Phenotypic Variability of Autosomal Dominant Myotonia Congenita in a Taiwanese Family with Muscle Chloride Channel (CLCN1) Mutation

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Abstract-

Background: Myotonia congenita (MC), whether inherited in autosomal dominant or recessive form, is caused by mutation of CLCN1 on chromosome 7 and associated with impaired skeletal muscle relaxation after contraction. The basic pathophysiology is the reduction of chloride conductance in skeletal muscles caused by various molecular mechanisms. The cause of the wide phenotypic variability in both dominant and recessive MC remains unclear.

Methods: A family clinically diagnosed with autosomal dominant myotonia congenita was enrolled. Three family members underwent a detailed neurological examination, eletromyography, and genetic analysis.

Results: A G230E mutation of CLCN1 was confirmed in these three family members. One of them was completely asymptomatic and the electromyographic studies were normal. A great variability of clinical presentation was found in these family members with MC.

Conclusions: We report the first autosomal dominant MC family with G230E mutation in oriental countries. Most of the previously reported MC families with G230E mutation were autosomal dominant pedigrees, and only 1 out of 20 heterozygous family members was asymptomatic. The reduced penetrance in this family indicates a less "dominant negative effect" of the G230E mutation.

Key Words: Myotonia congenita, CLCN1 mutation, Phenotypic variability

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INTRODUCTION

Myotonia congenita (MC), a hereditary non-dystrophic muscle disorder, is characterized by impaired skeletal muscle relaxation after contraction. This disorder can be inherited as either an autosomal dominant or an autosomal recessive trait. Autosomal dominant myotonia congenita (Thomsen's disease) was first described in 1876 by Julius Thomsen, while autosomal recessive myotonia congenita (Becker's disease) was

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proved by Peter Emil Becker in 1977. The Thomsen form is usually associated with an earlier onset and milder myotonic symptoms and muscular hypertrophy, whereas the Becker form is characterized by a later onset, more severe symptoms, significant muscular hypertrophy, and episodic or even permanent muscle weakness[1]. The myotonic symptoms frequently described as "muscle stiffness" are easily induced after prolonged resting and can be improved by repetitive exercise (warm up phenomenon). Myotonic discharges can be identified by electromyography (EMG).

The pathophysiological basis of myotonia congenita is reduction of the sacrolemmal chloride conductance. One of the important functions of the chloride channel in plasma membrane of most cells is to stabilize membrane potentials. In 1990, cloning of the voltage-gated chloride channel, CLC-0 from the electric organ of Tarpedo marmorata led to molecular identification of several members of the CLC family in mammals[2]. The major chloride channel of skeletal muscle is CLC-1. The CLC-1 proteins, which function as homodimers[3], are responsible for the high resting membrane conductance of skeletal muscle cells. Possible molecular mechanisms of CLC-1 dysfunction include altered gating properties[4-6], different open-close channel transition[7] and dramatic change in ion selectivity[8].

Both autosomal dominant and recessive human MC are caused by mutation of CLCN1, the gene encoding CLC-1 protein[9], on chromosome 7q35 linked to the *T* cell receptor β (TCRB) locus and containing 23 exons[10]. To date, over 80 different mutations in the CLCN1 gene with a predominance of autosomal recessive inheritance have been described[1]. Some mutations have been found in both autosomal dominant and recessive pedigrees, while some inheritances are unelucidated.

An important inquiry is whether the mutation is dominant or recessive in inheritance. Even with the same mutation in the same pedigree, phenotype often varies [11-14]. In this study, we report the clinical features, electrophysiological studies, and molecular genetic analysis of a Taiwanese family with autosomal dominant MC. The G230E mutation was identified in this pedigree and intrafamilial variations were observed among our patients. The differing molecular basis of CLC dysfunction in G230E and the reduced penetrance inducing phenotypic variability are discussed.

PATIENTS AND METHODS

The index patient III-2 was a 17-year-old man with difficulty in muscle relaxation since age 9. The patient experienced frequent muscle "spasms" and "stiffness", especially when initiating movements or when emotionally distressed. The discomfort could often be relieved by repetitive movements. However, he frequently suffered from falls or "freezing-up" with embarrassment during severe attacks. At the age of 15, he visited our hospital. Neurological examinations revealed percussion myotonia of both hands without muscular hypertrophy or weakness. Laboratory data showed normal values for creatine kinase (CK), complete blood count (CBC), liver function, and renal function.

Patient II-3, the 47-year-old father of the proband, first experienced stiffness and mild muscle "ache" at age 16 when initiating movement after prolonged immobilization or walking upstairs. These symptoms were usually aggravated during cold weather, and physical activity was helpful for relaxation. Neurological examinations revealed percussion myotonia. The patient also exhibited muscle wasting in the left leg since childhood due to poliomyelitis. Laboratory findings showed a slight increase in CK value (148 mg/dl, reference: 15-130 mg/dl). Otherwise data of CBC and liver function were unremarkable.

Patient I-1 and I-2, the mother and the aunt of patient II-3, had similar symptoms. Both of them complained of intermittent cramps, transient stiffness of calf muscles, and difficulty initiating movements since teenage. However, both declined further evaluation.

Patient III-1, the elder sister of the index patient III-2, was nineteen years old and asymptomatic. Her laboratory data, including CK, CBC and liver functions were within normal limits.

Genetic analysis

Venous blood 10 ml was drawn from patients II-3,

III-1, and III-2 after informed consents. Genomic DNA was extracted from peripheral blood lymphocytes by using a Puregene DNA isolation kit (GentraSystems, Minneapolis, MN, USA). The entire coding sequence of the CLCN1 gene was screened for mutations using polymerase chain reaction (PCR) followed by direct DNA sequencing analysis. Twenty-three sets of primers for all exons of CLCN1 and conditions of PCR were as described previously[15] with minor modifications. Abnormal conformers were sequenced by DNA sequencer (Promega BioScience, San Luis Obispo, CA, USA) as described previously[16].

Electromyographic studies

The needle EMG study was performed of selected muscles, including biceps, abductor pollicis brevis, quadriceps, and anterior tibialis muscles on patients II-3, III-1, and III-2.

RESULTS

Table 1 shows clinical data of the 5 family members. The EMG findings of patients II-3 and III-2 revealed myotonic discharges in all muscles tested. Fig. A shows the pedigree of this family and hereditary pattern for the affected subjects. However, the EMG myotonia was absent in patient III-1. Exon 5 sequencing in patients II-3, III-1, and III-2 revealed a nucleotide 689 G>A (G230E) mutation (Fig. B). A polymorphism of 2090 C>T (P697) was also detected in the molecular genetic analysis of patient III-2.

Slow progression of myotonic symptoms were noted in patient II-3 and patient III-2. Under treatment with mexiletine 300 mg daily, the muscle stiffness has improved in both patients and patient III-2 did not suffer from falls anymore.

DISCUSSION

The patients in this study are the first autosomal dominant MC Asian family with the G230E mutation. As compared with Becker type, Thomsen's disease is less common. By single-strand conformation polymorphism (SSCP) analysis, the molecular basis of Thomsen's disease is proved to have a G to A transition in the segment D3 exon in affected individuals with autosomal dominant MC[17]. This point mutation (G230E), the first reported Thomsen's disease mutation, replaces highly conserved neutral residue (glycine) with a negatively charged amino acid (glutamic acid). To our

Table 1. *Clinical data of the family members with myotonia congenita*

Probands	I-1	I-2	II-3	III-1	III-2
Age (years)	74	70	47	19	17
Sex	F	F	M	F	M
Age of onset (years)	teenage	teenage	16	-	9
Clinical manifestations					
Muscle stiffness	+	+	+	-	+
Myalgia	-	-	+	-	-
Falling	-	-	-	-	+
Myotonia					
Percussion	NA	NA	+	-	+
Action	NA	NA	+	-	+
Coldness	NA	NA	+	-	-
Improvement after exercise	+	+	+	-	+
Limb hypertrophy	NA	NA	-	-	-
Electromyographic myotonia	NA	NA	+	-	+
Mutation of *CLCN1* (site)	NA	NA	G230E (689G>A)	G230E (689G>A)	G230E (689G>A)

F: female; M: male; NA: not available; +: present; -: absent.

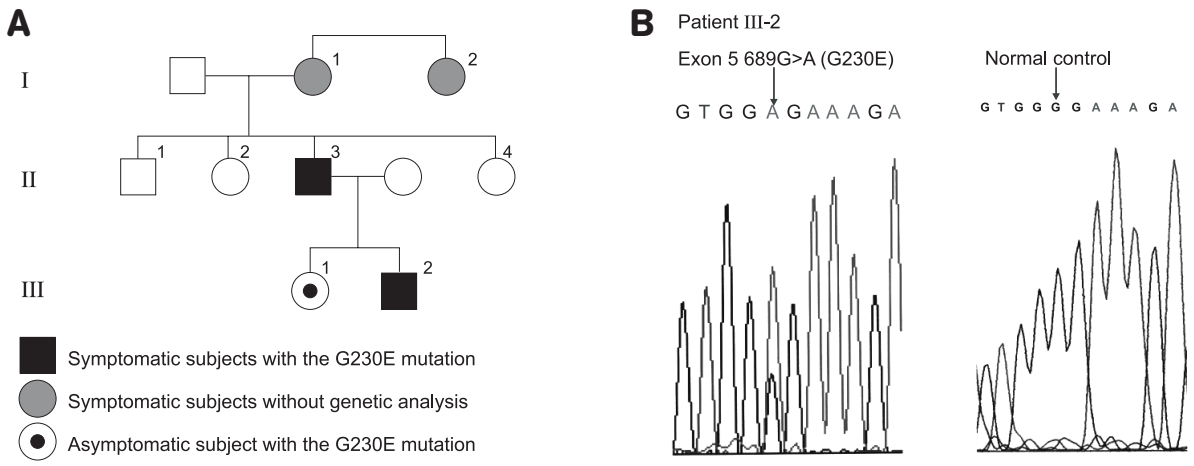

Figure. (A) A pedigree of the family with myotonia congenita, and (B) sequencing data revealing the G230E mutation of exon 5 in patient III-2 and exon 5 in a normal control.

Table 2. *Comparison of the present myotonia congenita family with previously reported families with G230E mutation*

Reference	Family No.	Nation	Inheritance	Numbers of patient with G230E mutation	Clinical myotonia	EMG myotonia
George et al.[17]	1	Canadian	AD	5	5	?
	2	Canadian	AD	3	3	?
	3	Canadian	AD	5	5	?
Koty et al.[19]	4#	American	AD	7	7	5
	5#	American	AD	5	5	3
	6#	American	AD	4	3*	3
Zhang et al.[18]	7	American	AR**	3	1	1
Plassart-Schiess[11]	8	French	AD	?	?	?
Brugnoni et al.[31]	9	Italian	AD	2	2	?
Current study	10	Taiwanese	AD	3	2	2

EMG, electromyography; +, positive.

#Family 4, 5, and 6 were believed to have a common affected ancestor with a founder effect.

*Only one proband (a 35 y/o woman) with G230E mutation in AD inheritance had no clinical symptoms.

**In this family, AR inheritance or AD with reduced penetrance was debatable.

knowledge, nine MC families with G230E mutation have been reported (Table 2). All G230E families had a dominant inheritance except for an American family, in which the only symptomatic proband had a compound heterozygote (G230E/R894X)[18]. In a latest review[1], G230E was found to have a dual inheritance. However, whether a truly recessive inheritance in G230E exists or a reduced penetrance in a dominant pedigree remains unclear. SSCP-mediated haplotyping was performed on 3 unrelated American families with the G230E mutation[19]. The results indicate a high probability of "founder effect", and suggest that the G230E families may share a common ancestor instead of distinct recurrent mutational events. However, the patients in this study are Taiwanese, whose ancestors migrated from mainland China approximately 400 years ago. Common founder effect should be questioned in this family. A detailed analysis of ancestry or SSCP-mediated haplo-

typing is needed for further confirmation. Review of the previously reported Taiwanese MC families indicated that some mutations including S471E, P575S, and D644G were discovered as novel mutations[15], and other mutations were also found elsewhere[16], which implied a high molecular heterogeneity in Taiwanese MC families.

Structure-function relationships of CLC-1 may explain how dominant or recessive traits work by the same mutant gene. Moreover, even patients with identical mutations may exhibit variations. Such phenotypic variability is also observed without exception in patients with the G230E mutation. In G230E heterozygous probands, some experienced severe myotonic symptoms, prominent muscle hypertrophy, and frequent falling; while others had minimal symptoms[19]. The hypothesized difference in allelic expression may partially account for this intrafamilial variation in dominant pedigrees[1,20]. Interestingly, a previous study reported a female patient with the G230E mutation in a dominant inheritance pedigree with neither clinical myotonia nor myotonic discharges on EMG[19]. This rare condition was also noted in our patient III-1, who had no myotonic symptoms but with a heterozygous G230E mutation of CLCN1. Gender might be a factor in this symptomatology. Although myotonia congenita did not exhibit gender differences, myotonic symptoms were reported more prominent in men[21,22]. However, recent studies indicated that autosomal recessive MC was more severe in men than in women[1]. Age is an additional consideration. Age of onset is variable widely from infancy to age 40 in myotonia congenita, although onset is generally earlier in dominant MC. The asymptomatic carrier in this study is only 19 years old. The patient requires close observation for subsequent clinical presentations, especially during pregnancy and immediately thereafter, since myotonia may worsen during pregnancy in female patients[23].

The molecular mechanism of G230E interference with chloride conductance is noteworthy. The G230E mutation is located at the D3 segment and belongs to the pore-forming segments of the chloride channel[24]. Unlike other CLCN1 mutation disturbing gating properties, G230E alters the relative permeability to cations and the selectivity for permeable anions to reduce chloride conductance[8]. By patch-clamp technique, residue K231 and G233 also have the greatest effect on anion-selectivity aside from G230[24]. One possibility is that compensatory mechanisms in this pore-forming segment occur when a single amino acid mutates (i.e. G230E) to maintain the pore properties. This may be related to the less "dominant negative effect" in G230E, which has been observed previously in co-expression assay[25]. To test this hypothesis, further investigation by patch-clamp technique is needed for a detailed simultaneous evaluation of channel function in the symptomatic patients as well as asymptomatic carriers.

Routine medical treatment for myotonia congenita is still equivocal and no guidelines for medication have been established[26]. Symptomatic control by medication should generally commence when patients suffer from disability or disturbance in daily life due to myotonia. Many drugs had been found effective in treating myotonia congenita, including phenytoin, carbamazepine, dantrolene, and anti-arrhythmic agents[27-29]. In our experience, mexiletine is also effective in our patients as other Taiwanese patients with MC[30], and patient's tolerance is acceptable. In patients II-3 and III-2, myotonic symptoms decreased dramatically under daily mexiletine 100mg-200mg. Furthermore, patient III-2 exhibited improvement in balance after treatment. Appropriate medication should be encouraged to improve quality of life in patients with myotonia congenita.

CONCLUSION

Thomsen's disease is a rare hereditary muscle disease caused by various CLCN1 mutations and has a great phenotypic variability. The G230E mutation reported here is the only known Asian pedigree of this point mutation. The founder effect, which commonly ocurred in Caucasians can not explain the G230E mutation of this family without haplotype analysis. In this study, one subject with this mutant gene was asymptomatic. Such variable clinical presentations remind us that some factors may be related to reduced penetrance and a less dominant negative effect of this gene. The location of G230E on pore-forming segments of CLC-1 may play

a role. A detailed analysis of molecular mechanisms by patch-clamp technique is recommended. In addition, mexiletine is effective and safe in patients with myotonic symptoms.

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