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SHORT COMMUNICATION

Mutations in *PRRT2* responsible for paroxysmal kinesigenic dyskinesias also cause benign familial infantile convulsions

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Paroxysmal kinesigenic dyskinesia (PKD (MIM128000)) is a neurological disorder characterized by recurrent attacks of involuntary movements. Benign familial infantile convulsion (BFIC) is also one of a neurological disorder characterized by clusters of epileptic seizures. The BFIC1 (MIM601764), BFIC2 (MIM605751) and BFIC4 (MIM612627) loci have been mapped to chromosome 19q, 16p and 1p, respectively, while BFIC3 (MIM607745) is caused by mutations in *SCN2A* on chromosome 2q24. Furthermore, patients with BFIC have been observed in a family concurrently with PKD. Both PKD and BFIC2 are heritable paroxysmal disorders and map to the same region on chromosome 16. Recently, the causative gene of PKD, the protein-rich transmembrane protein 2 (*PRRT2*), has been detected using whole-exome sequencing. We performed mutation analysis of *PRRT2* by direct sequencing in 81 members of 17 families containing 15 PKD families and two BFIC families. Direct sequencing revealed that two mutations, c.649dupC and c.748C>T, were detected in all members of the PKD and BFIC families. Our results suggest that BFIC2 is caused by a truncated mutation that also causes PKD. Thus, PKD and BFIC2 are genetically identical and may cause convulsions and involuntary movements via a similar mechanism.
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INTRODUCTION

Paroxysmal kinesigenic dyskinesia (PKD (MIM128200)) is a heritable paroxysmal movement disorder characterized by recurrent and brief attacks of involuntary movements triggered by sudden voluntary movements. PKD attacks consist of any combination of dystonic, choreoathetotic and ballistic components, often occur daily and frequently more than once a day, and usually last from a few seconds to 1–2 min.[1–3] Age of onset is usually during early adolescence. Most of PKD cases are usually inherited as an autosomal dominant trait, but many sporadic cases have also been reported.[2–4] In our previous study, we performed a genome-wide linkage and haplotype analysis and defined disease locus within the pericentromeric region of chromosome 16.[1,5] Subsequently, we performed mutation analysis on 229 genes between D16S3131 and D16S503; however, we failed to identify the causative gene.[5,6] Recently, Chen *et al.*[7] identified truncating mutations within protein-rich transmembrane protein 2 (*PRRT2*) in eight Han-Chinese families with histories of PKD using whole-exome sequencing.

Benign familial infantile convulsion (BFIC) is a clustered epileptic syndrome occurring from 3 to 24 months.[3] Seizures usually occur in clusters over a day, and four associated loci have been identified. BFIC1 (MIM601764), BFIC2 (MIM605751) and BFIC4 (MIM612627) have been mapped to chromosome 19q, 16p and 1p, respectively,[2,3] while BFIC3 is caused by the mutations in *SCN2A* (182390) on chromosome 2q24.[3] Patients with BFIC2 have also been observed within a family that concurrently had PKD.[3] Caraballo *et al.*[8] suggested that BFIC2, PKD, and infantile convulsion and paroxysmal choreoathetosis (ICCA; MIM 602066) might be allelic disorders because of mapping evidence and that these three conditions have

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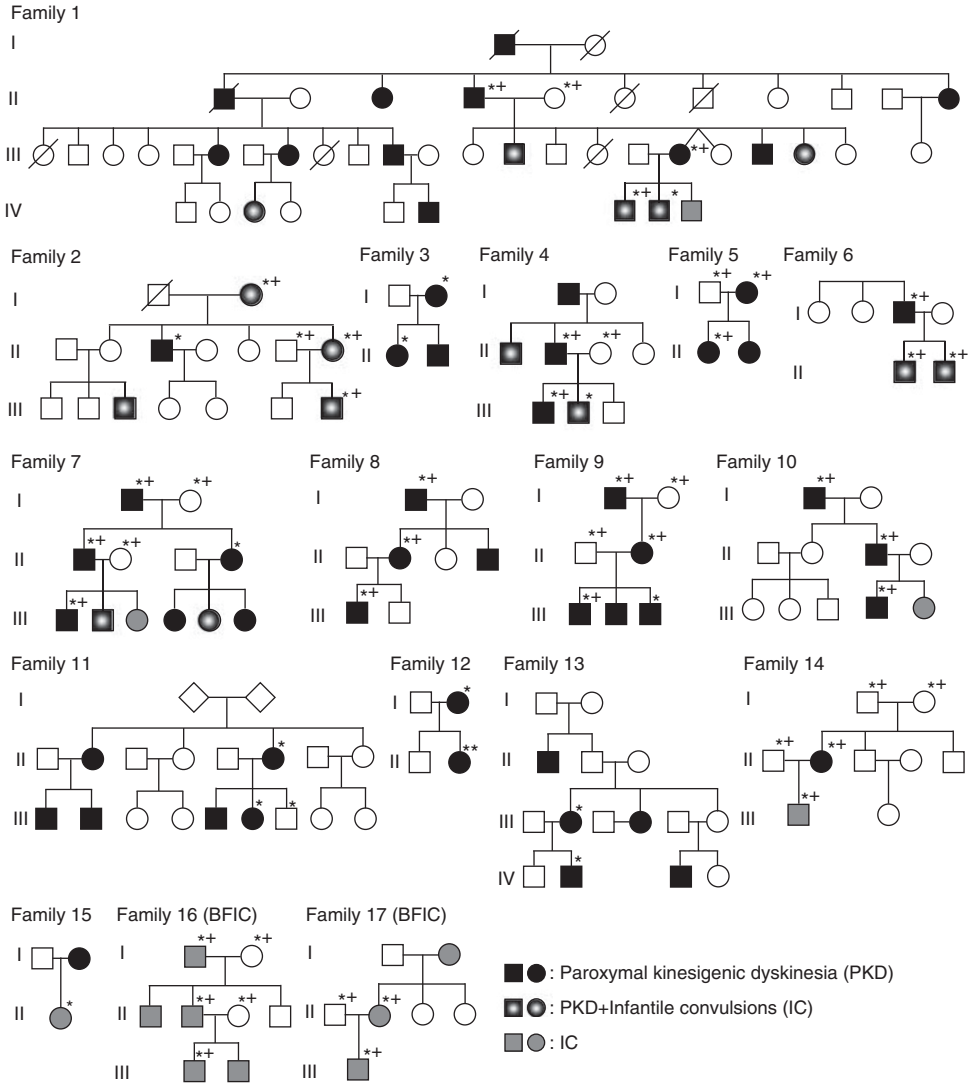

Figure 1 Pedigrees of the 17 families with paroxysmal kinesigenic dyskinesia (PKD) and/or benign familial infantile convulsion (BFIC). Filled-in symbols indicate individuals with PKD or PKD with infantile convulsions or BFIC. Empty symbols indicate unaffected individuals. * indicates individuals whose DNA was used in the mutation analysis. + indicates individuals whose DNA was used in the haplotype analysis.

been found in one family. However, the genetic background and clinical features of BFIC remain unclear.

Here, we describe the results of *PRRT2* mutation analyses in 15 PKD and 2 BFIC2 Japanese families.

MATERIALS AND METHODS

Subjects

We recruited 17 Japanese families for our study, comprised 15 PKD families and two BFIC families with only infantile convulsion patients (Figure 1). Among all these families, 68 patients were diagnosed with PKD and 13 patients were diagnosed with BFIC or infantile convulsion. Out of all 68 PKD patients, 16 patients were diagnosed with infantile convulsion as infants. Detailed information of all families is shown in Figure 1 and Table 1. Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Mini kit (QIAGEN, Hilden, Germany). Experimental procedures were approved by the Committee for Ethical Issues on Human Genome and Gene Analysis at Nagasaki University.

Mutation analysis

We carried out mutation analysis of *PRRT2* by direct sequencing after PCR amplification (primer sequences are available on request). Amplified fragments were directly sequenced using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and were run on an ABI PRISM 3130×l Genetic Analyzer (Applied Biosystems).

Haplotype analysis

Phased haplotypes were constructed with the Merlin software[9] using rs648559, rs7190132, rs183529, rs402720, rs235659, rs10204, rs1057451, rs9938630, rs4788186, rs1291771, rs12919612, rs1129700 and rs7201384. These single nucleotide polymorphisms spanned ~80 kb of the telomeric and ~100 kb of the centromeric sides flanking *PRRT2*. The rs11645263, which is located 53 kb on the telomeric side from (3′ end) of *PRRT2*, was a repeat length polymorphic marker instead of a single nucleotide polymorphism. Therefore, we genotyped the sequences using the GeneMapper (Applied Biosystems) after being run on an ABI PRISM 3130xl Genetic Analyzer.

RESULTS

The c.649dupC mutation, generating a truncated protein with only 223 amino acids, was found in 16 Japanese families with PKD and BFIC2 out of 17 families (Table 1). This mutation in Family 14 was confirmed as *de novo* (Figure 2a). Interestingly, the c.649dupC mutation found in PKD families was also detected in Family 16 and 17 with BFIC2 only. The c.748C>T (p.Q250X) mutation, which substitutes the codon for Q250 with a stop codon, was found in Family 15 (Figure 2b). Furthermore, these two mutations were not found in 288 ethnically matched normal controls.

Haplotype analyses indicated that 11/12 families have the common single nucleotide polymorphism haplotype that carried the mutation flanking *PRRT2*. This haplotype pattern consisted of all major alleles. However, these 11 families could be divided into six groups based on repeat length markers (Supplementary Figure).

DISCUSSION

We report three novel findings in this study. First, the c.649dupC mutation was found not only in PKD families but also in two BFIC2 families that lacked PKD patients. This mutation has been previously reported as the causative gene for PKD by two studies.[7,10] Several reports also indicate that PKD and BFIC2 may be allelic disorders because these two diseases occurred in the same families and map to the same region on chromosome 16.[11] To date, it has been recognized that these two diseases are closely related, yet non-identical, because the main symptom of BFIC2 is convulsion and that of PKD is involuntary movement.[3] Results of our study strongly suggest that PKD and BFIC2 are genetically caused by identical mutations. The difference between these two diseases may simply be due to phenotype differences caused by fluctuating PRRT2 mRNA level during aging. Another possible cause is the *cis*-acting polymorphisms or base changes around the mutation, because BFIC families without PKD (for example Family 16 and Family 17) are obviously observed. Additional mutation searches in many PKD and BFIC families and sporadic cases, along with further analysis of base alterations distinguishing PKD and BFIC, are needed to confirm these assumptions. Because the same mutation can cause the PKD or BFIC, we speculate that convulsions and involuntary movements might be caused by a similar mechanism, and thereby may help in the treatment of involuntary movements.

Second, the c.748C>T (p.Q250X) mutation, which was found in Family 15, is a new detected mutation in *PRRT2*. This mutation is located within the N-terminal extracellular domain, thereby generating a truncated protein, which is similar to the mutant protein generated by c.649dupC mutation in that it also lacks a transmembrane domain.[7,10] Chen *et al.* and Wang *et al.*[7,10] suggested that the truncated proteins caused by mutations located in the second transmembrane motif influence the function of ion channels and might thereby cause PKD.[7,10] Our results are congruent with this suggestion, given that all patients in our study had truncated *PRRT2* mutations.

Finally, the c.649dupC mutation observed in Family 14 was found to be a *de novo* mutation. In our study, the mutation was also observed in 16/17 families. This observation suggests that the mutation observed in our study is potentially a founder mutation. The results of haplotype analysis using repeat length markers, along with the observed *de novo* mutation in Family 14, led us to suggest that the mutation c.649dupC arose independently in most cases. This mutation is the most commonly found in patients with family based PKD, as supported by two previous reports.[7,10] Our results also suggest that the associated sequence, which has nine consecutive cytosines, is a mutational hotspot that has expanded one base to

Table 1 Mutations within *PRRT2* in patients with paroxysmal dyskinesia

Family	*Number of affected individuals (detailed information)*	*Nucleotide mutation*	*Protein alteration*
1	15 (PKD: 9, PKD+IC: 5, IC: 1)	c.649dupC	p.P217fsX7
2	5 (PKD: 1, PKD+IC: 4)	c.649dupC	p.P217fsX7
3	3 (PKD: 3)	c.649dupC	p.P217fsX7
4	5 (PKD: 3, PKD+IC: 2)	c.649dupC	p.P217fsX7
5	3 (PKD: 3)	c.649dupC	p.P217fsX7
6	3 (PKD: 1, PKD+IC: 2)	c.649dupC	p.P217fsX7
7	9 (PKD: 6, PKD+IC: 2, IC: 1)	c.649dupC	p.P217fsX7
8	4 (PKD: 4)	c.649dupC	p.P217fsX7
9	5 (PKD: 5)	c.649dupC	p.P217fsX7
10	4 (PKD: 3, PKD+IC: 1)	c.649dupC	p.P217fsX7
11	6 (PKD: 6)	c.649dupC	p.P217fsX7
12	2 (PKD: 2)	c.649dupC	p.P217fsX7
13	5 (PKD: 5)	c.649dupC	p.P217fsX7
14	2 (PKD: 1, IC: 1)	c.649dupC	p.P217fsX7
15	2 (PKD: 1, IC: 1)	c.748C>T	p.Q250X
16 (BFIC)	5 (IC: 5)	c.649dupC	p.P217fsX7
17 (BFIC)	3 (IC: 3)	c.649dupC	p.P217fsX7

Abbreviations: BFIC, benign familial infantile convulsion; IC, infantile convulsion; *PRRT2*, protein-rich transmembrane protein 2; PKD, paroxysmal kinesigenic dyskinesia.

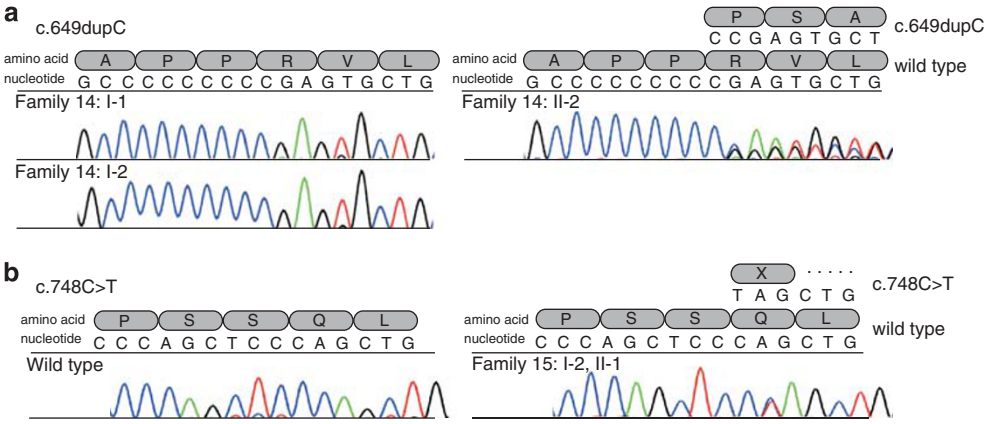

Figure 2 Base substitutions detected in individuals with PKD or infantile convulsion in Family 14 and 15. (**a**) The c.649dupC mutation was detected as a *de novo* mutation in Family 14. (**b**) The c.748C>T mutation was detected in Family 15 and generated a truncated protein with only 249 amino acids. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

result in frameshift. The observed poly C tract in the exon could explain the existence of many sporadic PKD cases. In accordance with this discussion, we clearly identified *de novo* c.649dupC mutation in Family 14. To date, we have analyzed familial PKD, BFIC or ICCR, however, we further need to perform mutation screening in such sporadic cases. Increased attention should be given to the homopolymer within the exon such as *PRRT2*, especially when searching for mutations using the PCR-direct sequencing method. This is because it is potentially difficult to analyze >9 or 10 homopolymers within the elctropherogram by the PCR-direct sequencing method due to slippage causing mixed signals during the PCR amplification, and that homopolymer sites could be mutation hot spots for the development of *de novo* mutations as observed in *PPRT2*.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)