

Paroxysmal kinesigenic dyskinesia and myotonia congenita in the same family: coexistence of a *PRRT2* mutation and two *CLCN1* mutations

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ABSTRACT

Paroxysmal kinesigenic dyskinesia (PKD) and myotonia congenita (MC) are independent disorders that share some clinical features. We aimed to investigate the sequences of *PRRT2* and *CLCN1* in a proband diagnosed with PKD and suspected MC. Clinical evaluation and auxiliary examinations were performed. Direct sequencing of the entire coding regions of the *PRRT2* and *CLCN1* genes was conducted. Haplotype analysis confirmed the relationships among the family members. The proband suffered choreoathetosis attacks triggered by sudden movements, and lower-limb weakness and stiffness that worsened in cold weather. Carbamazepine monotherapy completely controlled his choreoathetosis and significantly relieved his limb weakness and stiffness. His father, when young, had similar limb stiffness, while his mother and brother were asymptomatic. Genetic analysis revealed that the proband and his father harbored a *PRRT2* c.649dupC mutation, and *CLCN1* c.1723C>T and c.2492A>G mutations. His brother carried only the two *CLCN1* mutations. None of these mutations were identified in his mother and 150 unrelated controls. This is the first report showing the coexistence of

PRRT2 and *CLCN1* mutations. Our results also indicate that both the *PRRT2* and *CLCN1* genes need to be screened if we fail to identify *PRRT2* mutations in PKD patients or *CLCN1* mutations in MC patients.

Keywords: paroxysmal kinesigenic dyskinesia; myotonia congenita; *PRRT2*; *CLCN1*

INTRODUCTION

Paroxysmal kinesigenic dyskinesia (PKD) is an episodic movement disorder characterized by involuntary attacks that are usually precipitated by sudden movements. It is transmitted in an autosomal dominant pattern with high penetrance^[1]. PKD is clinically heterogeneous, manifesting as any combination of choreoathetosis, ballismus, and dystonia. It typically responds well to antiepileptic drugs, among which carbamazepine and phenytoin are usually considered the drugs of choice^[1,2]. Recent studies from independent groups have shown that mutations within *PRRT2* are causative for PKD^[3–6]. More than 50 *PRRT2* mutations have been documented so far, among which c.649dupC has been shown to be a mutation hotspot^[7–9].

Myotonia congenita (MC) is a muscle disorder distinguished by impaired relaxation after voluntary

contraction, and variable degrees of stiffness and hypertrophy^[10]. The warm-up phenomenon is a specific sign of MC, for the myotonia usually worsens after prolonged rest or in cold weather but improves with repetitive movements^[11]. MC can be transmitted as an autosomal dominant or recessive trait, known as Thomsen disease or Becker disease, respectively^[12]. Both forms are attributed to mutations within the *CLCN1* gene, which is mapped to chromosome 7q35 and encodes the skeletal muscle chloride channel^[13]. *CLCN1* mutations lead to dysfunction of the chloride channel and reduction of chloride conductance, increasing membrane hyperexcitability and delaying muscle fiber relaxation^[14]. So far, >150 *CLCN1* mutations have been reported to cause dominant or recessive MC (see www.hgmd.cf.ac.uk). However, certain mutations have been found to cause both forms^[13,15]. Sodium-channel blockers such as carbamazepine, phenytoin, and mexiletine are available for MC, usually resulting in a favorable effect^[16,17].

Although PKD and MC are independent disorders, they share some clinical features. For example, both present with stiffness or muscle weakness in the extremities, and these are especially pronounced on initiating an action. Moreover, they both commence in childhood, affect more males than females^[18,19], and respond favorably to carbamazepine or phenytoin^[17]. These findings can make it difficult to clinically distinguish PKD from MC.

In the current study, we present a Chinese patient affected with choreoathetosis attacks and muscle weakness and stiffness that were usually induced by sudden movements, and investigated the sequences of the *PRRT2* and *CLCN1* genes.

PARTICIPANTS AND METHODS

Participants

A two-generation family including the proband, his father who had limb stiffness between 15 and 20 years of age, and his asymptomatic mother and brother, were recruited from the Department of Neurology, First Affiliated Hospital of Fujian Medical University in November 2011. One hundred and fifty controls without a history of PKD or MC were enlisted from the Department of Neurology, Huashan Hospital, Fudan University. Written informed consent was given by all participants. The study was approved by the Ethics Committees of Huashan Hospital

and the First Affiliated Hospital. Clinical evaluation and physical examination were performed by two senior neurologists. Blood samples were collected from the family for investigations of the routine blood exam, liver function, kidney function, and myocardial enzymes. Electroencephalogram (EEG), electromyogram (EMG), and brain magnetic resonance imaging (MRI) were performed for the proband, his father, and his brother.

Mutation Analysis

Genomic DNA was extracted from peripheral blood using the standard protocol. Since the proband was diagnosed with PKD and suspected MC, we directly sequenced the entire coding regions of the *PRRT2* and *CLCN1* genes. Primers for *PRRT2* were described previously^[3], and primers for *CLCN1* were designed and are listed in Table 1. Amplification products were purified with shrimp alkaline phosphatase and exonuclease, followed by direct DNA sequencing using an ABI 3730 Automated DNA Sequencer (Applied Biosystems, Foster City, CA). The sequences were aligned with NCBI human genome reference MIM 614386 and MIM 118425, respectively.

Haplotype Analysis

To confirm that these four family members were genetically related, we conducted haplotype analysis based on 20 single nucleotide polymorphisms (SNPs) using the Sequenom MassArray system at Fudan-Van Andel Research Institute Center (School of Life Science, Fudan University, Shanghai). Alleles were detected using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry. Primers for 18 of the 20 SNPs were described previously^[8], and primers for the other two (rs3851760 and rs2071390) are listed in Table S1.

RESULTS AND DISCUSSION

The 21-year-old proband had an unremarkable birth, but suffered from febrile convulsions between the ages of two months and one year. When he was 11 years old, he developed attacks of choreoathetosis that were usually triggered by sudden movements. His lower extremities felt weak and inflexible during the attacks, which sometimes made him fall to the ground. After the first attack, the proband sought medical care in the local hospital. Diagnosed with epilepsy, he was prescribed phenytoin and

Table 1. Forward and reverse PCR primers for the *CLCN1* gene

Exon	Forward Primer (5'→3')	Reverse Primer (5'→3')	Size of PCR product (bp)	Annealing temperature (°C)
1	ATAAATAGCTGGAGGTGGGCAT	ACTTAAGTGAGCCCAGACTTTC	494	60
2	CAAAGTCACCCTGCATGCAGTC	AGTCTGAATGACAGAGTGAGAC	422	62
3	CACCCAAAGTAAAGTAGTGACTC	CTCTCTGCGCAATATTCGCTTC	402	60
4	AATGAGAGCAGCACCATCTCAG	GTGCAGGGTCAAGGTGAAGGT	463	60
5	CCATTCCCATATTCTGGACATTC	CTCAGTTGGAGGAACCTCCAAAG	378	60
6	CCTCTGTGTAACCTCCGTATTTTC	GATTAGTGCGATGCTGCTTCAG	334	60
7	TCTCTTGGCCTGGGAATCACAG	CTGGCACATAGCAAAGGCTTAC	379	60
8	GAGCATGGGAATCCAAGAGATC	TTTGTACATACACCCTTGGGCCT	425	60
9-10	AGTATATCCATGGAGGAGTGTG	TTTAATGCAAGCCACCCAGAG	698	60
11-12	ATGAGACTACGGTGGACTAAAG	GGTTGGATGAAGACCAAATGAAG	671	60
13-14	GACTTTCAGAAGGATCAGCTATC	AGCCATGGGTATGTTATCTGAG	647	60
15	ATGAGTATTGGCACTGACCAG	CAATATCTACTAGGTGCATGAG	475	60
16	CTCATGCACCTAGTAGATATTG	CTTGATACATAGCACATTGGATGG	369	60
17	TCCAGGAAGCTGAGAAAGATG	TGGCTTCTCAGTTACCAGAC	525	57
18	AAGGTTGCAGATGATGGTATCCT	TGCATGCAGGTCAAGGTCAGGT	360	60
19-20	GAGGACAGGGTTCTTATTCATC	ACTTCCCATCCAGACCACATTC	689	60
21-22	TTGCATGTTCCAGATTCTGGG	ATATTCTTCTGTCCCCACTGC	480	60
23	ATGTGGCTGCAGGTGGTCACT	ATTGGCATGACCTCGCCACATTC	752	60

valproate, which significantly reduced his choreoathetosis attacks but only slightly relieved his limb weakness and stiffness. His dyskinesias still occurred intermittently even when the dosage of medications was increased. Other anti-epileptic drugs such as phenobarbital, clonazepam, and haloperidol were tried but had unfavorable effects.

The neurological examination showed normal strength, muscle tone, and deep tendon reflexes in the extremities. The Babinski sign was negative bilaterally. Routine hematological tests revealed no significant results except a mild increase of creatine kinase (236 U/L; normal 38–174 U/L). EEG showed generalized slow waves, but no focal or lateralized spike or sharp-slow waves. The brain CT and MRI were unremarkable.

We made a diagnosis of PKD, performed direct sequencing of the *PRRT2* gene, and identified a c.649dupC (p.R217Pfs8) mutation (Fig. S1). In a previous study, we had found that PKD cases with the *PRRT2* mutation responded completely to 50 mg carbamazepine once

daily^[2], so this dose was prescribed. His choreoathetosis completely disappeared and he did not report any side-effects. However, he still complained of weakness and stiffness in his lower extremities when he started leg movements. We then increased the dosage of carbamazepine to 100 mg once daily, which had limited effects. However, a further increase to 100 mg twice daily controlled his limb weakness and stiffness. Clearly, the proband had an incomplete response to 50 mg carbamazepine, although he carried a *PRRT2* mutation.

We then made a detailed inquiry about his attacks. The proband said that the weakness and stiffness in his lower extremities worsened in cold weather but were relieved after repetitive movements. We thereby conjectured that he might coincidentally exhibit MC symptoms. No muscle hypotrophy was observed in his lower limbs. Percussion of his gastrocnemius muscle, however, showed a mild myotonia. EMG showed diffuse and abundant myotonic discharges. The nerve conduction study was normal. We

then screened for the *CLCN1* gene in the proband. The results showed that he also harbored two heterozygous *CLCN1* mutations, c.1723C>T (p.P575S) (Fig. S2) and c.2492A>G (p.Q831R) (Fig. 1). The c.1723C>T mutation has been reported to have recessive effects^[20], while c.2492A>G is a novel mutation. Q831 is located in the cystathionine beta-synthase 2 (CBS2) domain of CLC-1 and is highly conserved^[21] (Fig. S3). It has been suggested that mutations in the CBS domains affect the protein-protein interactions within CLC protein subunits and between the subunits of CBS dimers^[22]. The pathogenic status of the p.Q831R mutation was not investigated in this study. However, it was not found in the 150 normal individuals, arguing against its polymorphism.

His father suffered from limb stiffness between 15 and 20 years of age; this usually occurred after initiating a movement and worsened in cold weather. The father had not taken any medication because his symptoms were tolerable and disappeared after he was 20 years old. Neither his mother nor his brother was clinically affected. No remarkable changes in the physical and auxiliary examinations were found in his father, brother, and mother.

Evaluation of the *PRRT2* and *CLCN1* sequences in his family members revealed that his father harbored the

same *PRRT2* and *CLCN1* mutations as the proband, but his brother carried only the two *CLCN1* mutations. Neither the *PRRT2* nor *CLCN1* mutations were identified in his mother and the 150 unrelated controls. To ensure that the results of mutation screening were correct, we collected the samples again from the 4 family members, repeated the DNA sequencing, and obtained the same results.

We then performed haplotype analysis in this family and found that the proband inherited one haplotype from his mother and one from his father (Fig. 2). His brother inherited the same haplotype from his mother and a different haplotype from his father. The biological relationships among these family members were confirmed.

Here, we present a family carrying a *PRRT2* c.649dupC mutation and two *CLCN1* mutations, indicating the coexistence of PKD and MC. The proband had a predominant phenotype of choreoathetosis and additional signs of myotonia. We previously found that *PRRT2* mutation carriers respond completely to 50 mg carbamazepine^[2], but the case described here did not have a complete response. This might be partly due to the influence of the *CLCN1* mutations on the PKD phenotype, resulting in a less favorable response to carbamazepine.

The *PRRT2* c.649dupC mutation was also identified in the patient's father. However, the father did not appear to have PKD symptoms, suggesting incomplete penetrance. In addition, the proband's brother carried two *CLCN1* mutations, but he did not present MC symptoms either. This implied that the *CLCN1* mutations were not expressed, or the signs were too mild to be noticed in the brother. An EMG examination would be needed to detect myotonic discharges. The variable expressivity of the *PRRT2* and *CLCN1* mutations is poorly understood. It is possible that there are other genetic variants or epigenetic changes. Additional experiments to investigate the genetic modifiers in these individuals are needed.

Both the proband and his brother inherited the two heterozygous *CLCN1* mutations from their father, indicating that these mutations are located in the same chromatid. In addition, the proband and his father harbored a *PRRT2* mutation. Actually, the coincidence of a *CLCN1* mutation with other mutations such as *CNBP* expansion is not uncommon in the literature^[23-25]. Suominen *et al.* reported that 5% of myotonic dystrophy type 2 (DM2) patients, and 1% of DM1 patients in Germany co-segregate with the

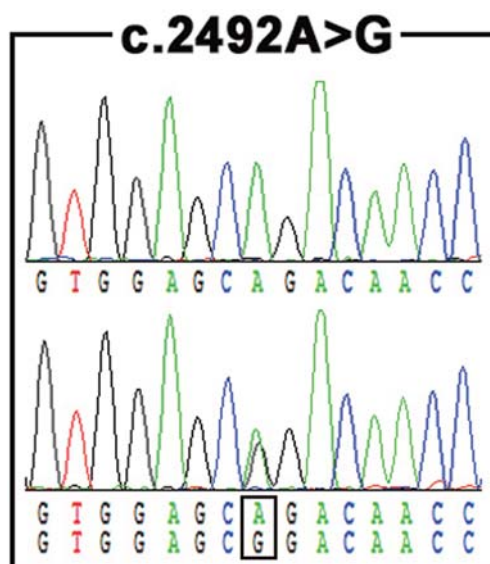


Fig. 1. Chromatogram of the novel c.2492A>G mutation within the *CLCN1* gene. The upper panel depicts the wild-type sequence, and the lower panel shows the heterozygous mutated sequence.

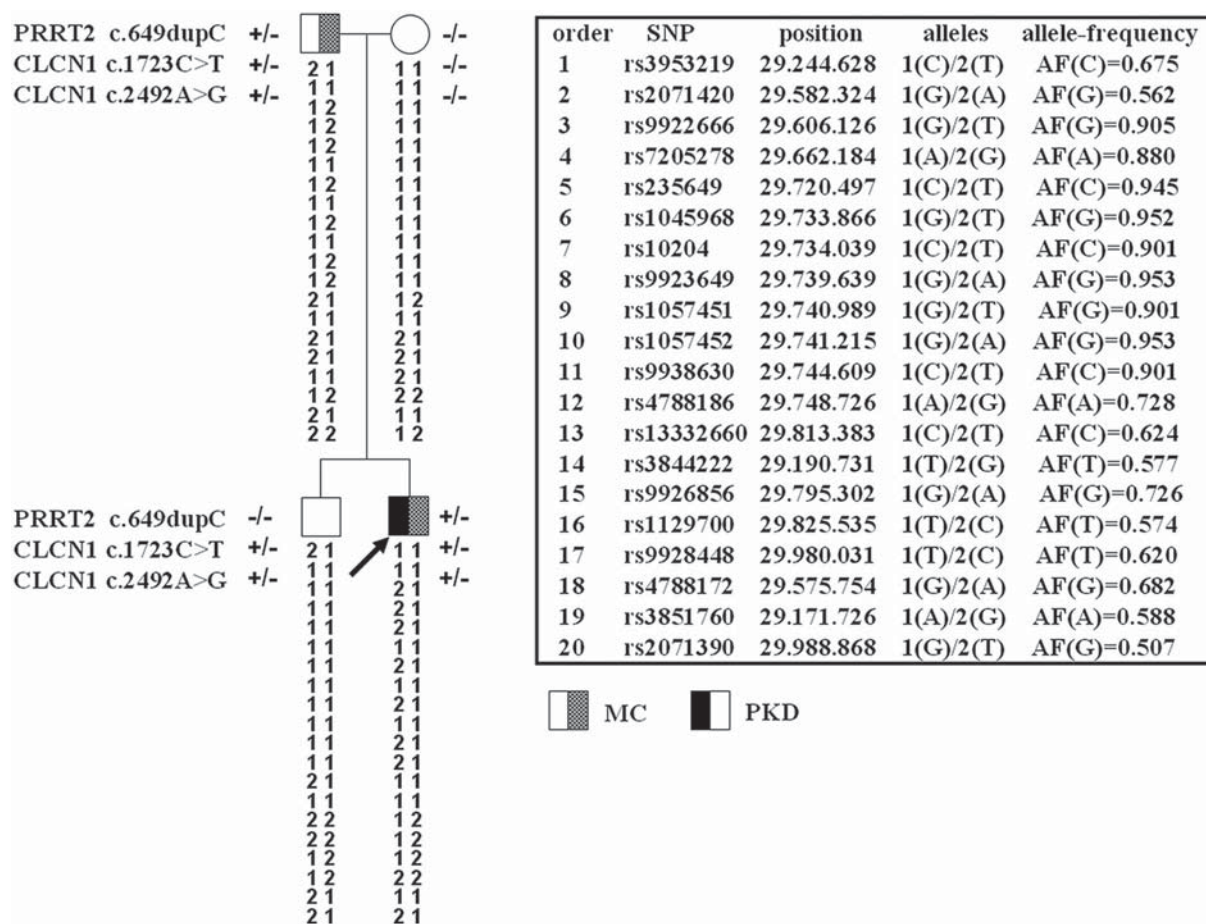


Fig. 2. Haplotype analysis based on 20 SNPs flanking the *PRRT2* gene. Right panel: reference ID, position, alleles, and allele-frequency for each of the 20 SNPs. Left panel: numbers below the squares or circles represent the genotype of each individual. 1 and 2 denote the specific allele for each SNP. The arrow denotes the proband. “+/-” denotes heterozygous mutations, “-/-” denotes absence of mutations.

CLCN1 p.R894X mutation^[23]. In addition, they found that 3% of Finnish DM2 patients are carriers of the *CLCN1* p.R894X mutation, and 2% carry the *CLCN1* p.F413C mutation^[23]. In a large Norwegian DM2 family, Sun *et al.* found that 6 patients carried *CNBP* expansion and the *CLCN1* p.F413C mutation, while 7 others harbored only *CNBP* expansion^[24]. The 6 affected carriers showed more severe symptoms and EMG myotonia. Thus, it seems that *CLCN1* mutation modifies the phenotypic effects of *CNBP* expansion and thereby exaggerates the severity of myotonia.

The pathological mechanism underlying PKD is still poorly understood. But it responds favorably to antiepileptic drugs, especially carbamazepine and phenytoin, which regulate ion channels^[1,2]. Hence, ion channel abnormality

might be one of the mechanisms underlying PKD^[26]. Yeast two-hybrid studies suggested that *PRRT2* protein interacts with synaptosomal associated protein 25 (SNAP25)^[27], a presynaptic membrane protein engaged in synaptic vesicle handling. SNAP25 plays a crucial role in the regulation of intracellular Ca^{2+} concentration and neuronal exocytosis^[28]. It is thus possible that mutation of *PRRT2* impairs its interaction with SNAP25, influencing Ca^{2+} dynamics and neurotransmitter release. However, solid evidence is still lacking.

To the best of our knowledge, this is the first report on the coexistence of *PRRT2* and *CLCN1* mutations in the same family. Since PKD and MC share some clinical features, cautious consideration is needed in diagnosis. We therefore suggest that researchers and clinicians screen for

both genes if they fail to identify *PRRT2* mutations in PKD patients or *CLCN1* mutations in MC patients.

SUPPLEMENTAL DATA

Supplemental data include one table and three figures and can be found online at <http://www.neurosci.cn/epData.asp?id=212>.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China (81330025 and 81125009). We sincerely thank the family for participating in this study.

Received date: 2014-01-16; Accepted date: 2014-04-28

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