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Role of *PRRT2* in common paroxysmal neurological disorders: a gene with remarkable pleiotropy

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ABSTRACT

Mutations in the gene *PRRT2* encoding proline-rich transmembrane protein 2 have recently been identified as the cause of three clinical entities: benign familial infantile epilepsy (BFIE), infantile convulsions with choreoathetosis (ICCA) syndrome, and paroxysmal kinesigenic dyskinesia (PKD). Patients with ICCA have both BFIE and PKD and families with ICCA may contain individuals who exhibit all three phenotypes. These three phenotypes were all mapped by linkage analyses to the pericentromeric region of chromosome 16, and were hypothesised to have the same genetic basis due to the co-occurrence of the disorders in some families. Despite considerable effort, the gene or genes for BFIE, ICCA, and PKD were not identified for many years after the linkage region was identified. Mutations in the gene *PRRT2* were identified in several Chinese families with PKD, suggesting that the gene may also be responsible for ICCA and BFIE in families linked to the chromosome 16 locus. This was demonstrated to be the case, with the majority of families with ICCA and BFIE found to have *PRRT2* mutations. The vast majority of these mutations are truncating and are predicted to lead to haploinsufficiency. *PRRT2* is a largely uncharacterised protein. It is expressed in the brain and has been demonstrated to interact with SNAP-25, a component of the molecular machinery involved in the release of neurotransmitters at the presynaptic membrane. Therefore, the *PRRT2* protein may play a role in this process. However, the molecular mechanisms underlying the remarkable pleiotropy associated with *PRRT2* mutations have still to be determined.

INTRODUCTION

Benign familial infantile epilepsy (BFIE), previously termed benign familial infantile seizures or benign familial infantile convulsions, is a self-limiting seizure disorder of infancy. Patients have non-febrile seizures with onset at between four and 12 months of age and offset by 2 years of age. Subsequent neurological development is usually normal. BFIE was originally described by Watanabe and colleagues¹ in 1987 and the phenotype was further described and named 'benign familial infantile convulsions' by Vigeveno and colleagues² in 1992.

Watanabe and colleagues¹ described nine infants with benign complex partial epilepsies characterised by the presence of clusters of seizures with motor arrest, decreased responsiveness, and automatisms. They observed that these infants had an apparently normal developmental outcome and that their seizures were easily controlled with antiepileptic drugs. This was in contrast to many cases of seizures in infancy, which were associated with

an unfavourable outcome and developmental delay. Watanabe and colleagues¹ also observed that four of the nine patients had a family history of benign infantile convulsions.

Vigeveno and colleagues² described a further five infants with clusters of partial seizures with secondary generalisation occurring between the ages of 4 and 7 months. The epilepsy was familial in all cases and had a favourable outcome. It was observed that the clinical features in these patients were similar to those seen in benign familial neonatal convulsions, apart from the age of onset. It was also observed that the features in these patients overlapped those described by Watanabe and colleagues.¹ Vigeveno and colleagues² proposed the name 'benign familial infantile convulsions' for the disorder. This nomenclature has since been updated and the disorder is termed BFIE in the most recent classifications of the International League Against Epilepsy.³

Paroxysmal kinesigenic dyskinesia (PKD), also called paroxysmal kinesigenic choreoathetosis, is a movement disorder characterised by sudden attacks of involuntary movement that are induced by a sudden movement from rest, such as rising from a chair or starting to walk, or by exercise. The attacks in PKD consist of dystonic posturing, chorea or athetosis.⁴ Diagnostic criteria for PKD were proposed by Bruno and colleagues⁵ and include attacks with an identified kinesigenic trigger, short duration (<1 min), no associated loss of consciousness or pain, normal neurologic examination, exclusion of other causes, and onset at between 1–20 years of age or a family history of PKD. The disorder was delineated in detail in 1967 by Kertesz,⁶ who described the phenotypes of 10 patients from six families and reviewed similar reports from the literature. It was observed in this study that the disorder was often familial and therefore a genetic cause was proposed. PKD usually has onset in late childhood or adolescence and may remit in adulthood. The disorder responds well to treatment with antiepileptic drugs, particularly carbamazepine or phenytoin, and patients are otherwise normal.^{5–7} PKD shows autosomal dominant inheritance in families and sporadic cases are also observed.^{5, 6}

Infantile convulsions and choreoathetosis (ICCA) is a syndrome in which BFIE and PKD co-occur in the same patient or family. The syndrome was first described as a distinct clinical entity in 1997 by Szepietowski and colleagues,⁸ who identified four French families with autosomal dominant inheritance of BFIE and PKD. Genome-wide linkage analysis was performed for these families and all were found to be linked to a 10 cM interval in the pericentromeric region of chromosome 16. Following

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this report, additional families with ICCA and linkage to the same region were described.^{9–10} Families were also described with BFIE or PKD alone that showed linkage to the same region.^{11–19} ICCA, BFIE, and PKD were therefore hypothesised to be allelic.¹¹ The minimal critical region (MCR) for the majority of these families corresponded to a 21.69 Mb (6 cM) region between *D16S690* and *D16S517* on chromosome 16 and was similar for ICCA, BFIE, and PKD (figure 1). A single BFIE family with a recombination event at *D16S685*,¹³ potentially reducing the MCR to a 2.7 Mb region between *D16S690* and *D16S685*, and a second PKD locus on the q-arm of chromosome 16²⁰ were also described (figure 1).

IDENTIFICATION OF MUTATIONS IN *PRRT2*

Despite the identification of the chromosome 16 BFIE/ICCA/PKD locus in 1997 and the subsequent extensive sequencing of candidate genes within the region,^{19–21} the causative gene was not identified for many years. In 2011, Chen and colleagues⁴ successfully employed a strategy combining linkage analysis and whole exome sequencing to identify mutations in an uncharacterised gene, *PRRT2*, in eight Chinese families with PKD. This finding was rapidly followed by many similar reports of mutations in the same gene in families from different ethnic backgrounds with PKD, ICCA, and BFIE.^{22–54} To date, over 330 families and individuals with mutations in *PRRT2* have been described. De novo mutations are observed in sporadic cases of PKD, ICCA, and BFIE. The vast majority of these families (over 80%) have the same recurrent frameshift mutation: *PRRT2* c.649–650insC; p.Arg217Profs*7. The remaining mutations in

PRRT2 are spread throughout the gene (table 1, figure 2). Most of them are nonsense, frameshift and splice site mutations predicted to lead to protein truncation. Several of these mutations have been demonstrated to cause altered cellular localisation of the *PRRT2* protein or loss of detectable protein expression in vitro.^{4–28} Fifteen different missense mutations have been reported.^{22–25 29 34 35 37 42 45 46 50 54} These alter amino acid residues clustered in and around two putative transmembrane domains located near the C-terminus of the protein (table 1, figure 2A). In addition to these substitution and small insertion or deletion mutations, three PKD or ICCA patients have been described with large sub-microscopic deletions encompassing a number of genes including *PRRT2* (figure 2B).^{55–57} These findings indicate the need for copy number variant analysis as well as sequencing of *PRRT2* to be included in a full diagnostic analysis of the gene.

Mutations in *PRRT2* have also been identified in families with hemiplegic migraine (HM) and other forms of migraine. This association was initially described in a family with heterogeneous paroxysmal phenotypes including infantile seizures, PKD, HM, and paroxysmal torticollis.³³ Migraine was also noted as a feature in some families where the primary phenotype was PKD, ICCA or BFIE.^{24 31 44 46 52 53} A small number of families with *PRRT2* mutations have been described in which migraine, most commonly HM, is the only phenotype observed.^{44 46} In contrast to the high mutation rate observed in BFIE, ICCA, and PKD patients, *PRRT2* mutations account for only a small proportion (0.7–3%) of cases of HM occurring without other paroxysmal disorders.^{44 45 47} The association of both migraine and seizure disorders with the same gene has been previously observed. A family with a mutation in *ATP1A2* causing both migraine and infantile seizures has been described⁵⁸ and mutations in *SCN1A*, which usually cause epilepsy, have been described in patients with HM.^{59 60}

Occasional families with *PRRT2* mutations have been described in which epilepsy phenotypes other than BFIE are observed.^{48 54} These phenotypes include febrile seizures, febrile seizures plus, and nocturnal convulsions. *PRRT2* mutations have not been associated with other phenotypes that include infantile seizures. In particular, no mutations have been identified in patients with convulsions with gastroenteritis (CwG) or benign familial neonatal epilepsy (BFNE).^{43 48 50} BFNE is most commonly caused by mutations in the potassium channel subunit genes *KCNQ2* and *KCNQ3*.⁶¹ The seizures in CwG show similar clinical characteristics to those seen in BFIE, but occur in the context of gastroenteritis, often caused by rotavirus infection.⁶²

There is no evidence of a genotype–phenotype relationship between *PRRT2* mutations and the three different phenotypes with which they are associated. All three phenotypes (BFIE, PKD, ICCA) are associated with the common insertion mutation (p.Arg217Profs*7) and all three phenotypes are also associated with other mutations, including the 15 missense mutations. The lack of a genotype–phenotype relationship is not unexpected given the phenotypic variability seen in families with ICCA, in which the same mutation can cause BFIE alone, PKD alone, or both syndromes in different individuals within the same family. This suggests that the expression of the phenotype is influenced by other genetic or environmental factors, rather than the particular *PRRT2* mutation carried by an individual. What these additional factors may be has yet to be determined.

The common c.649–650insC mutation occurs in a homopolymer tract of nine cytosine bases that are preceded by four guanines. This sequence has the potential to form a hairpin loop (as

Figure 1 Linkage regions identified for infantile convulsions with choreoathetosis (ICCA), benign familial infantile epilepsy (BFIE), and paroxysmal kinesigenic dyskinesia (PKD) shown against a physical map of the positions of microsatellite markers in the pericentromeric region of chromosome 16 from MapViewer, based on human genome build 37.1. Linkage intervals for ICCA (red), BFIE (light blue), and PKD (dark blue) from 13 publications are shown, along with the minimal critical region derived from these linkage intervals.

Table 1 Heterozygous mutations reported in *PRRT2* in families and patients with PKD, ICCA, BFIE, and HM

Mutation	Type	Associated phenotype(s)	Reference(s)
c.117delA; p.Val41Tyrfs*49	fs	PKD	54
c.133-136delCCAG; p.Pro45Argfs*44	fs	PKD	51
c.272delC; p.Pro91Glnfs*24	fs	PKD	34
c.291delC; p.Asn98Thrfs*17	fs	BFIE	29
c.369-370insG; p.Ser124Valfs*10	fs	PKD	23
p.Arg145Glyfs*31	fs	BFIE	44
c.487C>T; p.Gln163*	ns	ICCA	22
c.510-511insT; p.Leu171Serfs*3	fs	PKD	54
c.513-514ins; p.Leu171Leufs*3	fs	PKD	45
c.514-517delTCTG; p.Ser172Argfs*3	fs	PKD	4
c.516-517insT; p.E173*	fs	ICCA	28
c.562C>T; p.Gln188*	ns	ICCA	30
c.573-574insT; p.Gly192Trpfs*7	fs	ICCA	26
c.579-580insA; p.Gle194Argfs*6	fs	ICCA	54
c.595G>T; p.Glu199*	ns	PKD	34
c.604-607delTCAC; p.Ser202Hisfs*16	fs	PKD	34
c.629delC; p.Pro210Glnfs*18	fs	BFIE	29
c.629-630insC; p.Ala211Serfs*14	fs	PKD, ICCA	25 30
c.649C>T; p.Arg217*	ns	PKD, ICCA	30 31 35 38 40 54
c.649delC; p.Arg217Glufs*12	fs	PKD, ICCA, HM	30 31 34 47
c.649-650insC; p.Arg217Profs*8	fs	PKD, ICCA, BFIE, HM	4 22-36 39 41 43-54
c.718C>T; p.Arg240*	ns	ICCA, BIS, PKD	28 34 39 44
p.Ser248Alafs*65	fs	ICCA	44
c.748C>T; p.Gln250*	ns	ICCA	27
c.776insG; p.A260*	fs	PKD	41
c.796C>T; p.Arg266Trp	ms	PKD	22
c.824C>T; p.Ser275Phe	ms	ICCA	35
c.841T>C; p.Trp281Arg	ms	PKD	23
c.859G>A; p.Ala287Thr	ms	PKD	23
c.872C>T; p.Ala291Val	ms	PKD	54
c.879+1G>T	ss	BFIE	25
c.879+5G>A	ss	BFIE	25
c.880-2A>T	ss	ICCA	46
c.904-905insG; p.Asp302Glyfs*38	fs	ICCA	24
c.913G>A; p.Gly305Arg	ms	PKD	24 37
c.913G>T; p.Glu305Trp	ms	PKD	45
c.916G>A; p.Ala306Trp	ms	BFIE	46
c.917C>A; p.Ala306Asp	ms	ICCA	42
c.922C>T; p.Arg208Cys	ms	PKD	23 34
c.950G>A; p.Ser317Asn	ms	ICCA	25
c.964delG; p.Val322Trpfs*15	fs	PKS	23
c.968G>A; p.Gly232Glu	ms	BFIE	29
c.970G>A; p.Gly324Arg	ms	ICCA	46
c.971G>A; p.Gly324Glu	ms	BFIE	46
c.972delA; p.Val325Serfs*12	fs	PKD	4
c.980-981insT; p.Ile327Ilefs*14	fs	PKD	28
c.981C>G; p.Ile327Met	ms	BFIE	50
p.332insGAC	ifi	ICCA	45
c.1011C>T	ss	PKD	45
c.1011-1012delCG+1-9del9bp	ss	PKD	23
c.1012+2insT	ss	BFIE	43
0.54 Mb deletion at 16p11.2 affecting approximately 30 genes	del	PKD	55
0.6 Mb deletion at 16p11.2 affecting 27 genes	del	ICCA	56
0.43 Mb deletion at 16p11.2 affecting 30 genes	del	PKD	57

Mutations reported in multiple families are indicated in bold and the common insertion mutation is indicated in bold and underlined.

BFIE, benign familial infantile epilepsy; BIS, benign infantile seizures; del, large deletion; fs, frameshift; ifi, in-frame insertion; HM, hemiplegic migraine; ICCA, infantile convulsions with choreoathetosis; ms, missense; ns, nonsense; PKD, paroxysmal kinesigenic dyskinesia; ss, splice site.

Figure 2 (A) Diagram of the *PRRT2* protein, which contains 340 amino acid residues, showing the locations of known mutations in the gene. The two putative transmembrane domains are indicated in dark grey and the proline-rich region in medium grey. Frameshift mutations are indicated by stars, nonsense mutations by diamonds, missense mutations by crosses, splice site mutations by 12-pointed stars, and the in-frame insertion by a three-pointed star. Mutations associated with paroxysmal kinesigenic dyskinesia (PKD) are shown in dark blue, infantile convulsions with choreoathetosis (ICCEA) in red, and benign familial infantile epilepsy (BFIE) in light blue. Mutations marked in purple are either associated with other paroxysmal movement disorders, hemiplegic migraine alone or no phenotype was reported for them. (B) Map showing the extent of the three large deletions identified in patients with PKD or ICCEA and the genes involved in these deletions. *PRRT2* is indicated in red. The region affected by the deletions contains 26 other genes marked in grey and three pseudogenes marked as white boxes.

illustrated in figure 3), which may cause polymerase slippage and the insertion of an additional base during DNA replication.²⁵ The poly-C tract appears to be particularly prone to mutation: in addition to the common insertion mutation, a 1 bp deletion and a nonsense mutation affecting the same nucleotide have been reported several times (table 1). Furthermore, there are five single nucleotide polymorphisms (SNPs) altering bases within the poly-C tract reported in public databases (dbSNP and 1000 Genomes).

The high frequency of the c.649-650insC mutation in this homopolymer tract explains, at least in part, the failure of many previous efforts to identify the BFIE/ICCEA/PKD gene. Insertions in homopolymer tracts are less likely to be detected by next generation sequencing (NGS) technologies, due to the technical limitations of the chemistries and detection methods used by them.⁶³ Indeed, the failure of NGS methods to detect the common insertion mutation has been described twice.^{25 29} Homopolymer tracts can also affect the results of classical

Sanger sequencing. Polymerase slippage when reading through homopolymers leads to 'noisy' or ambiguous sequence following the homopolymer tract. This has the potential to mask the presence of insertion mutations in the homopolymer or other mutations in the downstream sequence, as has been noted.²⁷

FUNCTIONAL ROLE OF THE *PRRT2* PROTEIN

The full length *PRRT2* protein contains 340 amino acid residues with two putative transmembrane domains near the C-terminal end (figure 2A). The expression of the gene has begun to be characterised.^{4 25 28} The mouse orthologue has been shown by in situ hybridisation analyses to be localised throughout the brain, with the highest mRNA concentrations seen in the cerebral cortex.^{4 25} Reverse transcriptase PCR (RT-PCR) experiments also showed *Prprt2* expression in the brain, with lower values seen in spinal cord and no expression in other tissues tested.⁴ The *Prprt2* mRNA concentrations in mice were highest on postnatal day 14 (P14), which corresponds approximately to an age of 1–2 years in humans. Western blots of mouse tissues probed with anti-*PRRT2* antibody showed high expression in the brain, low expression in spinal cord, and no expression in other tissues tested,²⁸ reiterating the RT-PCR results described above. Overall, these data demonstrate convincingly that *PRRT2* codes for a protein with specific expression in the brain and nervous system. The expression of *PRRT2* peaks during postnatal development, consistent with its role in the pathogenesis of infantile seizures. Robust expression of the mRNA throughout the mouse brain is still seen at postnatal day 46,²⁵ approximately equivalent to adolescence in humans. The mRNA is present in the adult mouse brain, although the expression levels are approximately 50% of those seen at the peak of expression on P14.⁴ The expression of *PRRT2* into adulthood is consistent with its role in the pathogenesis of

Figure 3 Sequence surrounding the polycytosine tract which is the *PRRT2* mutation hotspot and illustration of a potential hairpin loop structure formed by it.

PKD, which has onset in late childhood or adolescence and sometimes continues during adult life.

Although *PRRT2* is largely an uncharacterised protein, the first steps have been taken in the understanding of its role in neuronal function. Yeast-2-hybrid studies suggested that it interacts with synaptosomal associated protein 25 (SNAP-25).⁶⁴ This interaction was confirmed by in vitro and in vivo co-immunoprecipitation experiments.²⁸ SNAP-25 is a member of the SNARE protein family. Proteins in this family are essential for the transport of vesicles through the Golgi apparatus and to the plasma membrane. SNAP-25 forms part of a complex involved in the release of neurotransmitters from synaptic vesicles at the pre-synaptic membrane. The SNAP-25 protein is located on the cytoplasmic surface of the plasma membrane in association with syntaxin-1, which is membrane bound. Neurotransmitter release is triggered by the influx of calcium ions resulting from an action potential. These activate synaptotagmin and synaptobrevin molecules on the cytoplasmic surface of the synaptic vesicle, causing the binding of synaptobrevin to syntaxin-1 and SNAP-25 and bringing the synaptic vesicle to the plasma membrane. The synaptic vesicle then fuses with the plasma membrane, releasing its contents into the synapse.⁶⁵ Co-immunostaining experiments using FLAG tagged *PRRT2* expressed in primary hippocampal neurones indicated that *PRRT2* co-localised with synapsin-1 at neuronal puncta.²⁸ Synapsin-1 associates with the cytoplasmic surface of synaptic vesicles and is involved in synaptogenesis and the modulation of neurotransmitter release.^{66 67} The localisation of *PRRT2* at neuronal puncta and its interaction with SNAP-25 suggest that it may also play a role in the modulation of neurotransmitter release. A reduction in the amount of *PRRT2* due to haploinsufficiency presumably leads to a dysregulation of this process. The seizures and dystonic posturing seen in BFIE and PKD could possibly result from either excessive neurotransmitter release at excitatory synapses or a reduction in the release of inhibitory neurotransmitters. Understanding which of these processes is affected will require a more precise understanding of the role of *PRRT2* in synaptic transmission. Increased understanding of the pathogenic mechanism underlying *PRRT2* mutations may also explain the particular effectiveness of some antiepileptic drugs—for example, carbamazepine, a sodium channel blocker—in treating the disorders associated with *PRRT2* mutations. Presently there is no obvious link between decreased sodium channel activity and the effective treatment of a disorder resulting from altered neurotransmission, but this may become apparent in the future.

CONCLUSION

Mutations in *PRRT2* account for between 40–100% of familial cases of BFIE,^{25 29 39 46 48} 33–100% of familial cases of ICCA,^{22 24 28 30 34 35 39 46 48} and 62–100% of familial cases of PKD^{4 22–24 28 30 34 35} in the various cohorts of patients that have been studied. The percentages of sporadic cases found to be *PRRT2* mutation positive are generally lower, with mutations found in 27–50% of PKD patients with no family history^{23 24 30 35} and 29–100% of cases of sporadic benign infantile seizures.^{25 29 39 46 48} The generally high frequency of mutations in familial cases of PKD and BFIE indicates that *PRRT2* mutations are the most common cause of both disorders. The frequency of mutations is particularly high in ICCA. It is possible that the rare mutation negative ICCA families have non-coding mutations or large deletions affecting *PRRT2*, as these would not have been detected by the sequence based screening methods used for the studies reviewed here. It is therefore possible that all cases of ICCA are caused by *PRRT2* mutations. The

mutation negative BFIE and PKD cases may also have these types of mutation, or may have mutations in other genes.

PRRT2 is the major gene for BFIE, ICCA, and PKD and contains the second highest number of reported mutations associated with epilepsy after *SCN1A*. The vast majority (95%) of mutations in the gene are truncating mutations predicted to lead to haploinsufficiency. A small number of missense mutations have been reported and these all alter amino acid residues clustered in two predicted transmembrane domains at the C-terminal end of the protein. *PRRT2* is predicted to code for a protein involved in the modulation of presynaptic neurotransmitter release, and a perturbation of this process is likely to be the cause of the seizure and movement disorder phenotypes associated with mutations in the gene. The identification of a heterozygous mutation in *PRRT2* can provide a definitive diagnosis for patients with suspected BFIE, ICCA or PKD. This molecular diagnosis can reduce or prevent the need for additional investigations in these patients and guide treatment for these disorders.

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