distinct repertoires of immunoglobulin (Ig) and T-cell receptor (TCR) genes through stochastic V(D)J rearrangements in the Ig and TCR gene clusters, respectively (Tonegawa, 1983; Wu et al., 2020). In the olfactory system, each olfactory sensory neuron expresses a single olfactory receptor (OR) out of more than...
MOLECULAR MECHANISMS FOR GENERATING DIVERSIFIED NEURAL IDENTITY CODES

Promoter Choice of cPcdhs in the Cell Nucleus

The unique genome organization of cPcdh genes has the potential to generate unparalleled diversities for neuron identity codes. The cPcdh locus contains three closely-linked gene clusters (Pcdha, Pcdhb, and Pcdhy) encompassing nearly one million base pairs at the human chromosome 5q31 region (Figure 1A) (Wu and Maniatis, 1999). Both the Pcdha and Pcdhy clusters contain tandem arrays of large variable exons, each of which is transcribed from its own promoter and then spliced to the common set of three downstream constant exons within the respective cluster (Figure 1A) (Wu and Maniatis, 1999; Tasic et al., 2002; Wang et al., 2002a). The Pcdhb cluster has no constant region and thus contains only variable exons (Figure 1A) (Wu and Maniatis, 1999; Wu et al., 2001). Each Pcdh α, β, and γ variable exon encodes an extracellular domain with 6 ectodomains (ECs), a transmembrane domain (TM), and a juxtamembrane variable cytoplasmic domain (VCD). The three constant exons encode a common membrane-distal cytoplasmic domain (αCD and γCD) (Figures 1B,C) (Wu and Maniatis, 1999). Based on phylogenetic analyses, the 53 human cPcdh genes are divided into five groups: Pcdha (α1 – α13), Pcdhb (β1 – β16), PcdhyA (γα1 – α12), PcdhyB (γβ1 – γβ7), and C-type (αc1, αc2, γc3, γc4, γc5) (Wu et al., 1997; Wu and Maniatis; Wu et al., 2001). Interestingly, different species, even between the closest relatives such as human and chimpanzee, do not have exactly the same number of cPcdh genes, suggesting that their absolute numbers are not important once reached a certain threshold (Wu, 2005).

The three Pcdh clusters contain tandem arrays of CTCF binding sites (CBS) in both gene promoters and enhancers (Figure 1A) (Golan-Mashiach et al., 2012; Guo et al., 2012, Monahan et al., 2012). CTCF/cohesin-mediated long-range chromatin interactions between these CBS elements determine the stochastic and combinatorial expression of diverse cPcdh repertoires on the cell surface (Wu and Jia, 2020). Differential expression of cPcdh genes in individual neurons is regulated by epigenetic modifications, such as DNA methylation and H3K9me3 histone modification in the promoter region (Guo et al., 2012; Toyoda et al., 2014; Chen et al., 2015; Jiang et al., 2017; Wada et al., 2018). In particular, DNA methylation precludes the binding of CTCF proteins (Wu et al., 2001; Guo et al., 2012; Yin et al., 2017). Without CTCF binding, cPcdh promoters cannot form long-range chromatin contacts with remote enhancers and thus cannot be activated (Guo et al., 2012; Canzio et al., 2019). It is this higher-order chromatin architecture that determines the Pcdh promoter choice in the cell nucleus (Wu and Jia, 2020).

Cell-Surface Delivery of cPcdhs Through Cytoplasm

Significant progress has been made on cell-surface delivery mechanisms of cPcdhs. In particular, Pcdhy proteins are initially detected in the cytoplasm as a mobile pool and their cell-surface delivery corresponds to the maturation of synapses (Fernandez-Monreal et al., 2009, 2010; LaMassa et al., 2020). For example, Pcdhya3 and Pcdhyb2 form juxtanuclear membrane tubules in the cytoplasm when overexpressed (Hanson et al., 2010). In contrast, PcdhyC4 accumulates as continuous sheets but does not form tubules (Hanson et al., 2010). Intracellular trafficking and tubulation of Pcdhya3 are determined by a 26-amino-acid VCD motif (O’Leary et al., 2011). Consistently, despite that the VCD...
domain is the most diverse region of cPcdhs (Figures 1C,D), there are several highly conserved residues within the VCD motif (Wu and Maniatis, 1999; Shonubi et al., 2015).

Pcdhα2 and isoforms of the Pcdh β and γ families (except γc4) can be delivered to the cell surface by themselves; by contrast, surface localization of Pcdhα (except α2) and Pcdhγc4 requires the co-expression with other isoforms of Pcdh β or γ, known as carrier isoforms (Murata et al., 2004; Bonn et al., 2007; Thu et al., 2014; Goodman et al., 2016b). The mechanism by which carrier isoforms facilitate the delivery of members of Pcdhα and Pcdhγc4 to the cell surface is not known but may require cis-dimerization on the ER membrane between cPcdh isoforms. This hypothesis is consistent with evidence of cis-dimerization or multimerization between cPcdh in transfection experiments (Schalm et al., 2010; Schreiner and Weiner, 2010; Biswas et al., 2012; Thu et al., 2014; Rubinstein et al., 2015). Finally, analyses of single-cell RNA-seq data of 23,178 cortical cells (Tasic et al., 2018) revealed that almost every cell (97.0%) expresses isoforms of Pcdh β or γ but only 74.5% cells express members of Pcdhα (Figure 1E). Moreover, every Pcdhα-expressing cell (99.4%)
co-express isoforms of either Pcdh β or γ, while only 76.7% of Pcdh β- or γ-expressing cells co-express members of Pcdha, consistent with the requirement of Pcdh β or γ for the cell-surface delivery of Pcdha (Figure 1F).

**Self-Recognition and Non-self Discrimination on the Cell Surface**

The cis-dimers of cPcdhs can engage in highly stringent homophilic trans-interactions to generate cell-recognition specificities (Thu et al., 2014). The trans-interactions of cPcdhs are mediated through EC1–EC4 domains, especially the most-divergent EC2 and EC3 domains, in an anti-parallel manner (Figures 1C,D, 2) (Wu, 2005; Schreiner and Weiner, 2010; Nicoludis et al., 2015, 2016, 2019; Rubinstein et al., 2015; Goodman et al., 2016a; Brasch et al., 2019). Remarkably, liposome-tethered ectodomains spontaneously assemble into a zipper-like lattice structure (Brasch et al., 2019). Thus, when neurites bearing the same cPcdh isoforms contact with each other, the cPcdhs are thought to form a zipper lattice through homophilic trans-interactions and trigger an intracellular signaling pathway that eventually leads to adhesion or repulsion (e.g., self-avoidance) through cytoskeletal remodeling (Molumby et al., 2016; Fan et al., 2018; Brasch et al., 2019).

Isoforms of Pcdha and Pcdhy could be cleaved by the metalloproteinase ADAM10 and γ-secretase to release the intracellular domain which translocates into the nucleus to regulate gene expression (Haas et al., 2005; Hambsch et al., 2005; Reiss et al., 2006; Bonn et al., 2007; Buchanan et al., 2010). Thus, diverse extracellular signals can converge on the same intracellular signaling pathway through the common CD shared by members of Pcdha or Pcdhy (Wu and Maniatis, 1999; Wu et al., 2001; Schalm et al., 2010; Fan et al., 2018) (reviewed in Wu and Jia, 2020). In addition, the intracellular domains of Pcdha and Pcdhy can bind to several kinases including FAK (focal adhesion kinase), Pyk2 (proline-rich tyrosine kinase 2), and Ret (receptor tyrosine kinase rearranged during transformation) (Figure 2) (Chen et al., 2009; Schalm et al., 2010; Garrett et al., 2012; Suo et al., 2012; Keeler et al., 2015). Finally, Pyk2 inhibits the activities of Rac1, which regulates lamellipodial dynamics and actin cytoskeletal remodeling (Figure 2) (Suo et al., 2012; Fan et al., 2018).

**BIOLOGICAL FUNCTIONS OF CLUSTERED Pcdhs**

**Homophilic Adhesion-Induced Repulsion Is Required for Dendrite Self-Avoidance**

During development, sister neurites avoid each other in innervating the receptive field, a phenomenon known as self-avoidance. Neurite self-avoidance requires neurones to discriminate self from non-self (Grueber and Sagasti, 2010; Zipursky and Grueber, 2013). In *Drosophila*, self-avoidance is mediated through diversified Dscam1 (Down syndrome cell adhesion molecule 1) generated by alternative splicing (Zipursky and Grueber, 2013). In vertebrates, it is thought to be achieved by diverse cPcdhs as supported by a growing body of evidence. In particular, knockout of *Pcdhy* causes collapses of dendrites, a defect of self-avoidance, of the same starburst amacrine cells (Figure 3A) (Lefebvre et al., 2012; Kostadinov and Sanes, 2015). When the entire Pcdhy cluster is replaced with a single Pcdhy isoform, self-avoidance defects are rescued. However, these cells recognize heteroneuronal dendrites as ‘self’ since neighboring starburst cells also express the same cPcdh isoform (Lefebvre et al., 2012). Similar to starburst cells, dendritic self-avoidance defects were also observed in cerebellar Purkinje cells in the absence of Pcdhy (Figure 3B) (Lefebvre et al., 2012; Toyoda et al., 2014). Subsequent studies demonstrated that Pcdha and Pcdhy function together to mediate dendritic self-avoidance of starburst and Purkinje cells (Ing-Esteves et al., 2018).

**Isoform Diversity Is Required for Neurite Coexistence**

In addition to a critical role in dendritic self-avoidance, cPcdh diversity plays a crucial role in dendrite coexistence (Lefebvre et al., 2012). Specifically, reducing the diversity of Pcdhy disrupts dendrite coexistence in retinal starburst amacrine cells (Lefebvre et al., 2012). The cPcdh proteins also function in patterning of axon terminals. For example, olfactory sensory neurons (OSNs) expressing identical OR genes project their axons to the same location in the olfactory bulb and converge to form a glomerulus (Mombaerts et al., 1996). Mechanistically, alternate cPcdh isoforms from all three Pcdh clusters are stochastically expressed in single OSNs, ensuring that axons from different OSNs do not share the same profile and thus can coexist in the same glomerulus of the olfactory bulb (Mountoufaris et al., 2017). Genetic deletion of the entire Pcdha cluster in mice results in abnormal sorting of OSN axons leading to the formation of ectopic small glomeruli (Hasegawa et al., 2008; Mountoufaris et al., 2017). Deletion of Pcdhβ or Pcdhy also affects OSN wiring (Hasegawa et al., 2016; Mountoufaris et al., 2017). Finally, deletion of all three Pcdh clusters in mice causes the most severe phenotype with axons from the same OR-expressing OSNs branching profusely, reminiscent of the axonal self-avoidance defects (Figure 3C) (Mountoufaris et al., 2017).

Gain-of-function experiments provide additional insights into the role of cPcdhs in OSN wiring. Overexpression of the same three cPcdh isoforms (one isoform from each cluster) in different OSNs, which overrides the endogenous cPcdh diversity and makes every OSN express the same set of cPcdh isoforms, results in their axons repelling each other and projecting diffusely (Mountoufaris et al., 2017). These axons cannot converge in the same location of the olfactory bulb to form proper glomeruli. The severity of convergence defects correlates with numbers of neurons overexpressing cPcdhs (Mountoufaris et al., 2017).

**PcdheC2 Regulates Axonal Tiling of Serotonergic Neurons**

Serotonin is a critical neurotransmitter that regulates diverse higher-order brain functions including mood, cognition, reward, learning and memory. Although there are only 300,000
FIGURE 2 | Molecular mechanisms for generating diverse neural identity codes. The stochastic and combinatorial expression of cPcdhs by CTCF/cohesin-mediated chromatin interactions generates vast repertoires of cell surface proteins. Most cPcdh genes are expressed monoallelically. The cPcdh proteins form cis-dimers and engage in specific homophilic trans-interactions. Through cytoplasmic common effectors of WRC, Pyk2, FAK, Ret and Rac1, cPcdhs regulate actin dynamics and cytoskeletal remodeling, eventually leading to various cellular functions, such as dendritic arborization and self avoidance, heteroneuronal neurite coexistence, axon tiling and myelination, neuronal survival and migration, and synaptogenesis.

serotonergic neurons in the human brain, serotonergic axon terminals innervate the entire brain with even spacing. This tilling pattern is required for the maintenance of constant serotonin levels since serotonin molecules can only function over a short distance from their releasing sites. Genetic evidence suggests that Pcdhα participates in the even spacing of serotonergic axons in mice (Chen et al., 2017; Katori et al., 2017). Specifically, deletion of the Pcdhα cluster, but not double deletion of the Pcdhβ and γ clusters, causes serotonergic axons to form clumps throughout the brain and results in insufficient terminal arborization in mice (Chen et al., 2017; Katori et al., 2017). In addition, labeling individual serotonergic neurons with brainbow technologies revealed that serotonergic axons from different neurons have tiling and self-avoidance defects upon constitutive Pcdhα deletion (Chen et al., 2017). Finally, these Pcdhα-deleted mice displayed depression-like behaviors, including
FIGURE 3 | Genetic evidence for C-Pcdh functions in neuronal wiring. Pcdhγ has critical function in sister dendrite self-avoidance of the retinal starburst amacrine cells (A) and cerebellar Purkinje neurons (B). Tri-cluster Pcdhs are required for olfactory sensory neurons to form glomeruli in the olfactory bulb (C). Pcdhαc2 is essential for the even spacing and tiling of serotonergic axons (D). Pcdhγc4 is the sole necessary and sufficient isoform for interneuron survival in the spinal cord (E).

increased immobility time in tail-suspension, forced-swimming, and contextual fear conditioning tests (Chen et al., 2017).

Conditional knockout of the entire Pcdha cluster in serotonergic neurons or their target fields revealed cell-autonomous functions of Pcdha in serotonergic neurons (Chen et al., 2017; Katori et al., 2017). However, deletion of α1-α12 of the Pcdha cluster results in no obvious defect in serotonergic wiring, suggesting an important role of C-type Pcdha genes in serotonin circuit formation. Consistently, deletion of both Pcdhac1 and Pcdhac2, or deletion of Pcdhac2 alone, causes the clumping of serotonergic axons similar to deletion of the entire Pcdha cluster (Chen et al., 2017; Katori et al., 2017). In addition, Pcdhac2 is predominately expressed in serotonergic neurons (Chen et al., 2017; Katori et al., 2017). Thus, among all members of the Pcdha cluster, only Pcdhac2 has been shown to be required for serotonergic axon wiring (Figure 3D) (Chen et al., 2017; Katori et al., 2017). This is a striking example that a neuronal cell type uses a single cPcdh isoform to achieve axonal tiling or even spacing in all of its projecting fields. Therefore, in contrast to the diversity generated by multi-cPcdh isoforms for cell–cell recognition of starburst cells, Purkinje cells, and OSNs, the single-isoform-mediated repulsion is a remarkable strategy adopted by serotonergic axons to tile brain regions with even spacing. However, since Pcdhγc4 and c5 are also expressed at lower levels in serotonergic neurons (Chen et al., 2017), it is difficult to rule out their partially redundant contribution in patterning the serotonergic axons. Combinatorial C-type gene deletion and rescue experiments are needed to fully address this important question.

Pcdhγc4 Is Required for Interneuron Survival

Experiments of in situ hybridization and immunohistochemistry showed that Pcdhγc4 is expressed in many brain regions as well as in spinal cord (Zou et al., 2007; Miralles et al., 2020). Detailed analyses of constitutive or conditional mutant mice lacking all Pcdh genes revealed the increased apoptosis of interneurons in the spinal cord, retina, cerebral and cerebellar cortex (Wang et al., 2002b; Lefebvre et al., 2008; Prasad et al., 2008; Chen et al., 2012; Hasegawa et al., 2016; Carriere et al., 2020; Mancia Leon et al., 2020). For example, in retina, Pcdhγ deletion results in interneuron apoptosis but does not affect survival of neighboring WT interneurons, suggesting a cell-autonomous effect (Lefebvre et al., 2008). In the spinal cord, however, the survival of Pcdhγ-deficient interneurons can be enhanced if they are surrounded by neurons expressing Pcdhγ (Prasad et al., 2008). This non-cell autonomous effect on survival was also observed for cortical interneurons (Mancia Leon et al., 2020). Taken together, these studies suggest that the effect of Pcdhγ on interneuron survival is cell- or tissue-type dependent.

Knockout of the three C-type isoforms (Pcdhγc3-c5) of the Pcdhγ cluster results in neuronal apoptosis that is indistinguishable from that observed in mice with deletion of the entire Pcdhγ cluster (Chen et al., 2012; Carriere et al., 2020; Mancia Leon et al., 2020). Moreover, deletion of only Pcdhγc4 fully recapitulates interneuron apoptosis caused by deletion of the
entire Pcdh cluster, suggesting that Pcdhγ4 is the only crucial isoform required for neuronal survival (Figure 3E) (Garrett et al., 2019). Although genetic evidence suggested that Pcdhγ4 contributes to interneuron survival, analyses of double or tri-cluster deletion mice revealed that Pcdhα and β clusters also have cooperative roles in neuronal survival. For example, cell apoptosis in the spinal cord of the Pcdhγ double-cluster or tri-cluster deletion mice is much more severe than that of the Pcdhγ-cluster deletion mice, although deletion of Pcdhα or Pcdhβ themselves reveals no apoptosis (Hasegawa et al., 2016). In addition, double deletion of Pcdhα and Pcdhγ also leads to more severe apoptosis of retina cells than the Pcdh deletion alone (Ing-Esteves et al., 2018). It’s possible that members of Pcdhβ and Pcdhγ contribute to neuronal survival by acting as carrier isoforms to facilitate Pcdhγ4 to be delivered to the cell surface.

A Role of Pcdhα in Myelination
Pcdhα has been observed to be strongly expressed in the developing, but not mature, axons at the late embryonic and early postnatal stages in mice. Specifically, Pcdhα expression is negatively correlated with the increasing expression levels of myelination markers during development (Morishita et al., 2004). In addition, deletion of the Pcdhα cluster in mice results in delayed oligodendrocyte maturation and defects in myelination (Yu et al., 2012). Moreover, imaging by transmission electron microscopy revealed the reduced ratio of myelinated nerve fibers and abnormal myelin sheaths in the Pcdhα deletion mice (Lu et al., 2018). Consistently, patients with a deletion in the 5q31.3 region, which covers the Pcdhα cluster, show delayed myelination (Shimojima et al., 2011). Further investigation of how cPcdhs participate in myelination processes is urgently needed.

Clustered Pcdhs Regulate Spine Morphogenesis and Synaptogenesis
The establishment of proper synaptic connections in the brain is central to information processing. Subcellular fractionation and microscopic imaging studies have shown that isoforms of Pcdhα and Pcdhγ are located at synaptic junctions (Kohmura et al., 1998; Wang et al., 2002b; Phillips et al., 2003; Lefebvre et al., 2008; Garrett and Weiner, 2009). In addition, two isoforms of Pcdhβ, β16 and β22, have been found to be enriched in subsets of synapses in the retina and cerebellum (Junghans et al., 2008; Puller and Haverkamp, 2011; Nuhn and Fuerst, 2014). Finally, isoforms of Pcdhγ are found to accumulate at axodendritic and dendrodendritic synapses (Fernandez-Monreal et al., 2009). These expression patterns suggest a role of cPcdhs in synaptogenesis.

Deletion of Pcdhγ leads to a significant loss of synapses in the mouse spinal cord (Wang et al., 2002b). To specifically investigate the role of cPcdhs in synapse development, apoptosis is blocked by BAX mutation in the Pcdh deletion mice. In this case, the spinal cord still displays decreased synaptic density, suggesting a direct role of Pcdhγ in synaptogenesis (Weiner et al., 2005). In contrast, blocking apoptosis in Pcdhγ-deficient retina by mutating BAX reveals normal synaptic numbers (Lefebvre et al., 2008). In addition, the decreased VGAT+ and increased VGLUT1+ synapses seen in the Pcdhγ-deficient spinal cord are secondary to interneuron apoptosis (Chen et al., 2012). Another study showed that synapse development requires Pcdhγ-mediated contacts between astrocytes and neurons (Garrett and Weiner, 2009). Specifically, deletion of Pcdhγ in astrocytes in vivo reduces both excitatory and inhibitory synapses in a contact-dependent mechanism (Garrett and Weiner, 2009). Finally, reciprocal synaptic connections between sister neurons derived from the same neural stem cell are impaired by deletion of the three Pcdh clusters (Tarusawa et al., 2016).

In the cortical neurons of Pcdhγ knockout mice, only thin spines are slightly increased (Molumbry et al., 2017). In addition, overexpression of a single Pcdhγ gene, γα1, significantly decreases spine density through repressing the postsynaptic cell adhesion molecule neurolgin-1 (Molumbry et al., 2017). However, conditional knockout of Pcdhγ in the olfactory granule cells decreases dendritic spines (Ledderose et al., 2013). In addition, in Pcdhα deletion mice, a significant decrease of spine density was observed in hippocampal Golgi staining in vivo and cultured hippocampal neurons in vitro (Suo et al., 2012). Similar phenotype was found in Pcdhδ knockout hippocampal neurons (Suo et al., 2012). In summary, cPcdhs are implicated in various aspects of synapse development in a cell-type dependent manner.

A SIGNALING PATHWAY LINKING CLUSTERED Pcdhs TO LAMELLIPodial FORMATION AND ACTIN CYTOSKELETAL DYNAMICS
A growing body of evidence supports the vital roles of cPcdhs in neuronal survival and migration, dendritic arborization and self-avoidance, spine morphogenesis and maturation, axonal targeting and tiling, as well as synaptogenesis (Figure 2) (Wang et al., 2002b; Weiner et al., 2005; Chen et al., 2012, 2017; Garrett et al., 2012; Lefebvre et al., 2012; Suo et al., 2012; Katori et al., 2017; Mountoufaris et al., 2017; Fan et al., 2018; Ing-Esteves et al., 2018; reviewed in Mountoufaris et al., 2018). These diverse functions of cPcdhs are all subserved by the intracellular signaling pathway via their conserved CDs. Interestingly, Pcdhβ cluster contains a WIRS (Wiskott-Aldrich syndrome family verprolin homologous protein (WAVE) interacting receptor sequence) motif in CD which can directly bind WRC (WAVE regulatory complex), linking to actin filament branching via the Arp2/3 complex, to regulate lamellipodial dynamics and cytoskeletal remodeling (Fan et al., 2018).

Pcdhβ also indirectly modulates actin dynamics through inhibiting Pyk2, which is a synaptic non-receptor tyrosine kinase. Pyk2, a known risk factor in neuropsychiatric diseases (Lambert et al., 2013), then regulates conformational changes of WRC via Rac1 (Fan et al., 2018). The Pcdh β and γ proteins, on the other hand, do not contain the WIRS motif. However, they may also modulate actin dynamics through forming cis-heterodimers with Pcdhα (Thu et al., 2014; Rubinstein et al., 2015; Goodman et al., 2017), or through the shared downstream effector Pyk2 (Chen et al., 2009; Suo et al., 2012) (reviewed in Wu and Jia, 2020).
Thus, this actin cytoskeletal dynamic regulation via Pyk2 and WRC is a general underlying mechanism for various functions of cPcdhs (Fan et al., 2018). It is known that dysregulation of actin dynamics and synaptic structural plasticity is closely related to neuropsychiatric diseases (Forrest et al., 2018). In the following four sections, we discuss the genetic, epigenetic, 3D genomic, and environmental dysregulations of cPcdhs in neuropsychiatric diseases.

GENETIC VARIANTS OF CLUSTERED Pcdhs IN MENTAL DISORDERS

Patients with the 5q31.3 deletion, which includes the three Pcdh clusters, show severe neurodevelopmental delay, encephalopathy associated with myelination defects, and hypotonia (Table 1) (Shimojima et al., 2011; Brown et al., 2013). In addition, human genetic studies revealed that deletion of a 5q31.3 fragment covering the Pcdha cluster is associated with poor music perception, suggesting its role in human higher cognitive functions (Table 1) (Ukkola-Vuoti et al., 2013).

Clustering Pcdh Genes in Autism Spectrum Disorders (ASD)

Autism spectrum disorders (ASD) are common neurodevelopmental conditions with social, communicational, and behavioral impairments (Betancur et al., 2009). Genetic studies have identified cPcdhs as candidate genes for ASD. First, whole exome sequencing identified de novo mutation of Pcdhβ4 D555H in sporadic autism probands (O’Roak et al., 2012). Second, five single-nucleotide polymorphisms (SNPs) of Pcdha are reported to have significant genetic associations with autism in a large cohort of 841 families (Anitha et al., 2013). Third, whole exome sequencing revealed that single nucleotide variants (SNVs) within the Pcdh clusters are associated with ASD (Iossifov et al., 2014; Krumm et al., 2015). Finally, abnormal CpG methylation patterns of Pcdha12 are found in a South African ASD cohort (Stathopoulos et al., 2020).

Clustering Pcdh Genes in Bipolar Disorders

The 5q31 region is found to be associated with bipolar disorders (Hong et al., 2004; Herzberg et al., 2006). Specifically, patients with bipolar disorders showed a striking increase in homozygosity of a minor allele of the Pcdha cluster, in which a SNP within the HS5-1 enhancer is found to be associated with aberrant Pcdha expression (Pedrosa et al., 2008).

Clustering Pcdh Genes in Dyslexia

A set of SNPs within the Pcdh cluster is dominantly inherited in a family with dyslexia, a neurodevelopmental disorder characterized by reading and writing difficulties (Naskar et al., 2018). Two of them alter amino acids within EC2 and EC3 domains of Pcdh γa3 and γa4, respectively (Naskar et al., 2018). These missense SNPs located in the two critical ectodomains required for homophilic interactions, which might alter cell recognition and contribute to aberrant neuronal circuits of dyslexia.

Clustering Pcdh Genes in Extreme Obesity with Behavioral Abnormality

In patients with extreme obesity, cPcdhs have a significantly higher frequency of rare variants than in general population, indicating an association between rare variants of the Pcdh clusters and extreme obesity (Table 1) (Mariman et al., 2014, 2015). In addition, copy number variations (CNVs) of two Pcdh genes, Pcdhβ7 and Pcdhβ8, are associated with increased body mass index (Moon et al., 2017). In mice, Pedh regulates hypothalamic feeding circuitry (Su et al., 2010), which is known to be involved in extreme obesity. In particular, conditional knockout of Pedh in mouse hypothalamic neurons causes excessive feeding behaviors and defects in energy homeostasis, leading to extreme obesity (Su et al., 2010). These studies suggest that cPcdhs may play an important role in regulating neural circuits controlling feeding behavior, resulting in extreme obesity.

EPigenetic Dysregulations of Clustered Pcdhs in MENTAL DISORDERS

Clustered Pcdhs in Down Syndrome

Down syndrome (DS) is a genetic disorder with intellectual disabilities caused by an extra copy of chromosome 21. In the developing DS brain tissues, 5’ alternate isoforms of the Pedhβ cluster are significantly hypermethylated (Table 1) (Mendioroz et al., 2015; El Hajj et al., 2016). In addition, in postmortem DS brains, 3’ alternate isoforms of the Pedhβ cluster retain the fetal-like methylation state (Almenar-Queralt et al., 2019). Consequently, expression levels of these isoforms remain high in adult brain tissues, similar to that of the fetal stage, supporting a model of delayed brain maturation in the DS patients (Almenar-Queralt et al., 2019). Moreover, human DS iPSC-derived astrocytes show a transcriptional profile between fetal and mature stages, further supporting the delayed maturation model of DS (Ponroy Bally et al., 2020). Furthermore, these DS astrocytes show reduced levels of the Pedhβ expression and have selective impairments in Pedhβ-mediated cell adhesion (Ponroy Bally et al., 2020). Finally, nearly all of the cPcdh genes are downregulated in trisomic iPSCs comparing to the genetically edited disomic iPSCs derived from the same DS patient (Gonzales et al., 2018). Considering the important functions of cPcdhs in brain development, it’s possible that dysregulated cPcdhs may be related to defective synaptogenesis and neurite growth seen in DS brains.

Clustered Pcdhs in Alzheimer’s Disease (AD)

In humans, the expression of cPedh genes is frequently found to be altered in AD. For example, Pcdhac2 and Pcdhγc5 are decreased while subsets of alternate Pedh isoforms are increased in the lateral temporal lobe of postmortem AD brains (Nativio
et al., 2018). Interestingly, APOE4, a prominent genetic risk allele for late-onset AD, causes cPcdh gene upregulation in iPSC-derived cerebral organoids (Meyer et al., 2019).

In mice, loss of excitatory or inhibitory synapses has been implicated in the pathogenesis of AD. For example, Pcdhγc5 specifically interacts with GABA receptors through its cytoplasmic domain to stabilize and promote inhibitory GABAergic synaptic transmission (Li et al., 2012). The expression levels of Pcdhγc5 in GABAergic synapses are increased in hyperexcitatory conditions. The increased Pcdhγc5 enhances synaptic inhibition and elevates GABAergic protein levels, which may contribute to pathogenic excitatory/inhibitory imbalance in an AD mouse model (Table 1) (Li et al., 2017). As a downstream effector of cPcdhs, the cell-adhesion kinase Pyk2 has been identified as a major susceptibility gene for late-onset AD (Lambert et al., 2013). Consistently, overexpression of Pyk2 results in synapse loss and learning deficit (Lee et al., 2019; Salazar et al., 2019). Therefore, dysregulation of cPcdhs or disruption of its intracellular signaling pathway may contribute to AD pathogenesis.

Clustered Pcdhs in Major Depressive Disorders (MDD)

Reduced serotonin levels have been implicated in the pathogenesis of MDD and selective serotonin reuptake inhibitors (SSRIs) are the most commonly used antidepressants (Belmaker and Agam, 2008). In humans, altered expression patterns of members of the Pcdha cluster are detected in serotonergic neurons derived from patients of SSRI-resistant MDD (Vadodaria et al., 2019). In addition, low expression levels of Pcdha7 and Pcdha8 in the prenatal brain are associated with MDD (Hall et al., 2020). Moreover, in rats with learned helplessness, a model of depression, members of the Pcdha cluster are upregulated in CA1 neurons (Garafola and Henn, 2014). Finally, in mice Pcdhc2 is specifically expressed in serotonergic neurons and knockout of the Pcdha cluster leads to depressive-like behaviors (Chen et al., 2017). In conjunction with the important role of cPcdhs in serotonergic axon wiring (Chen et al., 2017), these findings suggest that uneven distribution of serotonergic fibers by cPcdh dysregulation could lead to reduced serotonin signaling in various brain regions.

### Table 1 | Genetic/epigenetic dysregulation of the three Pcdh clusters causes various brain disorders.

| Disorder                        | Gene                  | Alteration         | References                                                                 |
|--------------------------------|-----------------------|--------------------|-----------------------------------------------------------------------------|
| Aging                          | Pcdh clusters         | Differential methylation | Rakyan et al., 2010; Bell et al., 2012; Salpea et al., 2012; Hannum et al., 2013; McClay et al., 2014; Slieker et al., 2016; Kim et al., 2018a |
| Alzheimer’s disease            | Pcdhγc5               | Altered expression  | Li et al., 2012 (rat); Li et al., 2017 (mouse)                             |
|                                | Pcdh clusters         | Altered expression  | Nativio et al., 2018; Meyer et al., 2019                                  |
| Antipsychotic medication       | Pcdh clusters         | Differential methylation | Melka et al., 2014; Nakazawa et al., 2017                                 |
| Autism                         | Pcdhα cluster         | SNP                | Anitha et al., 2013                                                        |
|                                | Pcdh clusters         | De novo mutations  | Iossifov et al., 2014                                                       |
|                                | Pcdh clusters         | SNV, CNV           | Krumm et al., 2015                                                          |
|                                | Pcdhγc12              | Differential methylation | Stathopoulos et al., 2020                                                  |
| Bipolar disorder               | Pcdh clusters         | Association        | Hong et al., 2004; Herzberg et al., 2006                                   |
|                                | Pcdhα cluster         | SNP                | Pedrosa et al., 2008                                                        |
| Child maltreatment             | Pcdh clusters         | Differential methylation | McGowan et al., 2011 (rat); Suderman et al., 2012 (rat and human)           |
| Developmental delay            | Pcdh clusters         | 5q31.3 microdeletion | Shimojima et al., 2011; Brown et al., 2013                                 |
| Down syndrome                  | Pcdhα cluster         | Differential methylation | Mendioroz et al., 2015                                                      |
|                                | Pcdhγc5               | Differential methylation | El Haj et al., 2016; Almenar-Queralt et al., 2019                          |
|                                | Pcdhγc1               | Altered expression  | Ponroy Bally et al., 2020                                                   |
|                                | Pcdhγ cluster         | Altered expression  | Gonzales et al., 2018                                                       |
| Dyslexia                       | Pcdhγc5               | SNP                | Naskar et al., 2018                                                         |
|                                | Pcdh clusters         | Deletion           | Su et al., 2010 (mouse)                                                      |
|                                | Pcdh clusters         | Rare variants      | Mariman et al., 2014; 2015; Moon et al., 2017                              |
| Fetal alcohol spectrum disorder| Pcdh clusters         | Differential methylation | Laufer et al., 2015, 2017                                                   |
| Poor music perception          | Pcdhα cluster         | 5q31.3 microdeletion | Ukkola-Vuoti et al., 2013                                                    |
| Major depressive disorder      | Pcdhγc5               | Differential methylation | Garafola and Henn, 2014 (rat)                                                |
|                                | Pcdhα cluster         | Deletion           | Chen et al., 2017 (mouse)                                                    |
|                                | Pcdhγc6, γ8           | Altered expression  | Vadodaria et al., 2019                                                       |
|                                | Pcdhγc7, γ8           | Altered expression  | Hall et al., 2020                                                            |
| Schizophrenia                  | Pcdh clusters         | Association        | Schwab et al., 1997; Straub et al., 1997; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Pardinas et al., 2018; Walker et al., 2019 |
|                                | Pcdh clusters         | 3D genome          | Rajajaran et al., 2018                                                       |
|                                | Pcdh clusters         | Altered Pcdh pathway | Shao et al., 2019                                                            |
|                                | Pcdhγc7, γ8           | Altered expression  | Hall et al., 2020                                                            |
Clustered Pcdhs in Schizophrenia

Clustered Pcdhs have been identified as susceptible loci for schizophrenia (Schwab et al., 1997; Straub et al., 1997; Pardinas et al., 2018; Walker et al., 2019). In particular, large-scale genome-wide association studies have revealed that the cPcdh locus is associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). In addition, high expression levels of members of the Pcdha cluster, Pcdha7 and Pcdha8, are associated with schizophrenia (Hall et al., 2020). Finally, cPcdhs and their downstream effector PKC are dysregulated in hiPSC-derived cortical interneurons from schizophrenic patients (Shao et al., 2019).

Epigenetic modifications, especially DNA methylation of gene promoters, play a major role in cPcdh regulation (reviewed in Wu and Jia, 2020). Antisense transcription of long non-coding RNAs leads to demethylation of cPcdh promoters (Canzio et al., 2019). CTCF then binds the unmethylated cPcdh promoters and brings them in close contacts with remote enhancers via long-distance chromatin interactions to activate cPcdh transcription (Guo et al., 2012). Collectively, these studies suggest that epigenetic dysregulation of cPcdhs is related to various mental disorders. Since epigenetic and higher-order chromatin regulations are a general phenomenon, it is very likely that these regulations also affect other cell-surface neural receptors.

3D GENOME DYSREGULATIONS OF CLUSTERED Pcdhs IN MENTAL DISORDERS

The proper expression of the cPcdh genes requires specific long-range enhancer-promoter contacts in the 3D nuclear space. Mutations in genes encoding 3D genome architectural proteins, such as CTCF, cohesin, MeCP2, SETDB1, and WIZ, dysregulate cPcdh genes through altering higher-order chromatin contacts (Figure 4). Therefore, it’s interesting to note that an increasing list of mutations in genes encoding 3D genome architectural proteins is linked to brain disorders. We suggest that many complex neuropsychiatric diseases of mutations of 3D genome regulators are ‘actually’ resulted from cPcdh dysregulations.

3D Genome Dysregulation of Clustered Pcdhs by CTCF

De novo mutations of CTCF in humans causes the type 21 of autosomal dominant mental retardation (MRD21) (Gregor et al., 2013; Bastaki et al., 2017; Hori et al., 2017; Chen et al., 2019; Konrad et al., 2019). This rare condition is first described in four individuals with intellectual disability, microcephaly, and growth retardation. Genetic studies revealed one individual with CTCF missense mutation, two with CTCF frameshift mutations, and one with CTCF deletion (Gregor et al., 2013). A subsequent study reported an additional individual of MRD21 with a different de novo CTCF frameshift mutation (Bastaki et al., 2017). Recent studies independently reported 2, 3, and 39 additional individuals of MRD21 with pathogenic variants of CTCF (Hori et al., 2017; Chen et al., 2019; Konrad et al., 2019). Finally, a human genetic study showed strong associations between CTCF SNPs and schizophrenia in multiple cohorts (Juraeva et al., 2014). Given that CTCF mutations or variants dysregulate cPcdh genes, it’s likely that this cPcdh dysregulation is responsible for complex brain disorders caused by CTCF mutations or variants.

In mice, conditional knockout of CTCF in cortical neurons downregulates nearly all of the cPcdh genes. These mice display abnormal behavior and growth retardation (Hirayama et al., 2012). Strikingly, every member of the cPcdh genes carrying CBS elements is downregulated, suggesting that long-distance chromatin looping between enhancers and promoters is impaired. Histological analyses revealed the loss of the functional somatosensory circuits and the reduction of dendritic arborizations in CTCF knockout mice, reminiscent of the phenotypes of cPcdh deletion (Hirayama et al., 2012). In addition, conditional CTCF knockout in excitatory or inhibitory neurons impairs long term memory and cortical synaptic plasticity (Kim et al., 2018b). Finally, RNA-seq experiments revealed a predominant dysregulation of the cPcdh genes (Kim et al., 2018b). In conjunction with the known role of CTCF in the cPcdh 3D regulation, it is possible that neuropsychiatric symptoms in human CTCF mutations are related to the dysregulation of cPcdh genes.

3D Genome Dysregulation of Clustered Pcdhs by Cohesin and its Regulators

The cohesin complex, which mediates chromatin loop extrusion and sister chromatid cohesion, is central for 3D genome folding. Mutations of cohesin or of its regulators have been shown to be associated with a large set of complex human diseases, collectively called cohesinopathies (Liu and Krantz, 2008). The symptoms of cohesinopathies vary in different mutations but share the same manifestations such as cognitive retardation and intellectual disability. Cornelia de Lange syndrome (CdLS), the best characterized cohesinopathy, is caused by mutations of cohesin regulators, such as NIPBL and HDAC8, or of cohesin subunits, including SMC1A, SMC3, and RAD21 (Bose and Gerton, 2010). Roberts syndrome, which is phenotypically related to CdLS, is caused by mutations of the cohesin regulator ESCO2 (Liu and Krantz, 2008). Similar to CTCF, knockdown of cohesin in cell lines significantly downregulates the expression of cPcdh genes (Guo et al., 2012). Interestingly, only the SA1 but not SA2 subunit of cohesin is located at cPcdh promoters and regulates their activities (Remeseiro et al., 2012). Collectively, cognitive impairments in cohesinopathies with mutations of cohesin subunits or of its regulators may result from their 3D genome dysregulations of the cPcdh genes.

3D Genome Dysregulation of Clustered Pcdhs by MeCP2

The Rett syndrome is a rare, severe type of ASDs caused by mutations in the X-linked MeCP2 (methyl-CpG binding protein 2) gene (Amir et al., 1999). In addition, Pcdhβ1 is upregulated in both the MeCP2 mutant mice and postmortem brains of Rett syndrome patients (Miyake et al., 2011). Moreover, in MeCP2 mutant mice, several members of the Pcdh clusters are
dysregulated (Chahrour et al., 2008). Finally, in the human cPcdh locus, each of the 15 Pcdhα, 16 Pcdhβ, and 22 Pcdhγ genes is associated with a CpG island (Wu et al., 2001) and methylation of a CpG dinucleotide within the cPcdh promoter CTCF sites precludes CTCF binding (Guo et al., 2012). Given the known role of MeCP2 in gene regulation via the binding to methylated CpG islands, MeCP2 and CTCF may bind mutually exclusively to the cPcdh promoters. Thus, the dysregulation of cPcdhs by MeCP2 mutations may result from altering 3D genome configuration via CTCF.

### 3D Genome Dysregulation of Clustered Pcdhs by SETDB1

Deletion of SETDB1 (SET domain bifurcated 1), which encodes a histone H3K9 methyltransferase, results in a five-hundred-fold increase of cPcdh transcripts in the mouse cortex compared to the rest of the genome (Jiang et al., 2017). SETDB1 protects the cPcdh locus from excessive CTCF binding to maintain the cPcdh superTAD structure (Jiang et al., 2017). Interestingly, the S’ boundary of the cPcdh superTAD, which enriches with H3K9me3 histone modifications (Figure 4), contains a haplotype significantly associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Jiang et al., 2017). This region regulates the expression of cPcdh genes through long-range chromatin loops across hundreds of kilobases (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Rajarajan et al., 2018). Within this region a schizophrenia-risk SNP (rs11896713) significantly affects the expression of Pcdh genes in a dosage-dependent manner (Fromer et al., 2016; Rajarajan et al., 2018).

Mutations of SETDB1 itself are also associated with psychiatric disorders such as autism (Cukier et al., 2012). In addition, the expression of SETDB1 is markedly increased in patients with Huntington’s disease (HD) (Ryu et al., 2006). In the mouse model of HD, 37 out of 58 cPcdh genes are strikingly dysregulated as the size of huntingtin CAG repeats increases (Langfelder et al., 2016). Together, cognitive defects associated with SETDB1 mutations may result from the dysregulation of cPcdhs.

### 3D Genome Dysregulation of Clustered Pcdhs by WIZ

WIZ (Widely interspaced zinc finger-containing protein) forms a heteromeric H3K9 methyltransferase complex with G9a and GLP. In autism patients, exon resequencing identified non-synonymous variants in WIZ and its partners G9a/GLP (Balan et al., 2014). Haploinsufficiency of GLP causes the Kleefstra syndrome, a multi-system syndrome associated with intellectual disability, neurodevelopmental delay, and neuropsychiatric diseases (Willemsen et al., 2012). WIZ is a modulator of chromatin loops and its genomic binding sites overlap with CTCF (Figure 4) (Isbel et al., 2016; Justice et al., 2020). Remarkably, only members of the Pcdhβ cluster are dysregulated in WIZ mutant mice (Isbel et al., 2016). Interestingly, these mice display an anxiety-like phenotype (Isbel et al., 2016), consistent with the requirement for cPcdhs in neuronal development and connectivity. All in all, these human and mouse genetic studies suggest that mutations of genome architectural proteins may be involved in pathogenesis of complex neural diseases through dysregulation of cPcdh genes (Figure 4).

### DYSREGULATIONS OF CLUSTERED Pcdhs IN MENTAL DISORDERS BY ENVIRONMENTAL FACTORS

#### Clustered Pcdhs and Child Maltreatment

Early life experiences are known to have long-term effects on mental health and behavior through epigenetic reprogramming, including alterations in stress responses. cPcdhs display differential methylations in response to maternal care. In children suffered from maltreatment or abuse, increased methylation levels are seen in promoters across the three Pcdh clusters, especially in Pcdhβ (Table 1) (Suderman et al., 2012). In rat pups who received less licking and grooming from mothers, Pcdhβ promoters are hypermethylated and their expression levels are downregulated (McGowan et al., 2011; Suderman et al., 2012). These studies suggest that early life experience
affects cPcdh expression through epigenetic modification of their promoters, which may contribute to the psychological abnormalities in abused children.

**Clustered Pcdhs and Fetal Alcohol Spectrum Disorders (FASD)**

Similar to early life experiences, prenatal alcohol exposure during pregnancy, which can cause lifelong alterations in cognition and behavior, also influences the methylation states of the three Pcdh clusters (Laufer et al., 2015, 2017). In mouse models of FASDs and in children suffered from prenatal alcohol exposure, there are similar methylation changes in the cPcdh genes (Laufer et al., 2015, 2017).

**Clustered Pcdhs and Antipsychotic Medication**

Olanzapine, an antipsychotic medication commonly used for treating bipolar disorders and schizophrenia, causes methylation changes in the cortex in a rat model. Interestingly, the promoter regions of cPcdhs showed prominent methylation alterations in the cerebrum and hippocampus (Table 1) (Melka et al., 2014). In addition, clozapine, the most effective medication for treatment-resistant schizophrenia, causes differential expression of cPcdh genes in iPSC-derived neurons (Nakazawa et al., 2017). Thus, antipsychotic drugs may exert their therapeutic effects through altering the regulation of cPcdhs.

**Clustered Pcdhs and Brain Aging**

Epigenetic alteration is a hallmark of aging. Interestingly, many age-related differentially methylated regions are found to be located within the three Pcdh clusters (Rakyan et al., 2010; Bell et al., 2012; Hannum et al., 2013; Sliker et al., 2016). In addition, cPcdh is prominently differentially methylated during aging (McCay et al., 2014). Moreover, epigenetic modifications in the three Pcdh clusters are reprogrammed during aging (Salpea et al., 2012). Finally, a 134-twin aging study revealed that methylation states of cPcdh promoters correlate with biological ages (Kim et al., 2018a). Altogether, various environmental factors can influence methylation states of the Pcdh clusters, leading to their abnormal expression patterns, which may be related to mental disorders and behavioral abnormalities.

**CONCLUDING REMARKS**

Clustered Pcdhs are thought to function as molecular identity codes for individual neurons to discriminate self from non-self (Lefebvre et al., 2012; Mountoufaris et al., 2018; Honig and Shapiro, 2020; Sanes and Zipursky, 2020; Wu and Jia, 2020). The tremendous diversity afforded by unique variable and constant genomic organization (Figure 1), the stochastic promoter choice realized by topological chromatin looping and balanced enhancer contacts (Figure 1A), and amazing structural recognition evolved through promiscuous cis-interactions and stringent homophilic trans-interactions (Figure 2) enable billions of neurons to achieve neurite self-avoidance and to establish proper connectivity in the brain. A key unresolved question is how cPcdh homophilic adhesions are transformed into neurite repulsion. This is likely achieved through common intracellular signaling transduction pathways with Pyk2, FAK, Ret, and WRC complex, eventually leading to actin dynamics and cytoskeletal remodeling (Figure 2). Shedding the extracellular domain by metalloproteinase may be essential for separating the homophilic-interaction-bridged plasma membranes. Finally, genetic studies provide strong evidence that cPcdh genes are central for neurodevelopment and neurite morphogenesis in the brain (Figure 3).

The diverse roles of cPcdh genes in neural circuit formation suggest that their mutations or dysregulations may be involved in complex brain disorders. Indeed, numerous studies reported mutations of cPcdh genes in Alzheimer’s disease, ASDs, bipolar disorders, major depressive disorders, schizophrenia, behavioral abnormalities, and several neurodevelopmental disorders with cognitive impairments (Table 1). In addition, mutations of 3D genome architectural proteins or regulators of cPcdh genes such as CTCF, cohesin, MeCP2, SETDB1, and WIZ cause a wide variety of brain disorders (Figure 4). These overwhelming data suggest that mutations or dysregulations of cPcdh genes play an important role in the pathogenesis of brain disorders.

In summary, growing lines of evidence suggest the important roles of cPcdh genes in complex mental or brain disorders. However, most brain and mind diseases have complex etiology and pathogenesis. How these diseases are initiated and progressed through genetic mutations and epigenetic dysregulations of cPcdh genes remain largely unknown. A deeper understanding of regulatory mechanisms and biological functions of cPcdh genes may be the key to understand the etiology and pathogenesis and to facilitate developing rational therapeutic strategies in the future.

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Both authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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