Urologic and gastrointestinal symptoms in the dystroglycanopathies

Cameron D. Crockett, BS
Laura A. Bertrand, MD
Christopher S. Cooper, MD
Riad M. Rahhal, MD, MS
Ke Liu, MS
M. Bridget Zimmerman, PhD
Steven A. Moore, MD, PhD
Katherine D. Mathews, MD

Correspondence to
Cameron D. Crockett:
cameron-crockett@uiowa.edu

ABSTRACT

Objective: To determine the frequency of urologic and gastrointestinal (GI) symptoms in a cohort of individuals with dystroglycanopathy compared with healthy household controls.

Methods: Participants in a North American dystroglycanopathy natural history study (NCT00313677) and other members of their households completed a questionnaire modified from validated instruments and clinical criteria. Urologic and GI symptom frequency, effect on patient life, and medications taken for these symptoms were assessed. Those younger than 4 years or not toilet trained were excluded. Healthy human bladder, esophagus, and duodenum from surgical specimens were immunostained for glycosylated α -dystroglycan.

Results: Thirty of 58 potential participants with dystroglycanopathy (51.7%) and 16 household controls participated. Subjects were aged 6 to 51 years (mean 26.7); 60.0% were female. Controls were aged 7 to 55 years (mean 34.6); 56.3% were female. The dystroglycanopathy cohort had higher frequency of urinary voiding symptoms ($p = 0.02$), higher urologic symptom scores ($p = 0.05$), and higher dysphagia symptom scores ($p = 0.04$). A correlation existed between urologic symptom score and effect on life ($r = 0.71$; 95% confidence interval 0.46, 0.85; $p < 0.0001$) and between dysphagia symptom score and effect on life ($r = 0.72$; 95% confidence interval 0.48, 0.86; $p < 0.0001$). Glycosylated α -dystroglycan was present in visceral smooth muscle of all normal tissues analyzed.

Conclusions: Urologic symptoms and dysphagia are reported more frequently by individuals with dystroglycanopathies than by household controls. These symptoms can cause a perceived negative effect on patient life. Our results suggest urologic and GI dysfunction may be part of the dystroglycanopathy phenotype, and that questions about these symptoms should be incorporated into routine care because they may influence medical management. *Neurology*® 2015;84:532–539

GLOSSARY

CI = confidence interval; **DMD** = Duchenne muscular dystrophy; **GI** = gastrointestinal; **Ig** = immunoglobulin; **REDCap** = Research Electronic Data Capture; **6MWT** = 6-Minute Walk Test.

The α - and β -dystroglycan proteins are encoded by the dystroglycan gene (*DAG1*) and constitute key components of the dystrophin-glycoprotein complex.¹ After extensive glycosylation, α -dystroglycan serves as a link between the transmembrane β -dystroglycan protein and a number of ligands within the extracellular matrix.¹ Disorders in α -dystroglycan glycosylation or mutations in the gene for the core DAG1 protein lead to a subset of muscular dystrophies known collectively as the dystroglycanopathies.^{1–4} The dystroglycanopathies have a heterogeneous clinical presentation that includes limb-girdle and congenital muscular dystrophies, with Walker-Warburg syndrome at the most severe end of the spectrum.^{4,5} Mental retardation with or without structural brain abnormalities may be part of the phenotype, and dilated cardiomyopathy can occur in some patients.^{4–6}

Anecdotally, we noted that patients with dystroglycanopathies reported difficulties with swallowing or voiding. Because these are not typically considered part of the dystroglycanopathy phenotype, we elected to systematically analyze the nature and frequency of symptoms of

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From the Departments of Pediatrics (C.D.C., C.S.C., R.M.R., K.D.M.), Urology (L.A.B., C.S.C.), Pathology (S.A.M.), and Neurology (K.D.M.), University of Iowa Carver College of Medicine, Iowa City; and Department of Biostatistics (K.L., M.B.Z.), College of Public Health, University of Iowa, Iowa City.

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urologic and gastrointestinal (GI) dysfunction in the dystroglycanopathy patient population using a questionnaire. In addition, we assessed α -dystroglycan glycosylation in healthy human bladder, esophagus, and duodenum. The results presented here are consistent with the clinical impression that some urologic and GI symptoms occur with increased frequency in patients with dystroglycanopathy and provide a preliminary exploration of the potential underlying pathophysiology.

METHODS **Participants.** All individuals older than 4 years who are enrolled in a North American dystroglycanopathy natural history study (NCT00313677, posted on clinicaltrials.gov) and who had indicated a willingness to be contacted for future research were invited to complete this questionnaire. Participants were enrolled in the natural history study on the basis of elevated creatine kinase and evidence of a dystroglycanopathy as determined by a review of muscle pathology, documented mutations in one of the known genes, or abnormal α -dystroglycan glycosylation in cultured fibroblasts. There were no predefined exclusion criteria for the natural history study. Participants were included in the final analysis group only if they had been toilet trained. Household members were enlisted as controls if older than 4 years, and were included in the final analysis if toilet trained.

Standard protocol approvals, registrations, and patient consents. Participant contact was initiated after receiving approval from the University of Iowa institutional review board. Return of a completed questionnaire indicated consent.

Questionnaire. The assessment tool developed for use in this study asked questions regarding the frequency and severity of various urologic and GI symptoms, the perceived effect of these symptoms on patient life, and medications taken for these symptoms. The questionnaire was developed to be applicable to a mixed adult and pediatric study population and of acceptable length. Questions on urologic function were adapted from multiple validated questionnaires including the American Urological Association Symptom Score, the International Prostate Symptom Score, and the Dysfunctional Voiding Symptom Score, with minor language modifications. The GI-related questions included variables needed to define the presence of chronic constipation according to the Rome III criteria.^{7,8} The questionnaire also included clinically relevant questions to assess for the presence and frequency of dysphagia symptoms. The full questionnaire is available in supplemental materials (e-Questionnaire on the *Neurology*[®] Web site at Neurology.org). Questionnaire administration, as well as data collection and management, were performed using Research Electronic Data Capture (REDCap) tools hosted at the University of Iowa.⁹ REDCap is a secure, Web-based application designed to support data capture for clinical research studies. Participants were contacted via e-mail or postal mail and all were given the opportunity to complete the questionnaire online. Those contacted by post were also provided a hardcopy questionnaire. For minor subjects, the family was asked to assist in filling out the questionnaire as necessary.

A randomized identification number linked questionnaires to individual participants in the dystroglycanopathy population. Mutation information, demographic data, and clinical function

data were available through the natural history study. Distance walked on previous 6-Minute Walk Test (6MWT), generally within 12 months before completion of the questionnaire, was used as a measure of mobility status and disease progression.¹⁰

Statistical analysis. All analyses were performed using SAS (version 9.3; SAS Institute, Cary, NC). Between-group comparisons were tested using Wilcoxon rank-sum test for the ordinal variables and Pearson χ^2 test for the categorical variables. Correlations between variables were assessed using Spearman correlations (with 95% confidence interval [CI]). Significance for all assessments was set at $p \leq 0.05$.

For analysis of items administered on a 7-point Likert scale, we converted responses to a 5-point scale consisting of "greater than 4 days per week," "1–3 days per week," "1–3 days per month," "less than 1 day per month," and "never." For each symptom, question results were scored on Likert scale response, from 0 (lowest frequency of occurrence) to 4 (highest frequency). Scores for individual questionnaire items related to urologic symptoms were summed for an overall urologic symptom score. Scores for individual questionnaire items about dysphagia were also summed for an overall dysphagia symptom score. In obtaining both urologic symptom score and dysphagia symptom score, any responses of "unable to answer" were given a score of 0. Urologic questionnaire items were also assessed by categorizing individual items as either storage (urgency, enuresis), voiding (hesitancy, intermittency), or postmicturition (double voiding, incomplete emptying) symptoms as previously proposed by the International Continence Society.¹¹ The symptom scores for these categories were derived from the Likert scale responses as detailed above for individual question items.

Immunostaining. Frozen samples of human bladder, duodenum, and esophagus collected during cancer resection procedures from patients without dystroglycanopathy were obtained from the University of Iowa Tissue Procurement Core. The samples were normal tissue grossly uninvolved by neoplasm and confirmed free of tumor by histopathologic evaluation. Cryosections of each sample and of normal human skeletal muscle (Iowa Wellstone Center Core B) were immunostained for dystrophin (rabbit polyclonal ab15277; Abcam, Cambridge, UK), α -dystroglycan (clone IIH6; Developmental Studies Hybridoma Bank), and β -dystroglycan (clone 7D11; Developmental Studies Hybridoma Bank). Secondary antibodies were goat anti-rabbit immunoglobulin G (IgG), goat anti-mouse IgM, or goat anti-mouse IgG labeled with AlexaFluor488 (Life Technologies, Carlsbad, CA).

RESULTS **Study population.** Fifty-eight individuals with dystroglycanopathy were eligible for participation. Of these, 33 (56.9%) returned a questionnaire. Three of 33 respondents were excluded because they were not toilet trained, resulting in a final analysis group of 30 participants (51.7%). Sixteen healthy household members completed questionnaires (control group). Participants with dystroglycanopathy ranged from 6 to 51 years of age (mean 26.7 ± 14.5); 18 (60.0%) were female. The age range of participants with dystroglycanopathy was similar to that of the total invited population, although the response group had a higher percentage of females. Controls ranged from 7 to 55 years of age (mean 34.6 ± 14.0); 9 (56.3%) were female. Of the 30 members of the dystroglycanopathy cohort, 25 (83.3%) had

mutations in *FKRP*. Of the remaining participants, one had a mutation in *POMT2*, one had a mutation in *POMGnT1*, one had a mutation in *ISPD*, and 2 had evidence of dystroglycanopathy based on review of muscle pathology not yet confirmed by genetic testing. Demographic data are summarized in the table. Only 3 (10.0%) of the participants were non-ambulatory. Two patients (6.7%) were cognitively impaired. Of the 30 participants, only 5 (16.7%) had their questionnaires filled out by a surrogate (parent or guardian). Of these, 3 had mutations in *FKRP* and 2 had mutations in other genes.

Urologic function. The dystroglycanopathy group reported higher individual symptom scores relating to urinary hesitancy (Wilcoxon $p = 0.05$) (figure 1A) and double voiding (Wilcoxon $p = 0.04$) (figure 1B). In addition, the data suggested increased difficulty with intermittency or weak stream in the dystroglycanopathy group compared with the control group that did not reach significance (Wilcoxon $p = 0.06$) (figure 1C). Individuals with dystroglycanopathy had an increase in overall urologic symptom score (Wilcoxon $p = 0.05$) compared with controls (figure 1D). The dystroglycanopathy population had a higher voiding symptom score (Wilcoxon $p = 0.02$) (figure 1E). Postmicturition symptom score was also higher in the dystroglycanopathy group, but was not significant (Wilcoxon $p = 0.06$) (figure 1F). There was no difference in storage symptom scores (Wilcoxon $p = 0.23$). There was a direct correlation between perceived effect on life and urinary storage symptoms (Spearman $r = 0.69$; 95% CI 0.43, 0.84; $p < 0.0001$), postmicturition symptoms (Spearman $r = 0.63$; 95% CI 0.33, 0.80; $p = 0.0001$), and total urologic symptom score (Spearman $r = 0.71$; 95% CI 0.46, 0.85; $p < 0.0001$) (figure 2A). Of the 30 respondents in the dystroglycanopathy group, 23 (76.7%) reported at least one urologic symptom,

while only 2 (6.7%) reported taking any medications for urinary issues either regularly or intermittently.

GI function. Of all questions related to esophageal function, only dysphagia with solids was more common in the dystroglycanopathy cohort than in controls (Wilcoxon $p = 0.04$) (figure 3A). Those with dystroglycanopathy had a higher dysphagia symptom score compared with controls (Wilcoxon $p = 0.04$) (figure 3B). The dystroglycanopathy and control groups were similar in reported effect of dysphagia on life, but dysphagia symptom score was correlated with perceived effect on life within the dystroglycanopathy group (Spearman $r = 0.72$; 95% CI 0.48, 0.86; $p < 0.0001$) (figure 2B).

The dystroglycanopathy and control groups did not differ in frequency of bowel-related symptoms. Because many respondents were taking medication continuously or intermittently for constipation, symptoms may have been masked by successful treatment. To account for this possible effect, those taking medicines (as needed or daily) were combined with those reporting constipation (defined either by self-report of constipation 1–3 days per month or more, passing less than 3 bowel movements per week, or painful bowel movements half the time or more) in each group. In the dystroglycanopathy group, 13 (43.3%) had constipation symptoms and/or took medications for constipation compared with 5 (31.2%) in the control group, indicating no difference between groups regarding constipation (Pearson $\chi^2 p = 0.42$).

Relationships between urologic and GI symptoms and disease features. The postmicturition symptom score was directly correlated with age (Spearman $r = 0.39$; 95% CI 0.04, 0.66; $p = 0.03$). We found no correlation between subject age and urologic symptom score, urinary voiding score, or dysphagia symptom score. Symptoms were present across genotypes, but small group numbers did not allow for statistical analysis of relationship between genotype and symptoms. Because of the variable clinical progression in individuals with dystroglycanopathy, age is not a good surrogate for disease progression. Because of this, we next examined relationships between symptoms and disease status as assessed by the 6MWT distance. There was no correlation between any urologic symptom category, urologic symptom score, or dysphagia symptom score and ambulation status. While constipation was not more common in subjects with dystroglycanopathy compared with controls, within the dystroglycanopathy cohort, there was a lower median distance on last 6MWT for those patients who reported constipation symptoms or treatment (317 m) compared with those who did not report these problems (409 m) (Wilcoxon $p = 0.03$).

Table	Demographic information		
	Dystroglycanopathy invited participants	Dystroglycanopathy final analysis group	Control participants
No. of participants	58	30 (51.7% of invited)	16
Age, y, mean $\pm$ SD (range)	21.9 $\pm$ 13.7 (4–51)	26.7 $\pm$ 14.5 (6–51)	34.6 $\pm$ 14.0 (7–55)
Sex, female, n (%)	25 (43.1)	18 (60.0)	9 (56.3)
Mutations			
FKRP, n (%)	44 (75.9)	25 (83.3)	NA
Other, ^a n (%)	14 (24.1)	5 (16.7)	NA

Abbreviation: NA = not applicable.

^a“Other” category includes both those who have had a genetic mutation identified in a gene other than *FKRP* and those who are included on the basis of muscle biopsy without genetic confirmation.

Figure 1 Urologic symptoms in dystroglycanopathy (DG) relative to household controls

Participants responded to individual questionnaire items on urinary hesitancy (A), double voiding (B), and urinary intermittency or weak stream (C). Responses to questions on individual symptoms were scored and summed to obtain overall urologic symptom score (D). Individual symptoms were grouped physiologically into categories including voiding (E) and postmicturition (F) symptoms.

α-Dystroglycan in normal human tissue. Immunofluorescence demonstrated that key components of the dystrophin-glycoprotein complex were present in healthy human visceral smooth muscle. The duodenum displayed positivity for α-dystroglycan (figure 4B), β-dystroglycan (figure 4D), and dystrophin (figure 4F) similar to that observed in the sarcolemma of normal human skeletal muscle (figure 4, A, C, and E). Immunofluorescence in the

duodenum was representative of that observed in the bladder and esophagus (data not shown).

DISCUSSION Symptoms of urologic and GI dysfunction occurred more frequently in our cohort of individuals with dystroglycanopathy than in household controls. The urologic symptoms encountered more often in those with dystroglycanopathy were classified as voiding symptoms (hesitancy and

Figure 2 Perceived effect of urologic symptoms and dysphagia on the life of patients with dystroglycanopathy

Participant response to question regarding perceived effect on participant life of urologic symptoms was compared against calculated urologic symptom score (A). Participant response to item on perceived effect on participant life exerted by swallowing symptoms was compared against calculated dysphagia symptom score (B). CI = confidence interval.

Figure 3 Gastrointestinal symptoms in dystroglycanopathy (DG) relative to household controls

Participants provided their level of agreement with questionnaire item on dysphagia to solids (A). Responses to individual questions relating to dysphagia were scored and summed to obtain overall dysphagia symptom score (B).

intermittency). Dysphagia with solids was the only GI symptom found to occur more regularly in those with dystroglycanopathy. Postmicturition symptoms (double voiding and incomplete emptying) trended toward a difference, while urinary storage symptoms (urgency and enuresis), heartburn/reflux, and constipation were similar between patients and controls.

Of all symptoms and symptom scores that reached a difference between groups, only postmicturition symptoms had a correlation with subject age, which raises the question of secondary causes of obstruction such as benign prostatic hypertrophy or pelvic organ prolapse. However, the young age (mean age 26.7 years) of our study population makes these possibilities less probable and emphasizes the importance of screening for these symptoms from an early age in patients with dystroglycanopathy.

The perceived effect of urologic and GI symptoms on the life of individuals in our cohort was mixed. Urologic symptoms were relatively frequent, and urologic symptom score was correlated with perceived effect on participant life, indicating that urinary dysfunction is a clinical problem for some individuals with dystroglycanopathy. However, relatively few in our cohort were taking medication for treatment of urologic conditions. We were not able to determine from this study why more participants were not taking medications for these symptoms. It is possible they chose not to take medication, they did not tolerate medication, or they have not discussed the symptoms with a physician. By contrast, dysphagia did not appear to change perceived effect on participant life overall. Even so, the observed correlation between perceived effect on the participant's life and dysphagia score suggests that when symptoms are severe, they have an increasingly negative effect on patients' lives.

Many of the findings from this study mirror observations in Duchenne muscular dystrophy (DMD), another common form of muscular dystrophy. A recent retrospective review of medical records noted as many as 50% of patients with DMD have at least one urologic symptom, while 28% had more than one, confirming findings previously suggested in a questionnaire-based study of patients with DMD.^{12,13} The most common symptom was urinary hesitancy, a voiding symptom that also differed from controls in our dystroglycanopathy population. Furthermore, it has been shown that urinary dysfunction in DMD is often amenable to medical intervention, both regarding specific symptom frequency and reported quality of life.¹⁴ In the DMD cohort, loss of ambulation was associated with more urinary symptoms.¹² We did not find a similar relationship between motor function and urologic symptoms in our dystroglycanopathy cohort using last 6MWT distance as a proxy for motor function. This could be an artifact of our small sample size. Alternatively, it could indicate different underlying pathophysiology. Additional studies, including urodynamic evaluations, will be required to clarify the physiology.

Dysphagia has been identified as a symptom of concern for a number of neuromuscular diseases.^{15–17} Dysphagia with solids more than liquids has also been reported in DMD.^{18–22} We identified similarly increased occurrence of dysphagia with solids, but did not detect progression of dysphagia with skeletal muscle weakness as reported in studies of DMD.^{20,22} While the underlying pathology of dysphagia in dystroglycanopathy is unknown, surface EMG studies of swallowing in a small series of patients with DMD suggest that the muscles involved in swallowing are weak, with dysphagic DMD patients exerting a greater proportion of maximal

Components of the dystrophin-glycoprotein complex are expressed in skeletal muscle (A, C, and E) and visceral smooth muscle, duodenum presented here (B, D, and F). Shown are α -dystroglycan (A and B), β -dystroglycan (C and D), and dystrophin (E and F). The scale bar is 120 μ m in A, C, and E, 60 μ m in B, D, and F.

activity with each swallow.²³ Our results indicate that dysphagia can be a component of the clinical phenotype of dystroglycanopathies, but additional studies, such as direct assessment of swallowing dynamics, will be required to elucidate the underlying pathophysiology.

While symptoms of constipation were common in the dystroglycanopathy population, they were also common in household controls, so although there was a trend toward more symptoms in the disease group, there was not a significant difference. This was true even when considering those receiving treatment for this symptom. We also found no increase in the urologic symptoms that are known to be associated with constipation (urologic storage symptoms, including urgency, frequency, nocturia, and urgency urinary incontinence).²⁴ Constipation is a known

concern for patients with myotonic dystrophy and is considered in routine care of patients with most neuromuscular diseases.^{15–17} This is at least in part related to inactivity, a relationship also observed in our dystroglycanopathy cohort. Further investigation into the role of α -dystroglycan in various tissue types may provide explanations for these findings.

The pathophysiology underlying the urologic and GI symptoms identified here is not known. Many of the symptoms investigated here have the potential for either skeletal or visceral smooth muscle contributions to dysfunction (such as the proximal vs distal components of swallowing or the interplay of urologic smooth muscle and striated pelvic floor and external sphincter muscles in urination). Our observations that these symptoms can occur relatively early in disease and were not highly correlated with a standard

measure of skeletal muscle weakness suggest a primary abnormality of visceral smooth muscle function, such as weak or poorly coordinated contraction. To begin to explore these possibilities, we demonstrated that glycosylated α -dystroglycan is present in normal human visceral smooth muscle. The potential role of aberrant α -dystroglycan glycosylation in visceral smooth muscle dysfunction is further supported by the previous demonstration of hypoglycosylated α -dystroglycan in the bladder smooth muscle of a mouse model of α -dystroglycanopathy, *Large^{myd}* mice.²⁵ Glycosylated α -dystroglycan is also found in vascular smooth muscle, where it may have a developmental role.^{25–27} Additional clinical and animal studies will be required to determine whether glycosylation of α -dystroglycan contributes to the symptoms reported by patients.

This study was limited by a relatively small sample size in this rare disease and incomplete response rate. The small sample size might have prevented identification of relationships between symptoms and other variables. The potential for some response bias exists, which may skew the results in favor of finding greater occurrence of symptoms. In addition, while the questionnaire used was developed from validated instruments and clinical assessment guidelines, it was not itself validated for use. The changes, although minor, may have affected the interpretation of questionnaire items by participants. Items derived from clinical assessment questions may similarly be misinterpreted. Finally, while parents or guardians were asked to assist pediatric participants if necessary, it is possible that younger participants who completed the questionnaire had a different understanding of the questions than the adult participants. The mixed population of this study necessitated the use of such an instrument, but the limited external validity that results must be noted.

The results presented here demonstrate that urologic and GI symptoms may be a component of the clinical phenotype of the dystroglycanopathies. These symptoms can negatively affect the lives of those with dystroglycanopathy, and may be amenable to medical intervention. We suggest that individuals with dystroglycanopathy should be asked about difficulties with swallowing or urination as part of routine care.

AUTHOR CONTRIBUTIONS

C.D. Crockett: study design, data interpretation, and manuscript drafting and revision. Dr. Bertrand: expertise on urology, study design, data interpretation, and manuscript revision. Dr. Cooper: expertise on urology, study design, data interpretation, and manuscript revision. Dr. Rahhal: expertise on gastroenterology, study design, data interpretation, and manuscript revision. K. Liu: statistical analysis and interpretation. Dr. Zimmerman: statistical analysis, interpretation, and manuscript revision. Dr. Moore: study design, data interpretation, and manuscript revision. Dr. Mathews: study conceptualization and design, data interpretation, and manuscript revision.

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