CHANNELS
2024, VOL. 18, NO. 1, 2349823
https://doi.org/10.1080/19336950.2024.2349823

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RESEARCH PAPER

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Clinical and genetic characteristics of myotonia congenita in Chinese population

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ABSTRACT

Myotonia congenita (MC) is a rare hereditary muscle disease caused by variants in the CLCN1 gene. Currently, the correlation of phenotype-genotype is still uncertain between dominant-type Thomsen (TMC) and recessive-type Becker (BMC). The clinical data and auxiliary examinations of MC patients in our clinic were retrospectively collected. Electromyography was performed in 11 patients and available family members. Whole exome sequencing was conducted in all patients. The clinical and laboratory data of Chinese MC patients reported from June 2004 to December 2022 were reviewed. A total of 11 MC patients were included in the study, with a mean onset age of 12.64 ± 2.73 years. The main symptom was muscle stiffness of limbs. Warm-up phenomenon and percussion myotonia were found in all patients. Electromyogram revealed significant myotonic charges in all patients and two asymptomatic carriers, while muscle MRI and biopsy showed normal or nonspecific changes. Fourteen genetic variants including 6 novel variants were found in CLCN1. Ninety-eight Chinese patients were re-analyzed and re-summarized in this study. There were no significant differences in the demographic data, clinical characteristics, and laboratory findings between 52 TMC and 46 BMC patients. Among the 145 variants in CLCN1, some variants, including the most common variant c.892 G>A, could cause TMC in some families and BMC in others. This study expanded the clinical and genetic spectrum of Chinese patients with MC. It was difficult to distinguish between TMC and BMC only based on the clinical, laboratory, and genetic characteristics.

ARTICLE HISTORY
Received 15 November 2023
Revised 13 March 2024
Accepted 22 March 2024

KEYWORDS
Myotonic congenital; *CLCN1*; Thomsen MC; Becker MC; skeletal muscle channelopathies

Introduction

Myotonia congenital (MC), caused by variants in the chloride voltage-gated channel 1 (*CLCN1*) gene, is characterized by myotonia and muscle weakness, which can manifest in all limbs, evenly affect the extraocular muscle, facial muscle, and tongue muscle. Myotonia is usually triggered by movement after rest such as taking the first step, standing up, or shaking hands. Common triggers include exposure to cold temperatures and stress. Based on genetic findings and inheritance patterns, the disorder is categorized into two forms: autosomal dominant-type Thomsen (TMC) (OMIM #160,800) and autosomal recessive-type Becker (BMC) (OMIM #255,700) [1].

Historically, TMC has been characterized by relatively mild myotonia with an early onset, predominantly affecting the upper limbs and facial muscles [2]. In contrast, BMC typically manifests as severe myotonia with a later onset, primarily involving the lower limbs, then followed by the upper limbs and face [3,4]. Patients with BMC may also experience myalgias and muscle weakness [3,4]. However, it's worth noting that there can be significant variations in phenotypic characteristics among different families [5]. The Mendelian model does not consistently predict the specific type of congenital myotonia, particularly in cases of incomplete penetrance and variable expressivity. Therefore, the heterogeneity in both phenotype and genotype presents considerable challenges when attempting to differentiate between TMC and BMC, particularly in sporadic cases or small families.

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/19336950.2024.2349823

The estimated global incidence of MC ranges from 0.2 to 7.3 cases per 100,000 individuals [6], while the incidence may be lower in Asian population [7–9]. Despite being a populous country, China has seen only a limited number of small-scale studies on MC patients. In this study, we initially collected and summarized the clinical and genetic data of 11 patients with MC in our center. Subsequently, we conducted a systematic review and analysis of clinical and genetic features in 87 Chinese MC reported in the literature. We aimed to compare the characteristics of phenotype-genotype relationships between TMC and BMC in Chinese patients.

Materials and methods

Subjects

A total of 11 patients with MC were enrolled in the department of neurology in the first affiliated hospital of Nanchang university between July 2017 and September 2023. The age of onset, clinical manifestations, and laboratory tests were retrospectively collected. This study was approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University, and all the patients or their guardians had signed informed consent to participate in the study.

Electrophysiological examination

Neuro electrophysiological examinations including nerve conduction velocity (NCV) in the extremities and needle electromyography (EMG) were performed in all patients and available family members. In generally, bilateral deltoid muscles, biceps brachii muscles, thenar muscles, vastus medialis muscles, and tibialis anterior muscles were usually selected for EMG examination.

Muscle MRI

Axial planes of the thigh and leg muscles were imaged in the four patients (Case 1, 3, 7and 10) using 3.0-T MR scanners. MRI conventional T1 weighted image (T1WI) sequences were obtained to observe fatty infiltration with the following parameters: repetition time (TR) = 500 ms, echo time (TE) = 8 ms, matrix 512 × 512. The short time inversion recovery (STIR) sequences were obtained to evaluate muscle edema with the following parameters: TR = 6100 ms, TE = 70 ms, inversion time = 180 ms, matrix 512 × 512. The slice thickness was 5 mm, with a slice gap of 1 mm, and the field of view was 36 × 48 cm. Refer to our previous work for detailed methods [10,11].

Pathological biopsy

Muscle biopsies were performed from the right bicep in 4 patients (Case 1,2,3, and 5). For histological examination, muscle samples were conducted on serially frozen sections (8 μm) using routine histological and histochemistry staining. The stains included hematoxylin and eosin (H&E), modified Gomori trichrome (MGT), periodic acidic Schiff (PAS), oil red O (ORO), nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), succinate dehydrogenase (SDH), cytochrome coxidase (COX), nonspecific esterase (NSE), and ATPase stain. Refer to our previous work for detailed methods [10,11].

Genetic analysis

Genomic DNA was extracted from peripheral blood samples of 11patients and their available relatives. The whole-exome sequencing (WES) was commercially supported by GrandOmics Lab (Beijing, China). Targeted exon enrichment was performed using SureSelect Human All Exon V5 (Agilent Technologies). The exon-enriched DNA libraries were subjected to paired-end sequencing with the Hiseq2000 platform (Illumina, Inc.). Sequence data were mapped with BWA and SAMTOOLS onto the hg19 human genome as a reference. Calls with variant quality less than 20 were filtered out. The pathogenicity of missense variants was predicted by Polyphen-2, SIFT, Mutation Taster, and CADD. Sanger sequencing was conducted to confirm the *CLCN1* variants in the patients and their family members. The pathogenicity of variants was classified based on the American College of Medical Genetics and Genomics (ACMG) guidelines [12]. Refer to our previous work for detailed methods [10,11].

Literature review

The literature was retrieved from several databases including PubMed, Web of Science, and

WanFang database using the keywords ("myotonia congenita" OR "*CLCN1*" OR "skeletal muscle chloride" OR "nondystrophic myotonia" OR "Chloride channel") AND (Chinese OR China OR Asia). All enrolled patients had a definitely genetic diagnosis. The clinical characteristics, laboratory results, and genetic characteristics of all patients were summarized and analyzed.

Statistical analysis

All statistical analyses were performed using SPSS 25 (SPSS Inc., Chicago, IL, USA). Numerical variables were presented as the mean ± standard deviations or median (interquartile range) and compared using the Student's t-test or Mann-Whitney U test. Categorical variables were compared using Chi-Squared test or Fisher exact test as appropriate. One-sided tests were used. Differences were considered statistical significance if $p < 0.05$.

Results

Clinical features

There were a total of 11 MC index patients (Figure 1), including 10 males and 1 female, with ages ranging from 13 to 32 years old. These patients had normal development milestones. The mean age of onset was 12.64 ± 2.73 years, with a range of 8.0 to 16.0 years. All 11 patients initially showed muscle stiffness of lower limbs, resulting in walking or running difficulties for nine patients and stair-climbing difficulties for two patients. Ten patients experienced muscle stiffness in upper limbs, characterized by an inability to quickly extend their arms or relax their hands after clenching their fists (Figure 2a,b). Facial muscle stiffness was observed in Case 3 and 11, leading to slow mouth closure after sneezing. The warm-up phenomenon was presented in all patients. Four patients occasionally experienced sudden muscle weakness during exercise. Clinical symptoms could be exacerbated by cold temperature in 5 patients, or by psychogenic stress in 3 patients (Table 1). Percussion myotonia was observed in all patients (Figure 2c–e). Eight patients showed mild limb-muscle hypertrophy, particularly in the lower limbs. Two patients suffered from anxiety or depression, while none had urinary or digestive system diseases, cataract, strabismus, diplopia, and other eye diseases.

Echocardiography revealed sinus arrhythmias in 2 patients, while the rest were normal. Laboratory tests showed normal levels of blood electrolytes in all patients. The level of serum creatine kinase (CK) was within the normal range for all patients expect for Case 3, who had a CK level of 764 IU/L (normal range, 40–200 IU/L) (Table 1).

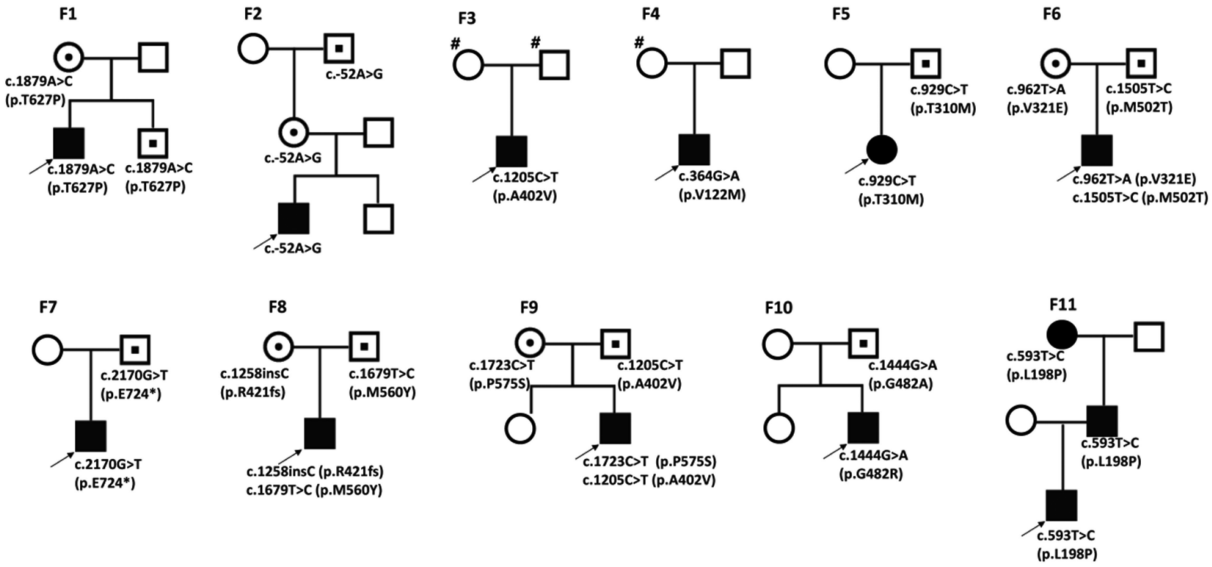

Figure 1. Pedigrees of the 11 index patients. The filled symbol represents affected individuals, the symbol "#" indicates individuals who have not undergone genetic testing and the symbol with a central black spot indicates the asymptomatic variant carrier, the arrow points to proband.

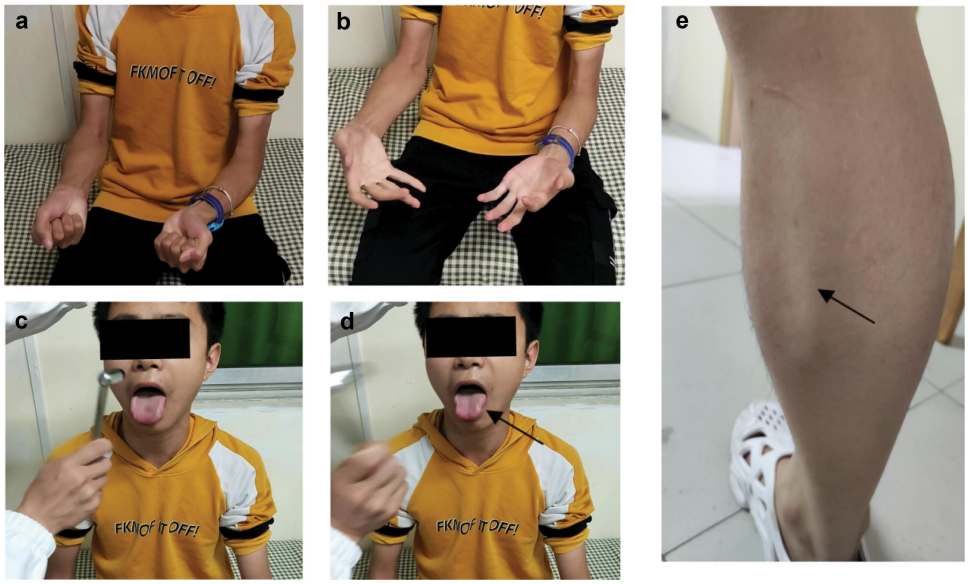

Figure 2. Illustrations about myotonia in case 1. Griping myotonia (a and b); percussion myotonia on tongue muscle (d and d) and gastrocnemius muscle (e).

Electrophysiological changes

Nerve conduction studies showed normal NCV data in 9 patients, while 2 patients had mild decrease of motor action potential amplitude in the peroneal nerves. Notably, EMG findings included myotonic potential bursts, characterized by typical sounds resembling motorcycle acceleration or a bomber dive (Supplemental Figure S1). Additionally, 10 patients displayed myogenic impairments, featured by a short-duration and low-amplitude motor unit action potential (Supplemental Table S1).

Muscle MRI changes

Muscle MRI of Case 7 revealed mild hypertrophy of thigh muscles, primarily affecting the vastus

Table 1. The clinical, laboratory and genetic results of 11 MC patients.

P	Sex	AOD/ AOO(years)	Clinical presentation and signs				CK	Variants	Inheritance	Exon	Amino acid	Variant source
			Initial symptom	SW	WU	CF						
1	M	16/9	Muscle stiffness of lower limbs.	+	+	-	normal	c.1879A>C	AD	16	p. T627P	mother
2	M	18/15	Muscle stiffness of lower limbs.	-	+	-	normal	*c.-52A>G	AD	-	-	mother
3	M	16/13	Muscle stiffness of lower limbs.	-	+	cold	764 U/L	c.1205C>T	AD	11	p. A402V	ND
4	M	16/11	Muscle stiffness of lower limbs.	+	+	tension	normal	*c.364 G>A	AD	3	p. V122M	father?
5	F	32/15	Muscle stiffness of lower limbs.	+	+	-	normal	c.929C>T	AD	8	p. T310M	father
6	M	16/13	Muscle stiffness of lower limbs.	-	+	cold, tension, agitation.	normal	*c.962T>A; *c.1505T>C.	AR	8; 14	p. V321E; p. M502T.	mother; father.
7	M	13/10	Muscle stiffness of lower limbs.	+	+	cold	normal	*c.2170 G>T	AD	17	p. E724*	father
8	M	18/16	Muscle stiffness of lower limbs.	-	+	cold	normal	*c.1258insC; c.1679T>C.	AR	12; 15	p.R421fs; p. M560T.	mother; father.
9	M	22/14	Muscle stiffness of lower limbs.	-	+	tension	normal	c.1205C>T; c.1723C>T.	AR	11,15	p.A402V; p.P575S.	father, mother.
10	M	18/15	Muscle stiffness of lower limbs.	-	+	cold	normal	c.1444 G>A	AD	13	p.G482A	father
11	M	14/8	Muscle stiffness of lower limbs.	-	+	tension, agitation.	normal	c.593T>C	AD	5	p.L198P	father

M: male; F: female; AOD: Age of diagnosis; AOO: age of onset; SW: sudden weakness; WU: warm-up; CF: contributing factors; AD: autosomal dominant; AR: autosomal recessive; ND: not done; * indicates a novel variant.

lateralis, vastus intermedius, adductor longus, and adductor magnus, while no abnormalities were observed in the other cases (Supplemental Figure S2). In addition, there were no changes in fat infiltration or edema.

Myopathological changes

Muscle sections from Case1 showed no abnormal changes (Figure 3a,b). The sections of Case 2 revealed cytoplasmic bodies in a single fiber, which were nonspecific changes (Figure 3c). In Case 3, with elevated CK, sections showed some fibers with fresh necrosis on HE staining (Figure 3d), and atypical tubular aggregation and vacuoles on NADH staining (Figure 3e). In Case 5, the sections showed cytoplasmic masses under the sarcolemma in a few fibers (Figure 3f).

Genetic variants

The index patients were initially screened by WES, and then were validated by Sanger sequence. All patients carried variants in the *CLCN1* gene, with eight patients having heterozygous variants (Case 1–5, 7, 10, and 11) and three patients having compound heterozygous variants (Case 6, 8, and 9) (Supplemental Figure S3).

Case 1 had a missense variant (c.1879A>C; p.Thr627Pro) in *CLCN1*, which was also detected in the asymptomatic mother and subclinical brother with electrophysiological myotonia. The variant c.-52A>G was detected in Case 2, asymptomatic mother, and symptomatic grandfather. The variant located at the upstream of initiation codon caused severe decrease of the transcription level of *CLCN1* mRNA (Supplemental Figure S4). The c.1205C>T (p.Ala402Val) variant was found in Case 3, but the parents were unavailable for genetic screening. The c.364 G>A (p.Val122Met) variant was identified in the Case 4, while his mother did not have the variant and his father did not test it. Case 5 and the asymptomatic father had the same c.929C>T (p.Thr310Met) variant in *CLCN1*. Case 6 had compound c.962T>A (p.Val321Glu) and c.1505T>C (p.Met502Thr) variants from the parent, respectively.ACMG Case 7 had c.2170 G>T (p.Glu724Ter) variant in *CLCN1*, and the asymptomatic father but with electrophysiological myotonia carried the same variant. Case 8 had compound heterozygous c.1679T>C (p.Met560Thr) and c.1258insC (p.Arg421Profs *9) variants from the parent, respectively. Case 9 had compound heterozygous c.1205C>T (p.Ala402Val) and c.1723C>T (p.Pro575Ser) variants from the parent, respectively. Case 10 and his asymptomatic father had the same c.1444 G>A (p.Gly482Ala) variant in *CLCN1*. Case 11

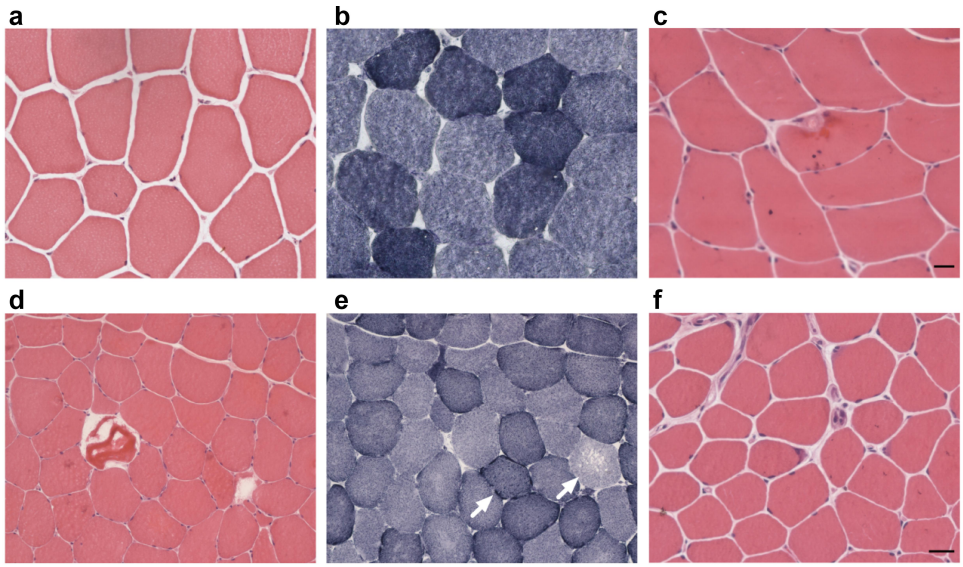

Figure 3. Myopathological changes in the MC patients. Muscle sections of case 1 showed no abnormal changes on HE (a) and NADH (b) stains. The sections of case 2 had cytoplasmic bodies on HE stain (c). In case 3, the sections showed some fibers with fresh necrosis on HE stain (d), and atypical tubular aggregation and vacuoles on NADH stain (arrow, e). In case 5, the sections showed cytoplasmic mass under the sarcolemma in a few fibers (f). a-c: 20 μm scale. e-f: 40 μm scale.

had a paternal heterozygous c.593T>C (p.Leu198Pro) variant, his father and grandmother had a history of similar myotonia (Figure 1).

In total, six variants had not been previously reported in GeneMatcher, including c.-52A>G, c.364 G>A, c.962T>A, c.1258insC, c.1505T>C, and c.2170 G>T. Additionally, the variants newly discovered in this study were not found in professional HGMD, 1000 genomes database, ExAC database, and gnomAD database. The pathogenicity of these variants on in silico tools was considered deleterious in SIFT, possibly damaging in PolyPhen, and disease-causing in Mutation Taster (Table 2). The amino acid residues of p.Val122, p.Thr310, and p.Met502 associated with missense mutations were relatively conserved evolutionarily (Supplemental Figure S5). The pathogenicity of variants was classified according to the ACMG guidelines.

Clinical and genetic characteristics of Chinese MC patients

A PRISMA flowchart depicting the literature search and selection process was shown in Supplemental Figure S6. Initially, 151 articles were identified, with 7 duplicates excluded. Screening of title and abstracts resulted in exclusion of 7 literatures. Then by reading the full text of the remaining articles, 112 were excluded for the follow reasons: no genetic data (60), other diseases (19), no full text (17), experimental study (7), and no available data (9). Finally, 25 articles were included to re-analyze and review the clinical and genetic characteristics of Chinses MC patients.

The 25 articles collectively reported on 87 independent Chinese MC patients, and comprehensive clinical data was summarized in the Supplemental Table S2. Therefore, a total of 98 patients were analyzed, including the 11 patients in this study. The patients included 70 males, 24 females, and 4 patients with unknown gender. Notably, 40.8% (40/98) of patients exhibited a positive family history. The median age of onset was 9.0 years (ranging 1 to 53.5 years) for the 52 TMC patients with AD inheritance or single heterozygous variants in *CLCN1*. In contrast, the median age of onset was 7.0 years (ranging from 1 to 26.0 years) for the 46 BMC patients with autosomal recessive inheritance or two heterozygous variants in

Table 2. The pathogenicity of variants was classified based on the ACMG guidelines.

Patients	Variants	Mutation Taster	PolyPhen-2	SIFT	Revel	Variant Classification (ACMG)
1	c.1879A>C	Polymorphism	Possibly damaging	Tolerated	Neutral	Uncertain (PM2_supporting + PP4)
2	*c.-52A>G	-	-	-	-	Pathogenic (PVS1 + PM2_supporting + PP4)
3	c.1205C>T	Disease causing	Probably damaging	Deleterious	Deleterious	Uncertain (PM2_supporting + PP3_moderate + PP4)
4	*c.364 G>A	Disease causing	Possibly damaging	Tolerated	Deleterious	Uncertain (PM2_supporting + PM6 + PP4)
5	c.929C>T	Disease causing	Probably damaging	Deleterious	Deleterious	Likely pathogenic (PM1 + PM2_supporting + PP3_moderate + PP4)
6	*c.962T>A; *c.1505T>C	Disease causing; Disease causing	Probably damaging; Probably damaging	Deleterious; Deleterious	Deleterious; Deleterious	Likely pathogenic (PM1 + PM2_supporting+ PM3 + PP3_strong + PP4); Uncertain (PM2 + PP4)
7	*c.2170 G>T	Disease causing	Probably damaging	-	-	Pathogenic (PVS1 + PM2_supporting + PM4 + PP4)
8	*c.1258insC; c.1679T>C.	Disease causing; Disease causing	-; Probably damaging	-; Tolerated	-; Deleterious	Pathogenic (PVS1 + PM2_supporting + PM3 + PM4 + PP4); Uncertain (PM2_supporting + PP3_strong+ PP4)
9	c.1205C>T; c.1723C>T	Disease causing; Disease causing	Probably damaging; Possibly damaging	Deleterious; Deleterious	Deleterious; Deleterious	Uncertain (PM2_supporting + PP3_moderate + PP4); Uncertain: PM2_supporting + PP3_moderate + PP4
10	c.1444 G>A	Disease causing	Probably damaging	Deleterious	Deleterious	Pathogenic (PS1 + PS3_moderate + PM2_supporting + PM3 + PP3_strong + PP4)
11	c.593T>C	Disease causing	Probably damaging	Deleterious	Deleterious	Pathogenic (PS3_moderate + PS4_moderate + PM2_supporting + PM5_supporting + PP4)

CLCN1 (Figure 4a). Muscle stiffness was a predominant symptom, occurring in 93.9% (92/98) of the upper limbs, 95.9% (94/98) of the lower limbs, and 31.6% (31/98) of the neck or face. A total of 62.2% (61/98) of patients reported specific triggers for myotonia or muscle weakness attacks. Furthermore, 94.9% (93/98) of patients exhibited the warm-up phenomenon. Muscle hypertrophy was evident in 63.3% (62/98) of patients. During the course of the disease, approximately 13.3% (13/98) experienced muscle weakness, and 9.2% (9/98) reported myalgia (Figure 4b). Statistical analysis indicated no significant differences in the aforementioned symptoms between TMC and BMC patients (Table 3). Among the 98 Chinese MC patients, 35 underwent muscle biopsy, all of which exhibited nonspecific mild pathological changes or were nearly normal, with no discernible differences between the 18 patients with dominant TMC and the 17 patients with recessive BMC.

Of the 98 Chinese MC patients, two had homozygous variants, 44 harbored compound heterozygous variants, and 52 carried with single heterozygous variants. In total, 145 variants were identified, including 110 missense variants, 23 frameshift variants, 8 nonsense variants, and 4 splice variants (Supplemental Table S2). Some variants might be associated with autosomal dominant MC in some families and a recessive form in others. These variants included c.214_215delAG, c.350A>G, c.762C>G, c.892 G>A, c.1024 G>A, c.1205C>T, c.1262insC, c.1679T>C, c.2330delG, and c.2527C>T (Figure 5). The most common

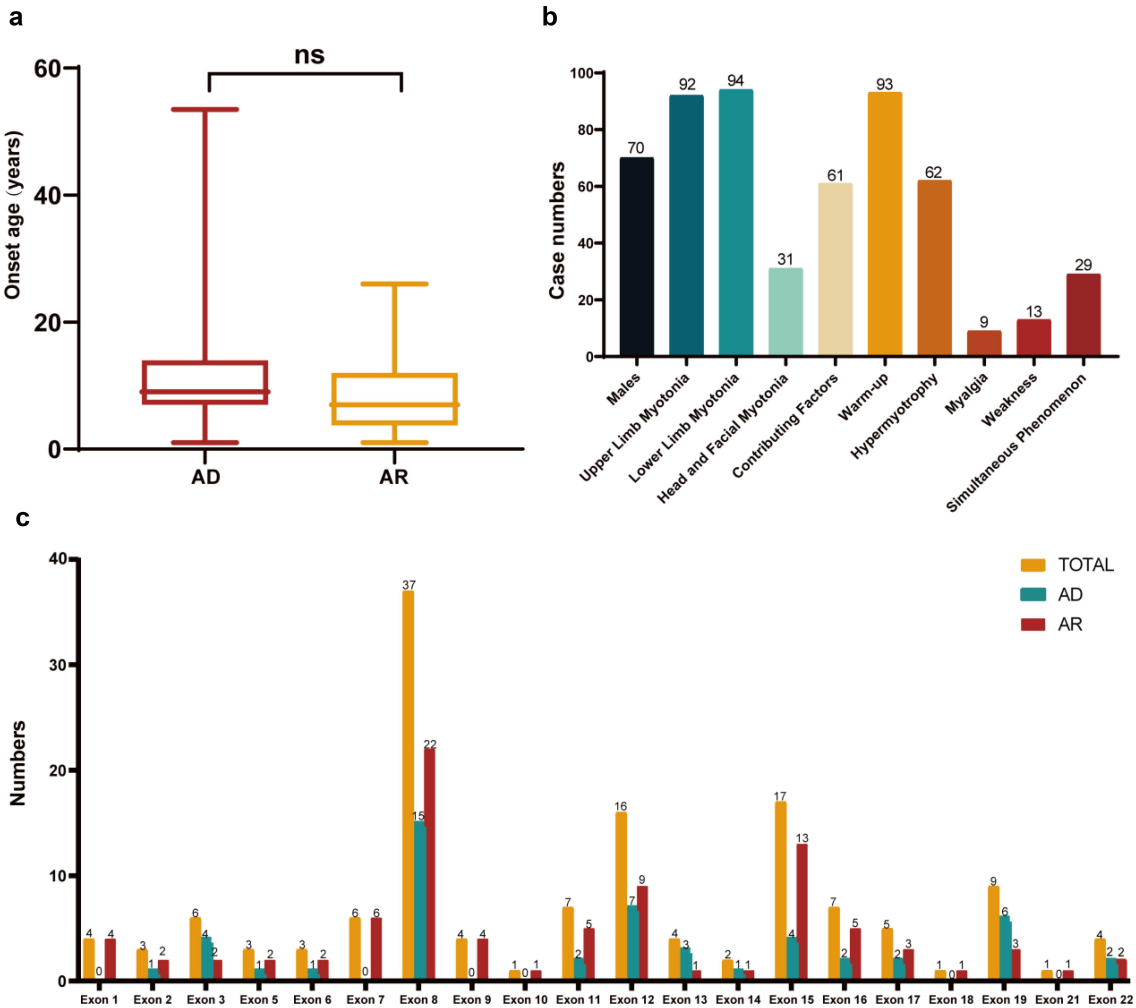

Figure 4. The summarization and comparison of Chinese MC patients. The onset age of AD patients (10.52 ± 8.59 years) was not significantly different from that of AR patients (7.84 ± 5.51), $p > 0.05$ (a). The symptom incidence of 98 Chinese MC patients (b). The hotspot variants located at the exons 8, 12, 15, and 19 in the Chinese MC patients (c).

Table 3. The comparison of clinical symptoms between TMC and BMC in Chinese patients.

	TMC ($n = 52$)	BMC ($n = 46$)	P value
Males	36/49 (73.5%)	34/45(75.6%)	0.819
Age of onset	9 (1–53.5 years)	7 (1–26 years)	0.160
Upper limb myotonia	49/49 (100%)	43/44 (97.7%)	0.473
Lower limb myotonia	49/49 (100%)	45/45 (100%)	1.000
Neck or face myotonia	15/34 (44.1%)	16/29 (55.2%)	0.382
Trigger factors	31/42 (73.8%)	30/36 (83.3%)	0.310
Warm-up	49/52 (94.2%)	44/45 (97.8%)	0.716
Hypermyotrophy	32/44 (72.7%)	29/35 (82.9%)	0.286
Myalgia	4/31 (13.3%)	5/25 (20.0%)	0.724
Muscle weakness	8/25 (32%)	5/22 (22.7%)	0.478

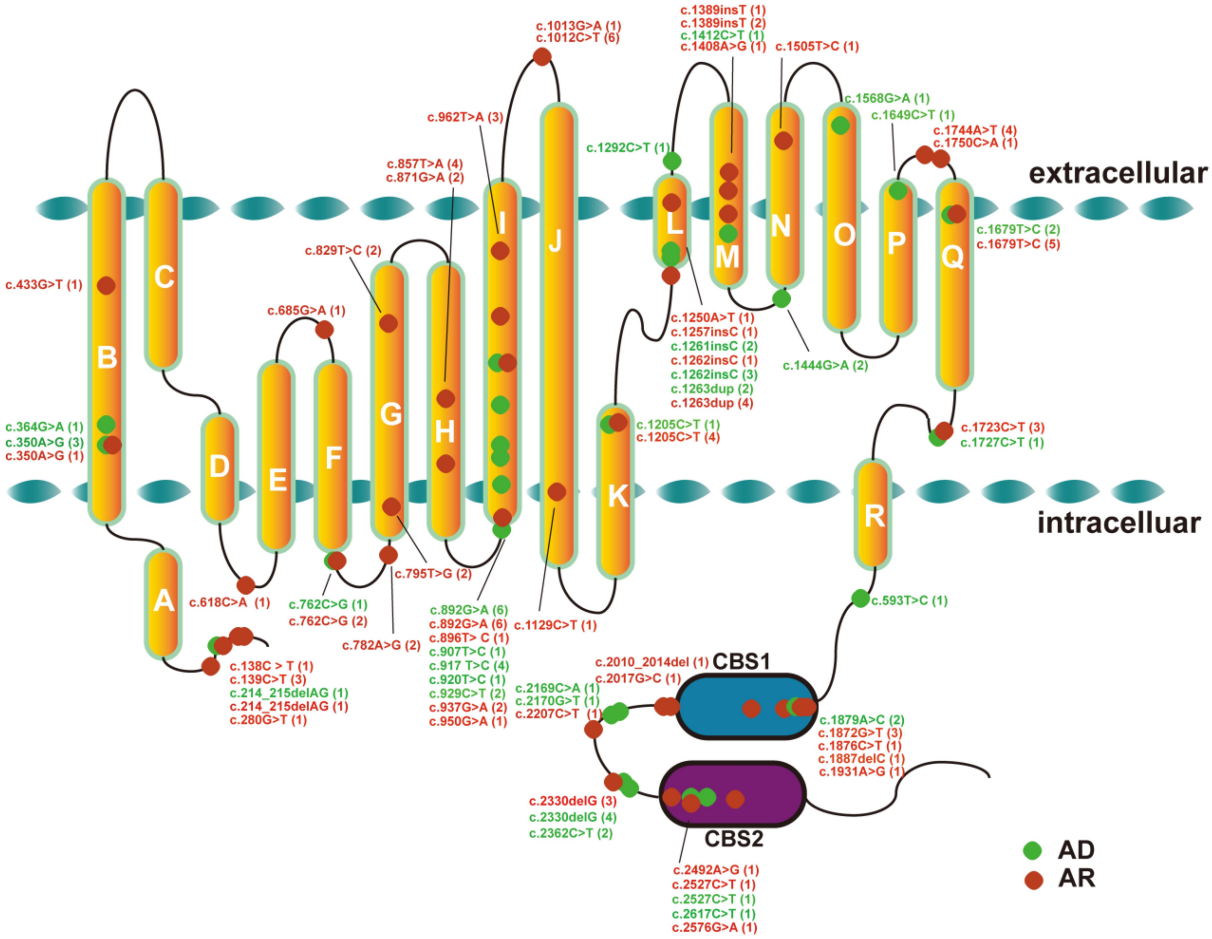

Figure 5. The variant distribution on the topology of chloride channel. The overall topology of chloride channel includes 17 helices (αB to αR), 2 cystathionine β-synthase domains. Numbers represented the frequency of variants reported in Chinese MC patients. The green fonts standed for dominantvariants, the red fonts standed for recessive variants.

hotspot variants included c.892 G>A (p. Ala298Thr) with an allele frequency of 12, c.1679T>C (p.M560T) with an allele frequency of 7, and c.2330delG (p.G777fs) with an allele frequency of 7. The framshift variant of p.R421fs was identified in 7 patients, including c.1261insC in 2 patients, c.1262insC in 4 patients, and c.1258insC in one patient. Of the reported variants, 140 were located on exons, of which 25.52% (37/145) were located on exon 8, 11.03% (16/145) on exon 12, 11.72% (17/145) on exon 15, and 6.21% (9/145) on exon 19 (Figure 4c). Therefore, the hotspot regions

of Chinese MC patients included the exon 8, 12, 15, and 19 of the *CLCN1* gene. Notably, both exon 8 and 12 served as hotspot regions for AD and AR inheritance, while exon 19 and exon 15are hotspot regions for AD and AR inheritance, respectively. Additionally, variants on exon 1, 7, 9, 10, 18 and 21 were exclusively found in patients with AR inheritance (Figure 4c).

Discussion

Myotonia congenita is the most common type of non-dystrophic myotonic disorder, originating from a genetic defect of the CLCN1 channel associated with skeletal muscle membrane excitability. Clinical and genetic characteristics of MC have been extensively documented in several large case series in Western countries [13–16]. However, the number of MC cases in China remains limited. In this study, we summarized the clinical and genetic characteristics of 11 newly identified Chinese patients, in addition to 87 cases previously reported in Chinese individuals with MC. Our study revealed six novel variants, including c.-52A>G, c.364 G>A, c.962T>A, c.1258insC, c.1505T>C, and c.2170 G>T, thereby broadening the genetic spectrum of MC patients in the Chinese population.

In Chinese MC patients, no substantial disparities were observed in the severity of myotonia and muscle weakness between dominant TMC and recessive BMC. It is worth noting that the median age of onset in our patients was slightly elder than that reported in previous Chinese patient studies [17–19]. The age of onset was extremely variable in MC patients, although typically BMC was considered to manifest later than TMC [5,8,13,14]. Interestingly, our findings suggest that the age of onset does not effectively differentiate between BMC and TMC in Chinese patients, indicating that the two phenotypes cannot be distinguished based on the onset age. Early studies suggested no gender discrepancy in the incidence of both dominant and recessive inheritance [20]. However, Nevertheless, an increasing number of studies, including our own, have indicated that MC was more prevalent in males than females [5,8,9,14,15]. There was significant in gender preference, with the 98 MC probands comprising 70 male and 28 female patients in this study. A similar finding, albeit in a smaller cohort, was recently reported in Chinese patients [17–19]. Intriguingly, female patients often experienced exacerbated myotonia symptoms during pregnancy or menstruation, indicating potential influences of sex hormones on chloride channel conduction and contributing to the observed gender disproportion [21,22].

According to the specific pattern of muscle involvement, muscle MRI changes have been demonstrated to help distinguish various myopathies [23]. However, there were limited studies on muscle MRI of *CLCN1*-related myotonia [24,25] partly due to the limited value of muscle MRI to channelopathy. In this study, muscle MRI in MC patients at the onset stage exhibited no apparent abnormalities. Conversely, Morrow et al. found that 10 out of 11 MC patients with mean age of 45 years had a central stripe of STIR hyperintensity in the medial gastrocnemius [26], which might be an additional aid to guiding genetic testing. It indicated that long-term muscle MRI could potentially monitor the trend of MC patients toward myopathy. As a muscle channelopathy, most MC patients had relatively normal changes of the muscle pathology [19]. Our patients also confirmed that the muscle biopsy of MC had mild nonspecific changes such as fiber atrophy, necrosis, and degeneration, while the dominant Case 3 of this study showed atypical tubular aggregation and vacuolar change, which had pathological diagnostic values to channelopathy. It should be noted that some patients with MC could show a phenotype of myopathy with myopathic changes in muscle biopsy, yet there was no apparent distinction between TMC and BMC in Chinese patients [2,27–29]. Therefore, muscle biopsy had little diagnostic significance for distinguishing between dominant TMC and recessive BMC.

In the majority of Chinese MC patients, myotonic discharge on EMG remained the most common electrodiagnostic finding, despite the simultaneous presence of myopathic changes in some patients. Only a few adult patients might show negative myotonic discharge on EMG due to the recovery of myotonia with age, indicating that EMG was the most valuable test for the diagnosis of MC. Latent myotonia, characterized by subclinical electrophysiological positivity, could

be detected in some individuals with heterozygous missense variants [30,31]. In this study, some asymptomatic individuals with TMC heterozygous variants had subclinical myotonic discharges. This indicated that latent myotonia in electrophysiological examinations could help detect subclinical myotonic signs and partially explain the low clinical expressivity observed in members of TMC families, despite seemingly negative family histories.

The variant p.Ala298Thr was not found in our 11 patients, while it still was the most common variant in the Chinese and Japanese patients [8]. Another common variant was p.R421fs, which was caused by several framshift variants with c.1258insC, c.1261insC, and c.1262insC, indicating a hotspot variant region in the exon 12 of *CLCN1* due to unsteady duplicated cytosine bases. Almost all causative MC variants were located at exons or splice sites, while c.-52A>G variant was located at the far away upstream of starting codon, with only one similar c.-59C>A variant but without functional investigation was reported in another MC family [32]. Transcription experiment revealed that c.-52A>G variant caused a severe decrease in *CLCN1* mRNA level, we suppose that this variant might affect the transcriptional regulatory element of *CLCN1*. Therefore, this study identified the first variant located within transcriptional regulatory element in MC patient.

Traditionally, TMC has been attributed to a heterozygous variant on one allele, while biallelic heterozygous variants inherited from both parents have been associated with BMC patients [2]. The CLCN1 chloride channel protein consisted of a homodimer structure, with each subunit containing its own pore [33,34]. Previous research indicated that TMC affected half of the subunits, whereas BMC impacted all subunits, potentially explaining the more severe phenotype of the latter [28,35,36]. However, our study revealed at least 10 variants that might lead to dominant TMC in some families and recessive BMC in others. This suggested that distinguishing between TMC and BMC based solely on variant types was unreliable. Of note, the dominant variant might exert a dominant negative effect [37], and could potentially be associated with reduced penetrance or incomplete dominance [3,38], which had also been observed in other Chinese MC patients with latent myotonia [39]. Nevertheless, it cannot be excluded that a second deep intronic variant or small deletion may be missed in patients with a heterozygous state due to technical limitation, potentially resulting in a false pattern of dominant inheritance.

In summary, this study expanded the clinical and genetic spectrum in Chinese MC patients. Compared to muscle MRI and biopsy, EMG was the most valuable examination for the diagnosis of MC. Conducting EMG on asymptomatic family members for latent myotonia not only helped to investigate the family history, but also aided to determine genetic pathogenicity. Importantly, this study suggested that distinguishing between TMC and BMC based on clinical and laboratory characteristics was challenging. Considering that some variants could cause dominant TMC in some families and recessive BMC in others, it was also unreliable to distinguish between TMC and BMC based on the inherited patterns and variant types.

Acknowledgments

We thank the families and control individuals for their cooperation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Natural Science Foundation of China (82160252 and 82271439), Jiangxi Provincial Natural Science Foundation (20202BAB206029, 20232BAB206064 and 20224ACB206015), Double thousand talents program of Jiangxi province (jxsq2019101021).

Ethics approval

The research was approved by the ethics committee of the first affiliated hospital of Nanchang university ((2023) CDYFYYLK (02–055)).

Author contributions

DJH and DDT conceptualized the study. YTH contributed to data collection, analysis and drafting of the manuscript. YSQ conducted the genetic experiments and analyzed the

data. YX, YS, KYJ, HCY, PCH, YZ, MZ and MHZ participated in clinical data acquisition and analysis. All authors revised the manuscript and approved the final version.

Consent for publication

All the patients or their guardians had signed informed consent to publish these details.

Data availability statement

All relevant data are within the paper and its Supplemental files.

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