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RESEARCH PAPER

Sequence *CLCN1* and *SCN4A* in patients with Nondystrophic myotonias in Chinese populations: Genetic and pedigree analysis of 10 families and review of the literature

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ABSTRACT

Myotonia congenita (MC), paramyotonia congenita (PC) and sodium channel myotonias(SCM) were belonged to Non-dystrophic myotonias, in which muscle relaxation is delayed after voluntary or evoked contraction. These diseases can not be simply distinguished only based on symptoms and signs but also on genetics: more than 100 mutations in the *CLCN1* gene have been associated with MC, while at least 20 mutations in the *SCN4A* gene have been associated with PC and SCM. Most of these genetics studies have been conducted outside China, only several MC, PC, and SCM families accepted gene scan were reported in China. Therefore we analyzed genetic mutations in *CLCN1* and *SCN4A* in 10 Chinese families clinically diagnosed with Non-dystrophic myotonias. Our result revealed 12 potential disease-causing mutations(3 mutations were novel) that were present in the probands and affected family members. We also reviewed all available literature on mutations linked to these 3 disease in Chinese populations. Our results may help identify genetic determinants as well as clarify genotype-phenotype relationships.

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Introduction

Myotonia congenita (MC), which in its dominant form is referred to as Thomsen's disease (OMIM 160800) and in its recessive form as Becker's disease (OMIM 255700), is belong to non-dystrophic myotonia, together with paramyotonia congenita (PC,OMIM 168300) and sodium channel myotonias (SCM,OMIM 608390). As myotonias, all diseases are characterized by delayed muscle relaxation after voluntary or evoked contraction.

The typical clinic characteristic of patients with MC including delayed relaxation after contraction, percussion myotonia, warm up phenomenon (myotonia relieved after repeated activity). MC is associated with dysfunction of the voltage-gated chloride channel CLC-1 in skeletal muscle. CLC-1 is encoded by the *CLCN1* gene, which is located at chromosome 7q35 and contains 23 exons. CLC-1 is important for normal repolarization of muscle action potentials, and certain mutations in *CLCN1* cause the protein to misfunction, resulting in plasma membrane hyper-excitation in skeletal muscle tissue and the "myotonic runs" typically seen in the electromyograms of myotonic patients.[1]

Mutations in the α-subunit of the sodium channel in skeletal muscle, encoded by the SCN4A gene located at chromosome 17q, can cause various forms of disease such as PC, SCM.[2] Patients with PC show cold sensitivity, myotonia worsens after repetitive activity, and episodic weakness.[3] While, patients with SCM have variable cold-sensitivity and not with episodic weakness.[3] This sodium channel is a heterodimer comprising a pore-forming α-subunit and a regulatory B1 subunit and the α subunit consists of 4 homologous domains, each containing 6 transmembrane segments. Certain mutations in the *SCN4A* gene are sufficient to cause repetitive discharges leading to myotonia.[4]

More than 100 mutations in *CLCN1* have been linked to MC, and more than 50 mutations have been identified in the *SCN4A* gene, of which about 20 have been linked to PC.[5] Most of these studies were

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conducted outside China, raising the question of how relevant they are to Chinese patients with MC, SCM, and PC. Indeed, in Chinese populations, fewer than 20 mutations in *CLCN1* have been associated with MC[6-11] and only 8 mutations in *SCN4A* have been associated with SCM or PC.[12-19] Clarifying the genotype-phenotype relationships specifically in Chinese patients is particularly important given that studies primarily in other ethnic groups have shown that mutations in the 2 genes can lead to clinically indistinguishable myotonias, while certain mutations in either gene can give rise to a spectrum of clinically heterogeneous phenotypes.[20,21]

To gain further insight into mutations that may contribute to MC, SCM and PC, as well as clarify genotype-phenotype relationships, we analyzed *CLCN1* and *SCN4A* in 10 families with Nondystrophic myotonias from southwest China. As a result, 5 families were confirmed as MC, 2 families as SCM and 3 families as PC.

Material and methods

Subjects

This study involved 10 probands clinically diagnosed with non-dystrophic myotonia at the Department of Neurology of West China Hospital, Sichuan University (Chengdu, China), as well as numerous affected and unaffected members of their families. Complete patient histories were obtained, and physical-neurological examinations were performed by neurologists. Diagnoses were confirmed by 2 neurologists based on the Diagnostic Criteria for Neuromuscular Disorders.[22] All patients showed non-dystrophic myotonia, and symptoms ranged from mild to severe. All probands and part of their familial members underwent electromyography and blood testing. In addition, 400 healthy Chinese people unrelated to the families involved in the present research were recruited as controls.

This study protocol was approved by the Ethics Committee of West China Hospital, Sichuan University. Written informed consent was obtained from patients or, if necessary, legal guardians, before blood samples were collected.

Mutational screening of CLCN1 and SCN4A

Peripheral blood lymphocytes were isolated from probands and from their affected and unaffected members of 10 families, and genomic DNA was extracted using classical phenol-chloroform extraction. The polymerase chain reaction (PCR), followed by direct sequencing, was used to scan for mutations across all 23 exons of *CLCN1*, 24 exons of *SCN4A*, exon-intron boundaries, untranslated regions and flanking regions. Primers for all *CLCN1* and *SCN4A* exons (Tables S1–2) were designed using on-line software (www.yeastgenome.org/cgi-bin/web-primer) and synthesized at the Molecular Pathology Center of The General Hospital of the Air Force of the PLA (Beijing, China). Amplicons of the exons were sequenced on an ABI PRISM 3730 DNA Sequencer (Applied Biosystems, Foster City, CA).

Results

Clinical characteristics

All probands in the 5 families with MC were male, and age of disease onset ranged from 1 to 26 y (Table 1). The pedigree is shown in Figure 1. The disease inheritance pattern was considered to be autosomal dominant in all families except family 2, which showed autosomal recessive inheritance. All patients complained of intermittent stiffness involving the masticatory muscles, tongue, limbs and trunk muscles. Only patient P2 from family 2 showed lid involvement. Obvious factors inducing stiffness could not be identified, though stiffness was exacerbated by cold, hunger, fatigue and nervous tension. Stiffness in all patients improved with exercise (warm-up phenomenon). Patients in all families except family 5 showed hypertrophy of affected muscles. Percussion myotonia was detectable in the thenar eminence muscles even in patients without obvious hypertrophy. Electromyography of all patients showed typical myotonic discharges. All the blood tests were normal.

Probands in Family 6, 7 with SCM, had cold/exercise-induced stiffness from 1 yr and 12 y respectively (Table 2). The disease inheritance pattern of the two families were autosomal dominant (Fig. 2). Lids, masticatory muscles, limbs and trunk muscles were involved with the progress of the disease. The probands and their affected familial members absent of intermittent periods of weakness, even after cold exposure. All 2 probands showed normal blood biochemistry, and electromyography showed typical myotonic discharges. Blood testing revealed elevated creatine kinase only in patient P6 from family 6 (712 μmol/L).

All probands in the 3 families with PC were male, and age of disease onset ranged from 1 to 15 y

Table 1. Clinical characteristics of probands from families with MC carrying mutations in *CLCN1*.

	Family 1	Family 2	Family 3	Family 4	Family 5
Patient	P1	P2	P3	P4	P5
Gene	*CLCN1*	*CLCN1*	*CLCN1*	*CLCN1*	*CLCN1*
Mutation	p.E291K, c.2172+4A>G	p.R338Q/p. R47W	p.A298T	p.A298T	p.D117G
Gender	M	M	M	M	M
Age at onset, yr	12	26	1.0	9	15
Age at admission, yr	16	32	4.0	17	29
Initial symptoms	Lower limb stiffness	Lower limb myotonia	Lower limb myotonia	Lower limb myotonia	Grip myotonia
Trigger	NA	NA	NA	NA	NA
Clinical myotonia					
Lid	−	+	−	−	−
Masticatory muscles	−	−	−	+	+
Tongue	−	−	−	+	+
Upper limb	+	+	+	+	+
Lower limb	+	+	+	+	+
Muscles of Trunk	+	−	+	+	−
Hypermyotrophy	+	+	+	+	−
Cold aggravation	+	+	+	+	+
Warm-up phenomenon	+	+	+	+	+
Percussion myotonia	+	+	+	+	+
Creatine kinase	N	N	N	N	NA
Potassium	N	N	N	N	NA
EMG	Myotonic discharges	Myotonic discharges	Myotonic discharges	Myotonic discharges	Myotonic discharges

Note. EMG, electromyography; NA, not applicable; MC, Myotonia congenita; N, normal.

(Table 2). The disease inheritance showed an autosomal dominant pattern in all 3 families (Fig. 2). Nearly all probands complained of cold- and/or exercise-induced stiffness involving masticatory muscles,lids, and limbs. Only patient P10 in family 10 reported the absence of cold- or exercise-induced stiffness of the lids. That patient's mother also showed a similar absence of such stiffness. All 3 probands showed normal blood biochemistry, and electromyography showed typical myotonic discharges.

The detailed clinic information of all the probands were listed in Tables 1,2.

Genetic analysis

Direct sequencing of all exons in *CLCN1* and *SCN4A* revealed 12 potential disease-causing mutations that were present in the probands and affected family members. Three of the 12 mutations were novel: a splice mutation in *CLCN1* associated with MC in family 2 (c.2172 + 4A > G), a missense mutation in *CLCN1* associated with MC in family 5 [c.350A > G (p.D117G)], and a deletion in *SCN4A* associated with PC in family 10 (c.2638_2640delAAG). We failed to find these 3 mutations in the ExAC database (www.exac.broadinstitute.org) or 1000 Genomes Project database (www.1000genomes.org). None of these 3 mutations was detected in any of the 400 healthy controls. The remaining 9 mutations have already been reported (Tables 1–2, Figs. 1–2).[8,23–28]

Discussion

The present study confirm 5 families with MC(Family 1–5), 2 families with SCM (Family 6–7) and 3 families with PC (Family 8–10) from southwest China segregated 1–2 candidate disease-causing mutations in probands and their familial members from all 10 families. All of the three diseases showed an autosomal dominant pattern of inheritance in nearly all families; the exception was family 2, in which MC showed an autosomal recessive pattern. Most disease-associated mutations that we detected were missense mutations; one splice mutation was detected in family 1 with MC, and one deletion was detected in family 10 with PC.

Of the 5 families with MC in our study, only family 1 showed the E291K mutation in *CLCN1*. This mutation was reported to be a recessive mutation in a German patient with Becker's disease.[24] In contrast, this mutation occurred in our population in one family showing an autosomal dominant pattern of disease. In addition, the proband and his affected father possessed the E291K mutation, his unaffected brother and mother lacked this mutation.[24] Our results appear to be the first report linking the E291K mutation to autosomal dominant MC. Our findings are consistent with reports that MC-associated mutations in *CLCN1* can be dominant and recessive. The presence of the mutation may not always predict the same clinical presentation: the proband showed severe myotonia involving upper and lower limbs,

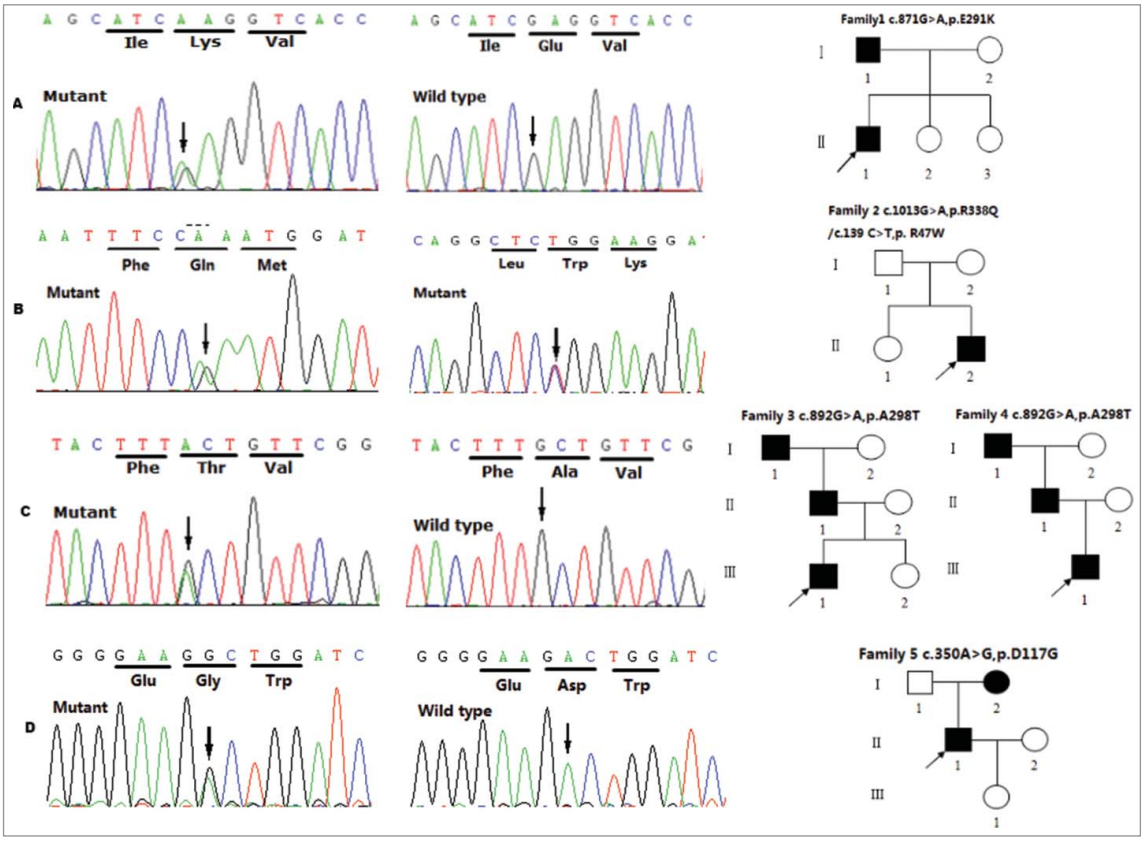

Figure 1. Sequencing chromatograms of mutations in *CLCN1*(Family 1–5) and related pedigrees of families with MC. Black arrows indicate mutations; dark squares, affected patients; arrows, the proband. (A) Sequencing chromatogram of the c.871G > A(p.E291K) mutation in *CLCN1* and the pedigree of family 1. The black arrow shows the position of a G-to-A transition at nucleotide 871 that replaces Glu with Lys at codon 291. The proband and his Father suffered from MC. (B) Sequencing chromatogram of the c.1013G > A(p.R338Q) and c.139C > T(p.R47W) mutation in *CLCN1* and the pedigree of family 2. The first black arrow shows the position of a G-to-A transition at nucleotide 1013 that replaces Arg with Gln at codon 338. The second black arrow shows the position of a C-to-T transition at nucleotide 139 that replaces Arg with Trp at codon 47. The proband suffered from MC. (C) Sequencing chromatogram of the c.892G > A(p.A298T) mutation in *CLCN1* and the pedigree of families 3 and 4. The black arrow shows the position of a G-to-A transition at nucleotide 892 that replaces Ala with Thr at codon 298. In both families, the proband's father and grandfather suffered from MC. (D) Sequencing chromatogram of the c.350A > G(p.D117G) mutation in *CLCN1* and the pedigree of family 5. The black arrow shows the position of an A-to-G transition at nucleotide 350 that replaces Asp with Gly at codon 117. The proband's mother suffered from MC.

masticatory muscles and trunk; in contrast, the proband's father showed only mild stiffness in the lower limbs, which was triggered by sudden initiation of movement. The more severe disease in the proband may reflect the presence of a novel intronic splice mutation c.2172 + 4A > G in *CLCN1*, which was not found in ExAC, 1000G as well as our 400 health controls. This splice mutation was presented in the proband and his mother, but not in his father or his 2 brothers. Interestingly c.2172 + 4A > G was near a reported splice mutation(c.2172 + 1G > T), which result in skipping of exon 17 and lead to recessive myotonia.[29] Thus we speculated a similar function between the 2 splice mutations. However, to verify our speculation,it will be important to do some further functional studies using a mini-gene assay[30] to confirm whether it affects splicing and interact with E291K to modulate disease severity.

Family 2 showed another mutation, c.1013G > A(p.R338Q), which has previously been reported as dominant and recessive.[31-33] This mutation was present in the proband and his mother, but the mother showed no myotonia symptoms. This difference may reflect the fact that the proband, but not the mother, also had the mutation c.139 C > T(p.R47W), which is reported to occur at a frequency of 0.00002529 according to the ExAC database. The same mutation has recently been reported in a Chinese patient with Becker's disease.[11] Unfortunately the proband's father, who showed no symptoms of MC, died before the study, so we could not obtain a DNA

Table 2. Clinical characteristics of probands from families with SCM and PC carrying mutations in *SCN4A*.

	Family 6	Family 7	Family 8	Family 9	Family 10
Patient	P6	P7	P8	P9	P10
Gene	*SCN4A*	*SCN4A*	*SCN4A*	*SCN4A*	*SCN4A*
Mutation	p.V445M	p.G1306V	p.R1448H	p.T1313M	p.E790del
Gender	M	M	M	M	M
Age at onset, yr	1	12	1	12	16
Age at admission, yr	27	17	19	17	17
Initial symptoms	Lower limb stiffness	Exercise-induced lower limb stiffness	Cold-induced lower limb stiffness	Cold-induced four limb stiffness	Cold-induced lower limb stiffness
Triggers	Cold	Exercise	Cold/exercise	Cold/exercise	Cold/exercise
Clinical myotonia					
Masticatory muscles	−	−	+	−	+
Lid	+	+	+	+	−
Tongue	+	−	−	−	+
Upper limb	+	−	+	+	+
Lower limb	+	+	+	+	+
Hypermyotrophy	+	+	−	+	+
Cold aggravation	+	+	+	+	+
Percussion myotonia	−	−	−	−	+
Warm-up phenomenon	−	−	−	−	−
Weakness	−	−	+	+	+
Creatine kinase	712	N	N	N	N
Potassium	N	N	N	N	N
EMG	Myotonic discharges	Myotonic discharges	Myotonic discharges	Myotonic discharges	Myotonic discharges

Note. EMG, electromyography; N, normal; PC, paramyotonia congenital; SCM, sodium channel myotonias.

sample in order to test whether the R47W mutation came from him. Since R338Q showed lower penetrance in this family, we conclude that the compound heterozygous mutation led to the proband's symptoms and that the proband had Becker's disease. However, whether the R47W was functional important, functional electrophysiology will be useful to assess the mutated channel function and demonstrate that it is not a polymorphism.

We detected the mutation c.892G > A (Ala298Thr) at exon 8 of *CLCN1* gene in families 3 and 4, and this mutation has previously been reported in a Chinese family with MC.[8] All patients in the 2 families carrying this mutation showed symptoms at an early age, ranging from 1 to 9 y. The mutation occurs at the junction between helices H and I in CLC-1, and its structural and functional effects remain unclear. One possibility is that the mutation causes the same effects as the nearby F297S mutation, which exerts a strong dominant-negative effect on wild-type channels, resulting in larger currents at strongly depolarized potentials.[34] The overall result is an increase in membrane excitability. Actually, variations in exon 8 are commonly associated with dominant MC making it more likely that A298T will act with a dominant negative effect.[34]

Our study detected the novel mutation c.350A > G (p.D117G) in family 5. Whether the proband received this mutation from his mother, also affected by MC, is unclear because we were unable to obtain DNA from her. D117 is located in transmembrane segment B of CLC-1 and is highly conserved across species. PolyPhen software predicted the effects of the D117G mutation to be 'probably damaging', SIFT software predicted its effects to be 'damaging' and Mutation Taster indicating 'disease causing'. However, future studies should examine whether this mutation affects the function of the chloride channel.

Family 6 was found to carry mutation c.1333G > A (p.V445M) in *SCN4A*, and this mutation was previously associated with MC. This mutation has previously reported been associated with MC in Caucasians from the US[35] and Dutch.[36] Indeed, as many as 20% of patients with MC without mutations in *CLCN1* possess mutations in *SCN4A* including the V445M mutation.[36] However, patients with *SCN4A* mutation and have a pure myotonic phenotype which is now named SCM and is not considered to be a MC phenotype. The V445M mutation is located in transmembrane segment 6 of domain 1 of the sodium channel,[37] and it impairs fast inactivation and enhances slow inactivation, thereby reducing the risk of depolarization-induced attacks of weakness. This may explain why patients with SCM and the V445M mutation do not suffer attacks of episodic weakness. Some Caucasian patients with the V445M mutation show debilitatingly painful myotonia and eyelid myotonia.[35,36] In contrast, the proband and other affected members of family 6 in our study did not show painful myotonia, consistent with

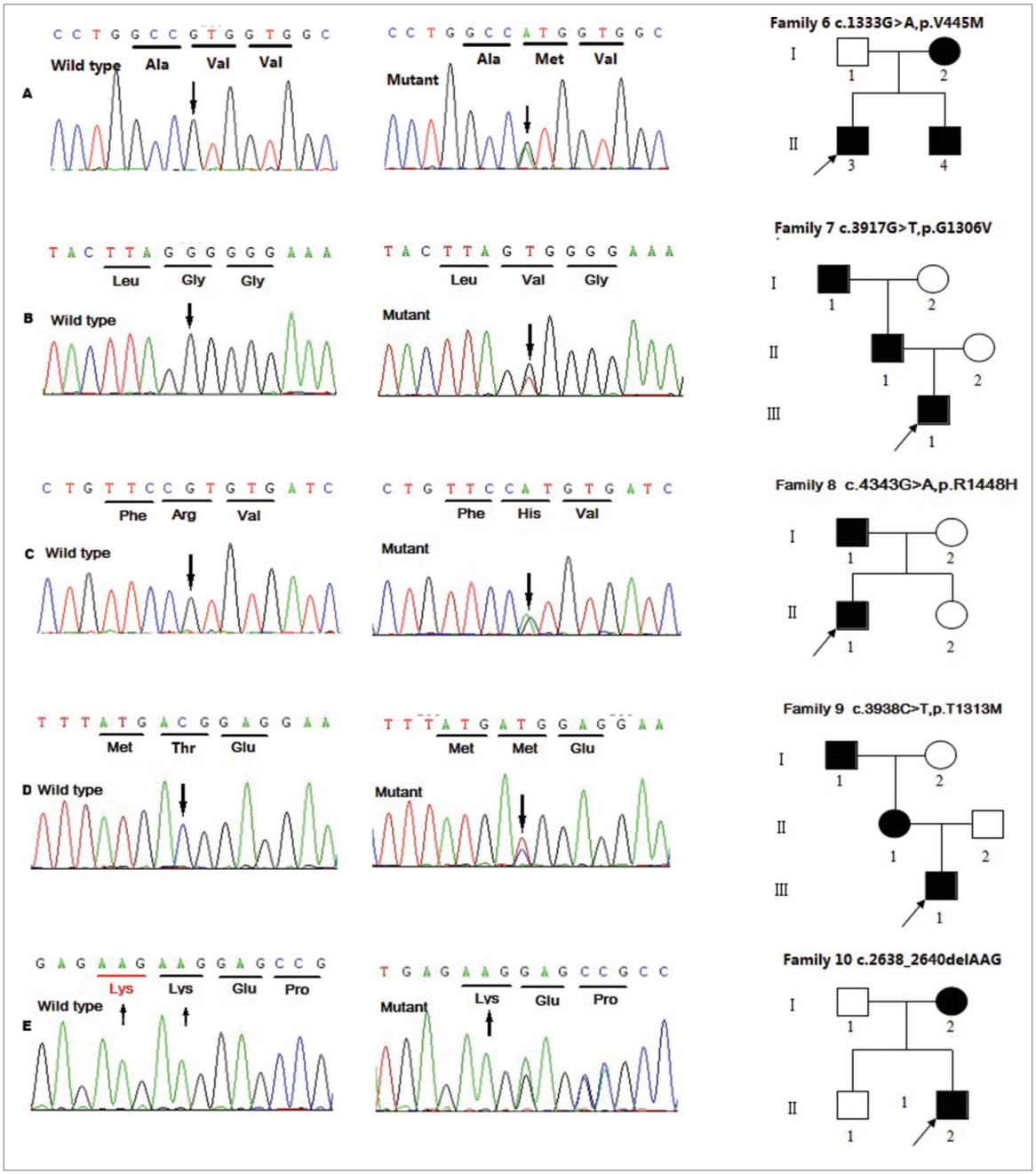

Figure 2. Sequencing chromatograms of *SCN4A* mutation and related pedigrees of SCM(Family 6–7) and PC(Family 8–10) families in which the mutations are present. Black arrows indicate mutations; dark squares, affected patients; arrows, the proband. (A) Sequencing chromatogram of the c.1333G > A(p.V445M) mutation in *SCN4A* and pedigree of family 6. The black arrow shows the position of an G-to-A transition at nucleotide 1333 that leads to the replacement of Val by Met at codon 117. The proband, his brother and Mother suffered from SCM. (B) Sequencing chromatogram of the c.3917G > T(p.G1306V) mutation at *SCN4A* gene and the pedigree of Family 7 who carried the p.G1306V mutation. The arrow shows the position of an G-to-T transition at nucleotide 3917 that leads to the replacement of Gly by Val at codon 1306. The proband, his Father and Grandfather suffered from SCM. (C) Sequencing chromatogram of the c.4343G > A(p.R1448H) mutation at *SCN4A* gene and pedigree of family 8. The black arrow shows the position of a G-to-A transition at nucleotide 4343 that leads to the replacement of Arg by His at codon 1448. The proband and his Father suffered from PC. (D) Sequencing chromatogram of the c.3938C > T(p.T1313M) mutationat *SCN4A* gene and the pedigree of Family 9. The arrow shows the position of an C-to-T transition at nucleotide 3938 at *SCN4A* gene that leads to the replacement of Thr by Met at codon 1313. The proband, his Mother and Grandfather suffered from PC. (E) Sequencing chromatogram of the c.2638_2640delAAG(E879del) mutation at *SCN4A* gene Family 10. The arrow shows the position AAG was deleted and leads to the deletion of Lys at codon 879. The proband and his Mother suffered from PC.

Table 3. Spectrum of mutations in *CLCN1* identified in Chinese families with MC.

Family no.	Gene	Exon/Intron	cDNA	Protein	Mutation type	Inheritance	Reference
1	CLCN1	Exon 8	c.871G>A	p.E291K	Missense	AD	This work
1	CLCN1	Intron17	c.2172+4A>G	—	Splice		This work
2	CLCN1	Exon 8	c.1013G>A	p.R338Q	Missense	AR	This work
2	CLCN1	Exon 1	c.139 C>T	p. R47W	Missense		This work
3,4	CLCN1	Exon 8	c.892G>A	p.A298T	Missense	AD	This work
5	CLCN1	Exon 3	c.350A>G	P.D117G	Missense	AD	This work
—	CLCN1	Exon 8	c.1024 G>A	p.A342T	Missense	AD	[6]
—	CLCN1	Exon 11	c.1292 C>T	p.A431V	Missense	Sporadic	[6]
—	CLCN1	Exon 8	c.905G>A	p.R317Q	Missense	AR	[7]
—	CLCN1	Exon 11	c.1205,C>T	p.A402V	Missense		[7]
—	CLCN1	Exon 7	c.782A>G	p.Y261C	Missense	AR	[8]
—	CLCN1	Exon 15	c.1679T>C	p.M560T	Missense		[8]
—	CLCN1	Exon 8	C.892G>A	p.A298T	Missense	AD	[8]
—	CLCN1	Exon 15	c.1744A>T	p.I553F	Missense	AR	[9]
—	CLCN1	Exon 15	c.1750,C>A	p.H555N	Missense		[9]
—	CLCN1	Exon 22	c.2617C>T	p.L844F	Missense	AD	[9]
—	CLCN1	Exon 15	c.1723C>T	p.P575S	Missense	AD	[10]
—	CLCN1	Exon 15	c.2492A>G	p.Q831R	Missense		[10]
T1	CLCN1	Exon 7	c.782A>G	p.Y261C	Missense	AD	[11]
T1	CLCN1	Exon 22	c.2576G>A	p.G859D	Missense		[11]
T2	CLCN1	Exon 14	c.1568G>A	p.G523D	Missense	AD	[11]
T3	CLCN1	Exon 15	c.1679T>C	p.M560T	Missense	AR	[11]
T4	CLCN1	Exon15	c.1679T>C	p.M560T	Missense	AR	[11]
T4	CLCN1	Intron 19	c.2364+2T>C	—	Splice		[11]
T5	CLCN1	Exon 1	c.139 C>T	p. R47W	Missense	AR	[11]
T5	CLCN1	Exon 5	c.685G>A	p.V229M	Missense		[11]

Note. AD, autosomal dominant; AR, autosomal recessive; MC, Myotonia congenita.

another study of Caucasians and a recently report in a Chinese family.[12,27] These findings suggest the possibility that the V445M mutation may be associated with different phenotypes depending on other factors, including geographic area and ethnicity.

Family 7 carried the mutation c.3917G > T (p. G1306V), which has been reported associated with PC.[38-40] All affected individuals from family 9 that were genotyped showed a similar phenotype that was consistent with previous reports associating this mutation: exercise-induced muscle stiffness that is aggravated by cold, without intermittent periods of weakness, even after cold exposure.[39] Thus, this family should be SCM as Family 6. The mutation G1306V was first reported in Chinese patients with SCM in the present research. These results suggest a robust genotype-phenotype correlation for this mutation that is independent of ethnicity or geographic region.

Among the 3 families with PC in our study, the most frequent reported mutations c.4343G > A (p. R1448H) and c.3938C > T(p.T1313M) were found in families 8 and 9 respectively. These mutations were associated with classic characteristics of PC: cold- and exercise-induced muscle stiffness as well as intermittent periods of weakness not necessarily related to cold or myotonia.[9,18,41-43] Functional experiments have shown that the mutations R1448H and T1313M impairs fast inactivation of sodium channels in a temperature-sensitive model, which may help explain the clinical phenotype of patients with PC who have these mutations.[44]

Family 10 possessed a novel deletion (c.2638_2640delAAG); both the proband and his

Table 4 Spectrum of mutations in *SCN4A* identified in Chinese families with SCM and PC

Family	Gene	Exon	cDNA	Protein	Mutation type	Diagnosis	Inheritance	Reference
6	SCN4A	9	c.1333G>A	p.V445M	Missense	SCM	AD	This work, [12]
7	SCN4A	24	c.4343G>A	p.R1448H	Missense	PC	AD	This work
8	SCN4A	22	c.3938C>T	p.T1313M	Missense	PC	AD	This work
9	SCN4A	22	c.3917G>T	p.G1306V	Missense	SCM	AD	This work
10	SCN4A	14	c.2638_2640delAAG	-	Del	PC	AD	This work
-	SCN4A	24	c.4765G>A	p.V1589M	Missense	PC	AD	[13]
-	SCN4A	14	C.3473C>T	p.P1158L	Missense	PC	AD	[14]
-	SCN4A	22	c.3938C>T	p.T1313M	Missense	PC	AD	[15]
-	SCN4A	24	c.4343G>A	p.R1448C	Missense	PC	AD	[16-17]
F6	SCN4A		C.2065C>T	p.L689F	Missense	PC	AD	[18]
-	SCN4A	24	c.4427T>C	p.M1476T	Missense	PC	AD	[19]

Note. AD, autosomal dominant; PC, paramyotonia congenital; SCM, sodium channel myotonias;

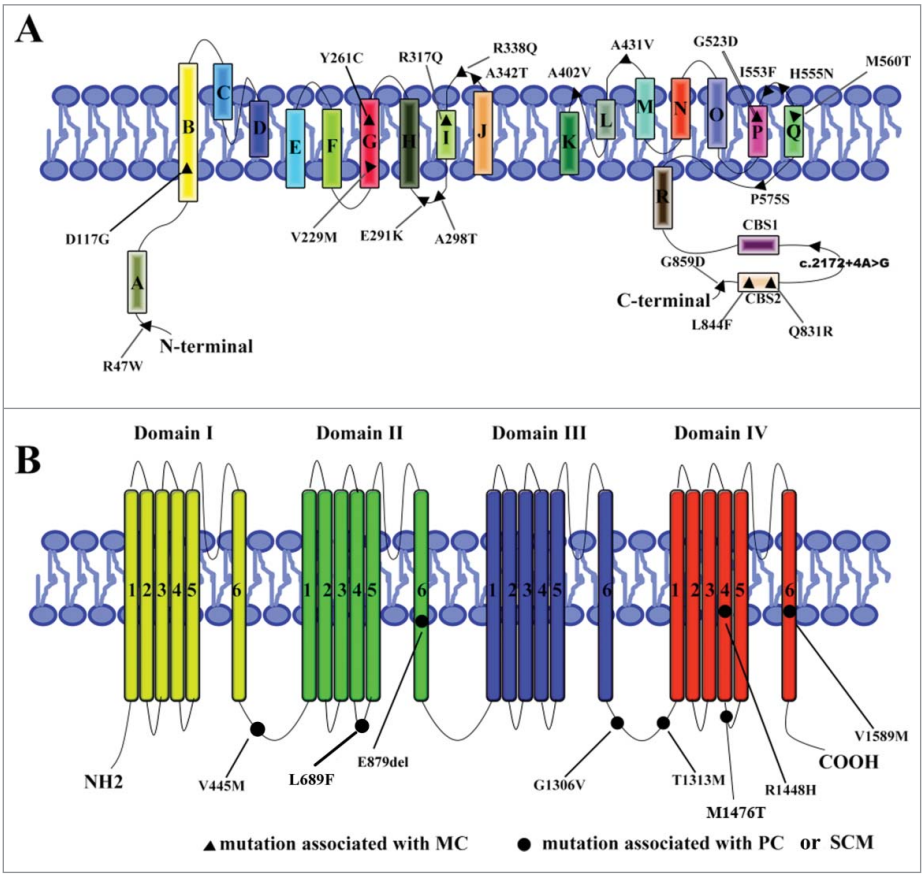

Figure 3. Presentation of (A) all mutations in *CLCN1* reported in Chinese patients with MC in the present study and in the literature, and (B) all mutations in *SCN4A* reported in Chinese patients with SCM or PC in the present study and in the literature.

mother had this mutation. Both the probands and his mother showed milder symptoms as well as generalized tonic-clonic seizure. Whether the seizure was associated with this mutation or was merely a coincidence is unclear. The SCN4A protein is found mainly in skeletal muscle, suggesting that its dysfunction should not lead to seizures. However,in a recent study a p.Gly1537Ser mutation in SCN4A was segregated in an Spanish dominant essential tremor family. In this family, 2 patients with ET also develop into epilepsy. Following functional analyses of this mutation demonstrated that the mutation can facilitated the conductance of both ammonium and potassium ions, which could increase the susceptibility to epilepsy and ET, respectively.[45] Furthermore, given that CLC-1 was originally thought to be expressed only in skeletal muscle and was later detected in human and murine brain and linked to epilepsy.[46] We are unaware of functional studies of the c.2638_2640delAAG deletion, though this mutation may not result in frameshift but it leads deletion of Lys residue may increases the probability that the mutant protein functions differently from the wild-type one.

Conclusion

This large-scale screening of mutations potentially linked to 10 Chinese families with nondystrophic myotonias has identified 3 novel mutations, including one missense mutation, one splice mutation and one deletion. Combining our results with the literature on Chinese populations indicates that 21 mutations in *CLCN1* have been associated with MC, while 7 mutations in *SCN4A* have been associated with PC, 2 mutations in SCN4A have been associated with SCM (Tables 3–4, Fig. 3). Review of published studies on Chinese populations suggests that MC shows autosomal recessive inheritance in 7 of 17 families (41.2%) and autosomal dominant inheritance in the remaining 10 (58.8%). This literature-based incidence of autosomal recessive disease is higher than the incidence of 14% reported in one previous study of a Chinese population, which was based only on clinical pedigree

analysis.[47] Some patients diagnosed with sporadic MC may actually have the autosomal recessive form of the disease, as the case in family 2, whose affected members contained compound heterozygous mutations. Our results highlight the importance of screening both *CLCN1* and *SCN4A* in genetic studies of Non-dystrophic myotonias. In addition, different mutations may play different roles in the pathogenesis of Non-dystrophic myotonias, and a given mutation may also correlate with a range of phenotypes. This highlights the strong possibility that epigenetic factors influence the clinical expression of certain mutations. Future studies should examine these factors in detail, and functional experiments such as electrophysiological experiment should clarify how disease-associated mutations contribute to phenotype. Such work may bring us closer to developing drugs to relieve the symptoms of Non-dystrophic myotonias.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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