

## SHORT REPORT

# Targeted genomic sequencing identifies *PRRT2* mutations as a cause of paroxysmal kinesigenic choreoathetosis

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## ABSTRACT

**Background** Paroxysmal kinesigenic choreoathetosis (PKC) is characterised by recurrent and brief attacks of involuntary movement, inherited as an autosomal dominant trait with incomplete penetrance. A PKC locus has been previously mapped to the pericentromeric region of chromosome 16 (16p11.2-q12.1), but the causative gene remains unidentified.

**Methods/results** Deep sequencing of this 30 Mb region enriched with array capture in five affected individuals from four Chinese PKC families detected two heterozygous *PRRT2* insertions (c.369dupG and c.649dupC), producing frameshifts and premature stop codons (p.S124VfsX10 and p.R217PfsX8, respectively) in two different families. Sanger sequencing confirmed these two mutations and revealed a missense *PRRT2* mutation (c.859G→A, p.A287T) in one of the two remaining families. This study also sequenced *PRRT2* in 29 sporadic cases affected with PKC and identified mutations in 10 cases, including six with the c.649dupC mutation. Most variants were truncating mutations, consistent with loss-of-function and haploinsufficiency.

**Conclusion** The present study identifies *PRRT2* as the gene mutated in a subset of PKC, and suggests that PKC is genetically heterogeneous.

## INTRODUCTION

Paroxysmal kinesigenic choreoathetosis (PKC, OMIM 128200) is the most frequently described type of paroxysmal dyskinesias disorder and is

characterised by recurrent and brief attacks of involuntary movement.<sup>1</sup> Familial and sporadic cases have been described. Familial PKC usually shows an autosomal dominant inheritance pattern with incomplete penetrance.

We previously performed linkage and haplotype analysis in four Chinese families (family 2 and family 4 with incomplete penetrance) with similar choreoathetosis clinical symptoms, and all mapped the disease locus to a region between D16S3093 and D16S3057 at 16p11.2-q12.1.<sup>2-3</sup> This PKC critical region was in accordance with other studies.<sup>4-5</sup> However, the causative gene remains unidentified. Recently, next generation sequencing has proven to be effective for discovering novel causal mutations in inherited diseases.

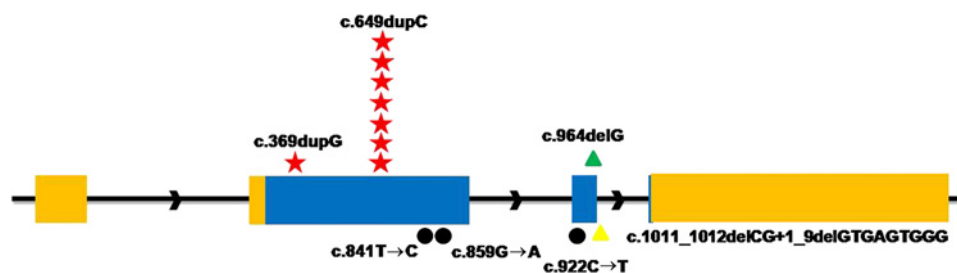
## METHODS

We sequenced the targeted region of linkage (chr16: 27 958 387–57 529 851), containing 237 RefSeq genes, in five individuals with PKC and one healthy control (did not carry the risk haplotype we previously identified) from four families in our previous report linked to this locus. The shotgun fragment libraries were hybridised to a custom NimbleGen array targeting the region and the eluate sequenced on the Illumina HiSeq 2000 platform. On average, 3.0 gigabases of data were generated per sample, to achieve at least 150-fold coverage of the mappable region (13 Mb) (details in supplementary table 1). We focused primarily

**Table 1** Mutations in the *PRRT2* gene by Sanger sequencing

Sample	Mutation	Exon	Amino acid change	SIFT and Polyphen predictions
Family 1	c.859G→A	2	p.A287T	Damaging
Family 2	c.649dupC	2	p.R217PfsX8	—
Family 4	c.369dupG	2	p.S124VfsX10	—
6	c.649dupC	2	p.R217PfsX8	—
11	c.649dupC	2	p.R217PfsX8	—
16	c.649dupC	2	p.R217PfsX8	—
17	c.649dupC	2	p.R217PfsX8	—
18	c.649dupC	2	p.R217PfsX8	—
22	c.1011_1012delCG+1_9delGTGAGTGGG	3	Splice-site	—
26	c.964delG	3	p.V322WfsX15	—
27	c.841T→C	2	p.W281R	Damaging
28	c.649dupC	2	p.R217PfsX8	—
29	c.922C→T	3	p.R308C	Damaging

**Figure 1** Identified mutations in the *PRRT2* gene. Relative positions of mutations are indicated by symbols. Red Stars, frameshift insertions; green triangles, frameshift deletions; black dots, missense; yellow triangles, splice site mutations.



on non-synonymous variants, splice site acceptor or donor variants, and coding insertions or deletions (NS/SS/I) that were most likely to be pathogenic (supplementary table 2).

## RESULTS

A total of 149–160 NS/SS/I variants were detected in 55 genes per affected individual. After filtering for variants found in dbSNP129, 1000 Genome Project and the healthy control sequencing data to remove shared variants, the number of variants was reduced approximately eightfold. A 0.60 Mb de novo microdeletion at 16p11.2 was identified in two sporadic PKC case reports,<sup>6,7</sup> which suggested loss-of-function mutations seemed to cause the disorder and narrowed down the candidate region. Only one gene located in this region of the microdeletion, *PRRT2*, which encodes proline-rich transmembrane protein 2, harboured two heterozygous frameshift insertions in two families: a cytosine insertion (c.649dupC) in family 2 causing a frameshift and premature stop codon (p.R217PfsX8); and a guanine insertion (c.369dupG) in family 4 causing a frameshift and premature stop codon (p.S124VfsX10). In each family, the independent heterozygous mutations were indeed present in all available affected individuals and healthy risk haplotype carries we previously identified using Sanger sequencing.

To confirm that mutations in *PRRT2* were responsible for PKC, we screened the whole sequences of *PRRT2* gene (exon, intron, 500 bp upstream of 5'UTR and 500 bp downstream of 3'UTR) in two other families (targeted genomic sequencing did not detect mutations likely due to the quality of sample) and 29 new PKC individuals. In family 1 (one of the two remaining families), a missense mutation (c.859G→A, p.A287T) in *PRRT2* completely cosegregated with PKC phenotype, and five different mutations were detected in 10 sporadic PKC individuals. Collectively, seven different mutations were identified in *PRRT2* by a combination of targeted genomic and Sanger sequencing (table 1, figure 1). None of these mutations was found in chromosomes from 192 ethnically matched control individuals.

## DISCUSSION

Our results strongly suggest that mutations in *PRRT2* are a cause of PKC. Little is known about *PRRT2* or the functions of its encoded protein. In UniProtKB database, *PRRT2* (Q7Z6L0) is predicted to be a multi-pass membrane protein consisting of an N-terminal extracellular domain containing a proline-rich domain and an N-glycosylation site, two transmembrane domains, and a C-terminal cytoplasmic domain. The N-terminal extracellular domain of *PRRT2* is similar to PRiMA (proline-rich membrane anchor), which targets acetylcholinesterase to membrane.<sup>8</sup> In addition, *PRRT2* is also highly expressed in brain (Unigene). Therefore, we speculate that *PRRT2* may serve as an anchor to connect with certain molecular at synapse.

In our Chinese PKC cohort, heterozygous mutations in *PRRT2* explain 39.4% (13/33) of cases. Most of the mutations were predicted to truncate the polypeptide. One mutation was

recurrent—c.649dupC—which was present in one family and six sporadic cases. Haploinsufficiency seems to be the most likely mechanism. Microdeletions of chromosome 16p11.2, encompassing *PRRT2*, have been reported in two sporadic PKC individuals.<sup>6,7</sup> Also, three pathogenic missense mutations identified in family 1 and two sporadic cases were present at a conserved site among different species (supplementary figure 1). Different types of mutations may be associated with different phenotype. Clinical characteristics should be defined strictly. However, for one family (family 3) linked to the pericentromeric region of chromosome 16 and 19 sporadic cases, we screened the whole sequences of the *PRRT2* gene and did not identify any mutations. It is possible that exonic deletions in *PRRT2* or other mutations in distal gene regulatory sequences, or an additional causative gene on the same genomic region, are responsible for PKC. Alternatively, PKC appears to be genetically heterogeneous,<sup>9,10</sup> and further analysis of these cases by exome sequencing may find additional genes.

Interestingly, PKC, benign familial infantile convulsions (BFIC2, OMIM 605751), infantile seizures and choreoathetosis (ICCA, OMIM 602066), and rolandic epilepsy with paroxysmal exercise induced dystonia and writer's cramp (RE-PED-WC, OMIM 608105) overlap across a pericentromeric region of chromosome 16,<sup>11</sup> suggesting that they may be allelic disorders caused by *PRRT2* mutations. Further functional studies of the *PRRT2* gene and these specific gene mutations are needed to provide important insights into the pathophysiology of PKC and other movement disorders.

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**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** This study was approved by the Ethics Committee of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

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Supplementary Table 1 Summary for targeted genomic sequencing data of five cases and one control

sample	Family 1			Family 2	Family 3	Family 4
	III-15*	II -4	III-14	IV-2	II -2	III-1
Bases (Mb)	2874.44	2922.26	2987.40	2635.44	2953.09	3390.09
Map bases (Mb)	2665.19	2725.40	2773.49	2450.51	2752.09	3144.94
Map bases rate (%)	92.7	93.3	92.8	93.0	93.2	92.8
Average read length (bp)	85.58	86.17	85.96	85.71	86.08	85.8
Average sequencing depth	184.67	187.97	191.45	168.72	189.87	216.92
Covered length (Mb)	13.18	13.18	13.18	13.18	13.18	13.17
Coverage at least 20× (%)	98.9	99.0	99.0	98.9	98.9	99.0

\* Healthy control did not carry the risk haplotype we previously identified.

Supplementary Table 2 Identification of the causal gene by targeted genomic sequencing

Filter	Family 1		Family 2	Family 3	Family 4
	II -4	III-14	IV -2	II -2	III-1
NS/SS/I	160	157	155	149	158
Not in dbSNP129	30	30	38	31	35
Not in dbSNP129, nor in 1000 Genomes	24	22	26	20	27
Not in dbSNP129, 1000 Genomes, nor in one control	15	21	15	17	22

NS, nonsynonymous variant; SS, splice-site acceptor or donor variants; I, coding insertions or deletions.

Variants were excluded by dbSNP129, 1000 Genomes, one healthy control (from family 1 did not carry the risk haplotype we previously identified) or all. With loss-of-function filter, two genes were remaining in more than one family.

Supplementary Table 3 Clinical characteristics of sp affected individuals

No.	Gender	Age	Age at onset	Trigger	Duration of attacks (s)	Involuntary movement	Response to anticonvulsants	Complication	Family history
1	F	18	11	SM	60	C	No treatment	None	None
2	F	22	12	SM	<10	D	CBZ+	None	None
3	M	21	15	SM	30	D	CBZ+	None	None
4	M	23	14	SM	2 -3	D	CBZ+	None	None
5	M	20	10	SM	10	D	No treatment	None	None
6	M	22	10	SM	10	C	No treatment	None	None
7	M	29	18	SM	7-8	C/D	CBZ+	None	None
8	F	22	13	SM	30	D	CBZ+	None	None
9	M	23	16	SM	15	C/D	VPA+	None	None
10	M	22	13	SM	3-4	C/D	Haloperidol+	None	None

11	M	24	7	SM	10	D	No treatment	None	None
12	F	21	10	SM	30	D	No treatment	None	None
13	M	26	15	SM	2-3	D	CBZ+	Epilepsy	None
14	M	35	9	SM	10-60	D	CBZ+	Epilepsy	None
15	F	19	11	SM	15	D	No treatment	Cerebral dysgenesis	None
16	M	32	15	S/SM	10	D	TPM+	None	None
17	M	20	5	SM	10-60	D	No treatment	IC	None
18	M	24	13	SM	5	D	CBZ+	None	None
19	M	23	12	SM	30	C	CBZ+	None	Brother
20	M	20	12	S/SM	30	D	CBZ+	Migraine, epilepsy	None
21	M	28	7	S/SM	60	C	CBZ+	None	None
22	M	15	8	SM	15	D	No treatment	None	None
23	F	18	12	SM	20	D	CBZ+	None	None
24	M	16	7	S/SM	15	D	No treatment	None	None
25	M	26	13	SM	10	C	No treatment	None	Maternal uncle
26	M	23	10	SM	20	D	No treatment	None	None
27	M	23	13	SM	10-30	D	CBZ+	None	None
28	M	32	10	SM	60	C/D	CBZ+	None	None
29	M	24	7	SM	3-5	C	CBZ+	None	None

F, female; M, male; S, startle; SM, sudden movement; C, choreoathetotic; D, dystonic; CBZ, carbamazepine; VPA, sodium valproate; TPM, topiramate; IC, infantile convulsion.

**c.841T>C**  
**p.W281R**

**c.859G>A**  
**p.A287T**

**c.922C>T**  
**p.R308C**

Human	...C	F	C	P	M	W	P	V	N	I	V	A	F	A	Y	A	V	...V	D	G	A	Q	R	L	G	R	V	A	...
Rhesus	...C	F	C	P	M	W	P	V	N	I	V	A	F	A	Y	A	V	...V	D	G	A	Q	R	L	G	R	V	A	...
Mouse	...C	F	C	P	M	W	P	V	N	I	V	A	F	A	Y	A	V	...V	D	G	A	Q	R	L	G	R	V	A	...
Dog	...C	F	C	P	M	W	P	V	N	I	V	A	F	A	Y	A	V	...V	D	G	A	Q	R	L	G	R	V	A	...
Elephant	...C	F	C	P	M	W	P	V	N	I	V	A	F	A	Y	A	V	...V	D	G	A	Q	R	L	G	R	V	A	...
Clawed toad	...C	F	C	P	M	W	P	V	N	I	V	G	F	A	Y	S	L	...I	D	G	A	F	R	L	G	R	V	A	...
Zebra fish	...C	F	C	P	V	W	P	I	N	I	V	G	F	A	Y	S	I	...V	D	G	A	R	R	L	G	R	V	A	...