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ABSTRACT

PCDH19 encodes protocadherin 19 on chromosome Xq22.3. This 1148 amino-acid protein, highly expressed during brain development, could play significant roles in neuronal migration or establishment of synaptic connections. *PCDH19* is composed of 6 exons, with a large first exon encoding the entire extracellular domain of the protein. Heterozygous *PCDH19* mutations were initially identified in epilepsy and mental retardation limited to females, a familial disorder with a singular mode of inheritance since only heterozygous females are affected whereas hemizygous males are asymptomatic. Yet, mosaic males can also be affected, supporting cellular interference as the pathogenic mechanism. Recently, mutations in *PCDH19*, mostly occurring *de novo,* were shown to be a frequent cause of sporadic infantile-onset epileptic encephalopathy in females. *PCDH19* mutations were also identified in epileptic females without cognitive impairment. Typical features of this new epileptic syndrome include generalized or focal seizures highly sensitive to fever, and brief seizures occurring in clusters, repeating during several days. Here we present a review of the published mutations in the *PCDH19* gene to date and report on new mutations. *PCDH19* has become the second most relevant gene in epilepsy after *SCN1A.*

KEY WORDS: PCDH19, Epilepsy, X-linked, females, male sparing, cellular interference

INTRODUCTION

Mutations in *PCDH19* (MIM# 300460) were originally identified in 2008 in rare large families in which female patients had variable degrees of epilepsy and intellectual deficits. This disorder, first reported as epilepsy and mental retardation limited to females (or epilepsy, female-restricted, with mental retardation, EFMR) and recently renamed Epileptic encephalopathy, early infantile, 9 (MIM# 300088), was transmitted via asymptomatic males, suggesting an unusual X-linked inheritance with selective involvement of females [Juberg and Hellman, 1971; Fabisiak and Erickson, 1990; Ryan et al., 1997; Dibbens et al., 2008].

Recently, *PCDH19*-related infantile epileptic encephalopathy turned out to be more frequent than first anticipated on the observation of families, *PCDH19* becoming the second most clinically relevant gene in epilepsy after *SCN1A* (MIM# 182389). Most patients with *PCDH19* mutations are sporadic cases or belong to families with few affected female patients, making the recognition of the inheritance pattern difficult. In addition, associated clinical features, including age at onset, seizure types and severity, and the degree of cognitive disability, are highly variable. However, clinical characteristics such as clustering of repeated seizures within short periods of time are now emerging as hallmark features that orient etiological diagnosis towards *PCDH19*. This mutation update summarizes the mutations identified in the gene and the recent findings in the field.

Background

Protocadherins (pcdhs) are transmembrane proteins primarily involved in calcium-dependent adhesion that constitute the largest subgroup of the cadherin superfamily. Mammalian genomes contain more than 70 *Pcdh* genes that are divided into two groups based on their genomic structure: clustered (~58 genes, *Pcdh*α, β, γ) and non-clustered *Pcdh* genes (~13 genes, *Pcdh*δ and other *Pcdhs*). Pcdhs typically have six or more extracellular calcium-binding cadherin repeats or ectodomains (EC domains), which are required for cell-cell homophilic or heterophilic interactions, and a divergent cytoplasmic domain [Yagi and Takeichi, 2000; Patel et al., 2003; Redies et al., 2005; Patel et al., 2006; Morishita and Yagi, 2007]. Protocadherins, like many other cadherins, are predominantly expressed in the brain, where they play significant roles in neurodevelopment, such as neuronal migration and synaptic plasticity [Frank and Kemler, 2002; Junghans et al., 2005; Redies et al., 2005; Morishita and Yagi, 2007]. The combinatorial expression of multiple cadherin and protocadherin genes could contribute to the molecular specification of the vast complexity of neurons in the cerebral cortex [Krishna et al., 2011].

PCDH19 (MIM# 300460), located on chromosome Xq22.3, encodes the 1148 amino acid protocadherin 19, and belongs to the δ2-subclass of non-clustered protocadherins that also includes *PCDH8*, *PCDH10*, *PCDH17* and *PCDH18* [Wolverton and Lalande, 2001; Redies et al., 2005]. The *PCDH19* gene has six coding exons, the first exon being unusually large and encoding the entire extracellular domain, composed of six EC domains (Fig. 1, based on the NM_001184880.1 reference transcript corresponding to the longest isoform). *PCDH19* expression is spatially and temporally regulated in the central nervous system during mammalian

development and shows a unique expression profile among protocadherins. In particular, protocadherin 19 is highly expressed in the developing brain, including the subventricular zone, the intermediate zone, the subplate, specific layers (layers II, IV, V and VI) of the cerebral cortex, the hippocampus and the subiculum [Vanhalst et al., 2005; Gaitan and Bouchard, 2006; Kim et al., 2007; Krishna et al., 2009; Kim et al., 2010; Hertel and Redies, 2011; Krishna et al., 2011]. Two other isoforms (NM_020766.1 and NM_001105243.1) resulting from the alternative splicing of exon 2 and the existence of two possible acceptor sites for intron 4 (adding a residue at the beginning of exon 5) exist in databases but their existence, distribution and respective roles need to be confirmed and studied at the molecular level.

The precise function of protocadherin 19 remains currently unknown. Yet, other δ2-protocadherins were reported to mediate calcium-dependent cell-cell adhesion *in vitro* and cell sorting *in vivo* [Redies et al., 2005] and could regulate the establishment of neuronal connections during brain development and/or remodeling of selective synaptic connections during the early postnatal stage [Kim et al., 2007]. However, in contrast to cadherins, which associate through strong homophilic interactions, the extracellular domain of *PCDH19* exhibits specific but weak homophilic adhesive properties, suggesting that the involvement of *PCDH19* in cell adhesion could involve heterophilic interactions [Biswas et al., 2010; Tai et al., 2010].

Variants

Pathogenic Mutations

More than 60 different mutations in *PCDH19* have been identified (Table 1A and Fig. 1). All types of DNA alterations are observed, including nonsense mutations, small nucleotide deletions and insertions, mutations altering the splice sites, missense mutations, and intragenic or whole

gene deletions. Half of the pathologic allelic variants result in the appearance of premature termination codons, either due to nonsense mutations (18.3%; 17/93 patients, based on review of the literature and personal data), small deletions or insertions leading to a frameshift (25.8%, 24/93) or splice site mutations (3.2%, 3/93). These mutation types are scattered along the *PCDH19* gene with the exception of exon 2, in which no variants or mutations have been reported so far. Whole gene or intragenic deletions have been reported in only 6 patients (6.5%, 6/93) [Depienne et al., 2009; Depienne et al., 2011; Vincent et al., 2011] but this mutation class has likely been underestimated since most studies used only direct sequencing, which misses heterozygous deletions, to screen *PCDH19* (Table 1C). Missense substitutions, which represent 46.2% (43/93) of the mutations are, contrary to other mutation types, all clustered in exon 1, where they affect highly conserved amino acids in the large extracellular domain of protocadherin 19. Several recurrent mutations have been reported in patients, the most frequent being the Asn340Ser missense mutation (Table 1A).

Mutations have been reported in the Leiden Open Variation Database (X chromosome gene database, PCDH19: http://www.lovd.nl/PCDH19).

Polymorphisms and variants of unknown significance

The *PCDH19* gene contains few known polymorphisms (Table 2). The most frequent natural variations reported correspond to synonymous substitutions located in exon 1. Rarer, nonpathogenic missense polymorphisms, all clustered in the intracellular domain of protocadherin 19 (exon 6), were occasionally found in patients and in ethnically matched control populations, suggesting that this domain, contrary to the extracellular domain, can tolerate some missense changes. One missense variant located in exon 3 has been identified in a patient with

mental retardation but the pathogenic effect of this variant remains uncertain (Table 1B) [Tarpey et al., 2009].

Biological Relevance

Mutations in *PCDH19*: a Loss of Function at the Cellular Level

The mutation spectrum in *PCDH19* is compatible with a loss of function of the mutated allele. Messenger RNAs with mutations introducing premature termination codons (PTC) have indeed been shown to be degraded via the nonsense-mediated mRNA decay (NMD) surveillance system of the cell in patients' fibroblasts [Dibbens et al., 2008]. The identification of whole gene deletions in three patients [Depienne et al., 2009; Depienne et al., 2011] also supports the notion that loss of function is the main consequence of the mutations. Missense mutations located in the extracellular domain could alter the adhesive properties of protocadherin 19 and also lead to a loss of function. In particular, the Asn340Ser and Glu414Gln mutations could specifically alter amino acids involved in calcium binding [Patel et al., 2006; Marini et al., 2010]. However, these hypotheses have yet to be formally tested.

Cellular Interference: a Gain of Function at the Tissue Level

Males with hemizygous *PCDH19* mutations show normal cognitive function and do not have seizures, although a subtle psychiatric phenotype was evoked in some asymptomatic carriers [Dibbens et al., 2008]. The absence of major symptoms in hemizygous males indicates that the constitutive loss of function of protocadherin 19 (i.e., the absence of functional protein in all the cells of an individual's body) is not pathogenic. Hence, although protocadherin 19 could be essential for early brain morphogenesis in other species [Emond et al., 2009], it is a non essential

protein in humans, its absence likely being compensated for or buffered by other proteins and pathways.

In contrast, females with heterozygous *PCDH19* mutations present with early intractable seizures and a variable degree of mental retardation. *PCDH19* is located in a region submitted to X-inactivation in females [Dibbens et al., 2008]. Random X-inactivation in mutated females is expected to lead to tissue mosaicism; i.e., co-existence of cells that have inactivated the mutated *PCDH19* allele and express the normal protein, and *PCDH19*-negative cells that have inactivated the normal allele [Depienne et al., 2009]. This mosaicism could account for the pathogenesis by altering cell-cell interactions [Ryan et al., 1997; Dibbens et al., 2008; Depienne et al., 2009]. The loss of function of protocadherin 19 at the level of the cell would thus result in a gain of function at the tissue level because of abnormal interactions between "mutated" and "normal" cells. A mechanism of this type was termed "cellular interference" [Wieland et al., 2004] in reference to the "metabolic interference" concept developed many years ago by William Johnson [Johnson, 1980]. According to this theory, mosaic males would be affected like mutated females. The identification of an affected male with a mosaic *PCDH19* deletion in his fibroblasts, strongly supports cellular interference as the pathogenic mechanism associated with *PCDH19* mutations [Depienne et al., 2009]. To definitely establish that cellular interference is the pathogenic mechanism, it would however be necessary to demonstrate that females homozygous for *PCDH19* mutations are also unaffected like hemizygous males. In the absence of human cases, the development of a *PCDH19*-deficient mouse model will be crucial to confirm this pathogenic mechanism, assuming that the pathogenic mechanisms are identical in both species.

Interestingly, another human disorder, craniofrontonasal syndrome (CFNS; MIM# 304110), caused by mutations in *EFNB1*, the gene encoding Ephrin B1, a ligand for Eph receptors (EphR)

on chromosome Xq12, has the same unusual X-linked pattern of inheritance [Wieland et al., 2004]. Ephrin B1/EphR signaling plays a role in cell migration and pattern formation during developmental morphogenesis [Klein, 2004], reminiscent of the possible function of protocadherin 19 in brain development. Cellular interference has been confirmed as the pathogenic mechanism in CFNS. Female mice heterozygous for mutations in Ephrin B1 have a mosaic expression of Ephrin B1 resulting in ectopic interactions between the Ephrin B1 ligand and EphB receptors that are sufficient to induce the skeletal defects [Compagni et al., 2003].

Clinical Relevance

Clinical Features of Patients with *PCDH19* Mutations

Female patients with heterozygous *PCDH19* mutations have epileptic phenotypes ranging from mild to severe in terms of seizure type and severity. Seizures usually begin in infancy or early childhood (mean age at onset: 12.9 months, median: 10 months; range: 4-60 months; n = 86 patients from the literature and unpublished personal data) and are highly sensitive to fever. Febrile seizures (FS) are the initial manifestation in approximately half of the cases and seizures are triggered or worsened by fever in ~90% of the patients [Scheffer et al., 2008; Depienne et al., 2009; Marini et al., 2010; Depienne et al., 2011]. Seizure types mostly consist in generalized tonic, clonic or tonic-clonic and/or focal seizures with or without secondary generalization. Atypical absences, atonic seizures and myoclonic jerks may also be part of the clinical picture, although they are more rarely observed [Scheffer et al., 2008; Depienne et al., 2009; Marini et al., 2010; Depienne et al., 2011; Specchio et al., 2011]. *Status epilepticus*, which can be inaugural, and prolonged seizures are frequently reported in *PCDH19*-positive patients but the most characteristic feature is the presence of brief seizure clusters lasting 1-5 minutes and

repeating up to or more than 10 times a day during several days [Depienne et al., 2009; Marini et al., 2010; Depienne et al., 2011; Specchio et al., 2011]. Seizures are resistant to treatment in most cases, especially during infancy and childhood, but their frequency and intractability tend to decrease naturally over time, some patients being sometimes free of seizures during adolescence or adulthood on monotherapy [Scheffer et al., 2008; Depienne et al., 2011; Specchio et al., 2011].

We and others have shown that the clinical spectrum associated with *PCDH19* mutations can overlap that of Dravet syndrome (DS, previously named severe myoclonic epilepsy of infancy or SMEI), a stereotyped epileptic encephalopathy also associating FS and epilepsy but due in 75% of cases to a *de novo* mutation in the *SCN1A* gene [Depienne et al., 2009; Marini et al., 2010; Depienne et al., 2011; Higurashi et al., 2011; Nabbout et al., 2011]. However, DS-like patients with *PCDH19* mutations slightly differ on average from *SCN1A*-positive classical DS patients: age at onset is slightly older (12.5 months [range: 4-60] versus 6.3 months [range: 0.5-14]), status epilepticus and occurrence of myoclonic jerks are less frequent, and long-term outcome is better in *PCDH19*-positive patients than in *SCN1A*-positive patients, most patients with *PCDH19* mutations fitting the definition of bordeline SMEI (SMEB) [Fukuma et al., 2004]. Furthermore, photosensitivity, frequently reported in classical DS, is exceptional in *PCDH19*-positive patients [Depienne et al., 2009; Marini et al., 2010].

Behavioral disturbances are frequent in patients with heterozygous *PCDH19* mutations and essentially manifest as autistic, obsessive or aggressive features [Scheffer et al., 2008; Depienne et al., 2009; Depienne et al., 2011; Marini et al., 2011]. In some patients, social withdrawal or personality disorders are even the most prominent and disabling feature when the patient becomes older [Depienne et al., 2011].

Intellectual outcome ranges from normal intellect (27.7%, 23/83), to mild (36.1%, 30/83), moderate (21.7%, 18/83), or severe (14.5%, 12/83) cognitive impairment [Scheffer et al., 2008; Depienne et al., 2009; Depienne et al., 2011; Marini et al., 2011; Specchio et al., 2011]. Interestingly, the cognitive prognosis does not appear to be clearly related to the severity of epilepsy [Depienne et al., 2011; Specchio et al., 2011]. Language delay is frequently associated with cognitive deficit.

Finally, neurological features such as ataxia can also be observed in some patients and are reminiscent of those observed in DS patients.

Sporadic Versus Familial Cases

Although *PCDH19* was first identified in large families where the mutations were inherited over several generations, *PCDH19*-related epileptic encephalopathy is more commonly sporadic or encountered in families with few affected females, making the recognition of the unusual pattern of inheritance difficult. *De novo* mutations account for most isolated cases (72%, 32/44) and represent 56% (32/57) of all mutations reported so far, in which transmission could be investigated [Depienne et al., 2009; Marini et al., 2010; Depienne et al., 2011; Specchio et al., 2011]. The remaining mutations found in sporadic cases were inherited by asymptomatic fathers (18%, 8/44), asymptomatic mothers (7%, 3/44) or by a mother who has had only FS (2%). In addition, parental mosaicism leading to the recurrence of the disease was demonstrated in two mothers (one being asymptomatic and one being affected) of unrelated families [Dibbens et al., 2011], a result reminiscent of mosaicism in DS caused by *SCN1A* mutations [Depienne et al., 2010].

Genotype-Phenotype Correlations

Since the clinical features (age at onset, severity of the epilepsy and cognitive outcome) are highly variable for a given mutation even within the same family, genotype-phenotype correlation studies will likely be uninformative. Strikingly, Higurashi and collaborators reported monozygotic twin sisters in which the p.Asn340Ser mutation was associated with different clinical pictures, confirming the existence of non-genetic modifiers [Higurashi et al., 2011]. An expected source of phenotypic variability is the status of X inactivation in females. Interestingly, skewing of X chromosome inactivation can occur in normal females and increases in tissues with age [Chagnon et al., 2005; Bolduc et al., 2008]. A totally skewed X inactivation situation would theoretically reproduce a non-pathogenic situation. Partially skewed X inactivation would represent intermediate situations in which cellular interference could be limited compared to balanced X inactivation, where it would be expected to be the highest. In this setting, the severity of the epilepsy and/or the intellectual disability could be correlated with the relative amount of inactivated neurons for each chromosome, and the female mutation carriers with a totally skewed pattern of inactivation in the brain would be asymptomatic. However, so far no correlations between the X-inactivation status in blood cells and the phenotypic expression have been found in support of this hypothesis [Marini et al., 2010]. Nonetheless, the X-inactivation status in lymphocytes does not reflect that of the neural tissues and studies investigating directly cerebral tissues, in mouse models for example, are important to further investigate this hypothesis.

Diagnostic Relevance

The identification of mutations of the *PCDH19* gene provides a definite diagnosis in female patients with infantile epilepsy. This result also makes it possible to calculate the risk of

recurrence of the disorder in the family, which is markedly different if the pathogenic mutation is *de novo* or if it has been inherited from a parent. Although diagnosis is made clinically, differentiating one epileptic condition from another is sometimes difficult, especially when the first symptoms appear. The molecular confirmation of the genetic defect underlying the epilepsy and an analysis of the parents' status are crucial to be able to give families appropriate genetic counseling.

Molecular testing of *PCDH19* should be considered in females with early-onset FS and/or epilepsy with or without cognitive impairment and family history. Some clinical features could help to prioritize the analysis of *PCDH19*, such as female patients presenting with seizure clusters, the presence of multiple affected females in the family with obligate male carriers, and, more generally, the presence of generalized and/or focal seizures beginning in infancy or early childhood, resistant to treatment and sensitive to fever. With regard to Dravet syndrome, screening for *PCDH19* mutations should be performed for female patients when analysis of *SCN1A* is negative. Molecular testing of *PCDH19* should include sequencing of the coding sequence of the gene as well as a method (quantitative PCR, multiplex ligation-dependent probe amplification or equivalent) able to identify heterozygous deletions. The percentage of *PCDH19*-positive cases in females with FS and epilepsy has been shown to range from 5 to 37% depending on the clinical criteria [Depienne et al., 2009; Marini et al., 2010; Depienne et al., 2011]. Molecular testing of *PCDH19* in sporadic affected males can be considered with the same indication, although the somatic mosaicism expected in this case can easily be missed if the analysis is performed from genomic DNA extracted from blood cells, which considerably complicates the interpretation and decreases the reliability and significance of the result.

Given the unusual mode of inheritance and the wide phenotypic variability associated with *PCDH19* mutations, genetic counseling appears delicate. In the case of mutations inherited from an asymptomatic father, all the daughters are expected to be affected. Considering the frequent poor outcome (mild to severe cognitive impairment in about 70% of *PCDH19*-positive females) of *PCDH19*-related epileptic encephalopathy, it is feasible to offer a prenatal diagnosis that could simply be based on fetal sex determination from maternal blood [Wright and Burton, 2009]. Female patients with *PCDH19* mutations have a 50% risk of transmitting the mutation but, as only the females would be affected, the overall risk would be 25%. In cases with *de novo* mutations, the risk of recurrence is expected to be low but the possibility of germinal mosaicism in one parent is still possible [Dibbens et al., 2011].

Future Prospects

Although the frequency and clinical features of *PCDH19*-related epileptic encephalopathies have become more precisely determined during the past 3 years, several challenges remain to fully understand the functional consequences of the mutations and the mechanisms by which they contribute to epileptogenesis and cognitive impairment.

Several steps were recently made towards elucidating the function of protocadherin 19 in zebrafish or chicken models. The zebrafish ortholog of protocadherin 19 (*pcdh19*) was shown to be crucial for early steps of brain morphogenesis. Partial depletion of the protein with morpholino oligonucleotides impairs the convergence cell movements of the anterior neural plate, where *pcdh19* is specifically expressed, a phenotype reminiscent of the n-cadherin (*ncad*) mutants [Emond et al., 2009]. Interestingly, the Pcdh19 and Ncad proteins directly interact *in vitro* and *in vivo* in this model [Biswas et al., 2010]. Together with the observation that Pcdh19 exhibits weak homophilic adhesive properties [Biswas et al., 2010; Tai et al., 2010], these results suggest that protocadherin 19 could preferentially interact with other members of the cadherin superfamily to regulate cell adhesion, neuronal migration or synapse formation. The development of mouse models is an important step to confirm these hypotheses as well as the cellular interference theory, and to investigate the genetic or epigenetic factors underlying the phenotypic variability observed in the human disorder.

So far, although mammalian genomes contain over 70 protocadherin genes, only two, *PCDH19* and *PCDH15* (causing autosomal recessive Usher syndrome) have been related to a human Mendelian disorder. Recent studies have suggested that defects in the expression or function of some other protocadherins may be related to neurodevelopmental disorders such as autism, schizophrenia and mental retardation [Bray et al., 2002; Dean et al., 2007; Morrow et al., 2008].

These findings suggest that protocadherins are likely to play many roles that have yet to be discovered in human disorders.

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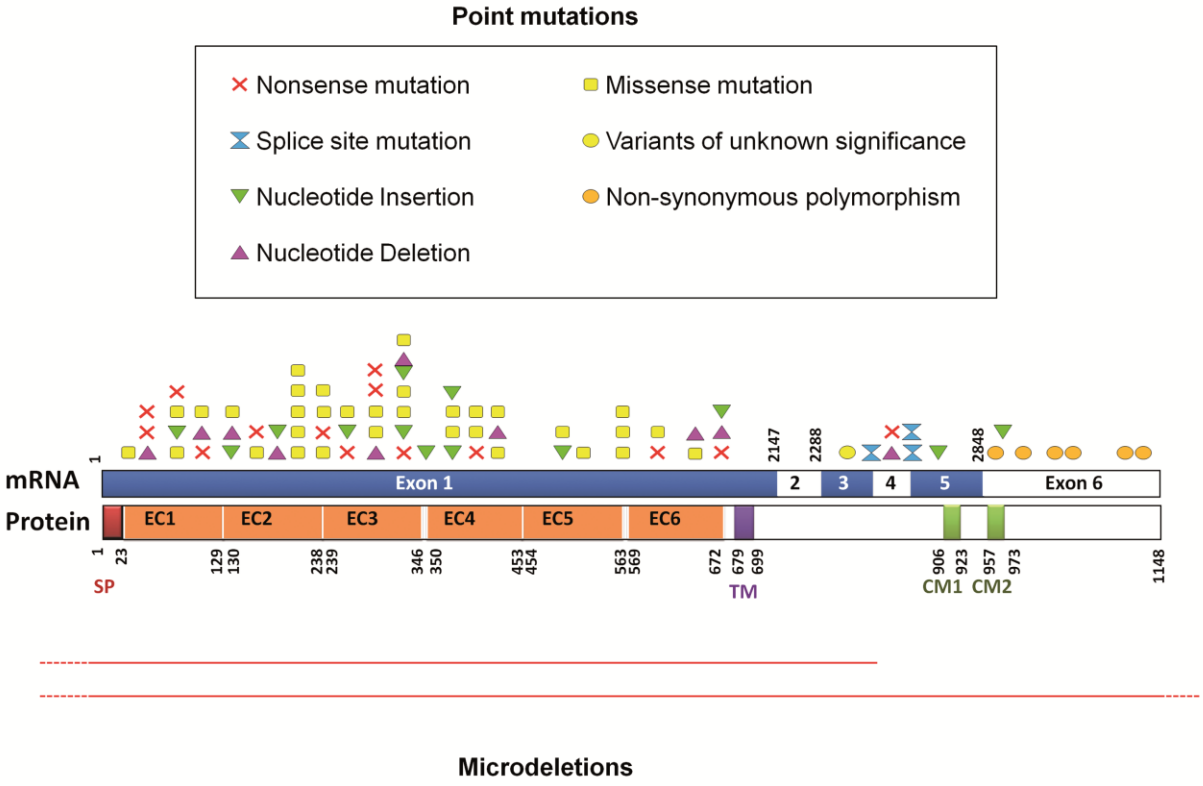

Figure 1. Schematic representation of the mutations, rearrangements and polymorphisms identified in the PCDH19 gene on the mRNA and protein. Point mutations (above): red crosses, nonsense mutations; yellow squares, missense mutations; hourglass, splice site mutations; green/pink triangles, small insertions/deletions leading to a frameshift; orange circles, non-synonymous polymorphisms; yellow circles, variants of unknown significance. Microrearrangements (below): red lines indicate deletions. Dashed lines indicate that the deletion continues farther than the gene.

TABLE 1A. Pathogenic mutations identified in the *PCDH19* Gene

Exon / intron	DNA variant	Predicted effect on the protein	Type	Recurrent mutation	Transmission	References
Exon 1	c.74T>C	p.Leu25Pro	Missense		Mosaic mother	Dibbens et al. 2011
Exon 1	c.78delG	p.Lys26AsnfsX4	Frameshift		De Novo	Jamal et al., 2010
Exon 1	[c.83C>A; c.90A>G]	p.Ser28X	Nonsense		De Novo	Marini et al., 2010
Exon 1	c.142G>T	p.Glu48X	Nonsense		Paternal inheritance	Depienne et al., 2009
Exon 1	c.215T>G	p.Val72Gly	Missense		Unknown	Higurashi et al., 2011
Exon 1	c.241dupC	p.Leu81ProfsX8	Frameshift		De Novo	This study
Exon 1	c.242T>G	p.Leu81Arg	Missense		Unknown	Depienne et al., 2011
Exon 1	c.253C>T	p.Gln85X	Nonsense	yes	Familial condition	Dibbens et al., 2008, this study
Exon 1	c.352G>T	p.Glu118X	Nonsense		De Novo	Depienne et al., 2009; this study
Exon 1	c.357delC	p.Lys120ArgfsX3	Frameshift		Familial condition	Dibbens et al., 2008
Exon 1	c.361G>A	p.Asp121Asn	Missense		Paternal inheritance	Depienne et al., 2009
Exon 1	c.415_423dup	p.Ser139_Ala141dup	In-frame duplication		Maternal inheritance	Depienne et al., 2011
Exon 1	c.424delG	p.Ala142ProfsX70	Frameshift		Unknown	Depienne et al., 2011

Exon 1	c.437C>G	p.Thr146Arg	Missense		Paternal inheritance	Depienne et al., 2011
Exon 1	c.457G>A	p.Ala153Thr	Missense		Paternal inheritance	This study
Exon 1	c.462C>A	p.Tyr154X	Nonsense		Unknown	Depienne et al., 2011
Exon 1	c.506delC	p.Thr169SerfsX43	Frameshift		De Novo	Depienne et al., 2009
Exon 1	c.514dupG	p.Glu172GlyfsX54	Frameshift		Unknown (not in the mother)	Depienne et al., 2011
Exon 1	c.569T>G	p.Leu190Arg	Missense		Unknown	This study
Exon 1	c.571G>C	p.Val191Leu	Missense		Unknown	Higurashi et al., 2011
Exon 1	c.595G>C	p.Glu199Gln	Missense		Unknown (not in the mother)	Depienne et al., 2009
Exon 1	[c.608A>C; c.617T>G]	[p.His203Pro; Phe206Cys]	Missense		De Novo	Marini et al., 2010
Exon 1	c.617T>A	p.Phe206Tyr	Missense		Maternal inheritance (mother asymptomatic)	Depienne et al., 2011
Exon 1	c.695A>G	p.Asn232Ser	Missense		Unknown	This study
Exon 1	c.697_700delinsTAAC	p.Asp233X	Nonsense		Unknown (not in the mother)	Depienne et al., 2011
Exon 1	c.701A>G	p.Asn234Ser	Missense		De Novo	This study
Exon 1	c.706C>T	p.Pro236Ser	Missense		De Novo	Specchio et al., 2011
Exon 1	c.729C>A	p.Tyr243X	Nonsense		De Novo	Jamal et al., 2010

Exon 1	c.730dupG	p.Ala244GlyfsX76	Frameshift		De Novo	This study
Exon 1	c.747A>T	p.Glu249Asp	Missense		Maternal inheritance (mother with FS)	Depienne et al., 2011
Exon 1	c.772_773delAT	p.Ile258ProfsX61	Frameshift		Unknown	Higurashi et al., 2011
Exon 1	c.785C>A	p.Ala262Asp	Missense		Unknown	This study
Exon 1	c.826T>C	p.Ser276Pro	Missense		De Novo	Hynes et al., 2010
Exon 1	c.840C>G	p.Tyr280X	Nonsense		De Novo	Higurashi et al., 2011
Exon 1	c.859G>T	p.Glu287X	Nonsense	yes	De novo (n=1); unknown (n=1)	Depienne et al., 2009
Exon 1	c.949C>T	p.Gln317X	Nonsense		Familial condition	Higurashi et al., 2011
Exon 1	c.958dupG	p.Asp320GlyfsX22	Frameshift		De Novo	Specchio et al., 2011
Exon 1	c.1019A>G	p.Asn340Ser	Missense	yes	De Novo (n=7); inherited from the mother (n=1), mosaic mother (n=1); unknown (n=1)	Depienne et al., 2009; Marini et al., 2010; Specchio et al., 2011;Dibbens et al. 2011; Higurashi et al., 2011 ; this study
Exon 1	c.1023C>G	p.Asp341Glu	Missense		De Novo	Depienne et al., 2011
Exon 1	c.1026_1027delinsAA	p.Asn342_Pro343delinsLysThr	Frameshift		Unknown	This study
Exon 1	c.1031C>G	p.Pro344Arg	Missense		Unknown	This study
Exon 1	c.1036_1040dup	p.Asn347LysfsX23	Frameshift		Familial condition	Depienne et al., 2009
Exon 1	c.1091dupC	p.Tyr366LeufsX10	Frameshift	yes	De Novo (n=1); familial condition (n=1) ; Paternal	Dibbens et al., 2008; Higurashi et al., 2011; this study

					inheritance (n=1)	
Exon 1	c.1129G>C	p.Asp377His	Missense		De Novo	Marini et al., 2010
Exon 1	c.1131C>A	p.Asp377Glu	Missense		De Novo	This study
Exon 1	c.1143dupT	p.Gly382TrpfsX19	Frameshift		Unknown	This study
Exon 1	c.1192G>T	p.Glu398X	Nonsense		Paternal inheritance	Marini et al., 2010
Exon 1	c.1211C>T	p.Thr404Ile	Missense		De Novo	Marini et al., 2010
Exon 1	c.1240G>C	p.Glu414Gln	Missense		Paternal inheritance	Marini et al., 2010
Exon 1	c.1298T>C	p.Leu433Pro	Missense		De Novo	Specchio et al., 2011
Exon 1	c.1300_1301delCA	p.Gln434GlufsX11	Frameshift		De Novo	Specchio et al., 2011
Exon 1	c.1322T>A	p.Val441Glu	Missense		Familial condition	Dibbens et al., 2008
Exon 1	c.1521dupC	p.Ile508HisfsX15	Frameshift		Unknown	Marini et al., 2010
Exon 1	c.1537G>C	p.Gly513Arg	Missense		De Novo	Specchio et al., 2011
Exon1	c.1628T>C	p.Leu543Pro	Missense		Paternal inheritance	Depienne et al., 2009
Exon 1	c.1671C>G	P.Asn557Lys	Missense		Familial condition	Dibbens et al., 2008; Hynes et al., 2010
Exon 1	c.1682C>G	p.Pro561Arg	Missense	yes	Paternal inheritance (n=1), unknown (n=1)	Depienne et al., 2011
Exon 1	c.1700C>T	p.Pro567Leu	Missense		Maternal inheritance (mother asymptomatic)	Depienne et al., 2011

Exon 1	c.1804C>T	p.Arg602X	Nonsense		De Novo	This study
Exon 1	c.1852G>A	p.Asp618Asn	Missense		Maternal inheritance (mother asymptomatic)	Depienne et al., 2011
Exon 1	c.1924G>A	p.Val642Met	Missense		Unknown	This study
Exon 1	c.1956_1959delCTCT	p.Ser653ProfsX6	Frameshift		Unknown	This study
Exon 1	c.2012C>G	p.Ser671X	Nonsense		Familial condition	Dibbens et al., 2008
Exon 1	c.2019delC	p.Ser674LeufsX2	Frameshift		De Novo	Depienne et al., 2011
Exon 1	c.2030dupT	p.Leu677PhefsX41	Frameshift		Familial condition	Dibbens et al., 2008
Intron 3	c.2617–1G>A	p.?	Misplicing (abolition of exon 4 acceptor site)		De Novo	Marini et al., 2010
Exon 4	c.2631_2634delTTTT	p.Phe878ThrfsX5	Frameshift		De Novo	Jamal et al., 2010
Exon 4	c.2656 C>T	p.Arg886X	Nonsense	yes	Familial condition (n=1);unknown (n=1)	Depienne et al., 2011; this study
Intron 4	c.2675+1G>C	p.?	Misplicing		Unknown (not in the mother)	This study
Intron 4	c.2676–6A>G	p.?	Misplicing (creation of a new acceptor site)		De Novo	Marini et al., 2010
Exon 6	c.2697dupA	p.Glu900ArgfsX8	Frameshift		De Novo	Marini et al., 2010
Exon 6	c.2903dupA	p.Asp968GlufsX18	Frameshift		De Novo	Marini et al., 2010

Mutation nomenclature is based on the PCDH19 cDNA reference sequence (NM_001184880.1). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence.

TABLE 1B. Variant in *PCDH19* of unknown significance (possibly pathogenic)

Exon / intron	DNA variant	Predicted effect on the protein	Type	Phenotype of the patient (gender)	Transmission	References
Exon 3	c.2454C>G	p.His817Gln	Missense	Mental retardation (unknown)	Unknown	Tarpey et al., 2009

Mutation nomenclature is based on the PCDH19 cDNA reference sequence (NM_001184880.1). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence.

TABLE 1C. Pathogenic rearrangements identified in the *PCDH19* Gene

Exon / intron	DNA variant	Predicted effect on the protein	Type	Recurrent mutation	Transmission	References
Exons 1-6	c.1-?_3447+?del	Absence of protein synthesis	Whole gene deletion	recurrent gene del with different BP	De Novo (n=3); unknown (n=2)	Depienne et al., 2009; Depienne et al., 2011 ; Vincent et al., 2011
Exons 1-3	c.1-?_2616+?del	Absence of protein synthesis	Deletion of exons 1, 2 and 3		De Novo	Depienne et al., 2011

BP: breakpoints

TABLE 2. Frequent and rare polymorphisms of the *PCDH19* Gene

Ex/intr	Nucleotide change	Protein consequence	rs number	Frequency in European population (other populations)	Ref
Exon1	c.6G>A	p.Glu2Glu		<2%	Hynes et al., 2010 ; ; Higurashi et al., 2011
Exon1	c.402C>A	p.Ile134Ile	rs41300169	7%	Hynes et al., 2010; Depienne et al., 2011 ; Higurashi et al., 2011
Exon1	c.531G>A	p.Glu177Glu		<1% (Japan population)	Higurashi et al., 2011
Exon1	c.655C>T	p.Leu219Leu		<1%	Hynes et al., 2010
Exon1	c.1137C>T	p.Gly379Gly	rs56277715	4%	Hynes et al., 2010; Depienne et al., 2011
Exon1	c.1627C>T	p.Leu543Leu		20%	Tarpey et al., 2009; Hynes et al., 2010; Depienne et al., 2011
Exon1	c.1683G>A	p.Pro561Pro		<1%	Tarpey et al., 2009; Hynes et al., 2010
Intron 3	c.2617-27C>A				This study
Intron 5	c.2849-28A>C				This study
Exon6	c.2873G>A	p.Arg958Gln		<1%	Tarpey et al., 2009; Hynes et al., 2010
Exon6	c.2938C>T	p.Arg980Cys	rs3764758	<1% (Asian population)	Hapmap data (phase II) ; Higurashi et al., 2011
Exon6	c.2994T>C	p.Thr998Thr		<1% (Japan population)	Higurashi et al., 2011

Exon6	c.3018C>T	p.Asp1006Asp	rs16983426	<1% (17%African population)	Tarpey et al., 2009; dbSNP
Exon6	c.3280C>G	p.Leu1094Val		<1%	This study ; Higurashi et al., 2011
Exon 6	c.3319C>G	p.Arg1107Gly		1- (2-3% in Japan population)	Depienne et al., 2009
Exon 6	c.3400A>C	p.Asn1134His		<1% (>10% in Japan population)	This study; Higurashi et al., 2011
3'UTR	c.3447+8T>C			<1%	This study

Mutation nomenclature is based on the PCDH19 cDNA reference sequence (NM_001184880.1). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence.