Congenital Myasthenic Syndrome Due to Compound Heterozygous Mutations in the GFPT1 Gene

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Abstract

Congenital myasthenic syndrome (CMS) is a heterogeneous group of hereditary neuromuscular disorders associated with neuromuscular junction (NMJ) dysfunction. Here, we report the genetic variants and clinical follow-up of one individual suffering from CMS. The proband presented with limb weakness, and symptoms worsened after limb activities. In addition, decreases in muscle action potential were observed with repetitive nerve stimulation. Thus, myasthenia gravis was initially suspected, but the patient did not experience drooping eyelids or blurred vision. Trio whole exome sequencing was performed for the proband and his parents. We found two different heterozygous missense variants (c.331C>T; p.Arg111Cys and c.1428G>C; p.Lys476Asn) in the gene encoding glutamine-fructose-6-phosphate transaminase 1 (GFPT1) in this patient with autosomal recessive CMS, of which one was novel. The new c.1428G>C; p.Lys476Asn variant has not been previously reported, and has not been recorded in the ClinVar dataset or the gnomAD global population dataset. The phenotypes of proximal muscle weakness and fatigue while ocular and facial involvement is only minimal of limb-girdle weakness and fatigue. A beneficial and sustained response to acetylcholinesterase inhibitor treatment of the proband were consistent with those previously reported for CMS. Our study provides important information for the diagnosis and treatment of patients with CMS.

1. Introduction

The neuromuscular junction (NMJ) is a chemical synapse between a motor neuron and a muscle fiber (1). The prerequisite for muscle contraction is efficient neuromuscular signal communication from the motor neuron to a skeletal muscle fiber at the NMJ (2). Congenital myasthenic syndrome (CMS) is a heterogeneous group of hereditary neuromuscular disorders characterized by fatigable weakness of the muscle (3, 4). CMS often manifests in infancy or childhood with fatigable muscle weakness, generalized hypotonia, ophthalmoparesis, respiratory distress, and apnea (5, 6). If left untreated, it can lead to loss of life (7). In addition, CMS can manifest in adults as generalized limb fatigability and weakness (3). CMS is classified into three subclasses: presynaptic, synaptic, or postsynaptic (8), with postsynaptic CMS being most common (9).

CMS arises from genetic defects affecting NMJ proteins (10, 11). Gene sequencing can provide a diagnostic modality for patients with CMS (10). CMS is caused by genetic changes in at least 30 genes including synaptotagmin 2, Munc13-1, myosin 9A, laminin alpha submit 5, rapsyn, vesicle-associated membrane protein 1, adenylate kinase, synaptobrevin-1, myosin-9A, solute carrier family member 18 member A3,

glutamine-fructose-6-phosphate transaminase 1 (GFPT1), alpha-1,3-mannosyltransferase,

GDP-mannose pyrophosphorylase B, choline acetyltransferase, synaptotagmin-2,

collagen type XIII alpha 1 chain, cholinergic receptor nicotinic epsilon subunit, downstream of tyrosine kinase 7, and low-density lipoprotein receptor-related protein 4 (11–18). Evidence has shown that GFPT1

leads to limb-girdle CMS by regulating the N-glycosylation pathway (18, 19). GFPT1 is expressed in skeletal muscle and motor nerve tissues (20). Since 2011, more than 50 GFPT1 mutations in patients have been identified (20, 21).

In this study, we report our findings in one patient with GFPT1-myasthenia, in whom we identified one novel GFPT1 mutation using whole-exome sequencing (WES).

2. Materials And Methods

2.1 Patient information

A two-generation three-member family was recruited for this study. Written informed consent was obtained from the proband.

2.2 Clinical material investigation

The level of serum creatine kinase (CK) in the proband was detected. Electromyography (EMG) including repetitive nerve stimulation (RNS) was performed on the patient. Muscle biopsies were taken from the right biceps brachii muscle by open biopsy. Cryosections were stained with hematoxylin and eosin (H&E) and modified Gomori trichrome (GT) stain. Magnetic resonance imaging (MRI) of the proband's thigh muscle was performed.

2.3 DNA isolation

The peripheral blood of the proband and his parents was drawn for DNA isolation. Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (Hilden, Germany) according to the manufacturer's procedure with slight modifications. Genomic DNA samples were quantified using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The isolated DNA was stored at -80°C until analysis.

2.4 WES

Trio WES was performed for the proband and his parents. Whole-exome target enrichment was performed using the Agilent SureSelectXT Human All Exon V6 Target Kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocols. Next-generation sequencing was performed on the NovaSeq 6000 Sequencing System with the 150 base pairs paired-end module (Illumina Inc., San Diego, CA, USA). Internal bioinformatics pipelines were applied to the WES data. Variants found in the proband and in his parents were compared and filtered.

2.5 Sanger validation

PCR and sequencing primers were generated using PrimePremier software, and the amplicons were sequenced on the ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The primer sequences are described in Table 1.

2.6 Data analysis

Skewer was used to remove the sequencing adapters (22). Trimmed reads were mapped to the human reference genome (hg19) with Burrows Wheeler Aligner-2 (23). To reduce the low-quality reads, those mapped to more than one location with identical mapping score and/or duplicates likely resulting from PCR amplification were removed. Genome Analysis Toolkit was used to detect small variants, and these variants were annotated using several public databases (24).

3. Results

3.1 Clinical features

The proband, a 30-year-old male, had longstanding limb weakness. The patient started experiencing limb weakness and fatigue after limb activities at 4 years of age. He felt drained of energy when climbing stairs, but could walk stably on level ground and lift his arms above his head. In addition, he tired easily with physical activities, but this symptom was relieved after rest. Muscle weakness was mildest in the morning and worst in the afternoon or evening. He was diagnosed with vacuolar myopathy by muscle biopsy at the age 5. At 29 years of age, he developed pneumonia and underwent tracheotomy due to difficulty breathing. He recovered completely after treatment. Neurologic examination revealed that his posture and gait were normal for his age. The muscle tension in his limbs was normal (Fig. 1A, B). Myodynamia of the proximal limb muscles and distant limb muscles was grade 4 and grade 5, respectively. Auxiliary examination revealed that his serum CK level was 425 U/L (normal 50–310 U/L) (Table 2). RNS at 3 Hz led to decremental responses in the abductor digiti minimi (–43.3%) and abductor pollicis brevis (–49%) (Table 2). EMG findings suggested myogenic damage.

3.2 Histochemical observations

Biopsy of the right biceps brachii muscle was performed in the proband. H&E staining revealed that the muscle fibers had altered diameters, and atrophic muscle fibers were visible in small angular, long, and round shapes. In addition, the muscle fibers were surrounded by hypertrophic muscle fibers (Fig. 1C). Moreover, lipid storage in some muscle fibers was found in the proband (Fig. 1C). Analysis of the muscle biopsy by GT staining showed nuclear migration, aggregation, and nuclear bag fiber (Fig. 1D). In addition, muscle biopsy showed no denatured, necrotic, or regenerated muscle fibers and inflammatory cell infiltration, but showed hyperplasia in the connective tissue of the myometrium (Fig. 1D). GT staining showed rimmed vacuoles in some muscle fibers, whereas ragged-red fibers were not seen in some muscle fibers (Fig. 1D). Muscle biopsy of the right biceps brachii muscle showed myopathic changes in the muscle fibers. MRI showed prominent fatty infiltration in the thigh muscles including the gluteus maximus, rectus femoris, vastus lateralis, vastus intermedius, sartorius, adductor longus, adductor magnus, and gracilis (Fig. 1E).

3.3 Therapy

The patient was treated with oral pyridostigmine 60 mg three times daily until now. After taking the medicine, the patient's muscle weakness began to gradually improve.

3.4 Genetic finding

Genetic analysis of genomic DNA by WES revealed two compound heterozygous missense variants in GFPT1: c.331C>T; p.Arg111Cys and c.1428G>C; p.Lys476Asn (Fig. 2A). The proband harbored the c.331C>T; p.Arg111Cys missense mutation in the paternal allele and c.1428G>C; p.Lys476Asn in the maternal allele. The parents were heterozygous carriers of the corresponding mutation. Sanger sequencing of GFPT1 identified two mutations, of which one was novel (Fig.2B). The c.331C>T; p.Arg111Cys variant has been previously reported in a compound heterozygous state (2, 20), and has been recorded in the ClinVar dataset with the clinically significant classification of 'pathogenic/likely pathogenic'. This variant is seen at a frequency of 0.05% in the Latino population in the gnomAD global population dataset. The c.1428G>C; p.Lys476Asn variant has not been previously reported, and has not been recorded in the ClinVar dataset or gnomAD global population dataset. Genomic analysis using the UCSC Genome Browser showed that this mutation led to a change in the amino acid sequence, in that a highly conserved lysine residue was replaced. In addition, this variant was found to occur at a nucleotide position that is highly conserved among 100 vertebrates.

4. Discussion

We report a patient with a typical presentation of CMS due to compound heterozygous mutations in GFPT1. The onset of CMS in patients harboring a GFPT1 mutation usually occurs under 10 years of age (2, 25). In this study, the patient started experiencing limb weakness at 4 years old accompanied by limb fatigue after exercise. RNS revealed decrements in abductor digiti minimi and abductor pollicis brevis, and EMG assessment suggested myogenic damage. The proband responded well to an *acetylcholinesterase inhibitor*, and the phenotypes of the proband were consistent with previous reports of CMS (26–28). Thus, myasthenia gravis or CMS was initially suspected. However, he did not experience drooping eyelids or blurred vision, and did not have alleviation of symptoms in the morning and aggravation of symptoms in the evening, which are important characteristics of myasthenia gravis (29). The proband had pneumonia and underwent tracheotomy due to difficulty breathing. Liu *et al.* (30) indicated that patients with CMS have repeated apnea and become ventilator-dependent, and need a tracheotomy to alleviate symptoms as was the case in our study. The proband and his parents tested negative for acetylcholine receptor (AChR) antibodies. Furthermore, MRI in the proband showed prominent fatty infiltration in the thigh muscles. Thus, the patient was diagnosed with CMS. Symptoms of CMS markedly improved with treatment of 60 mg pyridostigmine, which was taken orally three times a day.

CMS has several distinctive clinical features including the presence of tubular aggregates (TAs) on muscle biopsy, increased levels of serum CK, proximal limb muscle weakness and fatigability starting in childhood (31). It is still unclear whether TAs are pathological structures or show compensatory reactions to a variety of pathogenic events, such as familial myopathies and other myopathies of uncertain

etiology (32, 33). Previous results have paved the way for understanding the etiology of this rare neuromuscular disorder that may be considered “TA myopathy with synaptopathy” (20). In the study by Zhang *et al.* (34), muscle biopsy revealed massive TAs within the muscle fibers of a patient with CMS. However, Guergueltcheva *et al.* (35) reported two patients with clinical features of CMS and GFPT1 mutations, but in whom TAs were not conclusively detected. These two patients were diagnosed with CMS at 7 and 19 years old, respectively (35). In addition, Finlayson *et al.* (36) reported a patient diagnosed with CMS at the age of 6, while TAs in muscle biopsies were not present in this proband. Muscle biopsy of the 30-year-old proband in this study showed no TAs in the myofibers, indicating that TA expression may differ in different muscles or during the lifetime of patients.

A genetic test identified two missense mutations (c.331C>T; p.Arg111Cys and c.1428G>C; p.Lys476Asn) in GFPT1. The c.331C>T; p.Arg111Cys variant has been previously reported in a compound heterozygous state (2, 20), and has been recorded in the ClinVar dataset and the gnomAD global population dataset, whereas the c.1428G>C; p.Lys476Asn variant has not been recorded in these two datasets. The c.331C>T; p.Arg111Cys variant was first found in two families, while functional analysis showed that enzymatic activities in the wild-type GFPT1 group were not different from those in the mutant GFPT1 (c.331C>T; p.Arg111Cys) group (2). In addition, Bauche *et al.* (20) reported a patient with CMS due to compound heterozygous mutations in GFPT1 (c.331C>T; p.Arg111Cys). Moreover, the variant was classified as ‘pathogenic/likely pathogenic’ according to the ClinVar dataset, and was seen at a frequency of 0.05% in the Latino population in the gnomAD global population dataset. According to the current data, the variant was classified as a likely pathogenic variant. The novel mutation c.1428G>C; p.Lys476Asn occurred at a nucleotide position that is highly conserved among 100 vertebrates. In addition, this variant led to a change in the amino acid sequence, as a highly conserved lysine residue was replaced, according to analysis using the UCSC Genome Browser. Moreover, Splice AI software predicted that this mutation could result in a deficiency in a variable splice donor site. Previous studies have indicated that mutations that disrupt the splicing code may contribute to a variety of diseases (37, 38). In addition, the novel variant c.1428G>C; p.Lys476Asn in GFPT1 was predicted to likely be deleterious by using the Pathogenicity Island Prediction Software. However, the pathogenicity of the mutation has not been assessed, so we classified this variant as ‘uncertain significance’.

GFPT1 is the rate-limiting enzyme in the hexosamine biosynthetic pathway, which contributes to the production of UDP-N-acetylglucosamine (UDP-GlcNAc), a donor substrate for O-linked and N-linked glycosylation of various proteins (39). In addition, GFPT1 provides important glycosylated groups to proteoglycan, glycoprotein, and glycolipid synthesis (40). Muscle, skeletal receptor tyrosine-protein kinase, AChR subunits, and integrins are the key proteins in the NMJ, and exert biological functions via glycosylation (41). Bauche *et al.* (20) showed that GFPT1 mutations typically result in decreased GFPT1 levels, which lead to abnormal muscle protein glycosylation. Senderek *et al.* (2) found that GFPT1 knockdown led to histological changes in zebrafish muscle with delayed maturation of the NMJ. In addition, a previous study showed that inhibiting GFPT1 enzymatic activity or small interfering RNA-mediated silencing of GFPT1 expression downregulated AChR expression on the cell surface *in vitro*, thus affecting neuromuscular transmission at NMJs (19). However, the molecular basis of CMS is not fully

understood. Therefore, further studies on the molecular mechanisms of this disorder are needed, which may provide valuable information that inform current therapies.

CMS is a rare inherited condition, that is very difficult to diagnose (42). In this study, the patient began experiencing symptoms of CMS at 4 years old, but was not diagnosed with CMS until 30 years old. Here, we described the symptoms, evaluation, and management of a patient with CMS, and identified two compound heterozygous mutations. These findings may lead to the earlier diagnosis of this disease and an earlier start to treatment.

5. Conclusions

CMS is a rare inherited condition, that is very difficult to diagnose (42). In this study, the patient began experiencing symptoms of CMS at 4 years old, but was not diagnosed with CMS until 30 years old. Here, we described the symptoms, evaluation, and management of a patient with CMS, and identified two compound heterozygous mutations. These findings may lead to the earlier diagnosis of this disease and an earlier start to treatment.

Declarations

Ethics approval and consent to participate: The experiment was approved by the Ethics Committee of the First Hospital of Jilin University and Written informed consent was obtained from the proband.

Consent for publication: The authors agree this paper to be published

Availability of data and material: Please contact author for data requests.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: YFJ provides the funds needed for the research; SWL is responsible for collecting case information; CYW is responsible for performing Electromyography (EMG) including repetitive nerve stimulation (RNS) on the patient. YY and PZ are responsible for data querying and interpretation. XFY is responsible for the overall diagnosis and treatment of the patient. All authors read and approved the final manuscript.

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Tables

Table 1 Primer sequences.

Name		Primer sequences (5' – 3')
Chr2-69590659	F1	ACTCAAGCAATCCTCCCAACTC
	R1	TTCCAAGAGAGCAGCATTGTAA
Chr2-69565030	F1	TAGCCAGCAGAGACCTTTTCAA
	R1	TGTGTGTTATGGTTGGCCTACTT

Table 2 Summary of clinical features

Individual	The proband
Sex/age (years)	M/30
Age at onset (years)	4
First symptoms	Fatigue after limb activities
Limb-girdle weakness	No
Creatinine kinase levels	Normal
RNS: decrement at 3 Hz%/muscle	
abductor digiti minimi	43.3
abductor pollicis brevis	49

Figures

Figure 1

Patients with GFPT1-CMS and histochemical observations. (A, B) Photographs of the proband. Muscle biopsy from the biceps brachii in the proband showed mild changes on (C) H&E and (D) modified GT staining. Black arrow pointing to the thickened muscle fibers. Red arrow pointing to the edge cavitation. (E) T1-weighted muscle MRI scans from a patient with GFPT1-CMS. Bone and fat appear white, and muscle appears dark gray.

Figure 2

Identified mutations in the proband. (A) Sanger sequencing confirmed the WES genotypes of the GFPT1(c.331C>T; p.Arg111Cys and c.1428G>C; p.Lys476Asn) genes of the family members. Vertical arrows indicate the mutation site. (B) Schematic representation of GFPT1 protein with three domains and the position of the mutations identified in individuals suffering from CMS. The mutation identified in the proband reported here is underlined, and the novel one is in red.