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.
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., 2013a; 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., 2012a) 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 dyskinasias. 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., 2012a). 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., 2012a; 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 Ca2+-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.
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., 2012a). 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., 2012a). 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<sup>+/−</sup>) 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<sup>2+</sup>-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<sub>1.2</sub> 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
additional cytosine during DNA replication and thus to the common c.649dupC frameshift mutation (Li et al., 2013).

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.
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., 2012a; 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, 2012a; 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., 2012a; 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 anticonvulsant agents (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 anticonvulsant 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 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...
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 striopallidodentate calcinosis, moyamoya 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

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<th>Table 1 Demographic and genetic characteristics of PRRT2-associated PKD, PKD/IC and BFIE (total number of patients: 560, 206, 602)</th>
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<td><strong>Sex (M:F)</strong></td>
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<td>(n = 513; 186; 585)</td>
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<td><strong>Ethnicity</strong></td>
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<tr>
<td><strong>Family history</strong></td>
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<tr>
<td>(n = 560; 206; 602)</td>
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<tr>
<td><strong>Common PRRT2 mutations</strong></td>
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<td>(n = 560; 207; 602)</td>
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<td><strong>Familial PRRT2 mutation</strong></td>
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<td>(n = 560; 206; 602)</td>
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<td><strong>Family history</strong></td>
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<tr>
<td>(n = 560; 206; 602)</td>
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* Showing the five most common (please refer to Supplementary material for the complete dataset).

* Mutations affecting >1% are shown (please refer to Supplementary material for the complete dataset).
**Table 2** Primary diagnosis in PRRT2 mutation carriers without a diagnosis of PKD, PKD/IC or BFIE (n = 76)

<table>
<thead>
<tr>
<th>Movement disorders</th>
<th>Seizure disorders</th>
<th>Headache disorders</th>
<th>Developmental delay and intellectual disability</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNKD / ‘PNKD-like’ (n = 2)</td>
<td>Febrile seizures (n = 15)</td>
<td>Migraine with aura (n = 4)</td>
<td>Non-migrainous headaches (n = 1)</td>
</tr>
<tr>
<td>PED (n = 2)</td>
<td>Febrile seizures plus (n = 6)</td>
<td>Migraine without aura (n = 3)</td>
<td>Non-syndromic intellectual disability (n = 5)</td>
</tr>
<tr>
<td>Febrile seizures plus (n = 6)</td>
<td>Epilepsy (not specified) (n = 11)</td>
<td>Non-migrainous headaches (n = 1)</td>
<td></td>
</tr>
<tr>
<td>Generalized epilepsy (n = 1)</td>
<td>Nocturnal convulsions (n = 2)</td>
<td>Manifestation on the left side is evident in affected limbs.</td>
<td></td>
</tr>
<tr>
<td>West syndrome (n = 1)</td>
<td>Hemiplegic migraine (n = 19)</td>
<td>A precipitating aura preceding attacks by a variable time period.</td>
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</tr>
<tr>
<td></td>
<td>Migraine with aura (n = 4)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Migraine without aura (n = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-migrainous headaches (n = 1)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Non-syndromic intellectual disability (n = 5)</td>
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</table>

**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

<table>
<thead>
<tr>
<th>BFIE, n = 602</th>
<th>PKD/IC, n = 206</th>
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<tbody>
<tr>
<td>Age onset – Infantile seizures</td>
<td>Age at remission – Infantile seizures</td>
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<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<tr>
<td>(n = 401)</td>
<td>(n = 241)</td>
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<tr>
<td>6.0 ± 2.9 months</td>
<td>11.2 ± 1.55 months</td>
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<tr>
<td>6.3 ± 3.2 months</td>
<td>17.4 ± 13.4 months</td>
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**Response to treatment / clinical course**

<table>
<thead>
<tr>
<th>PKD, n = 560</th>
<th>PKD/IC, n = 206</th>
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</thead>
<tbody>
<tr>
<td>Age of onset - PKD (Mean ± SD)</td>
<td>Age at remission - PKD (Mean ± SD)</td>
</tr>
<tr>
<td>(n = 283)</td>
<td>(n = 108)</td>
</tr>
<tr>
<td>10.3 ± 4.9 years</td>
<td>9.1 ± 4.4 years</td>
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<tr>
<td>(n = 108)</td>
<td>(n = 108)</td>
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<tr>
<td>5.7% (16/283)</td>
<td>3.7% (4/108)</td>
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</table>

**Phenomenology Index**

- **Bilateral/unilateral:** 1.0/0.96/0.42
- **Sensory aura:** 11.4% (64/560)
- **Maximum duration of attacks:** 28.1 ± 23.1 s
- **Maximum frequency per day:** 11.0 ± 14.5

**Response to definite treatment**

- **Complete:** 92.7%
- **Partial:** 7.3%
- **Complete:** 98.1%
- **Partial:** 1.9%
Importantly, the question of the origin of PKD attacks and their pathophysiological implications remain limited. Pallido-thalamic circuitry although the diagnostic and functional movement disorders such as psychogenic non-epileptic seizures 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 videoEEG, 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., 2013b). 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., 2013b). 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.S202His*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 nonkinesigenic 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 comitant 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, 2012b). 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 PPKD (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. (2012b) 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., 2013b; 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., 2012b; 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., 2012a; Labate et al., 2012; Weber et al., 2013; Guerrero-Lopez et al., 2014; Delcourt et al., 2015). Of 21 individuals carrying either homozygous, compound-homozygous 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 (Sweny 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 overlap 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.

**Acknowledgements**

The authors thank Professor Nobumasa Kato (University of Tokyo) and Professor Norio Niikawa (Health Sciences University of Hokkaido) for kindly providing helpful explanations and comments on the history of PKD and the first description in the Japanese literature. The authors also thank Rumiko Adamowicz and David Hideo Adamowicz (University of California, San Diego) for translation of the original Japanese literature and Dr Lara Wahlsler and Dr Jonathan Lipton (both at Boston Children’s Hospital) for helpful discussions. Brain expression data were kindly provided by the UK Human Brain Expression Consortium (UKBEC), which comprises John A. Hardy, Mina Ryten, Daniah Trabzuni (UCL Institute of Neurology); Michael Weale, Adaikalavan Ramasamy (King's College London), and Colin Smith and Robert Walker (University of Edinburgh).

**Funding**

D.E.-F. acknowledges support from the Graduate Academy of the University of Heidelberg, the Young Investigator Award Program at Ruprecht-Karls-University Heidelberg Faculty of Medicine, the Daimler and Benz Foundation (Daimler und Benz Stiftung, Ladenburg, Germany) and the Reinhard-Frank Foundation (Reinhard-Frank-Stiftung, Hamburg, Germany). A.S. is supported by a scholarship from the German National Academic Foundation (Studienstiftung des Deutschen Volkes e.V.). A.W. received intramural funding (Application Package - Paketantrag) from the University of Lübeck and a research grant from the Fritz Thyssen Foundation. C.K. is the recipient of a career development award of the Hermann and Lilly Schilling Foundation.

**Supplementary material**

Supplementary material is available at *Brain* online.

**References**


