Role of *PRRT2* in common paroxysmal neurological disorders: a gene with remarkable pleiotropy

Sarah E Heron, Leanne M Dibbens

School of Pharmacy and Medical Sciences and Sansom Institute for Health Research, University of South Australia, Adelaide, Australia

Correspondence to
Dr Sarah E Heron, School of Pharmacy and Medical Sciences, University of South Australia, P4-47, City East Campus, GPO Box 2471, Adelaide, SA 5001, Australia; sarah.heron@unisa.edu.au

Received 7 November 2012
Revised 18 December 2012
Accepted 20 December 2012
Published Online First 23 January 2013

ABSTRACT

Mutations in the gene *PRRT2* encoding proline-rich transmembrane protein 2 have recently been identified as the cause of three clinical entities: benign familial infantile epilepsy (BFIE), infantile convulsions with choreoathetosis (ICCA) syndrome, and paroxysmal kinesigenic dyskinesia (PKD). Patients with ICCA have both BFIE and PKD and families with ICCA may contain individuals who exhibit all three phenotypes. These three phenotypes were all mapped by linkage analyses to the pericentromeric region of chromosome 16, and were hypothesised to have the same genetic basis due to the co-occurrence of the disorders in some families. Despite considerable effort, the gene or genes for BFIE, ICCA, and PKD were not identified for many years after the linkage region was identified. Mutations in the gene *PRRT2* were identified in several Chinese families with PKD, suggesting that the gene may also be responsible for ICCA and BFIE in families linked to the chromosome 16 locus. This was demonstrated to be the case, with the majority of families with ICCA and BFIE found to have *PRRT2* mutations. The vast majority of these mutations are truncating and are predicted to lead to haploinsufficiency. *PRRT2* is a largely uncharacterised protein. It is expressed in the brain and has been demonstrated to interact with SNAP-25, a component of the molecular machinery involved in the release of neurotransmitters at the presynaptic membrane. Therefore, the *PRRT2* protein may play a role in this process. However, the molecular mechanisms underlying the remarkable pleiotropy associated with *PRRT2* mutations have still to be determined.

INTRODUCTION

Benign familial infantile epilepsy (BFIE), previously termed benign familial infantile seizures or benign familial infantile convulsions, is a self-limiting seizure disorder of infancy. Patients have non-febrile seizures with onset at between four and 12 months of age and offset by 2 years of age. Subsequent neurological development is usually normal. BFIE was originally described by Watanabe and colleagues[1] in 1987 and the phenotype was further described and named 'benign familial infantile convulsions' by Vigevano and colleagues[2] in 1992.

Watanabe and colleagues[1] described nine infants with benign complex partial epilepsies characterised by the presence of clusters of seizures with motor arrest, decreased responsiveness, and automatisms. They observed that these infants had an apparently normal developmental outcome and that their seizures were easily controlled with antiepileptic drugs. This was in contrast to many cases of seizures in infancy, which were associated with an unfavourable outcome and developmental delay. Watanabe and colleagues[1] also observed that four of the nine patients had a family history of benign infantile convulsions.

Vigevano and colleagues[2] described a further five infants with clusters of partial seizures with secondary generalisation occurring between the ages of 4 and 7 months. The epilepsy was familial in all cases and had a favourable outcome. It was observed that the clinical features in these patients were similar to those seen in benign familial neonatal convulsions, apart from the age of onset. It was also observed that the features in these patients overlapped those described by Watanabe and colleagues.[1] Vigevano and colleagues[2] proposed the name 'benign familial infantile convulsions' for the disorder. This nomenclature has since been updated and the disorder is termed BFIE in the most recent classifications of the International League Against Epilepsy.[3]

Paroxysmal kinesigenic dyskinesia (PKD), also called paroxysmal kinesigenic choreoathetosis, is a movement disorder characterised by sudden attacks of involuntary movement that are induced by a sudden movement from rest, such as rising from a chair or starting to walk, or by exercise. The attacks in PKD consist of dystonic posturing, chorea or athetosis.[4] Diagnostic criteria for PKD were proposed by Bruno and colleagues[5] and include attacks with an identified kinesigenic trigger, short duration (<1 min), no associated loss of consciousness or pain, normal neurologic examination, exclusion of other causes, and onset at between 1–20 years of age or a family history of PKD. The disorder was delineated in detail in 1967 by Kertesz,[6] who described the phenotypes of 10 patients from six families and reviewed similar reports from the literature. It was observed in this study that the disorder was often familial and therefore a genetic cause was proposed. PKD usually has onset in late childhood or adolescence and may remit in adulthood. The disorder responds well to treatment with antiepileptic drugs, particularly carbamazepine or phenytoin, and patients are otherwise normal.[5–7] PKD shows autosomal dominant inheritance in families and sporadic cases are also observed.[5 6]

Infantile convulsions and choreoathetosis (ICCA) is a syndrome in which BFIE and PKD co-occur in the same patient or family. The syndrome was first described as a distinct clinical entity in 1997 by Szepetowski and colleagues,[8] who identified four French families with autosomal dominant inheritance of BFIE and PKD. Genome-wide linkage analysis was performed for these families and all were found to be linked to a 10 cM interval in the pericentromeric region of chromosome 16. Following

To cite: Heron SE, Dibbens LM. *J Med Genet* 2013;**50**:133–139.

this report, additional families with ICCA and linkage to the same region were described.[9 10] Families were also described with BFIE or PKD alone that showed linkage to the same region.[11–19] ICCA, BFIE, and PKD were therefore hypothesised to be allelic.[11] The minimal critical region (MCR) for the majority of these families corresponded to a 21.69 Mb (6 cM) region between *D16S690* and *D16S517* on chromosome 16 and was similar for ICCA, BFIE, and PKD (figure 1). A single BFIE family with a recombination event at *D16S685*,[13] potentially reducing the MCR to a 2.7 Mb region between *D16S690* and *D16S685*, and a second PKD locus on the q-arm of chromosome 16[20] were also described (figure 1).

IDENTIFICATION OF MUTATIONS IN *PRRT2*

Despite the identification of the chromosome 16 BFIE/ICCA/PKD locus in 1997 and the subsequent extensive sequencing of candidate genes within the region,[19 21] the causative gene was not identified for many years. In 2011, Chen and colleagues[4] successfully employed a strategy combining linkage analysis and whole exome sequencing to identify mutations in an uncharacterised gene, *PRRT2*, in eight Chinese families with PKD. This finding was rapidly followed by many similar reports of mutations in the same gene in families from different ethnic backgrounds with PKD, ICCA, and BFIE.[22–54] To date, over 330 families and individuals with mutations in *PRRT2* have been described. De novo mutations are observed in sporadic cases of PKD, ICCA, and BFIE. The vast majority of these families (over 80%) have the same recurrent frameshift mutation: *PRRT2* c.649-650insC; p.Arg217Profs*7. The remaining mutations in *PRRT2* are spread throughout the gene (table 1, figure 2). Most of them are nonsense, frameshift and splice site mutations predicted to lead to protein truncation. Several of these mutations have been demonstrated to cause altered cellular localisation of the *PRRT2* protein or loss of detectable protein expression in vitro.[4 28] Fifteen different missense mutations have been reported.[22–25 29 34 35 37 42 45 46 50 54] These alter amino acid residues clustered in and around two putative transmembrane domains located near the C-terminus of the protein (table 1, figure 2A). In addition to these substitution and small insertion or deletion mutations, three PKD or ICCA patients have been described with large sub-microscopic deletions encompassing a number of genes including *PRRT2* (figure 2B).[55–57] These findings indicate the need for copy number variant analysis as well as sequencing of *PRRT2* to be included in a full diagnostic analysis of the gene.

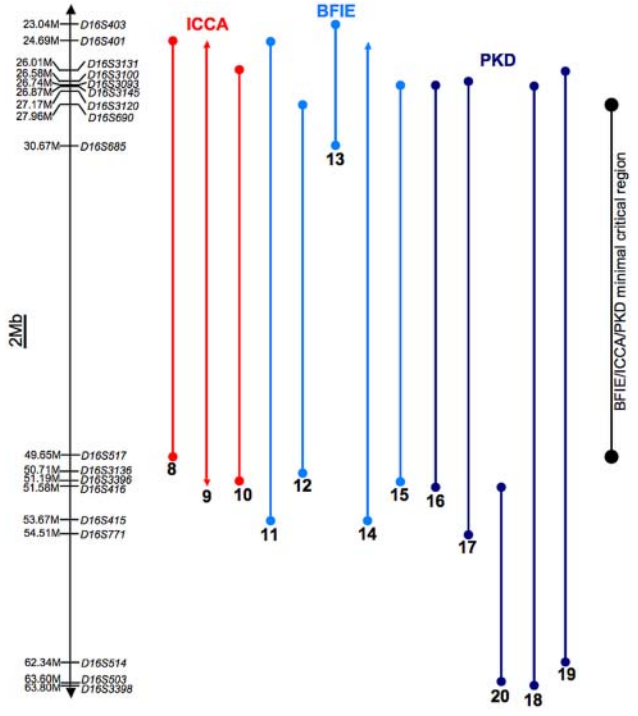

Figure 1 Linkage regions identified for infantile convulsions with choreoathetosis (ICCA), benign familial infantile epilepsy (BFIE), and paroxysmal kinesigenic dyskinesia (PKD) shown against a physical map of the positions of microsatellite markers in the pericentromeric region of chromosome 16 from MapViewer, based on human genome build 37.1. Linkage intervals for ICCA (red), BFIE (light blue), and PKD (dark blue) from 13 publications are shown, along with the minimal critical region derived from these linkage intervals.

Mutations in *PRRT2* have also been identified in families with hemiplegic migraine (HM) and other forms of migraine. This association was initially described in a family with heterogeneous paroxysmal phenotypes including infantile seizures, PKD, HM, and paroxysmal torticollis.[33] Migraine was also noted as a feature in some families where the primary phenotype was PKD, ICCA or BFIE.[24 31 44 46 52 53] A small number of families with *PRRT2* mutations have been described in which migraine, most commonly HM, is the only phenotype observed.[44 46] In contrast to the high mutation rate observed in BFIE, ICCA, and PKD patients, *PRRT2* mutations account for only a small proportion (0.7–3%) of cases of HM occurring without other paroxysmal disorders.[44 45 47] The association of both migraine and seizure disorders with the same gene has been previously observed. A family with a mutation in *ATP1A2* causing both migraine and infantile seizures has been described[58] and mutations in *SCN1A*, which usually cause epilepsy, have been described in patients with HM.[59 60]

Occasional families with *PRRT2* mutations have been described in which epilepsy phenotypes other than BFIE are observed.[48 54] These phenotypes include febrile seizures, febrile seizures plus, and nocturnal convulsions. *PRRT2* mutations have not been associated with other phenotypes that include infantile seizures. In particular, no mutations have been identified in patients with convulsions with gastroenteritis (CwG) or benign familial neonatal epilepsy (BFNE).[43 48 50] BFNE is most commonly caused by mutations in the potassium channel subunit genes *KCNQ2* and *KCNQ3*.[61] The seizures in CwG show similar clinical characteristics to those seen in BFIE, but occur in the context of gastroenteritis, often caused by rotavirus infection.[62]

There is no evidence of a genotype–phenotype relationship between *PRRT2* mutations and the three different phenotypes with which they are associated. All three phenotypes (BFIE, PKD, ICCA) are associated with the common insertion mutation (p.Arg217Profs*7) and all three phenotypes are also associated with other mutations, including the 15 missense mutations. The lack of a genotype–phenotype relationship is not unexpected given the phenotypic variability seen in families with ICCA, in which the same mutation can cause BFIE alone, PKD alone, or both syndromes in different individuals within the same family. This suggests that the expression of the phenotype is influenced by other genetic or environmental factors, rather than the particular *PRRT2* mutation carried by an individual. What these additional factors may be has yet to be determined.

The common c.649-650insC mutation occurs in a homopolymer tract of nine cytosine bases that are preceded by four guanines. This sequence has the potential to form a hairpin loop (as

Table 1 Heterozygous mutations reported in *PRRT2* in families and patients with PKD, ICCA, BFIE, and HM

Mutation	Type	Associated phenotype(s)	Reference(s)
c.117delA; p.Val41Tyrfs*49	fs	PKD	54
c.133-136delCCAG; p.Pro45Argfs*44	fs	PKD	51
c.272delC; p.Pro91Glnfs*24	fs	PKD	34
c.291delC; p.Asn98Thrfs*17	fs	BFIE	29
c.369-370insG; p.Ser124Valfs*10	fs	PKD	23
p.Arg145Glyfs*31	fs	BFIE	44
c.487C>T; p.Gln163*	ns	ICCA	22
c.510-511insT; p.Leu171Serfs*3	fs	PKD	54
c.513-514ins; p.Leu171Leufs*3	fs	PKD	45
c.514-517delTCTG; p.Ser172Argfs*3	fs	PKD	4
c.516-517insT; p.E173*	fs	ICCA	28
c.562C>T; p.Gln188*	ns	ICCA	30
c.573-574insT; p.Gly192Trpfs*7	fs	ICCA	26
c.579-580insA; p.Gle194Argfs*6	fs	ICCA	54
c.595G>T; p.Glu199*	ns	PKD	34
c.604-607delTCAC; p.Ser202Hisfs*16	fs	PKD	34
c.629delC; p.Pro210Glnfs*18	fs	BFIE	29
c.629-630insC; p.Ala211Serfs*14	**fs**	**PKD, ICCA**	25 30
c.649C>T; p.Arg217*	**ns**	**PKD, ICCA**	30 31 35 38 40 54
c.649delC; p.Arg217Glufs*12	**fs**	**PKD, ICCA, HM**	30 31 34 47
c.649-650insC; p.Arg217Profs*8	**fs**	**PKD, ICCA, BFIE, HM**	4 22–36 39 41 43–54
c.718C>T; p.Arg240*	**ns**	**ICCA, BIS, PKD**	28 34 39 44
p.Ser248Alafs*65	fs	ICCA	44
c.748C>T; p.Gln250*	ns	ICCA	27
c.776insG; p.A260*	fs	PKD	41
c.796C>T; p.Arg266Trp	ms	PKD	22
c.824C>T; p.Ser275Phe	ms	ICCA	35
c.841T>C; p.Trp281Arg	ms	PKD	23
c.859G>A; p.Ala287Thr	ms	PKD	23
c.872C>T; p.Ala291Val	ms	PKD	54
c.879+1G>T	ss	BFIE	25
c.879+5G>A	ss	BFIE	25
c.880-2A>T	ss	ICCA	46
c.904-905insG; p.Asp302Glyfs*38	fs	ICCA	24
c.913G>A; p.Gly305Arg	**ms**	**PKD**	24 37
c.913G>T; p.Glu305Trp	ms	PKD	45
c.916G>A; p.Ala306Trp	ms	BFIE	46
c.917C>A; p.Ala306Asp	ms	ICCA	42
c.922C>T; p.Arg208Cys	**ms**	**PKD**	23 34
c.950G>A; p.Ser317Asn	ms	ICCA	25
c.964delG; p.Val322Trpfs*15	fs	PKS	23
c.968G>A; p.Gly232Glu	ms	BFIE	29
c.970G>A; p.Gly324Arg	ms	ICCA	46
c.971G>A; p.Gly324Glu	ms	BFIE	46
c.972delA; p.Val325Serfs*12	fs	PKD	4
c.980-981insT; p.Ile327Ilefs*14	fs	PKD	28
c.981C>G; p.Ile327Met	ms	BFIE	50
p.332insGAC	ifi	ICCA	45
c.1011C>T	ss	PKD	45
c.1011-1012delCG+1-9del9bp	ss	PKD	23
c.1012+2insT	ss	BFIE	43
0.544 Mb deletion at 16p11.2 affecting approximately 30 genes	del	PKD	55
0.6 Mb deletion at 16p11.2 affecting 27 genes	del	ICCA	56
0.43 Mb deletion at 16p11.2 affecting 30 genes	del	PKD	57

Mutations reported in multiple families are indicated in bold and the common insertion mutation is indicated in bold and underlined.
BFIE, benign familial infantile epilepsy; BIS, benign infantile seizures; del, large deletion; fs, frameshift; ifi, in-frame insertion; HM, hemiplegic migraine; ICCA, infantile convulsions with choreoathetosis; ms, missense; ns, nonsense; PKD, paroxysmal kinesigenic dyskinesia; ss, splice site.

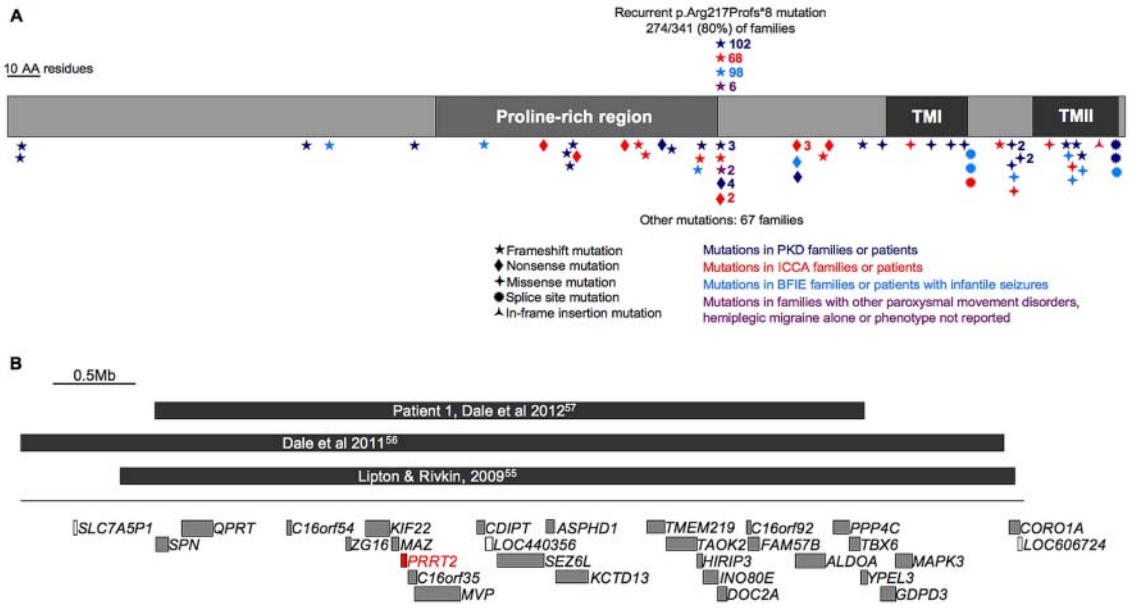

Figure 2 (A) Diagram of the *PRRT2* protein, which contains 340 amino acid residues, showing the locations of known mutations in the gene. The two putative transmembrane domains are indicated in dark grey and the proline-rich region in medium grey. Frameshift mutations are indicated by stars, nonsense mutations by diamonds, missense mutations by crosses, splice site mutations by 12-pointed stars, and the in-frame insertion by a three-pointed star. Mutations associated with paroxysmal kinesigenic dyskinesia (PKD) are shown in dark blue, infantile convulsions with choreoathetosis (ICCA) in red, and benign familial infantile epilepsy (BFIE) in light blue. Mutations marked in purple are either associated with other paroxysmal movement disorders, hemiplegic migraine alone or no phenotype was reported for them. (B) Map showing the extent of the three large deletions identified in patients with PKD or ICCA and the genes involved in these deletions. *PRRT2* is indicated in red. The region affected by the deletions contains 26 other genes marked in grey and three pseudogenes marked as white boxes.

illustrated in figure 3), which may cause polymerase slippage and the insertion of an additional base during DNA replication.[25] The poly-C tract appears to be particularly prone to mutation: in addition to the common insertion mutation, a 1 bp deletion and a nonsense mutation affecting the same nucleotide have been reported several times (table 1). Furthermore, there are five single nucleotide polymorphisms (SNPs) altering bases within the poly-C tract reported in public databases (dbSNP and 1000 Genomes).

The high frequency of the c.649-650insC mutation in this homopolymer tract explains, at least in part, the failure of many previous efforts to identify the BFIE/ICCA/PKD gene. Insertions in homopolymer tracts are less likely to be detected by next generation sequencing (NGS) technologies, due to the technical limitations of the chemistries and detection methods used by them.[63] Indeed, the failure of NGS methods to detect the common insertion mutation has been described twice.[25 29] Homopolymer tracts can also affect the results of classical Sanger sequencing. Polymerase slippage when reading through homopolymers leads to 'noisy' or ambiguous sequence following the homopolymer tract. This has the potential to mask the presence of insertion mutations in the homopolymer or other mutations in the downstream sequence, as has been noted.[27]

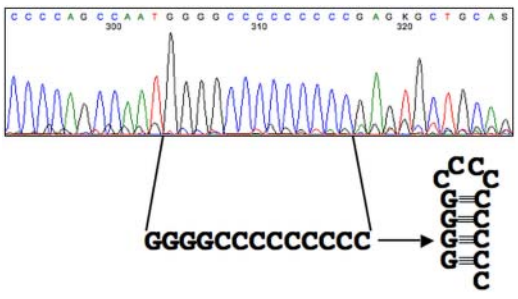

Figure 3 Sequence surrounding the polycytosine tract which is the *PRRT2* mutation hotspot and illustration of a potential hairpin loop structure formed by it.

FUNCTIONAL ROLE OF THE *PRRT2* PROTEIN

The full length *PRRT2* protein contains 340 amino acid residues with two putative transmembrane domains near the C-terminal end (figure 2A). The expression of the gene has begun to be characterised.[4 25 28] The mouse orthologue has been shown by in situ hybridisation analyses to be localised throughout the brain, with the highest mRNA concentrations seen in the cerebral cortex.[4 25] Reverse transcriptase PCR (RT-PCR) experiments also showed *Prrt2* expression in the brain, with lower values seen in spinal cord and no expression in other tissues tested.[4] The *Prrt2* mRNA concentrations in mice were highest on postnatal day 14 (P14), which corresponds approximately to an age of 1–2 years in humans. Western blots of mouse tissues probed with anti-*PRRT2* antibody showed high expression in the brain, low expression in spinal cord, and no expression in other tissues tested,[28] reiterating the RT-PCR results described above. Overall, these data demonstrate convincingly that *PRRT2* codes for a protein with specific expression in the brain and nervous system. The expression of *PRRT2* peaks during postnatal development, consistent with its role in the pathogenesis of infantile seizures. Robust expression of the mRNA throughout the mouse brain is still seen at postnatal day 46,[25] approximately equivalent to adolescence in humans. The mRNA is present in the adult mouse brain, although the expression levels are approximately 50% of those seen at the peak of expression on P14.[4] The expression of *PRRT2* into adulthood is consistent with its role in the pathogenesis of

PKD, which has onset in late childhood or adolescence and sometimes continues during adult life.

Although *PRRT2* is largely an uncharacterised protein, the first steps have been taken in the understanding of its role in neuronal function. Yeast-2-hybrid studies suggested that it interacts with synaptosomal associated protein 25 (SNAP-25).[64] This interaction was confirmed by in vitro and in vivo co-immunoprecipitation experiments.[28] SNAP-25 is a member of the SNARE protein family. Proteins in this family are essential for the transport of vesicles through the Golgi apparatus and to the plasma membrane. SNAP-25 forms part of a complex involved in the release of neurotransmitters from synaptic vesicles at the presynaptic membrane. The SNAP-25 protein is located on the cytoplasmic surface of the plasma membrane in association with syntaxin-1, which is membrane bound. Neurotransmitter release is triggered by the influx of calcium ions resulting from an action potential. These activate synaptotagmin and synaptobrevin molecules on the cytoplasmic surface of the synaptic vesicle, causing the binding of synaptobrevin to syntaxin-1 and SNAP-25 and bringing the synaptic vesicle to the plasma membrane. The synaptic vesicle then fuses with the plasma membrane, releasing its contents into the synapse.[65] Co-immunostaining experiments using FLAG tagged *PRRT2* expressed in primary hippocampal neurones indicated that *PRRT2* co-localised with synapsin-1 at neuronal puncta.[28] Synapsin-1 associates with the cytoplasmic surface of synaptic vesicles and is involved in synaptogenesis and the modulation of neurotransmitter release.[66 67] The localisation of *PRRT2* at neuronal puncta and its interaction with SNAP-25 suggest that it may also play a role in the modulation of neurotransmitter release. A reduction in the amount of *PRRT2* due to haploinsufficiency presumably leads to a dysregulation of this process. The seizures and dystonic posturing seen in BFIE and PKD could possibly result from either excessive neurotransmitter release at excitatory synapses or a reduction in the release of inhibitory neurotransmitters. Understanding which of these processes is affected will require a more precise understanding of the role of *PRRT2* in synaptic transmission. Increased understanding of the pathogenic mechanism underlying *PRRT2* mutations may also explain the particular effectiveness of some antiepileptic drugs—for example, carbamazepine, a sodium channel blocker—in treating the disorders associated with *PRRT2* mutations. Presently there is no obvious link between decreased sodium channel activity and the effective treatment of a disorder resulting from altered neurotransmission, but this may become apparent in the future.

CONCLUSION

Mutations in *PRRT2* account for between 40–100% of familial cases of BFIE,[25 29 39 46 48] 33–100% of familial cases of ICCA,[22 24 28 30 34 35 39 46 48] and 62–100% of familial cases of PKD[4 22–24 28 30 34 35] in the various cohorts of patients that have been studied. The percentages of sporadic cases found to be *PRRT2* mutation positive are generally lower, with mutations found in 27–50% of PKD patients with no family history[23 24 30 35] and 29–100% of cases of sporadic benign infantile seizures.[25 29 39 46 48] The generally high frequency of mutations in familial cases of PKD and BFIE indicates that *PRRT2* mutations are the most common cause of both disorders. The frequency of mutations is particularly high in ICCA. It is possible that the rare mutation negative ICCA families have non-coding mutations or large deletions affecting *PRRT2*, as these would not have been detected by the sequence based screening methods used for the studies reviewed here. It is therefore possible that all cases of ICCA are caused by *PRRT2* mutations. The mutation negative BFIE and PKD cases may also have these types of mutation, or may have mutations in other genes.

PRRT2 is the major gene for BFIE, ICCA, and PKD and contains the second highest number of reported mutations associated with epilepsy after *SCN1A*. The vast majority (95%) of mutations in the gene are truncating mutations predicted to lead to haploinsufficiency. A small number of missense mutations have been reported and these all alter amino acid residues clustered in two predicted transmembrane domains at the C-terminal end of the protein. *PRRT2* is predicted to code for a protein involved in the modulation of presynaptic neurotransmitter release, and a perturbation of this process is likely to be the cause of the seizure and movement disorder phenotypes associated with mutations in the gene. The identification of a heterozygous mutation in *PRRT2* can provide a definitive diagnosis for patients with suspected BFIE, ICCA or PKD. This molecular diagnosis can reduce or prevent the need for additional investigations in these patients and guide treatment for these disorders.

Acknowledgements The authors thank Associate Professor Paul Thomas for advice on the correlation of developmental ages in humans and mice.

Contributors Sarah Heron and Leanne Dibbens co-wrote this manuscript.

Funding This work was funded by National Health and Medical Research Council of Australia Training Fellowship 1016715 to SEH and Career Development Fellowship 1032603 to LMD.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Watanabe K, Yamamoto N, Negoro T, Takaesu E, Aso K, Furune S, Takahashi I. Benign complex partial epilepsies in infancy. *Pediatr Neurol* 1987;3:208–11.
2 Vigevano F, Fusco L, DiCapua M, Ricci S, Sebastianelli R, Lucchini P. Benign infantile familial convulsions. *Eur J Pediatr* 1992;151:608–12.
3 Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshé SL, Nordli D, Plouin P, Scheffer IE. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE commission on classification and terminology, 2005–2009. *Epilepsia* 2010;51:676–85.
4 Chen WJ, Lin Y, Xiong ZQ, Wei W, Ni W, Tan GH, Guo SL, He J, Chen YF, Zhang QJ, Li HF, Lin Y, Murong SX, Xu J, Wang N, Wu ZY. Exome sequencing identifies truncating mutations in *PRRT2* that cause paroxysmal kinesigenic dyskinesia. *Nat Genet* 2011;43:1252–5.
5 Bruno MK, Hallett M, Gwinn-Hardy K, Sorenson B, Considine E, Tucker S, Lynch DR, Mathews KD, Swoboda KJ, Harris J, Soong B-W, Ashizawa T, Jankovic J, Renner D, Fu Y-H, Ptacek LJ. Clinical evaluation of idiopathic paroxysmal kinesigenic dyskinesia. *Neurology* 2004;63:2280–7.
6 Kertesz A. Paroxysmal kinesigenic choreoathetosis. An entity within the paroxysmal choreoathetosis syndrome. Description of 10 cases, including 1 autopsied. *Neurology* 1967;17:680–90.
7 Goodenough DJ, Fariello RG, Annis BL, Chun RW. Familial and acquired paroxysmal dyskinesias. A proposed classification with delineation of clinical features. *Arch Neurol* 1978;35:827–31.
8 Szepetowski P, Rochette J, Berquin P, Piussan C, Lathrop GM, Monaco AP. Familial infantile convulsions and paroxysmal choreoathetosis: a new neurological syndrome linked to the pericentromeric region of human chromosome 16. *Am J Hum Genet* 1997;61:889–98.
9 Lee WL, Tay A, Ong HT, Goh LM, Monaco AP, Szepetowski P. Association of infantile convulsions with paroxysmal dyskinesias (ICCA syndrome): confirmation of linkage to human chromosome 16p12-q12 in a Chinese family. *Hum Genet* 1998;103:608–12.
10 Swoboda KJ, Soong B, McKenna C, Brunt ER, Litt M, Bale JF Jr, Ashizawa T, Bennett LB, Bowcock AM, Roach ES, Gerson D, Matsuura T, Heydemann PT, Nespeca MP, Jankovic J, Leppert M, Ptácek LJ. Paroxysmal kinesigenic dyskinesia and infantile convulsions: clinical and linkage studies. *Neurology* 2000;55:224–30.
11 Caraballo R, Pavek S, Lemainque A, Gastaldi M, Echenne B, Motte J, Genton P, Cersósimo R, Humbertclaude V, Fejerman N, Monaco AP, Lathrop MG, Rochette J, Szepetowski P. Linkage of benign familial infantile convulsions to chromosome 16p12-q12 suggests allelism to the infantile convulsions and choreoathetosis syndrome. *Am J Hum Genet* 2001;68:788–94.

12 Weber YG, Berger A, Bebek N, Maier S, Karafyllakes S, Meyer N, Fukuyama Y, Halbach A, Hikel C, Kurlemann G, Neubauer B, Osawa M, Püst B, Rating D, Saito K, Stephani U, Tauer U, Lehmann-Horn F, Jurkat-Rott K, Lerche H. Benign familial infantile convulsions: linkage to chromosome 16p12-q12 in 14 families. *Epilepsia* 2004;45:601–9.
13 Callenbach PMC, van den Boogerd EH, de Coo RFM, ten Houten R, Oosterwijk JC, Hageman G, Frants RR, Brouwer OF, van den Maagdenberg AMJM. Refinement of the chromosome 16 locus for benign familial infantile convulsions. *Clin Genet* 2005;67:517–25.
14 Striano P, Lispi ML, Gennaro E, Madia F, Traverso M, Bordo L, Aridon P, Boneschi F Martinelli, Barone B, dalla Bernardina B, Bianchi A, Capovilla G, De Marco P, Dulac O, Gaggero R, Gambardella A, Nabbout R, Prud'homme JF, Day R, Vanadia F, Vecchi M, Veggiotti P, Vigevano F, Viri M, Minetti C, Zara F. Linkage analysis and disease models in benign familial infantile seizures: a study of 16 families. *Epilepsia* 2006;47:1029–34.
15 Weber YG, Jacob M, Weber G, Lerche H. A BFIS-like syndrome with late onset and febrile seizures: suggestive linkage to chromosome 16p11.2-16q12.1. *Epilepsia* 2008;49:1959–64.
16 Tomita H, Nagamitsu S, Wakui K, Fukushima Y, Yamada K, Sadamatsu M, Masui A, Konishi T, Matsuishi T, Aihara M, Shimizu K, Hashimoto K, Mineta M, Matsushima M, Tsujita T, Saito M, Tanaka H, Tsuji S, Takagi T, Nakamura Y, Nanko S, Kato N, Nakane Y, Niikawa N. Paroxysmal kinesigenic choreoathetosis locus maps to chromosome 16p11.2-q12.1. *Am J Hum Genet* 1999;65:1688–97.
17 Bennett LB, Roach ES, Bowcock AM. A locus for paroxysmal kinesigenic dyskinesia maps to human chromosome 16. *Neurology* 2000;54:125–30.
18 Cuenca-Leon E, Cormand B, Thomson T, Macaya A. Paroxysmal kinesigenic dyskinesia and generalized seizures: clinical and genetic analysis in a Spanish pedigree. *Neuropediatrics* 2002;33:288–93.
19 Kikuchi T, Nomura M, Tomita H, Harada N, Kanai K, Konishi T, Yasuda A, Matsuura M, Kato N, Yoshiura K, Niikawa N. Paroxysmal kinesigenic choreoathetosis (PKC): confirmation of linkage to 16p11-q21, but unsuccessful detection of mutations among 157 genes at the PKC-critical region in seven PKC families. *J Hum Genet* 2007;52:334–41.
20 Valente EM, Spacey SD, Wali GM, Bhatia KP, Dixon PH, Wood NW, Davis MB. A second paroxysmal kinesigenic choreoathetosis locus (*EKD2*) mapping on 16q13-q22.1 indicates a family of genes which give rise to paroxysmal disorders on human chromosome 16. *Brain* 2000;123:2040–5.
21 Roll P, Sanlaville D, Cillario J, Labalme A, Bruneau N, Massacrier A, Délepine M, Dessen P, Lazar V, Robaglia-Schlupp A, Lesca G, Jouve E, Rudolf G, Rochette J, Lathrop GM, Szepetowski P. Infantile convulsions with paroxysmal dyskinesia (ICCA syndrome) and copy number variation at human chromosome 16p11. *PLoS One* 2010;5:e13750.
22 Wang JL, Cao L, Li XH, Hu ZM, Li JD, Zhang JG, Liang Y, San -A, Li N, Chen SQ, Guo JF, Jiang H, Shen L, Zheng L, Mao X, Yan WQ, Zhou Y, Shi YT, Ai SX, Dai MZ, Zhang P, Xia K, Chen SD, Tang BS. Identification of *PRRT2* as the causative gene of paroxysmal kinesigenic dyskinesias. *Brain* 2011;134:3493–501.
23 Li J, Zhu X, Wang X, Sun W, Feng B, Du T, Sun B, Niu F, Wei H, Wu X, Dong L, Li L, Cai X, Wang Y, Liu Y. Targeted genomic sequencing identifies *PRRT2* mutations as a cause of paroxysmal kinesigenic choreoathetosis. *J Med Genet* 2012;49:76–8.
24 Liu Q, Qi Z, Wan XH, Li JY, Shi L, Lu Q, Zhou XQ, Qiao L, Wu LW, Liu XQ, Yang W, Liu Y, Cui LY, Zhang X. Mutations in *PRRT2* result in paroxysmal dyskinesias with marked variability in clinical expression. *J Med Genet* 2012;49:79–82.
25 Heron SE, Grinton BE, Kivity S, Afawi Z, Zuberi SM, Hughes JN, Pridmore C, Hodgson BL, Iona X, Sadleir LG, Pelekanos J, Herlenius E, Goldberg-Stern H, Bassan H, Haan E, Korczyn AD, Gardner AE, Corbett MA, Gécz J, Thomas PQ, Mulley JC, Berkovic SF, Scheffer IE, Dibbens LM. *PRRT2* mutations cause benign familial infantile epilepsy and infantile convulsions with choreoathetosis syndrome. *Am J Hum Genet* 2012;90:152–60.
26 Cao L, Huang XJ, Zheng L, Xiao Q, Wang XJ, Chen SD. Identification of a novel *PRRT2* mutation in patients with paroxysmal kinesigenic dyskinesias and c.649dupC as a mutation hot-spot. *Parkinsonism Relat Disord* 2012;18:704–6.
27 Ono S, Yoshiura K, Kinoshita A, Kikuchi T, Nakane Y, Kato N, Sadamatsu M, Konishi T, Nagamitsu S, Matsuura M, Yasuda A, Komine M, Kanai K, Inoue T, Osamura T, Saito K, Hirose S, Koide H, Tomita H, Ozawa H, Niikawa N, Kurotaki N. Mutations in *PRRT2* responsible for paroxysmal kinesigenic dyskinesias also cause benign familial infantile convulsions. *J Hum Genet* 2012;57:338–41.
28 Lee HY, Huang Y, Bruneau N, Roll P, Roberson ED, Hermann M, Quinn E, Maas J, Edwards R, Ashizawa T, Baykan B, Bhatia K, Bressman S, Bruno MK, Brunt ER, Caraballo R, Echenne B, Fejerman N, Frucht S, Gurnett CA, Hirsch E, Houlden H, Jankovic J, Lee WL, Lynch DR, Mohamed S, Müller U, Nespeca MP, Renner D, Rochette J, Rudolf G, Saiki S, Soong BW, Swoboda KJ, Tucker S, Wood N, Hanna M, Bowcock A, Szepetowski P, Fu YH, Ptáček LJ. Mutations in the novel protein *PRRT2* cause paroxysmal kinesigenic dyskinesia with infantile convulsions. *Cell Rep* 2012;1:2–12.
29 Schubert J, Paravidino R, Becker F, Berger A, Bebek N, Bianchi A, Brockmann K, Capovilla G, Dalla Bernardina B, Fukuyama Y, Hoffmann GF, Jurkat-Rott K, Anttonen AK, Kurlemann G, Lehesjoki AE, Lehmann-Horn F, Mastrangelo M, Mause U, Müller S, Neubauer B, Püst B, Rating D, Robbiano A, Ruf S, Schroeder C, Seidel A, Specchio N, Stephani U, Striano P, Teichler J, Turkdogan D, Vigevano F, Viri M, Bauer P, Zara F, Lerche H, Weber YG. *PRRT2* Mutations are the major cause of benign familial infantile seizures. *Hum Mutat* 2012;33:1439–43.
30 Méneret A, Grabli D, Depienne C, Gaudebout C, Picard F, Dürr A, Lagroua I, Bouteiller D, Mignot C, Doummar D, Anheim M, Tranchant C, Burbaud P, Jedynak CP, Gras D, Steschenko D, Devos D, Billette de Villemeur T, Vidailhet M, Brice A, Roze E. *PRRT2* mutations: a major cause of paroxysmal kinesigenic dyskinesia in the European population. *Neurology* 2012;79:170–4.
31 Groffen AJ, Klapwijk T, van Rootselaar AF, Groen JL, Tijssen MA. Genetic and phenotypic heterogeneity in sporadic and familial forms of paroxysmal dyskinesia. *J Neurol* 2013;260:93–9.
32 Schmidt A, Kumar KR, Redyk K, Grünewald A, Leben M, Münchau A, Sue CM, Hagenah J, Hartmann H, Lohmann K, Christen HJ, Klein C. Two Faces of the Same Coin: Benign Familial Infantile Seizures and Paroxysmal Kinesigenic Dyskinesia Caused by *PRRT2* Mutations. *Arch Neurol* 2012;69:668–70.
33 Dale RC, Gardiner A, Antony J, Houlden H. Familial *PRRT2* mutation with heterogeneous paroxysmal disorders including paroxysmal torticollis and hemiplegic migraine. *Dev Med Child Neurol* 2012;54:958–60.
34 Lee YC, Lee MJ, Yu HY, Chen C, Hsu CH, Lin KP, Liao KK, Chang MH, Liao YC, Soong BW. *PRRT2* Mutations in Paroxysmal Kinesigenic Dyskinesia with Infantile Convulsions in a Taiwanese Cohort. *PLoS One* 2012;7:e38543.
35 van Vliet R, Breedveld G, de Rijk-van Andel J, Brilstra E, Verbeek N, Verschuuren-Bemelmans C, Boon M, Samijn J, Diderich K, van de Laar I, Oostra B, Bonifati V, Maat-Kievit A. *PRRT2* phenotypes and penetrance of paroxysmal kinesigenic dyskinesia and infantile convulsions. *Neurology* 2012;79:777–84.
36 Steinlein OK, Villain M, Korenke C. The *PRRT2* mutation c.649dupC is the so far most frequent cause of benign familial infantile convulsions. *Seizure* 2012;21:740–2.
37 Friedman J, Olvera J, Silhavy JL, Gabriel SB, Gleeson JG. Mild paroxysmal kinesigenic dyskinesia caused by PRRT2 missense mutation with reduced penetrance. *Neurology* 2012;79:946–8.
38 Wang K, Zhao X, Du Y, He F, Peng G, Luo B. Phenotypic overlap among paroxysmal dyskinesia subtypes: lesson from a family with *PRRT2* gene mutation. *Brain Dev* 2012 http://dx.doi.org/10.1016/j.braindev.2012.07.018 [Epub ahead of print].
39 Specchio N, Terracciano A, Trivisano M, Cappelletti S, Claps D, Travaglini L, Cusmai R, Marras CE, Zara F, Fusco L, Bertini E, Vigevano F. *PRRT2* is mutated in familial and non-familial benign infantile seizures. *Eur J Paediatr Neurol* 2012 http://dx.doi.org/10.1016/j.ejpn.2012.07.006 [Epub ahead of print].
40 van Strien TW, van Rootselaar AF, Hilgevoord AAJ, Linssen WHJP, Groffen AJA, Tijssen MAJ. Paroxysmal kinesigenic dyskinesia: cortical or non-cortical origin. *Parkinsonism Rel Dis* 2012;18:645–8.
41 Hedera P, Xiao J, Puschmann A, Momčilović D, Wu SW, LeDoux MS. Novel *PRRT2* mutation in an African-American family with paroxysmal kinesigenic dyskinesia. *BMC Neurol* 2012;12:93.
42 Cai C, Shi O, Li WD. Missense mutations of the proline-rich transmembrane protein 2 gene cosegregate with mild paroxysmal kinesigenic dyskinesia and infantile convulsions in a Chinese pedigree. *Parkinsonism Relat Disord* 2012 http://dx.doi.org/10.1016/j.parkreldis.2012.08.014 [Epub ahead of print].
43 Ishii A, Yasumoto S, Ihara Y, Inoue T, Fujita T, Nakamura N, Ohfu M, Yamashita Y, Takatsuka H, Taga T, Miyata R, Ito M, Tsuchiya H, Matsuoka T, Kitao T, Murakami K, Lee WT, Kaneko S, Hirose S. Genetic analysis of *PRRT2* for benign infantile epilepsy, infantile convulsions with choreoathetosis syndrome, and benign convulsions with mild gastroenteritis. *Brain Dev* http://dx.doi.org/10.1016/j.braindev.2012.09.006. [Epub ahead of print].
44 Cloarec R, Bruneau N, Rudolf G, Massacrier A, Salmi M, Bataillard M, Bolay C, Caraballo R, Fejerman N, Genton P, Hirsch E, Hunter A, Lesca G, Motte J, Roubertie A, Sanlaville D, Wong SW, Fu YH, Rochette J, Ptáček LJ, Szepetowski P. *PRRT2* links infantile convulsions and paroxysmal dyskinesia with migraine. *Neurology* 2012;79:2097–103.
45 Gardiner A, Bhatia KP, Stamelou M, Dale RC, Kurian MA, Schneider SA, Wali GM, Counihan T, Schapira AH, Spacay SD, Valente EM, Silviera-Moriyama L, Tieve HAG, Raskin S, Sander JW, Lees A, Warner T, Kullmann D, Wood NW, Hanna M, Houlden H. *PRRT2* gene mutations: from paroxysmal dyskinesia to episodic ataxia and hemiplegic migraine. *Neurology* 2012;79:2115–21.
46 Marini C, Conti V, Mai D, Battaglia D, Lettori D, Losito E, Bruccini G, Tortorella G, Guerrini R. *PRRT2* mutations in familial infantile seizures, paroxysmal dyskinesia, and hemiplegic migraine. *Neurology* 2012;79:2109–14.
47 Riant F, Roze E, Barbance C, Méneret A, Guyant-Maréchal L, Lucas C, Sabourand P, Trébuchon A, Depienne C, Tournier-Lasserve E. *PRRT2* mutations cause hemiplegic migraine. *Neurology* 2012;79:2122–4.
48 Scheffer IE, Grinton BE, Heron SE, Kivity S, Afawi Z, Iona X, Goldberg-Stern H, Kinali M, Andrews I, Guerrini R, Marini C, Sadleir LG, Berkovic SF, Dibbens LM. *PRRT2* phenotypic spectrum includes sporadic and fever-related infantile seizures. *Neurology* 2012;79:2104–8.
49 de Vries B, Callenbach PMC, Kamphorst JT, Weller CM, Koelwijn SC, ten Houten R, de Coo IFM, Brouwer OF, van den Maagdenberg AMJM. *PRRT2* mutation causes benign familial infantile convulsions. *Neurology* 2012;79:2154–5.

50 Okumura A, Shimojima K, Kubota T, Abe S, Yamashita S, Imai K, Okanishi T, Enoki H, Fukasawa T, Tanabe T, Dibbens LM, Shimizu T, Yamamoto T. PRRT2 mutation in Japanese children with benign infantile epilepsy. *Brain Dev* 2012. http://dx.doi.org/10.1016/j.braindev.2012.09.015 [Epub ahead of print].
51 Li HF, Ni w, Xiong ZQ, Xu J, Wu ZY. *PRRT2* c.649dupC mutation derived from *De Novo* in paroxysmal kinesigenic dyskinesia. *CNS Neurosci Ther* 2012; http://dx.doi.org/10.1111/cns.12034 [Epub ahead of print].
52 Sheerin U-M, Stamelou M, Charlesworth G, Shiner T, Spacey S, Valente EM, Wood MW, Bhatia KP. Migraine with aura as the predominant phenotype in a family with a PRRT2 mutation. *J Neurol* 2012 http://dx.doi.org/10.1007/s00415-012-6747-4 [Epub ahead of print].
53 Castiglioni C, López I, Riant F, Bertini E, Terracciano A. PRRT2 mutation causes paroxysmal kinesigenic dyskinesia and hemiplegic migraine in monozygotic twins. *Eur J Paediatr Neurol* 2012 http://dx.doi.org/10.1016/j.ejpn.2012.10.010 [Epub ahead of print].
54 Liu XR, Wu M, He N, Meng H, Wen L, Wang JL, Zhang MP, Li WB, Mao X, Qin JM, Li BM, Tang B, Deng YH, Shi YW, Su T, Yi YH, Tang BS, Liao WP. Novel *PRRT2* mutations in paroxysmal dyskinesia patients with variant inheritance and phenotypes. *Genes Brain Behav* 2012; http://dx.doi.org/10.1111/gbb.12008 [Epub ahead of print].
55 Lipton J, Rivkin MJ. 16p11.2-related paroxysmal kinesigenic dyskinesia and dopa-responsive parkinsonism in a child. *Neurology* 2009;73:479–80.
56 Dale RC, Grattan-Smith P, Fung VS, Peters GB. Infantile convulsions and paroxysmal kinesigenic dyskinesia with 16p11.2 microdeletion. *Neurology* 2011;77:1401–2.
57 Dale RC, Grattan-Smith P, Nicholson M, Peters GB. Microdeletions detected using chromosome microarray in children with suspected genetic movement disorders: a single-centre study. *Dev Med Child Neurol* 2012;54:618–23.
58 Vanmolkot KRJ, Kors EE, Hottenga JJ, Terwindt GM, Haan J, Hoefnagels WAJ, Black DF, Sandkuijl LA, Frants RR, Ferrari MD, van den Maagdenberg AMJM. Novel mutations in the Na^+, K^+-ATPase pump gene *ATP1A2* associated with familial hemiplegic migraine and benign familial infantile convulsions. *Ann Neurol* 2003;54:360–6.
59 Dichgans M, Freilinger T, Eckstein G, Babini E, Lorenz-Depiereux B, Biskup S, Ferrari MD, Herzog J, van den Maagdenberg AMJM, Pusch M, Strom TM. Mutation in the neuronal voltage-gated sodium channel *SCN1A* in familial hemiplegic migraine. *Lancet* 2005;366:371–7.
60 Castro MJ, Stam AH, Lemos C, de Vries B, Vanmolkot KRJ, Barros J, Terwindt GM, Frants RR, Sequeiros J, Ferrari MD, Pereira-Monteiro JM, van den Maagdenberg AMJM. First mutation in the voltage-gated $Na_v1.1$ subunit gene *SCN1A* with co-occurring familial hemiplegic migraine and epilepsy. *Cephalalgia* 2009;29:308–13.
61 Bellini G, Miceli F, Soldovieri MV, Miraglia del Giudice E, Pascotto A, Taglialatela M. Benign familial neonatal seizures. In: Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP, eds. *Gene reviews (internet)*. Seattle, WA: University of Washington, Seattle, 1993–2010 [updated 2011 Aug 04].
62 Specchio N, Vigevano F. The spectrum of benign infantile seizures. *Epilepsy Res* 2006;70S:S156–67.
63 Mardis ER. Next-generation DNA sequencing methods. *Annu Rev Genomics Hum Genet* 2008;9:387–402.
64 Stelzl U, Worm U, Lalowski M, Haenig C, Brembeck FH, Goehler H, Stroedicke M, Zenkner M, Schoenherr A, Koeppen S, Timm J, Mintzlaff S, Abraham C, Bock N, Kietzmann S, Goedde A, Toksöz E, Droege A, Krobitsch S, Korn B, Birchmeier W, Lehrach H, Wanker EE. A human protein-protein interaction network: a resource for annotating the proteome. *Cell* 2005;122:957–68.
65 Pang ZP, Südhof TC. Cell biology of Ca^{2+}-triggered exocytosis. *Curr Opin Cell Biol* 2010;22:496–505.
66 Evergren E, Benfanati F, Shupliakov O. The synapsin cycle: a view from the synaptic endocytic zone. *J Neurosci Res* 2007;85:2648–56.
67 Fornasiero EF, Bonanomi D, Benfenati F, Valtorta F. The role of synapsins in neuronal development. *Cell Mol Life Sci* 2010;67:1383–96.