Expanding the phenotype of GMPPB mutations

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Dystroglycanopathies are a heterogeneous group of diseases with a broad phenotypic spectrum ranging from severe disorders with congenital muscle weakness, eye and brain structural abnormalities and intellectual delay to adult-onset limb-girdle muscular dystrophies without mental retardation. Most frequently the disease onset is congenital or during childhood. The exception is FKRP mutations, in which adult onset is a common presentation. Here we report eight patients from five non-consanguineous families where next generation sequencing identified mutations in the GMPPB gene. Six patients presented as an adult or adolescent-onset limb-girdle muscular dystrophy, one presented with isolated episodes of rhabdomyolysis, and one as a congenital muscular dystrophy. This report expands the phenotypic spectrum of GMPPB mutations to include limb-girdle muscular dystrophies with adult onset with or without intellectual disability, or isolated rhabdomyolysis.

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Abbreviations: α-DG = alpha-dystroglycan; LGMD = limb-girdle muscular dystrophy
Introduction

Dystroglycanopathies are a heterogeneous group of diseases caused by mutations in different genes that all lead to defect in alpha-dystroglycan (α-DG) function (Muntoni et al., 2011). Most of them are due to defects in the glycosylation of α-DG, leading to secondary dystroglycanopathies. A mutation of dystroglycan itself, leading to a muscular dystrophy, has only been reported twice (Hara et al., 2011; Geis et al., 2013).

Dystroglycan is translated from DAG1 and latter cleaved into two subunits, α and β. Beta-dystroglycan has a transmembrane domain and associates with dystrophin and other intracellular proteins. Alpha-dystroglycan locates extracellularly, working as a linker to extracellular ligands and is part of the dystrophin-associated glycoprotein complex providing stability to the sarcolemma. Post-translational glycosylation of α-DG is essential for the binding of a range of extracellular ligands and for its function (Yoshida-Moriguchi et al., 2010).

Mutations in 15 genes are known to be associated with secondary dystroglycanopathies. Most of them were originally described in patients with congenital muscular dystrophies, affecting not only muscle but also associated with eye and brain abnormalities, with a clinical onset by the age of 6 months. However, mutations in some of these genes have subsequently been associated with milder phenotypes manifesting as a limb-girdle muscular dystrophy (LGMD) without brain abnormalities or mental retardation (Table 1). There is a wide phenotypic spectrum for mutations in most of these genes. Many are frequently associated with severe phenotypes, although FKRP often presents as an adult-onset LGMD (Boito et al., 2005).

GDP-mannose pyrophosphorylase B (GMPPB) catalyses the formation of GDP-mannose, which is required for the glycosylation of lipids and proteins (Maeda et al., 2005). Recently, Carss et al. (2013) described eight paediatric patients with a dystroglycanopathy due to mutations in GMPPB, all of them presenting by 4 years of age. As in other dystroglycanopathies, the muscle phenotype ranged from severe congenital muscular dystrophy to LGMD. Common associated features were hypotonia, epilepsy (40%), intellectual disability (80%), cataracts (40%) and cardiomyopathy (25%). In 30% of patients the brain MRI showed cerebellar hypoplasia.

Since that series only two siblings with congenital muscular dystrophy and epilepsy due to GMPPB mutations have been reported (Raphael et al., 2014). Here we describe eight patients from five families with mutations in GMPPB, presenting with a broad spectrum of severity, from a severe congenital muscular dystrophy with neonatal onset to a case of isolated episodes of rhabdomyolysis. The latter represents the mildest end of the clinical spectrum of the disease reported to date.

| Table 1 Gene defects in secondary dystroglycanopathies and associated LGMD syndromes (Carss et al., 2013; Nigro et al., 2014) |
|---|---|---|
| Gene symbol | MIM number | Limb-girdle syndrome |
| POMT1 | 607423 | LGMD 2K |
| POMT2 | 607439 | LGMD 2N |
| POMGNT1 | 606822 | LGMD 2O |
| DPM2 | 603564 | |
| DPM3 | 605951 | |
| DOLK | 610746 | |
| LARGE | 603590 | |
| B3GNT1 | 605581 | |
| B3GALNT2 | 610194 | |
| FKTN | 607440 | LGMD 2M |
| FKRP | 606596 | LGMD 2I |
| POMGNT2 (previously known as GTDC2) | 614828 | |
| ISPD | 614631 | LGMD 2U |
| TMEM5 | 605862 | |
| GMPPB | 615320 | LGMD 2T |

Materials and methods

Subjects and clinical assessments

The study was approved by the Human Research Ethics Committees of the University of Western Australia and Sydney Children’s Hospitals Network (10/CHW/45) and informed consent was obtained from all patients.

Histology and immunohistochemistry

Muscle biopsies were obtained using standard techniques and were then snap-frozen by immersion in liquid nitrogen-cooled isopentane.

The following primary antibodies were used for immunohistochemistry using standard procedures: dystrophin (DY51, rod domain; DYS2, C-terminal and DYS3, N-terminal), α-sarcoglycan (50 DAG), β-sarcoglycan (b-SARC), γ-sarcoglycan (35 DAG), δ-sarcoglycan (d-SARC), β-dystroglycan (43 DAG) and spectrin (SPECT) all from Novocastra Laboratories (NCL) and α-DG (IIH6C4; UpState/Millipore), merosin (Chemicon) and caveolin-3 (Transduction Laboratories). For Patients 5 to 8, immunolabelling for α-DG was performed using the VIA4-1 clone. For each patient sample, age-matched healthy control samples were also labelled at the same time and images were taken using the same magnification and exposure settings to allow for direct comparison of the immunofluorescence between control and patient sections.

Immunoblot

Western blotting was performed as described previously (Cooper et al., 2003; Ravenscroft et al., 2007). For Patients
Genomic DNA was extracted from blood using standard procedures. Mutations in FKRP were excluded by Sanger sequencing in Families 1, 2 and 5 before they were submitted for next generation sequencing. Variations in GMPPB found by next generation sequencing were confirmed by Sanger sequencing and the exons harbouring the mutations were also Sanger sequenced in unaffected and affected relatives to show cosegregation of the mutations with the disease.

Whole exome sequencing was performed for Patients 1 and 5 as described previously (Ravenscroft et al., 2013). Variants were called using LifeScopeTM 2.5 (Life Technologies) and filtered using ANNOVAR (Wang et al., 2010) against the ENCODE GencodeV14 (Harrow et al., 2006). Two custom variant filtering steps were used: (i) against the 1000 Genomes database, variants with a minor allele frequency >0.5% excluded; and (ii) against the dbSNP137common database.

DNA from Patients 3 and 7 was submitted for neurogenetic sub-exomic supercapture for the targeted capture and next generation sequencing (ProtonTM, Life Technologies) of 336 genes. The 336 genes included 254 disease genes listed in the Neuromuscular Disorders Gene Table at the end of December 2012 (www.musclegetatable.fr) in which the known disease-causing mutations were detectable by next generation sequencing, a small number of then unpublished neuromuscular disease genes, candidate disease genes and 59 cardiomyopathy disease genes (Yau, 2014). The same ANNOVAR pipeline, described above, was used to annotate and filter variants.

Results

Clinical findings

Family I

Patient 1 was an Australian male (Fig. 1), born to a non-consanguineous couple, who presented at the age of 15 years with difficulty running and jumping. Pregnancy and delivery were normal. He started to walk independently at 12 months of age. He had experienced muscle cramps since childhood, especially after long walks or swimming in cold water. He had behavioural problems since early childhood, and breath-holding attacks had occurred from the age of 18 months to 6 years. As a teenager and adult he had recurrent depression.

Examination at the time of presentation revealed mild proximal weakness in upper and lower limbs involving deltoid, biceps, triceps, ilio-psoas, quadriceps and hamstring muscles. Deep tendon reflexes were present. There was no facial or bulbar weakness, and strength in distal limb muscles was normal. His calves were hypertrophic, and to a lesser extent, also his quadriceps. He had a positive Gowers’ sign. Creatine kinase was 5000 IU. An EMG, performed at the age of 13 years, showed a myopathic pattern in proximal muscles, associated with normal nerve conduction studies. ECGs performed routinely were normal.

Over the years there was very slow progression of his muscle weakness. Weakness was restricted to proximal muscles of limbs and he remained able to walk and climb stairs until he died from unrelated causes at 25 years of age.

Patient 2 complained of painful muscle cramps from the age of 6 years. He was first examined at the age of 14 after his brother, Patient 1, was diagnosed with muscular dystrophy. At that stage he had normal strength but enlarged calves. His creatine kinase was 3645 IU. At the age of 24 he started noticing fatigability at the end of the day, although strength was normal to examination. At age 26 he was first found to have mild weakness in iliopsoas and quadriceps muscles. His creatine kinase fluctuated between 3640 and 17703 IU. From the age of 26 he had a right bundle branch block on ECG. Echocardiography at that time was normal. His condition deteriorated very slowly. He continued to have enlarged calves and muscle cramps, although quinine reduced the number and intensity of cramps. At 24 years he developed insulin-dependent diabetes mellitus. He died from an unrelated cause at 30 years of age.

Family II

Patient 3 is an Italian female born to non-consanguineous parents (Fig. 1) who presented in her late twenties with slowly progressive limb-girdle weakness. Initially she noticed difficulties rising from a chair, and later developed difficulties walking. The examination at the age of 42 showed hypertrophic calves. She had weakness (4+/5) of proximal muscles in upper and lower limbs, with preservation of strength in distal muscles. Deep tendon reflexes were absent. By the age of 48 years she needed help to stand from the seated position and had started using a wheelchair for longer distances, but was still able to walk. By the age of 68 years she was wheelchair-bound. Examination at age 71 years showed 3+/5 strength in deltoid, biceps and triceps, 4+/5 in wrist extensors, with finger flexors, finger extensors, abductor pollicis brevis and interossei of normal strength. In lower limbs, iliopsoas and quadriceps were 3−/5, and tibialis anterior and gastrocnemius were 4+/5.

At the age of 42 she had an EMG showing a myopathic pattern in deltoid and biceps muscles. Nerve conduction studies were normal. Echocardiography at age 70 was
normal, and a respiratory assessment at age 70 revealed severe impairment of ventilatory capacity with a restrictive pattern. A muscle CT scan performed at the age of 71 years is shown in Fig. 2.

Patient 4 is a male sibling of Patient 3 who presented at the age of 35 with weakness in the upper and lower limbs. On examination he had obvious calf hypertrophy and a waddling gait. He had weakness in sternocleidomastoid (4/5), neck flexors (4+/5), deltoids (4+/5), biceps (4+/5), triceps (4+/5), iliopsoas (4/5), quadriceps (4/5), hamstrings (4/5), abductors of the thighs (4/5), adductors of the thighs (4+/5), and tibialis anterior (4+/5). His deep tendon reflexes were present. By the age of 50 he had deteriorated moderately and was unable to run or lift heavy objects. He could climb four or five stairs and walk 1.5 miles. At 35 years he was diagnosed with sino-atrial block, with atrial ectopics and aberrant ventricular conduction. From that time he had daily episodes of an irregular heart beat lasting ~15 min. Creatine kinase fluctuated between 800 and 1500 UI.

**Family III**

Patients 5 and 6 are Caucasian siblings born to non-consanguineous parents (Fig. 1) who presented in their early twenties with mild proximal weakness. Pregnancy was normal for both siblings but the older male sibling (Patient 5) was noted to have significant learning difficulties with autistic spectrum behaviour, and attended a special needs school. On examination at 46 years there was no facial weakness. There was minor right-sided scapular winging, with 4/5 neck extension and flexion. Proximal muscles were graded 3-/5 in the upper limbs and 2/5 in the lower limbs. There was minor tibialis anterior weakness (4+/5). Deep tendon reflexes were present. Creatine kinase was 1400.

The younger sister (Patient 6) had mild cognitive difficulties and required assistance with learning in a mainstream school. On examination at 46 years her proximal muscles were graded 3/5 in both upper and lower limbs, with preserved distal power.

**Family IV**

Patient 7 is a 19-year-old male born to non-consanguineous parents (Fig. 1) who presented with recurrent episodes of rhabdomyolysis from the age of 13 years. Severe muscle rigidity and stiffness accompanied the episodes. Pregnancy and delivery were uncomplicated. There was a delay in speech development and episodic nocturnal enuresis up to 15 years, but overall cognitive function was normal. During childhood he had several seizures and was diagnosed with Rolando epilepsy. He reported two to three episodes of rhabdomyolysis per year triggered by exercise. The examination between episodes was normal. The highest recorded creatine kinase was 35 000 IU with a baseline of ~700 IU when well. An *in vitro* contracture test was performed for malignant hyperthermia, which was negative to halothane and caffeine.

**Family V**

Patient 8 is a female born to non-consanguineous Caucasian parents (Fig. 1). Muscle tone was normal at birth with no contractures, but she required a feeding tube for 3 days due to a poor suck. Creatine kinase was noted to be elevated at 6 months of age. Gross motor milestones were delayed, sitting at 2 years, crawling at 2.5 years, and walking at 3 years of age. She was never able to run. Speech was also delayed. Examination showed generalized muscle weakness, strabismus and toe walking.
Abnormal choreoathetotic movements were also noted. No contractures or scoliosis were present. Frequent cramps and episodes of rhabdomyolysis, with creatine kinase rising up to 10,000 IU, occurred repeatedly without obvious triggers. A brain MRI showed cerebellar atrophy (Fig. 2).

**Muscle biopsy**

Muscle biopsies were available from all patients, except Patient 6. Findings, muscles sampled and patients’ age are summarized in Table 2. They all showed a mild to moderate dystrophic pattern (Fig. 3). Immunohistochemistry for α-DG was performed in Patients 4, 5, 7 and 8 and showed reduced patchy staining in Patients 4, 5 and 7. In Patient 8, immunohistochemistry for α-DG was performed in two different muscle samples obtained at the ages of 2 and 9 years. While the first one showed a normal result, the biopsy taken at 9 years of age showed a marked reduction of α-DG (Fig. 3). Otherwise the remainder of the immunohistochemistry, detailed in Table 2, was normal in all patient biopsies.

**Immunoblotting**

Western blotting for α-DG was performed in muscle from Patients 4, 5, 7 and 8. Patients 5 and 7 again showed a reduced amount of protein compared to control muscles. For Patient 8, the first biopsy, performed at 2 years of age, showed a similar amount of α-DG to control samples, while in the second biopsy (taken at 9 years of age) α-DG was markedly reduced. In the muscle from Patient 4, no obvious reduction of α-DG was detected (Fig. 4). Western blotting for calpain-3 and dysferlin was performed in muscle from Patient 2 and were normal compared to controls (data not shown).

**Genetic analysis**

Whole exome sequencing for Patient 1 resulted in 85.5 million on-target reads, giving 90-fold average coverage, with 79.8% of targets covered to >20-fold. Two heterozygous mutations in GMPPB were identified, both previously reported as pathogenic by Carss *et al.* (2013): c.79G > C (p.Asp27His) and c.95C > T (p.Pro32Leu). Screening of the sibling (Patient 2), parents and an unaffected brother showed co-segregation of the mutations with the disease.

Neurogenetic sub-exomic supercapture in Patient 3 resulted in 117-fold average coverage with 93.2% of targets covered to >20-fold and showed the presence of two heterozygous variants in GMPPB: c.79G > C (p.Asp27His) and c.797G > A (p.Cys266Tyr). The c.797G > A mutation is novel. The affected amino acid is conserved in all species (Fig. 5). The mutation is predicted to be disease-causing by MutationTaster (score 1), deleterious by Provean (score –9.864), damaging by SIFT (score 0.00) and Polyphen-2 (score 0.985). The variation is not present in the Exome Variant Server (http://evs.gs.washington.edu/EVS/), 1000 Genomes (www.1000genomes.org) or in the Exome Aggregation Consortium (http://exac.broadinstitute.org; accessed October, 2014) databases, meaning that it is not present in 63,000 normal control exomes. The patient’s unaffected daughter was screened for both mutations and carries the c.79G > A but not the c.79G > C mutation, suggesting that the variants are heteroallelic in Patient 3. Patient 3’s affected sibling, Patient 4, was screened by Sanger sequencing and found to harbour both mutations.

Whole-exome sequencing for Patient 5 resulted in an average read-depth of >80 and 10-fold coverage in >90% of the coding regions of the human genome and showed the compound heterozygous mutations c.79G > C (p.Asp27His) and c.1036C > A (p.Arg346Ser) in GMPPB. Screening of Patient 6, both parents and unaffected siblings showed co-segregation of the mutations with the disease.
Figure 3 Immunohistochemistry for \(a\)-dystroglycan. (A) A representative image for normal healthy control muscle. (B) Patient 4 showing patchy staining in some myofibres. (C) Patient 5 showing areas of reduced patchy staining. (D) Patient 7 showing reduced and irregular staining of some fibres. (E) Patient 8 at 2 years of age showing normal sarcolemma staining compared to control. (F) Patient 8 at 9 years of age showing an even reduction in the intensity of the labelling of the sarcolemma. Scale bar = 50 \(\mu\)m.

Figure 4 Immunoblotting for \(a\)-dystroglycan. Immunoblotting for \(\alpha\)-DG (ADG), performed at two different facilities, using the IIH6C4 antibody on patients (Pt) from this study, age-matched normal controls (NC) and an affected control (AC, from a patient previously communicated in the original report from Carss et al., 2013). Coomassie staining of the myosin heavy chain (MHC) band is shown to demonstrate similar loading of muscle protein across each blot. Patients 5, 7 and 8 at 9 years of age show reduced amount of protein compared to controls. Sample from Patient 8 at 2 years of age and Patient 4 show similar amount of /C11-DG as normal controls. L = protein ladder.

Figure 5 Amino acid conservation across species at the position of the novel substitutions.
Table 2 Muscle biopsies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Muscle</th>
<th>Age</th>
<th>Findings</th>
<th>Immunohistochemistry performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Left vastus lateralis</td>
<td>15</td>
<td>Increased variability in fibre sizes, splitting, central nucleation.</td>
<td>Dystrophin, β-dystroglycan, α, β, δ, γ-sarcoglycan, spectrin, merosin, caveolin-3</td>
</tr>
<tr>
<td>1</td>
<td>Right vastus lateralis</td>
<td>22</td>
<td>Marked variation in fibre size. Internal nucleation. Increased endomysial connective tissue. Necrotic fibres.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Left vastus lateralis</td>
<td>21</td>
<td>Central nucleation. Increased endomysial connective tissue. Necrosis.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Right quadriceps</td>
<td>42</td>
<td>Necrosis, regeneration, central nucleation. Increased endomysial connective tissue. Predominance of type 1 fibres.</td>
<td>Dystrophin, α, β-dystroglycan, α-sarcoglycan spectrin</td>
</tr>
<tr>
<td>4</td>
<td>Left vastus lateralis</td>
<td>35</td>
<td>Central nucleation, necrotic fibres.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Left triceps</td>
<td>50</td>
<td>Variation in fibres size, internal nucleation, split fibres, increase in fat and connective tissue. Occasional necrosis and regeneration.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Left quadriceps</td>
<td>22</td>
<td>Active degeneration with occasional fibre splitting. Internal nuclei. Minimal interstitial inflammatory infiltrate.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Left vastus medialis</td>
<td>18</td>
<td>Mild variation in fibre size. Occasional fibres with multiple central nuclei. One regeneration fibre. Acid phosphatase activity was increased in some of the small muscle fibres. Type 2 fibre predominance.</td>
<td>Dystrophin, α-dystroglycan, α-sarcoglycan, caveolin-3, telethonin, spectrin α-dystroglycan</td>
</tr>
<tr>
<td>8</td>
<td>Quadriceps</td>
<td>2</td>
<td>High variability in fibres size, internal nuclei, small round fibres.</td>
<td>α-dystroglycan, spectrin</td>
</tr>
<tr>
<td>8</td>
<td>Quadriceps</td>
<td>9</td>
<td>Dystrophic features. Focal areas of aggregated degenerate cells. Type 1 fibre predominance</td>
<td>α-dystroglycan, spectrin</td>
</tr>
</tbody>
</table>

The second is a novel mutation, involving a highly conserved amino acid (Fig. 5), not present in 1000 Genomes, Exome Variant Server or Exome Aggregation Consortium databases and predicted to be disease-causing by MutationTaster (score 0.99), deleterious by Provean (score −5.2) and damaging by SIFT (score 0).

Neurogenetic sub-exomic supercapture in Patient 7 resulted in 147.1-fold average coverage with 93% of targets covered to >20-fold, revealing compound heterozygous mutations in GMPPB. A c.458C>T (p.Thr153Ile) change has not been previously reported as a disease-causing mutation and is absent from the 1000 Genomes, Exome Variant Server and Exome Aggregation Consortium databases. In silico tools predicted functional changes in the protein, including MutationTaster (disease causing, score 0.89), Provean (deleterious, score −3.178), SIFT (damaging, score 0.01) and PolyPhen-2 (possibly damaging, score 0.81). The variation involves an amino acid conserved up to mouse (Fig. 5). The second mutation is c.860G>A (p.Arg287Gln), previously reported (Carss et al., 2013).

Neurogenetic sub-exomic supercapture in Patient 8 resulted in 275.4-fold average coverage, and identified heterozygous c.95C>T (p.Pro32Leu) and c.860G>A (p.Arg287Gln) mutations, both previously described to be pathogenic (Carss et al., 2013). Mutations and associated clinical findings in each patient are summarized in Table 3.

Discussion

Dystroglycanopathies are a genetically and phenotypically diverse group of disorders. The clinical spectrum spans from the severe Walker-Warburg syndrome with cerebral and ocular involvement with prenatal onset or onset at birth to muscle-eye-brain/Fukuyama congenital muscular dystrophy (MEB/FCMD)-like, to congenital muscular dystrophy with cerebellar involvement, and congenital muscular dystrophy and LGMD with or without mental retardation with a structurally normal brain and age of onset ranging from congenital forms to late adulthood (Godfrey et al., 2007). The phenotypic spectrum described in patients with mutations in GMPPB varies from MEB/FCMD-like syndrome to LGMD (Carss et al., 2013; Raphael et al., 2014). In the Carss et al. (2013) report, seven of the eight patients had mental retardation and all had onset of muscular weakness by 4 years of age.

Our study reports a second cohort showing a much wider phenotypic spectrum, ranging from severe congenital muscular dystrophy with rhabdomyolysis episodes, to learning difficulties with late-onset muscular weakness, to adult-onset LGMD with normal cognition, and a patient with isolated episodes of rhabdomyolysis but otherwise asymptomatic between episodes. Interestingly, two of the patients described had cardiac conduction defects, although both were benign. Neither of the two patients that reached their eighth decade had major conduction defects or cardiomyopathy.
Reduced glycosylated α-DG was seen by immunohistochemistry in the muscle of our patients carrying novel mutations (Patients 4, 5 and 7). Accordingly, muscle from Patients 5 and 7 showed a reduction in the amount of glycosylated α-DG protein by immunoblotting. This supports the idea that the pathogenic mechanism in these milder patients is the same as that of the patients with previously described mutations. However, in muscle from Patient 4 at the age of 35 years no reduction of α-DG was noted by western blotting although the immunohistochemistry showed mildly reduced patchy staining of the sarclemma. Muscle from his affected sibling was not available to perform additional analysis. This result differs from that published by Carss et al. (2013) in paediatric patients. However, this is not surprising, as the reduction of α-DG seen in paediatric patients with GMPPB mutations is variable, but never complete. Patient 4 did not present until the age of 35 years, and thus was much milder phenotypically than the paediatric patients described by Carss et al. (2013). This may correlate with the mild reduction of α-DG. It is recognized that the reduction of α-DG in patients with dystroglycanopathies due to defects in FKRPO varies with the clinical severity and can be apparently normal in some patients (Brown et al., 2004). Moreover, we have shown in our most severe patient that the expression of α-DG can be normal at an early stage both by immunohistochemistry and western blotting, and show a severe reduction later on in the disease course. Therefore, normal α-DG staining does not necessarily exclude genes that are known to interfere with glycosylation of α-DG.

It is interesting to note that the mildest patient reported by Carss et al. (2013) (Patient 7) and six of our patients share the p.Asp27His substitution. C2C12 myoblasts transfected with GMPPB harbouring this mutation did not show mislocalization of the enzyme in the cytoplasm or abnormal aggregation, as opposed to myoblasts transfected with other GMPPB mutations detected in more severely affected patients (Carss et al., 2013). Taken together, these findings may suggest that the p.Asp27His substituted protein retains more functional activity than other mutants.

In conclusion, the clinical spectrum of dystroglycanopathies due to defects in GMPPB spans from MEB/FCMD-like disease to adult onset LGMD with normal cognition, and also isolated episodes of rhabdomyolysis, and is therefore similar to other dystroglycanopathies such as FKRPO mutations. This study suggests that GMPPB should be considered a candidate gene in patients with adult-onset LGMD even when α-DG staining appears normal.

### Table 3 Mutations identified and associated clinical features

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Mutation 1 (cDNA and chromosomal position)</th>
<th>Mutation 2 (cDNA and chromosomal position)</th>
<th>Age at onset and presenting muscle feature</th>
<th>Other associated features</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>c.79G &gt; C (p.Asp27His) chr3:49761081</td>
<td>c.95C &gt; T (p.Pro32Leu) chr3:49761065</td>
<td>15. Proximal limb weakness</td>
<td>Frequent cramps, enlarged calves. Behavioural problems</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>c.79G &gt; C (p.Asp27His) chr3:49761081</td>
<td>c.797G &gt; A (p.Cys266Tyr) chr3:49759552</td>
<td>Late 20s. Proximal limb weakness</td>
<td>Enlarged calves. Wheelchair-bound at age 68. Respiratory involvement at age 70</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>c.79G &gt; C (p.Asp27His) chr3:49761081</td>
<td>c.797G &gt; A (p.Cys266Tyr) chr3:49759552</td>
<td>35. Proximal limb weakness</td>
<td>Enlarged calves. Sino-atrial block</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>c.79G &gt; C (p.Asp27His) chr3:49761081</td>
<td>c.1036C &gt; A (p.Arg346Ser) chr3:49759313</td>
<td>Early 20s. Proximal limb weakness</td>
<td>Learning difficulties with autistic-spectrum behaviour. Scapular winging, neck flexors, extensors and tibialis anterior weakness</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>c.79G &gt; C (p.Asp27His) chr3:49761081</td>
<td>c.1036C &gt; A (p.Arg346Ser) chr3:49759313</td>
<td>Early 20s. Proximal limb weakness</td>
<td>Learning difficulties</td>
</tr>
</tbody>
</table>

Bold text indicates novel mutations; CK = creatine kinase.

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References


