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Case Report

Phenotypic variation of Val1589Met mutation in a four-generation Chinese pedigree with mild paramyotonia congenitia: case report

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Abstract: Four generations of a Chinese family with a mild form of paramyotonia congenital was characterized in phenotype and genotype. For each member, clinical history, physical examination, laboratory tests, electrophysiological and gene analyses were recorded and carried out. A potassium loading, exercise and cold provocation were further tested to diagnose the clinical differentiation. All members shared the characteristics of mild muscle cramp and stiffness induced by exercise or exposed to cold. The symptoms were relieved after rest and warming. A Val1589Met mutation at exon 24 of the SCN4A gene appears in affected subjects, while healthy members had a point mutation at position 1513 at exon 24 of the SCN4A gene. The mild phenotype of the paramyotonia congenital in the family had a Val1589Met mutation in the SCN4A gene. Various phenotypes can exist among different families, indicating that family, individual, genetic or environmental factors influence symptoms.

Keywords: Paramyotonia congenita, Val1589Met, phenotype, SCN4A, myotonia

Introduction

Paramyotonia congenita (PMC) is a disorder that affects skeletal muscle. It first presents during infancy or early childhood when patients experience attacks of sustained myotonia that prevent muscles from relaxing normally after activity. Myotonia causes muscle stiffness that often appears after sustained exercise and can also be elicited by muscle cooling. Unlike other forms of myotonia, the muscle stiffness associated with paramyotonia congenita is exacerbated by repeated movements and exertion. Exposure to cold initially causes muscle stiffness, with prolonged exposure producing temporary episodes of mild to severe muscle weakness that can last for several hours [1, 2].

PMC is classified as one of a group of skeletal muscle sodium channelopathies that include PMC with hyperkalemic periodic paralysis, isolated hyperkalemic periodic paralysis, potassium-aggravated myotonia (PAM), and rarely hypokalemic periodic paralysis [2-7] The disorder is caused by mutations in the skeletal muscle sodium channel gene which prolongs the channel's opening time, producing a higher-than-normal degree of muscle excitation. It is produced by a defective gene inherited by one parent and is therefore an autosomal dominant disease. The skeletal muscle voltage-gated sodium channel gene SCN4A is located on chromosome 17q23-25 [8, 9] Although sodium channel disorders have a relatively low prevalence [10], more than fifty kinds of mutation have been identified in the SCN4A gene, of which about twenty can lead to PMC [11, 12]. There is only a limited amount of data available about the Val1589Met mutation in the gene [1, 11]. Previously, we were unable to establish a specific relationship between phenotypes and genotypes of the SCN4A gene mutation. However, in the present investigation, for the first time the clinical and genetic characteristics of a four generation Chinese family with mild phenotype paramyotonia congenita attributed to a Val1589Met mutation have been characterised and the phenotype and genotype features analyzed in detail.

Material and methods

The ethics committee of the People's Hospital of Zhengzhou University approved the study.

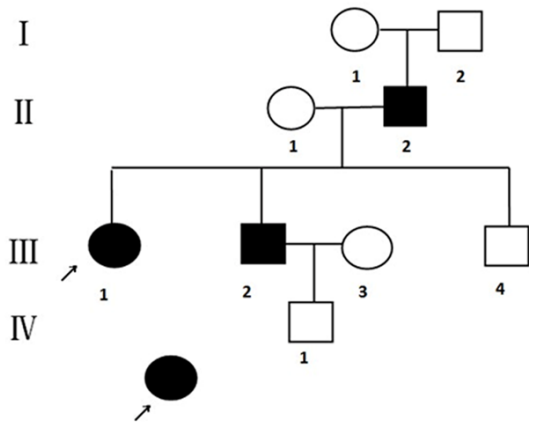

Figure 1. Genetic pedigree of the paramyotonia congenita family. The arrow indicates the proband.

Written consents were obtained from all participating patients and family members or their legal guardians.

In this four generations family, the proband was a twenty-five year old woman who complained of muscle cramps and stiffness after undergoing long periods of severe running/riding or if she had been exposed to cold temperatures (**Figure 1**). The symptoms involved all four limbs, especially the lower limbs, which were more seriously affected and exhibited notable weakness after recovery from muscle cramps and stiffness. As far as she was aware, the first attack occurred when she was four years old. The symptoms occurred once or twice a month as a child and at about the same frequency as an adult. Occasionally, she felt upper eyelid stiffness when exposed to cold wind or water, but all the symptoms were very mild and infrequent, and relieved simply by rest or warming for 1 to 2 hours. These symptoms were not triggered or exacerbated by eating fruits containing high potassium levels or by foods such as bananas or watermelons. The proband underwent a complete neurological examination, muscle strength was grade 5 (Medical Research Council Scale, graded 0-5) [13], the tendon reflex was slightly reduced, no apparent muscle atrophy or hypertrophy had occurred and no muscle ball after mechanical percussion. The father of the proband had the onset of symptoms when he was seven years old and her younger brother at age six. They both exhibited similar but milder symptoms in their four limbs than the proband, excluding their eyelids, which did not interfere with their daily work and lifestyles. Strikingly, the father did not suffer from apparent muscle weakness after the stiffness subsided. All neurological examinations conducted in the hospital including superficial sensation, deep sensation, myodynamia, dystonia and tendon reflexes were normal, and Babinski's sign was negative in a non-attack period.

Basic information

After completing three patients' clinical attack histories, the physical examinations included consciousness, physical activity, chest abdomen watching, touching, knocking, listening and laboratory tests. A number of blood samples were taken: 1 mL of peripheral blood for routine examination by ABX120; 3 mL for liver, renal function tests, glucose, serum creatine kinase and electrolyte level determinations using a ADVIA 2400 (Siemens, Germany), 2 mL for thyroid function tests using a Cenpaur XP (Siemens, Germany), chest X-rays (Raynova DRsc, China), and electrocardiograms using a ECG-1550P (NIHON KOHDEN, Japan). Moreover, we checked the serum creatine kinase and serum electrolyte levels at the onset of an attack.

Potassium loading test

Each subject was given 10% potassium chloride 29.8 mL or 40 mmol orally and the serum potassium levels determined every 30 minutes in conjunction with an assessment of clinical symptoms and a neurological examination.

Exercise test

After strenuous exercise of the lower limbs for ten minutes, the clinical symptoms were recorded and a further neurological examination carried out.

Cold provocation test

Each subject's two lower limbs were immersed in ice water (10-12°C) for 30 minutes and the degree of stiffness, weakness and neurological signs assessed.

Electrophysiological examination (M-800C)

All patients and healthy family members of the pedigree received a comprehensive electrophysiological study by EMG/EP MZB-2300C

Table 1. Primers designed for PCR

Exons	Sequence of the primers		Tm	Length of fragment (bp)
1	F	CACCCTTCTGGTCTCTGAGC	59	367
	R	CAGACAGGCAGACAGATGGA		
2-3	F	GAGATTGAGGAGCCCGAAC	55	592
	R	TCAGGGAGCAGGGAGACTTA		
4	F	ACTGTCCTTCCCAACCCTTG	59	408
	R	TATGTCCAGGCTTTGCTGTG		
5	F	GCCCTGATTTCTGTCCTACC	55	404
	R	TGAGTCGAACCCGTACCACC		
6	F	CCAGATTGAGGATGTGAACAGA	64.5	588
	R	AAATGTGGAGTCAGAGGCTACC		
7	F	GGGAACATCCGTTTGGTTTA	57	345
	R	ATTCCGTCAGTTCTGCCTGT		
8	F	GAAGGTGGGAGCTGATTAGAGA	64.5	540
	R	AAGAATTAAAGCTGTGGCTTGG		
9	F	TGAGCCAGGAGACCAGAAAC	62.5	558
	R	TGCTCCTTCTGCCTCAAAAC		
10	F	CACCCTGAAGGACTCTGGAA	64.5	297
	R	AGGCTCCACCCCTACCTAAG		
11	F	TGAGTGGTGGTGGAACTTGA	55	644
	R	CATCCTGCCCATGAATGA		
12	F	AAATGAATGGGAACGGGTCT	55	623
	R	CCAGCAGCCCTATGAAAGAA		
13	F	TTAGCAGAAGGGCACACTGA	55	599 (633)
	R	GAGAGGATGTGTTGGGGAGA		
14	F	TTTGGATCTGTAGACCGAGGA	56	645
	R	GGACTTAGGGCTTGCTCCAG		
15	F	TTCTTCCCTTCCCTTCTGGT	64.5	409
	R	TGGATCTTGCACCACAGTTC		
16	F	CTGGAGAAATGTCCTTGTCCAT	57	684
	R	TGCCATGTTAGAAAGTCAGCAC		
17	F	CACTGGGAAACGCTCTCAT	55	611
	R	TGGCAGGGATGGTAAGTAGC		
18	F	TCCTACAGGTACTGGGACAGGT	57	639
	R	AACACCACATAAACCTGCCTTC		
19	F	GAGAGGCACTGGCAATGGAC	53	349
	R	CTCCATCCAGGTTCCCGGCA		
20	F	ATCTCCGAGGTCAACAACAAGT	57	548
	R	CAAGTCTCCCTCCTGTCTTGAG		
21	F	AGGGAGAAGCCAGGTGCAAAC	55	205
	R	GGGGCTGGGCTGATACTCAT		
22	F	CAGCGAGATGTTGATGAAAAGT	54	600
	R	ACCAAGTGAGGGTAAAGCACTC		
23	F	TTGAAGGGAGGACCATGAAC	59	603
	R	AGGTGTGTCCGTGTGAGGAT		
24-1	F	CTGAGACTTGAGCAGAGCACAC	54	329
	R	ACATGCCGAAGATGGAGTAGAT		
24-2	F	ATCTACTCCATCTTCGGCATGT	56	515
	R	AAGTCCAGTGTGATGAGCTTGA		

(NIHON KOHDEN, Japan), including routine nerve conduction studies (excluding nerve disorders) and needle electromyography of the gastrocnemius muscle at rest and during exercise.

Genetic analysis

Five mL of peripheral blood was taken from the proband to obtain DNA and 24 pairs of polymerase chain reaction (PCR) primers were designed as listed in **Table 1**.

The primers were used to amplify 24 exons of the SCN4A gene (GeneAmp 9700, Perkin Elmer, USA) and their PCR products sequenced using an automated DNA sequencer (ABI 3730, Perkin Elmer, USA). Subsequently, we extracted DNA from the other two patients and all healthy subjects, and then sequencing their PCR products targeted at 24 exons of the SCN4A gene.

Results

In the proband, laboratory tests including routine blood values, liver, renal and thyroid function tests, blood glucose, serum creatine kinase and electrolyte levels were generally within normal limits. During attacks of stiffness, serum creatine kinase and electrolyte levels increased slightly, but were still just within normal limits. The chest X-ray and electrocardiogram were also normal. The proband had an attack of mild muscle cramp and stiffness approximately twenty minutes after strenuous exercise and about fifteen minutes after being exposed to cold water. Exercise and the cold-water test produced positive signs of mild cramp and stiffness, while the potassium-loading test showed a negative result. The results of electrophysiological examinations revealed that there was no abnormity in nerve conduction but we found myotonic potentials using needle electromyography at rest and after cold provocation and exercise. Neurological examination of the proband was undertaken when the attack muscle strength reached grade 4 (lower than in rest). The tendon reflex was slightly less than normal. All of the above examinations and tests were car-

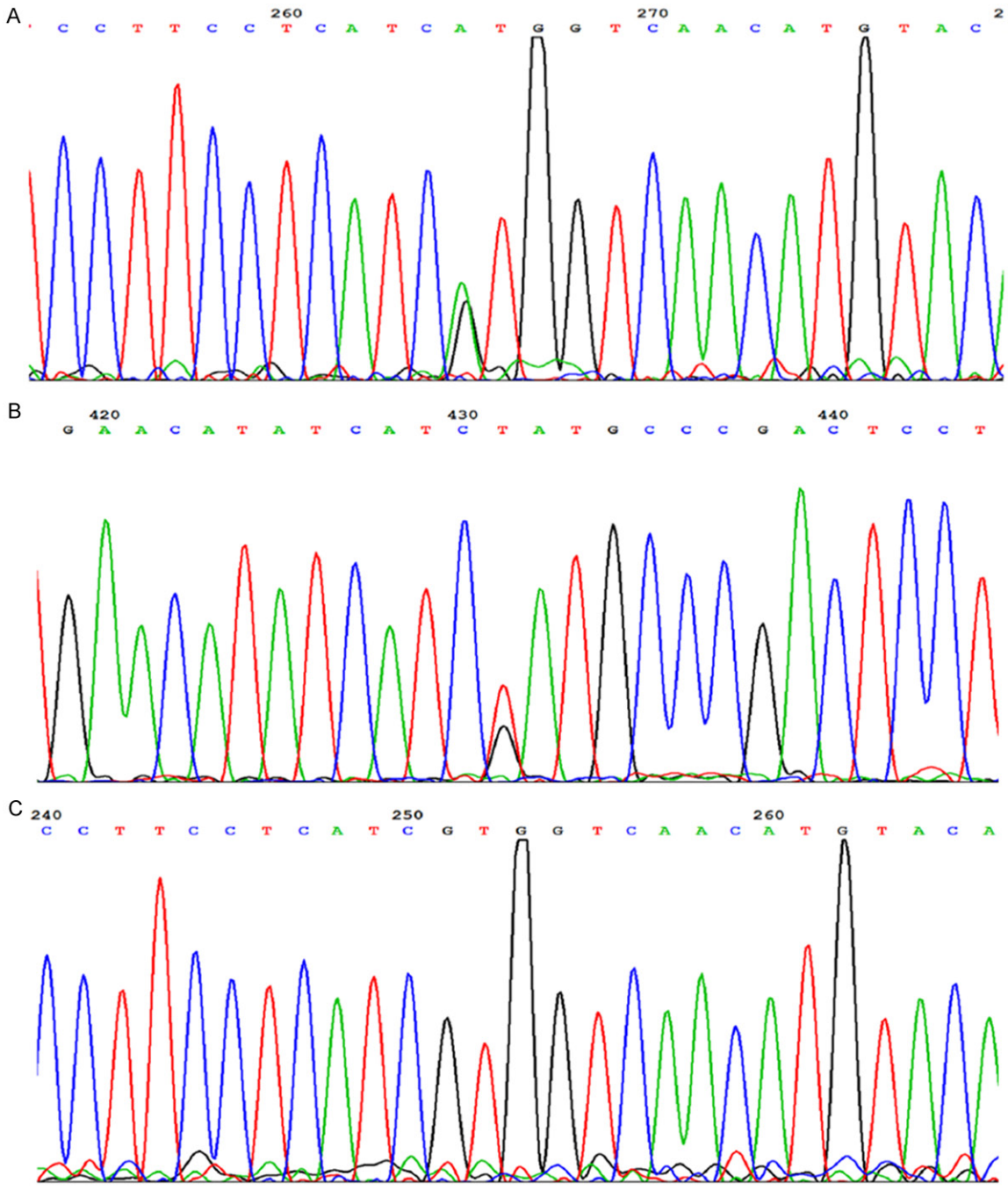

Figure 2. DNA sequence of SCN4A gene at position exon 24. A. G-A transition at the 4,765th position of SCN4A gene exon 24 in patient II 2, II 1 and III 2 in this family. B. T-G transition at the 1513th position of SCN4A gene exon 24 in family member II 1. C. No mutation of the SCN4A gene in other healthy people in this family at the same position.

ried out on the proband's father and younger brother with similar results; both neurological examinations were normal.

A mutation of the SCN4A gene was detected in the DNA of all three patients, at position 4,765 of the 24th exon, whereas there was no mutation in the six healthy people in this pedigree. We found a point mutation at position 1,513 in exon 24 of the SCN4A gene in the proband's mother (**Figure 2**).

Discussion

In the present study we have determined the clinical and genetic characteristics of a four

generation Chinese family with mild phenotype paramyotonia congenita caused by a Val1589Met mutation. Patients with PMC have the classical characteristic of stiffness and accompanied skeletal muscle weakness after exercise or being exposed to cold and may exhibit a paradoxical stiffness [14]. However, there are also various kinds of atypical phenotypes, for example, accompanying stiffness without weakness, muscle stiffness in a warm environment accompanied by a period of paralysis [15-17]. In this family, all the affected members shared characteristics of mild muscle cramp and stiffness, which were induced by strenuous exercise or exposure to cold, occasionally accompanied by muscle weakness. In addition, it has been shown that the ingestion of food containing high potassium levels will not induce an attack; these foods include bananas and watermelons. The clinical symptoms were mild and infrequent, lasting less than two hours following rest or when the subject returned to a warm environment. Electromyography examinations detected myotonic potentials at rest, even after excise or immersion in cold water [18]. According to clinical criteria, stiffness after exercise or cold exposure, muscle weakness, paradoxical stiffness and myotonic potentials [19-21] strongly suggest a diagnosis of PMC. However, it was not very obvious that symptoms of mild cramp were present and all other clinical symptoms were mild and occurred more infrequently than previously reported [11, 17, 21]. DNA sequencing analysis unequivocally demonstrated that a Val1589Met mutation was present in the SCN4A gene of all patients with PMC but not in unaffected family members. The mutation is a heterozygous guanine-to-adenine transition at position 4,765 in exon 24 of SCN4A [22], causing a methionine to valine mutation located within transmembrane segment S6 of the sodium channel repeat IV, close to the cytoplasmic surface. This gene encodes the skeletal muscle sodium channel α-subunit and the mutation is adjacent to a Met1592Val mutation which leads to hyperkalemic periodic paralysis [23, 24]. Studies of the pathophysiology associated with the Val1589Met mutation indicate that the mutation causes structural abnormalities of the Na^+ channel at the cytoplasmic surface of the cell. The change results in depolarization and hyperexcitability of the cytoplasmic surface, producing stiffness [25, 26]. Moreover, the mutation may well decrease the rate of inactivation, speed recovery from inactivation, and slow the rate of deactivation of the channel which will lengthen the duration of the muscle action potential [27, 28]. Finally, the increased period of depolarization will cause hypoexcitability due to prolonged inactivation of the muscle sodium channel leading to muscle weakness after stiffness. In the Chinese family studied in the present investigation, all three patients had a mild phenotype of stiffness, muscle cramp and weakness, so we hypothesise that it was related to individual or environmental factors, but this hypothesis will require further study.

There is a paucity of data available in the literature about a Val1589Met mutation. The published papers mainly report two kinds of clinical diagnosis, PMC and PAM [11]. Heine et al. described a German family who exhibited characteristic potassium/cold aggravated myotonia without weakness or paralysis who were diagnosed with PAM and had a mutation of Val1589Met. Ferriby reported a French PMC family with a Val1589Met mutation [17]. PMC is characterized by a marked worsening of myotonia induced by cold or repeated exercise, and episodes of muscle weakness. PAM is characterized by episodes of muscle stiffness that are aggravated by the intake of potassium, and is notable for the absence of episodic weakness but may have cold sensitivity. PAM belongs to the pure myotonic phenotypes and is generally known as myotonia fluctuans, myotonia permanens or acetazolamide-responsive myotonia. Both PAM and PMC may or may not have raised serum electrolyte levels but muscle hypertrophy occurs infrequently [26, 29-31]. Nevertheless, these clinical features may well serve as important points for a differential diagnosis.

Finally, it is worth noting that the three affected members of this family exhibited different clinical symptoms. All four limbs and the upper eyelids were affected in the proband, while her father and younger brother had more mild and infrequent symptoms just in the limbs, which had no debilitating effects on their daily work and lifestyles. Thus, different phenotypes likely existed intra family, indicating that family, individual, genetic or environmental factors may influence the appearance of this disorder,

which requires further research [32, 33]. Detailed analysis of clinical as well as genetic data of additional families with the Val1589Met mutation is required.

Conclusion

The clinical and genetic characteristics of a Chinese family with PMC were studied who had a Val1589Met mutation. They exhibited the characteristics of a mild phenotype and an uncommon Val1589Met mutation in contrast to previous reports. Phenotypes of Val1589Met mutation mainly include PMC and PAM, and various types of phenotypes exist inter/intra family, indicating that family, individual, genetic or environmental factors are important.

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Disclosure of conflict of interest

None.

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