

## **TITLE**

Clinical features of the myasthenic syndrome arising from mutations in GMPPB

## **AUTHOR'S NAMES AND AFFILIATIONS**

Pedro M Rodríguez Cruz,<sup>1,2</sup> Katsiaryna Belaya,<sup>1</sup> Keivan Basiri,<sup>3</sup> Maryam Sedghi,<sup>4</sup> Maria Elena Farrugia,<sup>5</sup> Janice L. Holton,<sup>6,7</sup> Wei Wei Liu,<sup>1</sup> Susan Maxwell,<sup>1</sup> Richard Petty,<sup>5</sup> Timothy J. Walls,<sup>8</sup> Robin Kennett,<sup>2</sup> Matthew Pitt,<sup>9</sup> Anna Sarkozy,<sup>7</sup> Matt Parton,<sup>7</sup> Hanns Lochmüller,<sup>10</sup> Francesco Muntoni,<sup>11</sup> Jacqueline Palace,<sup>2</sup> David Beeson,<sup>1</sup>

<sup>1</sup> Neurosciences Group, Nuffield Department of Clinical Neurosciences, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, OX3 9DS, UK

<sup>2</sup> Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, Oxford, OX3 9DU, UK

<sup>3</sup> Neurology Department, Neuroscience Research Centre, Isfahan University of Medical Sciences, Isfahan, Iran

<sup>4</sup> Medical Genetics Laboratory, Alzahra University Hospital, Isfahan University of Medical Sciences, Isfahan, Iran

<sup>5</sup> Department of Neurology, Institute of Neurological Sciences, The Queen Elizabeth University Hospital, Glasgow, G51 4TF, UK.

<sup>6</sup> Department of Molecular Neurosciences, UCL Institute of Neurology, London, UK

<sup>7</sup> MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, Queen Square, London, UK

<sup>8</sup> Department of Neurology, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, UK

<sup>9</sup> Department of Clinical Neurophysiology, Great Ormond Street Hospital for children  
NHS foundation trust, London WC1N 3JH

<sup>10</sup> Institute of Genetic Medicine, John Walton Muscular Dystrophy Research Centre,  
MRC Centre for Neuromuscular Diseases, Newcastle University, Newcastle upon Tyne,  
NE1 3BZ, UK

<sup>11</sup> Dubowitz Neuromuscular Centre & MRC Centre for Neuromuscular Diseases, UCL  
Institute of Child Health, London, WC1N 1EH, UK

### **CORRESPONDING AUTHOR**

Professor David Beeson

Nuffield Department of Clinical Neurosciences, Neurosciences Group, Weatherall  
Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, UK

E-mail address: david.beeson@ndcn.ox.ac.uk

Tel.: +44 1865 222311

Fax: +44 1865 222402

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## **ABSTRACT**

**Background:** Congenital myasthenic syndrome (CMS) due to mutations in *GMPPB* has recently been reported confirming the importance of glycosylation for the integrity of neuromuscular transmission.

**Methods:** Review of case notes of patients with mutations in *GMPPB* to identify the associated clinical, neurophysiological, pathological and laboratory features. In addition, serum creatine kinase (CK) levels within the Oxford CMS cohort were retrospectively analysed to assess its usefulness in the differential diagnosis of this new entity.

**Results:** All patients had prominent limb-girdle weakness with minimal or absent craniobulbar manifestations. Presentation was delayed beyond infancy with proximal muscle weakness and most patients recall poor performance in sports during childhood. Neurophysiology showed abnormal neuromuscular transmission only in the affected muscles, and myopathic changes. Muscle biopsy showed dystrophic features and reduced  $\alpha$ -dystroglycan glycosylation. In addition, myopathic changes were present on muscle MRI. CK was significantly increased in serum compared to other CMS subtypes. Patients were responsive to pyridostigmine alone or combined with 3,4-diaminopyridine and/or salbutamol.

**Conclusions:** Patients with *GMPPB*-CMS have phenotypic features aligned with CMS subtypes harboring mutations within the early stages of the glycosylation pathway. Additional features shared with the dystroglycanopathies include myopathic features, raised CK levels and variable mild cognitive delay. This syndrome underlines that CMS can occur in the absence of classic myasthenic manifestations such as ptosis,

ophthalmoplegia or facial weakness, and links myasthenic disorders with dystroglycanopathies. This report should facilitate the recognition of this disorder, which is likely to be under-diagnosed and can benefit from symptomatic treatment.

## **INTRODUCTION**

Congenital myasthenic syndromes (CMS) are disorders caused by mutations in genes encoding proteins that are essential for maintaining the integrity of neuromuscular transmission [1]. All syndromes share the clinical features of fatigable weakness, but age at onset, presenting symptoms, distribution of weakness, and response to treatment vary depending on the molecular mechanism resulting from the genetic defect. At present, mutations in at least 21 different genes are known to cause CMS [2]. Most of these genes encode proteins with a direct role in neuromuscular transmission such as the acetylcholine receptor (AChR) subunits and in the development or maintenance of the NMJ integrity such as DOK7 [3,4]. More recently, an unexpected relationship between defects in the glycosylation of proteins and CMS was found by using next-generation sequencing technology [5–7]. Mutations in *ALG2*, *ALG14* [5], *DPAGT1* [6] and *GFPT1* [7] were shown to produce a CMS with a predominantly limb girdle pattern of muscle weakness and minimal facial and ocular involvement.

Glycosylation is a ubiquitous pathway defined by the sequential attachment of sugar moieties to the polypeptide backbone (Figure 1). Post-translational modification of proteins is crucial for protein folding, transport and functionality [8]. In CMS, one suggested mechanism is through glycosylation of AChR subunits, which is required for the correct assembly of AChR pentamers and for efficient export to the cell surface [9] and so, abnormal glycosylation results in a reduction of AChRs at the muscle endplates and a reduced synaptic response to acetylcholine [7]. Many other key NMJ proteins such as MuSK, agrin, integrins and neural cell adhesion molecules (NCAM) are also

glycosylated [10] and therefore their abnormal glycosylation could contribute to the pathogenic effect. It is not known why defects in this ubiquitous process result in a dysfunction mainly restricted to the NMJ.

*GMPPB* is a recently reported CMS-associated gene [11]. *GMPPB* encodes GDP-mannose pyrophosphorylase B that catalyzes the conversion of mannose-1-phosphate and GTP to GDP-mannose. *GMPPB* contributes to both the N-glycosylation and the O-mannosylation pathways (Figure 1). Mutations within the O-mannosylation pathway were originally identified in patients with a form of muscular dystrophy within the spectrum of dystroglycanopathies [12]. These disorders are characterized by reduction in  $\alpha$ -dystroglycan glycosylation, which is the most well characterized and functionally relevant O-mannosylated protein. Patients with autosomal recessive mutations in *GMPPB* had reduced glycosylation of  $\alpha$ -dystroglycan in skeletal muscle and variable degree of clinical severity ranging from congenital muscular dystrophies with associated structural brain involvement, to milder limb-girdle muscular dystrophy variants of late childhood onset [13]. Recently, we showed that autosomal recessive mutations in *GMPPB* can also cause CMS and bridge myasthenic disorders with dystroglycanopathies [11]. This increased the spectrum of disorders caused by *GMPPB* mutations and confirmed the importance of glycosylation for the integrity of neuromuscular transmission.

Here we describe in detail the clinical features and investigation results of eight patients with CMS due to mutations in *GMPPB* and investigate CMS syndrome-specific CK values within our cohort to confirm its usefulness in the differential diagnosis.

## **METHODS**

Next-generation sequencing and Sanger screening identified the underlying genetic mutations in *GMPPB* in 8 individuals from 6 different kinships (Table 1) with similar clinical features and a decremental response of compound muscle action potentials to repetitive nerve stimulation as described previously [11]. Cases 9-11, present in the report by Belaya et al. [11], who have a muscular dystrophy dystroglycanopathy due to mutations in *GMPPB* but no clear defect in neuromuscular transmission are not included. Here we reviewed the case notes of these GMPPB-CMS patients in order to produce a detailed clinical picture allowing improved recognition of this condition. We also retrospectively obtained the serum creatine kinase (CK) values in the Oxford CMS cohort, to confirm that measuring CK can be useful in differentiating GMPPB-CMS from other CMS subtypes (using the local upper limit of normal range 200 IU/L).

### *Patients*

Patients 1-5 are White European originating from the UK. Patients 6-8 belong to the same kinship and originate from Iran. Consent for publication of data was obtained from all patients. Ethics approval for analysis of DNA and tissue samples was obtained (OXREC B: 04.OXB.017 and Oxfordshire REC C 09/H0606/74).

## **RESULTS**

### Clinical features

#### *Clinical presentation*

Pregnancy and early-life features were uneventful in all patients except in case 4 with congenital hip dislocation and resulting delayed motor milestones. None of the

patients were ventilated as infants or had feeding difficulties. The median age of onset was 22 years (range 1.5 - 50 years). Case 1 presented at age 24 years with limited walking tolerance following an influenza illness. Previously her gait was unrestricted. Case 2 was unable to run during her teens and had lumbar hyperlordosis noted from her 20s. Case 3 was unable to get up off the floor and had difficulty lifting heavy objects at age 20 years. She also had decreased walking tolerance and had to rest after 5 minutes of walking. Case 4 had difficulty climbing-up ramps and walking long distances noted from her mid-20s. Case 5 presented at 18 months with an acute episode of generalised weakness and persistently raised CK. Subsequently, she remained clumsier and slow at school compared to peers. Cases 6-8 presented with gait disturbance (waddling), difficulty climbing stairs and muscle cramps at the ages of 16, 22 and 31 years, respectively. Of note, even though most patients had onset in adult life, they recall being slower than peers at school and not good at sports.

#### *Pattern of weakness at assessment*

All patients had a predominantly limb-girdle pattern of muscle weakness, which was more severe in the lower than in the upper limbs, with moderate to severe impairment and marked waddling gait in some cases. Mild distal weakness was present only in cases 5 and 8. Facial and eye movements were normal in all patients, and only case 6 had mild fatigable ptosis. Two patients complained of occasional double vision (cases 2 and 6), although no ophthalmoparesis was elicited during examination. Neck muscles were mild to moderately weak in 4 patients without manifest flexor/extensor preponderance. Axial weakness was present in 6 patients with moderate to severe lumbar hyperlordosis in 5. Mild swallowing difficulties, in the absence of speech

abnormalities, were reported by case 7. Only case 2 suffered from shortness of breath on exertion, although there was a background history of asthma. None of the patients have required respiratory support.

#### *Additional clinical features*

Episodes of muscle aches and cramps were reported (case 3, 5 and 6-8), mainly in the calves, and activity-induced, such as with walking. Cramps were occasional but variable in frequency depending on the period, and usually subsided by extending the affected muscle. Case 4 had hypertrophic calves and thighs on physical examination. Case 5 was diagnosed with cataracts aged 9. Contractures were found only in case 5 with tightness of Achilles tendons bilaterally. Unlike other myasthenic syndromes, most of the patients did not have easily demonstrable short-term fatigability of muscle strength. Case 3 had mild learning difficulties in school. None of the patients had known cardiac involvement.

#### *Course of disease*

The long-term course of disease was generally progressive with gradual deterioration in muscle strength and mobility over time. In addition, there were significant deteriorations during intercurrent illnesses. Case 1 currently walks only for short distances with assistance and requires physical assistance in terms of activities of daily living and self-care. Case 2, with onset in her teens, has been unable to climb stairs for the last decade, and has difficulties in standing from a seated position. Although she could lift loads during her adult life, she developed significant proximal arm weakness in her fifth decade. Case 3 is currently stable on treatment, and mainly finds it difficult

to hold heavy objects and walk long distances. Case 4 had a slowly progressive deterioration of her waddling gait, with more recent requirement of a walking stick. Case 5 gradually deteriorated during childhood and adolescence with proximal weakness and mobility problems. Prior to treatment, she had limited walk tolerance, and on a bad day she could only walk around the house and used a wheelchair for distance. Cases 6-8 still walk independently although have had progressive deterioration in their gait over the years. Fluctuations of muscle strength over the course of days and weeks were reported in cases 1-5. They were commonly related to intercurrent viral infections, although they also occurred following no particular precipitant (case 1 and 5). Cases 3, 4 and 6 reported significant worsening of symptoms with menstruation. Case 6 also reported worsening of symptoms during pregnancy.

#### *Response to treatment*

All patients treated with pyridostigmine had a positive response to treatment. The effect was notable in cases 1,3,5,6,7 and 8 but mild in cases 2 and 4. Case 3 increased her endurance so that she can walk up to 10 minutes without fatigue and is able to manage stairs at home independently. She recalls omitting her medication by accident on a few occasions and being much weaker afterwards. For Case 4, walking improved such that she stopped using her walking stick. Her proximal strength in the upper and lower limbs is now graded MRC 5 from a previous MRC score of 4. Case 5 reported improvement in her muscle strength and walking speed, with less fatigability and reduced wheelchair use. After pyridostigmine, Case 6 improved her long distance walking and improvements of ptosis and dysphagia were noted. Cases 1 reported

additional benefit from 3,4-DAP (30 mg/day) and salbutamol (0.1 mg/Kg/day), and cases 2 and 4 reported benefit from additional salbutamol (0.05-0.2 mg/Kg/day).

## Investigations

### *Neurophysiology*

All patients had findings consistent with abnormal neuromuscular transmission on electrophysiological testing (Table 2). Of note, repetitive nerve stimulation (RNS) and single fibre EMG (SFEMG) abnormalities were present only in proximal muscles, while testing of distal and facial muscles was normal in 4/4 cases tested, in keeping with the clinical absence of facial and distal involvement (Figure 2). Repetitive discharges were not observed in any case. Concentric needle EMG examination of affected muscles showed mild to moderate myopathic changes in 7/7 subjects tested. Of note, case 1 had 70% increment in CMAP amplitude following voluntary contraction and high frequency stimulation. Mild post-exercise facilitation was also seen in cases 2, 3 and 6.

### *Muscle biopsy*

Cases 1, 3, 4, and 5 underwent muscle biopsy (Table 2). All cases showed dystrophic features and reduced labeling for  $\alpha$ -dystroglycan when immunohistochemical staining was performed. Secondary reduction of laminin- $\alpha$ 2 chain on immunoblotting was noted in case 3 and 4. Cases 3 and 5 showed sparse inflammation and focal upregulation of MHC-I complex, mostly within regenerating fibres. Tubular aggregates, which have previously been described in some forms of CMS due to glycosylation pathway defects, were not found in any of the samples. The muscle biopsy from case 3 is described in detail in Figure 3A-I.

### *Muscle MRI*

Muscle MRI from cases 2 and 4, at age 66 and 42 years respectively, showed extensive changes in T1-weighted sequences in pelvis and thighs, with predominant involvement of the posterior compartment of the thighs and relative sparing of certain muscle groups (Figure 4A-B). Case 2 had MRI of the calves that showed additional involvement<sup>11</sup>. Case 5 had a normal muscle MRI at age 10 years.

### *Serum CK*

We reviewed the serum CK values of 133 available patients with CMS (Figure 5). All GMPPB-CMS patients tested had raised creatine kinase levels in serum ( $2350 \pm 660$  UI/L; range 418-4807 UI/L). The mean serum CK value of GMPPB-CMS patients was 10.74 times above the upper limit of normal and was significantly higher than in any other CMS subtype (one-way ANOVA;  $P < 0.001$ , Dunnett's multiple comparison test). Occasionally, patients with DOK7-CMS, Slow Channel Syndrome and GFPT1-CMS had modestly raised CK values.

## **DISCUSSION**

We describe the clinical and complementary features of a new CMS subtype due to mutations in *GMPPB*, and show that high serum CK values are a key marker for differential diagnosis from other CMS. GMPPB-CMS shares the features of fatigable muscle weakness, significant decrement on RNS and a positive response to cholinesterase inhibitors typical of the majority of patients with CMS. However, there are features that may help differentiate it from the more common CMS subtypes. First,

a later clinical onset; second, presentation is with proximal muscle weakness and, unlike many CMS, with sparing of ocular, bulbar and respiratory muscles; and third, the presence of additional clinical features such as raised CK, dystrophic changes on muscle biopsy, activity-induced muscle cramps, and variable association with cognitive impairment and cataracts.

Most, though not all of the more common forms of CMS, present earlier in life and have characteristic features such as severe ophthalmoplegia (AChR-mutations), respiratory crisis (Rapsyn-CMS, ChAT-CMS and fast channel syndrome) and worsening with pyridostigmine (slow channel syndrome and COLQ-CMS). DOK7-CMS can have a late presentation with proximal weakness [14], but lacks the positive response to pyridostigmine and the dystrophic changes on muscle biopsy. Slow channel syndrome [15] and DOK7-CMS [16] can have a myopathic component with non-specific changes on muscle biopsy (such as increased variation in fibre size and core-like areas) and mildly raised CK levels, but these are rarely above 5 times the upper limit of normality. Cramps can also be seen in patients with DOK7-CMS, and may worsen on treatment with  $\beta$ 2-adrenergic agonists.

CMS due to other glycosylation pathway defects (GFPT1[7], DPAGT1[17] and ALG2/14 [5]) have clinical similarities to GMPPB-CMS, including clinical presentation, pattern of weakness, and response to treatment. However, GMPPB-CMS has a greater degree of myopathic involvement as shown by the higher CK levels and dystrophic changes on muscle biopsy. This is likely due to the additional role of GMPPB in the O-mannosylation pathway and glycosylation of  $\alpha$ -dystroglycan. GFPT1, DPAGT1 and ALG2-CMS can have tubular aggregates on muscle biopsy [18]. These have not been

detected in GMPPB-CMS although electron microscopy, which is a more sensitive method to detect tubular aggregates, was only performed in one case. GFPT1-CMS can display modestly raised CK values [19] but in our experience these are not as high as seen in GMPPB-CMS. Extensive abnormalities in muscle MRI have also been seen in GFPT1, DPAGT1 and ALG2/14-CMS, but not consistently in the **more** common CMS subtypes, although some patients with slow channel syndrome and *DOK7* and *RAPSN* mutations can show a wide range of fatty infiltration [20]. It is, therefore, understandable that most of these disorders were classified previously as non-specific myopathies. Cases 3 and 5 were initially misdiagnosed as autoimmune myopathies and treated with immunosuppression without improvement. Serum CK levels are significantly higher in patients with GMPPB-CMS, and so will be helpful in the differential diagnosis. Biochemical studies of transferrin glycosylation on serum samples have been found to be abnormal in only a few of our patients with *DPAGT1* mutations [17], but could be abnormal in GMPPB-CMS. However we believe that a combination of decrement of CMAP with repetitive nerve stimulation on EMG with measurement of serum CK will give simpler and better diagnostic pointer.

We have not seen heart abnormalities in 3 patients with GMPPB-CMS undergoing cardiac studies. However, a small study showed mild cardiac abnormalities in CMS patients with mutations in *GFPT1* and *DPAGT1*, including abnormal electrocardiography (repolarization and deep S waves or marked ventricular hypertrophy by voltage criteria), myocardial fibrosis, diastolic dysfunction and impaired energetics by cardiovascular magnetic resonance, despite normal systolic function[21].

Mutations in *GMPPB* underlie a wide clinical spectrum. At the severe end of the spectrum, mutations in *GMPPB* lead to CMD with eye and brain abnormalities (Walker-Warburg syndrome and muscle-eye-brain disease) and congenital LGMD and CMD with mental retardation[13]. At the mild end of the spectrum, *GMPPB* mutations cause adult onset LGMD[22] and CMS [11], with mild or absent intellectual disability, and isolated rhabdomyolysis [22]. In the middle, there are patients with onset during childhood with variable degrees of weakness and cognitive involvement [23]. We have not observed a clear genotype/phenotype correlation and thus what drives the striking differences in the phenotype remains to be determined.

All subjects harboring *GMPPB* mutations present with consistent muscle abnormalities. In skeletal muscle, the dystroglycan complex works as a link between the extracellular matrix and the cytoskeleton, providing structural integrity in muscle tissue [24]. Post-translational glycosylation of  $\alpha$ -dystroglycan is essential for its function and binding to extracellular ligands [25]. A reduction in the glycosylation levels disrupts molecular interactions and contributes to sarcolemma instability and eventual muscle damage. In many cases *GMPPB* mutations do not lead to abnormal neuromuscular transmission [11]. Several *GMPPB*-CMS patients had mild facilitation in RNS that could indicate additional presynaptic dysfunction. The clinical deterioration seen in *GMPPB*-CMS during intercurrent illnesses is also of interest. This is a classic feature of CMS, but clearly not unique to CMS, as it is also seen in dystroglycanopathies due to *FKTN* mutations [26] and other gene defects (F. Muntoni, personal observation). The pathogenesis underlying this phenomenon is unknown.

In conclusion, we describe the clinical and complementary features of a new CMS subtype due to mutations in the glycosylation gene *GMPPB*. Patients with GMPPB-CMS have a comparable phenotype to other CMS subtypes with mutations affecting the early stages of the glycosylation pathway. They harbor additional features, shared with the dystroglycanopathies, include dystrophic changes on muscle biopsy and raised CK levels. We find that this CMS occurs in the absence of classic myasthenic manifestations such as ptosis, ophthalmoplegia or facial weakness, and therefore is likely to be under-diagnosed. We would suggest performing RNS on weak muscles in patients with the phenotype we describe (or in patients in whom *GMPPB* mutations have been detected) to determine if there is involvement of neuromuscular transmission. This report adds to the expanding clinical spectrum of *GMPPB* mutations and should facilitate the recognition of a subset of this genetic disorder, which can be treated symptomatically.

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### **CONTRIBUTORSHIP**

Pedro M Rodríguez Cruz was involved in the literature search, manuscript design and preparation. Katsiaryna Belaya, Susan Maxwell, Maryam Sedghi and Wei Wei Liu contributed to the genetic studies. Robbin Kennett and Matthew Pitt performed the neurophysiological studies. Janice L. Holton characterized the muscle biopsy. Keivan Basiri, Maria Elena Farrugia, Richard Petty, Timothy J. Walls, Anna Sarkozy, Matt Parton, Hans Lochmüller, Francesco Muntoni and Jacqueline Palace were involved in patients' care. All authors contributed to the critical review and approval of the final manuscript. David Beeson participated in the design of the study, has full access to all data in the study and had final responsibility for the decision to submit for publication.

#### **COMPETING INTERESTS**

- Pedro M Rodríguez Cruz reports no disclosures relevant to the article
- Kate Belaya is a fellow of the Wellcome Trust-funded OXION: Ion Channels and Disease Initiative.
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- Matt Parton reports no disclosures relevant to the article
- Francesco Muntoni has served on scientific advisory boards for Acceleron Pharma, Genzyme, AVI BioPharma, the Debiopharma Group, GlaxoSmithKline, Prosensa, Servier, Summit and Santhera Pharmaceutical. He receives research support from Trophos and Biomarin, PTC, Roche, Sarepta and Pfizer. He is board advisor for Pfizer.
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- David Beeson holds MRC Programme Grant MR/M006824/1.

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## **FIGURES LEGENDS**

**Figure 1: Schematic representation of the N-glycosylation pathway and CMS-causing genes (red colour).**

Initial steps of N-linked protein glycosylation take place in the ER. It starts with the assembly of the core glycan on the lipid dolichol, and follows the incorporation of

sugar moieties by a series of glycosyltransferases, first on the cytoplasmic face of the ER and then in the ER lumen, once the product is flipped. The core glycan is then released from dolichol and transferred to asparagine residues of nascent proteins by the multimeric oligosaccharyl transferase complex (OST). GMPPB catalyzes the formation of GDP-mannose from GTP and mannose-1-phosphate; GFPT1 synthesizes UDP-GlcNAc (Uridine diphosphate N-acetylglucosamine). While ALG2, ALG13/14 and DPAGT1 are glycosyltransferases implicated specifically in the N-linked glycosylation of proteins, GFPT1 and GMPPB are also involved in other glycosylation pathways (green). Of note, GMPPB is involved in the O-mannosylation pathway, which is required for the glycosylation of  $\alpha$ -DG, and thus is of special relevance to the dystroglycanopathies. DOLK, dolichol kinase; DPM, dolichol-phosphate mannose synthase; Fru-6-P, fructose-6-phosphate; GlcN-6-P, glucosamine-6-phosphate; Glu-6-P, glucose-6-phosphate.

**Figure 2: Neurophysiological studies.** Repetitive nerve stimulation in case 5 was normal in orbicularis oculi (A) compared to 25% decrement in *trapezius* (B) The stimulated SFEMG study of *orbicularis oculi* (C) was normal (mean MCD 16.2  $\mu$ s; 62% of the upper limit of normal) compared to the examination of *extensor digitorum communis* (D), which showed instability of the neuromuscular junction (mean MCD 54.7  $\mu$ s; 210% of the upper limit of normal), as seen in the superimposed traces.

**Figure 3: Muscle biopsy.** Muscle biopsy from *quadriceps femoris* in case 3 at age 25 years, showing variation in fibre size with occasional atrophic fibres and increase in internal nucleation with hematoxylin and eosin staining (A). There is a focal increase in endomysial collagen (B), occasional necrotic fibres (C) and regenerating fibres (D).

NADH staining shows mild disturbance of the internal architecture in a number of fibres (E). Numerous fibres express neonatal myosin (F). There is a pattern of slow fibre predominance with these being of generally small size (G). Immunohistochemical staining shows reduced labeling for  $\alpha$ -dystroglycan (H) compared to  $\beta$ -dystroglycan (I). Ultrastructural examination shows lack of accumulation of granular material, rimmed vacuoles or tubular aggregates (J). Range of fibre size A-I = 30-120  $\mu\text{m}$ ; bar in J = 5  $\mu\text{m}$

**Figure 4: Muscle MRI.** Individual muscles are labelled on the left side of the figure and coloured according to severity into spared/mild (green), moderate (yellow) and severe (red); complete T1 weighted sequences are shown on the right side. In the pelvis, there is prominent involvement of the gluteal muscles, *tensor fasciae latae* and *obturator internus* muscles (A). In the thighs, there is prominent involvement of the hamstrings and adductors and, relative sparing of the anterior thigh musculature, *sartorius* and *gracilis* muscles (B). AI, *adductor longus*; AM, *adductor magnus*; BF, *biceps femoris*; GM, *gluteus maximus*; Gr *Gracillis*; Obt, *obturator internus*; RF, *rectus femoris*; SM, *semimembranosus*; ST, *semitendinosus*; St, *sartorius*; TFL *tensor fascia latae*; VI, *vastus intermedius*; VL, *vastus lateralis*; VM, *vastus medialis*.

**Figure 5: CMS subtype-specific serum creatine kinase levels.** The figure shows Individual serum CK values for each CMS subtype together with mean  $\pm$  SEM for each CMS subtype. Patients with GMPPB-CMS had greater CK values in serum than any other CMS subtype (one-way ANOVA;  $P < 0.001$ , Dunnett's multiple comparison test). Numbers of patients included from each CMS category are displayed in brackets. The

local upper limit of normal range 200 was IU/L. Mean  $\pm$  SD values are provided as supplementary data (Table S1).

**Table 1: Clinical features of patients with GMPPB CMS**

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	
Mutation, DNA	c.79G>C c.859C>T	c.781C>T c.130-3C>G	c.79G>C c.760G>A	c.79G>C c.907C>T	c.559C>T c.578T>C	c.308C>T homozyg	c.308C>T homozyg	c.308C>T homozyg	
Mutation, protein	p.Asp27His p.Arg287Trp	p.Arg261Cys Splicing	p.Asp27His p.Val254Met	p.Asp27His p.Leu303Phe	p.Gln187* p.Ile193Thr	p.Pro103Leu	p.Pro103Leu	p.Pro103Leu	
Gender	F	F	F	F	F	F	F	M	
Age (y)	Presentatio n	24	15	20	25	1.5	16	22	31
	Current	48	68	28	44	18	43	45	35
Presenting symptoms	Limited march tolerance (following influenza)	Unable to run.	Unable to get up from the floor and lift weights.	Unable to climb up ramps.	Acute episode of weakness	Difficulty in climbing stairs, cramps.	Difficulty in climbing stairs, cramps.	Difficulty in climbing stairs, cramps.	
Ptosis	No	No	No	Mild, not fatigable*	No	Mild	No	No	
Ophthalmoplegia	No	No	No	No	No	Mild	No	No	
Facial weakness	No	No	No	No	No	No	No	No	
Bulbar weakness	No	No	No	No	No	Mild	No	No	
Neck weakness	Mild	No	Mild	Mild	Moderate	No	No	No	
Proximal weakness	UL	Moderate	Moderate	Moderate	Moderate	Moderate	Mild	Mild	No
	LL	Severe	Severe	Moderate	Moderate	Severe	Moderate	Moderate	Mild
Distal weakness	UL	No	No	No	No	Mild	No	No	No
	LL	No	No	No	No	Mild	No	No	No
Axial weakness	Moderate	Moderate	No	Mild	Severe	Severe	Severe	No	
Respiratory weakness	No	No	No	No	No	No	No	No	
Cognitive impairment /learning difficulties	No	No	Mild	No	No	No	No	No	
Other features			Cramps	Calves hypertrophy	Cataracts Cramps	Cramps	Cramps	Cramps	
Treatment	P, D, S	P, S	P	P, S	P	P	P	P	

Footnote: D, 3,4-DAP; LL, lower limbs; P, pyridostigmine; S, salbutamol; UL, upper limbs. \* Ptosis appeared at age 26 after developing thyrotoxicosis and receiving treatment for it.

**Table 2: Clinical investigations of patients with GMPPB CMS**

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
RNS * Muscle Decrement (%)	Anconeus (42%)	Anconeus (14%)	ADM (<10%) Anconeus (50%)	ADM (<10%) Trapezius (30%)	EDC (37%) FCU (17%) Nasalis (4%) OO (5%) Trapezius (25%)	APB (<10%) Nasalis (<10%) Trapezius (20%)	-	-
SFEMG	EDC Abnormal	EDC Abnormal	OO Normal EDC Abnormal	EDC Abnormal	OO Normal EDC Abnormal	-	-	-
Cardiac studies	-	Normal EKG	-	Normal EKG	Normal echo	-	-	-
Myopathic EMG	Yes	Yes	Yes	Yes	Yes	Yes	-	-
CK (Normal ≤ 200)**	2800	418	1600	2668	4807	701	-	-
Biopsy	Muscle	Quadriceps	-	Quadriceps	Quadriceps	Quadriceps	-	-
	Age	27	-	25	35	10	-	-
	Features	Dystrophic	-	Dystrophic	Dystrophic	Dystrophic	-	-
	Alpha-DG	-	-	Reduced	Reduced	Reduced	-	-
Muscle	Age (y)	-	66	-	42	8	-	-
MRI	Result	-	Abnormal	-	Abnormal	Normal	-	-

ADM, *abductor digiti minimi*; APB, *abductor pollicis brevis*; CK, creatine kinase; EDC, *extensor digitorum communis*; FCU, *flexor carpi ulnaris*; OO, *orbicularis oculi*. \* A decrement of more than 10% in CMAP amplitude indicates a defect in neuromuscular transmission; \*\*The specific reference range for CK in serum depends on individual laboratories but values greater than 200 are in general considered abnormal.