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PRRT2 mutation in Korean patients with paroxysmal kinesigenic dyskinesia: A clinico-genetic analysis

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Abstract

Background & Objective: Recently, mutations in *PRRT2* have been found to cause paroxysmal kinesigenic dyskinesia (PKD). However, only several reports have described the detailed clinical features of patients with the *PRRT2* mutation compared to those without the mutation. Furthermore, 16p11.2 microdeletions including *PRRT2* also have been reported in patients with PKD; however, it is unknown to what extent the *PRRT2* deletion contributes to the development of PKD. *Methods:* We performed mutation screening in 29 Korean patients with PKD analyzing the sequence and gene dosage of *PRRT2* and their clinical features.
Results: Overall, genetic abnormalities in *PRRT2* were identified in 7 patients (24%): 3 from the 6 familial cases (50%) and 4 from the 23 sporadic cases (17%). The previously reported c.649dupC and c.649delC were found in 5 and 1 patient, respectively, and a novel mutation c.323_324delCA was found in 1 patient. No patients had deletions involving the *PRRT2* gene. Compared with the mutation-negative cases, the age of PKD onset was earlier in the mutation-positive cases. However, there were no differences in the other clinical features. A dystonia-only phenotype was reported only in the mutation-negative cases. Contrary to common belief that patients with PKD have an excellent response to carbamazepine, 3 mutation-positive patients taking carbamazepine reported only a partial response.
Conclusions: PRRT2 is a common causative gene for Korean patients with PKD. Our results show that the incomplete response to carbamazepine does not exclude the *PRRT2* mutation.

INTRODUCTION

Paroxysmal dyskinesias are characterized by recurrent attacks of involuntary movements such as choreoathetosis or dystonia. Both familial and sporadic forms exist, and they can be classified into three categories: paroxysmal kinesigenic dyskinesia (PKD), paroxysmal nonkinesigenic dyskinesia (PNKD) and paroxysmal exercise-induced dyskinesia (PED), among which PKD is the most common.[1]

Recently, it has been found that mutations in *PRRT2* cause PKD and infantile convulsions with choreoathetosis, an allelic disorder of PKD, in many ethnic groups.[2-6] Subsequently, *PRRT2* mutations have been implicated in other paroxysmal disorders including PNKD, PED, episodic ataxia, hemiplegic migraine, and migraine with aura suggesting a wide clinical spectrum associated with *PRRT2* mutations.[7,8] In addition, 16p11.2 microdeletions including the *PRRT2* gene also have been reported in patients with PKD.[9,10] However, it is unknown to what degree the *PRRT2* deletion contributes to the development of PKD.

In this study, we performed mutation screening in 29 Korean patients with PKD analyzing the sequence and gene dosage of *PRRT2* and their clinical features.

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METHODS

Twenty-nine (26 men) consecutive unrelated Korean patients diagnosed with PKD according to the standard criteria[11] were recruited between October 2011 and November 2012 from the Movement Disorder Clinic at Seoul National University Hospital (SNUH). Most of the patients were already under regular follow-up at the time of recruitment, and some were new patients. All patients were personally seen by one of the authors (HJK or BSJ). Clinical features were obtained by interviewing each patient using a detailed questionnaire. The differences between the groups were analyzed using the Mann-Whitney *U* test or the c^2 test as appropriate. The protocol of this study was approved by the Institutional Review Board of SNUH.

PRRT2 mutation analysis

After informed consent, blood was drawn from the subjects, and total genomic DNA was extracted from peripheral blood using the Gentra PureGene DNA isolation kit (Gentra Systems, Inc. Minneapolis, MN). PCR was performed with primers designed to flank the splice junctions of the coding exons (Table 1). Amplified products were sequenced on an ABI 3730XL analyzer (Applied Biosystems, Foster City, CA). Sequences were analyzed with the Sequencher software (Gene Codes, Ann Arbor, MI) and Mutation Surveyor (Softgenetics, State College, PA).

Gene dosage at the *PPRT2* gene was determined by quantitative PCR with fluorescently labeled primers. The labeled primer pairs for two target regions within *PRRT2* (exons 2 and 3) and two control loci (HBB and B2MG genes) were designed to yield different sized amplicons. PCR was done in triplicate for each patient limiting the PCR cycles to 18, and the amplified products were electrophoresed on an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA) to measure their fluorescence intensity. The target to control locus ratio was calculated for each triplicate PCR, and the average of the three ratios was compared to that of a normal subject to determine the *PRRT2* gene dosage.

RESULTS

The clinical features and *PRRT2* mutational status of the patients are summarized in Table 2. Six patients had a family history of PKD, and 23 patients were sporadic cases. Overall, genetic abnormalities in *PRRT2* were identified in 7 patients (24%), of which 3 were from the 6 familial cases (50%) and 4 from the 23 sporadic cases (17%). The previously reported c.649dupC (p.Arg217Profs*8) and c.649delC (p.Arg217Glufs*12) were found in 5 and 1 patient, respectively, and a novel mutation c.323_324delCA (p.Thr108Serfs*24) introducing a premature stop codon was found in 1 patient (Case 1). The gene dosage analysis showed that no patients had deletions involving the

Table 1: Primer information in the study

Primer	Sequence	Tm	Size	Purpose
PRRT2-2-1F PRRT2-2-1R	5'-CAGGAATGTGGCCCAATTGG-3' 5'-ACCACTGCCCCATTCTCTTG-3'	59.5 60.0	699	Sequencing
PRRT2-2-2F PRRT2-2-2R	5'-CCATGCCAAGAAACAGTGTCC-3' 5'-ATTGGGGAGGTGGGATCCAT-3'	59.7 60.3	699	Sequencing
PRRT2-3,4F PRRT2-3,4R	5'-CCCCTTCACTCCTCCTTCCT-3' 5'-GAAGCTGTAAACAAGGCCGC-3'	60.3 60.1	591	Sequencing
2-1F 2-1R	5'-FAM-GAGGAGAGTCCCAAGGTTCC-3' 5'-GGGTCTCTGTGGTTTCTGGA-3'	60 60	181	Gene dosage
3F 3R	5'-FAM-TTTCCACCTGATCCCTTCTG-3' 5'-CCCACTCACCGCCTAAGTT-3'	60 60	216	Gene dosage
HBB-186-F HBB-186-R	5'-FAM-ATGCCTCTTTGCACCATTCT-3' 5'-AATCCAGCCTTATCCCAACC-3'	60 60	186	Gene dosage
B2MG-190-F B2MG-190-R	5'-FAM-CTCACGTCATCCAGCAGAGA-3' 5'-AGTGGGGGTGAATTCAGTGT-3'	60 60	190	Gene dosage

Table 2: Clinical features and *PRRT2* mutational status of the 29 cases

Case	Sex	Age at onset, y	Phenotype	Familial history	*PRRT2* mutation
1	M	9	PKD, Sz	PKD	c.323_32delCA
2	M	12	PKD	PKD	c.649dupC
3	M	10	PKD	PKD	c.649dupC
4	F	11	PKD	No	c.649delC
5	M	9	PKD, Sz	No	c.649dupC
6	M	12	PKD	No	c.649dupC
7	M	7	PKD, Sz	No	c.649dupC
8	M	5	PKD	PKD	No
9	M	13	PKD	PKD	No
10	F	8	PKD, Sz	PKD	No
11	M	15	PKD	No	No
12	M	14	PKD, Sz	No	No
13	M	15	PKD	No	No
14	M	16	PKD	No	No
15	M	14	PKD, Sz	No	No
16	M	15	PKD	No	No
17	M	15	PKD	No	No
18	M	15	PKD	No	No
19	M	14	PKD	No	No
20	M	14	PKD, Sz	No	No
21	M	12	PKD	No	No
22	M	13	PKD	No	No
23	M	17	PKD	No	No
24	M	15	PKD, Sz	No	No
25	M	15	PKD	No	No
26	F	14	PKD	No	No
27	M	13	PKD	No	No
28	M	16	PKD	No	No
29	M	15	PKD	No	No

PKD, paroxysmal kinesigenic dyskinesia; Sz, seizure.

PRRT2 gene. Previously reported missense variants c.412C>G (p.Pro138Ala, rs79182085) and c.439G>C (p.Asp147His, rs79568162) were found in 5 and 2 patients, respectively. One patient had a novel variation c.236C>T (p.Ser79Leu), which is probably benign because the amino acid substituted is poorly conserved and predicted to be benign by Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml) and MutationTaster (http://www.mutationtaster.org) but deleterious by SIFT (http://sift.bii.a-star.edu.sg/index.html).

Compared with the mutation (-) cases, the age of onset for PKD was earlier in the mutation (+) cases (Table 3). However, there were no differences in the other clinical features. Interestingly, 29% of the mutation (+) patients and 45% of the mutation (-) patients reported provocation by only thinking about movement without any real movement. While 45% of the mutation (-) patients expressed

Table 3: Comparison between mutation (+) and mutation (-) cases

	Mutation(+) (n=7)	Mutation(-) (n=22)	All (n=29)
Age at onset, y	10.0±1.8	13.8±2.7	12.9±2.9
Attack frequency			
>20/day	2 (28.6)	6 (27.3)	8 (27.6)
1-20/day	5 (71.4)	13 (59.1)	18 (62.1)
1/week – 1/day	0	3 (13.6)	3 (10.3)
Duration of attack			
<5sec	2 (28.6)	9 (40.9)	11 (37.9)
5-30sec	4 (57.1)	13 (59.1)	17 (58.6)
30-60sec	1 (14.3)	0	1 (3.4)
Premonitory symptoms			
Always	4 (66.7)	10 (47.6)	14 (51.9)
Sometimes	2 (33.3)	8 (38.1)	10 (37.0)
Never	0	3 (14.3)	3 (11.1)
Speech involvement			
Always	1 (14.3)	11 (52.4)	12 (42.9)
Sometimes	3 (42.9)	6 (28.6)	9 (32.1)
Never	3 (42.9)	4 (19.0)	7 (25.0)
Laterality			
One side only	1 (14.3)	3 (13.6)	4 (13.8)
Bilateral	6 (85.7)	19 (86.4)	25 (86.2)
Provocations (multiple choice)			
Sudden movement	7 (100)	22 (100)	29 (100)
Startle	0	8 (36.4)	8 (27.6)
Intention to move	2 (28.6)	10 (45.5)	12 (41.4)
Hyperventilation	0	2 (9.1)	2 (6.9)
After brief movement	1 (14.3)	2 (9.1)	3 (10.3)
Phenotype			
Dystonia-only	0	10 (45.5)	10 (34.5)
Choreoathetosis-only	4 (57.1)	3 (13.6)	7 (24.1)
Mixed	3 (42.9)	9 (40.9)	12 (41.4)
Suppressibility			
Always	0	0	0
Sometimes	3 (42.9)	13 (59.1)	16 (55.2)
Never	4 (57.1)	9 (40.9)	13 (44.8)
Medication response			
Complete remission	3 (42.9)	9 (40.9)	12 (41.4)
Inner sensation of symptom, without overt	1 (14.3)	7 (31.8)	8 (27.6)
Dyskinesia			
Symptom continues with reduced frequency	3 (42.9)	6 (27.3)	9 (31.0)
No benefit at all	0	0	0

% in parenthesis.
Note that in some cells total number is smaller than 7 and 22 for mutation(+) and mutation(-) respectively, due to no answers.

the dystonia-only phenotype, none of the mutation (+) patients presented with the dystonia-only phenotype.

All patients were receiving pharmacological treatment, most of which was carbamazepine except in three patients receiving oxcarbazepine. Contrary to the common belief that patients with PKD have an excellent response to carbamazepine, 3 mutation (+) patients taking carbamazepine (Cases 1, 3, and 5) reported that the symptom continued with reduced frequency. The doses of carbamazepine in these 3 patients were 200,

400, and 300 mg per day, respectively, and not lower than those in the 3 mutation (+) patients with complete remission. Case 1 reported that his symptom only slightly improved with 100 mg/day, when the serum level of carbamazepine was 2.8 μg/ml. With 200 mg/day, the frequency of attacks was much improved, but he still had two or three attacks per day. Case 3 reported that he had attacks when he was stressed. Although the serum level of carbamazepine was not measured in patients 3 and 5, we could rule out non-compliance by interview with these patients. None of these three patients were taking other medications.

A history of migraine was reported in 1 patient with a mutation and in 2 patients without a mutation. The interictal electroencephalogram was normal in all patients studied (n=20). Brain MRIs were normal in all patients except in one patient without a mutation (Case 12) who showed several subcortical small high signal intensity lesions in the T2 weighted images. The significance of these lesions was not clear.

DISCUSSION

In our study, 7 out of 29 patients were found to have *PRRT2* mutations. Given that the proportion of mutation (+) patients in familial cases was as high as 100% in previous studies[12-14], the proportion of mutation (+) patients in the familial cases of our study appears to be somewhat lower than expected, showing a greater role for other genetic factors than just that of the *PRRT2* mutation in a Korean population. Similarly, some recent studies have also reported that only 50-60% of familial cases had *PRRT2* mutations.[15-17] The genetic heterogeneity of PKD has long been suggested[5,18,19], and mutations in other genes await identification.

Although the age of onset in our patients was similar to those in previous reports[15,16,20], it was earlier in the mutation (+) cases compared with the mutation (-) cases, which is inconsistent with previous clinicogenetic studies on PKD.[13,17,21] This difference was more prominent when the age of onset in the mutation (+) cases and mutation (-) familial cases (n=10) were compared with the mutation (-) sporadic cases (n=19) (9.6±2.5 vs. 14.6±1.2, $p<0.001$). Interestingly, all the mutation (-) sporadic cases had an age of onset of 12 years or greater. These findings suggest that environmental or acquired factors play at least some role in sporadic PKD although a genetic etiology cannot be excluded. However, in an individual patient, the age of onset did not seem to be a predictor of genetic cause, given an age of onset of 15 years or greater is not uncommon in mutation (+) patients[15,16], and even an age of onset of 40 years has been reported in a patient with the *PRRT2* mutation.[12]

No significant differences in the other clinical features, shown in Table 3, also show that the mutation (+) and mutation (-) patients cannot be differentiated based solely on the clinical features. Of note, no mutation (+) patients presented the dystonia-only phenotype in agreement with a recent report[13], although this difference was not found in another report.[17] Association of the *PRRT2* mutation with bilateral symptoms and premonitory sensation shown in previous reports[13,17] was not detected in our patients.

In a recent study that included 81 PKD patients[13], all *PRRT2* mutation (+) patients showed a complete response to 50 mg/day carbamazepine. However, in our patients, three of the seven mutation (+) patients reported an incomplete response for as high as 400 mg/day carbamazepine, which was not because of non-compliance. Our results indicate that an incomplete response to carbamazepine does not exclude the *PRRT2* mutation.

DISCLOSURE

Conflict of Interest: None

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