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REVIEW ARTICLE

The evolving spectrum of *PRRT2*-associated paroxysmal diseases

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Next-generation sequencing has identified mutations in the *PRRT2* (proline-rich transmembrane protein 2) gene as the leading cause for a wide and yet evolving spectrum of paroxysmal diseases. *PRRT2* mutations are found in the majority of patients with benign familial infantile epilepsy, infantile convulsions and choreoathetosis and paroxysmal kinesigenic dyskinesia, confirming a common disease spectrum that had previously been suggested based on gene linkage analyses and shared clinical features. Beyond these clinical entities, *PRRT2* mutations have been described in other childhood-onset movement disorders, different forms of seizures, headache disorders, and intellectual disability. *PRRT2* encodes a protein that is expressed in the central nervous system and is thought to be involved in the modulation of synaptic neurotransmitter release. The vast majority of mutations lead to a truncated protein or no protein at all and thus to a haploinsufficient state. The subsequent reduction of PRRT2 protein may lead to altered synaptic neurotransmitter release and dysregulated neuronal excitability in various regions of the brain, resulting in paroxysmal movement disorders and seizure phenotypes. In this review, we examine the genetics and neurobiology of *PRRT2* and summarize the evolving clinical and molecular spectrum of *PRRT2*-associated diseases. Through a comprehensive review of 1444 published cases, we provide a detailed assessment of the demographics, disease characteristics and genetic findings of patients with *PRRT2* mutations. Benign familial infantile epilepsy (41.7%; $n = 602$), paroxysmal kinesigenic dyskinesia (38.7%; $n = 560$) and infantile convulsions and choreoathetosis (14.3%; $n = 206$) constitute the vast majority of *PRRT2*-associated diseases, leaving 76 patients (5.3%) with a different primary diagnosis. A positive family history is present in 89.1% of patients; and *PRRT2* mutations are familial in 87.1% of reported cases. Seventy-three different disease-associated *PRRT2* mutations (35 truncating, 22 missense, three extension mutations, six putative splice site changes, and seven changes that lead to a complete *PRRT2* deletion) have been described to date, with the c.649dupC frameshift mutation accounting for the majority of cases (78.5%). Expanding the genetic landscape, 15 patients with biallelic *PRRT2* mutations and six patients with 16p11.2 microdeletions and a paroxysmal kinesigenic dyskinesia phenotype have been reported. Probing the phenotypic boundaries of *PRRT2*-associated disorders, several movement, seizure and headache disorders have been linked to *PRRT2* mutations in a subset of patients. Of these, hemiplegic migraine emerges as a novel *PRRT2*-associated phenotype. With this comprehensive review of *PRRT2*-associated diseases, we hope to provide a scientific resource for informing future research, both in laboratory models and in clinical studies.

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Abbreviations: BFIE = benign familial infantile epilepsy; PED = paroxysmal exercise-induced dystonia; PKD = paroxysmal kinesigenic dyskinesia; PKD/IC = paroxysmal kinesigenic dyskinesia with infantile convulsions; PNKD = paroxysmal non-kinesigenic dyskinesia; SNARE = soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptor

Introduction

The advent and increasing diagnostic availability of next-generation sequencing technologies has considerably advanced our understanding of the genetics behind both common and rare neurologic diseases (Yang *et al.*, 2013*a*; Biesecker and Green, 2014; Srivastava *et al.*, 2014). The rapid discovery of novel disease genes through unbiased whole-exome sequencing is beginning to reshape the boundaries between neurological diseases as we move from a phenotype-based syndromic categorization to a classification based on shared genetic and pathophysiological mechanisms. Along these lines, putative pathogenic mutations in patients with atypical clinical manifestations outside the well-described phenotypic spectrum of a given genetic condition, in many cases, argue for a broader phenotype than previously appreciated.

By combining classic linkage analysis with whole-exome sequencing, mutations in the *PRRT2* gene have been identified as the cause of paroxysmal kinesigenic dyskinesia (PKD) (Chen *et al.*, 2011; Wang *et al.*, 2011). More recently, *PRRT2* mutations have also been found to cause infantile convulsions and choreoathetosis (ICCA) (Heron *et al.*, 2012; Lee *et al.*, 2012*a*) and benign familial infantile epilepsy (BFIE) (Heron *et al.*, 2012; Ono *et al.*, 2012) confirming a continuous disease spectrum with shared molecular mechanisms that had been suspected based on clinical grounds and previous linkage analyses (Szepetowski *et al.*, 1997; Tomita *et al.*, 1999).

Curiously, the spectrum of *PRRT2*-associated diseases has evolved beyond these entities and now includes a number of other paroxysmal diseases covering movement and headache disorders. The shared paroxysmal nature of symptoms and genetic findings in *PRRT2*-associated diseases suggest a common pathophysiology that might involve *PRRT2*'s putative function in synaptic vesicle release and neuronal excitability. *PRRT2*-associated diseases therefore provide a unique opportunity to identify shared molecular mechanisms with implications for paroxysmal diseases and synaptic biology in general. We herein review the evolving clinical and molecular spectrum of *PRRT2*-associated paroxysmal diseases.

A historical perspective and current terminology

More than a century ago, the prominent Japanese psychiatrist Shuzo Kure provided a first detailed description of what was later termed paroxysmal kinesigenic dyskinesia (PKD) (Kure, 1892). Short attacks of purposeless, irregular and often-bizarre involuntary movements triggered by sudden voluntary motion were recognized as salient clinical features (Kure, 1892; Kato *et al.*, 2006). To illustrate the paroxysmal and kinesigenic character of involuntary movement in PKD, Professor Kure's seminal manuscript featured reproductions of photographs of a 23-year-old patient taken during an attack (Fig. 1A). The chair shown in the image on the right (Fig. 1A) suggests that attacks were triggered by a manoeuvre in which the patient is asked to quickly rise from a chair, an examination technique that is still commonly used to elicit attacks (Kure, 1892; Kato *et al.*, 2006). In the more recent English literature, Andrew Kertesz is credited for coining the term 'paroxysmal kinesigenic choreoathetosis' through his detailed description of patients with 'short paroxysms of unilateral or generalized tonic, choreiform, and athetoid movements and posturing' that were 'usually precipitated by movement' (Kertesz, 1967). 'Paroxysmal choreoathetosis' was introduced as a disease entity by Mount and Reback almost two decades earlier, although their case descriptions likely referred to a different disease, which is called paroxysmal non-kinesigenic dyskinesia (PNKD) today (Mount and Reback, 1940). Kertesz, however, documented the importance of a kinesigenic trigger for involuntary movements in his patients. The current classification, introduced by Demirkiran and Jankovic in 1995, clearly distinguishes PKD, grossly corresponding to Kertesz's paroxysmal kinesigenic choreoathetosis, from PNKD (Demirkiran and Jankovic, 1995). Paroxysmal exercise-induced dyskinesia (PED), described by Lance (1977), exists as a separate entity within the paroxysmal dyskinesias. In 2004, seminal work by Bruno *et al.* (2004) defined diagnostic clinical criteria for PKD (Box 1). These include the presence of an identifiable kinesigenic trigger, short duration of attacks (<1 min), no loss of consciousness or pain during attacks, a normal interictal neurological exam, exclusion of other

Box 1 Diagnostic criteria for PKD modified from Bruno et al. (2004)

- Identified kinesigenic trigger for the attacks
- Short duration of attacks (< 1 min)
- No loss of consciousness or pain during attacks
- Exclusion of other organic diseases and normal neurologic examination
- Control of attacks with phenytoin or carbamazepine, if tried
- Age at onset between 1 and 20 years, if no family history of PKD
- *New: Identification of a PRRT2 mutation with putative pathogenicity (optional)*

causes, and an onset between 1–20 years of age or a positive family history.

Historically, PKD has been described as a peculiar form of reflex epilepsy, an idea that was corroborated by the fact that a number of affected individuals had a history of afebrile infantile seizures. This particular type of seizure disorder in infants is now known as BFIE and was first described by Watanabe *et al.* (1987). Overlapping clinical features and emerging linkage studies strongly suggested that BFIE and PKD are allelic disorders, which led to the term infantile convulsions with choreoathetosis (ICCA) to label patients who present with both BFIE during infancy and PKD later in life (Szepetowski *et al.*, 1997; Tomita *et al.*, 1999; Swoboda *et al.*, 2000; Caraballo *et al.*, 2001). In an attempt to provide a more uniform terminology, ICCA has been termed paroxysmal kinesigenic dyskinesia with infantile convulsions (PKD/IC) in the more recent literature. The continuous spectrum of BFIE, PKD/IC and PKD constitutes the core of *PRRT2*-associated diseases as discussed in the following sections.

The neurobiology of PRRT2

The *PRRT2* gene is located on chromosome 16p11.2 and consists of four exons encoding a 340-amino acid protein, the proline-rich transmembrane protein 2 (PRRT2). PRRT2 is predicted to contain a proline-rich domain (amino acids 131–216) within its N-terminal extracellular region (amino acids 1–268) and two putative transmembrane domains at the C-terminal end (amino acids 268–289 and 318–338) and is thus likely membrane-bound (Fig. 1B). Secondary to alternative splicing at the 3' terminus, six splice variants and three different isoforms exist (www.ensemble.org; ENSG00000167371). As nearly all known pathogenic mutations in *PRRT2* locate to a conserved region covered by all splice variants, the significance of the different isoforms for *PRRT2*-associated diseases remains unclear. *PRRT2* is expressed throughout the CNS with high expression levels detected in the cortical layers of the cerebral cortex, basal ganglia and cerebellum (Fig. 2A and B) (Chen *et al.*, 2011; Heron *et al.*, 2012). Expression in other organs seems negligible, highlighting the CNS-specific function of this protein (Lee *et al.*, 2012*a*). Examination of PRRT2's expression pattern in the developing murine nervous system revealed a marked increase during early postnatal stages and declining levels during adulthood (Chen *et al.*, 2011). Age-dependent expression changes might bear significance for disease manifestations in different age groups in humans. Longitudinal analysis of mRNA levels during human brain development revealed a rapid increase of *PRRT2* expression in the striatum, neocortex, hippocampus, and thalamus until ~100 days post-conception, when a plateau is reached and levels even start to decline in thalamic regions (Fig. 2A; data were retrieved from the Human Brain Transcriptome database; Johnson *et al.*, 2009; Kang *et al.*, 2011). In the adult human brain, mRNA expression profiling confirms higher *PRRT2* expression in cerebellar, occipital, frontal, and temporal cortex, the putamen, and hippocampus compared to other brain regions, such as the substantia nigra, thalamus, inferior olivary nucleus, or intralobular white matter (Fig. 2B; microarray analysis; Trabzuni *et al.*, 2011). These findings are based on human post-mortem brain tissue from the UK Human Brain Expression Consortium dataset based on profiling of 1231 samples from 10 brain areas originating from 134 individuals.

At the subcellular level, PRRT2 is mainly detected in axons but not in dendrites of neurons. In axons it is thought to specifically localize to glutamatergic synapses (Lee *et al.*, 2012*a*; Li *et al.*, 2015) and associates with the glutamate receptor GRIN1A, a member of the AMPA receptor family (Schwenk *et al.*, 2012). In a two-hybrid screen, PRRT2 was also found to interact with the synaptic t-SNARE protein SNAP25, pointing to a possible role in synaptic vesicle docking and neurotransmitter release (Stelzl *et al.*, 2005) (Fig. 2C). Our functional association analysis, based on the available experimental evidence, confirms the association of PRRT2 and SNAP25. In addition, based on co-expression, co-localization, or shared protein domains, a dense network of functionally related proteins becomes readily apparent, many of which are involved in synaptic development, maturation, and signalling (Fig. 2D shows the top 20 functionally associated proteins, see Supplementary Fig. 1 for more details). SNAP25 is predominantly expressed in neurons and neuroendocrine cells and is enriched in presynaptic terminals. Here it modulates Ca^{2+}-triggered exocytosis of synaptic vesicles by at least three mechanisms: (i) it acts as a t-SNARE protein and is thus a crucial component of the molecular machinery required for synaptic vesicle fusion (Sudhof, 2013); (ii) it is involved in endocytosis at synapses and thus in replenishing the pool of available neurotransmitter vesicles (Xu *et al.*, 2013); and (iii) it negatively regulates voltage-gated ion channels, thus silencing SNAP25 results in increased channel activity in glutamatergic neurons (Pozzi *et al.*, 2008; Condliffe *et al.*, 2010) (Fig. 2C).

How this relates to disease mechanisms in *PRRT2*-associated diseases is yet unclear. The interaction of

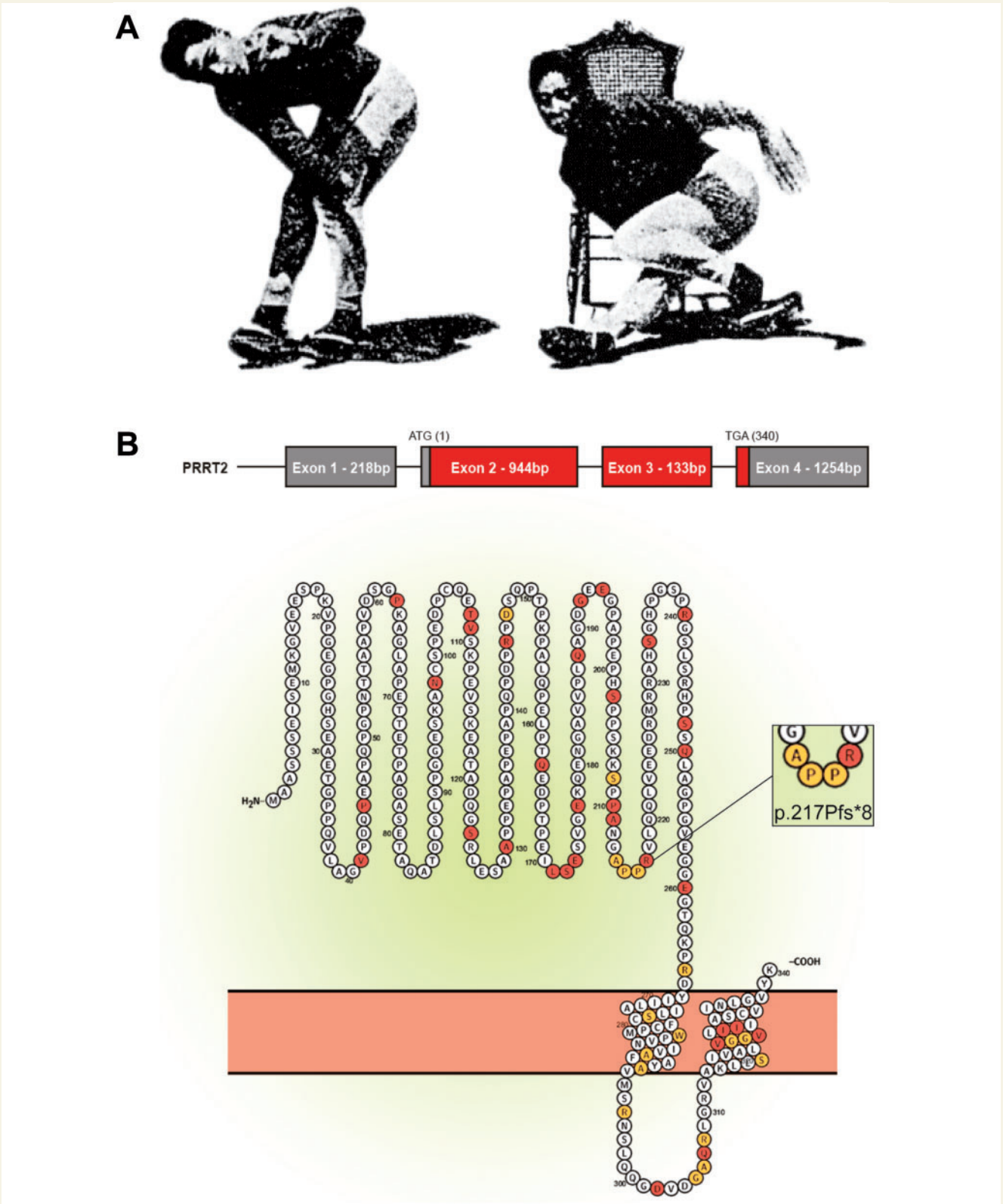

Figure 1 From the first description of PKD to the discovery of the *PRRT2* gene and protein. (**A**) First description of PKD in the medical literature. In 1892, Shuzo Kure published a first description of PKD in his manuscript entitled 'An atypical case of Thomsen's disease'. This seminal paper featured these handmade tracings of photographs of a patient taken during an attack. The paroxysmal character of PKD attacks is highlighted by the chair in the right image, suggesting that attacks could be triggered when the patient is asked to quickly rise to a standing position, a common manoeuvre to elicit attacks. Modified from Shuzo Kure, Tokyo Igakukai Zasshi [Journal of the Tokyo Medical Association] 1892;6(11): 505–14. Also see Kato *et al.* (2006). (**B**) Schematic representation of the *PRRT2* gene and protein with mutations highlighted. *Top:* Diagram of the *PRRT2* gene,

(continued)

PRRT2 with SNAP25; however, has been confirmed by immunoprecipitation experiments *in vitro* in cultured cell lines and *in vivo* in brain extracts from mice (Lee *et al.*, 2012*a*). One possible hypothesis is that PRRT2 acts as a chaperone protein to SNAP25 and thus might influence its abundance or engagement in synaptic neurotransmission. Although this remains to be tested, a similar chaperone-based regulation has been described: the CSPα–Hsc70–SGT chaperone complex acts as a chaperone specific to monomeric SNAP25 and keeps it in a state competent to engage in SNARE-complex formation (Chandra *et al.*, 2005; Sharma *et al.*, 2011, 2012).

In line with a similar mechanism, mutations in *PRRT2* lead to reduced membrane localization and impair the interaction with SNAP25 (Chen *et al.*, 2011; Lee *et al.*, 2012*a*). The latter could be consistent with a dominant-negative effect of selected missense mutations: mutant PRRT2 may still preserve a certain binding capacity to interacting proteins and thus may attenuate their binding to residual wild-type PRRT2. For the majority of *PRRT2* mutations, however, a loss of function mechanism (haploinsufficiency as discussed above) seems to be the primary mechanism by which they cause disease. The consequence of both, a dominant-negative effect or a haploinsufficient state, would be an impaired or diminished interaction of PRRT2 and SNAP25.

Reduced SNAP25 expression in transgenic heterozygous mice (*Snap25*$^{+/-}$) is associated with motor hyperactivity, frequent spikes on EEG, and increased susceptibility to kainate-induced seizures (Corradini *et al.*, 2014). Resembling some of the phenotypes of *PRRT2*-associated diseases, these abnormalities are improved by treatment with antiepileptic drugs and abate in adult animals (Corradini *et al.*, 2014). Developing glutamatergic synapses in *Snap25* heterozygous mice also show impaired short-term presynaptic plasticity that might be secondary to selectively enhanced glutamatergic neurotransmission (Antonucci *et al.*, 2013). This could potentially contribute to intellectual disability and learning disabilities found in a number of *PRRT2* patients (see below). In summary, *PRRT2* mutations likely interfere with Ca^{2+}-triggered presynaptic neurotransmitter release through an as yet unidentified mechanism. Enhanced neurotransmitter release from excitatory synapses or reduced release of inhibitory neurotransmitters may lead to a state of hyperexcitability. This could predispose to basal ganglia dysfunction or disruption of cortical circuits, which may give rise to paroxysmal disorders associated with *PRRT2*. This is very much in line with the few post-mortem studies of PKD that revealed no overt abnormalities, signifying that molecular deficits at certain synapses rather than gross anatomical changes account for PKD (Stevens, 1966; Kertesz, 1967).

Although *PRRT2*-associated diseases are not considered 'channelopathies' in the classic sense, similarities to episodic diseases with mutations in ion channels are evident. Age-dependent expression of PRRT2 in relevant neuronal subpopulations might be able to explain the age-dependence of different disease manifestations, a concept that has been introduced for genetic channelopathies such as $Na_v1.2$ mutations in benign familial neonatal-infantile seizures (Liao *et al.*, 2010).

In summary, little is known about PRRT2's physiological function and the molecular disease mechanisms in *PRRT2* mutation-associated paroxysmal disorders. Critical questions remain unanswered and will need to be addressed in future studies (Box 2).

The genetics of PRRT2

To date, 1444 patients with ~70 different *PRRT2* mutations have been reported (for a comprehensive list of all published PRRT2 mutations please refer to the Supplementary material and Supplementary Tables 1 and 2). Of these, 5.5% (79/1444) occurred *de novo* while 87.1% (1258/1444) are familial in origin (Supplementary Table 3). The vast majority of patients, 78.5% (1134/1444), carry the same frameshift mutation (c.649dupC; p.Arg217Profs*8) that leads to a premature stop codon. The nature of the DNA sequence in this 'mutational hotspot' region is that of a homopolymer of four guanine bases followed by nine consecutive cytosine bases. This peculiar sequence seems particularly prone to mutations given the frequent duplication mutation in addition to a deletion mutation and several point mutations in the same cytosine stretch (c.641–c.649) (Supplementary Table 1). Although experimental evidence is yet missing, it has been speculated that the repeats of cytosine bases might facilitate formation of a hairpin structure and 'slippage' of the DNA polymerase, leading to an insertion of an

Figure 1 Continued

which consists of four exons encoding a 340 amino acid protein. Protein coding regions are shown in red, non-coding untranslated regions in grey. *Bottom:* Schematic representation of the PRRT2 protein. PRRT2 (Uniprot Entry #Q7Z6L0, uniprot.org) contains two putative helical transmembrane domains near the C-terminal end (predicted by TMHMM Server version 2.0, http://www.cbs.dtu.dk/services/TMHMM). Residues 1–268 are extracellular [and contain a putative proline-rich domain (residues 131–216)], 269–289 and 318–338 are transmembrane and 290–317 are cytoplasmic, leaving the very end of the C-terminus (residues 339–340) in the extracellular space. Published disease-associated mutations map to different regions of the protein but a cluster in and around the two transmembrane domains is apparent (residues shown in red represent truncating or truncating/frameshift mutations, residues shown in yellow represent missense mutations). The c.649dupC (R217Pfs*8) frameshift mutation is by far the most frequent mutation, accounting for > 75% of all published cases. Putative splice site changes and extension mutations are not shown. The protein topology plot was generated using Protter version 1 (http://wlab.ethz.ch/protter; Omasits *et al.*, 2014).

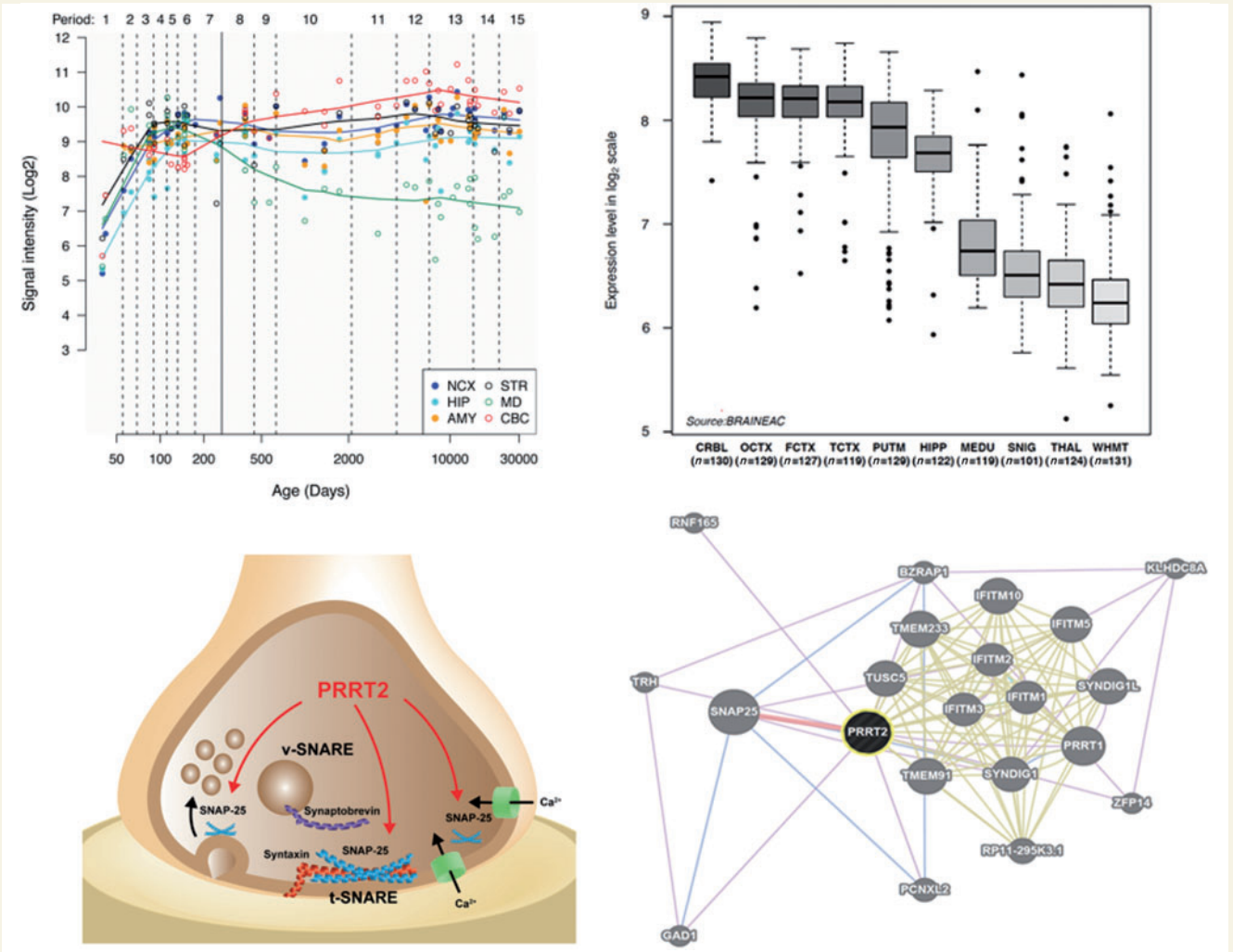

Figure 2 Expression, function and interaction network of PRRT2. (**A**) Expression pattern of *PRRT2* in the developing human brain. The graph shows longitudinal *PRRT2* mRNA expression levels in six brain regions [striatum (STR), amygdala (AMY), neocortex (NCX), hippocampus (HIP), mediodorsal nucleus of the thalamus (MD), and cerebellar cortex (CBC)] during the course of human brain development. Expression levels are based on exon array experiments and are plotted on a $\log_2$ scale (*y*-axis). A rapid increase of *PRRT2* expression during human brain development in the striatum, neocortex, hippocampus, and mediodorsal nucleus of the thalamus is seen until ~100 days post-conception, when levels reach a plateau in the striatum, neocortex, and hippocampus, and even start to decline in the thalamus. (**B**) Expression levels of *PRRT2* in adult human brain. Box plots represent *PRRT2* mRNA expression levels in 10 adult brain regions: cerebellar cortex (CRBL), occipital cortex (OCTX), frontal cortex (FCTX), temporal cortex (TCTX), putamen (PUTM), hippocampus (HIPP), medulla (specifically inferior olivary nucleus, MEDU), substantia nigra (SNIG), thalamus (THAL), and intralobular white matter (WHMT). The expression levels are based on exon array experiments and are plotted on a $\log_2$ scale (*y*-axis). The plot indicates higher *PRRT2* expression in cerebellar, occipital, frontal, and temporal cortex, putamen, and hippocampus, in comparison to levels in the inferior olivary nucleus of medulla, substantia nigra, thalamus, or intralobular white matter. The fold change between cerebellar cortex and intralobular white matter is 4.3 ($P = 7.4 \times 10^{-89}$). (**C**) Schematic depiction of PRRT2's role at the synapse. PRRT2 localizes to axons and associates with glutamatergic synapses. Here it is known to interact with SNAP25, a protein that is involved in Ca^{2+}-triggered neurotransmitter release through (i) its function as a t-SNARE protein; (ii) its role in synaptic endocytosis, which impacts the availability of synaptic vesicles at the readily releasable pool; and (iii) its regulation of voltage-gated ion channels. Loss-of-function mutations in *PRRT2* might lead to altered synaptic vesicle release and increased neuronal excitability, although the molecular mechanisms still remain to be determined. (**D**) Network-based *PRRT2* function analysis. Scheme showing putative PRRT2 functional partners based on physical interaction (red), co-expression (violet), co-localization (blue), and shared protein domains (yellow) was generated using the GeneMANIA algorithm (Mostafavi *et al.*, 2008).

additional cytosine during DNA replication and thus to the common c.649dupC frameshift mutation (Li *et al.*, 2013*a*).

In addition to this frequent mutation, about three-quarters of all published *PRRT2* mutations are truncating (either nonsense or *PRRT2* reading frame-shifting insertions and deletions) and three mutations affect the termination codon, thus extending the protein. Using the recently developed Combined Annotation Dependent Depletion (CADD) tool (Kircher *et al.*, 2014) for scoring the deleteriousness of *PRRT2* mutations, we established

Box 2 Critical open questions on *PRRT2* and challenges for future investigation

- **What is the precise temporal and spatial expression pattern of *PRRT2* in the human CNS?**

Hypothesis I: *PRRT2* expression is highest in regions clinically implicated in *PRRT2*-associated diseases, such as cortical regions and the basal ganglia. This is corroborated by data showing that, in the adult human brain, *PRRT2* is expressed throughout the CNS with high levels detected in cortical layers of the cerebral cortex, basal ganglia and cerebellum (Fig. 2A and B).
Hypothesis II: The expression pattern and/or protein levels of *PRRT2* shift from cortical to subcortical regions during human brain development and aging. This is corroborated by data on *PRRT2*'s expression pattern in the developing murine nervous system that show a peak expression during early post-natal stages and declining levels during adulthood. A temporal shift in *PRRT2* protein levels in relevant neuronal populations might account for the age-dependent manifestation of BFIE and PKD and for their remission at a certain stage of brain maturation (e.g. in BFIE seizures remit by 2 years of age; in many PKD patients the frequency of attacks declines markedly in adulthood).

- **What are the functional domains of the *PRRT2* protein?**

Hypothesis: The two transmembrane domains are essential to *PRRT2*'s structure and/or putative function. This is corroborated by the fact that these regions are evolutionarily conserved from zebrafish to humans, highlighting their structural and/or functional importance. In addition, the majority of missense mutations are clustered in these regions and essentially all of the frameshift changes upstream or within the transmembrane domains cause their loss.

- **How, when and where do *PRRT2* mutations alter synaptic function?**

Hypothesis: In cortical regions and the basal ganglia, *PRRT2* mutations alter synaptic function through impairing the protein's ability to bind interacting proteins involved in regulating synaptic transmission, e.g. in neurotransmitter release. This is corroborated by the identification of *PRRT2*'s interaction with GRIN1A and SNAP25. Another precedence is provided by similar mechanisms in related diseases such PNKD (or MR1)-related PNKD. Like *PRRT2*, the PNKD protein associates with membranes and is expressed in neurons where it enriches in pre- and postsynaptic preparations. Through an interaction with synaptic active zone proteins Rab3-interacting molecule (RIM) 1 and 2, PNKD modulates neurotransmitter release as recently demonstrated.

- **Do *PRRT2* mutations act by a loss-of-function or dominant-negative mechanism?**

Hypothesis: A loss-of-function mechanism is the cause in the majority of *PRRT2*-associated diseases. This is corroborated by the fact that the mRNA containing the frequent c.649dupC mutation is degraded by nonsense-mediated decay and is therefore not translated into a protein. The other frameshift changes are predicted to have the same effect or to lead to a truncated protein that might undergo rapid degradation and is unable to bind interacting proteins, such as SNAP25. Whether a dominant-negative mechanism exists with some of the other mutations (e.g. missense changes, mutations causing *PRRT2* extension, or truncating changes affecting exon 3 that are unlikely to undergo nonsense-mediated decay) remains to be tested experimentally. One could envision a scenario where a dysfunctional, truncated or misfolded protein product would interfere with the function (e.g. with functionally relevant interactions with other proteins) of the full-length wild-type protein encoded by the unaffected allele.

- **What are interacting proteins of *PRRT2* and how does *PRRT2* regulate 'excitability' of synapses?**

Hypothesis: Loss-of-function mutations in *PRRT2* lead to increased neuronal excitability in vulnerable brain regions through a decreased interaction with proteins involved in synaptic transmission. This is corroborated by data showing that *PRRT2* localizes to glutamatergic synapses and associates with the glutamate receptor GRIN1A, a member of the AMPA receptor family. In addition, *PRRT2* has been shown to interact with the synaptic t-SNARE protein SNAP25, pointing to a possible role in synaptic vesicle docking and neurotransmitter release. Parallels can be drawn to other genetic paroxysmal diseases such as PNKD (or MR1)-associated PNKD as discussed above. Another precedence for dysregulated neuronal excitability in paroxysmal diseases is set by the genetic channelopathies that can cause epilepsy or movement disorders.

- **How can the same mutation cause seizures in infants and movement and headache disorders in adults and what predicts these age-dependent cortical and subcortical disease manifestations?**

Hypothesis: The age-dependent manifestations of *PRRT2*-associated diseases correlate with the spatial and temporal expression pattern of *PRRT2* and/or functionally relevant interacting proteins. A switch in factors that influence neuronal excitability and synaptic transmission in general, such as for example age-dependent changes in expression of ion channel subsets in relevant brain regions, may also influence the clinical manifestation of *PRRT2* mutations. A precedence for this is set for example by genetic channelopathies such as benign familial neonatal-infantile seizures, where the temporal expression pattern of mutant $Na_v1.2$ has been shown to drive the age-dependent, in this case the neonatal, manifestations of seizures.

(continued)

Box 2 Continued

- **What genetic, epigenetic or environmental factors influence clinical expression, penetrance and pleiotropy?**

  Hypothesis: Genetic and/or environmental factors critically influence the clinical expression of *PRRT2*-associated diseases. This is corroborated by the concordance of disease manifestations in the very few twin pairs reported with *PRRT2* mutations. Clear genotype–phenotype correlations, however, have thus far not been established in *PRRT2*-associated disease, but might become evident as the phenotypic spectrum is further delineated. Environmental factors that directly influence the clinical expression of *PRRT2*-associated diseases remain to be identified.

- **Do novel *PRRT2*-associated diseases readily respond to treatment with anticonvulsants in the same way that classic PKD attacks do?**

  Hypothesis: The benefit of anticonvulsants in *PRRT2*-associated diseases can be attributed to a global suppression of neuronal hyperexcitability, rather than a *PRRT2* specific molecular mechanism. However, it remains to be established whether novel *PRRT2*-associated diseases, such as for example hemiplegic migraine attacks, respond to classic anticonvulsants in the same way that classic PKD attacks do. Anecdotal evidence, mostly from small retrospective studies, is emerging.

that all but two of the reported changes are likely pathogenic (with CADD scores >15, Supplementary Table 1). About two-thirds of the mutations have very high scores ranging from 25 to 37.

Although frameshift mutations spread across the entire *PRRT2* gene, a cluster of missense changes involving the two putative transmembrane domains is readily apparent (Fig. 1B). Due to the order of domains within PRRT2, truncating changes that localize to the proline-rich domain will subsequently also affect the transmembrane regions. Therefore, the number of missense mutations in a given domain better reflects the functional importance of this region: only seven missense mutations (changing five amino acid residues) affect the proline-rich region (CADD scores between 16.61–27.30) versus 14 (changing 12 amino acid residues) in the transmembrane regions (CADD scores between 22.80–33.00) suggesting that the latter plays an important role in PRRT2's biological function. Of note, of the 22 reported missense mutations, 16 are not found or are found only once in more than 60 000 exomes from the ExAC database (http://exac.broadinstitute.org/), indicating the high likelihood of their pathogenicity. Six missense changes, however, all affecting the putative proline-rich region (p.Asp147His, p.Ala214Pro, p.Pro215Arg, p.Pro216Arg, p.Pro216His, p.Pro216Leu), are present multiple times (109, 97, 77, 11, 38 and 764 times in a heterozygous state, respectively). Thus, it remains unclear whether these changes indeed represent pathogenic mutations or simply polymorphisms with functional significance (as indicated by their high CADD scores, Supplementary Table 1).

Most mutations, including the most prevalent c.649dupC mutation, lead to unstable messenger RNA or a truncated protein product that undergoes rapid degradation (Chen *et al.*, 2011; Lee *et al.*, 2012*a*; Wu *et al.*, 2014). This haploinsufficient state is consistent with a loss-of-function mechanism in *PRRT2*-associated diseases. Rare cases of large contiguous microdeletions that affect *PRRT2* (Supplementary Table 4) further support this hypothesis (Lipton and Rivkin, 2009; Dale *et al.*, 2011, 2012*a*; Silveira-Moriyama *et al.*, 2013; Weber *et al.*, 2013; Termsarasab *et al.*, 2014). Interestingly, except for these rare cases, PKD is not frequently reported in patients with 16p11.2 deletion syndrome, who may present with complex phenotypes of multiple congenital abnormalities, developmental delay, epilepsy, autism spectrum disorder and are at increased risk for obesity and psychiatric diseases later in life (Weiss *et al.*, 2008; Jacquemont *et al.*, 2011; Guha *et al.*, 2013; Reinthaler *et al.*, 2014; Hanson *et al.*, 2015).

While most *PRRT2* mutations are localized to protein-coding parts of the gene, a few intronic or splice site mutations have been reported (Supplementary Table 1). Completing the spectrum of possible *PRRT2* mutations, 15 patients with homozygous or compound-heterozygous mutations have been reported (Supplementary Table 5) (Najmabadi *et al.*, 2011; Labate *et al.*, 2012; Liu *et al.*, 2013; Chen *et al.*, 2014; Delcourt *et al.*, 2015). Interestingly, many of these carry a homozygous c.649dupC mutation ($n = 10$) or c.649dupC in combination with a deletion of the other *PRRT2* allele ($n = 1$) and are thus predicted to lack PRRT2 completely.

Further delineation of the clinical and genetic spectrum of *PRRT2* mutations in humans through next-generation sequencing and advanced phenotyping is clearly necessary. In addition, generation of suitable disease models for *in vitro* (e.g. neuronal cells differentiated from patient-derived induced pluripotent stem cells) and *in vivo* (knock-in or knock-out rodent models) studies will be instrumental for moving the field forward. New lines of experiments will become available and will delineate the molecular mechanisms that lead to neuronal hyperexcitability. Findings in *PRRT2*-associated diseases might thus benefit research into related diseases including different forms of seizure disorders, hyperkinetic movement disorders and primary headache disorders.

PRRT2 mutations and the BFIE–PKD–PKD/IC spectrum

The three diseases BFIE, PKD and PKD/IC form a continuous disease spectrum and the core of *PRRT2*-associated

disorders (Chen *et al.*, 2011; Wang *et al.*, 2011; Heron *et al.*, 2012; Lee *et al.*, 2012*a*; Ono *et al.*, 2012) (Fig. 3, Table 1 and Supplementary Table 3). *PRRT2* mutations account for the majority of familial BFIE, PKD and PKD/IC patients, thus genetic testing can support a clinical diagnosis in atypical or complex cases. Of 1444 reported *PRRT2* patients, 1368 (94.7%) have a diagnosis within the BFIE–PKD/IC–PKD spectrum, leaving only 76 (5.3%) patients with an alternative primary diagnosis (Table 2). *PRRT2* mutations in BFIE and PKD are inherited in an autosomal-dominant manner and affect both females and males in populations with various ethnic backgrounds (Table 1). Interestingly, more male patients have been reported in PKD [62.8% (322/513) males versus 37.2% (191/513) females], while PKD/IC and BFIE distribute more evenly between male and female patients (Table 1). A positive clinical and genetic family history is present in ~80% of PKD (446/560 and 427/560, respectively) and >95% of BFIE patients (586/602 and 583/602, respectively) and the vast majority carry the c.649dupC mutation (Table 1). We herein discuss general clinical features of BFIE–PKD/IC–PKD and summarize disease characteristics of all published *PRRT2*-associated cases (see Supplementary material and Supplementary Table 2 for a description of the methodology, selected references and additional data).

Benign familial infantile epilepsy

BFIE (OMIM #605751) is a self-limiting seizure disorder in infants characterized by non-febrile seizures that usually begin between 3 and 12 months of age (6.0 ± 2.9 months) and remit by 2 years of age (11.2 ± 15.5 months for *PRRT2*-associated cases, Table 3). Interestingly, the latter seems to be shifted towards a later remission of seizures in cases of PKD/IC (17.4 ± 13.4 months). BFIE-related seizures usually occur in clusters of complex-partial or generalized tonic-clonic seizures (Watanabe *et al.*, 1987). Brief spells with motor arrest, eye or head deviation, cyanosis, generalized hypertonia and limb jerks are commonly reported. The ictal EEG will often show parieto-occipital epileptic activity that may eventually generalize (Callenbach *et al.*, 2002). The interictal neurological exam, EEG and MRI studies are usually normal. In the majority of cases, seizures will readily respond to treatment with conventional anticonvulsive drugs (Table 3). Remission rates for treated cases are ~98% in BFIE and ~89% in PKD/IC leaving only a small subset with a partial response to therapy (Table 3). Whether this reflects the natural disease history (which is likely) or can be attributed to treatment with anticonvulsive agents remains unknown. Therapy with phenobarbital, carbamazepine or valproate has been frequently reported and a combination of two or more anticonvulsants is rarely necessary. BFIE has no known long-term neurological sequelae and development is usually normal.

Mutations in *PRRT2* have been identified in the majority of families with BFIE, resulting in 602 patients with

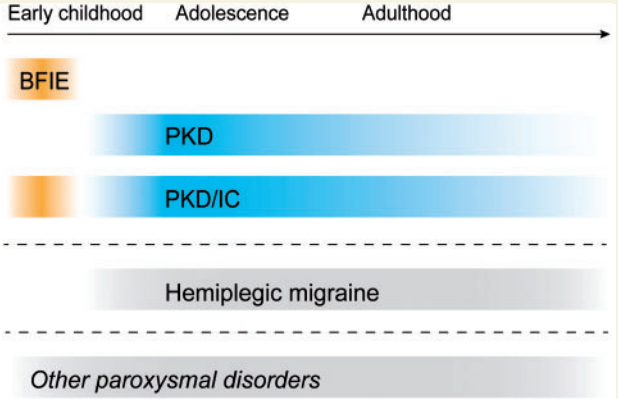

Figure 3 Natural history of *PRRT2*-associated diseases. While BFIE, PKD and PKD/IC form the core of *PRRT2*-associated diseases, hemiplegic migraine emerges as novel associated disease. *PRRT2*-associated BFIE manifests with non-febrile seizures in the first year of life with an average age of onset ~6 months (6.0 ± 2.9 months, *n* = 401). Onset of *PRRT2*-associated PKD peaks in early adolescence (10.3 ± 4.9 years, *n* = 283) with PKD/IC patients showing a trend towards earlier onset of PKD symptoms (9.1 ± 4.4 years, *n* = 108). PKD attacks often decrease in frequency during adulthood and may remit completely in mid- or late adulthood. To date, *PRRT2* mutations have been reported in 34 patients with hemiplegic migraine, with an average onset of 11.5 (±3.7) years in the 14 patients with data available. Other movement, seizure or headache disorders may also manifest in patients with *PRRT2* mutation carriers although a firm association still remains to be established.

PRRT2-associated BFIE reported to date (Table 1 and Supplementary Table 3). These mainly come from large cohorts from Europe, China, Japan and Australia (70.5% Caucasian, 28.5% Asian; Table 1). The c.649dupC frameshift mutation is found in ~81.4% of cases. Other common mutations include a deletion at the same position (c.649delC; ~4%) and at a more proximal residue c.291delC (~2%). The remaining 30 less frequent pathogenic, BFIE-associated *PRRT2* mutations can be found in Supplementary Table 6. Mutations run in families in >95% of cases and only a small subset of *de novo* sporadic mutations has been discovered (~1.3%, Table 1). At present, there is no evidence to suggest any genotype-phenotype correlations.

Paroxysmal kinesigenic dyskinesia

PKD (OMIM #128200) is a paroxysmal movement disorder that affects ~1:150 000 in the general population (Spacey and Adams, 2013). Although considered rare overall, PKD is the most frequent type of paroxysmal dyskinesia. Symptoms most commonly manifest shortly before or during puberty in *PRRT2* mutation carriers, with an average age of onset of 10.3 ± 4.9 years for isolated PKD (range 1–42 years) and 9.1 ± 4.4 years for patients with PKD/IC (range 0–20 years) (Table 3). In a mixed Asian population with a high proportion of sporadic cases, *PRRT2* mutation carriers manifested at an earlier age

Table 1 Demographic and genetic characteristics of *PRRT2*-associated PKD, PKD/IC and BFIE (total number of patients: 560, 206, 602)

	PKD	PKD/IC	BFIE
Sex (M:F) (n = 513; 186; 585)	62.8% (322):37.2% (191)	57.5% (107):42.5% (79)	46.5% (272):53.5% (313)
Ethnicity (n = 443; 167; 393)	Asian 58.5% (259) Caucasian 33.9% (150) Black 4.7% (21) Mixed 2.9% (13)	Caucasian 50.3% (84) Asian 45.5% (76) Mixed 2.4% (4) Black 1.8% (3)	Caucasian 70.5% (277) Asian 28.5% (112) Mixed 1.0% (4)
Country of origin (n = 436; 165; 420)[a]	China 39.7% (173) USA 12.4% (54) UK 11.2% (49) Japan 8.9% (39) Taiwan 3.7% (16)	China 20.0% (33) Italy 13.3% (22) Taiwan 12.7% (21) USA 9.7% (16) Finland 8.5% (14) Japan 8.5% (14)	Germany 21.2% (89) Italy 17.6% (74) China 13.8% (58) Japan 11.4% (48) Australia 11.0% (46)
Family history (n = 560; 206; 602)	Yes 79.7% (446) No 12.3% (69) Unknown 8.0% (45)	Yes 93.2% (192) No 3.9% (8) Unknown 2.9% (6)	Yes 97.3% (586) No 1.5% (9) Unknown 1.2% (7)
Common *PRRT2* mutations (n = 560; 207; 602)[b]	c.649dupC 80.5% (451) c.649delC 1.8% (10) c.718C>T 1.8% (10) c.649C>T 1.6% (9)	c.649dupC 73.8% (152) c.579dupA 3.9% (8) c.649C>T 3.4% (7) c.291delC 2.9% (6) c.604_607delTCAC 2.9% (6) c.649delC 2.9% (6) c.718C>T 1.9% (4) 16p11.2 microdeletion 1.5% (3) c.649dupC (biallelic) 1.5% (3) c.487C>T 1.0% (2) c.516dupT 1.0% (2) c.904dupG 1.0% (2)	c.649dupC 81.4% (490) c.649delC 4.3% (26) c.291delC 1.8% (11) c.879+5G>A 1.3% (8) c.718C>T 1.2% (7)
Familial *PRRT2* mutation (n = 560; 206; 602)	Yes 76.3% (427) No 9.6% (54) Unknown 14.1% (79)	Yes 90.8% (187) No 3.4% (7) Unknown 5.8% (12)	Yes 96.9% (583) No 1.3% (8) Unknown 1.8% (11)

[a] Showing the five most common (please refer to Supplementary material for the complete dataset).
[b] Mutations affecting ⩾1% are shown (please refer to Supplementary material for the complete dataset).

than non-mutation carriers (mean of 10 versus 12.5 years) (Tan *et al.*, 2014). A similar relationship was found in a European population (Meneret *et al.*, 2012). Only ∼5% of all published *PRRT2*-associated cases have an onset after 18 years of age, putting the paediatric neurologist at the forefront of diagnosing and treating PKD (Table 3). Like BFIE, PKD is an autosomal-dominant disease making it a familial disease in the majority of cases (in ∼75%, Table 1). Sporadic cases have also been described, and are potentially even more frequent than currently reported (∼10%, Table 1) since gene identification studies preferentially focus on familial cases. Disease penetrance is estimated at 60–90% (van Vliet *et al.*, 2012), leading to unaffected or only mildly affected family members despite the presence of a known pathogenic mutation or a full manifestation in an index case. In addition to primary familial PKD (termed PxMD-*PRRT2* according to novel guidelines, Marras *et al.*, unpublished), sporadic cases with PKD or PKD-like phenotypes have been reported secondary to a wide range of neurological diseases that affect basal ganglia integrity. This includes CNS tumours, stroke, traumatic brain injury, encephalitis, demyelinating diseases, perinatal hypoxic encephalopathy, and rare cases of pseudohyperparathyroidism with basal ganglia calcification, idiopathic striopallidodendate calcinosis, moya-moya disease or central pontine myelinolysis (Roos *et al.*, 1991; Demirkiran and Jankovic, 1995; de Seze *et al.*, 2000; Baba *et al.*, 2003; Gonzalez-Alegre *et al.*, 2003; Chiesa *et al.*, 2008; Diaz *et al.*, 2010; Thomas *et al.*, 2010; Chung *et al.*, 2012).

Clinically, PKD is characterized by short and frequent attacks of unilateral or bilateral hyperkinetic movements that are precipitated by sudden voluntary movements such as initiation of walking, rising from a chair, or being startled. Diagnostic criteria for PKD have been proposed by Bruno *et al.* (2004) (Box 1) and emphasize the presence of an identifiable kinesigenic trigger. The attacks consist of brief and stereotypical episodes of dystonia, chorea and athetosis, often in combination. In

Table 2 Primary diagnosis in *PRRT2* mutation carriers without a diagnosis of PKD, PKD/IC or BFIE (*n* = 76)

Movement disorders	PNKD / 'PNKD-like' (*n* = 2)
	PED (*n* = 2)
Seizure disorders	Febrile seizures (*n* = 15)
	Febrile seizures plus (*n* = 6)
	Epilepsy (not specified) (*n* = 11)
	Dravet syndrome (*n* = 4)
	Generalized epilepsy with febrile seizures plus (*n* = 1)
	Nocturnal convulsions (*n* = 2)
	West syndrome (*n* = 1)
Headache disorders	Hemiplegic migraine (*n* = 19)
	Migraine with aura (*n* = 4)
	Migraine without aura (*n* = 3)
	Non-migrainous headaches (*n* = 1)
Developmental delay and intellectual disability	Non-syndromic intellectual disability (*n* = 5)

PRRT2-associated cases of PKD and PKD/IC, dystonia is the most commonly reported manifestation followed by chorea and athetosis (Table 3). Interesting less frequently described phenotypes include ballism (Cloarec *et al*., 2012; Fusco *et al*., 2014) and hemiballism (Zhang *et al*., 2015), tongue movements, perioral dyskinesias, clawing of the hands (Silveira-Moriyama *et al*., 2013) or frozen gaze (Weber *et al*., 2013). A more detailed characterization of *PRRT2*-associated movement phenotypes might reveal additional clinical manifestations and will need to parallel advances seen in genetic investigations, as many genetic studies report on the most common motor phenotypes only.

Most patients experience bilateral attacks (~3.5-fold more common than unilateral in PKD) although a greater relative proportion of unilateral manifestations are reported in PKD/IC compared to PKD (Table 3). Regardless of the laterality, the upper limbs are most commonly affected, followed by the lower limbs, face (PKD) or trunk (PKD/IC). The neck is the least commonly affected body region (Table 3). A tendency towards a more frequent manifestation on the left side is evident in affected limbs. Attacks involving the face and neck region are important as they can lead to speech disturbance in the form of dysarthria or even anarthria (Houser *et al*., 1999).

A precipitating aura preceding attacks by a variable time period is frequently reported (11.4% in PKD, 6.8% in PKD/IC) and can help the patient to anticipate or even control arising attacks (Table 3). Aura symptoms can consist of a crawling sensation in the affected limb, paraesthesias, or non-specific epigastric discomfort (Bruno *et al*., 2004). Stress, sleep deprivation and anxiety are consistently being reported as factors that increase the likelihood for PKD episodes and sometimes even the mere intention to move, without an actual appreciable voluntary movement, can trigger attacks. Attack frequency ranges from more than 100 per day to as few as one every couple of weeks. In *PRRT2* mutation carriers with PKD or PKD/IC

Table 3 Clinical characteristics of seizures in *PRRT2*-associated BFIE and PKD/IC, and of PKD in *PRRT2*-associated PKD and PKD/IC

	BFIE, *n* = 602	**PKD/IC, *n* = 206**
Age of onset – Infantile seizures (Mean ± SD)	6.0 ± 2.9 months (*n* = 401)	6.3 ± 3.2 months (*n* = 108)
Age at remission – Infantile seizures (Mean ± SD)	11.2 ± 15.5 months (*n* = 241)	17.4 ± 13.4 months (*n* = 52)
Treatment	PB (63); CBZ (40); VPA (35); PRI (11); PHE (7); LD (4); VPA and PB (4); VPA and PHE (3); VPA and CBZ (3); LEV (1); STM (1); TPM (1); PHE and STM (1); CBZ and STM (1); VPA and ETX (1); CBZ and PHE (1)	PB (18); CBZ (15); VPA (9); PHE (3); LTG and AZ (2); PB and PHE (1); PB and PHE and VPA (1)
Response to treatment / clinical course	Remission 97.8% Partial response 2.2% (*n* = 90)	Remission 88.9% Partial response 11.1% (*n* = 18)
	PKD, *n* = 560	**PKD/IC, *n* = 206**
Age of onset - PKD (Mean ± SD)	10.3 ± 4.9 years (*n* = 283)	9.1 ± 4.4 years (*n* = 108)
Adult onset (⩾18 years of age)	5.3% (15/283)	3.7% (4/108)
Phenomenology index[a] dystonia/chorea/athetosis	1.0/0.96/0.42	1.0/0.75/0.21
Bilateral:unilateral	3.7:1 (*n* = 173)	1.6:1 (*n* = 37)
Involved body area (*n*)	UL (65) > LL (52) > Face (25) > Trunk (22) > Neck (17)	UL (22) > LL (18) > Trunk (8) > Face (5) > Neck (2)
Upper limbs – R:L ratio	1:1.2	1:0.9
Lower limbs – R:L ratio	1:1.3	1:1.1
Sensory aura	11.4% (64/560)	6.8% (14/206)
Maximum duration of attacks (mean ± SD)	28.1 ± 23.1 s (*n* = 173)	27.4 ± 21.7 s (*n* = 62)
Maximum frequency per day (mean ± SD)	11.0 ± 14.5 (*n* = 107)	14.2 ± 26.0 (*n* = 49)
Definite treatment (*n*)	CBZ (122); PHE (8); LTG (2); VPA (2); PB and PHE (2); CLZ (1); OXC (1); PB (1); CBZ and VPA (1); LEV and LCM (1); OCX and LTG (1)	CBZ (42); PHE (8); VPA (8); OXC (5); LTG (3): PHE (3); CBZ and CLZ (2); LGT and AZ (2); TPM (1); CBZ and PHE (1); PHE and PB (1)
Response to definite treatment	Complete 92.7% Partial 7.3%	Complete 98.1% Partial 1.9%

[a]Phenomenology Index shows the relative frequency of motor phenotypes in relation to the most prevalent phenotype.

AZ = acetazolamide; CBZ = carbamazepine; CLZ = clonazepam; ETX = ethosuximide; L = left; LCM = lacosamide; LD = lidocaine; LEV = levetiracetam; LL = lower limbs; LTG = lamotrigine; OXC = oxcarbazepine; PB = phenobarbital; PHE = phenytoin; PRI = primidone; R = right; STM = sulthiame; TPM = topiramate; UL = upper limbs; VPA = valproate.

the mean maximum frequency per day is 11.0 ± 14.5 (range <1 to 100) and 14.2 ± 26.0 (range <1 to 100), respectively (Table 3). A decline in attack frequency is usually seen along the natural history of the disease and many patients report infrequent attacks or even a complete remission during adulthood. Improvement during pregnancy has also been observed in one study (Bruno *et al.*, 2004). Attacks usually last for less than a minute with an average ~30 s in PKD (range 2–180 s) and PKD/IC (range 5–60 s) (Table 3). Longer attacks, although rarely reported in PKD, point to other types of paroxysmal dyskinesia or a completely unrelated aetiology.

Importantly, PKD attacks do not occur during sleep and never involve loss of consciousness, pain or weakness. Although infrequent, falls and injuries can occur and may help to distinguish PKD from functional movement disorders which are a common, yet sometimes challenging, differential diagnosis, particularly when occurring as a 'phenocopy' within families with classic PKD (Ebrahimi-Fakhari *et al.*, 2014). The interictal exam in PKD is usually unremarkable, underscoring the importance of a thorough history and an exam that includes triggering manoeuvres. If no attacks can be elicited during the office visit, patients should be encouraged to videotape characteristic episodes. Diagnostic tests such as EEG and MRI do not show any distinct abnormalities in primary PKD. The few reported ictal and interictal functional, perfusion and diffusion tensor imaging studies (Ko *et al.*, 2001; Shirane *et al.*, 2001; Joo *et al.*, 2005; Zhou *et al.*, 2010; Kim *et al.*, 2011, 2015) point to abnormalities in the cortico-striato-pallido-thalamic circuitry although the diagnostic and pathophysiological implications remain limited. Importantly, the question of the origin of PKD attacks (cortical versus subcortical) remains unanswered.

In addition to secondary causes of PKD, which can be distinguished based on laboratory or imaging studies, conditions exist that mimic a PKD phenotype. This includes the other paroxysmal dyskinesias, PNKD, PED and paroxysmal hypnogenic dyskinesia, although they lack the kinesigenic trigger and have other unique characteristics as discussed below. Additional differential diagnoses that can present with paroxysmal hyperkinetic movements and mimic PKD include seizures, metabolic diseases such as thyrotoxicosis, tics and stereotypies, Sydenham chorea, hemifacial spasms, and perhaps most importantly functional disorders such as psychogenic non-epileptic seizures and functional movement disorders (Ebrahimi-Fakhari *et al.*, 2014). If uncertainties remain despite a thorough history and clinical exam, additional tests including video-EEG, cranial MRI and laboratory tests may help to facilitate an alternative diagnosis.

Rapid response of attacks to anticonvulsants is another important diagnostic clue, as this is characteristic for the vast majority of PKD patients. Carbamazepine is the drug of choice and dosages that are much lower than those used to treat epilepsy (e.g. 50–200 mg/day) are usually sufficient. Other anticonvulsive agents may also be effective, including phenytoin, valproate, oxcarbazepine, lamotrigine, levetiracetam or topiramate. In *PRRT2*-associated cases, carbamazepine is the most frequently used drug and a complete response is seen in 92.7% of PKD and 98.1% of PKD/IC patients (Table 3). A recent study involving patients who carried one of four *PRRT2* mutations (c.649dupC, c.514_517delTCTG, c.972delA, c.649delC) set out to investigate whether a correlation between *PRRT2* mutation status and a response to carbamazepine might exist (Li *et al.*, 2013*b*). Twelve *PRRT2* mutation carriers were treated with carbamazepine and readily responded to a dose of 100 mg/day and remained asymptomatic even when the dosage was reduced to 50 mg/day (Li *et al.*, 2013*b*). Of the PKD patients in this study in whom no *PRRT2* mutation could be identified, 20 received carbamazepine at 100 mg/day but only two patients achieved complete remission. Although larger studies are clearly necessary to confirm this, these findings, in addition to observations from smaller cohorts (Mao *et al.*, 2014) and anecdotal evidence, suggest that patients with common *PRRT2* mutations are more likely to achieve remission through treatment with carbamazepine and might even require lower dosages. Interestingly, a few rare historic patients (Loong and Ong, 1973), along with a more recent atypical 16p11.2 microdeletion-associated case (Lipton and Rivkin, 2009), showed improvement to levodopa although no response was seen in others (Garello *et al.*, 1983).

Since attack frequency decreases with age and no long-term neurological sequelae have been reported, treatment may eventually be tapered or even discontinued in adult patients. Anecdotal experience suggests that patients are often willing to accept an occasional attack in return for discontinuing anticonvulsants. In any case, the patient's individual circumstances and expectations should be discussed and the therapy plan should be tailored accordingly.

Paroxysmal kinesigenic dyskinesia with infantile convulsions

PKD/IC (OMIM #602066) is the third disease within the BFIE–PKD–PKD/IC spectrum and is characterized by the presence of BFIE and PKD in the same patient. More than 200 PKD/IC patients with *PRRT2* mutations have been reported to date (Table 1 and 3B). Disease characteristics and genetic features largely overlap with those of isolated cases of BFIE and PKD, as discussed above. Interestingly, the c.579dupA duplication mutation seems to be more commonly present in PKD/IC. A typical case history of a patient with PKD/IC is illustrated in the Supplementary material.

In summary, BFIE, PKD and PKD/IC form a continuous disease spectrum and the core of *PRRT2*-associated diseases (Fig. 3). The natural evolution from infantile seizures to a paroxysmal movement disorder in adolescents is peculiar. However, both ends of the spectrum share many key features, namely their paroxysmal and stereotypical character, benign nature, excellent response to anticonvulsants and favourable prognosis.

Genotype–phenotype correlations in *PRRT2*-associated BFIE–PKD–PKD/IC

Despite the discovery and extensive characterization of *PRRT2* mutations, no clear evidence for genotype-phenotype correlations exists for any of the three phenotypes. The many families in which the same *PRRT2* mutation can cause BFIE alone, PKD alone or PKD/IC exemplify the lack of such a correlation (see case vignette in the online Supplementary material). Age of onset and disease severity vary considerably, too. This strongly suggests that other factors critically influence disease expressivity (Box 2). Interestingly, the 604-607delTCAC (p.S202Hfs*25) frameshift mutation reported in one three-generation Han-Chinese family (Zhang *et al.*, 2015) seems to cause PKD/IC with an early onset of PKD at ~4 years of age (3.75 ± 2.2, $n = 4$), although this awaits confirmation in additional kindreds. PKD and PKD/IC patients with homozygous (Labate *et al.*, 2012; Delcourt *et al.*, 2015) or compound-heterozygous (Liu *et al.*, 2013; Chen *et al.*, 2014; Delcourt *et al.*, 2015) mutations may also show a distinct phenotype as they more often present with additional symptoms (Supplementary Table 5). Biallelic mutations lead to increased disease severity with persistent PKD attacks and additional manifestations such as intellectual disability, attention-deficit hyperactivity disorder, absence epilepsy, migraine, and in some cases paroxysmal non-kinesigenic dyskinesias or episodic ataxia (Supplementary Tables 5, 7, 8 and 9) (Labate *et al.*, 2012, Delcourt *et al.*, 2015). The clustering of paroxysmal diseases in patients with biallelic loss of *PRRT2* suggests a gene dosage effect, although experimental proof through transgenic animal models is needed to formally test this hypothesis. Curiously, two patients with homozygous mutations showed cerebellar atrophy on MRI (Delcourt *et al.*, 2015).

In addition to *PRRT2*, other genetic causes of PKD (and BFIE) are suspected given that not all familial or sporadic PKD cases have been linked to the *PRRT2* locus on chromosome 16p11.2 (Valente *et al.*, 2000; Spacey *et al.*, 2002; Zhou *et al.*, 2008; Chen *et al.*, 2011; Cao *et al.*, 2012; Gardiner *et al.*, 2012; Groffen *et al.*, 2013). However, these putative causative genes remain to be identified.

Given the high prevalence of *PRRT2* mutations among patients with BFIE, PKD or PKD/IC, sequence analysis can be considered to establish or confirm a clinical diagnosis (Box 1). If no pathogenic sequence variant is found, testing for deletions or duplications should follow. Confirmation of *PRRT2* mutations in patients with typical infantile seizures can re-assure parents and caregivers that the seizures are likely going to be self-limited. Establishing an early genetic diagnosis can also help to appropriately advise and educate families about the possibility of PKD later in life and can thus facilitate an early diagnosis and treatment. Despite the high yield of genetic testing, however, the diagnosis of PKD is still made on clinical grounds and failure to identify a pathogenic *PRRT2* mutation does not exclude the diagnosis (Box 1). A diagnostic algorithm that includes genetic testing has been introduced recently (Erro *et al.*, 2014).

PRRT2 mutations in other movement disorders

Although PKD is by far the most common movement disorder phenotype associated with mutations in *PRRT2*, the clinical spectrum has expanded to include other paroxysmal movement disorders (Supplementary Table 7). Perhaps most importantly, *PRRT2* mutations have been found in a few patients clinically described as having PNKD or PED, sometimes in addition to PKD. PNKD is characterized by paroxysmal attacks of dystonic or choreiform movements that show no discernible kinesigenic trigger and are often of much longer duration (minutes to hours) than attacks in PKD. PNKD attacks occur at rest and are frequently precipitated by emotional stress, caffeine or alcohol consumption. While these features can readily distinguish PNKD and PKD, patients may sometimes present with attacks of both kinds. Although no diagnosis of PNKD was established, 8 of 11 childhood-onset, *PRRT2*-associated PKD patients had attacks at rest in addition to those triggered by sudden movements (Silveira-Moriyama *et al.*, 2013). A PNKD-like phenotype was described in one family carrying the c.649dupC mutation (Liu *et al.*, 2012) and a second family with the same mutation had attacks somewhat consistent with PNKD as they occurred at rest (Wang *et al.*, 2013). PNKD-like attacks were reported in a female patient with a c.884G>A mutation and late-onset PKD in her forties, in addition to two PKD/IC patients who had concomitant PKD and PNKD attacks since adolescence (Becker *et al.*, 2013). Finally, a patient with a homozygous loss-of-function mutation in *PRRT2* had PNKD attacks (Delcourt *et al.*, 2015). Despite these anecdotal reports (Supplementary Table 7), *PRRT2* mutations were absent in four PNKD cases (Groffen *et al.*, 2013). While the relationship between *PRRT2* mutations and PNKD thus remains unclear, similarities in the pathophysiology exist. Mutations in the *PNKD* gene (formerly *MR1*) are the established genetic cause for the majority of PNKD cases (Lee *et al.*, 2004; Rainier *et al.*, 2004). The encoded PNKD protein associates with membranes and is expressed in neurons where it enriches in pre- and postsynaptic preparations (Lee *et al.*, 2004, 2012*b*). Very recently, PNKD was demonstrated to interact with synaptic active zone proteins Rab3-interacting molecule (RIM) 1 and 2 and to modulate neurotransmitter release (Shen *et al.*, 2015). Overexpression of PNKD mitigated neurotransmitter release, and mutant PNKD protein was found to be less effective than wild-type protein at inhibiting exocytosis. Based on these findings, altered release of synaptic neurotransmitter vesicles and increased neuronal hyperexcitability, similar to the scenario in *PRRT2*-associated diseases (Fig. 2C), has been

postulated as the main disease mechanism in PNKD (Lee *et al.*, 2015).

Among the diseases that have been mapped to the same pericentromeric region of chromosome 16 as PKD, Rolandic epilepsy with PED and writer's cramp (RE-PED-WC, OMIM #608105) has similarities to PKD/IC (Guerrini *et al.*, 1999). In addition, the co-occurrence of either PED or writer's cramp with *PRRT2*-associated PKD suggests clinical overlaps between these paroxysmal diseases, although only a few patients have been reported to date (Supplementary Table 7) (Liu *et al.*, 2012; van Vliet *et al.*, 2012; Youn *et al.*, 2014). Beyond the paroxysmal dyskinesias, through screening 182 patients with episodic ataxia, one patient was identified to carry the common c.649dupC mutation (Gardiner *et al.*, 2012). Curiously, the same patient manifested with hemiplegic migraine, a potential novel *PRRT2*-associated phenotype as discussed below. Interestingly, 4 of 11 patients with homozygous *PRRT2* mutations presented with episodic ataxia (Supplementary Tables 5 and 7) (Labate *et al.*, 2012; Delcourt *et al.*, 2015). Concluding the current spectrum of movement disorders potentially associated with *PRRT2*, Dale *et al.* (2012*b*) reported a patient with the c.649dupC mutation who manifested with transient paroxysmal torticollis and later with BFIE.

PRRT2 mutations in other seizure disorders

The association of movement disorders and epilepsy is well established in CNS channelopathies indicating overlapping disease pathways and clinical manifestations (Kullmann and Waxman, 2010; Russell *et al.*, 2013; Lee *et al.*, 2015). While BFIE is characterized by non-febrile seizures, the relatively frequent history of febrile seizures in patients with *PRRT2* mutations has gained considerable attention (Marini *et al.*, 2012; Scheffer *et al.*, 2012; Schubert *et al.*, 2012; van Vliet *et al.*, 2012; Labate *et al.*, 2013; Okumura *et al.*, 2013; Yang *et al.*, 2013*b*; Djemie *et al.*, 2014; He *et al.*, 2014; Zheng *et al.*, 2014). Including one case of generalized epilepsy with febrile seizures plus (GEFS+) and six cases of febrile seizures plus, febrile seizures have been reported in 51 of 1444 *PRRT2* patients (3.5%; Supplementary Table 8). This matches the general prevalence of febrile seizures reported for children between 6 months and 5 years of age (2–5%; Hauser, 1994). Thus it appears that the association of *PRRT2* mutations with febrile seizures is more coincidental than causal. In addition, febrile seizures are more common in patients with epilepsy suggesting that even if a slight increase in febrile seizures existed, this could be explained by higher rates of epilepsy (BFIE and others) rather than by an actual effect of *PRRT2* mutations.

Whether *PRRT2* mutations lower the threshold for other kinds of seizures or epilepsy in general is another interesting question. In 53 *PRRT2* patients seizures occurred in contexts other than BFIE or febrile seizures. Of these, epilepsy in 42 patients was not further specified and mostly reflected a history of complex-partial seizures or more commonly generalized tonic-clonic seizures (Supplementary Table 8). Interestingly, five patients were diagnosed with absence seizures and six had non-convulsive seizures. Of those with additional information available, four were diagnosed with severe myoclonic epilepsy of infancy (SMIE or Dravet syndrome; all from one study) (He *et al.*, 2014), one patient had West syndrome (Djemie *et al.*, 2014) and one was diagnosed with Rolandic epilepsy (Cloarec *et al.*, 2012) (Supplementary Table 8). Studies that screened for *PRRT2* mutations in benign familial and sporadic infantile epilepsies almost uniformly conclude that *PRRT2* mutations are not a common cause for infantile epilepsies other than BFIE (Scheffer *et al.*, 2012; Ishii *et al.*, 2013; Okumura *et al.*, 2013; Zara *et al.*, 2013; Grinton *et al.*, 2015). Investigating the presence of *PRRT2* mutations in a broad spectrum of epilepsies, no association between *PRRT2* mutations and epileptic encephalopathies could be established (Heron and Dibbens, 2013; Djemie *et al.*, 2014). This is an important finding that can inform the rational use of genetic testing. In summary, *PRRT2* mutations are the cause of BFIE and PKD/IC but do not seem to play a major role in other types of epilepsy.

PRRT2 mutations in headache disorders

Neuronal hyperexcitability is a common theme in paroxysmal headache disorders. In large kindreds, co-segregation of different forms of migraines with PKD or PKD/IC has been noted for a long time (Singh *et al.*, 1999; Bruno *et al.*, 2004). Among *PRRT2* mutation carriers, 32 patients with migraine with aura and 36 with migraine without aura have been reported in detail, although this is likely an under-representation (Supplementary Table 9). In six PKD and PKD/IC families migraines co-segregated with *PRRT2* mutations (Cloarec *et al.*, 2012). Expanding this relationship, three other groups, at the same time, reported migraine-type headaches in a substantial number of *PRRT2* patients (Gardiner *et al.*, 2012; Marini *et al.*, 2012; Riant *et al.*, 2012). The majority of *PRRT2* patients with migraine had a concomitant diagnosis of PKD rather than BFIE, likely reflecting an age bias, as migraines tend to manifest in adolescence or young adulthood (Supplementary Table 9). Of those with aura, a visual aura was by far the most common manifestation followed by aphasia and speech disturbance. Common to migraines with aura, a considerable number of patients reported multiple kinds of auras. Triggers for migraine attacks included stress, anxiety, sleep deprivation, and exercise. While the link between *PRRT2* and paroxysmal headache disorders is compelling, whether *PRRT2* mutations predispose to migraines is difficult to discern given the high prevalence

in the general population (Jensen and Stovner, 2008). The high detection rates in some kindreds, however, suggests parallels to the well-established increased prevalence of migraines in PNKD (Bruno *et al*., 2007). A detailed and standardized assessment of migraine types in large and well-characterized cohorts of *PRRT2* mutation carriers, in addition to screening for *PRRT2* mutations in well-defined cohorts of patients with migraine will be able to definitively answer the question whether *PRRT2* mutations and subsequent neuronal hyperexcitability might manifest as migraine in a subset of patients.

Besides common forms of migraine, *PRRT2* has been associated with hemiplegic migraine, a peculiar, rare form of migraine characterized by the presence of a motor aura in the form of transient motor deficits, typically weakness (Russell and Ducros, 2011). Hemiplegic migraine can occur as a sporadic or autosomal-dominant familial disease. Typical hemiplegic migraine attacks begin during childhood or adolescence and, in addition to hemiparesis, can involve gradually worsening visual, sensory, or basilar-type symptoms, always accompanied by headaches (Russell and Ducros, 2011). Mutations in three ion transporter subunits (*CACNA1A*, *ATP1A2*, and *SCN1A*) are known to cause hemiplegic migraine, but do not cover a substantial portion of patients (Thomsen *et al*., 2007; Ferrari *et al*., 2015). This is particularly the case for sporadic cases in which the genetic aetiology often remains elusive. Previous reports and the paroxysmal nature of hemiplegic migraine prompted several investigators to study a possible role for *PRRT2* in this condition (Cloarec *et al*., 2012; Dale *et al*., 2012*b*; Gardiner *et al*., 2012; Marini *et al*., 2012; Riant *et al*., 2012; van Vliet *et al*., 2012; Castiglioni *et al*., 2013; Silveira-Moriyama *et al*., 2013; Djemie *et al*., 2014; Pelzer *et al*., 2014; Delcourt *et al*., 2015). Thirty-four hemiplegic migraine patients from different kindreds with *PRRT2* mutations have been identified to date (Supplementary Table 9). Of these, 19 did not have BFIE or PKD, thus hemiplegic migraine is the most common primary diagnosis in *PRRT2* mutation carriers in whom no diagnosis of BFIE or PKD can be established (Table 2). Frequent comorbidities in *PRRT2*-associated hemiplegic migraine include migraine with and without aura. Reported attack frequencies range from a maximum of three per week to one every 2 weeks and triggers are similar to those for migraine with aura. Interestingly, all hemiplegic migraine-associated *PRRT2* mutations locate to the c.649/650 residue: A c.649dupC mutation was found in 23 patients, the c.649delC mutation occurred in four and the c.650delG in another six patients, making this location the hotspot for hemiplegic migraine-associated *PRRT2* mutations. Providing evidence for a strong co-segregation of PKD and hemiplegic migraine, a phenotype consisting of typical PKD and hemiplegic migraine was found in monozygotic twin brothers carrying the c.649dupC mutation (Castiglioni *et al*., 2013). An interesting observation is the response of hemiplegic migraine attacks to carbamazepine therapy in one of the brothers, which is supported by a recent report of a patient with a similar beneficial response (Dale, 2014). Whether a response to carbamazepine is seen in other hemiplegic migraine patients with *PRRT2* mutations is currently unknown and warrants further investigations.

In summary, the increasing detection of hemiplegic migraine in families with *PRRT2*-associated PKD or PKD/IC argues in favour of a non-incidental association of hemiplegic migraine with familial PKD and *PRRT2* mutations. A great number of studies in genetic disease models provide evidence that gene mutations in familial hemiplegic migraine increase neuronal excitability and predispose to cortical spreading depression (Ferrari *et al*., 2015), suggesting common disease pathways with *PRRT2*-associated diseases. The clinical association and pathophysiological similarities between *PRRT2*-associated diseases and hemiplegic migraine are remarkable and might justify adding the *PRRT2* gene to the list of genes associated with hemiplegic migraine (Fig. 3). Large-scale screening studies in sporadic and familial hemiplegic migraine cohorts will aid in further discerning the role of *PRRT2* in hemiplegic migraine. On a cautionary note, a careful exclusion of other disease-causing gene mutations has to be assured in order to unequivocally attribute new phenotypes, like hemiplegic migraine, to the spectrum of *PRRT2*-associated diseases. This was exemplified recently in a family in which BFIE was likely caused by a *PRRT2* mutation while hemiplegic migraine could be attributed to a co-segregating *ATP1A2* mutation (Pelzer *et al*., 2014).

PRRT2 in intellectual disability and neurodevelopmental disorders

In 2011, an Iranian family with five individuals presenting with severe non-syndromic intellectual disability was described to carry a homozygous *PRRT2* mutation (c.649dupC) (Najmabadi *et al*., 2011). This substantiates observations in cases with rare biallelic mutations or 16p11.2 microdeletions that often show a severe, syndromal phenotype involving cognitive and neurodevelopmental deficits in addition to classic paroxysmal movement disorders and seizures (Dale *et al*., 2012*a*; Labate *et al*., 2012; Weber *et al*., 2013; Guerrero-Lopez *et al*., 2014; Delcourt *et al*., 2015). Of 21 individuals carrying either homozygous, compound-heterozygous or microdeletion mutations reported in the literature, 52.4% (11/21) present with intellectual disability and 14.3% (3/21) with learning difficulties, whereas only 0.6% (8/1423) of individuals with heterozygous *PRRT2* mutations have intellectual disability and 0.8% (12/1423) reported learning disabilities (Supplementary Table 10).

As both deletions and duplications at the 16p11.2 locus, which cover *PRRT2*, increase the risk for autism spectrum

disorder, 431 individuals with autism spectrum disorder and 186 controls were screened for *PRRT2* mutations. However, no enrichment of deleterious *PRRT2* mutations could be ascertained, arguing that *PRRT2* mutations are not associated with increased susceptibility to autism spectrum disorder (Huguet *et al.*, 2014).

Taken together, the increased presence of intellectual disability in patients with biallelic *PRRT2* mutations indicates a gene-dosage effect in which a strongly reduced or absent PRRT2 protein leads to a more severe phenotype with additional disease manifestations. This further highlights the importance of *PRRT2* for synaptic function and might suggest an additional role during neuronal development.

Conclusions and future challenges

Findings in *PRRT2*-associated diseases exemplify the virtues of next-generation gene sequencing techniques and a break with the 'one gene mutation–one disease phenotype' paradigm. Parallels can be drawn to findings in CNS channelopathies, which bridge a similar range of disease entities. A comparable pleiotropy is for example seen in *ATP1A3* mutation-associated diseases, which consist of at least four paroxysmal phenotypes, namely (i) rapid-onset dystonia-parkinsonism; (ii) alternating hemiplegia of childhood; (iii) CAPOS (cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss) syndrome (Sweney *et al.*, 2015); and (iv) catastrophic early life epilepsy, episodic prolonged apnoea, and postnatal microcephaly (Paciorkowski *et al.*, 2015). *PRRT2*-associated diseases cover an evolving continuum of paroxysmal diseases from benign infantile epilepsy, to paroxysmal dyskinesias and paroxysmal headache disorders such as hemiplegic migraine. Although a great number of cases with *PRRT2* mutations have been reported to date, the spectrum of *PRRT2*-associated diseases is almost certainly broader than we currently recognize (Fig. 3). The identification of mutations in *PRRT2* as the genetic cause for a range of diseases is a crucial entry point to a better understanding of the molecular mechanisms behind paroxysmal diseases. Extensive genetic and phenotypic characterization will elucidate the boundaries of a wide phenotypic spectrum. Carefully designed clinical trials will help to clarify whether novel *PRRT2*-associated paroxysmal diseases readily respond to treatment with classic anticonvulsants as it is the case for classic attacks of PKD in the vast majority of patients. In addition, development of disease models will help to clarify the neurobiology of *PRRT2* and implications for normal CNS function and disease. Given the clinical and molecular overlaps with other paroxysmal diseases, advanced understanding of synaptic vesicle release and neuronal hyperexcitability in *PRRT2*-associated diseases will cross-fertilize research in epilepsy, movement-, headache- and behavioural disorders associated with synaptic dysfunction.

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Supplementary material

Supplementary material is available at *Brain* online.

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