PRRT2 Mutations and PRRT2 Disorders

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Abstract

The recent discovery of PRRT2 mutations in Paroxysmal Kinesigenic Dyskinesia (PKD) has spurred a number of studies on PRRT2 mutations and PRRT2 disorders including PKD, Benign Familial Infantile Epilepsy (BFIE), and Infantile Convulsions with Choreoathetosis (ICCA). Mutated PRRT2 is also implicated in several other paroxysmal neurological disorders, indicating a wide phenotypic spectrum of PRRT2 mutations. Although the correlations between PRRT2 mutations and specific phenotype have not been well investigated, our previous studies have shown that PRRT2 mutations carriers may have a distinct clinical feature and drug response with non-PRRT2 mutations carriers. Yeast two-hybrid studies have suggested that PRRT2 interacts with synaptosomal associated protein 25 (SNAP25), which is involved in synaptic vesicle handling and neuronal exocytosis. Therefore, the pathogenesis of PRRT2 disorders is probably associated with synaptic dysfunction. Further studies are needed to elucidate the role of PRRT2 and the potential mechanism of PRRT2 disorders.

Keywords: PRRT2; Paroxysmal Kinesigenic Dyskinesia (PKD); Benign Familial Infantile Epilepsy (BFIE); Infantile Convulsions with Choreoathetosis (ICCA)

Introduction

PRRT2 encoding proline-rich transmembrane protein 2 is a poorly characterized gene before the discovery of its role in Paroxysmal Kinesigenic Dyskinesia (PKD), the most common subtype of Paroxysmal Dyskinesias (PDs). PRRT2 is located on chromosome 16p11.2 and consists of 4 exons. The full length PRRT2 protein contains 340 amino acid residues with a proline-rich region in the N-terminal and two putative transmembrane domains near the C-terminal. Combining whole-exome sequencing and Sanger sequencing, Chen et al. identified three truncating mutations within PRRT2 in 8 Chinese PKD families but not in 1000 matched control subjects, indicating that PRRT2 was a causative gene of PKD [1]. This finding is of great importance, spurring a number of studies focused on PRRT2 mutations and PRRT2 disorders including PKD [2-5], Benign Familial Infantile Epilepsy (BFIE) [6-8] and Infantile Convulsions with Choreoathetosis (ICCA) syndrome [9-12]. These studies have provided convincing evidence that PRRT2 is a major causative gene for PKD, BFIE, and ICCA. Furthermore, substantial progress has been made to further define the phenotypic spectrum of PRRT2 mutations. It has been reported that PRRT2 mutations are also implicated in several other paroxysmal neurological disorders, such as hemiplegic migraine (HM) [13,14], Paroxysmal Non-kinesigenic Dyskinesia (PNKD) [15,16], Paroxysmal Exertion-induced Dyskinesia (PED) [15], Febrile Convulsions (FC) [12,17,18], Episodic Ataxia (EA) [13], and paroxysmal torticollis [19]. In this review, we summarize recent advance regarding PRRT2 mutations, PRRT2 disorders, and the genotype-phenotype correlations. We will also discuss the potential mechanism underlying PRRT2 disorders.

Various Phenotypes Associated with PRRT2 Mutations

PKD, also termed Paroxysmal Kinesigenic Choreoathetosis (PKC), is an autosomal dominant movement disorder characterized by episodic attacks of dyskinesia which are usually triggered by sudden movement [20]. PKD attacks can clinically present as any combination of chore, athetosis, ballism or dystonia. Onset usually commences during childhood or early adolescence and the frequency and severity generally diminish in adulthood. Since the first description by Kertesz in 1967, the culprit gene of PKD has been elusive until recently, when Chen et al. identified that PRRT2 mutations were causative for PKD [1]. This finding was rapidly replicated by several studies with different populations [2-4]. PKD is the first identified and the major phenotype of PRRT2 mutations, regardless of ethnic backgrounds. Importantly, 60-100% of familial PKD cases were detected to harbor PRRT2 mutations [21]. To date, more than 30 PRRT2 mutations have been identified in PKD cases. However, PRRT2 mutations were much less common in sporadic PKD cases, suggesting that sporadic PKD may be etiologically distinct with familial PKD. Other causative genes of PKD require to be investigated in the future.

BFIE is an autosomal dominant epilepsy syndrome distinguished by frequent nonfebrile seizure with onset at between 4 and 12 months of age [22]. Seizures tend to occur in clusters but remit spontaneously by 2 years of age. Electroencephalogram (EEG) and magnetic resonance imaging (MRI) usually produce unremarkable results. In general, the prognosis of BFIE is favorable, because subsequent neurological development is usually normal. Heron et al. first reported that PRRT2 was mutated in 14 of 17 BFIE families [6]. Thereafter, Schubert et al. detected PRRT2 mutations in 42 index cases of 49 families with BFIE [7]. Recent studies have revealed that PRRT2 mutations were also identified in sporadic benign infantile epilepsy (BIE), which is a disorder identical to BFIE except for apparent family history [8,11,23]. Approximately, 40-100% of BFIE cases harbored PRRT2 mutations [21]. As BFIE and sporadic BIE are clinically indistinguishable, PRRT2 mutations should be screened in both familial and sporadic cases. Benign familial neonatal epilepsy (BFNE), which is similar to BFIE but exclusively affect neonates, however, seems not to be associated with PRRT2 mutations [11,18]. The lack of mutation in BFNE negates the
role of PRRT2 in this phenotypically similar seizure disorder in early infancy.

ICCA is a syndrome combining the occurrence of infantile convulsions (IC) at the age of 3-12 months and variable paroxysmal choreoathetosis (CA) in young childhood [24]. Kinesigenic choreoathetosis is the most common type, although non-kinesigenic or exercise-induced type has also been reported [25]. The phenotype encompassed by the ICCA is actually the same to PKD/IC. As ICCA share the suspected locus with BFIE and PKD, these three entities are hypothesized to be allelic [26,27]. In a relative large cohort of PKD/IC patients, Lee et al. identified 52/103 of the index cases harbored PRRT2 mutations [9]. Other studies also showed that PRRT2 is a causative gene of ICCA [6,10-12]. Taken together, the published data showed that PRRT2 mutations account for 33-100% familial cases of ICCA [21], indicating that ICCA is one of the major phenotypes associated with PRRT2 mutations.

HM is a rare subtype of migraine, in which attacks start in the first or second decade of life and are associated with transient weakness or hemiparesis [28]. Recently, PRRT2 mutations have been identified in families with HM. Collecting 101 cases with HM that started before age of 20 years, Riant et al. identified PRRT2 mutations in 4 patients [14]. In another study, 1 of 128 HM cases was detected to carry PRRT2 mutation [13]. These results suggest that PRRT2 mutations account for only a small proportion of pure HM. However, HM or migraine was commonly noted as accompanying feature in several PRRT2 mutations carriers whose primary phenotype was PKD, ICCA or BFIE [13,17,29]. Despite the low frequency, we believe PRRT2 may still be a candidate gene for familial HM.

PNKD and PED are two subtypes of Paroxysmal Dyskinesias (PDs). They share some manifestations with PKD but have different trigger factors. PNKD is precipitated by ingestion of alcohol or coffee, hunger, fatigue, stress and menstruation, while PED is triggered by sustained exertion [30]. Recent studies have reported that PRRT2 mutations carriers represented PNKD or PED phenotype, although the documented cases can be numbered. Liu et al. found PRRT2 mutations in 1 PNKD family and 2 sporadic PED cases [15]. In addition, there was one reported PRRT2 mutation carrier who suffered PNKD and infantile non-convulsive seizures [16]. Wang et al. also described a family with a phenotype overlapping PKD, PNKD and PED, and with a PRRT2 c.649C>T (p.R217X) mutation [31]. However, a Dutch study failed to identify any PRRT2 mutations in 4 PNKD cases and 1 PED case [32]. These studies suggest that PRRT2 mutations may be a rare cause of PNKD and PED. Alternatively, the recorded PNKD or PED cases may actually be PKD patients but were misdiagnosed with PNKD or PED, because these three disorders clinically exhibit many similar features. Further investigations are needed to determine the prevalence of PRRT2 mutations in PNKD and PED cases.

Occasionally, families with PRRT2 mutations were reported to represent epilepsy phenotypes, such as febrile seizures, absence, and nocturnal convulsions (NC) [16,18]. One c.649dupC mutation carrier was reported to exhibit both PKD and generalized tonic-clonic seizures [16]. Febrile seizures were also described in several families with PRRT2 mutations [12,17,18]. In a BFIC family carrying c.649dupC mutation, one of affected members developed a febrile seizure and died of probable sudden unexplained death in epilepsy (SUDEP) [33]. Furthermore, a sporadic BIE case with p.R240X nonsense mutation was described to present with absence seizures [16]. These together indicate that PRRT2 mutations are associated with different epilepsy phenotype.

In addition to the phenotypes described above, PRRT2 mutations are also implicated in EA and paroxysmal torticollis. In one study, one out of 182 individuals with EA was detected to carry PRRT2 c.649dupC mutation [13]. In another study, EA was part of a complex phenotype in two siblings with c.649dupC mutation [34]. Paroxysmal torticollis, however, was described only in one PRRT2 mutation carrier. Dale et al. recently reported a pedigree with c.649dupC mutation, in which the proband developed transient infantile paroxysmal torticollis followed by BIE, his father exhibited PKD and migraine, and his two brothers had HM with onset in childhood [19]. Since the associations of PRRT2 mutations with EA and paroxysmal torticollis are rarely documented, we speculate these two disorders may not be the phenotypes of PRRT2 mutations but be an accompanying feature of other disorders such as PKD, BFIE, or ICCA. This hypothesis is based on the high frequency of PRRT2 mutations in these disorders and the incomplete penetrance of them [3,6,7].

**PRRT2 Mutations, Inheritance and Penetrance**

To date, over 330 families and individuals have been reported to carry PRRT2 mutations and more than 50 mutations have been described in these cases [21]. The mutations spread throughout the PRRT2 gene, but are clustered in the second half of the exon 2 and in exon 3. A vast majority (>95%) of PRRT2 mutations are frameshift mutations, predicted to result in premature truncation of the protein [16]. It is thus believed that loss of function may be the underlying pathogenic mechanisms of mutated PRRT2. Other forms of PRRT2 mutations, such as missense mutations, nonsense mutations, and splice site mutations, however, account for a small percentage. Additionally, large sub-microscopic deletions encompassing PRRT2 gene have also been described in patients affected with PKD or ICCA [35,36], indicating that copy number variant analysis should also be considered.

Generally, PRRT2 mutations are autosomal dominantly inherited, which are shown in almost all studies. However, Liu et al. reported a proband carried a compound heterozygous PRRT2 mutation (c.510dupT and c.647C>G), while his asymptomatic father and mother harbor each mutation respectively [16]. This may indicate a pattern of autosomal recessive inheritance. In addition, de novo mutagenesis of PRRT2 mutations was also observed in sporadic cases affected with PKD, BFIE, and ICCA [4,5,7,37]. These together indicate that the inheritance of PRRT2 is heterogeneous. The penetrance of PRRT2 mutations is incomplete, as observed in previous studies reporting families with low penetrance [3,6,7]. This hints that PRRT2 mutations may be underestimated, because asymptomatic mutation carriers will not go to hospital and be recruited for sequencing of PRRT2 gene. The heterogeneous inheritance and incomplete penetrance of PRRT2 mutation suggest that particular attention should be paid by genetic counselors.

Of note, c.649dupC (p.R217Pfs*7) is the most frequently encountered mutation within PRRT2, present in approximately 80% of mutations carriers [10]. This mutation has been identified in different ethnicity and different phenotypes including PKD, BFIE, and ICCA. It is mainly inherited in an autosomal dominant manner, although several reports have shown c.649dupC could derive from de novo mutagenesis [4,38]. Furthermore, several different haplotypes linked to the c.649dupC have been reported in isolated groups [1,6,10]. Therefore, the c.649dupC mutation does not arise from a founder effect but is a hotspot mutation. Why c.649dupC occur in such a high frequency? Firstly, this mutation occurs in a homopolymer tract of 9 cytosine (C) bases, representing a highly unstable DNA sequence. Secondly, the homopolymer tract is preceded by 4 guanine (G) bases and therefore
has the potential to form a hairpin loop. Such sequence context is easy to result in DNA-polymerase slippage and insertion of an extra C base during DNA replication. In addition to the common insertion mutation, c.649delG and c.649C>T affecting the same nucleotide have also been reported several times [4,32,39].

Taken together, we suggest an effective strategy to rapidly screen PRRT2 mutations in cases affected with PKD, BFIE, or ICCA. For clinical practice, c.649dupC mutation should be screened first, because it is a mutation hotspot, accounting for a percentage as high as 80%. Direct sequencing of this cytosine stretch will be an easy and available approach with a high chance to detect the mutation. If negative, sequencing of exons 2 and 3 can be performed, as most PRRT2 mutations are clustered in these two exons. Sequencing of exons 1 and 4, and splice site should be performed at last. For laboratory researchers, however, Copy Number Variations (CNVs) may also be considered.

Correlations of PRRT2 Mutations with Clinical features and Drug Response

The association of PRRT2 genotypes with specific phenotypes and drug responses has not been well investigated by now. Patients with PRRT2 mutations may have a distinct phenotype to these without PRRT2 mutations. Collecting 81 PKD cases, we observed that PRRT2 mutation carriers have a lower age at onset and longer attacks during each episode, compared to non-PRRT2 mutation carriers. All PRRT2 mutation carriers in our study expressed bilateral choreoathetosis phenotype. Non-PRRT2 mutation carriers, however, presented with dystonia or choreoathetosis phenotype, and bilateral attacks occur in only 42% of cases [40]. We also found that all PRRT2 mutation carriers responded completely to low-dose (50 mg/d) carbamazepine, while 94% of the non-PRRT2 mutation carriers did not have a full response to carbamazepine, even after the dose was increased [40]. These demonstrate a clear-cut correlation between PRRT2 mutations and phenotypes. However, the limitation of our study is small number of patients and single-centre research. The results should be verified by further studies in the future.

In comparison to PRRT2 frameshift mutations, missense mutations seem to be associated with mild phenotype. In a Chinese pedigree, Cai et al. detected two heterozygous PRRT2 missense mutations (p.P138A and p.A306D) in 6 affected members who presented with mild PKD/ICCA symptoms [41]. A missense c.913G>A mutation was also reported in a PKD family, in which 2 siblings had infrequent symptoms requiring carbamazepine rarely, and the mother was a nonsymptomatic carrier, indicative of mild phenotype and reduced penetrance [42]. We speculate PRRT2 missense mutations may lead to partial loss of PRRT2 function and less pathogenicity.

Homozygous PRRT2 mutations may be associated with a more severe phenotype including intellectual disability. Using homozygosity mapping and next-generation sequencing, Najmabadi et al. identified a homozygous frameshift PRRT2 mutation in 5 affected members of an Iranian family who exhibited severe intellectual disability [43]. In another consanguineous Italian family with ICCA, two cases carrying homozygous c.649dupC mutation were reported to suffer mental retardation, episodic ataxia, and absences [34]. This suggests that double dose of PRRT2 mutation have an additive effect on brain pathology and function.

Mechanisms of PRRT2 Disorders

The PRRT2 protein is widely expressed in the nervous system with high concentrations in the cerebral cortex, hippocampus and cerebellum [1]. The levels of PRRT2 mRNA in mice brain were highest on postnatal day 14 (P14), corresponding approximately to an age of 1-2 years in humans. However, PRRT2 mRNAs levels decline to a relatively low level in the adult brain of mice [1], which is consist with another in situ hybridization study [6].

PRRT2 mutations are associated with a variety of paroxysmal disorders. With wider screening over time, we believe that the clinical spectrum of PRRT2 mutations is likely to expand to encompass other childhood neurological diseases. How mutations within PRRT2 cause different phenotypes and even the same PRRT2 mutation (e.g. c.649dupC) leads to phenotypic variability remain to be investigated. The clinical variability could be explained partly by high expression of PRRT2 in the brain. Alternatively, wild-type PRRT2 allele might modify the phenotypic expression of the mutant allele and thus contribute to heterogeneity in affected families. Moreover, other factors such as unidentifed genetic variants or epigenetic changes in PRRT2 may also exist. The potential mechanisms underlying the remarkable pleiotropy of PRRT2 mutations remain to be determined.

Although the PRRT2 disorders are quite heterogeneous, most of them are benign, mildly affecting the quality of life. For example, PKD, BFIE, and ICCA usually have an early onset and release gradually in adulthood, indicative of a self-limited, age-dependent course. Most of these disorders respond well to anticonvulsant drugs, especially carbamazepine or phenytoin [30]. As age influences the clinical features and the severity, age-dependent expression of PRRT2 may play a role in these phenotypic variations. A similar phenomenon has been observed in benign familial neonatal-infantile seizures (BFNIS) caused by mutated SCN2A encoding Na+ channel Nav1.2, whose expression is transient during development [44]. Because of the nature of non-specific and multiweak binding sites of proline-rich proteins [45], the effect of mutated PRRT2 may be limited, which can explain partly the incomplete penetrance and recessive inheritance of PRRT2 mutations.

The associations of PRRT2 mutations with various phenotypes such as PKD, BFIE, ICCA, and HM suggest that these PRRT2 disorders may have a common pathway. Wild PRRT2 is located on the cytoplasmic membrane, while c.649dupC PRRT2 lost membrane targeting and localization [1]. Yeast two-hybrid studies have suggested that PRRT2 interacts with synapsosomal associated protein 25 (SNAP25) [46], which is a presynaptic membrane protein engaged in synaptic vesicle handling. SNAP25 plays a crucial role in the regulation of intracellular Ca2+ dynamics as well as in the neuronal exocytosis [47]. In PRRT2 mutation carriers, the interaction between PRRT2 and SNAP25 may be impaired, affecting the normal properties of Ca2+ channels [48]. It is known that SNAP25 assembles with syntxin-1 and syntaxotrobin to form SNARE complex in neuron. We hypothesized that PRRT2 may have functional connectivity with SNARE complex, even be a component of the complex. The pathogenesis of PRRT2 disorders is probably associated with synaptic dysfunction. PRRT2 may serve as an anchor to connect SNAP25 to the presynaptic membrane. The interactions of PRRT2 with SNAP25 suggest that mutated PRRT2 impaired synaptic transmission during the initial stage of voluntary movements.

Conclusion

In conclusion, PRRT2 mutations are causative for a wide spectrum of paroxysmal neurological disorders including PKD, BFIE, ICCA, and HM. The correlations of PRRT2 mutations with phenotypes remain to be investigated in the future. Cases carrying PRRT2 mutations may
respond favorably to carbamazepine, which need further studies to verify. The mechanisms underlying PRRT2 disorders may be associated with synaptic dysfunction. Further experiments and investigations are required to elucidate the role of PRRT2 mutations in PRRT2 disorders.

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References


