Original Article

Mutation Analysis of *MR-1*, *SLC2A1*, and *CLCN1* in 28 *PRRT2*-negative Paroxysmal Kinesigenic Dyskinesia Patients

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Abstract

Background: Paroxysmal kinesigenic dyskinesia (PKD) is the most common subtype of paroxysmal dyskinesias and is caused by mutations in *PRRT2* gene. The majority of familial PKD was identified to harbor *PRRT2* mutations. However, over two-third of sporadic PKD patients did not carry any*PRRT2* mutation, suggesting an existence of additional genetic mutations or possible misdiagnosis due to clinical overlap.

Methods: A cohort of 28 Chinese patients clinically diagnosed with sporadic PKD and excluded *PRRT2* mutations were recruited. Clinical features were evaluated, and all subjects were screened for *MR-1*, *SLC2A1*, and *CLCN1* genes, which are the causative genes of paroxysmal nonkinesigenic dyskinesia (PNKD), paroxysmal exertion-induced dyskinesia, and myotonia congenita (MC), respectively. In addition, 200 genetically matched healthy individuals were recruited as controls.

Results: A total of 16 genetic variants including 4 in *MR-1* gene, 8 in *SLC2A1* gene, and 4 in *CLCN1* gene were detected. Among them, *SLC2A1* c.363G>A mutation was detected in one case, and *CLCN1* c.1205C>T mutation was detected in other two cases. Neither of them was found in 200 controls as well as 1000 Genomes database and ExAC database. Both mutations were predicted to be pathogenic by SIFT and PolyPhen2. The *SLC2A1* c.363G>A mutation was novel.

Conclusions: The phenotypic overlap may lead to the difficulty in distinguishing PKD from PNKD and MC. For those *PRRT2*-negative PKD cases, screening of *SLC2A1* and *CLCN1* genes are useful in confirming the diagnosis.

Key words: *CLCN1*; *MR-1*; Paroxysmal Kinesigenic Dyskinesia; *PRRT2*; *SLC2A1*

Introduction

Paroxysmal kinesigenic dyskinesia (PKD) is an episodic movement disorder characterized by recurrent and brief attacks of involuntary movements or dystonia, without alteration of consciousness.[1] Attacks are usually triggered by sudden movement or change in velocity, presenting with choreoathetosis, dystonia, or ballism.[2] Neurological examinations between attacks usually disclose no remarkable findings. The episodes of PKD usually start in childhood or early adolescence and tend to remit with age. PKD cases usually have a good response to antiepileptic drugs, such as carbamazepine and phenytoin.[3]

PKD is often familial with an autosomal dominant inheritance. Sporadic PKD cases are also reported, which are considered to be attributed to incomplete penetrance or *de novo* mutations.[4] *PRRT2* encoding proline-rich transmembrane protein 2 has been identified as a causative gene for PKD by several independent groups.[5-7] A hotspot mutation c.649dupC has been found in PKD cases from different ethnic origins.[8,9] However, *PRRT2* mutations do not account for all PKD cases, especially for those sporadic ones. It was revealed that the frequency of *PRRT2* mutations in sporadic PKD was 12.5–50.0%.[10-12] The low frequency of

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PRRT2 mutations in sporadic PKD suggested an existence of additional gene mutation or possible misdiagnosis due to overlapping clinical manifestations.

Of note, *PRRT2* mutations were not only implicated in PKD but also identified in other paroxysmal disorders, such as benign familial infantile seizures,[13,14] paroxysmal nonkinesigenic dyskinesia (PNKD),[15,16] and paroxysmal exertion-induced dyskinesia (PED).[15,17] This demonstrated a phenotypic overlap between PKD and other paroxysmal neurological disorders. However, it is still elusive whether the causative genes of other paroxysmal disorders are involved in the etiology of *PRRT2*-negatives poradic PKD. In this study, we collected a cohort of 28 Chinese patients who were clinically diagnosed with sporadic PKD and were excluded *PRRT2* mutations. We investigated the genetic variants of *MR-1*, *SLC2A1*, and *CLCN1*, which are the causative genes of PNKD, PED, and myotonia congenita (MC), respectively, in these cases.

Methods

Subjects

This study was approved by the Medical Ethics Committee of Huashan Hospital, Fudan University. The informed consents were obtained from participants or the parents of minors. Twenty-eight Chinese patients with a presumptive diagnosis of sporadic PKD were recruited in this study [Table 1]. All patients were primary PKD and met the diagnostic criteria proposed by Bruno *et al*.[1] Among these PKD cases, 19 individuals (Patient 1–19) had been reported in our previous study.[3] The other nine patients (patient 20–28) were collected between August 2012 and December 2012. Two of them are female whereas the others are males. The mean age at onset was 12.3 ± 3.6 years (range 6–18 years). None of them was accompanied by infantile febrile, hemiplegic migraine, migraine or episodic ataxia. All patients reached developmental milestones appropriately. *PRRT2* mutations were excluded in all these cases. In addition, 200 healthy individuals of Chinese ancestry were recruited as control subjects.

Mutation analysis

Genomic DNA of 28 subjects was extracted from 3 ml peripheral blood by a standard protocol using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). Specific primers were designed using the web-based Primer 3.0 program. All exons of the targeted genes (*MR-1*, *SLC2A1*, and *CLCN1*) and their respective intron-exon boundaries were amplified by the polymerase chain reaction (PCR). Primer sequences and the amplification conditions were listed in Supplementary Tables 1–3. PCRs were carried out in a final volume of 20 μl, containing 40 ng of DNA and 12.5 pmol of both forward and reverse primers. The purified PCR products of 28 samples were sequenced using an ABI 3730 Automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The sequencing results were aligned to the NCBI human reference DNA sequence of *MR-1*, *SLC2A1*, and *CLCN1*, respectively (Ensembl gene ID: ENSG00000153029, ENSG00000117394, and ENSG00000188037). Amino acid and nucleotide changes were identified and numbered corresponding to their position within the *PRRT2* mRNA. SIFT and PolyPhen2 were used to predict the pathogenicity of the identified mutations.

Results

Mutation analysis

After sequencing in the 28 patients with presumptive diagnosis of sporadic PKD, we identified 16 genetic variants, including 4 in *MR-1* gene, 8 in *SLC2A1* gene, and 4 in *CLCN1* gene [Table 2]. Among these variants, 7 *SLC2A1* variants (c.45C>T, c.399C>T, c.588G>A, c.987G>A, c.1011C>T, c.1065A>G, and c.1372C>A) and 3 *CLCN1* variants (c.261C>T, c.2154C>T, and c.2180C>T) were proved to be nonpathogenic variants. However, we detected 2 heterozygous missense mutations, including *SLC2A1* c.363G>A (p.Met121Ile) [Figure 1a] in one patient and *CLCN1*c.1205C>T (p.Ala402Val) [Figure 1b] in two other cases. The *SLC2A1* c.363G>A was absent in 1000 Genomes database, ExAC database, and our in-house 200 controls. It was predicted to be deleterious by SIFT and PolyPhen2. The allele frequency of *CLCN1*c.1205C>T was reported at 0.03% in 1000 Genomes database. It was predicted to be deleterious by both SIFT and PolyPhen2 too. Sequencing of *CLCN1* in our 200 controls also did not reveal the presence of *CLCN1* c.1205C>T. Moreover, it was identified as a causative mutation in a sporadic MC case by Fialho *et al*.[18]

Clinical features of the case with *SLC2A1* mutation

The clinical features of 28 presumptive PKD cases are summarized in Table 2. The patient (patient 17) with *SLC2A1* c.363G>A mutation was a 15-year-old girl, who had 8 years history of dystonia triggered by sudden movement. At the age of 7, she developed attacks of involuntary dystonia and choreoathetotic movements. The attacks were usually triggered by sudden standing or running and sometimes by fatigue. Each episode lasted 30 s to 1 min, but occasionally up to 3–5 min. The attacks initially occurred once or twice per week, but increased to 2–10 times/day when she was 12 years old. Her consciousness was preserved throughout the episodes. Between the events, her neurologic examinations were normal. Electroencephalogram disclosed unremarkable findings during the attacks. Brain computed tomography and magnetic resonance imaging (MRI) scanning were uninformative. At the onset, she was diagnosed with epilepsy in local hospital and sodium valproate (100 mg/d) was prescribed. However, her symptoms were not improved. She then sought her medical care in our hospital when she was 14 years old. A diagnosis of PKD was rendered, and carbamazepine was prescribed at a dose of 100 mg daily. The frequency of attacks was dramatically reduced from 2–10 times/day to 1–2 times/week.

Clinical features of the cases with *CLCN1* mutations

A 14-year-old boy (patient 8) with the *CLCN1* c.1205C>T mutation developed paroxysmal dystonia in lower limbs when

Table 1: Clinical features and genetic investigations of 28 presumptively sporadic PKD patients

Case (gender)	Age (years)	Age at onset (years)	Trigger	Duration (s)	Frequency (/day)	Phenotype	*MR-1*	*SLC2A1*	*CLCN1*
1 (male)	16	14	SM/S	5–10	0–4	D	–	–	–
2 (male)	15	13	SM/Fa	10–30	1–3	D/Ch	–	–	–
3 (male)	18	14	SM/S	5–10	3–10	D	–	–	–
4 (male)	21	18	SM/S	10–20	2–4	Ch	–	–	–
5 (male)	13	10	SM/S	5–15	0–3	D/St/W	–	–	–
6 (male)	23	8	SM	20–40	5–10	Ch	–	–	–
7 (male)	17	11	SM/Fa	10–15	2–8	Ch	–	–	–
8 (male)	14	12	SM/S	5–60	1–3	D/St/W	–	–	c.1205C>T
9 (male)	24	17	SM	20–40	3–5	Ch	–	–	–
10 (male)	12	8	SM/Co	30–60	5–10	D/St	–	–	c.1205C>T
11 (male)	13	6	SM/S	20–30	1–5	D	–	–	–
12 (male)	22	17	SM	10–15	5–10	D	–	–	–
13 (female)	18	15	SM/S	5–15	0–5	D/Ch	–	–	–
14 (male)	14	7	SM/Co	10–20	1–3	Ch	–	–	–
15 (male)	22	12	SM/S	15–30	2–4	D/Ch	–	–	–
16 (male)	18	15	SM	30–40	0–3	D	–		–
17 (female)	15	7	SM/Fa	30–60	2–5	D	–	c.363G>A	–
18 (male)	21	11	SM	10–15	10–15	Ch	–	–	–
19 (male)	17	15	SM	10–15	2–3	Ch/D	–	–	–
20 (male)	23	15	SM/S	10–60	0–3	Ch	–	–	–
21 (male)	16	13	SM/Fa	5–15	1–5	D/St	–	–	–
22 (male)	15	7	SM/S/Fa	20–40	1–10	D	–	–	–
23 (male)	19	13	SM/Co	30–60	10–15	D/Ch	–	–	–
24 (male)	22	18	SM/S	10–20	2–5	Ch/D	–	–	–
25 (male)	13	8	SM/S	15–20	2–8	D/St	–	–	–
26 (male)	22	12	SM	30–40	1–5	D	–	–	–
27 (male)	16	15	SM/S	20–30	2–3	Ch	–	–	–
28 (male)	17	14	SM	5–15	0–5	Ch	–	–	–

SM: Sudden movement; S: Shifting position; Fa: Fatigue; Co: Cold; Ch: Choreoathetosis; D: Dystonia; St: Stiffness; W: Weakness; PKD: Paroxysmal kinesigenic dyskinesia.

Table 2: Sixteen variants identified in the coding regions and intron-exon boundaries of *MR-1*, *SLC2A1*, and *CLCN1* gene

Gene	Variant	Protein	Type
MR-1	c.353-24CT	–	Intron variant
	c.465+65CA	–	Intron variant
	c.617+86CG	–	Intron variant
	c.781+52CT	–	Intron variant
SLC2A1	c.45C>T	p.Ala15Ala	Synonymous
	c.363G>A	p.Met121Ile	Missense mutation
	c.399C>T	p.Cys133Cys	Synonymous
	c.588G>A	p.Pro195Pro	Synonymous
	c.987G>A	p.Glu329Glu	Synonymous
	c.1011C>T	p.His337His	Synonymous
	c.1065A>G	p.Leu355Leu	Synonymous
	c.1372C>A	p.Arg458Arg	Synonymous
CLCN1	c.261C>T	p.Thr87Thr	Synonymous
	c.1205C>T	p.Ala402Val	Missense mutation
	c.2154C>T	p.Asp718Asp	Synonymous
	c.2180C>T	p.Pro727Leu	Missense variant

he was 12 years old. The episodes usually occurred at the moment he began to walk, shift position, or ascend the stairs. The attacks occurred several times a month, and each spell lasted 10–20 s. Consciousness remained undisturbed for the duration of attacks. During the intermission of attacks, he was totally normal. Over the next 1 year, the frequency of attacks increased to 5–10 times/day. In addition, he complained that the attacks occurred more frequently and longer in winter or cold weather. His cognitive function was normal. Physical examinations revealed unremarkable findings. Brain MRI demonstrated normal results. He was diagnosed with PKD 6 months after the onset. Carbamazepine was prescribed at a dosage of 100 mg once daily. This monotherapy resulted in a noticeable reduction of the attacks. However, he still complained occasional rigidity and muscle weakness. Moreover, the attacks seemed to be more frequent in cold weather. During the follow-up period, we made a cold water test which showed muscle relaxation delayed in this case. However, no muscle atrophy or hypertrophy was seen. His creatine kinase (CK) was 207 U/L (normal < 145 U/L). Electromyography (EMG) examination was refused because the patient believed his symptoms were tolerable.

The other patient (patient 10) with the *CLCN1* c.1205C>T mutation was a 12-year-old boy, who began to experience intermittent rigidity of his left leg at the age of 8. When the

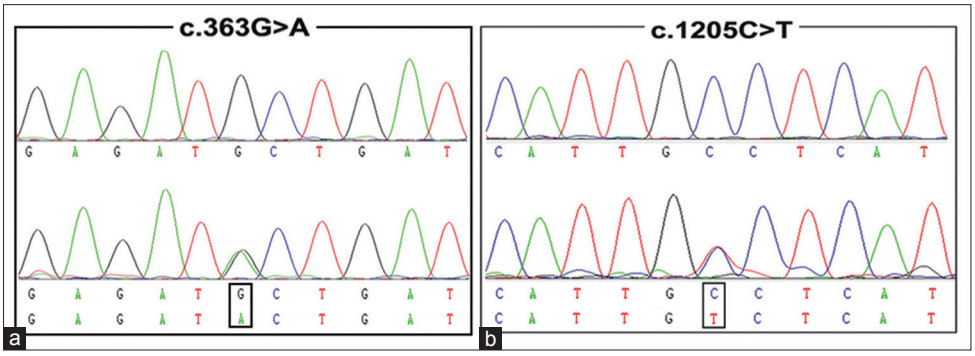

Figure 1: Chromatogram of c.363G>T mutation in *SLC2A1* and c.1205C>T mutation in *CLCN1*. Direct sequencing revealed *SLC2A1* c.363G>T mutation (a) in one case and *CLCN1* c.1205C>T mutation (b) in two cases with presumptive paroxysmal kinesigenic dyskinesia. The upper panel for each chromatogram depicts the wild-type sequence whereas the lower panel represents heterozygous mutated sequence.

attacks occurred, his leg was stiffened and could not walk normally. The spells often occurred the moment he tried to run or change position. It usually lasted approximately 20 s and occurred 10 times a day. His birth history was normal. There was no history of neurologic or muscle diseases in his family. Cranial MRI was normal. He sought his medical care in our hospital 3 years ago and was diagnosed with PKD. Carbamazepine was prescribed 100 mg twice daily, which showed mild alleviation of his symptoms. CK test revealed 185 U/L. Unfortunately, EMG was unavailable.

Discussion

In the present study, we screened *MR-1*, *SLC2A1*, and *CLCN1* genes in 28 patients who were diagnosed with sporadic PKD but not carrying *PRRT2* mutations. Among these cases, 26 of them are males and the remaining are females, which is consistent with the male predominance of PKD. We identified *SLC2A1* c.363G>A in one case and *CLCN1* c.1205C>T in two cases. The *SLC2A1* c.363G>A mutation was absent in 1000 Genomes database and was predicted to be deleterious by SIFT and PolyPhen2. It has not been reported so far and is therefore identified as a novel mutation. The *CLCN1* c.1205C>T was previously reported as a causative mutation in a sporadic MC patient.[19] The absence of 200 controls and the predicted deleteriousness demonstrated that *CLCN1* c.1205C>T mutation was responsible for this case.

We identified *SLC2A1* and *CLCN1* mutations in three patients who were diagnosed with PKD. This did not elucidate that *SLC2A1* or *CLCN1* were additional causative gene of PKD. Misdiagnosis could be a reasonable interpretation. Typically, PKD was characterized by a kinesigenic trigger, sudden attacks of involuntary movements, preservation of consciousness, remission with age, and good response to antiepileptic drug.[2] However, some cases also present with an atypical PKD features.[19] Also, a variety of diseases can mimic the phenotype of PKD, such as PED, tics, seizures, pseudoseizures, and functional movement disorders,[19] increasing the diagnostic challenge in clinical practice. Both PKD and PED are subgroups of paroxysmal dyskinesias (PxDs).[2] They have similar attacks but different trigger and duration. PKD is usually triggered by sudden movement while PED was precipitated by prolonged exercise.[2] The clinical overlap between PKD and PED sometimes make it difficult to distinguish these two diseases. Wang *et al.* described a family with a phenotype overlapping PKD, PNKD, and PED, and with a *PRRT2* c.649C>T mutation.[20] In this study, the patient with *SLC2A1* c.363G>A mutation met the diagnostic criteria proposed by Bruno *et al.* However, an alternative trigger of prolonged exercise and duration of up to 5-min attacks were not typical for PKD and prone to PED. Therefore, a diagnosis of PxDs or *SLC2A1*-related disease might be more appropriate for this case. In addition, we identified *CLCN1* c.1205C>T mutation in two cases, both of whom exhibited paroxysmal stiffness of limbs. Since both of them had a precipitating factor of sudden movement, childhood onset, and preserved consciousness during attacks, we diagnosed them with PKD. However, the 14-year-old boy's symptoms were worsened in cold weather whereas the 12-year-old boy had a mild response to carbamazepine. These were not typical clinical features of PKD but mimicked the manifestations of MC. Regrettably, we did not perform the EMG test for these two cases, which is the limitation of our study.

We did not identify any *MR-1* exon mutation in our cohort of 28 sporadic PKD cases. This is consistent with another study, in which *MR-1* mutations were not identified in 57 no-*PRRT2* associated PKD cases.[21] In fact, *MR-1* mutations produce a highly homogenous phenotype of PNKD.[22] In one previous study, *PRRT2* mutation was identified in one family with PNKD.[15] We conjectured that that case was possibly misdiagnosed as PNKD. Therefore, these reports demonstrate that there are much overlapping between PKD and other paroxysmal disorders.

In summary, we identified *SLC2A1* and *CLCN1* mutations in three cases who were diagnosed with PKD. Our results suggested that the phenotypic overlap may lead to the difficulty in distinguishing PKD from PNKD and MC. Also, our report revealed that misdiagnosis is a rational explanation for the low frequency of *PRRT2* mutation in sporadic PKD. For these patients who had atypical phenotype of PKD or

negative *PRRT2* mutation, screening of *SLC2A1* or *CLCN1* may be helpful.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: Primers and annealing temperatures for *MR-1*

Exon	Oligonucleotide primer (5' → 3')	Length (bp)	Annealing temperature (°C)
Exon 1	F: AAGAGTAGTTCTCCTGGGTCC	321	60
	R: AAAGTGTGGGGAGGAACCAAG		
Exon 2	F: ACTTCAGACTGCAGTCACTCC	393	60
	R: ATTCAGCTCGCCACCTGAAAC		
Exon 3	F: AGGCATCACGAAGGAGTCTAG	201	60
	R: TGAGGTAGCTGTAGTTGTCCG		
Exon 4	F: TCAATGGTGAGCTCTGACTGC	232	66
	R: TGGAGCATATGGGACACATCC		
Exon 5	F: TCCATCCCTTTCCTCTGCTTC	338	60
	R: TGCTGGTGTCCAGGTGACTTC		
Exon 6	F: AACAAAGGCCTAGAGGTGTGG	387	60
	R: AGTGCCAGTCTTCAGATAGGC		
Exon 7	F: ATGGAAGCCCACTCTCTTGTG	396	60
	R: TAACTGCCACGGCAACATCTG		
Exon 8	F: GGTCTGGAAAGTGCACAGTCA	352	60
	R: AGGTGGAGCTGTGTCGCTTC		
Exon 9	F: AGACTGTTCTGACTGTACCACC	389	60
	R: AGAGAAGCTGGGAAATGGACC		
Exon 10	F: TCAGAACTCCTGAACTCCAGG	371	60
	R: TGGTGGTGTTAGCGATGAGAAG		

F: Forward; R: Reverse.

Supplementary Table 2: Primers and annealing temperatures for *SLC2A1*

Exon	Oligonucleotide primer (5' → 3')	Length (bp)	Annealing temperature (°C)
Exon 1	F: AATGGCCGGGGTCCTATAAACG	519	62
	R: TAGATCCGAAGCCCATCCC		
Exon 2	F: AAAGACTGGTGTGGTGCCAAG	348	66
	R: AGAGGCAGACAGTACAGTGC		
Exon 3	F: TGAATCTGTGGCAGAGCCAG	440	66
	R: TCAGGCCAGTGCCCACATTC		
Exon 4	F: AGGTAGGGGAGACTTATCTGC	412	64
	R: AGATCCGAGAGCCACTGAAG		
Exon 5	F: ACAAAGTAGGGAAGGCCACTC	384	66
	R: TGCAGGTCATGGGTCACGTC		
Exon 6	F: TTCCTGCTCATCAACCGCAAC	439	60
	R: AGGGAAACTTCTTCGGCAGAG		
Exon 7	F: TTTACTCATCCTGGGTCCCAC	297	66
	R: AGAGCTGAGAAAGTCACAGGG		
Exon 8	F: ATCTTCCCCACTGGTCTTTGC	336	66
	R: AGAATGCAGCCACCAAAAGGC		
Exon 9	F: TTCTCGCATAGCTCTGCTCTG	416	64
	R: TACCCTCAGTTTCCTCCTCAG		
Exon 10	F: TTCAGCTCAAAGGCCCAAAGG	446	60
	R: TCTGGACATCATTGCTGGCTG		

F: Forward; R: Reverse.

Supplementary Table 3: Primers and annealing temperatures for *CLCN1*

Exon	Oligonucleotide primer (5' → 3')	Length (bp)	Annealing temperature (°C)
Exon 1	F: ATAAATAGCTGGAGGTGGGCAT	494	60
	R: ACTTAAGTGAGCCCAGACTTTC		
Exon 2	F: CAAAGTCACCCTGCATGCAGTC	422	62
	R: AGTCTGAATGACAGAGTGAGAC		
Exon 3	F: CACCCAAAGTAAAGTAGTGACTC	402	60
	R: CTCTCTGCGCAATATTCGCTTC		
Exon 4	F: AATGAGAGCAGCACCATCTCAG	463	60
	R: GTGCAGGGTCAAGGTGAAGGT		
Exon 5	F: CCATTCCCATATTCTGGACATTC	378	60
	R: CTCAGTTGGAGGAACTTCCAAAG		
Exon 6	F: CCTCTGTGTAACTCCCGTATTTC	334	60
	R: GATTAGTGCGATGCTGCTTCAG		
Exon 7	F: TCTCTTGGCCTGGGAATCACAG	379	60
	R: CTGGCACATAGCAAAGGCTTAC		
Exon 8	F: GAGCATGGGAATCCAAGAGATC	425	60
	R: TTTGTCATACACCCTTGGGCCT		
Exon 9–10	F: AGTATATCCATGGAGGAGTGTG	698	60
	R: TTTAATGCAAGCCACCCAGAG		
Exon 11–12	F: ATGAGACTACGGTGGACTAAAG	671	60
	R: GGTTGGATGAAGACCAAATGAAG		
Exon 13–14	F: GACTTTCAGAAGGATCAGCTATC	647	60
	R: AGCCATGGGTATGTTATCTGAG		
Exon 15	F: ATGAGTATTGGCACTGACCAG	475	60
	R: CAATATCTACTAGGTGCATGAG		
Exon 16	F: CTCATGCACCTAGTAGATATTG	369	60
	R: CTTGTACATAGCACATTGGATGG		
Exon 17	F: TCCAGGAAGCTGAGAAAGATG	525	57
	R: TGGCTTTCTCAGTTACCAGAC		
Exon 18	F: AAGGTTGCAGATGATGGTATCCT	360	60
	R: TGCATGCAGGTCAAGGTCAGGT		
Exon 19–20	F: GAGGACAGGGTTCTTATTCATC	689	60
	R: ACTTCCCATCCAGACCACATTC		
Exon 21–22	F: TTGCATGTTCCCAGATTCTGGG	480	62
	R: ATATTCCTTCTGTCCCCACTGC		
Exon 23	F: ATGTGGCTGCAGGTGGTCACT	752	60
	R: ATTGGCATGACCTCGCCACATTC		

F: Forward; R: Reverse.