PRRT2 Gene Mutations in Indian Paroxysmal Kinesigenic Dyskinesia Patients

To Editor,

Paroxysmal kinesigenic dyskinesia (PKD) is an involuntary movement disorder belonging to the category of Paroxysmal Dyskinesias (PDs). PKD is one of the rare movement disorders characterized by brief, episodic hyperkinetic movement abnormalities like dystonia, chorea, hemiballismus, or with the combination. The abnormal movements usually induced by sudden body movement such as running, walking, standing, or turning. The attacks are usually short, lasting seconds or minutes with preserved consciousness.[1,2] In specific to PKD, the abnormal body movements and posturing are quite similar to other kinds of neurological disorders and may mimic seizures. For this reason, PKD reported as "frequently misdiagnosed movement disorders" in medical science.[3] The diagnosis is primarily based on family history, clinical findings (symptoms and stimuli), neurological investigations like EEG, CT, and MRI and serum electrolyte test, and response to antiepileptic drug and its dosage. Evidently, neurological investigations and other clinical findings are not sufficient to classify the PKD condition in many patients. Hence, to eliminate cognitive biases in clinical practice, genetic investigations are required in complex movement disorders like PKD.[4] Two different studies based on whole-genome sequencing in families affected either with PKD or PKD/infantile convulsions (IC) has identified the PRRT2 gene as the first causative gene of PKD, including the infantile convulsions and choreoathetosis syndrome.[5,6] PRRT2 gene consists of four exons (exon1-exon4) encoding the proline-rich transmembrane protein-2, which encompasses 340 amino acids and contains two predicted transmembrane domains mainly localized in neurons.[7] The hotspot mutation c.649-650insC position of PRRT2 gene reported in several studies and could be a cause of PKD in many ethnic races.

In this study, the four suspected PKD with complex multiple conditions referred by neurologist were selected for genetic testing (PRRT2 gene mutation analysis). The informed consent from patients and their family members was obtained for genetic analysis. The study was approved by institutional ethics committee of RajaRajeswari medical college & Hospital. The neurologist examined the disease etiology and clinical features, and the patient's clinical data were recorded as per the standard classification.[8] In the study cohort, there was no family history of PKD in all of the four cases, but other pathological conditions like mild tremors and seizures were recorded. The proband age in different pedigrees varied as 15, 26, 13, and 5 years old in cases 1, 2, 3, and 4, respectively. In family 2 and 4, a possible seizure in family history was recorded. Further, in case 4, the neurologist was facing ambiguity in distinguishing PKD from other neurological disorders in the proband. Consequently, the alternative clinical diagnosis was considered to find out whether nonorganic psychogenic or functional neurological disorder was present in this case. The parents were asymptomatic in all of the cases, and father of case

2 proband had deceased; however, deceased father's history revealed the occurrence of seizers and was in anticonvulsant drug Benzodiazepines treatment for 10 years. Accurately, PKD, or any other movement disorders were not recorded in family history in case 2. Hence, in this study, all of the cases were majorly classified as sporadic/idiopathic PKD by the neurologist before the genetic test as there were no PKD specific symptoms recorded in the family history.

The detailed information about four different cases is summarized below.

CASE 1: 15-year-old male patient having abnormal movements since the age of 5 or 6. These symptoms are stimulated or induced by the sudden movement of the body. Clinical investigation findings suggested that there is no focal deficit; neurological examination by EEG and MRI was found to be normal but clinically diagnosed as PKD.

CASE 2: 26-year-old male patient having abnormal posturing of limbs left to right, from 10 years. The symptoms stimulated while suddenly standing up, and the frequency of effect is 30-40 times/day. Neurological examinations by EEG and MRI was found to be normal. The clinical finding showed spontaneous posturing of all limbs, and there was a family history of seizures, as shown in the pedigree and clinically diagnosed as PKD. Also, a swiftly changing position from sitting to standing trigged multifocal dystonia as recorded by the patient shown in Figure 1.

CASE 3: 13-year-old boy was clinically diagnosed as PKD having abnormal posturing of the left upper limb, and lower limb, with facial contractions since the last one year two months at the time of admission. The symptoms were induced by a sudden movement of the body. EEG and the MRI of the brain and spine were normal.

CASE 4: A five-year-old girl was diagnosed as possible PKD and was also with an ambiguity of having another neurological syndrome. The symptoms having abnormal posturing of limbs lasting for 2 minutes-3 days; her symptoms started at the age of 2 years. In family history, probable paternal side inheritance was recorded. The patient's neurological examination by MRI and EEG was found to be normal.

We sequenced the PRRT2 gene in four suspected PKD patients and their asymptomatic family members along with two healthy participants as controls by following a standard protocol.[9] The exons of PRRT2 gene sequencing in four PKD patients disclosed the presence of the most common PRRT2 mutation (hotspot mutation), i.e., c. 649-650insC; p.Arg217Profs*7 in only two cases, from family I and family II. Surprisingly, in family I, the presence of c. 649-650insC was observed; p.Arg217Profs*7 mutation was observed in the asymptomatic father of proband. In the case of family II, the proband father DNA could not be tested, as he was deceased. Hitherto (till this point), the asymptomatic mother was tested and was found negative for PRRT2 mutations. In family III and IV, the proband and their family members were

Figure 1: Showing video segment of a 26-year-old PKD male patient. Dystonia triggered by sudden movement, from A to H showing multifocal dystonia posturing of limbs' left to right after standing up

found negative for PRRT2 gene mutation. The pedigree for all analyzed samples is given in Figure 2. The chromatogram for PRRT2 hot spot mutation (c. 649-650insC) detected by Sanger sequencing is shown in Figure S1 (Supplementary information), and details of detected mutation of each family are shown in Table S1 [Supplementary information].

Our sequencing results of the PRRT2 gene disclosed the presence of PRRT2 hotspot mutation in case 1 proband and his asymptomatic father. These results show that there is a family history of PRRT2 gene mutation, and hence, this case further classified as familial paroxysmal kinesigenic dyskinesia. In this case, we also enquired about the father for his history on any movement disorders since sometimes the symptoms of PKD diminish with age,[10] but PKD or any other movement disorder symptoms not recorded in his life. In PKD, variant inheritance like incomplete penetrance of the PRRT2 gene leads to clinically unaffected members of the family even though in presence of hot spot mutation (c.649-650insC). Hence, the father of proband in Case 1 PKD condition was classified as familial, autosomal dominant inheritance with incomplete penetrance. The nature of the incomplete penetrance of the PRRT2 gene leads to some biases in the classification of PKD. Therefore, PRRT2 gene mutation analysis for the previous three generations of the proband is essential for proper counseling and patient care. Our study has disclosed the incomplete penetrance of the PRRT2 gene in the asymptomatic parent, which will help the neurologist for proper classification. Further, this study may help the clinician in personalized medicine. Previous studies showed that PKD with a PRRT2 mutation will respond well and completely attenuate the episodes of PKD with low-dose (50 mg/d) carbamazepine.[11,12] The present study also showed that PRRT2 mutation in suspected PKD patients helped in the treatment and care. However, it is crucial to conduct a genetic test even for asymptomatic parents for the proper classification of disorders. Although in monogenic form of diseases the complete mechanism behind the genetics of reduced penetrance and variable expressivity is not clearly understood, and it is a complex system with an influence of several other factors like premutation, sex limitation, modifier gene, a trans arrangement of alleles, and environmental factors, which may increase the chance of reduced penetrance.

In case 2, the mutational analysis clearly shows the presence of PRRT2 hotspot mutation in proband but absent in his asymptomatic mother. Unfavorably, the father's sample was not collected, as he was deceased. Case 2 results further confirm that PRRT2 hotspot mutation is the primary cause of PKD. Besides, the patient's pedigree revealed the presence of a family history of possible seizures (convulsion) in father, and he was under treatment with Benzodiazepines. Further,

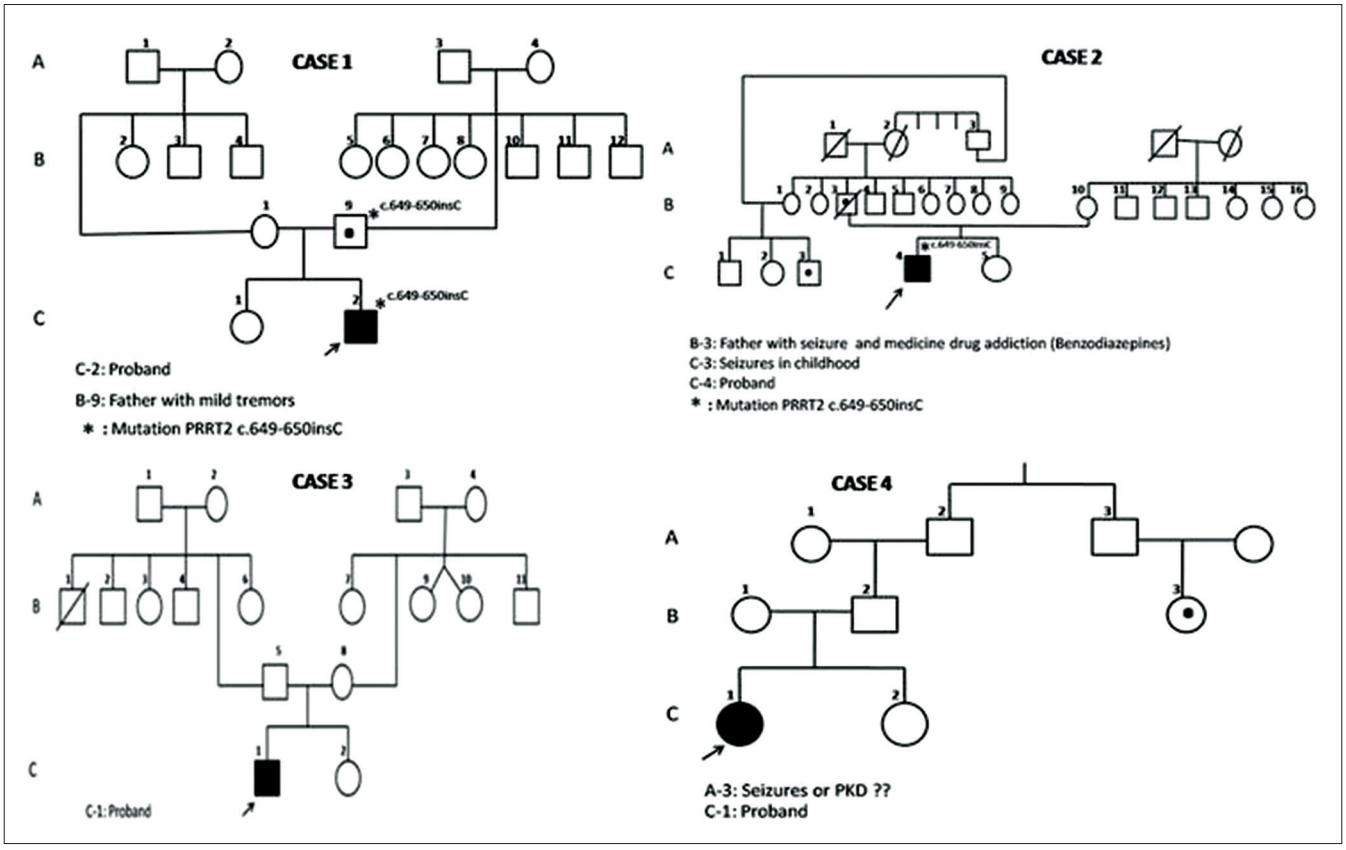

Figure 2: Pedigree of four separate PKD cases – Squares are male, circles are female, squares or circles with slash represents deceased (dead) individuals. Affected individuals are represented as black filled squares or circles, centered dot represents special conditions, dizygotic twins are represented as two offsprings originating from a single node, and proband is denoted with an arrow

one of the distant relatives of the proband also showed possible seizures in history. In several cases, the attacks of PD and epileptic seizures have several characteristics in common: both are paroxysmal with a tendency to spontaneous remission, and a subset of PD also responds well to anticonvulsant drugs.[13] Apart from the intricate inheritance patterns like incomplete penetrance and variable expressivity, the hot spot mutations of PRRT2 c.649-650insC; p.Arg217Profs*7 gene well documented as pathogenic for approximately 80% of the PKD cases in different ethnic groups.[5,9]

In case 3 and case 4, the probands and their asymptomatic parents were found negative for PRRT2 mutation. However, the results of phenotype-genotype comparisons of PKD patients based on the presence and absence of PRRT2 mutations revealed that around 57% of the familial and 46% of sporadic cases carried PRRT2 mutations.[14] Notably, in case 4, the neurologist doubts the pathophysiology of the patient to diagnose PKD or another neurological syndrome. Hence, PRRT2 gene sequencing results considered for eliminating the ambiguity of overlapping symptoms of movement disorder and neurological syndrome. In this case, very little chance for proband to have PKD as the genetic test did not show any mutation in the PRRT2 gene, and this overlapping symptom was maybe because of some other neurological syndrome. To resolve the genetics of PKD patients without PRRT2 gene mutations, researchers should consider for further screening of mutations in other possible genes. There are 23 preliminary-evidence genes (Gene Panel) listed for test against group of movement disorders; in which the genes *KCNMA1*, *PNKD*, *PRRT2*, *SLC2A1* are recommended specifically to *Paroxysmal Movement Disorders*.[8,15,16] In some of the cases, clinical PKD patients have shown negative for all associated genes. In such situation, the whole exome sequencing is required for further screening of new linked genes and mutations. In relevance to this, Ding Liu *et al.*, using polymorphic markers has identified a novel gene locus on chromosome 3 among PRRT2-mutation-negative PKD patients.[17] Screening of such polymorphic markers across the patient genome will further have importance in contributing accurate molecular diagnosis.

In specific, PRRT2 gene mutations are the most pathogenic factor in causing PKD. Besides, PRRT2 mutations also have a significant role on the phenotype of PKD patients. The recent Chinese genotype-phenotype correlation study showed that proline-rich transmembrane protein 2-mutated patients will be susceptible to symptoms at early age and have longer attack duration, bilateral limb involvement with varied dyskinesia, and family history. In addition, high-knee-exercise efficiently induced attacks of PKD.[12] Similarly, in our present study, the two PRRT2 positive patients have shared these common symptoms. In case 1, the patient has shown symptoms from age 5, and the family history of PRRT2 mutation was disclosed in genetic testing (Father was positive). Similarly in case 2, the patient has shown symptoms from age 16 and the family history of seizures was disclosed in deceased father. The frequency of attack was very high and seems to be the involvement of bilateral limb posturing. In addition, the multifocal dystonia attacks were induced specifically position shift from sitting to standing, which shows that involvement of knee pressure in inducing attacks in patient.

In this report, we have also checked the correlation between drug response in PKD with and without PRRT2 mutations. All of the patients were treated with carbamazepine, and they responded well. One patient, albeit responded to CBZ (carbamazepine), but did not tolerate it. Hence, he was started on Lacosamide and had been having reasonable control with it. In the overall study, we did not find novel mutations or 5'UTR mutations, as reported in our previous study.[9] This study confirms the significance of PRRT2 gene hot spot mutation in the diagnosis of PKD and disclosing the other special conditions like PRRT2 gene incomplete penetrance in asymptomatic parents. This work also suggests that PRRT2 gene screening for three generations is necessary for correct disease classification. In addition, this also contributes to the genotype-phenotype correlation in Indian PKD patients with PRRT2 mutations. Besides, the mutation analysis of PRRT2 gene will assist the neurologist in proper classification and disease management in complicated movement disorders. Further, this study may help the clinician in personalized medicine. In overall, our study will help the neurologist to eliminate bias in the classification of PKD from other overlapping neurological syndromes.

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Conflicts of interest

There are no conflicts of interest.

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References

1. Lotze T, Jankovic J. Paroxysmal kinesigenic dyskinesias. Semin Pediatr Neurol 2003;10:68-79.
2. Jain RS, Prakash S, Nagpal K, Handa R. Paroxysmal kinesigenic dyskinesia: A rare neurological disorder posing diagnostic challenge but therapeutic rewards. Austin J Clin Neurol 2015;2:1077.
3. Junewar V, Sahu R, Shukla R. Paroxysmal kinesigenic dyskinesia: A frequently misdiagnosed movement disorder. BMJ Case Rep 2014;2014:bcr2014203681. doi: 10.1136/bcr-2014-203681.
4. Klein JG. Five pitfalls in decisions about diagnosis and prescribing. BMJ 2005;330:781-3.
5. Chen WJ, Lin Y, Xiong ZQ, Wei W, Ni W, Tan GH, *et al.* Exome

sequencing identifies truncating mutations in PRRT2 that cause paroxysmal kinesigenic dyskinesia. Nat Genet 2011;43:1252-5.
6. Szepetowski P, Rochette J, Berquin P, Piussan C, Lathrop GM, Monaco AP. Familial infantile convulsions and paroxysmal choreoathetosis: A new neurological syndrome linked to the pericentromeric region of human chromosome 16. Am J Hum Genet 1997;61:889-98.
7. Wang JL, Cao L, Li XH, Hu ZM, Li JD, Zhang JG, *et al.* Identification of PRRT2 as the causative gene of paroxysmal kinesigenic dyskinesias. Brain 2011;134:3493-501.
8. Bruno MK, Hallett M, Gwinn-Hardy K, Sorensen B, Considine E, Tucker S, *et al.* Clinical evaluation of idiopathic paroxysmal kinesigenic dyskinesia: New diagnostic criteria. Neurology 2004;63:2280-7.
9. Prabhakara S, Anbazhagan K. Molecular analysis of PRRT2 gene in a case of paroxysmal kinesigenic dyskinesia patient. Ann Indian Acad Neurol 2014;17:459-62.
10. Unterberger I, Trinka E. Diagnosis and treatment of paroxysmal dyskinesias revisited. Ther Adv Neurol Disord 2008;1:4-11.
11. Li HF, Chen WJ, Ni W, Wang KY, Liu GL, Wang N, *et al.* PRRT2 mutation correlated with phenotype of paroxysmal kinesigenic dyskinesia and drug response. Neurology 2013;80:1534-5.
12. Huang XJ, Wang SG, Guo XN, Tian WT, Zhan FX, Zhu ZY, *et al.* The phenotypic and genetic spectrum of paroxysmal kinesigenic dyskinesia in China. Mov Disord 2020. doi: 10.1002/mds.28061.
13. Rochette J, Roll P, Szepetowski P. Genetics of infantile seizures with paroxysmal dyskinesia: The infantile convulsions and choreoathetosis (ICCA) and ICCA-related syndromes. J Med Genet 2008;45:773-9.
14. Becker F, Schubert J, Striano P, Anttonen AK, Liukkonen E, Gaily E, *et al.* PRRT2-related disorders: Further PKD and ICCA cases and review of the literature. J Neurol 2013;260:1234-44.
15. Ncbi.nlm.nih.gov. 2020. Paroxysmal Movement Disorders Panel-Tests-GTR-NCBI. [online] Available from: https://www.ncbi.nlm.nih.gov/gtr/tests/522511/[Last accessed on 2020 May 24].
16. Invitae.com. 2020. [online]. Available from: https://www.invitae.com/en/physician/tests/03351/. [Last accessed on 2020 May 24].
17. Liu D, Zhang Y, Wang Y, Chen C, Li X, Zhou J, *et al.* Novel locus for paroxysmal kinesigenic dyskinesia mapped to chromosome 3q28-29. Sci Rep 2016;6:25790.

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Table S1: PRRT2 gene mutations in PKD affected patients and their families; presence of the mutation is denoted as positive and the absence of the mutation is denoted as negative. Unaffected male and female participants are considered as controls

Groups	Relationship	PRRT2 Gene Mutation	Position of mutation
Controls Unaffected	Healthy control Female	–	—
	Healthy control Male	–	—
Family I (Case 1)	Patient	+	c.649-650insC
	Father	+	c.649-650insC
	Mother	–	—
Family II (Case 2)	Patient	+	c.649-650insC
	Mother	–	—
Family III (Case 3)	Patient	–	—
	Father	–	—
	Mother	–	—
	Younger sister	–	—
Family IV (Case 4)	Patient	–	—
	Father	–	—
	Mother	–	—

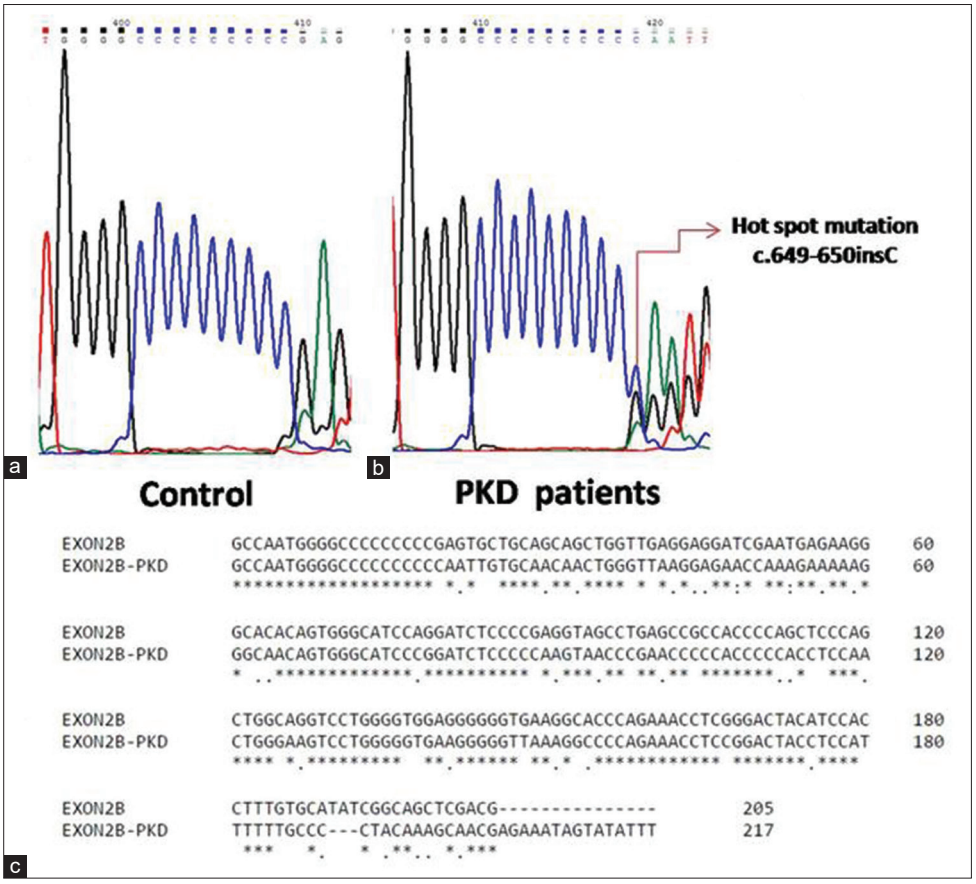

Figure S1: DNA sequence chromatogram of PCR-amplified PRRT2 gene-EXON2 from healthy control and from a PKD patient (a) showing presence of 9 cytosine as normal, (b) showing hot spot mutation c.649-650insC (presence of 10 cytosine) in proband of PKD positive, (c) showing DNA pairwise alignment PRRT2-EXON2 of normal and c. 649-650insC hotspot mutation sequences (frame shift mutation) in PKD patients