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Non-dystrophic myotonia Chilean cohort with predominance of the *SCN4A* Gly1306Glu variant

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Abstract

Non-dystrophic myotonias are a group of rare neuromuscular diseases linked to *SCN4A* or *CLCN1*. Among the subtypes, myotonia permanens, associated with the Gly1306Glu variant of *SCN4A*, is a relatively less frequent but more severe form. Most reports of non-dystrophic myotonias describe European populations. Therefore, to expand the genetic and phenotypic spectrum of this disorder, we evaluated 30 Chilean patients with non-dystrophic myotonias for associated variants and clinical characteristics. *SCN4A* variants were observed in 28 (93%) of patients, including 25 (83%) with myotonia permanens due to the Gly1306Glu variant. Myotonia permanens was inherited in 24 (96%) patients; the mean age of onset was 6 months, and the initial symptoms were orbicularis oculi myotonia in 17 (74%) patients and larynx myotonia in 12 (52%) patients. The extraocular muscles were involved in 11 (44%) patients, upper limbs in 20 (80%), and lower limbs in 21 (84%). Thirteen (52%) patients experienced recurrent pain and 10 (40%) patients reported limitations in daily life activities. Carbamazepine reduced myotonia in eight treated patients. The high frequency of the Gly1306Glu variant in *SCN4A* in Chilean patients suggests a founder effect and expands its phenotypic spectrum.

Keywords: Myotonia Congenita; Founder Effect; Apnoea; Strabismus; Laryngospasm; Carbamazepine.

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1. Introduction

Non-dystrophic myotonias are muscle channelopathies caused by pathogenic variants in *CLCN1* or *SCN4A*. The specific phenotype or subtype, treatment, and inheritance pattern of non-dystrophic myotonias depend on the involved gene and pathogenic variant of the same gene [1–3].

Patients with non-dystrophic myotonias typically experience a reduced quality of life, including disability and difficulty finding a job, although the severity tends to be lower in patients with *CLCN1* variants compared to in those whose disease is caused by *SCN4A* variants [3]. In addition, some younger patients exhibit severe neonatal episodic laryngospasm (SNEL), which is linked to specific *SCN4A* variants [4]. Differential diagnosis is based on clinical and genetic evaluation. An electrophysiology test, the Repeated Short Exercise Test (RSET), allows the identification of Fournier patterns I–III, which can help to distinguish between *SCN4A* or *CLCN1* variants and even among different variants of the same gene. Fournier patterns are defined by variations in the compound muscle action potential amplitude of the abductor digiti minimi muscle after a set of three isometric exercises in this muscle at room temperature and after cooling. Pattern I is characterized by a progressive decrease in the compound muscle action potential amplitude after each exercise that does not recover after a period of rest; pattern II by a decrease immediately after exercise that recovers after a period of rest; and pattern III by no significant decrement. Cold temperatures may aggravate or unmask patterns I–II. Fournier patterns I and III are typically associated with *SCN4A*, whereas Fournier pattern II is more commonly associated with variants in *CLCN1* [5].

Myotonia permanens is among the most severe phenotypes of non-dystrophic myotonia, which is associated with the Gly1306Glu variant of *SCN4A*, accounting for 0.6% of all cases [6]. Myotonia permanens is a rare entity with only sporadic case reports and a recent report of a 10-patient cohort published to date, demonstrating features of severe disease, high mortality, and frequent SNEL in European patients [7].

To further understand this disease entity on a global scale, we characterized the clinical, electrophysiological, and genetic findings from a Chilean cohort of adult and paediatric patients with non-dystrophic myotonias and evaluated the predictive value of the RSET in these patients. We aimed to determine the mutational spectrum of our population and their associated phenotypes to provide diagnosis, treatment, and prognosis that is more specific, in clinical practice in our patients and other populations. We observed a striking predominance of the Gly1306Glu variant in *SCN4A* and describe the natural history and specific phenotypic findings of the largest cohort of patients carrying this variant published to date to contribute to clinical management of affected patients.

2. Patients and methods

2.1. Patient selection and inclusion/exclusion criteria

Patients with non-dystrophic myotonia diagnosed by an experienced neuromuscular diseases child neurologist, with available electromyography (EMG) results showing myotonic discharges and RSET performed in at least one family member were recruited for this study from three paediatric neuromuscular disorders reference centres in Chile between January 2013 and June 2019, including Complejo Asistencial Dr. Sótero del Río, Hospital de Niños Roberto del Río, and Red de Salud UC-Christus. The pedigree of each patient was obtained and involved family members were invited to participate in the study during consultation. Clinical, electrophysiological, and genetic evaluations were performed. Patients who could not cooperate in relaxation time (RT) assessments because of difficulty in understanding commands or because they were experiencing severe discomfort with evoked myotonias were excluded from the RT study. In addition, patients with a medical condition impeding electromyography or those refusing to undergo electromyography were excluded from this test. Patients younger than 5 years or older than 45 years of age were also excluded from the electromyography study because of difficulties in following commands and the higher prevalence of comorbidities, respectively.

This study was approved by the Ethics Committees of the three sites (Comité Etico Científico del Servicio de Salud Metropolitano Sur Oriente, Comité de Etica de la Investigación del Servicio de Salud Metropolitano Norte/Comité de Coordinación de Investigación del Hospital de Niños Roberto del Río, and Comité Etico Científico de la Facultad de Medicina de la Pontificia Universidad Católica de Chile). Informed consent was obtained from all patients or family members if the patients were minors.

2.2. Clinical evaluation

Medical records were reviewed, and disease history and physical examination data were obtained (Table 1). We grouped myotonia according to its localization, as follows: extraocular, laryngeal, upper (arm, forearm, or hand) or lower limbs (thigh, leg, or foot). Upper or lower limbs included any muscle or muscle group in the corresponding limb (one or more muscles could be involved). We also observed for night or nocturnal myotonia, which refers to those events that were severe enough to wake up the patient from sleep at night. The height of the patients was measured, and the percentile was calculated according to Centre of Disease Control growth charts.

Table 1
Demographic characteristics, first symptoms, and disease course in 30 patients with non-dystrophic myotonia according to the pathogenic variant.

	SCN4A		*CLCN1*		Total
	Gly1306Glu	Ile693Thr	Gly188Val/Glu788Pro	Intron 10	
Demographic characteristics					
Patients, n	25	3	1	1	30
Families, n	10	1	1	1	13
Inheritance, AD/AR	25/0	3/0	1/0	0/1	29/0
Age at evaluation, years (median, IQR)	19, 12–37	23, 7–54	13	20	20, 13–36
Age at EMG, years (median, IQR)	17, 12–30	38	7.9	20	19, 12–30
Gender F/M, *n*	16/9	1/2	0/1	0/1	17/13
Height percentile (median, IQR)	31, 17–85	21, 16–36	7	40	37, 15–72
Point prevalence	0.81	0.09	0.03	0.03	0.97
First symptoms					
First symptoms, months (mean, range)	6, 0–12	20, 0–36	60	108	15, 0–108
Gait acquisition, months (mean, range)	13, 10–18	11, 10–12	13	12	12, 12–18
First myotonia localization, *n* (%)					
• *Eyelids*	17 (74)	3 (100)	0	0	20 (71)
• *Extraocular muscles*	1 (4)	0	0	0	1 (3)
• *Larynx*	12 (52)	0	0	0	12 (43)
• *Superior limbs*	2 (9)	0	0		2 (7)
• *Inferior limbs*	4 (17)	1 (33)	1 (100)	1 (100)	7 (25)
Symptoms and signs during disease course					
Strabismus[a], *n* (%)	11 (44)	0	0	0	11 (36)
Apnoea/dyspnoea, *n* (%)	16 (64)	1 (33)	0	0	17 (56)
Superior limbs[a], *n* (%)	20 (80)	3 (100)	1 (100)	1 (100)	26 (86)
Inferior limbs[a], *n* (%)	21 (84)	3 (100)	1 (100)	1 (100)	25 (83)
Episodic muscle weakness[a], *n* (%)	0	0	0	1	1
Nocturnal myotonia, *n* (%)	16 (89)	2 (100)	0	0	18 (78)
Triggering factors, *n* (%)					
• *Cold*	24 (98)	3 (100)	1	0	28 (93)
• *Exercise*	20 (80)	2 (67)	1	1	24 (80)
• *K+ rich food*	9 (36)	2 (67)	0	0	11 (37)
• *Anaesthesia related complications*	3/7* (43)	0	0	0	3/7[b]
CK at baseline, IU/L (median, IQR; $n=7$)	1045, 489–2395	UA	UA	UA	1045, 489–2395

[a] These findings were observed in clinical examination, other symptoms were referred by patients
[b] $n=7$
UA, unavailable; IQR, interquartile range; CK, creatine kinase; EMG, electromyography; AD, autosomal dominant; AR, autosomal recessive.

2.3. RT

The percussion- and action-myotonia RT was measured in several muscles/muscle groups for 16 patients. Patients were seated on an examination table with legs resting on a footstool. Both sides were evaluated, except for the tongue, and the average of both sides was calculated. Percussion-induced myotonia was evaluated in the tongue, deltoids, biceps, triceps, anterior forearm, posterior forearm, thenar/hipothenar eminence, quadriceps, hamstring muscles, tibialis anterior, and gastrocnemius using a reflex hammer. Action-myotonia after 10 s of maximum isometric voluntary contraction was manually evaluated in the same muscles (except for the tongue), along with the orbicularis oculi, hand flexor muscles, and iliopsoas. Orbicularis oculi and hand flexors were evaluated after forced closure. Patients were asked to rapidly relax the evaluated muscle at the end of the 10 s contraction. The time from percussion or from the end of voluntary contraction to a fully relaxed muscle was determined using a digital stopwatch. We obtained the mean RT after 10 s of exercise of all the above-mentioned muscles (all_muscles), orbicularis oculi, and of all muscles excluding the orbicularis oculi (non_orbicularis) in 16 patients. Two patients were under antimyotonic treatment; thus they were excluded from RT analysis. Paradoxical action myotonia was not systematically tested given that it was poorly tolerated due to the long RT of most subjects in our cohort.

2.4. Electrophysiology evaluation

Needle electromyography was performed in the tibialis anterior or first dorsal interosseous muscle with a concentric disposable 30-G needle using a Cadwell Sierra® Summit™ (Kennewick, WA, USA) or Cadwell Sierra® Wave™ (Seattle, WA, USA) machine. Two insertions were made in each muscle with 30 s evaluation per insertion [8]. In contrast to other protocols designed to evaluate adults that perform 10 insertions [9], we minimized the number of insertions because our group included minors who would not be able to tolerate a long-duration evaluation. If myotonic discharges were present, an RSET was performed at the ulnar nerve and abductor digiti minimi at room temperature in one hand and

after cooling with an ice pack for 10 min in the opposite hand. We classified the RSET patterns as I, II, and III as described by Fournier [5].

2.5. Genetic analysis

Variants in *SCN4* or *CLCN1* were detected according to the determined RSET pattern in the index case. Between January 2013 and December 2017, genetic tests were performed at Unité de Cardiogénétique et Myogénétique, Groupe Hospitalier Pitié-Salpêtrière, Assistance Publique Hôpitaux de Paris, France. Ten of the 24 coding exons of *SCN4A* were analysed if Fournier pattern I or III was observed (exons 5, 9, 12, 13, 18, 19, 21–24). If Fournier pattern II was present, the 23 *CLCN1* coding exons were sequenced [5]. If no pathogenic variants were observed in the first analysis, the alternative gene was sequenced.

Given the high frequency of a pathogenic variant in exon 22 of *SCN4A* detected in our cohort, from January 2018 to June 2019, if Fournier pattern I or III was observed, only this exon was sequenced. If negative, or if Fournier pattern II was detected, *SCN4A* and *CLCN1* full-gene sequencing was performed in a commercial laboratory. After the index case variant was obtained, it was searched in the family members.

2.6. Statistical analysis

Clinical characteristics are presented as the mean and standard deviation (SD) or median and interquartile range (IQR).

3. Results

3.1. General clinical and genetic evaluation

We recruited 30 patients from 13 different families, including 17 females and 13 males with a median age of 20 years (IQR = 13–36 years). There was a high variability in these characteristics according to the involved gene and according to variants within each affected gene (Table 1).

Most patients (n = 28, 93%) had pathogenic variants in *SCN4A*, with only two patients (7%) harbouring a variant in *CLCN1*. We found two different pathogenic variants in *SCN4A*, both of which showed an autosomal dominant inheritance pattern. The Gly1306Glu variant in *SCN4A* was by far the most frequent variant detected (n = 25, 83%) (Table 1).

All families except for one were of Chilean ancestry up to the third generation. The exception was a family harbouring the Gly1306Glu variant, whose first affected ancestry four generations prior was of Italian origin.

3.2. Electrophysiology

Twenty-four patients, including at least one from each family, were evaluated by needle EMG and RSET at a median age of 19 years (12–30 years); 23 of these patients underwent electrophysiology examination before genetic testing, and the other patient was confirmed to harbour the *SCN4A* Gly1306Glu variant prior to the electrophysiology test. All patients showed frequent myotonic discharges and all of those with Gly1306Glu variants had permanent myotonic discharges. Patients with *CLCN1* and the Ile693Thr variant of *SCN4A* had periods of silence at rest (5–10 s). Both patients with *CLCN1* variants and one patient with Gly1306Glu in *SCN4A* showed Fournier pattern II; the other 22 patients with *SCN4A* variants showed Fournier pattern III. Excluding the patient who had previous genetic results, we obtained a 100% positive predictive value and 66% negative predictive value for the association of Fournier pattern III with *SCN4A* variants. We obtained pattern II mirror values for patients with *CLCN1* variants.

3.3. SCN4A

3.3.1. Gly1306Glu variant

For patients harbouring the *SCN4A* c.3917G>A, p.Gly1306Glu variant, the age at symptom onset varied from a few days to the first decade of life, but symptoms manifested in the first year of life in most cases (median = 6 months, IQR = 0–12 months). The most frequent symptoms at onset were orbicularis oculi (74%) and laryngeal (52%) muscles myotonia, leading to apnoea episodes. Laryngeal muscles myotonia was triggered by crying in infants and small children, but it was also spontaneous during sleep. In older children and adults, it was triggered by drinking cold drinks, eating ice cream, coughing, or yawning. It was more frequent during upper respiratory tract infections. Typical episodes of larynx muscles myotonia presented with apnoea and sometimes perioral cyanosis, and they lasted several seconds and were self-limited. None of them led to unconsciousness. Myotonia evolved over time, involving the upper (80%) and lower (84%) limbs as well as the extraocular muscles (44%).

Recurrent pain associated with myotonia episodes was observed in 52% (n = 13) of patients. Myotonia limited daily life activities in 40% (n = 10) of patients. Among them, school-aged children were impeded from fully participating in gymnastic classes and had difficulty with handwriting in their notebooks. Among the adults, manual jobs such as cooking, typing, driving, and holding heavy objects were affected, both in personal and professional activities.

There were no deaths during the study period. In addition, there were no myotonia-related deaths in infants in other non-evaluated family members. No patients had severe SNEL requiring hospitalization, oxygen, or positive-pressure ventilation. Seven patients underwent surgery with general anaesthesia, without malignant hyperthermia precautions, as the disease had not been diagnosed at surgery; three of these patients experienced side effects.

Seven of the female patients had children. Myotonia was more frequent during pregnancy, but there were no further complications, and delivery was also normal. In addition to myotonia and myotonia-related symptoms, the babies did not

Table 2
Relaxation time after 10 s of exercise in 14 non-treated patients with non-dystrophic myotonia.

	Relaxation time [mean (SD), seconds]			
	SCN4A		*CLCN1*	
	Gly1306Glu	Ile693Thr	Gly188Val/ Gln788Pro	Intron 10
All_muscles	13.9 (20.0)	5.2 (15.8)	2 (4.2)	2.7 (2.6)
Orbicularis oculi	73.3 (37.7)	55 (NA)	10 (NA)	5 (NA)
Non_orbicularis	8.5 (7.7)	0.6 (2.2)	1.1 (3.3)	2.5 (2.6)

NA, non-aplicable

have other diseases or complications. One patient who had very frequent episodes of myotonia even before pregnancy was treated with carbamazepine during pregnancy, but no teratogenic adverse events were observed in her child.

At examination, the height was within the normal range (median = 31st percentile, IQR = 17th–85th percentiles). Most patients had an athletic body type or mild generalized muscle hypertrophy, but no severe hypertrophy of specific muscle groups was observed. Percussion-induced myotonia was more frequent in the tongue, deltoids, thenar and hypothenar eminence, and ankle extensors, with an RT of less than 10 s at most sites. No patients showed percussion-induced myotonia of the hamstring muscles (Fig. S1). Action-myotonia was widely distributed in different muscle groups and was more prominent in the orbicularis oculi, with a mean RT of 73.3 s in this muscle; however, the iliopsoas was spared (Fig. S2, Table 2).

These patients were mainly treated with carbamazepine or acetazolamide. Carbamazepine reduced myotonia in all eight treated patients but was interrupted in one patient because of excessive daytime somnolence. Acetazolamide was effective in four of the six treated patients, two of whom experienced adverse effects (Table 3).

3.3.2. Ile693Thr variant

Patients harbouring the c.2078T>C, p.Ile693Thr variant of *SCN4A* experienced the first symptoms of disease in early childhood (mean age = 20 months, IQR = 0–36 months) as orbicularis oculi myotonia in all cases (100%). Subsequently, laryngeal (33%) and limbs myotonia (100%) were observed, and no patients showed extraocular muscles involvement. Two patients had frequent falls due to transient weakness in lower limbs, and one had paramyotonia (myotonia augmented by exercise). At clinical examination, percussion-induced myotonia was localized in the thenar eminence, and action-myotonia was observed in the orbicularis oculi in one patient, whereas the other patient showed a more widespread pattern of action-myotonia (Table 2, Figures S1 and S2). One patient was treated with acetazolamide, but the symptoms persisted.

3.4. CLCN1

Two patients from two different families harboured *CLCN1* variants. Myotonia started in the lower limbs in the first decade of life (mean age = 17 years, IQR = 13–20 years), and neither patient showed extraocular muscles or laryngeal involvement. Percussion-induced myotonia was observed in the thenar and hypothenar eminences, and action-myotonia was observed in the orbicularis oculi and intrinsic hand muscles in both patients with a mean RT of all_muscles of 2 and 2.7 s in each patient (Table 2, Figs. S1 and S2).

One patient showed an autosomal dominant inheritance pattern according to the pedigree. He harboured two heterozygote variants (c.563G>T, p.Gly188Val and c.2363A>C, p.Gln788Pro), but his parents refused clinical and genetic evaluations. In his family, the men were more severely affected than the women who were symptomatic only during pregnancy. The other patient showed an autosomal recessive inheritance pattern (c.1167-10C>T, intronic homozygous variant). In addition to myotonia, he experienced brief muscle weakness when starting movements (Table 1). The first patient was treated with carbamazepine, which decreased the incidence of myotonias, and the other patient was treated with acetazolamide, but his symptoms did not improve.

4. Discussion

We present the clinical and genetic characteristics of a cohort of patients with non-dystrophic myotonia in a South American (Chilean) population, with a high predominance of *SCN4A*-related cases, mostly comprising cases of myotonia permanens linked to the *SCN4A* Gly1306Glu variant. This is the largest myotonia permanens cohort linked to Gly1306Glu reported to date, and its high frequency suggest a founder effect in this population.

In our cohort, disease onset in patients with the *SCN4A* Gly1306Glu variant occurred during the first months of life or even in the neonatal period, with prominent orbicularis oculi or laryngeal muscles myotonia, and frequent limbs, orbicularis oculi, extraocular muscles, and night myotonias observed during the disease course. Patients with the *SCN4A* Ile693Thr variant had similar symptoms but no extraocular muscle involvement. In the two patients with a *CLCN1* variant, symptom onset occurred later in life, mostly in the limbs, and there were no night myotonias and no extraocular muscles or pharyngeal involvement. An earlier onset has previously been described in patients with *SCN4A* rather than *CLCN1* variants [3]. Extraocular muscles involvement has also been described in patients with congenital myotonia specifically linked to *SCN4A* variants [10,11], and the Ile693Thr variant in *SCN4A* has previously been observed in patients with periodic paralysis and paramyotonia congenita [12].

Myotonia permanens linked to the sodium channel was first described by Lerche et al. [13] in 1993 in severely compromised patients with respiratory involvement. In 2006, Cølding-Jorgensen et al. [14] reported two Chilean patients residing in Denmark belonging to an extended family with myotonia permanens showing a milder phenotype. Several cases of SNEL associated with the Gly1306Glu variant have also been reported [4,11]. Recently, a European cohort

Table 3
Pharmacologic treatment in patients with non-dystrophic myotonia.

		SCN4A		*CLCN1*	
		G1306E	I693T	Gly188Val/Gln788Pro	Intron10
Carbamazepine, $n=9$	Daily dose, mg (median, IQR)	200, 200–300		400	
	Effectivity, *n* (%)	8 (100)		1 (100)	
	Adverse effects, *n* (%)	1 (12)		1 (100)	
Acetazolamide, $n=8$	Daily dose, mg (median, IQR)	250, 125–1062[a]	80[b]		UK
	Effectivity, *n* (%)	4 (66)	0 (0)		1 (100)
	Adverse effects, *n* (%)	2 (33)	1 (100)		0 (0)
Phenytoin, $n=1$	Daily dose, mg	UK			
	Effectivity, *n* (%)	1 (100%)			
	Adverse effects, *n* (%)	0 (0)			
Mexiletine, $n=1$	Daily dose, mg	400			
	Effectivity, *n* (%)	1 (100%)			
	Adverse effects, *n* (%)	0 (0)			

IQR, interquartile range; UK, unknown
[a] One paediatric patient was excluded from the dose calculation
[b] Paediatric patient treated with a 5 mg/kg daily dose

of 10 non-related patients with myotonia permanens was described [7]. Although similar in many aspects to those described in the European population [7], our patients showed a milder phenotype, closer to those of patients described in the first Chilean family [14]. Moreover, in contrast to previously reported series, our patients did not experience severe SNEL episodes, although laryngeal myotonia, leading to apnoea episodes, was frequent in infants. None of our patients or their relatives died of myotonia or anaesthetic complications, although adverse reactions to anaesthesia were observed in nearly half of our patients. Extraocular muscles involvement was quite frequent in contrast to previous reports in these patients. Although it is currently unclear whether these differences are due to genetic or environmental factors, a genetic modifier appears more likely given that the Chilean patients residing in Europe also showed a milder phenotype compared to those reported for other European patients [14]. A polymorphism or modifier gene may be a protective or aggravating factor. Thus, more in-depth genetic analysis such as whole-gene, exome, or genome sequencing is needed to explain this difference.

Recurrent episodes of myotonia-associated pain and limitation in daily life activities, at school, and at work were observed in nearly half of the children and adults with myotonia permanens. Unemployment because of the disease has previously been reported in 3% and 14% of patients with *CLCN1*- and *SCN4A*-associated myotonias, and disability was reported in 6% and 23% of cases, respectively [3].

Pregnancy in patients with myotonia permanens was previously described in only one patient to our knowledge [7]. In our cohort, seven women went through pregnancy and delivery, describing augmentation in the severity of myotonia but no further complications in the mother or child. Similarly, aggravation of myotonia episodes with no obstetric or neonatal complications was previously described in women with other forms of *SCN4A* or *CLCN1*-related myotonias [15].

One patient with congenital myotonia had an autosomal dominant inheritance according to his pedigree but he had two pathogenic variants in *CLCN1* (Gly188Val and Gln788Pro). His parents refused genetic evaluation, and therefore, the phase of these variants is unknown. The Gly188Val [16] and Gln788Pro [17] variants have been observed in Spanish patients with autosomal recessive myotonia congenita.

With respect to the RT, percussion-induced myotonia was longer in the tongue, thenar and hyptothenar eminences, and gastrocnemius, sparing the hamstring muscles, and was typically less than 10 s. There was no evident association of RT with the genetic variant. We found no reports of RT after percussion in different genetic groups, although the above-mentioned regions are typically most commonly affected in both myotonic dystrophy and non-dystrophic myotonias [18]. RT after 10 s of exercise was found to be longer in all_muscles in the myotonia permanens group. Action-myotonia was also longer in the orbicularis oculi than in non_orbicularis in all genetic variants. Myotonia RT after 3 or 5 s of exercise is generally measured in the orbicularis oculi and hand flexors, and RT after 5 s of exercise is currently used as an outcome measure in clinical trials [19,20]. However, no studies have reported the RT of other muscle groups or RT after 10 s of exercise. A previous study showed that although orbicularis oculi myotonia is present in patients with either *CLCN1* or *SCN4A* variants, the RT is significantly longer in the latter group [21]. Moreover, our patients showed a longer RT in the orbicularis oculi than that previously reported, possibly because of the longer duration of the exercise test (10 vs. 5 s) or may reflect a longer RT in patients with myotonia permanens [21]. We performed a manual clinical evaluation with intrinsic limitations in comparison to other methods such as a hand-grip myometer, which is able to quantify RT precisely [22].

The creatine kinase (CK) level was elevated by 10-fold in seven patients with myotonia permanens. Although CK may be generally elevated in patients with non-dystrophic

myotonias, it is typically no more than 2-fold the normal value in patients with *CLCN1* variants and slightly higher in patients with *SCNA4* variants [23]. In a previously reported series, 20–25% of patients in the *SCNA4* variant group showed elevated CK [24,25]. In contrast, all patients with myotonia permanens in the present cohort had elevated CK levels, similar to the two Chilean patients previously reported [14]. However, the CK level was not reported for previous series of patients with myotonia permanens [7].

From an electrophysiology perspective, continuous or permanent myotonic activity was observed, which was used to define myotonia permanens. It is uncertain if patients with other types of non-dystrophic myotonia might also have permanent myotonic discharges; accordingly, we have considered that genetic tests should always be performed to confirm the diagnosis of myotonia permanens. Pattern III in RSET showed excellent (100%) positive predictive value for *SCN4*, but a poor negative predictive value with mirror values for pattern II and *CLCN1*. This is likely because of the high prevalence of *SCN4A* variants in this population.

With respect to treatment, both acetazolamide and carbamazepine reduced the severity of clinical myotonia, although carbamazepine was clearly more effective and was better tolerated by our patients in all genetic groups, including those with myotonia permanens. Phenytoin was also used for two patients, which reduced myotonia, and mexiletine was used for one patient. Treatment of myotonia permanens may be challenging in some patients. Acetazolamide [14] and flecainide [7] have been described as effective antimyotonic medications for this disorder, but a recent report showed that flecainide possibly triggered Brugada syndrome in one patient, which is a severe side effect that may limit its use in patients with myotonia permanens [26]. Therefore, carbamazepine may be considered a useful alternative. We were able to use mexiletine only in one patient, given that this treatment is not available in our country. We do not know if mexiletine, the level-1 treatment of non-dystrophic myotonia [9,19], might be more or as effective as carbamazepine in this sub-group of patients.

The main study limitations were that the history was obtained retrospectively, including data from several years ago. Additionally, the group with *CLCN1* variants was small, preventing broader statistical comparison between the clinical characteristics of the different genetic groups. Additionally, we did not always sequence the whole genes to identify other modifying variants and whole-exome sequencing to identify modifying genes was not performed. The efficacy of pharmacologic treatment was assessed only by patients' reports and clinical examination, lacking objective and blinded evaluation. Finally, life quality and functional scales could not be used to further explore the impact of the disease in our patients.

We present a large cohort of patients with non-dystrophic myotonias with a predominance of *SCN4A* Gly1306Glu myotonia permanens as a subgroup, demonstrating a possible founder effect. Our patients showed frequent strabismus, but the symptoms were less severe than those described in most previous reports, and most patients responded well to antimyotonic drugs, particularly carbamazepine. Our findings expand the phenotypic spectrum of myotonia permanens associated with the Gly1306Glu variant in *SCN4A*.

A prospective natural history study, including other family members, life expectancy, severe complications such as SNEL and anaesthesia side effects, and their management may further clarify the phenotype and long-term prognosis of myotonia permanens. Exploring specific pharmacological treatments in this unique homogeneous cohort of patients with non-dystrophic myotonia through a randomized controlled trial may clarify the efficacy of different agents, particularly that of carbamazepine. The individual treatment response to different anti-myotonic treatments could be investigated using multiple cross-over periods in an aggregated N-of-1 trials, an innovative design that has proved to be efficient in assessing the treatment of non-dystrophic myotonia [9]. Evaluating the prevalence of myotonia permanens in other South American countries to determine the broader founder effect of Gly1306Glu in *SCN4A* or for other frequent variants will also improve the understanding and lead to specific care of patients with non-dystrophic myotonia.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2020.04.006.

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