PCDH19 interplay with GABA(A) receptors: a window to DEE9 pathogenetic mechanisms

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Developmental and epileptic encephalopathy 9 (DEE9): The gene PCDH19 (Xq22.1), which encodes the calcium-dependent cell adhesion protein protocadherin-19 (PCDH19), is nowadays considered as one of the most important genes in monogenic epilepsy (Depienne and LeGuern, 2012). Mutations in PCDH19 are responsible for DEE9 (OMIM #300088), a severe neurodevelopmental disorder characterized by early-onset clustering epilepsy, various degrees of cognitive impairment and neuropsychiatric comorbidities, like autism spectrum disorder (ASD) and behavioural problems. DEE9 patients start suffering from seizures around the age of 10 months until adolescence, when seizures tend to reduce or even disappear, while the psychiatric symptoms persist (Depienne and LeGuern, 2012; Kolc et al., 2018).

DEE9 is a peculiar X-linked disorder, since only the heterozygous females are affected, while hemizygous males are spared. Despite the increasing number of DEE9 patients, little is known about the pathogenetic mechanisms responsible for this syndrome, even if cellular mosaicism has been proposed as the putative cause. According to this hypothesis, the co-presence of neurons expressing a wild type form of PCDH19 and neurons expressing a mutated form of PCDH19, or not expressing it at all, would scramble cell-cell communication, leading to the onset of the disorder. The identification of some PCDH19 mosaic male patients, due to early somatic mutations in PCDH19, seems to confirm this hypothesis (Depienne and LeGuern, 2012; Kolc et al., 2018; Niazi et al., 2019).

PCDH19 consists of six exons encoding PCDH19, a member of the cadherins superfamily. PCDH19 is composed by an extracellular domain with six conserved cadherin repeats that mediate adhesion, a transmembrane region, and an intracellular C-terminal domain (Gerosa et al., 2018; Niazi et al., 2019). Up to now, more than 175 pathogenic variants have been reported, most of which fall in exon1, encoding the extracellular domain. Mutations, either inherited or de novo, include whole gene deletion, large deletions and duplications, small insertions/deletions, and point mutations, either missense or nonsense. Since there is no clear genotype-phenotype correlation, all pathogenic variants are thought to cause PCDH19 loss of function (Depienne and LeGuern, 2012; Kolc et al., 2018; Niazi et al., 2019).

PCDH19 is predominantly expressed in the central nervous system, especially in limbic system and cortex, both during development and adulthood (Gerosa et al., 2019). Multiple PCDH19 functions are emerging in the brain. PCDH19 has been reported to regulate neuronal progenitors differentiation, cell adhesion, neuronal migration and cell-sorting, and synaptic transmission. These functions can be understood in the light of PCDH19 interacting partners, such as the cell-adhesion molecule N-cadherin, the WRC complex, which regulates cytoskeletal dynamics, and the γ-aminobutyric acid type A (GABA(A)) receptor (GABA(A)R) (reviewed in Gerosa et al., 2019).

PCDH19 and GABA(A)R: The identification of PCDH19 interaction with GABA(A) receptors (GABA(A)Rs) (Bassani et al., 2018), paved the way for a new investigation field on DEE9 pathogenetic mechanisms.

GABA(A)Rs are ligand-gated channel receptors permeable to chloride ions and, to less extent, to bicarbonate ions. GABA binding causes the channel opening and the flux of chloride ions according to their transmembrane gradient, which changes during neurodevelopment causing the switch of polarity in the GABAergic transmission. Early in brain development, chloride ions efflux from the neurons causes their depolarization and the activation of calcium signaling on which the trophic action of GABA relies. In this period, GABAergic signaling drives neuronal progenitors differentiation, neuronal migration and maturation. After the so-called GABA switch, chloride influx hyperpolarizes the plasma membrane, thus conferring to the GABA its well-known inhibitory function (Braat and Kooy, 2015).

These different roles explain the involvement of the GABAergic signaling and of GABA(A)Rs in epilepsy and, more in general, in neurodevelopmental disorders and their characteristic cognitive and psychiatric symptoms, in primis intellectual disability (ID) and ASD. Examples of neurodevelopmental syndromes in which the involvement of GABA(A)R signaling has been demonstrated are Dravet syndrome, Rett syndrome, Fragile X syndrome, and Prader-Willi syndrome (Braat and Kooy, 2015). Our recent studies suggested that DEE9 is part of the list.

We provided the first experimental evidence that PCDH19 downregulation in hippocampal neurons affects both components of GABA(A)R-mediated transmission, i.e., phasic and tonic inhibition. Primary rat excitatory neurons expressing PCDH19 shRNA exhibited reduced frequency and altered kinetic of spontaneous miniature inhibitory postsynaptic currents as well as reduced tonic currents (Bassani et al., 2018; Serratto et al., 2020; Figure 1).

What are the mechanisms? GABA(A)Rs are pentameric receptors generally composed of 2 alpha, 2 beta and 1 gamma or delta subunit, and we found that PCDH19 intracellular C-terminal domain is able to bind a conserved region of the alpha subunits. We hypothesized that, through this interaction, PCDH19 might regulate GABA(A)Rs trafficking. This is consistent with the well-known expression of protocadherins in intracellular vesicles and the association of PCDH19 with cytoskeletal components, and especially with the reduced expression of GABA(A)Rs that we observed on the surface of neurons expressing PCDH19 shRNA (Bassani et al., 2018).

Notably, we found that PCDH19 binding region on alpha subunit maps in the intracellular loop that connects the third and fourth transmembrane domains and is known to regulate GABA(A)R gating properties. Accordingly, PCDH19 downregulation affected GABA(A)R biophysical properties, as demonstrated by an altered proportion of long and brief openings in favor of the latter, thus resulting in the flickering behavior of channels (Serratto et al., 2020). Reduced number of surface receptors and their altered kinetics offer nonexclusive- most
likely cooperative-explanations for the reduced inhibitory transmission (Figure 1).

What are the consequences of altered GABAergic transmission? Neurons in which PCDH19 was downregulated displayed reduced rheobase and increased firing frequency (Figure 1) and we reported a causal link between reduced GABA(A)R currents and enhanced neuronal excitability in vitro (Serratto et al., 2020). Furthermore, in utero electroporation of PCDH19 shRNA in the hippocampus of rats increased their seizure susceptibility in the first postnatal period. Interestingly, PCDH19 shRNA-expressing neurons showed altered positioning in the hippocampus and abnormal dendritic arborization (Bassani et al., 2018). Even though our experiments did not allow directly ascribing these migratory and morphological effects to the altered GABA(A)R transmission, the contribution of a reduced trophic action of GABA is likely.

GABA(A)R and DEE9 treatments: We wondered how our recent findings can be inserted in the context of current DEE9 therapies. It must be said that the treatment of DEE9 is very difficult, and clinicians are still looking for a shared therapeutic protocol. In most of the cases, epilepsy tends to be refractory to treatments and patients require cocktails of different antiepileptic drugs to try controlling seizures. The extremely high heterogeneity of DEE9 symptoms, polytherapy, and the natural evolution of the pathology do not help (Samanta, 2020). Epileptic seizures, in which most of the therapeutic effort is put, as they represent the most debilitating and life threatening symptom during infancy, follow a temporal evolution in terms of onset and offset, distribution in clusters, and sensitivity to external triggers such as fever (Depienne and LeGuern, 2012; Kolc et al., 2018; Samanta, 2020).

This complicates the evaluation of any drug efficacy. However, among classic antiepileptic treatments, some of the best results were obtained with clobazam and bromide (Samanta, 2020). Notably, clobazam is an allosteric modulator of GABA(A)Rs that binds the alpha-gamma2 subunits interface and increases the frequency of channel openings thus favoring the chloride ions influx. Similarly, bromide ions, which permeate GABA(A)R more efficiently than chloride ions, enhance GABAergic transmission providing the membrane hyperpolarization that counters hyperexcitability (Suzuki et al., 1994). By contrast, controversial results were obtained by using voltage-gated sodium channels blockers, such as carbamazepine, phenytoin and oxcarbazepine, which counteract high frequency neuronal spiking. Some patients benefitted from these drugs, some others experienced no effect and some underwent seizure exacerbation, and the reasons remain unknown (Samanta, 2020).

In addition to the use of classic antiepileptic drugs, a hypothesis-based drug clinical trial is underway to test the efficacy of ganaxolone (Samanta, 2020), a synthetic analog of allopregnanolone, which is a neurosteroid with a potent positive allosteric modulator effect on GABA(A)Rs. In fact, it had been observed that DEE9 patients display altered levels of hormone-metabolizing enzymes in their skin fibroblasts and low levels of allopregnanolone in the blood (Tan et al., 2015). Altogether, these results and those of our recent studies suggest that the GABAergic system is doubly affected in patients with DEE9: on one hand there is a reduced level of GABA(A)Rs positive allosteric modulators, on the other hand there are low levels of surface receptors, which display a flickering behavior.

We have hypothesized that PCDH19 might directly affect GABA(A)Rs kinetics via its binding to the channel (Serratto et al., 2020). However, we cannot exclude alternative mechanisms. If PCDH19 loss of function influences neurosteroids synthesis, via a mechanism not yet characterized, PCDH19 might affect GABA(A)Rs kinetics indirectly, through neurosteroids. In fact, neurosteroids influence channel kinetics without affecting conductance. They have been shown to increase GABA(A)Rs mean open time, increase the contribution of long openings, and decrease close time duration (Akk et al., 2004). Hence, we cannot exclude that neurosteroids reduction might have contributed to the flickering behavior observed in PCDH19-shRNA expressing neurons, even though we did not observe any change in closing time properties in these neurons (Serratto et al., 2020). Alternatively, the PCDH19-GABA(A)R complex could facilitate the access of neurosteroids to their binding sites on GABA(A)Rs.

PCDH19-GABA(A)R association and the downstream GABAergic functional effects provide the key to interpret the therapeutic effectiveness of drugs that target the GABAergic transmission in DEE9, but many questions remain open, some of which will be briefly discussed in the next paragraph.

Open questions and concluding remarks: With our studies on the PCDH19-GABA(A)R association we have unveiled the tip of the iceberg, which means there is still much to be done to understand the
complex role of PCDH19 in the modulation of the GABAergic system.

For instance, up to now we focused on the postsynaptic side of hippocampal excitatory neurons; it might be relevant to look at the presynaptic site. How do inhibitory neurons perform upon PCDH19 loss of function? Inhibitory transmission might be compromised at both sides of synapses. Furthermore, different subpopulations of neurons in different brain areas and developmental stages might show a different susceptibility to PCDH19 loss.

Another field of future investigation regards the relationship between the GABAergic system dysfunctions and the mosaicism hypothesis (Depienne and LeGuer, 2012). In our recent paper (Serratto et al., 2020), we speculated that a mosaic expression of PCDH19 might translate in a mosaic of excitatory levels, which in turn might introduce a bias in cellular wiring and memory trace formation. Hence, there is the urgency to shift from the single cell level to the network level in order to understand how normal and hyperexcitable cells wire together, and the resulting excitability level of the entire network. This might theoretically help understand the controversial effects in DEE9 of drugs that target neuronal spiking activity, such as sodium channels blockers. Furthermore, it might shine a light on DEE9 cognitive and behavioral aspects, which becomes the most debilitating symptoms in adult patients, after seizure decrease.

Finally, in light of DEE9 unique inheritance, it would be interesting to investigate whether the relationship between PCDH19 and GABA(A)Rs might contribute to explaining the different gender susceptibility of DEE9. Indeed, it is known that sex shapes the features of the GABAergic system, which in turn influences the brain development.

In conclusion, we argue that GABA(A)Rs and inhibitory transmission represent an important field for future investigations. Providing an answer to these and other questions will help reveal the multifaceted role of PCDH19 in GABAergic transmission. The gained knowledge will provide the key to understand the effectiveness of ongoing DEE9 treatments and predict which drugs are most likely to be effective and which are not. More importantly, we hope it will actively drive the development of new and more specific treatments for DEE9 and related neurodevelopmental syndromes.

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References

Akk G, Bracamontes JR, Covey DF, Evers A, Dao T, Steinbach JH (2004) Neuroactive steroids have multiple actions to potentiate GABA(A) receptors. J Physiol (Lond) 558:59-74.

Bassani S, Cwetsch AW, Gerosa L, Serratto GM, Folci A, Hall IF, Mazzanti M, Cancedda L, Passafaro M (2018) The female epilepsy protein PCDH19 is a new GABAAR-binding partner that regulates GABAergic transmission as well as migration and morphological maturation of hippocampal neurons. Hum Mol Genet 27:1027-1038.

Braat S, Kooy RF (2015) The GABA receptor as a therapeutic target for neurodevelopmental disorders. Neuromol Med 17:119-130.

Depienne C, Le Guern E (2012) PCDH19-related infantile epileptic encephalopathy: an unusual x-linked inheritance disorder. Hum Mutat 33:627-634.

Gerosa L, Francolini M, Bassani S, Passafaro M (2019) The role of protocadherin 19 (PCDH19) in neurodevelopment and in the pathophysiology of early infantile epileptic encephalopathy-9 (EIEE9). Dev Neurobiol 79:75-84.

Kolic KL, Sadleir LG, Scheffer IE, Ivanovic A, Roberts R, Pham DH, Gecz J (2018) A systematic review and meta-analysis of 271 PCDH19-variant individuals identifies psychiatric comorbidities, and association of seizure onset and disease severity. Mol Psychiatry 24:241-251.

Niazi R, Fanning EA, Depienne C, Sarmady M, Abou, Tayoun AN (2019) A mutation update for the PCDH19 gene causing early-onset epilepsy in females with an unusual expression pattern. Hum Mutat 40:243-257.

Samanta D (2020) PCDH19-related epilepsy syndrome: a comprehensive clinical review. Pediatr Neurol 105:3-9.

Serratto GM, Pizzi E, Murru L, Mazzoleni S, Pelucchi S, Marcello E, Mazzanti M, Passafaro M, Bassani S (2020) The epilepsy-related protein PCDH19 regulates tonic inhibition, GABAAR kinetics, and the intrinsic excitability of hippocampal neuron. Mol Neurobiol 57:5336-5351.

Suzuki S, Kawakami K, Nakamura F, Nishimura S, Yagi K, Seino M (1994) Bromide, in the therapeutic concentration, enhances GABA-activated currents in cultured neurons of rat cerebral cortex. Epilepsy Res 19:89-97.

Tan C, Sharif C, Ranieri E, Hynes K, Pham DH, Leach D, Buchanan G, Corbett M, Shoubridge C, Kumar R, Douglas E, Nguyen LS, Mcmahon J, Sadleir L, Specchio N, Marini C, Guerrini R, Moller RS, Depienne C, Haan E, et al. (2015) Mutations of protocadherin 19 in female epilepsy (PCDH19-EE) lead to aloopregnanolone deficiency. Hum Mol Genet 24:5250-5259.

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