

De Novo Mutation in *SCN4A* Gene Detected in Polish Patient with Paramyotonia Congenita Phenotype

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Abstract

A 16-year-old Polish male child was referred to confirm the diagnosis of myotonia congenita. For several years he had experienced cold-induced myotonia and muscle stiffness. DNA analysis of the *SCN4A* gene showed a C to T transition at nucleotide position 3938 in exon 22 of *SCN4A* (Thr1313Met) in patient but not in his parents. Based on his symptoms and results of DNA analysis, paramyotonia congenita was diagnosed, which prevalence is very low in Poland.

Keywords: Missense Mutation; Paramyotonia Congenita *SCN4A* gene; Sodium Channel

Introduction

Paramyotonia congenita (PMC; OMIM #168300), first described by Eulenburg [1] represents a skeletal muscle sodium channelopathy due to episodic membrane hyperexcitability of the skeletal muscle cells. This disorder has an autosomal dominant mode of inheritance with a variable penetration. Clinical symptoms are episodic and from one patient to another may vary in severity, duration and frequency. Symptomatically, PMC is characterised by paradoxical and cold-induced myotonia, defined as increased stiffness during repeated activities and cold-induced muscle stiffness. PMC usually affects the neck, face and distal upper extremity muscles. This muscle stiffness is induced by exercise or exposure to cold. However, the phenotype of PMC is very heterogenous; in some patients paramyotonia occurs during exercise even under warm temperatures, some others have cold-induced paralytic attacks without stiffness whilst in others stiffness can be induced by cold temperature without weakness [2-6]. PMC is caused by a mutation in the *SCN4A* gene. *SCN4A* gene is located on chromosome 17q23-25 and comprises 24 exons with a 5.5-kb open reading frame. This gene encodes a skeletal muscle voltage-gated sodium channel, Nav 1.4. The protein encoded by the *SCN4A* gene comprises 1836 amino acids and mediates the voltage-dependent permeability of the excitable membrane for sodium ion. The ex-

pression of Nav 1.4 has been detected in all skeletal muscle cells. During the past 30 years more than 50 different types of *SCN4A* mutation from several populations and with several clinically different forms of myotonia have been identified, most of which are single-base substitutions producing missense mutations. Studies have shown that certain *SCN4A* mutations are associated with a specific phenotype [7-10].

Case Study

The patient was 16-year old male child that was referred to us from another clinic to confirm the diagnosis of paramyotonia congenita. He was the only child and at age 12 years he began to experience muscle stiffness that affected his face and upper and lower extremities. Both muscle stiffness and weakness were provoked and worsened by muscle cooling (such as being outdoors in winter temperatures of 5°C-10°C) and muscle activity. Routine laboratory tests were performed including complete blood count, liver and renal function tests, electrolytes and serum Creatine Kinase (CK) level. The laboratory findings were all nearly normal, serum sodium potassium and chloride levels were normal, although CK level was elevated at 502 U/L, two months later at 1008 U/L, and after one year at 914 U/L (normal range: 0.00–171.00 IU/L). Electromyography revealed prominent myotonic discharges. The patient was suspected as having paramyotonia on the basis of clinical findings and electromyography examination. Subsequently, he was clinically diagnosed as having PMC. In order to confirm this

clinical diagnosis, the patient and his parents were included in a molecular genetic study.

Material and Methods

After obtaining informed consent, we performed a mutational analysis of *SCN4A* gene in the patient and his parents. Whole blood samples for analysis were collected using tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA). Total genomic DNA was extracted from peripheral blood leukocytes using High Pure PCR Template Preparation Kit (ROCHE Diagnostic, Mannheim, Germany). Sequences of primers specific to the *SCN4A* gene presented according to Dian K. Nurputra et al. 2012 as previously described. The 24 *SCN4A* coding exons and adjacent intronic regions were amplified by polymerase chain reaction (PCR) with Fast Start PCR Master Kit (ROCHE Diagnostic). PCR was carried out using a PCR System Nexus Gsx 1e (Eppendorf). The sequences were determined on both DNA strands from at least two independent PCR products. The PCR products were purified with High Pure PCR Product Purification Kit (ROCHE Diagnostic) and sequenced by dideoxynucleotide chain termination using Big Dye Terminator v.3.1 Cycle Sequencing Kit (Thermofisher, Foster City, CA, USA). The products of this cycle sequencing were purified by unbound fluorescent dyes with Big Dye XTerminator Purification Kit (Thermofisher, Foster City, CA, USA) and separated in an automated DNA sequencer 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were analysed by using AB DNA Sequencing Analysis Software v.5.2. (Applied Biosystems) and then were compared to the *SCN4A* gene reference (NCBI Gene Bank Reference Sequence Accession Number: NM_000334.4 and ENSEMBL database Accession Number: ENST00000578147.5).

Results

Direct nucleotide sequence analysis of PCR products from the patient showed a C to T transition at nucleotide position 3938 in exon 22 c.[3938C>T]; [3938C=] of the *SCN4A* gene, resulting in the substitution of threonine for methionine at amino acid position 1313 in the *SCN4A* gene p.[Thr1313Met]; [Thr1313=] Figure 1. This heterogenous missense mutation as described above. All the nucleotides in that gene were examined, but no other mutations were detected (data not shown). The results of our analysis in the patient's mother and father showed no mutation in exon 22 of *SCN4A* Figure 1. Finally, based on these DNA results together with the observation of clinical symptoms the patient was diagnosed with PMC caused by a *de novo* mutation.

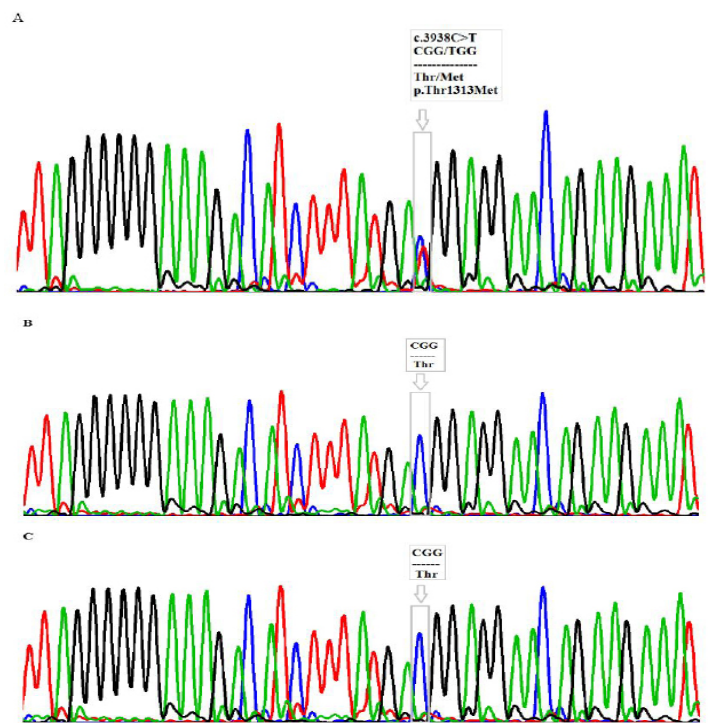


Figure 1: *SCN4A* sequencing chromatograms showing position 3939 of the coding DNA sequence (indicated by the grey frame). **Graph A** shows a *de novo* mutation in exon 22 identified in the proband where a substitution of cytosine to thymidine at position 3938 changes the codon for threonine at position 1313 into methionine. **Graph B** shows the *SCN4A* gene sequence chromatogram showing the nucleotide C at codon 3938 in the proband's mother. **Graph C** shows the *SCN4A* gene sequence chromatogram showing the nucleotide C at codon 3938 in the proband's father.

Our patient with PMC phenotype has been shown to have the heterozygous missense *de novo* mutation c.[3938C>T]; [3938C=], p.[Thr1313Met]; [Thr1313=] in the *SCN4A* gene. This finding confirmed the clinical diagnosis of PMC in the patient. The mutation was present only in the patient and not in other family members (mother and father). Although it is known that PMC is a predominantly inherited neuromuscular disorder, our patient seems to be a sporadic case with no positive family history. It suggests that the disease in the patient was caused by a mutation that might have occurred in germ-line cells in one of the parents. Two mutations predominant in PMC in European populations: Thr1313Met (45% of cases) [11] and Arg1448Cys (35% of cases)

[12]. Thr1313Met mutation was previously described and is known as being predominant in the population of France. In the Polish population, our diagnosis is the first such report of a mutation in *SCN4A* in a patient with PMC.

The *SCN4A* gene encodes the alpha subunit which plays a crucial role as a principal pore-forming and voltage-sensing subunit in the skeletal muscle sodium channels. Ion channels are transmembrane proteins that allow ions to flow into and out of the cells, creating electrical currents. The alpha-subunit comprises four repeated domains (from DI to DIV) as showed on Figure 2. Each domain consists of six transmembrane segments termed S1-S6. The fourth transmembrane segment (S4) of each domain contains positively charged amino acid and functions as voltage sensor for channel activation. In each domain, there is also an extracellular P-loop between the S5 and S6 segments. These P-loops together create ion-conducting pore, which acts as a selectivity filter. These filters are very important for ion permeability. The most crucial region of the alpha-subunit of the muscle sodium channel is an intracellular loop located between DIII and DIV domains. This is responsible for the rapid inactivation of this channel [13]. The alpha-subunit is also associated with the beta-subunit which has not been linked to any muscle human phenotype.

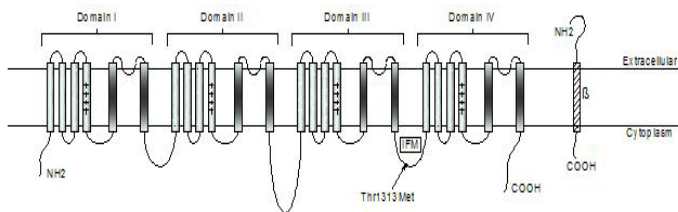


Figure 2: Two-dimensional structure of the rat Nav1.4 channel. A sodium channel is composed of four domains (I – IV), each consisting of six transmembrane segments. The -helical S4 segment, which possesses many positive charges, is the voltage sensor. The loop between domains III and IV is the inner mouth of the channel.

The mutations in the *SCN4A* gene alter different features of the alpha-subunit of Nav 1.4. It has been shown that the principal defect of gating (opening and closing) is disruption to fast inactivation, rendering it delayed or incomplete. This results in channel re-openings and intracellular Na⁺ accumulation, which depolarises muscle cells and generates repetitive action potentials. A mild depolarisation causes a long-lasting hyperexcitability, which leads to myotonia [9,10,12].

In the PMC phenotype disruption to fast activation is the predominant abnormality of sodium channel gating. This fast inactivation is responsible for limitation of the action potential duration which initiates repolarization of the muscle fibers. The Thr1313Met mutation is situated in part of the protein relevant

for channel inactivation in the intracellular loop between DIII and DIV domains. It thus causes the defective inactivation of the sodium channel. This results in myotonia and muscle stiffness. Moreover, in our patient both muscle stiffness and weakness were exacerbated by cold weather. It has been reported previously that Thr1313Met mutation results in temperature-sensitive behaviour of the sodium channel. In cultured human muscle cells was demonstrated that muscle fibers from PMC patients have normal membrane properties at 32°C while at 27°C, they are inexcitable, exhibit increased conductivity of Na, and have a reduced resting membrane potential of -40 mV [14].

In conclusion, in the Polish population, sodium channelopathy such as PMC is relatively rare. By mutational analysis of *SCN4A* gene, we identified a C to T transition at nucleotide position 3938 in exon 22 p.[Thr1313Met]; [Thr1313=] and we confirmed a diagnosis of PMC in a 16-year-old male suffering from cold-induced myotonia and stiffness. This is the first recorded diagnosis in the Polish population.

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Conflict of Interest Disclosures

The authors report no financial or other conflict of interest relevant to the subject of this article.

Ethic Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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