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Characteristics of patients with benign partial epilepsy in infancy without *PRRT2* mutations

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ABSTRACT

Mutations in the proline-rich transmembrane protein 2 gene (*PRRT2*) are known to cause clinical symptoms of paroxysmal kinesigenic dyskinesia (PKD), benign partial epilepsy in infancy (BPEI), and infantile convulsions with choreoathetosis (ICCA) syndrome; however, not all patients with BPEI have *PRRT2* mutations, and the genetic backgrounds for such patients are still unknown. To characterize BPEI patients without *PRRT2* mutations, we analyzed unrelated 63 patients with BPEI. Sanger sequencing identified *PRRT2* mutations in 33 probands (52%). The most common insertion, c.649dup, was identified in 28 probands. Two novel truncation mutations, c.232dup and c.503_504del were identified independently. 16p11.2 microdeletion was not detected in patients without *PRRT2* mutations. *PRRT2* mutation detection rates were 21/31 (68%) and 12/32 (38%) in probands who were positive or negative for family history, respectively, indicating a significant difference between the two groups. In this study, 20 probands with BPEI were negative for family history of BPEI and negative for *PRRT2* mutation. BPEI in these probands may be due to complex genetic predispositions. Because the possibility remains that a second gene contributes to BPEI, further studies are necessary in patients with BPEI but no *PRRT2* mutation, especially in Asian people.

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1. Introduction

There are several types of infantile seizures. The first description of benign infantile convulsion (BIC) was reported in Japan by Fukuyama (1963). Watanabe et al. (1987) proposed the term of "benign partial epilepsy in infancy" (BPEI). They reported nine infants who showed clusters of seizures that consisted of motion arrest, decreased responsiveness, and automatisms. These infants showed normal developmental, and the seizures were easily controlled with antiepileptic drugs. Four of the patients had a family history of the same symptoms. Vigevano et al. (1992) proposed a new epileptic syndrome as "benign familial infantile epilepsy" (BFIE) with five infants who had a family history of similar partial seizures, with secondary generalization occurring between the ages of 4 and 6 months. Their seizure outcomes were favorable. Vigevano et al. (1992) concluded that the clinical features of the patients overlapped with those of BPEI patients.

Because BPEI is inherited as an autosomal dominant trait, many researchers have analyzed chromosomal loci using linkage mapping to investigate genetic etiologies. Through such examination, a relationship between BPEI and paroxysmal choreoathetosis was suggested, and the centromeric region of chromosome 16 was designated as a possible locus (Szepetowski et al., 1997). In spite of such efforts, the gene responsible for BPEI was not identified until the use of massive parallel sequencing. In 2011, comprehensive genomic analysis using next-generation sequencing identified truncating mutations in the gene encoding the protein-rich transmembrane protein 2 (*PRRT2*) in eight Han Chinese families with histories of paroxysmal kinesigenic dyskinesia (PKD) (Chen et al., 2011). Subsequently, heterozygous *PRRT2* mutations were identified in 14 of 17 families affected by BFIE (Heron et al., 2012), indicating that *PRRT2* mutations are the most frequent cause of this disorder. Identification of *PRRT2* mutations in patients with infantile convulsions and/or PKD confirmed that both BPEI and PKD in

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adolescence are included in the broad clinical entity proposed by Watanabe et al. (1987). Now, this is recognized as infantile convulsions and choreoathetosis syndrome (ICCA) (Heron et al., 2012).

It is generally accepted that *PRRT2* is a major cause of BPEI (Schubert et al., 2012); however, many BPEI patients do not have *PRRT2* mutations, and the genetic backgrounds of such patients are still unknown, especially in Asian patients (Ishii et al., 2013; Liu et al., 2013; Okumura et al., 2013). In this study, we attempted to characterize BFIE patients without any *PRRT2* mutation as a step toward future discovery of the second gene responsible for BPEI.

2. Patients and methods

2.1. Ethics statement

This study was performed with the approval of the ethics committee of Tokyo Women's Medical University. After obtaining written informed consent from patients or their families, peripheral blood samples were obtained from the probands. In cases of patients with family history, blood samples were also obtained from affected individuals under informed consent. Patients' medical information and that of their family members were provided by their attending doctors.

2.2. Patients and sample collection

Generally, BPEI is diagnosed when patients meets all of the following conditions (Okumura et al., 2006): (1) focal seizures and/or secondary generalized seizures; (2) seizures onset less than 24 months of age; (3) normal psychomotor development and neurologic finding before seizure onset; (4) normal interictal electroencephalograms (EGGs); (5) normal cranial computed tomography (CT) and magnetic resonance imaging (MRI) findings; (6) no seizures during the first 4 weeks of life; (7) normal psychomotor development after 5 years of age; (8) potential PKD onset in adolescence.

2.3. Molecular analysis

We extracted genomic DNA from blood samples. *PRRT2* sequencing analysis was performed by Sanger sequencing using BigDye terminator and a 3130xl Genetic Analyzer (Life Technologies), as described previously (Okumura et al., 2013).

Chromosomal microarray testing for 16p11.2 microdeletion and any other chromosomal rearrangement was performed using Agilent Hmn 60K kit (Agilent Technologies, Santa Clara, CA) for patients who were negative for *PRRT2* mutation and showed homozygous patterns of single nucleotide variants (SNVs) in the *PRRT2* region (Shimojima et al., 2009).

2.4. Statistical analysis

Difference between the BPEI patients with and without *PRRT2* mutations were evaluated using the Wilcoxon signed-rank test for continuous and ordinary variables, while Pearson's chi-square test was used for categorical variables. The level of statistical significance was set a $p < 0.05$. All statistical analyses were conducted using the statistical discovery software JMP Pro 11 (SAS Institute Japan, Tokyo, Japan). In this study, probands whose parents and/or siblings had histories of BPEI are described as being "positive" for family history.

3. Results

A total of 63 unrelated Japanese families with BPEI were analyzed in this study (Table 1). This included 16 families who participated in our prior research (Okumura et al., 2013). A total of unrelated 63 representative proband (33 men and 30 women) from each family was enrolled. All families of the probands were non-consanguineous.

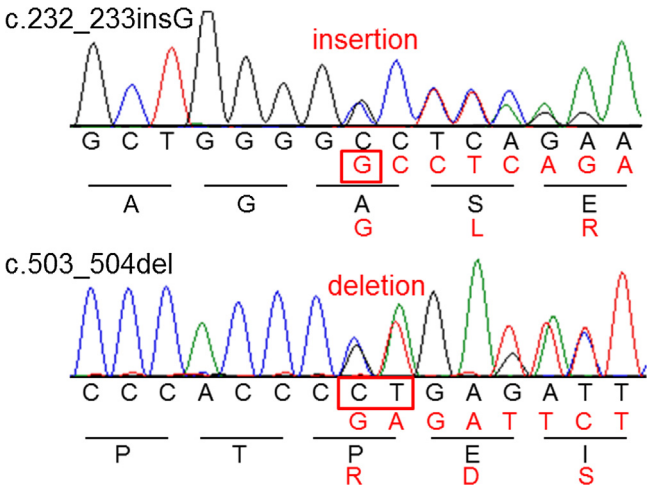

Fig. 1. Electropherograms produced by Sanger sequencing. The two truncation mutations firstly identified in this study are shown. Reference sequences and normal amino acids are shown in black. Altered sequences and affected amino acids are in red (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

Single nucleotide variants of *PRRT2* were identified in 33 probands (52%). The single nucleotide insertion, NM_001256442.1 (PRRT2_v001):c.649dup [NM_001256442.1(PRRT2_i001):p.(Arg-217Profs*8)], which was the most common variants in BPEI, was identified in 28 probands in this study. Two families (proband with ID #20 and #42; Table 1) were found to have a non-synonymous SNV; c.981C > G [p.(Ile327Met)], which was reported previously (Okumura et al., 2013). A SNVs, c.841T > C [p.(Trp281Arg)], which was already identified in a patient with PKD (Li et al., 2012), was identified in a family of proband #15. In this study, two truncation mutations, c.232dup [p.(Ala78Glyfs*56)] and c.503_504del [p.(Pro168Argfs*5)] (Fig. 1), were newly identified in two unrelated probands, #33 and #1, respectively. The novel insertion c.232dup was confirmed to be *de novo* origin, because neither parents of the proband 33 showed this insertion.

A c.439G > C [p.(Asp147His)] was identified in the family of proband #25. This variant is a known SNP registered as rs79568162 in dbSNP build 138, with a minor allele frequency of 0.276%. The scores of SIFT (http://sift.jcvi.org/www/SIFT_help.html) and Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) are 0.021 and 0.327, indicating "DAMAGING" and "benign", respectively. Thus, we concluded that this variant may not be related to BPEI and this variant is not listed in Table 1.

Thirty-one probands were positive for family history, and 32 probands were negative for family history (Table 1). Among them, *PRRT2* mutations were detected in 21 (68%) and 12 (38%), respectively. Probands negative for family history showed a statistically significant lower incidence of *PRRT2* mutations.

Probands with or without *PRRT2* mutations showed median ages of BPEI onset at 5 months (2–14 months) and 6 months (0–15 months), respectively, indicating no significant differences in the age of onset.

Within the total cohort, when *PRRT2* mutation was detected in a proband, the respective parents were subsequently tested for the presence of the mutation; parental mutation was detected in 21 cases. Only 9 of the parents were affected with BPEI, suggesting that the penetrance of the *PRRT2* mutations was 9/21 (43%).

Table 1
Summary of the analyzed patients.

Patient ID	Gender	Family history		Onset (months)	*PRRT2* mutation	Genetically confirmed parental origin
#1	F	+	Sib	2	c.503_504del	Maternal
#2	M	+	Mother	3	c.649dup	Maternal
#3	M	+	Father	3	c.649dup	Paternal
#4	M	+	Mother	4	c.649dup	Maternal
#5	F	+	Sib	4	c.649dup	Paternal
#6	F	+	Sib	4	c.649dup	Unknown
#7	M	+	Mother	4	c.649dup	Maternal
#8	M	+	Father	4	c.649dup	Unknown
#9	F	+	Sib	4	c.649dup	Maternal
#10	M	+	Mother	4	c.649dup	Unknown
#11	M	+	Father, Sib	5	c.649dup	Paternal
#12	F	+	Father, Sibs	5	c.649dup	Paternal
#13	F	+	Father	5	c.649dup	Unknown
#14	M	+	Father, Sibs	5	c.649dup	Unknown
#15	F	+	Father, Sib	5	c.841T>C	Paternal
#16	F	+	Father, Sib	6	c.649dup	Unknown
#17	M	+	Father, Sib	6	c.649dup	Unknown
#18	M	+	Sib	6	c.649dup	Maternal
#19	M	+	Father, Sibs	7	c.649dup	Unknown
#20	F	+	Mother	10	c.981C>G	Maternal
#21	F	+	Sib	14	c.649dup	Paternal
#22	M	+	Mother, Sib	1	–	N/A
#23	M	+	Father	3	–	N/A
#24	F	+	Mother	3	–	N/A
#25	M	+	Father	5	–	N/A
#26	F	+	Mother, Sib	5	–	N/A
#27	M	+	Sib	6	–	N/A
#28	M	+	Mother	7	–	N/A
#29	F	+	Mother	9	–	N/A
#30	F	+	Mother	10	–	N/A
#31	F	+	Sib	11	–	N/A
#32	F	–		3	c.649dup	Maternal
#33	F	–		3	c.232dup	de novo
#34	M	–		4	c.649dup	Unknown
#35	F	–		4	c.649dup	Paternal
#36	M	–		5	c.649dup	Unknown
#37	M	–		5	c.649dup	Maternal
#38	F	–		5	c.649dup	Unknown
#39	M	–		5	c.649dup	Maternal
#40	M	–		5	c.649dup	Maternal
#41	M	–		6	c.649dup	Unknown
#42	M	–		7	c.981C>G	Maternal
#43	F	–		8	c.649dup	Maternal
#44	F	–		0	–	N/A
#45	M	–		1	–	N/A
#46	M	–		3	–	N/A
#47	M	–		3	–	N/A
#48	F	–		3	–	N/A
#49	M	–		3	–	N/A
#50	F	–		5	–	N/A
#51	F	–		5	–	N/A
#52	F	–		6	–	N/A
#53	M	–		6	–	N/A
#54	M	–		6	–	N/A
#55	M	–		7	–	N/A
#56	M	–		7	–	N/A
#57	F	–		7	–	N/A
#58	M	–		8	–	N/A
#59	F	–		9	–	N/A
#60	F	–		10	–	N/A
#61	F	–		12	–	N/A
#62	M	–		14	–	N/A
#63	F	–		15	–	N/A

F, female; M, male; N/A, not applicable.

Thirty probands lacked *PRRT2* mutations; haploinsufficiency of *PRRT2* due to 16p11.2 microdeletions may be related. Thus, we analyzed whether probands without *PRRT2* mutations were heterozygous at the *PRRT2* locus by retrospectively checking benign SNV status of *PRRT2* coding regions. Fourteen probands were heterozygous for benign SNVs. The remaining 16 probands – who showed homozygous patterns of benign SNV in *PRRT* – may have *PRRT2* haploinsufficiency. This was explored by chromosomal microarray testing, but no pathogenic copy number variation was identified in whole chromosomal regions (including 16p11.2 region).

4. Discussion

In this study, we identified *PRRT2* mutation in 33 probands (52%). The most frequently observed mutation was the recurrent c.649dup [p.(Arg217Profs*8)], which was identified in 28 probands

(85%). This rate is similar to rates reported in worldwide. Among the other five mutations, two were novel truncating mutations; c.503_504delCT and c.232dup. Thus, the total frequency of truncating mutation was 91%. A de novo origin of c.232dup, which is quite rare in *PRRT2*, was confirmed in this study.

Since the first report on *PRRT2* mutation, incomplete penetrance of *PRRT2* has been recognized (Heron et al., 2012). So far, studies by van Vliet et al. (2012) and Liu et al. (2013) reported 61% and 73.5% incomplete penetrance of *PRRT2* mutation, respectively. Compared to these previous reports, the present study demonstrated lower penetrance of 43%. To calculate the percentage of penetrance, in this study, we focused on the obligate carrier as the subjects. However, it is often challenging to obtain accurate data regarding the history of the infantile seizures in present day adults, making it difficult to determine the precise penetrance of the mutation (Heron et al., 2012). This is possibly the reason behind such low penetrance percentage calculated in this study.

The *PRRT2* mutation detection rate of 52% reported here is similar to that reported in other research from Asian countries, 48% by Liu et al. (2013) and 61% by Ishii et al. (2013). At present, we do not know why reported *PRRT2* mutation detection rates are lower in Asian countries than in Western countries (Heron et al., 2012; Schubert et al., 2012).

A previous report described a patient with 16p11.2 microdeletion who met the criteria of BPEI and PKD (Weber et al., 2013). *PRRT2* is located in this common microdeletion region. Most of the identified *PRRT2* mutations are truncation mutation, which are predicted to cause loss-of-function of *PRRT2*. Therefore, it is reasonable that haploinsufficiency of *PRRT2* derived from 16p11.2 microdeletion may be masked in patients without *PRRT2* mutation. Based on the hypothesis that probands without *PRRT2* mutation may have microdeletions in *PRRT2* region, we analyzed the genomic copy number aberrations using microarray. However, no probands had microdeletions in the 16p11.2 region.

In this study, only one mutation (c.232dup) was of *de novo* origin. As a result, the incidence of family history in probands with *PRRT2* mutations was 64% (21/33), despite penetrance being 43%. This would explain why family history is frequently observed in BPEI probands with *PRRT2* mutations. In comparison, family history was less frequent in probands without *PRRT2* mutations (30% [10/30]). From these findings, we hypothesized that there might be a second BPEI subgroup that is genetically distinguishable from *PRRT2*-positive BPEI.

Previously, we performed whole-exome sequencing in a three-generation family with BPEI but no *PRRT2* mutation, and identified a SNV in the chloride channel voltage-sensitive 6 gene (*CLCN6*); however, subsequent cohort study identified only two other *CLCN6* variants, indicating quite low incidence (Yamamoto et al., 2015). In the present study, ten probands were positive for family history, but showed no *PRRT2* mutations. Among them, four women (40%) were positive for family history in their mothers. This may be related to X-linked dominant inheritance. The 20 probands were negative for family history and negative for *PRRT2* mutation. BPEI of these probands may be due to complex genetic predispositions. Because the possibility remains that a second gene contributes to BPEI, further studies are necessary in patients with BPEI but no *PRRT2* mutation, especially in Asian people.

Conflict of interest

The authors declare that they have no conflict of interest.

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