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Case Report

Molecular analysis of *PRRT2* gene in a case of paroxysmal kinesigenic dyskinesia patient

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Abstract

Paroxysmal kinesigenic dyskinesia (PKD) is an abnormal involuntary movement that is episodic or intermittent, with sudden onset, and the attacks are induced by sudden movement. Mutations in proline-rich transmembrane protein 2 (*PRRT2*) gene have been implicated in the cause of this disorder. This study presents a case of PKD on the basis of clinical findings supported and evidences obtained through a mutational analysis. Sequencing of all the exons of *PRRT2* gene revealed a frameshift mutation (p.R217Pfs*8) in exon 2 and a novel transition mutation (c.244C > T) in 5'-untranslated region (UTR). Though mutations in *PRRT2* gene are well-established in PKD, this study for the first time presents a novel transition mutation in the exon 2 region.

Key Words

Mutation analysis, paroxysmal kinesigenic dyskinesia, proline-rich transmembrane protein

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Introduction

Paroxysmal kinesigenic dyskinesia (PKD) is a rare, autosomal, dominant type of disorder. PKD is an abnormal involuntary movement that is episodic or intermittent, with sudden onset, and the attacks are induced by sudden movement.[1] Based on etiology, PKD can be classified as primary or secondary PKD.[2] Based on their hereditary nature, primary PKD can be further classified as idiopathic or familial PKD. Cases of familial PKD are the most common and are usually inherited in an autosomal dominant manner.[3] Secondary PKD is majorly caused by multiple sclerosis, head injury, metabolic derangements, or cerebral perfusion insufficiency.[2] The diagnostic criteria for primary idiopathic PKD includes sudden movements as the trigger, a short duration of attacks (a few seconds to a few minutes), lack of loss of consciousness or pain during attacks, a good response to antiepileptic drug treatment, exclusion of other organic diseases, and age of onset between 1 and 20 years.[1] Antiepileptic drugs such as, carbamazepine or phenytoin has proven to be effective for PKD patients.

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The gene associated with PKD has been mapped to chromosome 16, and mutations in *PRRT2* gene was identified to cause PKD.[4-6] *PRRT2* encodes a proline-rich transmembrane protein of unknown function. However, it has been reported to interact with the presynaptic plasma membrane associated protein (t-SNARE), synaptosomal associated protein 25 kDa (SNAP25).[7] *PRRT2* was found to be mainly expressed in the basal ganglia, a brain area possibly involved in the PKD pathogenesis.[4] Cases of PKD have been reported in India on the merits of clinical features, but not with extensive studies relating the disease to the molecular basis to support the diagnosis. Hence, it is considered worthwhile to take up genetic investigations in a clinically suspected case of PKD in an Indian context.

Case Report

A 27-year-old gentleman, born of nonconsanguineous parentage from south India, presented with history of episodic involuntary abnormal dystonic posturing of either trunk or limbs (upper/lower) lasting for few seconds to minutes, precipitated by brisk walking or sudden movements (such

as shaking of hand, etc.). These symptoms have been noticed since more than 10 years and used to occur at a frequency of three to four attacks per month [Figure 1a]. There was no other associated significant history and also there was no history of similar symptoms in family members except for probable history of similar symptoms in his paternal aunt (not assessed clinically) [Figure 1b]. Workup for his symptoms including blood counts, electrolytes, calcium, thyroid functions, vasculitic workup, magnetic resonance imaging (MRI) brain, and electroencephalogram (EEG) were all within normal limits. Clinically, he was considered to have PKD as per Bruno's criteria.[1] Symptoms completely remitted with usage of carbamazepine. After obtaining consent for mutational analysis from the proband, deoxyribonucleic acid (DNA) was extracted from the peripheral blood by standard salting out method. DNA purity and quantification was done by using the Perkin Elmer Lambda 35 Spectrometer. A total of 5 ml (200 ng) of the DNA was added to each 50 ml polymerase chain reaction (PCR) mix. Primers were designed for all the exons of *PRRT2* gene (Procured from Eurofins Genomics India Pvt Ltd). The primers and annealing temperature for the PCR used in this study is provided in Table 1. PCR was carried out as per Lee *et al.*, with minor modifications according to our lab conditions[7] using C1000 Touch Thermal Cycler (Bio-Rad). PCR product was purified by using Qiaquick PCR purification kit (Qiagen) method. DNA sequencing was carried out as per Sanger *et al.*[8] Sequencing reaction was performed with 50 ng of DNA and the products were purified by ethanol/ethylenediaminetetraacetic acid (EDTA)/sodium acetate precipitation and analyzed on an ABI 3500 Genetic Analyzer. The sequences obtained were compared with the genomic DNA sequence of *PRRT2*, and nucleotide changes were numbered corresponding to their position in *PRRT2* mRNA (Ensembl gene ID: ENSG00000167371). On DNA sequencing of all four exons, we identified an insertion of C at 650 bp of coding sequence (exon 2) leading to frameshift mutation [p.R217Pfs*8; Figure 2a] in the PKD patient, but not in the control subject. In addition, we also identified a novel point mutation [c.244C > T; Figure 2b] at exon 2 in the patient, but not in the control subject.

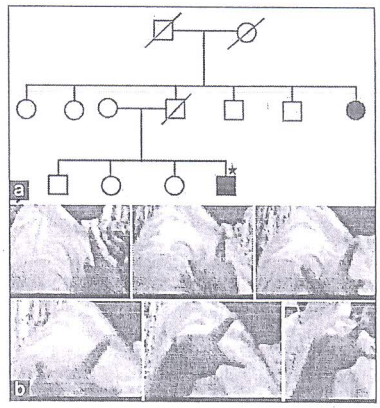

Figure 1: (a) Family pedigree — filled rectangle or circles are individuals affected with paroxysmal kinesigenic dyskinesia (PKD). Open rectangle or circles are unaffected individuals. Star mark represents proband in the current study (b) Short family video segment shows dystonic posturing of right hand with skewing of elbow, precipitated soon following a hand shake.

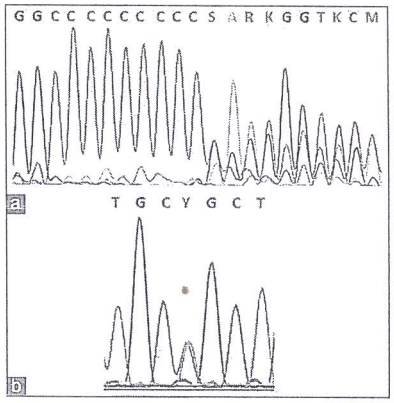

Figure 2: Mutations identified in the patient by deoxyribonucleic acid (DNA) sequencing. (a) Frameshift mutation (p.R217Pfs*8) (b) C to T transition at nucleotide 244 bp; c.244C > T

Discussion

PRRT2 is a recently identified gene and the mutations in this gene are implicated in PKD. This gene consists of four exons encoding the proline-rich transmembrane protein 2, which encompasses 340 amino acids and contains two predicted transmembrane domains [Figure 3]. *PRRT2* is highly expressed in the developing nervous system, and a truncating mutation alters the subcellular localization of the *PRRT2* protein.[5] The studies have showed that truncating mutations within *PRRT2* in Han Chinese families with histories of PKD. These truncating mutations co-segregated exactly with the disease in these families and were not observed in control subjects of matched ancestry.[4,5] The study by Li *et al.*, (2012) detected truncating or missense mutations in *PRRT2* gene in Chinese PKD families and none of these mutations was found in ethnically-matched control individuals.[6] Lee *et al.*, (2012) also showed truncating (frameshift or nonsense) mutations in *PRRT2* gene in samples from six well-defined PKD with infantile convulsions (PKD/IC) families.[7]

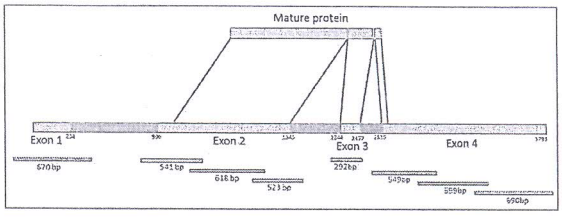

Figure 3: Structure of *PRRT2* gene and respective polymerase chain reaction (PCR) amplicon size

A genetic study of a large family of PKD from India, which was conducted in a western lab, has added significantly to the present understanding of the molecular genetics of PKD over the last decade.[9-12] In the present study, the mutational analysis of the *PRRT2* gene of the proband has revealed an insertion of 'C' at position 650 bp resulting in frameshift mutation starting from amino acid position 217 (p.R217Pfs*8).

Table 1: Primer sequences and annealing temperature used in PCR

Primer sequences	Annealing temperature (°C)	Amplicon size	Target exon
F: CATCTTCCTTGCCCTCCTGCTCGTA	56	670 bp	Exon 1 (5'-UTR)
R: CTGGTTATCTCCTTCGATCCCCTGT			
F: AACCCCAACTTTTCTTCCTCTC	63.5	541 bp	Exon 2a
R: CTCACTTCTGGTTTGGACACTG			
F: GCTCCAGAAACCACAGAGACC	63.5	618 bp	Exon 2b
R: GATGGCAAGGATGATGTAGTCC			
F: TCTGAGAGTGTAGGGGAAAAGC	62.3	523 bp	Exon 2c
R: CTAGGGAGAGGCAAACAAAGG			
F: TCTGGATGACTTTTCCACCTG	62.3	292 bp	Exon 3
R: AGGGGCAGGGGTTAGTAAAG			
F: TCTCCTGTCTGTCCTCCTT	64	549 bp	Exon 4a
R: GAGGACTGAGGGTTGAGAGATG			
F: CAGGCTCCTCTGCCAACTGTAG	64	599 bp	Exon 4b (3'-UTR)
R: AACCAGTACTCGCCACCCTCAC			
F: CCAGGCTCGGACTGTTCTCTGCT	68.1	690 bp	Exon 4c (3'-UTR)
R: TATACCACCCAGTAAGGTTCTGCCA			

PCR = Polymerase chain reaction, UTR = Untranslated region

This mutation could have resulted into a truncated protein with 223 amino acid length instead of the 340 amino acid residues contributing to a defective *PRRT2* gene product manifesting the disease. The cytosine (C) tract at nucleotides 641-649 of the *PRRT2* cDNA (5'-CCCCCCCCC-3') may be the hotspot for a single C duplication in PKD subjects.[5,6] This mutation (c.649dupC, p.R.217Pfs*8) was also reported in African-American family.[13] The present study reports a similar mutation as observed by earlier workers.[5,6,13] It may also be noted that such a mutation has been reported by Gardiner *et al.*, in an Indian patients implying that the p.R.217Pfs*8 mutation may be a cause for PKD.[12] However, the other mutation (C to T transition at nucleotide 244 bp; c.244C > T) observed in the present study in the exon 2 at the 5'-untranslated region (UTR) region needs further enquiry to invoke any relationship to the phenotype. Since there are reports stating the importance of 5'-UTR mutations in causing diseases,[14,15] it is likely that c.244C > T 5'-UTR mutation may play a role in predisposition of PKD.

PKD is often misdiagnosed clinically as epilepsy. Hence, genetic investigations might be of relevance in the diagnosis of PKD. It would also help in genetic counseling for patients suffering from PKD. To the best of our knowledge, this is the first study in an Indian laboratory on the molecular implications for the development of PKD.

Recent studies also showed that mutations in *PRRT2* have also identified in families with episodic ataxia, hemiplegic migraine, and other forms of migraine.[12] Liu *et al.*, 2012 showed that *PRRT2* gene mutations also causes paroxysmal non-kinesigenic dyskinesia and paroxysmal exercise-induced dyskinesia, apart from PKD.[16] Heron *et al.*, 2012 showed that *PRRT2* mutations also cause benign familial infantile epilepsy and infantile convulsions and choreoathetosis syndrome.[17]

It is therefore we propose to conduct extensive molecular biological investigations of paroxysmal non-kinesigenic dyskinesia, paroxysmal exercise-induced dyskinesia, and benign familial infantile epilepsy subjects apart from PKD in our laboratory. The outcome of the present study is an affirmation for the presence of the mutations in *PRRT2* gene in the Indian population as a cause for PKD as observed in the western and Chinese population. The study also records for the first time a 5'-UTR mutation in the exon 2 region, which needs to be investigated further in more cases of PKD to implicate its role either in the molecular diagnosis of PKD or for differential diagnosis of various forms of paroxysomal dyskinesias.

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