

PRRT2 Gene Analysis of Paroxysmal Kinesigenic Dyskinesia (PKD) in Thai Children

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Abstract:

Objective: To examine the frequency of the proline-rich transmembrane protein-2 (*PRRT2*) gene mutation in Thai patients with paroxysmal kinesigenic dyskinesia (PKD).

Material and Methods: A retrospective study of children aged 0–18 years with a diagnosis of PKD at Siriraj Hospital. The genetic analyses of the *PRRT2* gene were done by bidirectional Sanger sequencing.

Results: Twelve patients with PKD were included. The known *PRRT2* mutation, c.649dupC (p.Arg217Profs*8), was identified in three of the patients (25.0%), one of the nine sporadic cases (11.1%) and two of the three familial cases (66.6%), all from different families. PKD had a complete response to carbamazepine treatment regardless of *PRRT2* mutation status.

Conclusion: Our study provided the new details of the clinical phenotypes and *PRRT2* gene analysis findings for Thai PKD. *PRRT2* mutations were identified in our Thai PKD patients with increased detection rates in the familial PKD cases. The c.649dupC (p.Arg217Profs*8) was also found to be a hot-spot mutation in our Thai PKD patients. Furthermore, this study demonstrates the importance of *PRRT2* gene analysis in order to properly diagnose and treat these patients.

Keywords: paroxysmal kinesigenic dyskinesia, PKD, *PRRT2* gene, Thai children

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Introduction

Paroxysmal kinesigenic dyskinesia (PKD) is a rare, heterogenous group of movement disorders. The estimated prevalence is approximately 1 per 150,000.^{1,2} PKD can be sporadic or familial in an autosomal dominant inheritance. Sporadic PKD was reported in 58.8–71.4% of the cases in two previous studies.^{3,4} The clinical presentation is characterized by brief involuntary movements triggered by a sudden or changed direction of movement. Such movements can be caused by dystonia, chorea, ballism, or a combination of these. The symptoms respond well to low-dose carbamazepine (CBZ) and tend to decrease with age.^{2,5}

Mutations of the proline-rich transmembrane protein-2 (*PRRT2*) gene located on chromosome 16p11.2 have been identified as a cause of PKD.⁶ Various studies have reported that a *PRRT2* mutation was found in 60.0–92.8% of the familial and 13.6–45.0% of the sporadic PKD cases.^{3,4,7} Another study reported that 78.5% of the cases had a c.649dupC frameshift mutation.⁸ The clinical spectrum of *PRRT2*-related diseases involves benign familial infantile convulsions (BFIC), infantile convulsions with choreoathetosis (ICCA), hemiplegic migraine, and episodic ataxia.⁸ Apart from the *PRRT2* mutations, other causative genes of PKD have been reported, such as *SLC2A1*¹, *SCN8A*⁹, *KCNMA1*, *KCNA1*, *DEPDC5*¹⁰ and a deletion of chromosome 16p11.2.¹¹

A study of clinical phenotypes and genetic testing in Thai PKD patients has never been done. Our study aimed to assess the frequency of *PRRT2* mutations and characterize the clinical features of PKD along with the diagnostic impact of *PRRT2* gene mutations in Thai patients.

Material and Methods

This was a retrospective study which included children aged 0–18 years with a diagnosis of PKD who met Bruno's criteria⁶ between January, 2007 and May, 2019. The demographic data, clinical phenotypes, and results of

genetic and neuroimaging studies and treatment outcomes were collected by chart review. The protocol for this study was approved by the Siriraj Institutional Review Board, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Genomic deoxyribonucleic acid (DNA) samples from peripheral blood were extracted using the standard salting-out procedure. A mutation analysis of the *PRRT2* gene was carried out by Sanger sequencing. Then Polymerase Chain Reaction (PCR) with three newly designed primer pairs was performed to amplify the entire coding sequence of the *PRRT2* gene. The PCR amplified fragments were treated with ExoSAP-IT (Applied Biosystems, USA) and subsequently sequenced in both directions using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA) with the same primers used in the PCR reactions. The sequencing products were run on an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The obtained sequences were compared with the reference sequence (NM_145239.3). Sequence-variants identification was reported based on the Human Genome Variation Society nomenclature¹² and the classified pathogenicity according to the American College of Medical Genetics and Genomics (ACMG) guidelines.¹³

The data were described by descriptive statistics. The continuous data were reported by mean and standard deviation in a normal distribution or a median and inter-quartile range in a non-normal distribution. Categorical data were reported by percentage.

Results

A total of 12 patients with a diagnosis of PKD during the study period were recruited. Of these, nine were male and three had a family history of first-degree relatives with PKD. One patient (in the familial PKD group) had a history of BFIC. The clinical characteristics, investigations, and treatment results are summarized in Table 1.

Table 1 Clinical characteristics of study paroxysmal kinesigenic dyskinesia patients

| No. | Sex | Onset (age) | Family history | PRRT2 mutation | Movement | Duration (sec) | Frequency of attack (times/day) | Underlying disease | EEG | MRI brain | Treatment | Response |
|--------------|-----|-------------|----------------|----------------|----------|----------------|---------------------------------|--------------------|----------|-----------|------------------|----------|
| Familial PKD | | | | | | | | | | | | |
| 1 | M | 11.3 | PKD | c.649dupC | D/B | <30 | <10 | - | N | ND | CBZ | CR |
| 2 | M | 7.5 | PKD, BFIC | c.649dupC | D, C/B | <30 | 10-20 | E | CT spike | N | LEV,CLZ, VPA>CBZ | CR |
| 3 | M | 8.5 | PKD | - | D/U | <30 | 10-20 | - | N | ND | CBZ | CR |
| Sporadic PKD | | | | | | | | | | | | |
| 4 | M | 7.3 | - | - | D/B | <60 | >20 | - | N | N | VPA | CR |
| 5 | F | 14.4 | - | - | D/B | <60 | <10 | - | N | N | CBZ | CR |
| 6 | M | 12.9 | - | - | D/B | <30 | <10 | ASD, ID, E | N | N | VPA | PR |
| 7 | F | 9.3 | - | - | D/U | <30 | <10 | IC | N | ND | CBZ | CR |
| 8 | M | 14.2 | - | - | D/B | <30 | <10 | IC | N | ND | CBZ | CR |
| 9 | M | 11.0 | - | - | D/U | <30 | <10 | ADHD, ID | N | ND | TPM | PR |
| 10 | M | 13.4 | - | - | D/U | <30 | <10 | - | N | ND | CBZ | CR |
| 11 | F | 12.1 | - | c.649dupC | D, C/B | <30 | <10 | - | N | ND | VPA>CBZ | CR |
| 12 | M | 13.6 | - | - | D/U | <30 | >20 | - | N | ND | CBZ | CR |

ADHD=attention deficit hyperactivity disorder, ASD=autistic spectrum disorder, B=bilateral, BFIC=benign familial infantile convulsion, C=chorea, CBZ=carbamazepine, CLZ=clonazepam, CT spike=centrotemporal spike, CR=complete response, D=dystonia, E=epilepsy, F=female, IC=infantile convulsion, ID=intellectual disability, LEV=levetiracetam, M=male, N=normal, ND=not done, PKD=paroxysmal kinesigenic dyskinesia, PR=partial response, TPM=topiramate, VPA=valproic acid, U=unilateral

Clinical characteristics

The mean age of onset was 11.3 ± 2.6 years, and the median time to diagnosis was 1.1 (IQR, 0.2–1.5) years. All patients had dystonia triggered by the initiation of movement, and in three patients (25.0%) the dystonia was triggered by excitement. Two of them also had chorea. Seven (58.3%) patients had bilateral dyskinesia. Aurae such as general unease and/or tingling sensation were reported in nine patients (75.0%). Two individuals had an intellectual disability.

Investigation results

Pathogenic *PRRT2* mutations were identified in three patients: two of the three in the familial and one out of nine in the sporadic PKD groups. All mutations were of the heterozygous frameshift type of c.649dupC (p.Arg217Profs*8).

We performed electroencephalography (EEG) in all patients and their results were normal, except for one patient (no. 2), who had a bilateral centro-temporal spike. Four patients had magnetic resonance imaging of the brain, and all were normal.

Treatment results

Nine patients had a complete response to CBZ, with a mean dose of 3.8 ± 3.0 mg/kg/day. Other antiseizure medications (ASMs) were given to patients who had a positive HLA-B*1502 screening test. Four patients received sodium valproate, and there was a complete response in one of them and more than a 50.0% reduction of attacks in three. The average dose of sodium valproate was 20.0 ± 11.7 mg/kg/day. One patient received topiramate and had a partial response.

A misdiagnosis of PKD was made on patient no. 2. He had briefly developed focal to bilateral, generalized tonic-clonic seizures when he was five years old. His EEG showed a bilateral centrotemporal region with a bilateral,

generalized 2–3 Hz spike-wave complex during sleep. His seizures were controlled with phenobarbital. At age seven, he started having paroxysmal events characterized by a brief focal tonic stiffening of the limbs, with preserved consciousness. Due to his previous diagnosis of epilepsy and abnormalities in his EEG, he had been misdiagnosed as epilepsy for two years after the new symptoms started. Multiple ASMs were given to him, sodium valproate, clonazepam, levetiracetam, and topiramate, but without effect. He was then transferred to Siriraj Hospital for further evaluation. The paroxysmal events were captured during a study of his EEG and revealed no ictal EEG activity. It was later determined that all of his paroxysms were triggered by the initiation of movement. *PRRT2* gene sequencing was then obtained, and revealed heterozygous c.649dupC. Low-dose CBZ was given to him, and this resulted in rapid and complete treatment response.

Discussion

Our study reviewed the clinical phenotypes and the results of *PRRT2* gene sequencing in Thai patients with PKD. Overall, the clinical phenotypes were similar to those reported in other studies.^{3,4,7} Male predominance was observed in our study. All of our patients typically presented with dystonia during late childhood or adolescence. Our treatment results showed that all PKD patients who received low-dose CBZ had a good response. This supports prior research projects which concluded that CBZ is the most effective medication for PKD patients.³ In patients with positive HLA-B*1502 screening, other ASMs such as sodium valproate or topiramate were used and led to either a complete or partial response.

PKD can be familial or sporadic. Most of our patients were sporadic PKD cases (75.0%), which is similar to previous studies (58.8–71.4%).^{3,4} *PRRT2* mutations were reported in 60.0–92.8% of familial and 13.6–45.0% of sporadic cases.^{3,4,7} In our study, *PRRT2* gene mutations

were found in 3/12 (25.0%) of all the patients: 2/3 (66.7%) in the familial and 1/9 (11.1%) in the sporadic cases. The ratio of *PRRT2* mutations in familial PKD was higher than among the sporadic PKD cases. These findings correspond well with three Korean and one Chinese studies.^{7,14}

More than 80 different *PRRT2* mutations have been identified¹⁰, including truncating, missense, and splice-site mutations and a complete *PRRT2* deletion. Of these, the c.649dupC frameshift mutation has been most commonly found (80.5%) in PKD patients.⁸ The pathogenesis can be explained by the nature of the nucleotide sequence, in which nine consecutive cytosines (C) are located at positions 649–657, thus facilitating the slippage of DNA polymerase during the DNA replication process, and leading to an insertion of one C. This frameshift mutation introduces a premature stop codon which causes truncated *PRRT2* proteins with a consequent loss of function.^{15,16} In our study, all mutations were of the c.649dupC (p.Arg217Profs*8) type. This finding corresponds with that of other studies – namely, that this site is a hot-spot mutation in Thai PKD.^{7,8}

Although the *PRRT2* gene is the most common gene identified in patients diagnosed PKD, *PRRT2* mutations have not been detected in up to 40.0% of the familial and most of the sporadic PKD cases. Therefore, other causative PKD genes could be found in patients with negative *PRRT2* mutations including *SLC2A1*¹, *SCN8A*⁹, *KCNMA1*, *KCNA1*, *DEPDC5*¹⁰ and a deletion of chromosome 16p11.2.¹¹

As PKD is a paroxysmal disorder, it can be misdiagnosed as a seizure disorder, especially when the EEG reveals abnormalities. The patients, then, may be treated as epilepsy with various ASMs, usually at a higher dose than what is used for patients with PKD⁸, as was evident with our patient no. 2. When the diagnosis of PKD was confirmed with a *PRRT2* mutation, a low-dose CBZ was given and the paroxysms were completely controlled. An abnormal EEG has been reported in a similar case in a Korean study.¹⁷ This kind of case emphasizes the need to review the patient's medical history thoroughly, along with

the benefit of genetic testing, in order to make a correct diagnosis and provide the appropriate treatment.

This study had some limitations. We enrolled a small number of PKD cases, especially involving familial PKD. This may have affected the detection rate of *PRRT2* mutations in our study. Furthermore, we performed only *PRRT2* gene sequencing. Therefore, further investigations, such as whole exome sequencing, might help to identify other causative genes. Another point is that our familial cases were only defined by history taking without family genetic testing. Accordingly, it was difficult to show the inheritance pattern. Further genetic testing in families needs to be done.

Conclusion

Our study provided the new details of the clinical phenotypes and *PRRT2* gene analysis findings for Thai PKD. The ratio we identified of *PRRT2* mutations corresponds with that in other Asian studies while the familial PKD had a higher detection rate regarding *PRRT2* mutations. The finding of c.649dupC (p.Arg217Profs*8) as a hot-spot mutation suggests the priority of detecting c.649dupC in Thai patients with PKD. Although negative genetic findings cannot exclude a diagnosis of PKD, the detection of a *PRRT2* mutation could help physicians to make a correct diagnosis, especially when the clinical presentation mimics epileptic seizures. Importantly, a thorough medical history in patients with dyskinesia triggered by initiation of movement is the key to distinguish PKD from epilepsy, which leads to proper management.

Conflict of interest

None of the authors has any conflict of interest to declare.

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