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# Novel mutations in the C-terminal region of GMPPB causing limb-girdle muscular dystrophy overlapping with congenital myasthenic syndrome

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**Abstract**

Mutations in the *GMPPB* gene may underlie both limb girdle muscular dystrophy (LGMD) and congenital myasthenic syndrome (CMS). Forty-one cases have been reported to date and hotspot mutations are emerging in the Caucasian population. Clinical and pathological features of 5 patients with compound heterozygous GMPPB mutations were collected and retrospectively reviewed. In vitro functional analysis was performed to investigate the pathogeneity of GMPPB variants. The patients presented with proximal limb weakness in their first to second decades. Fluctuating muscle weakness, myalgia and calf hypertrophy were the major complaints. Myogenic changes on electromyography and marked attenuation on 3 Hz repetitive nerve stimulation were observed in all patients. Four reported a beneficial response to pyridostigmine. Muscle MRI showed selective involvement in the calf in case 1. Immunolabeling of α-dystroglycan was abnormal for case 1 and case 2. Four novel missense mutations in the C-terminal region of GMPPB were identified, with p.(Arg357His) being present in all the cases. In vitro functional assays demonstrated that these variants did not markedly reduce the amount of GMPPB, but gave rise to an increased propensity for protein aggregation. Increasingly, patients with GMPPB mutations are found to present with an overlapping LGMD/myasthenic syndrome. The mutation spectrum in Chinese patients may differ from that of European populations, with the mutation p.(Arg357His) most frequently found. These mutations may lead to abnormal folding of GMPPB leading to protein aggregates in the cytoplasm rather than an overall loss in protein expression.
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*Keywords:* Limb girdle muscular dystrophy; Congenital myasthenic syndrome; GMPPB; Mutation hotspot; Muscle MRI

## 1. Introduction

Limb girdle muscular dystrophy (LGMD) represents a group of genetically heterogeneous muscle diseases characterized by progressive proximal muscle weakness and atrophy. Emerging DNA technologies have broadened the genotypic spectrum [1]. A major subgroup of genes, for instance FKRP, POMT1, Fukutin, POMT2 and POMGnT1, are involved in the glycosylation of α-dystroglycan (α-DG). In 2013, new LGMD-causing mutations were identified within the gene encoding guanosine diphosphate mannose (GDP-mannose) pyrophosphorylase B (GMPPB) and thus the LGMD2T subtype was defined [2]. Later in 2015, GMPPB mutations were also found to be a cause of a congenital myasthenic syndrome (CMS) [3]. To date, at least 41 cases of GMPPB variants have been reported. The two mutations most frequently seen in the Caucasian population are c.79G>C p.(Asp27His) and c.860G>A p.(Arg287Gln) [2–7].

Here we identify four novel mutations in the C-terminal region of GMPPB genes in five Chinese patients from three kinships. All affected individuals carry a common mutation, c.1070G>A, p.(Arg357His).

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## 2. Materials and methods

### 2.1. Subjects and clinical assessments

All patients were of Chinese Han origin. Their first visits to our hospital were between 2014 and 2016 and since then they were followed up for clinical progression and treatment response. The study was approved by the Ethics Committees of Huashan Hospital, Fudan University. Informed consent was obtained from all patients.

### 2.2. Histology and immunohistochemistry

Muscle biopsies were obtained from the first two cases (Case 1 and Case 2) using standard techniques and were then snap-frozen by immersion in liquid nitrogen-cooled isopentane.

The following primary antibodies were used for immunohistochemistry with standard procedures: α-DG (VIA4-1 and IIH6C4, Millipore), ß-DG (Millipore), dystrophin (DYS1:Rod domain; DYS2:C-terminus; DYS3:N-terminus, Novocastra, Newcastle-upon-Tyne, UK), dysferlin (NCL-Hamlet, Novocastra), sarcoglycan (NCL-γ-SARC, NCL-α-SARC, NCL-β-SARC, NCL-δ-SARC, Novocastra), merosin (Chemicon, Massachusetts, USA). For each sample, a normal control was labeled at the same magnification and exposure to allow direct comparison between the patient and control sections.

### 2.3. Muscle imaging

We performed whole-body muscle MRI for case 1 and the thigh and calf muscle MRI for 3 cases (cases 2, 4 and 5). T1WI and T2WI sequences were employed on a 3-T MR scanner (Ingenia; Philips Medical Systems, The Netherlands). Each study was done using a 15-channel head coil, 12-channel posterior coils, and two 16-channel anterior Torso coils. The coronal series was fused to obtain composite whole-body coronal images, and then the transverse sections were analyzed and staged according to abnormal muscle bulk and signal intensity on T1-weighted images [8].

### 2.4. Genetic analysis

Genomic DNA was extracted from blood using standard procedures. The DNA samples were screened for targeted next-generation sequencing (NGS) of 157 genes related to neuromuscular diseases (Supplementary Table 1). All the mutations identified by NGS were subsequently confirmed by Sanger sequencing.

### 2.5. GMPPB protein expression studies

To explore the effect of novel variants, the corresponding mutations were introduced into cDNA by site-directed mutagenesis using QuikChange® kit from Stratagene. All sequences were confirmed by Sanger sequencing. HEK293 cells were transfected with wild-type or mutant constructs in combination with cDNA encoding GFP to monitor transfection efficiency. Forty-eight hours after transfection, the cells were harvested and lysed by rotating in cold lysis buffer for 1 h. Cell extracts were centrifuged and resuspended into protein loading buffer. Protein extracts were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membrane. The membrane was incubated respectively with primary anti-GMPPB antibody (Abcam, ab154061), anti-α-Tub (Sigma, T5168), anti-GFP antibody (Abcam, ab6556) and secondary anti-rabbit antibody conjugated to horseradish peroxidase (Dako).

At the same time, the transfected HEK293 cells were left to express for 48 hours, then fixed with 3% paraformaldehyde, permeabilized with 1% TX100 in PBS for 5 minutes at room temperature, and labeled with an anti-GMPPB antibody at room temperature for an hour. Cells were incubated with a secondary fluorescent antibody (Life Technologies Alexa Flour 568 goat anti-rabbit) for an hour at room temperature then washed and mounted for fluorescent light microscopy. The number of cells harboring punctate aggregates was counted for wild type and for each mutant GMPPB construct from within the transfected population of cells expressing both GMPPB and GFP (~50% of cells).

## 3. Results

### 3.1. Clinical findings

**Case 1 (Family 1).** A 29-year-old Chinese girl, born to a non-consanguineous couple was admitted to the hospital complaining of progressive proximal muscle weakness in lower limbs for 3 years, accompanied by muscle cramps and mild exercise intolerance. Fluctuation in muscle strength was also notable. She had normal motor milestones until 15 years of age. Cognitive function was normal. Physical examination in the morning disclosed prominent muscle weakness with 3/5 in neck flexors and 4/5 in proximal limbs, together with a waddling gait. On the same afternoon, her muscle strength was restored to near-normal. Facial, ocular and bulbar muscles were largely spared. Serum creatine kinase (CK) was elevated (2548 U/L, normal: <175 U/L). Electromyography (EMG) revealed myogenic changes. Further assessment of 3 Hz repetitive nerve stimulation (RNS) demonstrated a decrement in amplitude of compound muscle action potential (CMAP) especially in deltoid and trapezius (Table 1). Forced expiratory vital capacity (FVC) with 78.5% of predicted value was noted. Ultrasonic cardiography (UCG) and electrocardiogram (ECG) were normal. Muscle involvement on MRI was prominent in bilateral adductor magnus and longus, peroneus longus, medial and lateral gastrocnemius. A mild asymmetry was observed in the gastrocnemius (Fig. 1). One of the paraspinal muscles, multifidus muscle was selectively involved. The patient's weakness partially responded to pyridostigmine administration at an oral dose of 60 mg three times per day.

**Case 2 (Family 2).** A 19-year-old female patient born to a non-consanguineous family was admitted because of post-exercise myalgia and muscle cramps and slight difficulty in climbing upstairs first noticed in 2010 and exacerbated to persistent myalgia and limb weakness since 2014. She had delayed motor milestone and did not perform well on PE

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