Paramyotonia congenita in a Slovak population: Genetic and pedigree analysis of 3 families

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Background. Paramyotonia congenita is a non-dystrophic myotonia, in which muscle relaxation is delayed after voluntary or evoked contraction. This condition cannot be distinguished on the basis of symptoms and signs alone. It requires consideration of genetics as more than 100 mutations in the CLCN1 gene and at least 20 mutations in the SCN4A gene are associated with the clinical features of the non-dystrophic myotonias. Only a few families with the described features but no genetic testing have been reported in Slovakia. This prompted us to investigate genetic mutations in the SCN4A gene in 3 Slovak families clinically diagnosed with paramyotonia.
Subjects and Methods. Genomic DNA of the family members was extracted from peripheral blood and amplified by polymerase chain reaction. SCN4A variants were screened by Sanger sequencing.
Results. Our results revealed 2 potential disease-causing mutations present in the probands and affected family members – mutations c.3938C > T (p.T1313M) in two families and mutation c.2111C>T (p. T704M) in one family.
Conclusion. Our results may help to identify genetic determinants as well as clarify genotype-phenotype relationships in patients with paramyotonia in Slovakia.

Key words: paramyotonia, periodic paralysis, genetics

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INTRODUCTION

Paramyotonia congenita (PMC) is an autosomal dominant disorder with paradoxical myotonia, defined as increased stiffness on repeated activities, and cold-induced muscle stiffness[1]. In most cases, PMC is caused by mutations in the SCN4A gene on chromosome 17q23-3. The SCN4A gene comprises 1836 amino acids and mediates the voltage-dependent sodium ion permeability of excitable membrane[2]. The sodium channel is a heterodimer consisting of a pore-forming α-subunit and a regulatory β1 subunit. The α-subunit consists of 4 homologous domains, each containing 6 transmembrane segments. Some mutations in the SCN4A gene cause repetitive discharges leading to myotonia[3]. More than 50 different SCN4A mutations have been reported, most of them are missense mutations. Diseases caused by SCN4A mutations have diverse clinical phenotypes, not only PMC, but also sodium channel myotonia, hyperkalemic and hypokalemic periodic paralysis, potassium-aggravated myotonia, and congenital myasthenia syndrome[4]. It has also been reported that human CLCN1 gene in chromosome 7q34 that encodes the skeletal muscle chloride channel was responsible for PMC (ref.[5]).

PMC usually presents in the first decade of life and presents with myotonia or weakness in the hand, face, and neck muscles with less involvement of lower extremity muscles. Parents of patients may report that their child has pain or stridor, is clumsy, or has an eye that ''sticks'' after periods of prolonged crying. Symptoms are exacerbated by cold or exercise. Other triggers include pregnancy, hypothyroidism, potassium injection, or fasting. Patients with PMC experience paradoxical exacerbation of myotonia by exercise; specifically, hand-grip relaxation or opening the eyes are progressively delayed with repetition. In adults, cold exposure may produce transient disabling paralysis reversed by warming of the affected muscles. On examination, patients often appear athletic. Paradoxical hand and eyelid myotonia and also grip myotonia and weakness can be demonstrated with muscle cooling. Needle electromyographic (EMG) examination shows widespread myotonic discharges that are most pronounced in distal muscles. When cooling the muscle, fibrillation potentials and electrical myotonia become more obvious as grip strength declines. When temperature declines to 28 °C, fibrillation potentials recede, and at 20 °C an electrically silent contracture occurs and spontaneous and voluntary EMG activity cease. Repetitive stimulation at 5 Hz can show a compound muscle action potential (CMAP) amplitude decrement. Single-fiber EMG may show increased jitter, fiber density and blocking. Exercise tests (especialy short exercise) show characteristic amplitude drops. Patients need to avoid triggers, especially cold coupled with exercise[6]. Paramyotonia congenita is a

rare disease. The first Slovak patient with this diagnosis was described in 1980 (ref.[7]). In this report we present 3 Slovak families with PMC history. We analysed their SCN4A genes and identified a disease-causing mutations.

MATERIAL AND METHODS

Subjects

This study involved 3 probands clinically diagnosed with non-dystrophic myotonia at the Department of Neurology of Slovak Health University Bratislava and Department of Neurology, University Hospital Martin, Slovakia, 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 according to the Diagnostic Criteria for Neuromuscular Disorders[8]. All patients showed non-dystrophic myotonia, symptoms ranged from mild to severe. All probands and several of their family members underwent electromyography and blood testing.

Mutational screening of SCN4A

Genomic DNA was extracted from peripheral blood leukocytes by the standard salting-out method and amplified by PCR. Sequences of primers for amplification of all exons and adjacent intron sequences are available on request, as well as the conditions of particular PCRs. PCR products were directly sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and analysed on the ABI 3130xl Genetic Analyzer (Applied Biosystems). The resulting sequences were compared with the *SCN4A* reference sequence NG_011699.1 (NM_000334.4).

RESULTS

Clinical characteristics

All probands in the 3 families with PMC were female, and the age of disease onset ranged from 1 to 15 years (Table 1). All 3 probands showed normal blood biochemistry (patient 3 had mild elevation of creatine kinase) and their EMG showed typical myotonic discharges. The disease inheritance showed an autosomal dominant pattern in all 3 families. Among the 3 families with PC in our study, one of the most frequent reported mutations c.3938C>T (p.T1313M) was found in Families 1 and 2. In Family 1 the mutation is associated with classic characteristics of PMC: exercise-induced muscle stiffness as well as intermittent periods of weakness not necessarily related to cold or myotonia. Clinical presence of symptoms in this family is also apparent in the proband´s father, grandmother and half sister (Fig. 1). In Family 2 the mutation was present in the proband herself, her mother and uncle, her mother's cousin and his daughter, the proband's grandmother and grandmother's sister, and the proband's great-grandfather (Fig. 2). Clinical presentation in all genetically affected members was uniform - cold- and exercise-induced muscle stiffness with no periods of weakness. The same clinical presentation we saw in Family 3 – the proband, her father, grandmother and cousin, but with mild weakness in some members (Fig 3). The detailed information of the probands are listed in Table 1.

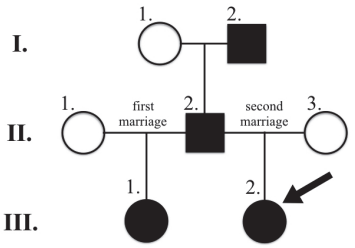

Fig. 1. Pedigree of Family 1. The family comprised four affected members in three generations. Roman numerals (I-III) give the generation, arabic numerals (1-3) state the individuals within one generation. Square - male, circle - female, filled symbol - affected subject, blank symbol - unaffected subject, arrow - proband.

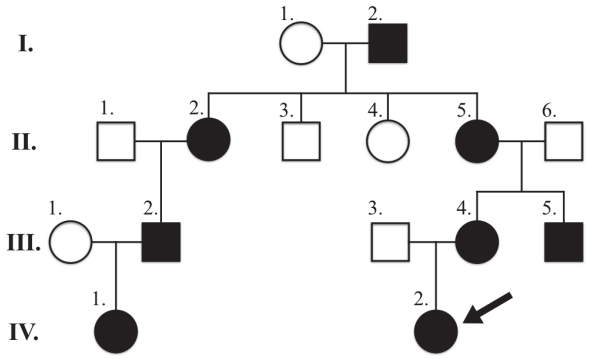

Fig. 2. Pedigree of Family 2. The family comprised eight affected members in four generations. Roman numerals (I-IV) give the generation, arabic numerals (1-6) state the individuals within one generation. Square - male, circle - female, filled symbol - affected subject, blank symbol - unaffected subject, arrow - proband.

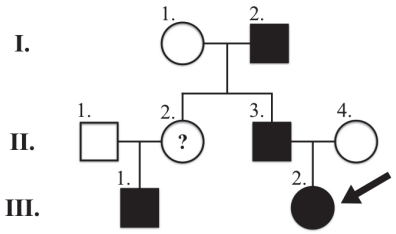

Fig. 3. Pedigree of Family 3. The family comprised eight affected members in four generations. Roman numerals (I-III) give the generation, arabic numerals (1-4) state the individuals within one generation. Square - male, circle - female, filled symbol - affected subject, blank symbol - unaffected subject, arrow - proband, question mark - unknown information.

Genetic analysis

Direct sequencing of all exons in SCN4A revealed 2 pathogenic sequence variants that were present in the probands and affected family members. Both pathogenic variants have already been reported[9,10], see Table 1.

Table 1. Clinical characteristics of probands from families with PMC carrying mutations in SCN4A gene.

Patient	H.V.	L.R.	I.P.
Gene	SCN4A	SCN4A	SCN4A
Mutation	c.3938C>T, p. Thr1313Met	c.3938C>T, p. Thr1313Met	c.2111C>T, p. Thr704Met
Gender	F	F	F
Age at onset	4 y	6 y	3 y
Age at admission	23 y	8 y	22 y
Initial symptoms	Cold induced four limb stifness	Cold induced acral limb stifness	Cold induced stifness of fingers
Triggers	exercise	exercise, cold	exercise, cold
Clinical myotonia	yes	yes	yes
Masticatory muscles	Yes	yes	yes
Eyelids	Yes	no	yes
Upper limbs	Yes	yes	yes
Lower limbs	Yes	yes	yes
Muscular hypertrophy	no	no	no
Cold aggravation	no	yes	yes
Warm-up phenomenon	no	no	no
Weakness	yes	no	yes
Creatine kinase	N	N	Mild elevation
EMG	Myotonic discharges	Myotonic discharges	Myotonic discharges

DISCUSSION

In most known cases, PMC is caused by a mutation in the *SCN4A* gene which encodes the α-subunit of the skeletal muscle sodium channel. The SCN4A gene is located on chromosome 17q23-3 and consists of 24 exons with a 5.5-kb open reading frame. The SCN4A protein comprises 1836 amino acids and mediates the voltage-dependent sodium ion permeability of excitable membrane. The protein has four homologous domains (DI, DII, DIII, and DIV). Each domain consists of six transmembrane α-helical segments (S1, S2, S3, S4, S5, and S6). The S4 segment in each domain contains four to seven repeated three-residue motifs of a positively charged amino acid (usually arginine) followed by two hydrophobic amino acids. The high concentration of positive charge in this α-helical segment suggests that the S4 segment is involved in voltage-dependent gating[11].

This study reports on 3 families with paramyotonia congenita. All three families showed an autosomal dominant pattern of inheritance. The mutation c.3938C>T (p.T1313M), which is the most frequently reported mutation in literature, was present in 2 of our families. This mutation is located in the DIII–DIV linker and is associated with classic characteristics of PMC: cold– and exercise-induced muscle stiffness as well as intermittent periods of weakness not necessarily related to cold or myotonia[12,13]. T1313 residue is located next to the COOH-terminal end of the IFM motif, which is thought to serve as an inactivation particle that blocks the pore during fast inactivation[14]. Mutagenesis experiments on brain type II Na channels suggest that both structure and polarity of this threonine residue are important factors for stability of fast inactivation. Methionine is a non-polar hydrophobic amino acid as opposed to polar hydrophilic threonine, but threonine has shorter side chains than methionine. This is supported by functional experiments that have shown that the mutation T1313M (loss of amino acid polarity) impairs fast inactivation of sodium channels in a temperature-sensitive model, which may help explain the clinical phenotype of patients with PMC who have this mutation[15]. Other gating properties of T1313M mutant channels, such as slow inactivation and deactivation, do not seem to be significantly altered[16]. To explain the link between Na channel fast inactivation defects and the clinical phenotype, Hayward et al. proposed a model in which large persistent Na current should lead to paralysis, whereas a depolarizing shift in voltage and slowed fast inactivation time without large sustained current should cause myotonia[17]. EMG results of exercise trials performed at room temperature or after cooling the muscles in patients with typical mutation T1313M showed exercise-induced decrease in CMAP amplitude that was further pronounced with cooling[1].

In Family 3 we found mutation T704M. This mutation alters Na channel activation – voltage dependence of the peak Na conductance is shifted to hyperpolarized potentials[18]. This shift reflects the fact that mutant channels open more readily, with less depolarization, than wild-type ones. From the functional standpoint, impairment of fast inactivation and augmentation of activation both result in a "gain-of-function", whereas mutant channels have a higher probability of being open and conducting Na current. The extent of slow inactivation was also reduced in T704M probands – 50% of the mutant channels recover within 20 milliseconds, indicating that half of the mutant channels failed to undergo slow inactivation[19].

CONCLUSION

In our study screening of mutations in 3 Slovak families with non-dystrophic myotonias has identified 2 mutations in the SCN4A gene associated with PMC. Our

results highlight the importance of screening SCN4A in genetic studies of non-dystrophic myotonias. Different mutations may play different roles in the pathogenesis and one mutation may correlate with a range of phenotypes – this highlights the possibility that epigenetic factors influence clinical expression. Future studies are needed to examine these factors and clarify how disease-associated mutations contribute to phenotype to bring us closer to effective treatment.

ABBREVIATIONS

CLCN1, Chloride voltage-gated channel 1 gene; CMAP, Compound muscle action potential; EMG, Electromyography; PCR, Polymerase chain reaction; PMC, Paramyotonia congenita; SCN4A, Sodium voltage-gated channel alpha subunit 4 gene.

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