Clinical and electrophysiological evaluation of myasthenic features in an alpha-dystroglycanopathy cohort (FKRP-predominant)

Paloma Gonzalez-Perez b, Cheryl Smith b, Wendy L. Sebetka c, Amber Gedlinske d, Seth Perlman c, d, 1, Katherine D Mathews c, d

a Department of Neurology, Massachusetts General Hospital, Boston, MA 02114, United States
b Department of Neurology, West Virginia University Hospitals, Morgantown, WV 26506, United States
c Department of Neurology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, United States
d Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, United States

Received 25 August 2019; received in revised form 16 January 2020; accepted 21 January 2020
Available online xxx

Abstract

A postsynaptic dysfunction of the neuromuscular junction has been reported in patients with alpha-dystroglycanopathy associated with mutations in guanosine diphosphate (GDP)-mannose pyrophosphorylase B gene (GMPPB), some of whom benefit from symptomatic treatment. In this study, we determine the frequency of myasthenic and fatigue symptoms and neuromuscular junction transmission defects in a fukutin-related protein (FKRP)-predominant alpha-dystroglycanopathy cohort. Thirty-one patients with alpha-dystroglycanopathies due to mutations in FKRP (n = 25), GMPPB (n = 4), POMGNT1 (n = 1), and POMT2 (n = 1) completed a six-question modified questionnaire for myasthenic symptoms and the PROMIS Short Form v1.0-Fatigue 8a survey, and they underwent 3 Hz repetitive nerve stimulation of spinal accessory nerve-trapezius and radial nerve-anconeus pairs. Results showed that fatigue with activity was common; 63% of the cohort reported fatigue with chewing. A defective postsynaptic neuromuscular junction transmission was not identified in any of the patients carrying FKRP mutations but only in one mildly affected patient with GMPPB mutations (c.79 G>C, p.D27H and c.402+1G>A, splice site variant). We conclude that symptoms of fatigue with activity did not predict abnormal neuromuscular junction transmission on electrophysiological studies in this cohort and that, unlike GMPPB subgroup, a defective neuromuscular junction transmission does not appear to be present in patients with FKRP-associated muscular dystrophies.

Published by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license. (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Keywords: Fukutin-related protein (FKRP); Fatigue; Myasthenia; Neuromuscular junction.

1. Introduction

Alpha-dystroglycan related muscular dystrophies (or α-dystroglycanopathies) are a heterogenous group of autosomal recessive-inherited muscular dystrophies characterized by hypoglycosylation of alpha-dystroglycan. To date, mutations in 18 genes have been identified to cause, either directly or indirectly, this glycosylation defect [1]. The phenotypic spectrum of this group of muscular dystrophies is strikingly broad ranging from congenital muscular dystrophy (CMD) with severe brain involvement to limb girdle muscular dystrophy (LGMD) [2–5].

The clinical spectrum of α-dystroglycanopathies was recently further expanded by the recognition of a myasthenic-type dysfunction of the neuromuscular junction (NMJ) in patients with guanosine diphosphate (GDP)-mannose pyrophosphorylase B gene (GMPPB) mutations, and their potential benefit from symptomatic treatment with pyridostigmine, salbutamol, or 3,4 diaminopyridine [6,7]. Furthermore, an important role of dystroglycans in both the formation and maintenance of the NMJ, and more
specifically in the clustering of the postsynaptic acetylcholine receptors, has been demonstrated [8,9]. Here, we determine if a neuromuscular transmission defect is unique to those with \textit{GMPPB} mutations or is also seen in a cohort of patients with \textit{α-dystroglycanopathies}, most of them with \textit{FKRP} mutations. We further investigated whether the presence of myasthenic symptoms and fatigue were associated with electrophysiologic evidence of a NMJ transmission defect in this cohort.

2. Patients and methods

Patient’s (or legal guardian’s) written consent was obtained in all cases. This study was approved by the University of Iowa institutional review board. All patients enrolled in the Iowa Wellstone Dystroglycanopathy Natural History Study who were seen during a 3-month period were invited to participate in this study. A total of 31 patients from 27 unrelated families agreed to participate. Of these, 25 patients (21 unrelated families) had \textit{FKRP} mutations, four unrelated patients had \textit{GMPPB} mutations, one patient had \textit{POMGNT1} mutations and another patient had \textit{POMT2} mutations. A six-question modified questionnaire to screen for myasthenic symptoms was administered to all participants except to patient#22 [10]; four of these questionnaires were filled in by the patient’s caregivers due to the severe intellectual disability of these subjects. In addition, 23 participants completed the Patient-Reported Outcomes Measurement Information System (PROMIS) Short Form v1.0-Fatigue 8a survey. Total raw PROMIS score for each participant was converted to standardized T-score with a mean = 50 (average for US general population) and standard deviation of 10.

Repetitive nerve stimulation (RNS) using the lowest supramaximal stimulus was performed on two nerve-muscle groups: right radial nerve-anconeus muscle and right spinal accessory nerve-trapezius muscle. The surface stimulating and recording electrodes were placed as previously reported [11,12]. These two muscles were selected based on their high sensitivity to detect a neuromuscular transmission defect and to duplicate the approach previously used in patients with \textit{GMPPB} mutations [13]. A train of 7 supramaximal stimuli were delivered at a frequency of 3Hz at rest, immediately after brief muscle activation of 10 sec, and after muscle activation of 1-minute duration. A 10 or higher percent decrement in the amplitude of the compound muscle action potential (CMAP) between the first and fourth, or first and fifth, responses was considered suggestive of a defect in neuromuscular transmission. All the RNS studies were performed with the same electromyography equipment (9400 Nihon Koken EMG system) and by the same two trained physicians.

The six-question modified questionnaire of myasthenic symptoms shows that fatigue with activity is common in this cohort. None of the participants reported diplopia, however, 19 (63.3%) reported fatigue with chewing, 8 (26.7%) fatigue with repeated swallowing, and 4 (13.3%) eyelid drooping. Most of the patients (n = 24, 80%) stated that their fatigue worsened in the afternoons or evenings. Of those, 18 (75%) also reported that they felt weaker later in the day. Chewing fatigability was reported in 14 out of 25 (56%) \textit{FKRP} patients, and in all \textit{POMGNT1}, \textit{POMT2}, and \textit{GMPPB} patients who participated in this questionnaire (Table 2). Chewing fatigability was the only symptom reported by Patient #23 whose phenotype was episodic exercise-induced rhabdomyolysis associated with \textit{GMPPB} mutations.

Individual T-scores for the 23 adults who participated in the PROMIS Short Form v1.0-Fatigue 8a scale are represented in Fig. 1A. Patients #23, #5 and #18 (the phenotype of the latter two was mild LGMD associated with \textit{FKRP} mutations) had the lowest fatigue T-scores. The PROMIS T-score of our \textit{α-dystroglycanopathy} cohort as a group was comparable to the reported T score for other adult chronic conditions (Fig. 1B).

Manual muscle testing (MMT) of right elbow extension revealed MRC ≤ 3, 4- to 4+, and 5 in 20%, 30%, and 50% of patients, respectively. Likewise, MMT of right elbow flexion showed MRC ≤ 3, 4- to 4+, and 5 in 30%, 60%, and 10% of patients, respectively. A total of 30 and 28 patients underwent RNS at rest of the anconeus and trapezius muscles, respectively. The mean CMAP amplitude at baseline of the anconeus and trapezius muscles were 4.624 mV (SD = 3.164 CI (95%) = 3.42−5.827) and 3.353 mV (SD = 1.799 CI (95%) = 2.641−4.065), respectively. A significant decrement (>10%) in the CMAP amplitude between the first and fourth, and first and fifth, responses was observed in Patient #23 only (Fig. 2) whose baseline CMAP amplitudes for anconeus and trapezius were 4.1 and 7.84 mV, respectively, and within normal range according to reported reference values [11,14–16]. This patient was treated with pyridostigmine 60 mg twice per day and prn, but he did not experience any significant benefit during two months on treatment and elected to discontinue it. Post-facilitation RNS of the anconeus and trapezius muscles were performed on 26 and 24 patients, respectively. No significant increment of the CMAP amplitude suggestive of presynaptic neuromuscular transmission defect was identified.

Of note, 3 patients declined RNS of spinal accessory-trapezius pair, a CMAP could not be obtained in one patient on RNS of radial-anconeus pair; 5 patients were not able to perform muscle activation due to intellectual disability or severe muscle weakness, and when patients were not able to maintain muscle activation for 1 min a minimum of 30 sec of exercise was accepted to obtain muscle exhaustion.

4. Discussion

We investigated an \textit{α-dystroglycanopathy} cohort (\textit{FKRP}-predominant) for electrophysiologic evidence of
neuromuscular transmission defects and myasthenic and myopathic fatigue symptoms. We identified a single subject (Patient #23), with GMPPB mutations (c.79 G>C, p.D27H and c.402+1G>A, splice site variant) who had electrophysiologic evidence of postsynaptic NMJ dysfunction on 3Hz RNS studies of spinal accessory and radial nerves, however, none of FKRP patients presented such defect. While NMJ transmission on RNS was normal in most of this cohort, symptoms of fatigue with activities and even eyelid drooping were surprisingly common; fatigue with chewing was reported in more than half of patients. These symptoms that are often associated with myasthenic syndromes did not predict electrophysiologic evidence of NMJ transmission defect in our study. On the other hand, patient #23 who did have a defective NMJ transmission on RNS reported chewing fatigability as only symptom on myasthenic questionnaire, his PROMIS T-score for fatigue was comparable to the average T-score for the US population, and his neurologic exam was normal between his episodes of exercise-induced rhabdomyolysis.

In our series, 80% of patients reported fatigue at the end of the day and most of these also reported worsened muscle weakness later in the day (although that was not reported by our single patient with postsynaptic NMJ impairment on RNS studies). Fatigue is a complex symptom that not only refers to subjective physical tiredness but also intellectual exhaustion. Muscle fatigue is defined by loss of muscle force that is restored by rest; whereas muscle weakness is the loss of muscle force that is not reversible by rest. Determining the degree of muscle fatigue in patients with muscle weakness due to muscular dystrophy is challenging for the clinician.

Our series shows that electrophysiologic evidence of defective neuromuscular transmission is not likely to be seen in patients with FKRP mutations and might be absent or rare in α-dystroglycanopathy patients with congenital muscular dystrophy. Together with previous reports, our work suggests that the myasthenic phenotype might be uniquely associated with GMPPB mutations. We note that we did not perform single fiber-electromyography but limited RNS studies to increase subject participation and tolerance, and that it is possible that minor defects in the neuromuscular transmission are present more commonly than we detected here. On the other hand, single fiber-electromyography, although it is very sensitive, is not specific and abnormal results may be secondary to the myopathic process these patients have rather than a superimposed NMJ transmission defect.

We found, as others have reported, that not all patients with GMPPB mutations have abnormal neuromuscular transmission on electrophysiologic studies and that those with abnormal neuromuscular transmission have relatively mild or fluctuating skeletal muscle phenotypes [6,7]. The phenotype of GMPPB-associated dystroglycanopathies is strikingly broad [17–19]. It has been suggested that there is a continuum of GMPPB-associated phenotypes with myasthenic syndrome at the mild end and congenital muscular dystrophy involving eye and brain at the more severe end. Some

Table 1
Summary of the phenotypic-genotypic spectrum of this α-dystroglycanopathy cohort.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CMD</th>
<th>LGMD</th>
<th>Exercise-induced rhabdomyolysis</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>FKRP (n=25)</td>
<td>25</td>
<td>2</td>
<td>0</td>
<td>C.826 C&gt;A (hm) (n=17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.826 C&gt;C&gt;T (n=2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.826 C&gt;A/1050_1027delins TCAA (n=2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.826C&gt;A/p.494_496delC (n=1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.826C&gt;A/c.856G&gt;C (n=1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.826 C&gt;A? (n=1)</td>
</tr>
<tr>
<td>GMPPB (n=4)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>C.860 G&gt;A/C.895C&gt;T, c.1069 G&gt;A/c.931 C&gt;T</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.79G&gt;C/c.760G&gt;A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.79G&gt;C/c.402+1G&gt;A</td>
</tr>
<tr>
<td>POMT2 (n=1)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>C.1997A&gt;G/c.1116+1G&gt;A</td>
</tr>
<tr>
<td>PMGNT1 (n=1)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>C.1895+1G&gt;T/c.626C&gt;T</td>
</tr>
</tbody>
</table>

CMD, congenital muscular dystrophy; LGMD, limb-girdle muscular dystrophy; hm, homozygous.

Three out of the four patients with GMPPB mutations were previously reported as patient P8 (CMD), P3 (LGMD), and P4 (exercise-induced rhabdomyolysis) [16].

Table 2
Six-question modified questionnaire of myasthenic symptoms.

<table>
<thead>
<tr>
<th>Question</th>
<th>FKRP (n=25)</th>
<th>GMPPB (n=3)</th>
<th>POMT2 (n=1)</th>
<th>PMGNT1 (n=1)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Have you ever noticed one or both eyelids drooping?</td>
<td>YES 2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td>2) Have you ever had double vision?</td>
<td>YES 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>3) Do you tire with repeated swallowing?</td>
<td>YES 5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8 (26.6%)</td>
</tr>
<tr>
<td>4) Does your jaw tire from chewing?</td>
<td>YES 14</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>19 (63.3%)</td>
</tr>
<tr>
<td>5) Is your weakness worse in the afternoons/evenings?</td>
<td>YES 14</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>18 (60%)</td>
</tr>
<tr>
<td>6) Is your fatigue worse in the afternoons/evenings?</td>
<td>YES 20</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>24 (80%)</td>
</tr>
</tbody>
</table>
genotype-phenotype correlations are apparent [19], but exactly how these relate to the presence of electrophysiological neuromuscular transmission dysfunction is still unclear [20]. More than 20 patients with GMPPB mutations and associated myasthenic syndrome (with or without electrophysiologic evidence of abnormal neuromuscular transmission) have been reported [6,7,20–24]. Of those, five belong to a Chinese cohort with the most frequent mutation being c.1070G>A (p.R357H) [20]. Up to 10 of the remaining patients (including Patient #23 in this cohort) have at least one copy of the c.79G>C variant (p.D27H) [6,21–24].

There is growing and compelling evidence to support the role of glycosylated dystroglycan in the formation and maintenance of the NMJ [8,9,25–27]. GMPPB acts very early in the process of glycosylation, catalyzes the formation of GDP-mannose, and contributes to both O-type and N-type glycosylation pathways. Thus, it is possible that GMPPB plays a specific role in dystroglycan glycosylation at the NMJ that is not shared by proteins more distal in the glycosylation pathway, which would support that a defective neuromuscular transmission had been identified only in patients with α-dystroglycanopathy associated with mutations in GMPPB gene. Another possibility is that GMPPB is involved in the glycosylation of key proteins that maintain the normal function of NMJ such as acetylcholine receptor (AChR), agrin, low-density lipoprotein receptor-related protein 4 (LRP4) or muscle-specific kinase (MusK); which are well known targets of the autoimmune response in myasthenia gravis [28]. Furthermore, it is plausible that the defect in neuromuscular transmission associated with GMPPB mutations is detected by electrodiagnostic studies in early stage of the disease and dissipates as the muscle becomes more dystrophic; this would explain why such defect is mostly found in mildly affected GMPPB patients.

The available data here and in prior reports indicate that clinicians should consider screening individuals with mutations in GMPPB, and relatively mild muscle weakness, with or without symptoms of fluctuating weakness, for a neuromuscular transmission defect. Although our patient did not benefit from symptomatic treatment, benefit has been previously reported [6,7]. On the other hand, we do not consider necessary to perform such screening in patients with FKRP mutations since we could not identify any with a defective NMJ transmission defect in this study. Whether patients with other α-dystroglycanopathies may benefit from NMJ assessments is uncertain and requires further investigations. Our clinical impression is that muscle fatigue is common and multifactorial in patients with severe muscular dystrophy. Thus, to better determine the presence and contributory role of a defective neuromuscular junction...
transmission as independent factor to account for patient’s muscle fatigue we favor to perform clinical and electrical longitudinal studies in patients with mild phenotypes (even pre-symptomatic patients when possible) in whom muscles are at early dystrophic stage.

Acknowledgments

We thank patients and their families for their participation in this study. We thank Carrie Stephan for her contributions in data collection and coordination of patient visits.

Funding sources

This study was supported by Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center (U54 NS053672). The clinical evaluations were carried out with the support of the Institute for Clinical and Translational Science at the University of Iowa (NIH U54TR001356). Dr. Paloma Gonzalez-Perez was funded by R25 Award from National Institutes of Health (5R25NS079173).

References


Please cite this article as: P. Gonzalez-Perez, C. Smith and W.L. Sebetsk et al., Clinical and electrophysiological evaluation of myasthenic features in an alpha-dystroglycanopathy cohort (FKRP-predominant), Neuromuscular Disorders, https://doi.org/10.1016/j.nmd.2020.01.002