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Novel compound heterozygous *GFPT1* mutations in a family with limb-girdle myasthenia with tubular aggregates

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Abstract

Limb-girdle myasthenia with tubular aggregates, a subtype of congenital myasthenic syndrome, is an extremely rare autosomal recessive genetic disease characterized by prominent limb-girdle weakness and good response to acetylcholinesterase inhibitor therapy. Herein, we reported two novel mutations of *GFPT1* gene in a Chinese pedigree. Two siblings presented with fatigue, weakness of limb-girdle and decrement of the muscle action potential with repetitive nerve stimulation. Thus, myasthenia gravis was initially suspected, but anti-AChR antibodies were negative. Two novel missense mutations (p.Lys154Asn and p.Asn363Ser) in *GFPT1* were identified through genetic testing conducted on 167 well-established genes associated with muscular diseases by targeted high throughput sequencing. Both mutations have not been recorded in the dsSNP database, Exome Aggregation Consortium database and 1000 Genomes Project database. The mutation sites were co-segregated with the phenotype and conserved between the different species. The mutations were not found in the 200 unrelated normal controls. Muscle biopsies revealed tubular aggregates, in accordance with previous reports with *GFPT1* mutations. Subsequently, dramatic improvement in strength occurred following anti-cholinesterase therapy. Our study will be helpful for the diagnosis and treatment for Limb-girdle myasthenia with tubular aggregates.

Keywords: GFPT1; Limb-girdle myasthenia; Tubular aggregates; AChR.

1. Introduction

Congenital myasthenic syndrome (CMS) is a clinically and genetically heterogeneous group of inherited disorders characterized by defects in neuromuscular transmission resulting from mutations in numbers of genes [1–3]. CMS is clinically similar to the autoimmune disorder myasthenia gravis, leading to transient muscle weakness and fatigue [4,5]. Herein, we described the clinical features of two siblings in a Chinese family, who presented with transient weakness of the limb-girdle and fatigue, but the result of anti-acetylcholine receptor antibodies assay was negative and the facial or extraocular muscles were not involved. Through high throughput sequencing methods commonly referred to as next generation sequencing (NGS) targeting the exons of genes associated with muscular diseases, we identified novel compound heterozygous mutations of the glutamine-fructose-6-phosphate transaminase 1 (*GFPT1*) gene, which have been described in limb-girdle myasthenia with tubular aggregates (LGM with TAs). And muscle biopsies revealed tubular aggregates in the proband. Subsequently, dramatic improvement occurred in the two siblings by anti-cholinesterase therapy.

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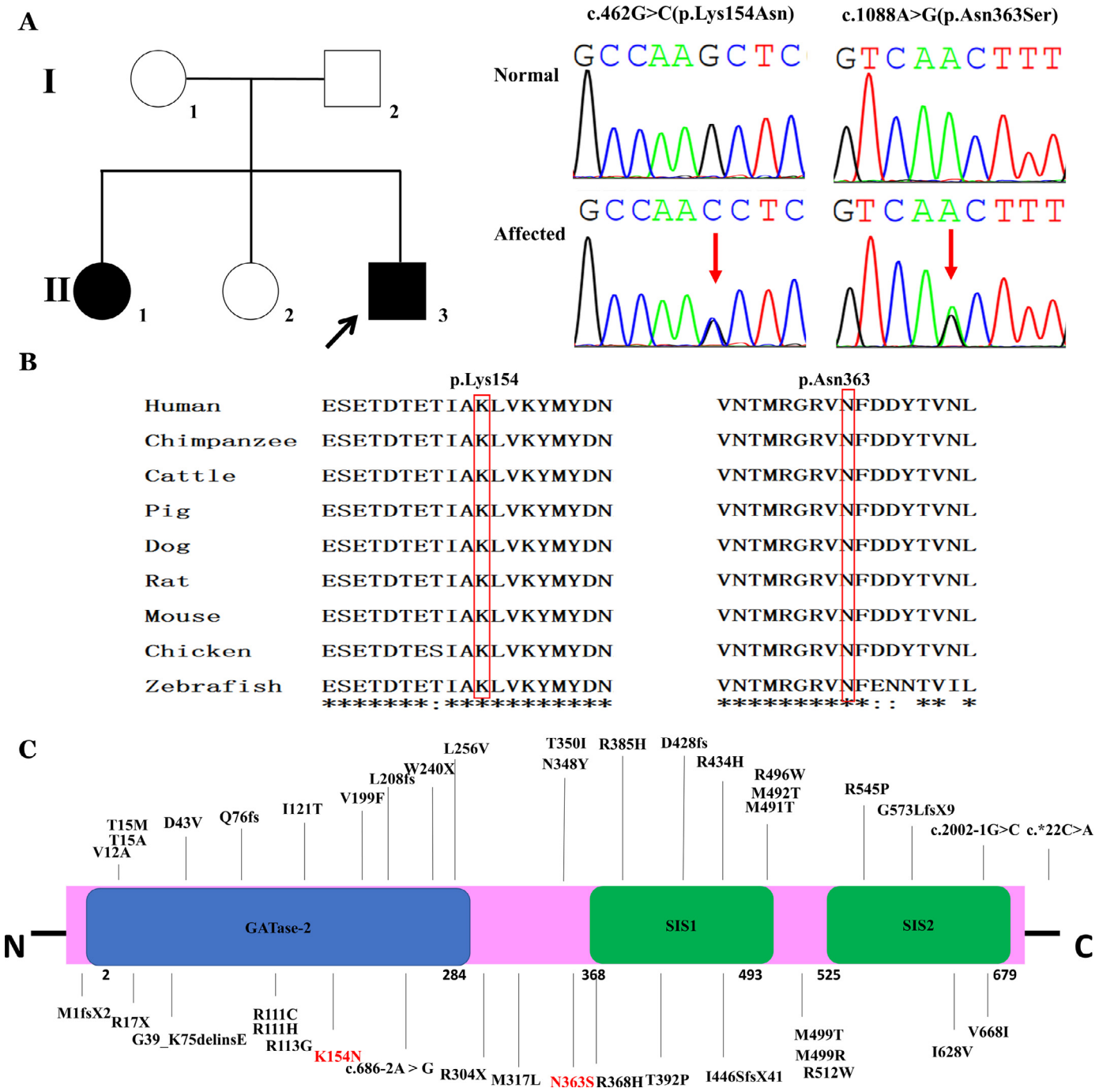

Fig. 1. Pedigree of the family affected by GFPT1 mutations. (A) Pedigree (left Panel). Open symbols, unaffected; filled symbol, affected; arrow, proband; sequencing chromatograms (right panel). Vertical arrows indicate the mutation site. (B) Conservation of the missense mutations. (C) Schematic representation of GFPT1 protein domains and the position of the mutations identified in the patients. The mutations of the present study are in red.

2. Materials and methods

2.1. Ethics statement

A 2-generation 5-member family with a history of CMS was recruited for this research (Fig. 1(A)). This study was approved by the local research ethics committee. Each family member provided informed consent for the participation in the study. Consent has also been obtained for publishing any recognizable persons in photographs.

2.2. Clinical material investigation

The family was from the Henan province, in the central territory of China. In addition to formal clinical assessment, serum creatine kinase levels and titres of anti-AChR

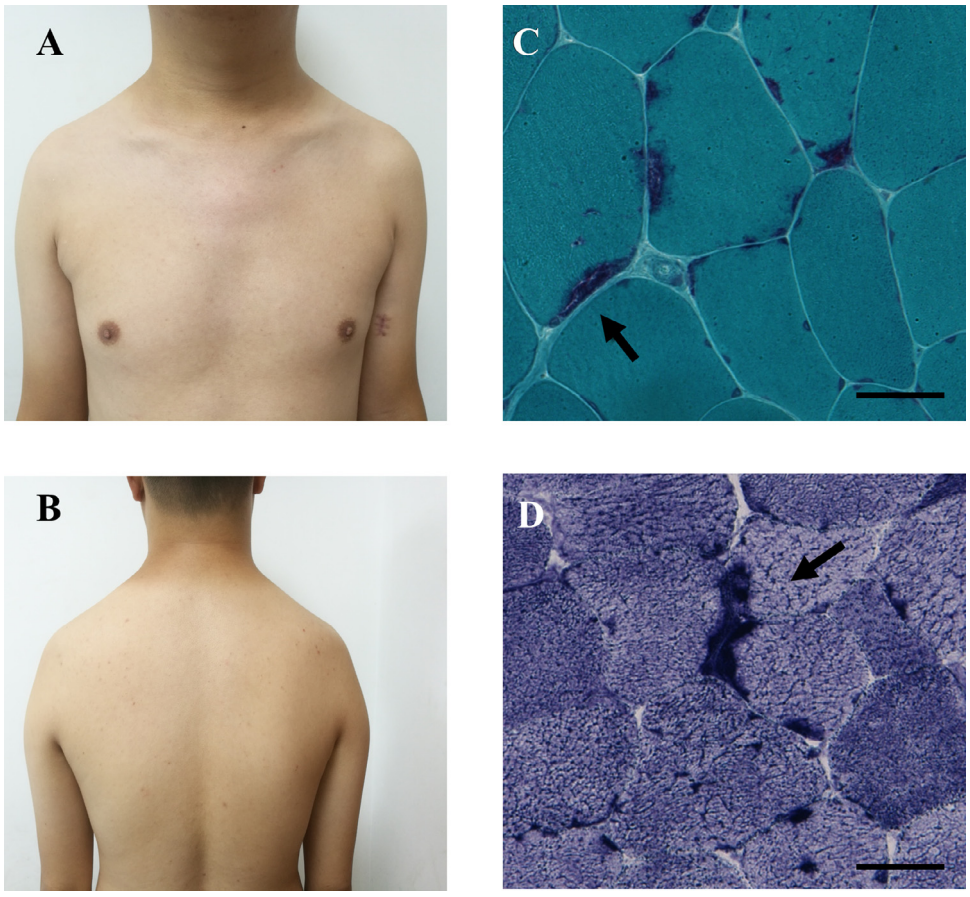

Fig. 2. Photographs and muscle biopsy sections of the individuals with *GFPT1* mutations. (A) and (B) Photographs of the proband. Muscle wasting and transient weakness of the shoulder. (C) Tubular aggregates stained dark purple by modified Gomori trichrome (II-3). (D) Tubular aggregates shown by NADH dehydrogenase reaction (II-3). Bars in (C) and (D) = 50 μm.

antibodies were measured in all patients. Electromyography (EMG), nerve conduction studies and repetitive nerve stimulation were performed using standard techniques. Muscle biopsy from the biceps brachii muscle was performed by open technique. Cryosections were stained with hematoxylin and eosin (H&E), NADH tetrazolium reductase (NADH-TR), modified Gomori trichrome, and succinate dehydrogenase (SDH).

2.3. Mutation screening

Genomic DNA was extracted from the peripheral white blood cells of the five family members by conventional methods. Genetic testing for the proband was performed on 167 genes known to be associated with muscular diseases by targeted high throughput sequencing. Genomic DNA was sheared to an approximate mean fragment length of 300 bp using the Covaris LE220 AFA instrument (Covaris Inc., Woburn, MA). The enrichment of targeted exons was performed using the Agilent SureSelect Target Enrichment System Kit (Agilent, 5190-7324). The pooled sample library was sequenced on NEXTSEQ 500 (Illumina) following the manufacturer's protocol. Subsequently, sequences from the sample were then aligned to the GRCh37.1 (hg19) genome build. Basic variant annotations were generated using PolyPhen-2.2.2 and ANNOVAR. Sanger sequencing targeting the relevant mutations was performed in the family and 200 unrelated normal controls.

3. Results

3.1. Clinical feature

The proband (II-3), a 23-year-old male, started experiencing weakness at the age of 5 years. The main manifestation of his disease were transient muscle weakness and fatigue. The weakness, difficulties with running and keeping his arms up, typically increased with exercise and repetitive muscle use (fatigue), and he was much better in the morning than in the evening. The neurologic examination revealed a waddling gait. The muscles of his proximal limbs were all weak (Fig. 2(A) and (B)), without ptosis and with intact extraocular movements (Table 1). The creatine kinase in serum was normal. Repetitive stimulation of the left ulnar nerve at 3 Hz revealed decrement in the peroneal nerve (Table 1). The similar symptoms were also observed in his older sister (II-1), while the creatine kinase in serum was increased to 380 U/L (10–190 U/L). Thus, myasthenia gravis

Table 1
Summary of clinical features in the family.

Individual	II-1	II-3
Sex/age (years)	F/32	M/23
Age at onset (years)	7	5
First symptoms	Fatigability	Fatigability
Symptoms course	Stable	Stable
Limb-girdle weakness	Yes	Yes
Fluctuation	Yes	Yes
CK levels*	2N	1N
Deep tendon reflexes	Diminished	Diminished
RNS: decrement at 3 Hz%/muscle		
Trapezius	31	55
Quadriceps	56	62
Abductor digiti minimi	27	46
Tubular aggregates in muscle biopsy#	Yes	NT
Response to AChEI	Positive	Positive

* N, Normal.
# NT, not test.

was initially suspected, but all patients tested negative for the anti-AChR antibodies. A biopsy of the left biceps brachii muscle was performed on the proband. NADH reaction (Fig. 2(D)) revealed TAs in a few fibers (arrow) and TAs were stained in red-purple (arrow) by modified Gomori trichrome (Fig. 2(C)).

3.2. Genetic finding

The targeted high throughput sequencing identified c.462G>C(p.Lys154Asn) and c.1088A>G(p.Asn363Ser) mutations (Fig. 1(A)) in the *GFPT1* gene (RefSeq: NM_00124710.1). The mutations have not been recorded in the dsSNP database, Exome Aggregation Consortium database and 1000 Genomes Project database. Sanger sequencing confirmed that the mutations were fully co-segregated with the phenotype in the pedigree. The proband (II-3) and his affected older sister (II-1) carried the p.Lys154Asn missense mutation in the paternal allele, and p.Asn363Ser missense mutation in the maternal allele, which was inherited down to his healthy older sister (II-2). The mutation sites and surrounding amino acid sequences were conserved between the different species (Fig. 1(B)). And the mutations were not found in the 200 controls.

3.3. Response to therapy

Subsequently, the patients were treated with oral pyridostigmine 60 mg 3 times daily, and a dramatic improvement in strength occurred. After 3 months of treatment, the patients had nearly no limitations in daily activities, although they can present with weakness after working for long hours.

4. Discussion

We reported a family with a typical presentation of LGM with TAs due to novel compound heterozygous mutations in *GFPT1*. The clinical features of the patients included prominent limb-girdle weakness, frequent tubular aggregates in the skeletal muscle and good response to acetylcholinesterase inhibitor therapy. Electrophysiological assessment showed signs of myopathy and repetitive stimulation revealed decrement in the peroneal nerve. The phenotypes of the patients were in accordance with previous reports of LGM with TAs [6–9] and our molecular findings are in complete agreement with the diagnosis of LGM with TAs.

Due to fatigue, prominent limb-girdle weakness and decrement after repetitive stimulation in electrophysiological study, myasthenia gravis was initially suspected. However, all patients tested negative for the anti-AChR antibodies. Subsequently, novel missense mutations (p.Lys154Asn and p.Asn363Ser) in *GFPT1* were identified. Both mutations have not been recorded in the dsSNP database, Exome Aggregation Consortium database and 1000 Genomes Project database. Analysis of available individuals within the family revealed that the mutations of the *GFTP1* gene consistently segregated with the disease. The novel mutation sites were well conserved between the different species and were not found in the 200 controls. The novel variant c.462G>C(p.Lys154Asn) in GFPT1 was predicted to be deleterious by SIFT (score: 0.01) and PolyPhen-2 (score: 1.0). The novel variant c.1088A>G(p.Asn363Ser) in GFPT1 was predicted to be disease causing by MutationTaster, while benign in SIFT (score: 0.027) and PolyPhen-2 (score: 0.012). Muscle biopsies revealed tubular aggregates by NADH dehydrogenase reaction, and the two siblings responded well to anticholinesterase medication. These are in accordance with previous reports with *GFPT1* mutations. According to ACMG standards and guidelines, the variants were classified as likely pathogenic variants [10].

GFPT1, the first and rate-limiting enzyme of the hexosamine biosynthetic pathway [11], leads to UDP-N-acetylglucosamine, serving as the common precursor for amino sugars which are essential for protein and lipid modifications [12,13]. Post-translational modifications are crucial for lots of key molecules at the neuromuscular junction including AChR [14,15]. Previous findings strongly suggested that disturbed amino sugar metabolism by *GFPT1* mutations is responsible for LGM with TAs [2]. The *GFPT1* mutations generally lead to reduced GFPT1 levels and decreased cell-surface AChR expression in the cultured muscle cells and patient muscle biopsies [5]. In addition, a morpholino-induced GFPT1 knock-down in zebrafish resulted in altered muscle histology with delayed maturation of the neuromuscular junction [2]. Both inhibiting the GFPT1 enzymatic activity and siRNA silencing of GFPT1 expression in vitro resulted in a lower cell-surface expression of AChR [16]. A further potential mechanism for the impaired neuromuscular transmission might be associated with the altered glycosylation of the AChR-subunit resulting from either reduced expression or reduced catalytic activity of GFPT1 [16]. The impaired AChR- subunit glycosylation results in reduced steady-state levels of AChR α, δ, ε

subunits and inefficient export of AChR to the cell surface membrane. In contrast, the molecular basis of the disorder remains incompletely understood. Indeed, it remains a puzzle that *GFPT1* mutations selectively affect the neuromuscular transmission and muscle fibre architecture, while glycosylated proteins are widely distributed in many tissues and organs [3,17]. Further studies should be conducted to address the underlying mechanism.

In conclusion, we reported a family presenting with LGM with TAs, and identified novel compound heterozygous *GFPT*1 gene mutations. Our study will be helpful for the diagnosis and treatment for LGM with TAs patients.

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