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ORIGINAL ARTICLE

PRRT2 c.649dupC Mutation Derived from *De Novo* in Paroxysmal Kinesigenic Dyskinesia

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Keywords

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SUMMARY

Aims: *PRRT2* was recently identified as a causative gene for paroxysmal kinesigenic dyskinesia (PKD), and the c.649dupC mutation was shown to be a "high frequency" mutation. This mutation was also identified in many sporadic cases. This might be attributed to the incomplete penetrance of c.649dupC. Alternatively, c.649dupC might derive from *de novo*. The aim of this study is to elucidate the possibility concerning *de novo* mutagenesis of *PRRT2* mutations in PKD. **Methods:** Nine sporadic Chinese PKD patients including one Mongolian patient were recruited. Direct sequencing of *PRRT2* was performed in them and their parents. Haplotype analysis was conducted to confirm the biological relationship. **Results:** A novel mutation, c.133_136delCCAG, was identified in one Han patient and his unaffected mother. The c.649dupC mutation was detected in another Han patient and his unaffected father. To our interest, c.649dupC was detected in the Mongolian patient but not in his parents. Haplotype analysis confirmed the biological relationship among the trio. No mutations were identified in the remaining six patients. **Conclusion:** These findings demonstrate the heterogeneity of PKD, and the *de novo* mutagenesis of *PRRT2* gene might indicate the genetic instability of this region.

Introduction

Paroxysmal kinesigenic dyskinesia (PKD) is an autosomal dominant movement disorder with incomplete penetrance and characteristics of involuntary attacks precipitated by sudden movements [1]. Attacks consist of choreoathetosis, dystonia or ballism with a brief duration and a frequency ranging from one per month to 100 per day [2,3]. Despite being a hereditary disorder, PKD is both clinically and genetically heterogeneous. It can co-occur with other episodic disorders, such as benign familial infantile epilepsy or infantile convulsions [4,5]. In clinical practice, PKD is often misdiagnosed as epilepsy because of its episodic, paroxysmal features and favorable response to antiepileptic drugs (AEDs), among which carbamazepine and phenytoin are widely considered as drugs of choice.

Recently, *PRRT2*-encoding proline-rich transmembrane protein 2 was identified as a causative gene for PKD by several independent groups [6–8]. Our previous study showed that c.649dupC mutation was identified in six of eight Chinese Han families, accounting for a percentage as high as 75% [6]. The high frequencies of c.649dupC mutation were also observed in two independent studies in China [7,8]. Moreover, the majority of reported patients affected by PKD with infantile convulsions (PKD/IC), infantile convulsions with choreoathetosis syndrome, or benign familial infantile convulsions were also detected to harbor c.649dupC mutation [9–12]. These together implied that c.649dupC mutation is a probable mutation "hot spot" within *PRRT2*. However, the potential molecular mechanism of c.649dupC mutagenesis remains unclear.

In addition, the high frequencies of c.649dupC mutation were also identified in sporadic PKD cases. This phenomenon might be attributed to the incomplete penetrance of c.649dupC mutation. Alternatively, c.649dupC mutation might derive from *de novo* mutagenesis in sporadic cases. To test this hypothesis, we screened *PRRT2* mutations in nine patients with sporadic PKD and their parents. In one family with Mongolian background, we detected c.649dupC mutation in the proband, but failed to detect it in his parents. Haplotype analysis confirmed the biological relationship among the trio, indicating the *de novo* origin of c.649dupC mutation in this case. To the best of our knowledge, this is the first

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report concerning *de novo* mutagenesis of *PRRT2* mutations in Chinese PKD.

Patients and Methods

Patients

The protocols of this study were approved by the ethics committees of Huashan Hospital, Fudan University. Nine patients with sporadic PKD who met Bruno criteria [1] and their parents were recruited in Neurology Clinic of Huashan Hospital between April 20 and August 6, 2012. One patient was of Mongolian descent and the other eight were Chinese Han. They are from Jiangsu (four patients), Zhejiang (two patients), Jiangxi (two patients), and Inner Mongolia (one patient).

Screening of *PRRT2* Mutations

Peripheral blood was collected after obtaining informed consents from all the patients and their parents. Genomic DNA was extracted using a Blood Genomic DNA Extraction kit (Tiangen, Beijing, China). To analyze *PRRT2*, fragments encompassing each of the four coding exons and corresponding splice junctions were amplified by polymerase chain reaction (PCR). Sequences of forward and reverse PCR primers and the annealing temperatures have been previously described [6]. PCRs were carried out in a final volume of 20 μL, containing 40 ng of genomic DNA and 12.5 pmol of both forward and reverse primers. Amplified products were purified, and DNA direct sequencing was performed using an ABI 3730 Automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The resulting sequences were aligned to the NCBI human reference DNA sequence of *PRRT2* (Ensembl gene identification number ENSG00000167371), and nucleotide changes were numbered corresponding to their position within the *PRRT2* mRNA (NCBI Reference Sequence: NM_145239.2).

Haplotype Analysis

To address the possibility that the family members were genetically related, we performed haplotype analysis based on 18 single nucleotide polymorphisms (SNPs). Among these SNPs, rs1045968 and rs10204 were located within *PRRT2*, while others (rs3953219, rs2071420, rs9922666, rs7205278, rs235649, rs9923649, rs1057451, rs1057452, rs9938630, rs4788186, rs13332660, rs3844222, rs9926856, rs1129700, rs9928448, and rs4788172) flanked *PRRT2*. All 18 SNPs were genotyped using Sequenom MassARRAY system at Fudan-Van Andel Research Institute (VARI) Center (School of Life Science, Fudan University, China). We used MassARRAY Assay Design 3.0 software (Sequenom, San Diego, CA, USA) to design the PCR primers used in the genotyping. Sequences of the forward and reverse PCR primers for these 18 SNPs were shown in Table 1. The resulting products were desalted and transferred to a 384-element SpectroCHIP array (Sequenom). Allele was detected using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The probability of the patient being biological son of the alleged parents was calculated by reverse parentage testing, as previously reported [13]. Paternity Index (PI) was calculated for each genetic locus and then multiplied together to obtain a combined paternity index (CPI) for all the genetic loci examined. By definition, we had CPI = p/(1−p) with p representing the probability of the child being the biological son of the alleged parents. P is then calculated as CPI/(1 + CPI).

Results

Identification of *PRRT2* Mutations

After conducting sequencing of *PRRT2* gene in nine sporadic patients, two patients (case 4 and 7) were detected to carry the most common *PRRT2* mutation, c.649dupC, and one (case 8) carry

Table 1 PCR primers for haplotype analysis based on 18 SNPs

SNP_ID	Allele	Forward primer(5′→3′)	Reverse primer (5′→3′)	Extension primer
rs3953219	C/T	ACGTTGGATGCTTAGAATATTAAGAGCGCAG	ACGTTGGATGGTCATTATAATTCTCTGCAAC	TAATTCTCTGCAACAAAACTA
rs2071420	G/A	ACGTTGGATGTGGAATCTAAGCTGCTGAGG	ACGTTGGATGAATGACAGAGGAACAGATGC	CCATCAGCCCCATCC
rs9922666	G/T	ACGTTGGATGCTGGCCATTGAGTTTTCTGC	ACGTTGGATGGCCAATATTTGGCCGTTTTG	GACCAATAAAAGCAGCA
rs7205278	A/G	ACGTTGGATGTGCTCCACCCTACAGAAATG	ACGTTGGATGAACTACATCTGCTGCTCACC	CCTCTCCTAAAGGGAAGAT
rs235649	C/T	ACGTTGGATGGCTACTCCTTCCTGATTCTG	ACGTTGGATGACAGGAGAGCAAGTCAGATG	CAGATGGGAACATCAGTATA
rs1045968	G/T	ACGTTGGATGAAATTCTCTGGACCTCCACC	ACGTTGGATGAAGAAGGGAAGGAAGAGAGG	TGAAAGTTGGGAGGAGG
rs10204	C/T	ACGTTGGATGCCCAAGCCTCGTCTAAGTG	ACGTTGGATGCATCTCCCTTAAGCATCCTC	GGTAACCGTTTACTCTGCAAAA
rs9923649	G/A	ACGTTGGATGACCGATCTAGGGCTAAGATG	ACGTTGGATGTCAGGCCTCTCTCTGACTTC	TCACCTGGGAAATCAGGTGG
rs1057451	G/T	ACGTTGGATGGCTCAGTCCTATGATTAGGC	ACGTTGGATGAGAACCGCCTAGATCAGAAG	GGAACCAGGCTTCAG
rs1057452	G/A	ACGTTGGATGCAGCCTGTTTTCACCCAGC	ACGTTGGATGGAGACACATTCTCAATTTCC	CTAAAGGGGGTAAACCA
rs9938630	C/T	ACGTTGGATGCTGGGAACAGCCTCCATTTG	ACGTTGGATGAGATGGTGGAGAGGCCTCAG	TGGTGTTGGGGAGATTATCCA
rs4788186	A/G	ACGTTGGATGGCTAGGATTCGGCCATAAAC	ACGTTGGATGCAGCCCAGGATTACTAAAGC	AAGCATAGGAAAGAGGC
rs13332660	C/T	ACGTTGGATGCAGATGAGTAATCAGGATGG	ACGTTGGATGTTAGGTCCCTTGGCTATGTG	CCCCCGCTGGTGTTTC
rs3844222	T/G	ACGTTGGATGTTCTCATGGTCTGCTGCTTG	ACGTTGGATGATGAGGGAGAGGCCAAGAG	TACGCATCCTCTCTCCA
rs9926856	A/G	ACGTTGGATGAAACTCAGATGCCGCAGAGG	ACGTTGGATGCTCTTTCTTGCACCGACCTG	CCCTGTTTCCTCGTCTCA
rs1129700	T/C	ACGTTGGATGTTCATTGCATGGCACAGCTC	ACGTTGGATGGGCCGGAATGTACCATGTTG	TGTTAGCAATGGGGTTGGGA
rs9928448	C/T	ACGTTGGATGCACTGGATAGACCAGTTCTC	ACGTTGGATGCCTGGAGGAAACAATGTCTG	GCAGCGTGTCCTTAGAG
rs4788172	A/G	ACGTTGGATGTGGAAGGGCAGAAGCAACG	ACGTTGGATGCACTCAGGGTAGAAGCCAAG	CTGACCCCTGCCTACCTCT

SNPs, single nucleotide polymorphisms.

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a novel mutation, c.133_136delCCAG (Figure 1). The novel mutation causes a deletion of Pro[44] and a frameshift within the codon for Glu[45], resulting in the substitution of arginine for proline and the addition of 43 additional novel amino acid residues prior to the termination at a stop codon within the new reading frame (p.Pro45Argfs*44). No nucleotide changes were identified in the other six patients.

Furthermore, we screened *PRRT2* mutations in the parents of three patients (case 4, 7, and 8) carrying *PRRT2* mutations. We also detected the c.649dupC mutation in the unaffected father of case 7 and the c.133_136delCCAG mutation in the unaffected mother of case 8, indicating an incomplete penetrance. However, in the case 4 with Mongolian background, we failed to identify any variants within *PRRT2* in both his father and his mother. This implies that the mutation in this patient may derive from *de novo*. Thus, we had to confirm the biological relationship among them.

Haplotype Analysis

As shown in Figure 2, the patient with Mongolian background inherited one haplotype from his mother and another one from his father. Using reverse parentage testing, we obtained a CPI 1378.25 in this family. The P value was therefore calculated as 0.9993, reflecting a 99.93% probability that the patient was the biological son of the alleged parents. This confirmed the biological relationship among the trio and further indicated that the c.649dupC mutation in this patient did derive from *de novo* mutagenesis.

Clinical Presentations

Among nine sporadic patients, two are women and the other seven are men. Age at onset ranged from 3 to 18 years. Electroencephalogram (EEG) and brain magnetic resonance imaging (MRI) revealed normal results in all of them. The clinical features of these nine sporadic PKD cases were summarized in Table 2.

The clinical manifestations of the case 4 were described in detail as follows because of his *de novo* mutagenesis. The prenatal history of this case was negative and his developmental milestones were normal. He had no significant medical history. The attacks were first evident at the age of 12 when the patient stood up quickly to answer teacher's question in class. His head turned to one side with his face twisted, his arms extended at the elbow and abducted at the shoulder, and his hands flexed at the wrist. He then began ballistic flailing, followed by athetotic movements of the torso. The attacks lasted about 20 seconds, making all of his classmates as well as his teacher stunned by his abnormal behaviors. He was fully aware of his surrounding during the attacks, which made him very embarrassed. He then sought his medical care in local hospital and was diagnosed with epilepsy, although his general physical and neurological examinations disclosed unremarkable findings and brain MRI, 24-h video-EEG revealed normal results. Valproate and clonazepam were prescribed to treat his attacks but showed null effect. In the following months, he developed similar dyskinesias triggered by a variety of sudden movements such as standing up, running, or jumping. The events usually lasted from 10 to 30 seconds and occurred up 3–5 times daily at the beginning and increased to 5–10 times per day at the age of 13–15. Stress and anxiety seemed to lower the threshold for attacks, heightening the likelihood and frequency of spells. He was misdiagnosed with epilepsy in the following 9 years and was prescribed various AEDs such as valproate, phenobarbital, gabapentin, phenytoin, and clonazepam. He took these AEDs irregularly and his dyskinesia was not completely controlled. Symptoms were significantly relieved, but the attacks still occurred intermittently. Day after day, he learned to act slowly to prevent the attacks when he felt the episodes were occurring.

Five months ago, he came to our hospital for further treatment. A diagnosis of PKD was made and he was prescribed carbamazepine at a low dosage of 50 mg once daily. A complete resolution of signs was achieved 2 days after initiation of carbamazepine. No side effects were reported. But even so, the carbamazepine was still necessary for him, because discontinuation of carbamazepine for 3 days resulted in a return of the attacks.

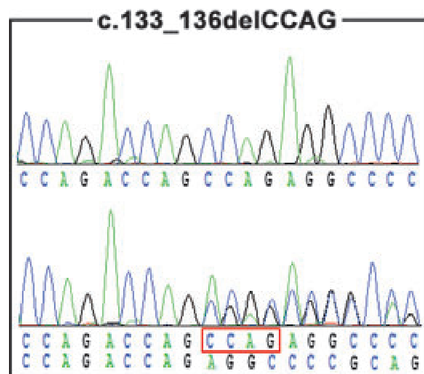

Figure 1 Chromatogram of the novel c.133_136delCCAG mutation within PRRT2 gene. The upper panel is the normal sequence, whereas the lower panel represents heterozygous mutated sequence.

Discussion

Paroxysmal kinesigenic dyskinesia represents a group of episodic movement disorders that are usually triggered by sudden movements. Since the first description by Kertesz in 1967 [14], immense effort has been made to detect the causative locus for PKD. No susceptibility gene, however, was detected until recently, when several independent groups showed that mutations in *PRRT2* were causative for PKD [6–8]. By now, more than 20 *PRRT2* mutations have been identified in patients affected with PKD or PKD/IC [6–12], and c.649dupC mutation accounted for a percentage approximate 80% [11]. However, the molecular mechanisms of c.649dupC mutation await to be elucidated.

In the current study, we collected nine sporadic PKD cases and identified the c.649dupC mutation in two cases and a novel c.133-136delCCAG mutation in one case. We further confirmed that the c.649dupC mutation derived from *de novo* in the case with Mongolian background. This finding implies that the site between bases 649 and 659 is unstable. We conjecture that the mutagenesis of c.649dupC is a consequence of the nature of the DNA sequences adjacent to cytosine[649], where it is one of nine consecutive cytosine (C) bases. It is likely that this run of C-residues facilitates "slippage" of DNA polymerase, leading to an insertion of a C base

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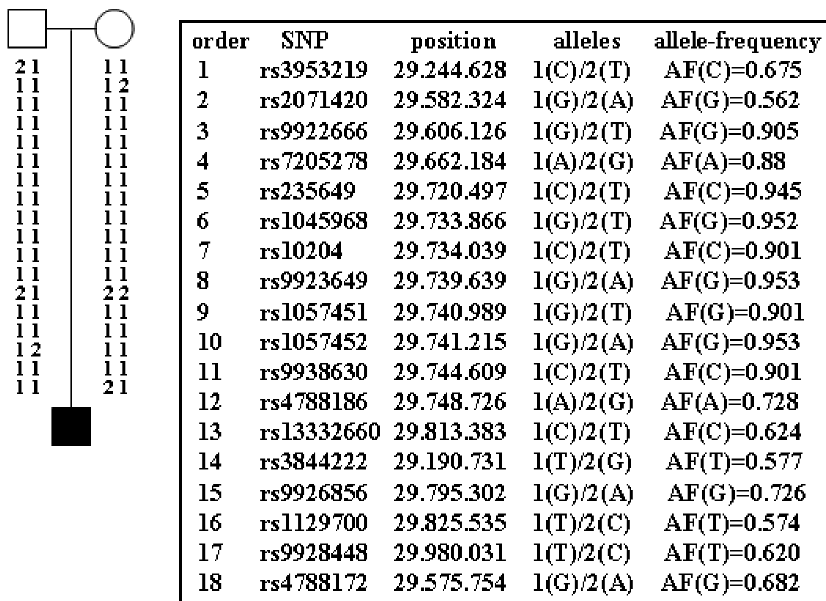

order	SNP	position	alleles	allele-frequency
1	rs3953219	29.244.628	1(C)/2(T)	AF(C)=0.675
2	rs2071420	29.582.324	1(G)/2(A)	AF(G)=0.562
3	rs9922666	29.606.126	1(G)/2(T)	AF(G)=0.905
4	rs7205278	29.662.184	1(A)/2(G)	AF(A)=0.88
5	rs235649	29.720.497	1(C)/2(T)	AF(C)=0.945
6	rs1045968	29.733.866	1(G)/2(T)	AF(G)=0.952
7	rs10204	29.734.039	1(C)/2(T)	AF(C)=0.901
8	rs9923649	29.739.639	1(G)/2(A)	AF(G)=0.953
9	rs1057451	29.740.989	1(G)/2(T)	AF(G)=0.901
10	rs1057452	29.741.215	1(G)/2(A)	AF(G)=0.953
11	rs9938630	29.744.609	1(C)/2(T)	AF(C)=0.901
12	rs4788186	29.748.726	1(A)/2(G)	AF(A)=0.728
13	rs13332660	29.813.383	1(C)/2(T)	AF(C)=0.624
14	rs3844222	29.190.731	1(T)/2(G)	AF(T)=0.577
15	rs9926856	29.795.302	1(G)/2(A)	AF(G)=0.726
16	rs1129700	29.825.535	1(T)/2(C)	AF(T)=0.574
17	rs9928448	29.980.031	1(T)/2(C)	AF(T)=0.620
18	rs4788172	29.575.754	1(G)/2(A)	AF(G)=0.682

Figure 2 Haplotype analysis based on 18 single nucleotide polymorphisms (SNPs) flanking the PRRT*2*. The large box lists the reference ID, position, alleles, and allele frequency for each of 18 SNPs included in the analysis. Numbers below the squares or circles represent the genotypes of the 18 SNPs for each individual (1 and 2 denote the specific allele for each SNP). The probability of the patient being biological son of the alleged parents was calculated as 99.93%.

Table 2 Clinical features of nine sporadic cases affected with paroxysmal kinesigenic dyskinesia

Case (gender)	Age at Onset	Age at Present	Duration (seconds)	Frequency (per day)	Trigger	Phenotype	Nucleotide change	Amino acid change
1(M)	12	17	5–10	1–3	SM/SP	D	–	–
2(M)	18	29	5–15	3–5	SM/SP	D	–	–
3(F)	16	25	5–10	1–8	SM	C/D	–	–
4(M)	12	27	10–30	5–10	SM	C	c.649dupC	p.Arg217Profs*8
5(M)	9	13	30–40	10–15	SM	C/D	–	–
6(M)	15	22	5–10	2–5	SM/SP	D	–	–
7(M)	18	20	15–20	10–20	SM/SP	C	c.649dupC	p.Arg217Profs*8
8(M)	3	17	5–20	5–6	SM	C	c.133_136delCCAG	p.Pro45Argfs*44
9(F)	13	22	10–20	1–5	SM/SP	C/D	–	–

SM, sudden movements; SP, shifting position; C, choreoathetosis; D, dystonia.

during DNA replication. Therefore, insertion of a C base in any position among the nine C bases would result in a frameshift mutation at this locus. Why cytosine^{649} is particularly susceptible to duplication, however, remains to be explained.

Although the PKD causative gene has been cloned, the pathophysiology and the function of *PRRT2* protein remain to be elucidated. *PRRT2* is a proline-rich transmembrane protein, containing two predicted transmembrane domains within its C terminus. Expression has been detected primarily in the central nervous system, especially in developing brain [6]. More importantly, a yeast two-hybrid study suggests that *PRRT2* interacts with the synaptosomal-associated protein 25 kDa (SNAP25) [15], which plays a crucial role in calcium-triggered neurotransmitter release from synaptic vesicles [16]. There is a possibility that *PRRT2* regulates ion channels during vesicular release [10,17], although this has yet to be experimentally confirmed. As most reported *PRRT2* mutations are truncating mutations, we speculate that truncated *PRRT2* proteins cannot anchor to the membrane and therefore loses its ability to interact with SNAP25, thus disturbing synaptic functions. Additional studies will be required to elucidate the properties of the *PRRT2* protein as well as its functions.

In summary, we present a *de novo* origin of the c.649dupC mutation and a novel c.133_136delCCAG mutation in this study. To the best of our knowledge, this is the first report concerning *de novo* mutagenesis of c.649dupC in Chinese PKD, which might generate much benefit to understand the molecular mechanism of PKD.

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Conflict of Interest

The authors declare no conflict of interest.

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