PRRT2 mutations in familial infantile seizures, paroxysmal dyskinesia, and hemiplegic migraine

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ABSTRACT

Objective: To perform a clinical and genetic study of a family with benign familial infantile seizures (BFIS) and, upon finding a *PRRT2* gene mutation, to study a cohort of probands with a similar phenotype. We extended the study to all available family members to find out whether *PRRT2* mutations cosegregated with additional symptoms.

Methods: We carried out a clinical and genealogic study of a 3-generation family and of 32 additional probands with BFIS (11 families), infantile convulsions and paroxysmal choreoathetosis (ICCA) (9 families), BFIS/generalized epilepsy with febrile seizures plus (5 families), and sporadic benign neonatal or infantile seizures (7 probands/families). We performed a genetic study consisting of linkage analysis and *PRRT2* screening of the 33 probands/families.

Results: We obtained a positive linkage in the 16p11.3-q23.1 chromosomal region in the large BFIS family. Mutation analysis of *PRRT2* gene revealed a c.649dupC (p.Arg217Profs*8) in all affected individuals. *PRRT2* analysis of the 32 additional probands showed mutations in 10, 8 familial and 2 sporadic, probands. Overall we found *PRRT2* mutations in 11 probands with a mutation rate of 11 out of 33 (33%). BFIS co-occurred with migraine and febrile seizures in 2 families, with childhood absence epilepsy in one family and with hemiplegic migraine in one family.

Conclusion: Our results confirm the predominant role of *PRRT2* mutations in BFIS and expand the spectrum of *PRRT2*-associated phenotypes to include febrile seizures, childhood absence seizures, migraine, and hemiplegic migraine. **Neurology® 2012;79:2109-2114**

GLOSSARY

BFIS = benign familial infantile seizures; **ICCA** = infantile convulsions and choreoathetosis.

Benign familial infantile seizures (BFIS) is an autosomal dominant disorder characterized by the occurrence of afebrile focal seizures, often in clusters, beginning between the 3rd and 12th months of life in otherwise normal infants.^{1,2} Seizures have a very benign outcome and disappear by age 2 years in most children who have normal psychomotor development. During childhood or adolescence, some patients may develop paroxysmal kinesigenic choreoathetosis.³ The term infantile convulsions and paroxysmal choreoathetosis (ICCA) has been used to describe patients in whom seizures and paroxysmal choreoathetosis co-occur.⁴

Linkage studies of informative families with autosomal dominant paroxysmal kinesigenic choreoathetosis, ICCA, and BFIS identified a locus on the pericentromeric region of chromosome 16p11.2-q12.1.⁴⁻⁸ Recently, using whole exome sequencing, mutations in the proline-rich transmembrane protein 2 (*PRRT2*) gene were identified in 8 families with autosomal dominant paroxysmal kinesigenic choreoathetosis.⁹ *PRRT2* gene maps to 16p.11.2 and encodes for the proline-rich transmembrane protein 2, which seems to be involved in the synaptic vesicle membrane docking and fusion pathway, interacting with SNAP25.⁹ Additional mutations in *PRRT2* have been recently identified in families with ICCA and BFIS.¹⁰⁻¹⁴

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using Kaviar (<http://db.systemsbiology.net/kaviar/cgi-pub/Kaviar.pl>) excluded that the identified variants had been reported as polymorphisms. The possible pathogenic effect of the identified variants has been evaluated using Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/index.shtml>), Mutation Taster (<http://www.mutationtaster.org/>), and SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html) for missense mutations and the BDGP Splice Site Prediction (http://www.fruitfly.org/seq_tools/splice.html), the Human Splicing Finder (<http://www.umd.be/HSF/>), and the NetGene2 Server (<http://www.cbs.dtu.dk/services/NetGene2/>) for the splice site mutation.

RESULTS Patients. A brief summary of relevant clinical information of affected individuals, including 9 familial and 2 sporadic patients, is summarized in the table.

Family 1 included 9 clinically affected individuals who presented with clusters of focal, afebrile seizures, at a mean of age 7 months (range 5–8) (figure 1). All affected individuals, with an age at the time of the study ranging from 2.5 to 44 years, mean 19 years, are at present seizure-free.

We also studied 32 probands, either sporadic or familial, whose phenotypes were 1) BFIS in 11 probands; 2) ICCA in 9 probands; 3) BFIS/generalized epilepsy with febrile seizure plus spectrum in 5 probands; 4) sporadic benign infantile seizures in 4 probands; and 5) neonatal seizures in 3 probands. We identified *PRRT2* gene mutations in 10 probands (figure 2), bringing the total number of probands with mutations to 11/33 (33%).

Overall, the whole cohort of families/patients carrying *PRRT2* mutations (including family 1) had a total of 27 individuals who had had focal seizures (figure 3) at a mean age of 7 months (median 7, range 3.5–8 months), 11 individuals with paroxysmal dyskinesia migraine, or both, and hemiplegic migraine and 4 in whom infantile seizures were variably associated with migraine, paroxysmal dyskinesia, and hemiplegic migraine (figure 2). A single individual (figure 2, family 8) had had only febrile seizures. Seizures manifested in clusters in all probands. In 5 of them, they were characterized by unresponsiveness followed by perioral cyanosis, staring, and hypotonia, lasting 30 to 60 seconds. During seizures, 3 of these 5 probands had lateral eye deviation and 2 also exhibited oral automatisms associated to a hiccup-like sound. In the remaining 6 probands, following initial unresponsiveness and staring, a hypertonic phase ensued, with a diffuse vibratory tonic component. In addition to focal seizures, 4 of the 11 probands also exhibited tonic-clonic seizures that were reported to be generalized from onset. All affected individuals had normal developmental milestones and cognition.

Nine of the 11 probands had a family history of seizures or other paroxysmal disorders, or both, consistent with a diagnosis of 1) BFIS in 6 families, one of which had a family member who had absence seizures in childhood and one with hemiplegic migraine in 5 individuals; 2) ICCA in 3 families with migraine

Table Families and patients with *PRRT2* mutations

Proband	Clinically affected family members	Age range at the time of the study, y	Age range at seizure onset	Seizure type	Paroxysmal dyskinesia	Other features	Diagnosis	<i>PRRT2</i> mutation
1	9	2-adulthood	6–8 mo	Focal	No	—	BFIS	c.649dupC p.Arg217Profs*8 familial
2	4	2–36	4 mo	Focal	2	—	ICCA	c.970G>A p.Gly324Arg familial
3	2	3–42	8 mo	Focal, absence	No	—	BFIS and CAE	c.649dupC p.Arg217Profs*8 familial
4	3	2–32	7 mo	Focal + GTCS	No	—	BFIS	c.649dupC p.Arg217Profs*8 familial
5	—	1-adulthood	6–8 mo	Focal + GTCS	No	—	BFIS	c.649dupC p.Arg217Profs*8 de novo
6	3	3–39	3.5–6 mo	Focal + GTCS	No	—	BFIS	c.971G>A p.Gly324Glu familial
7	—	4-adulthood	5–9 mo	Focal + GTCS	No	—	BFIS	c.649dupC p.Arg217Profs*8 NA
8	10	13-adulthood	6 mo	Focal, FS	8	FS, migraine	ICCA, FS, migraine	c.649dupC p.Arg217Profs*8 familial
9	2	4-adulthood	7–8 mo	Focal	No	—	BFIS	c.916G>A p.Ala306Thr familial
10	1	5-adulthood	7 mo	Focal	1	Migraine	ICCA, migraine	c.880-2A>T familial
11	7	7–65	7 mo	Focal	No	HM	BSIF, HM	c.649dupC p.Arg217Profs*8 familial

Abbreviations: BFIS = benign familial infantile seizures; CAE = childhood absence epilepsy; FS = febrile seizures; GTCS = generalized tonic-clonic seizure; HM = hemiplegic migraine; ICCA = infantile convulsions and paroxysmal choreoathetosis; NA = not available.

Figure 2 Pedigrees of the available families with benign familial infantile seizures (BFIS), infantile convulsions and choreoathetosis (ICCA), and additional features

and febrile seizures in 2. There were also 9 additional healthy individuals from family 1 (figure 1) and one unaffected obligate carrier from family 11 (figure 2) carrying *PRRT2* mutations.

Linkage analysis. Linkage analysis, performed in family 1, revealed a maximum 2-point lod score of 3.31 at marker D16S415, at $\theta = 0.0$ (table e-2), while multi-point lod score analysis reached a maximum value of 2.71 at marker D16S415 (figure e-1). The highest probability haplotype inferred using HaploPainter¹⁶ was consistent with key recombinant events between markers D16S3046 and D16S3068 in patient III-14 and between markers D16S5415 and D16S503 in patient III-24, outlining the common haplotype shared by all affected members (figure 1).

Mutation screening. Sequencing analysis of the *PRRT2* gene in the proband of family 1 demonstrated the duplication of a single base (c.649dupC) in exon 2, resulting in a frameshift with the insertion of a premature stop codon (p.Arg217Profs*8). The same mutation was identified in all affected individuals, in 1 obligate carrier, and in 8 asymptomatic individuals (figure 1). The p.Arg217Profs*8 is a recurrent frameshift, loss of function

mutation¹³ that has also been identified in families and in sporadic patients with paroxysmal kinesigenic dyskinesias with or without infantile convulsions and benign familial infantile epilepsy.^{9–14} The analysis of additional BFIS families led us to identify the c.649dupC in 3 additional familial cases and in the 2 sporadic cases (table, figure 2). In one of the 2 sporadic patients, the mutation occurred de novo. No DNA could be obtained for the parents of the other sporadic patient. In the remaining families, we found 3 novel missense mutations (c.916G > A; p.Ala306Thr, c.970G > A; p.Gly324Arg, and c.971G > A; p.Gly324Glu) and one novel splicing mutation (c.880-2A > T).

DISCUSSION Linkage analysis in family 1, with 9 affected individuals, showed a significant lod score in the 16p12.3-q23.1 region, partially overlapping previously published BFIS, ICCA, and autosomal dominant paroxysmal kinesigenic choreoathetosis loci.² Screening of the *PRRT2* gene in available members of family 1 identified a frameshift mutation, p.Arg217Profs*8, in all affected individuals, in one obligate carrier, and in 8 asymptomatic individuals. The identification of asymptomatic individuals carrying the mutation is not

Figure 3 Video-polygraphic recording of a focal seizure in an 8-month-old girl with *PRRT2* mutation

Video-polygraphic recording of a focal seizure in an 8-month-old girl with *PRRT2* mutation showing a rhythmic ictal discharge originating from the left parieto-tempo-occipital region (A), spreading to involve, 40 seconds from its onset, the whole left hemisphere and, 60 seconds later, the contralateral regions (B). The seizure ends 2 minutes after onset. The discharge is followed by small-amplitude asymmetric slow activity, corresponding to the postictal phase. Clinically the patient is unresponsive and stares for 60 seconds, then becomes stiff, manifesting some clonic, asynchronous jerking of the limbs. In the postictal phase the patient remains still for a few minutes, exhibiting intermittent oral automatisms.

surprising, since a reduced penetrance for BFIS has been already described.² The p.Arg217Profs*8 mutation, which is a loss of function mutation,¹³ is emerging as a very recurrent mutation in patients with paroxysmal kinesigenic dyskinesia, with or without infantile convulsions and BFIS.^{10–14}

After identifying the c.649dupC mutation in family 1, we assembled and studied a cohort of 32 probands with BFIS, ICCA, and other idiopathic infantile seizures, either familial or sporadic, and identified *PRRT2* mutations in 10 additional probands (11/33; 33%). Family history revealed that 8 probands had a familial segregation: 5 had pure BFIS, 1 had ICCA, 1 had a co-occurrence of BFIS and childhood absence epilepsy, 1 had a cosegregation of BFIS and hemiplegic migraine, and 2 had a combination of BFIS, febrile seizures, paroxysmal dyskinesia, and migraine. The 2 sporadic patients had benign infantile seizures with de novo *PRRT2* in one, whereas DNA could not be obtained for the parents of the other sporadic patient. In our study, there were 6/11 BFIS families carrying *PRRT2* mutations, corresponding to 54.5%, and 3/8 with ICCA, corresponding to 37.5%. These figures are smaller than previously reported, in which 80% of BFIS and ICCA families carried *PRRT2* mutations.¹⁴

Of the 34 affected individuals carrying a *PRRT2* mutation, 3 (from 2 different families) manifested migraine and 5 (from a single family) had hemiplegic migraine consisting of yearly attacks of a tingling sensation in one arm, leg, and face followed by the inability to move the affected limbs and aphasia. Attacks,

sometimes accompanied by vomiting, were associated with severe headache and lasted several hours. These episodes are therefore clearly distinguishable from epileptic seizures. The association with hemiplegic migraine is remarkable and suggests that *PRRT2* gene might be added to the list of genes associated with this disorder including *SCN1A*, *ATP1A2*, and *CACNA1A*.¹⁷ These 3 best known hemiplegic migraine genes all encode ion transporters, suggesting that disturbances in ion and neurotransmitter balances in the brain are responsible for this migraine type. *PRRT2*, which is involved in the synaptic vesicle membrane docking and fusion pathway, could cause migraine through a different, yet unknown mechanism. The c.649dupC mutation identified in the family with BFIS and hemiplegic migraine was also identified in 5 other families in our series (figure 2) and in several already published families.^{10–14} However, hemiplegic migraine was present only in this family (figure 2, family 11), possibly suggesting an interfamilial phenotypic variability and the interaction of *PRRT2* with genetic modifiers.

We found the c.649dupC mutation in 6/10 mutated families and in a sporadic patient. This finding is in agreement with previous reports and confirms that the nucleotide c.649 may be considered as a mutation hot-spot in *PRRT2*. In addition, we found 4 novel mutations (3 missense and a splicing mutation). Two missense mutations (c.970G > A; p.Gly324Arg and c.971G > A; p.Gly324Glu) affect the Gly324 residue and the remaining missense mutation (c.916G > A) the

residue Ala306. The transmembrane Gly324 residue is highly conserved and the 2 mutations affecting it cause nonconservative amino acid changes that are predicted to be highly pathogenic. The Ala306Thr substitution is predicted to be more tolerated, since alanine and threonine share similar chemical and physical properties. However, the Ala306 residue has a high evolutionary conservation which, taken together with its segregation in both affected family members on family 9 (figure 2), supports its pathogenic role. The fourth novel mutation we identified (c.880-2A > T) affects the canonical acceptor splice site of the exon 3 and is likely to alter the splicing process, leading to the skipping of exon 3 resulting in a truncated protein.

Our findings confirm that screening for *PRRT2* gene mutations is indicated both in familial and sporadic cases of infants presenting with clusters of afebrile seizures with normal psychomotor development, normal background EEG activity, and normal imaging. Although in our series seizure onset was within the first year of life, with peak at around the 7th month, later onset is possible, usually within the 18th month.¹⁴ Familial cosegregation of other paroxysmal neurologic disorders such as paroxysmal dyskinesia, migraine, or hemiplegic migraine is strongly suggestive of a *PRRT2* gene-related disorder. Infants without clear evidence of additional affected family members should also be tested since seizures occurring very early in infancy may not be on record. To reach a genetic diagnosis is important in order to avoid expensive and invasive investigations and for genetic counseling.

AUTHOR CONTRIBUTIONS

Study concept and design: Dr. Marini, Dr. Conti, D. Mei, and Dr. Guerrini. Acquisition of data: Dr. Marini, Dr. Conti, D. Mei, Dr. Battaglia, Dr. Lettori, Dr. Losito, Dr. Bruccini, Dr. Tortorella, and Dr. Guerrini. Analysis and interpretation of data: Dr. Marini, Dr. Conti, Dr. Battaglia, Dr. Lettori, Dr. Losito, and Dr. Guerrini. Drafting of the manuscript: Dr. Marini, Dr. Conti, and Dr. Guerrini. Critical revision of the manuscript for important intellectual content: Dr. Guerrini. Obtained funding: Dr. Marini. Administrative, technical, and material support: Dr. Conti, D. Mei, Dr. Battaglia, Dr. Bruccini, Dr. Tortorella, and Dr. Guerrini. Study supervision: Dr. Guerrini.

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