PRRT2 phenotypic spectrum includes sporadic and fever-related infantile seizures

Ingrid E. Scheffer, MBBS,
PhD*

Bronwyn E. Grinton, BSc
(Hons)*

Sarah E. Heron, PhD

Sara Kivity, MD

Zaid Afawi, MD

Xenia Iona, Dip

BioMedSci

Hadassa Goldberg-Stern,
MD

Maria Kinali, MD

Ian Andrews, MBBS

Renzo Guerrini, MD

Carla Marini, MD, PhD

Lynette G. Sadleir,

MBCbB, MD

Samuel F. Berkovic, MD

Leanne M. Dibbens, PhD

ABSTRACT

Objective: Benign familial infantile epilepsy (BFIE) is an autosomal dominant epilepsy syndrome characterized by afebrile seizures beginning at about 6 months of age. Mutations in *PRRT2*, encoding the proline-rich transmembrane protein 2 gene, have recently been identified in the majority of families with BFIE and the associated syndrome of infantile convulsions and choreoathetosis (ICCA). We asked whether the phenotypic spectrum of *PRRT2* was broader than initially recognized by studying patients with sporadic benign infantile seizures and non-BFIE familial infantile seizures for *PRRT2* mutations.

Methods: Forty-four probands with infantile-onset seizures, infantile convulsions with mild gastroenteritis, and benign neonatal seizures underwent detailed phenotyping and *PRRT2* sequencing. The familial segregation of mutations identified in probands was studied.

Results: The *PRRT2* mutation c.649-650insC (p.R217fsX224) was identified in 11 probands. Nine probands had a family history of BFIE or ICCA. Two probands had no family history of infantile seizures or paroxysmal kinesigenic dyskinesia and had de novo *PRRT2* mutations. Febrile seizures with or without afebrile seizures were observed in 2 families with *PRRT2* mutations.

Conclusions: *PRRT2* mutations are present in >80% of BFIE and >90% ICCA families, but are not a common cause of other forms of infantile epilepsy. De novo mutations of *PRRT2* can cause sporadic benign infantile seizures. Seizures with fever may occur in BFIE such that it may be difficult to distinguish BFIE from febrile seizures and febrile seizures plus in small families.

Neurology® 2012;79:2104-2108

GLOSSARY

BFIE = benign familial infantile epilepsy; **FS** = febrile seizures; **FS+** = febrile seizures plus; **GEFS+** = genetic epilepsy with febrile seizures plus; **ICCA** = infantile convulsions and choreoathetosis; **PKD** = paroxysmal kinesigenic dyskinesia.

Mutations in the proline-rich transmembrane protein 2 gene (*PRRT2*) have recently been found to cause approximately 80% of 2 autosomal dominant infantile-onset seizure disorders, benign familial infantile epilepsy (BFIE) and infantile convulsions choreoathetosis (ICCA) syndrome,^{1,2} following the discovery of *PRRT2* mutations in autosomal dominant paroxysmal kinesigenic dyskinesia (PKD).³⁻⁶ A variety of mutations in *PRRT2* have been reported, with the most common mutation in all 3 disorders being the c.649-650insC (p.R217fsX224) frame-shift mutation.

In BFIE (MIM 605751), seizures begin in a normal infant at a mean age of 6 months and typically cease by 2 years. Seizures commonly comprise clusters of focal or convulsive attacks. Interictal EEG and MRI studies are usually normal.⁷⁻⁹ In ICCA (MIM 602066), family

Correspondence & reprint
requests to Dr. Berkovic:
s.berkovic@unimelb.edu.au

Editorial, page 2086

See pages 2097, 2109,
2115, 2122, and 2154

Supplemental data at
www.neurology.org

Supplemental Data

*These authors contributed equally to this work.

From the Florey Neuroscience Institutes (I.E.S.), Melbourne; Department of Medicine (I.E.S., B.E.G., S.F.B.), The University of Melbourne, Melbourne Brain Centre, Austin Health, Heidelberg; Department of Paediatrics (I.E.S.), The University of Melbourne, Royal Children's Hospital, Melbourne; Epilepsy Research Program (S.E.H., X.I., L.M.D.), School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, Australia; Schneider Children's Medical Center of Israel (S.K., H.G.-S.), Petach Tikvah; Sackler Faculty of Medicine (Z.A.), Tel-Aviv University, Tel-Aviv, Israel; Department of Paediatric Neurology (M.K.), Chelsea and Westminster Hospital Foundation Trust, Imperial College, London, UK; Department of Neurology (I.A.), Sydney Children's Hospital, Sydney, Australia; IRCCS Stella Maris Foundation Pisa (R.G.), Pisa; Child Neurology Unit and Laboratories (C.M.), Pediatric Hospital A. Meyer, University of Florence, Florence, Italy; and Department of Paediatrics (L.G.S.), University of Otago, Wellington, New Zealand.

Study funding: Funding information is provided at the end of the article.

Go to Neurology.org for full disclosures. Disclosures deemed relevant by the authors, if any, are provided at the end of this article.

members have either infantile seizures or child/adolescent onset paroxysmal kinesigenic dyskinesia (PKD)¹⁰ (MIM 128200), or both.

Here we ask whether the phenotypic spectrum of *PRRT2* mutations extends beyond BFIE and ICCA. In addition to expanding our published cohort of classical BFIE and ICCA, we studied individuals with sporadic benign infantile seizures and families with atypical features for BFIE such as later seizure onset or offset, more severe seizures (such as episodes of status epilepticus), and multiple seizure types. We also investigated sporadic and familial benign neonatal seizures and infantile convulsions with mild gastroenteritis.^{11–13}

METHODS Individuals and families with infantile-onset seizure disorders were recruited from pediatric epilepsy and neurology clinics and by referral to the investigators over a 20-year period. All affected individuals underwent detailed electroclinical assessment including a validated seizure questionnaire.¹⁴ In particular, seizure-related triggers such as the presence of fever, gastroenteritis, and viral illnesses were examined. A careful history for movement disorders was also noted in the proband and family members. Where a family history of seizures or movement disorders was present, all available affected family members were phenotyped.

Standard protocol approvals, registrations, and patient consents. All participants and their parents/legal guardians in the case of minors gave informed consent. The study was approved by the Human Research Ethics Committees of Austin Health, Royal Children's Hospital, Melbourne, and the Women's and Children's Health Network, Australia.

Molecular analysis of the *PRRT2* gene. The open reading frame of *PRRT2* (NM_145239.2) was analyzed by direct Sanger sequencing of genomic DNA from each proband. Family members were then analyzed for the c.649-650insC mutation

by direct sequencing or by amplification using the labeled primers 5'-HEX-TCACTCACCACCCTCAAA-3' and 5'-CATTC-GATCCTCCTCAAC-3', followed by separation of the products by capillary electrophoresis on the ABI 3131 genetic analyzer. For the latter assay, normal samples have a single peak at 85 bp while the insertion mutation is detected as a double peak. The primer sequences used for mutation screening are listed in appendix e-1 on the *Neurology*[®] Web site at www.neurology.org.

RESULTS Of the 44 probands, 30 had infantile onset seizures, 5 had convulsions with mild gastroenteritis between 8 months and 3 years, and 9 had neonatal onset seizures (phenotypes listed in the table and the figure).

PRRT2 mutations were identified in 11 probands: 4 had a family history consistent with BFIE, 5 with ICCA, and 2 had no family history of infantile seizures or PKD (figure). The 11 mutation-positive probands all had the common *PRRT2* frameshift mutation, c.649-650insC (p.R217fsX224). Each sporadic case was confirmed to have a de novo mutation that was absent in their parents. Although families A and E were otherwise typical, 4 individuals had seizures with and without fever and were initially classified as febrile seizures plus.¹⁵ Family E also had 4 individuals with febrile seizures, of which 2 had the *PRRT2* mutation. One phenocopy was observed (family C) and 3 nonpenetrant carriers (families A, B, and C).

Nineteen of the 44 probands had a family history of seizures, but the familial pattern fell outside BFIE or ICCA due to a more severe seizure course than expected in BFIE such as seizures continuing into childhood, episodes of status epilepticus, more frequent seizures than typically seen in BFIE, later onset seizures, or multiple seizure types in the relatives (table). None had *PRRT2* mutations.

No *PRRT2* mutations were found in 5 probands with convulsions with mild gastroenteritis nor in 9

Table Clinical and genetic details of the 44 probands

Proband phenotype	No. of probands	Age at onset range	<i>PRRT2</i> mutations	Comments
BFIE	6	4 mo–8 mo	R217fsX224 identified in 4 families	All had family history consistent with BFIE
ICCA	5	4 mo–9 mo	R217fsX224 identified in all families	All had family history consistent with ICCA
Sporadic benign infantile epilepsy	2	4 mo–6 mo	Both cases de novo for R217fsX224	
Convulsions with mild gastroenteritis	5	8 mo–3 y	Nil	
Benign infantile epilepsy with variable family history	5	4 mo–9 mo	Nil	Family history of FS in 2; focal epilepsy in 1; GEFS+ in 1; variable epilepsies in 1
Infantile onset epilepsy with atypical features	12	3 mo–18 mo	Nil	Family history of infantile onset epilepsy in 7; generalized epilepsy in 2; focal epilepsy in 1; variable syndromes in 1
Benign neonatal seizures	9	1 d–3 wk	Nil	All probands negative for mutations in <i>KCNQ2</i> , <i>KCNQ3</i> , and <i>SCN2A</i>

Abbreviations: BFIE = benign familial infantile seizures; FS = febrile seizures; GEFS+ = genetic epilepsy with febrile seizures plus; ICCA = infantile convulsions with choreoathetosis.

Figure Pedigrees of families with *PRRT2* mutations

Pedigrees of the 11 mutation-positive families showing segregation of the *PRRT2* mutation. BFIE = benign familial infantile epilepsy; ICCA = infantile convulsions and choreoathetosis.

probands with benign neonatal seizures where no other molecular cause was found.

by examining other familial and sporadic infantile-onset seizures.

We found that 2 boys with sporadic benign infantile seizures had the common *PRRT2* mutation which was shown to have arisen de novo rather than having been inherited from a parent. This highlights that isolated as well as familial cases of benign infantile epilepsy can be due to mutations in *PRRT2*. De novo mutations were suspected to contribute to benign

infantile epilepsy due to the many independent mutational events in the *PRRT2* gene which have thus far been reported across many ethnicities, with the frameshift mutation p.R217fsX224 accounting for the majority of these events. This common mutation inserts an additional cytosine (C) in a homopolymer of 9 cytosines, creating a hairpin loop predicted to cause DNA polymerase slippage and resulting in a mutation “hot-spot.”² Probands with sporadic benign infantile seizures and BFIE may be clinically indistinguishable^{16–18} and *PRRT2* mutations should be considered in sporadic cases as they carry genetic counseling and prognostic implications.

A further issue with diagnosis of ICCA is the subtlety of the family history of PKD. The history of PKD in relatives was often not recognized or considered related to the seizures in infants at presentation. It was only ascertained by specific questioning, often allowing treatment for symptoms that had been present over many years and had affected daily life in adults with PKD who had not sought medical treatment.

We identified mutations in 4/6 new families with BFIE and 5/5 new families with ICCA. Thus, taken together with the families we previously reported, we have detected *PRRT2* mutations in 18/23 (78%) BFIE and 10/11 (91%) ICCA families of our cohort.²

Interestingly, although BFIE and ICCA classically comprise afebrile seizures in infancy, we observed 8 individuals from 3 families with *PRRT2* mutations who had phenotypes that were considered to be febrile seizures (FS) or febrile seizures plus (FS+) (families A and E; individual 16-II-2 in reference 2). FS+ is defined by FS that continue past the age of 6 years or where afebrile convulsions occur with FS. It is one of the phenotypes that form part of the familial epilepsy syndrome of genetic epilepsy with FS plus (GEFS+).¹⁹ When we reported family E as family CL in 1999,¹⁵ we described 2 individuals as FS+ and 4 as FS. The finding that 5 of these family members have the *PRRT2* mutation suggests that additional FS genes exist in this family. As FS or FS+ may begin in the first year with offset in early childhood, these phenotypes overlap with BFIE if fever is present. Thus BFIE is a differential diagnosis in certain families with FS and FS+ under 2 years of age that may have been conceptualized as GEFS+ families, and mutational analysis of *PRRT2* should be considered. In smaller families with only a few affected individuals, this distinction may be even more difficult. Modifier genes may explain the predisposition to seizures with fever in these *PRRT2* families with evidence for additional genetic variants being present in family E and family 16² where members not carrying the familial *PRRT2* mutation had FS.

As 6 cases from 5 of our *PRRT2* BFIE and ICCA families had some or all of their seizures with gastroenteritis, we examined 5 additional patients

with convulsions with mild gastroenteritis, an increasingly recognized syndrome.^{12,13} None had *PRRT2* mutations. Our findings suggest that BFIE and sporadic cases of convulsions with gastroenteritis have distinct causes.

No *PRRT2* mutations were identified in families with “atypical” infantile seizures or probands with a family history of later onset seizures. We included 19 with infantile seizures falling outside the BFIE phenotype so it is possible that testing larger cohorts of patients will reveal mutations in novel phenotypes. Given the referral bias to our study of epilepsy genetics, it is unlikely that we would be biased against those with *PRRT2* mutations as we are more likely to ascertain those cases with a family history. As both of our 2 infants with “sporadic” self-limited infantile seizures had de novo *PRRT2* mutations, a key question is the importance of *PRRT2* in a large cohort of similar cases.

Previously, overlap has been demonstrated between the ages at seizure onset in families with BFIE and benign familial neonatal seizures. We therefore studied babies with sporadic and benign familial neonatal seizures not associated with potassium channel mutations in *KCNQ2* and *KCNQ3*, but no *PRRT2* mutations were identified. These observations suggest that *PRRT2* is associated with a specific self-limited, age-dependent epilepsy syndrome likely relating to developmental expression and function of the PRRT2 protein.

As little is known about the function of PRRT2 it is difficult to hypothesize how the same mutation of this gene can cause both epilepsy and a movement disorder either in the same individual or family, or in separate families. Here, the number of probands tested was relatively small so a definitive statement regarding lack of involvement of *PRRT2* in specific phenotypes cannot be made. There is, however, a striking difference between the high mutation rate in BFIE and ICCA and the absence of mutations in other phenotypes, showing that *PRRT2* is not a frequent cause of these additional phenotypes.

Confirmation of *PRRT2* mutations in patients with infantile seizures provides families and clinicians with reassurance that the seizures are likely to be self-limited with an excellent outcome. Families can also be forewarned about the possibility of paroxysmal kinesigenic dyskinesia developing later in childhood or adolescence to enable rapid diagnosis and appropriate treatment.

AUTHOR CONTRIBUTIONS

Study concept and design were performed by I.E. Scheffer, B.E. Grinton, S.E. Heron, and S.F. Berkovic. Analysis or interpretation of data were performed by I.E. Scheffer, B.E. Grinton, S.E. Heron, S. Kivity, Z. Afawi, X. Iona, H. Goldberg-Stern, M. Kinali, I. Andrews, C. Marini, L.G. Sadleir, S.F. Berkovic, and L.M. Dibbens. Drafting and revising the

ACKNOWLEDGMENT

The authors thank the patients and their families for participating in the research.

STUDY FUNDING

Supported by the National Health and Medical Research Council of Australia (program grant 628952 to S.F.B., I.E.S., and L.M.D., Australia Fellowship 466671 to S.F.B., Practitioner Fellowship 1006110 to I.E.S., and Training Fellowship 1016715 to S.E.H.) and SA Pathology. L.M.D. is an MS McLeod Research Fellow.

DISCLOSURE

I.E. Scheffer has served on scientific advisory boards for UCB and Janssen-Cilag EMEA; may accrue future revenue on a pending patent WO61/010176: Therapeutic compound that relates to discovery of *PCDH19* gene as the cause of familial epilepsy with mental retardation limited to females; has received speaker honoraria and funding for travel from Athena Diagnostics, Biocodex, GlaxoSmithKline, Janssen-Cilag EMEA, and UCB, and receives/has received research support from the National Health and Medical Research Council of Australia, NIH, Australian Research Council, Health Research Council of New Zealand, The University of Melbourne, American Epilepsy Society, the Jack Brockhoff Foundation, the Shepherd Foundation, and the Perpetual Charitable Trustees. B.E. Grinton, S.E. Heron, S. Kivity, Z. Afawi, X. Iona, H. Goldberg-Stern, M. Kinali, I. Andrews, C. Marini, and L. Sadleir report no disclosures. S. Berkovic has served on scientific advisory boards for UCB and Janssen-Cilag; may accrue future revenue on pending patent WO61/010176: Therapeutic compound that relates to discovery of *PCDH19* gene as the cause of familial epilepsy with mental retardation limited to females; is one of the inventors listed on a patent held by Bionomics Inc on diagnostic testing of using the *SCN1A* gene, international publication number WO2006/133508, filed 16/06/2006; has received speaker honoraria from UCB; has received unrestricted educational grants from UCB, Janssen-Cilag, and Sanofi-Aventis; and receives/has received research support from the National Health and Medical Research Council of Australia and National Institute of Neurological Disorders and Stroke. L. Dibbens may accrue future revenue on a pending patent WO61/010176: Therapeutic compound that relates to discovery of *PCDH19* gene as the cause of familial epilepsy with mental retardation limited to females. **Go to Neurology.org for full disclosures.**

Received January 29, 2012. Accepted in final form May 10, 2012.

REFERENCES

1. Lee HY, Huang Y, Bruneau N, et al. Mutations in the gene *PRRT2* cause paroxysmal kinesigenic dyskinesia with infantile convulsions. *Cell Rep* 2011;1:1–11.
2. Heron SE, Grinton BE, Kivity S, et al. *PRRT2* mutations cause benign familial infantile epilepsy and infantile convulsions with choreoathetosis syndrome. *Am J Hum Genet* 2012;90:152–160.
3. Wang JL, Cao L, Li XH, et al. Identification of *PRRT2* as the causative gene of paroxysmal kinesigenic dyskinesias. *Brain* 2011;134:3490–3498.
4. Chen WJ, Lin Y, Xiong ZQ, et al. Exome sequencing identifies truncating mutations in *PRRT2* that cause paroxysmal kinesigenic dyskinesia. *Nat Genet* 2011;43:1252–1255.
5. Li J, Zhu X, Wang X, et al. Targeted genomic sequencing identifies *PRRT2* mutations as a cause of paroxysmal kinesigenic choreoathetosis. *J Med Genet* 2012;49:76–78.
6. Liu Q, Qi Z, Wan XH, et al. Mutations in *PRRT2* result in paroxysmal dyskinesias with marked variability in clinical expression. *J Med Genet* 2012;49:79–82.
7. Fukuyama Y. Borderland of childhood epilepsy, with special references to febrile convulsions and so-called infantile convulsions. *Seishin Igaku* 1963;5:211–223.
8. Vigeveno F, Fusco L, Di Capua M, Ricci S, Sebastianelli R, Lucchini P. Benign infantile familial convulsions. *Eur J Pediatr* 1992;151:608–612.
9. Vigeveno F. Benign familial infantile seizures. *Brain Dev* 2005;27:172–177.
10. Bruno MK, Hallett M, Gwinn-Hardy K, et al. Clinical evaluation of idiopathic paroxysmal kinesigenic dyskinesia: new diagnostic criteria. *Neurology* 2004;63:2280–2287.
11. Morooka K. Convulsions and mild diarrhea (in Japanese). *Shonika* 1982;23:131–137.
12. Uemura N, Okumura A, Negoro T, Watanabe K. Clinical features of benign convulsions with mild gastroenteritis. *Brain Dev* 2002;24:745–749.
13. Saadeldin IY. Electroclinical features of benign infantile seizures with mild gastroenteritis. *Epileptic Disord* 2011;13:8–17.
14. Reutens DC, Howell RA, Gebert KE, Berkovic SF. Validation of a questionnaire for clinical seizure diagnosis. *Epilepsia* 1992;33:1065–1071.
15. Singh R, Macdonell RA, Scheffer IE, Crossland KM, Berkovic SF. Epilepsy and paroxysmal movement disorders in families: evidence for shared mechanisms. *Epileptic Disord* 1999;1:93–99.
16. Caraballo RH, Cersosimo RO, Espeche A, Fejerman N. Benign familial and non-familial infantile seizures: a study of 64 patients. *Epileptic Disord* 2003;5:45–49.
17. Saadeldin IY, Housawi Y, Al Nemri A, Al Hifzi I. Benign familial and non-familial infantile seizures (Fukuyama-Watanabe-Vigeveno syndrome): a study of 14 cases from Saudi Arabia. *Brain Dev* 2010;32:378–384.
18. Espeche A. Benign infantile seizures: a prospective study. *Epilepsy Res* 2010;89:96–103.
19. Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus: a genetic disorder with heterogeneous clinical phenotypes. *Brain* 1997;120:479–490.