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Dystrophinopathy muscle biopsies in the genetic testing era: one center's data

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Abstract

Introduction—Comprehensive genetic testing for dystrophinopathy can detect ~95% of pathogenic variants in *DMD* and is often the preferred diagnostic approach.

Methods—We reviewed pathology reports for muscle biopsies evaluated at the University of Iowa with a pathological diagnosis of dystrophinopathy based on dystrophic histopathology and abnormal immunofluorescence staining: reduced to absent dystrophin, expression of utrophin, and loss of nNOS.

Results—The percentage of muscle biopsies with dystrophinopathy has been stable since 1997. Of 2298 biopsies evaluated between 2011 and 2016, 72 (3.1%) had pathologic features of dystrophinopathy. Median age at biopsy was 8 years (range 0.66–84). Half had undergone *DMD* genetic testing prior to biopsy. Clinical phenotypes recorded on requisitions were typical of muscular dystrophy for 57 (79%) biopsies.

Discussion—Muscle biopsy continues to play an important role in the diagnosis of dystrophinopathy, particularly in patients with later symptom onset, comorbidities, or normal *DMD* genetic testing.

Keywords

Duchenne-Becker muscular dystrophy; dystrophinopathy; muscle biopsy; pathology; diagnosis; indication

Introduction

Dystrophinopathy (Duchenne-Becker muscular dystrophy) is an X-linked recessive disorder with a prevalence of 1.38 per 10,000 males ages 5–24 in the United States.[1] Patients with

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Duchenne muscular dystrophy (DMD) typically present between the ages of 1.2 and8 years, with muscle weakness and delayed motor development.[2] In contrast, the mean age of onset in Becker muscular dystrophy (BMD) is 12 years, and patients with BMD have slower progression.[3]

The preferred diagnostic approach to a patient with probable dystrophinopathy is genetic testing followed by muscle biopsy if genetic testing is normal.[4] Deletion/duplication testing and sequencing of *DMD* can detect 95–98% of pathogenic *DMD* variants.[5, 6] The University of Iowa (UIHC) is a referral center for muscle biopsy evaluation. We evaluated how the role of muscle biopsy in dystrophinopathy has changed with the improvements in and availability of genetic testing.

Materials/Methods

This study was approved by the University of Iowa Institutional Review Board. The total number of muscle biopsies (male and female) evaluated at UIHC and number of biopsies diagnosed with dystrophinopathy was determined by searching the past 20 years of anatomic pathology cases in the laboratory information system. Complete pathology reports issued for dystrophinopathy cases between 2011 and 2016 were reviewed to verify there was dystrophic histopathology and an abnormal pattern of immunofluorescence staining characteristic of a dystrophinopathy: reduced to absent dystrophin, expression of utrophin, and loss of nNOS. Prior to 2005, the anti-dystrophin (NCL-DYS1, NCL-DYS2, and NCL-DYS3) mouse monoclonal antibodies were from Novocastra/Leica Biosystems. Beginning around 2005, the expression of dystrophin was initially evaluated with four antibodies: the rabbit polyclonal anti-C-terminus antibody ab15277 (Abcam) and mouse monoclonal anti-dystrophin antibodies [Developmental Studies Hybridoma Bank (DSHB), The University of Iowa] directed at the rod domain exon 50 (MANEX50), rod domain exon 46 (MANEX46B), and amino terminus exons 7/8 (MANEX7B). Additional anti-dystrophin antibodies directed at exons 1, 8, 10–12, 20/21, 27, 31/32, 38/39, 43, 45, 47, 48, and 48–50 (all from DSHB) were utilized as needed to better characterize dystrophin expression. Anti-utrophin (NCL-DRP2) and anti-nNOS (NCL-NOS1) antibodies were purchased from Novocastra/Leica Biosystems. Data was abstracted from these reports, including the indication(s) for biopsy, referring physician, clinical information, and genetic testing results. For each of the last 20 years, we determined the percent of total biopsies with a pathological diagnosis of a dystrophinopathy together with 95% confidence intervals.

We grouped biopsies based on whether *DMD* genetic testing was done prior to biopsy, and subdivided groups by clinical information abstracted from the reports. Typical DMD presentation was classified as age at biopsy (presumed to be near the time of presentation to a neurologist) between 1.2–8 years[2] with weakness, delayed motor development, elevated CK, and/or hypertrophic calves.[2, 3] We attempted to supplement the biopsy report-derived information by contacting referring physicians by mail. Of the 55 requests for information, 3 responded. Their responses are included in our results.

Results

20-year trend

The University of Iowa evaluated 5999 muscle biopsies (56% male) from 1997–2016, and 247 (4.1%) of those were diagnosed with dystrophinopathy by muscle pathology and immunofluorescence. The absolute number of muscle biopsies diagnosed annually with dystrophinopathy has been relatively stable (Figure 1a), and the annual percentage of dystrophinopathy biopsies has generally been between 2–5%. Confidence intervals overlap with the exception of the year 2000 (12.1%) (Figure 1b).

2011–2016 Cohort

There were 2298 muscle biopsies evaluated at UIHC from 2011–2016, and 72 (3.1%) had pathologic features of dystrophinopathy. Of these, half had *DMD* genetic testing prior to biopsy. Four biopsied patients had non-*DMD* genetic testing prior to biopsy (*CAPN3* sequencing, *LAMA2* sequencing, myotonic dystrophy panel, and limb-girdle muscular dystrophy (LGMD) panel without including *DMD*). Eleven biopsied patients had normal *DMD* genetic testing prior to biopsy. In 25 patients, a *DMD* variant was identified prior to biopsy. See Figure 2a for details.

Clinical information was available in 70 (97%) dystrophinopathy pathology reports from 2011–2016. Fifty-seven (79%) had a clinical phenotype reported that suggested dystrophinopathy. Of these, 32 reports had a DMD phenotype; 22 (69%) had *DMD* genetic testing prior to biopsy. The other 25 reports described a later onset presentation of typical muscular dystrophy; 12 (48%) had *DMD* genetic testing prior to biopsy. The median age of biopsy was 8 years (range 8 months-84 years), and 16 (22%) biopsies were done when the patient was > 18 years (Figure 2b). Clinical features that are not typical of dystrophinopathy based on sex or comorbid conditions were reported for 13 biopsied patients, summarized in Table 1. Neuropathy was the most common comorbid condition in this series.

DMD genetic testing results were received after the biopsy for 8 patients: 3 were pathological *DMD* variants, 1 was a *DMD* variant of unknown significance (see supplemental table), and *DMD* intronic variants were identified by RNA analysis for the remaining four.

Referring health professionals named on requisitions were predominantly neurologists and pathologists. With rare exceptions, referral biopsies did not have immunohistochemistry done locally; biopsies were sent to UIHC for this testing.

Discussion

Our results show that muscle biopsy continues to be a part of patient diagnosis and care for a subset of individuals with dystrophinopathy. The annual absolute number and percentage of muscle biopsies evaluated at the University of Iowa with a pathological diagnosis of dystrophinopathy has not changed substantially over the past 20 years, despite the advances in genetic testing. From 2011–2016, over two-thirds of the biopsy reports suggesting a typical DMD presentation also reported *DMD* testing prior to biopsy. However, less than

half of those reporting a later onset of typical muscular dystrophy had *DMD* testing prior to biopsy, suggesting dystrophinopathy was not the suspected diagnosis. This is consistent with the broader range of diagnostic possibilities outside of early childhood.[7] Nearly 25% of patients with a dystrophinopathy pathologic diagnosis were older than 18 years when biopsied. Dystrophin abnormalities have previously been reported in 17% of all subjects and 31% of male subjects with a limb-girdle muscular dystrophy (LGMD) presentation.[8] The clinical findings in these older individuals often do not allow distinction between BMD and LGMD, particularly if there is no known family history of muscular dystrophy. According to the LGMD practice parameter, muscle biopsy is the preferred diagnostic approach in this population.[7] However with the increasing availability of next generation sequencing panels, this diagnostic approach might be in evolution.

Muscle biopsy was sometimes requested to provide prognostic information or to understand the phenotype by quantifying dystrophin in muscle. For example, a referral biopsy case had an out-of-frame *DMD* deletion so was expected to have a DMD phenotype,[9–11] but his clinical progression was slower than expected. Biopsy was done to reconcile these findings. As genotype-phenotype relationships and the role of modifier genes are better defined, [6, 12–14] we predict that fewer dystrophinopathy biopsies will be ordered for prognostic information.

Finally, 2–5% of individuals with dystrophinopathy have *DMD* mutations that cannot be detected by deletion/duplication analysis or sequencing.[5, 6] Eleven (15%) biopsies in our series had normal *DMD* testing prior to muscle biopsy, and 5 subjects had a variant of unknown significance in *DMD* prior to biopsy. Muscle biopsies are still required for the diagnosis of dystrophinopathy in these patients both to determine dystrophin expression and to allow research-based analysis of RNA for identification of mutations in noncoding regions. Establishing a genetic diagnosis for patients with dystrophinopathy remains important for genetic counseling and patient management, particularly in the era of emerging genetic therapies for dystrophinopathy.[6, 9]

Some patients with typical presentation of DMD had a muscle biopsy prior to any genetic testing. We hypothesize that barriers to genetic testing, such as cost or insurance restrictions on genetic testing might explain some of these biopsies. In others, physicians might simply prefer to do the biopsy to guide genetic testing, particularly if the clinician is less comfortable with ordering and interpreting genetic tests.

Limitations of our study include sparse clinical history and lack of a detailed rationale for performing the biopsy available for some muscle biopsies. Biopsies are often referred by a pathologist, and requisitions are completed by the pathologist or support personnel. The brevity of clinical summaries may well be the result of this practice. We had little success in contacting the referring clinicians for additional information.

While the preferred diagnostic approach to a patient with probable dystrophinopathy is genetic testing followed by muscle biopsy if genetic testing is normal,[4] the UIHC experience reported here indicates that muscle biopsies still play a role in diagnosis or management of dystrophinopathy. Muscle pathologists should use continuing vigilance for dystrophinopathy

in their assessment of biopsies. Immunostaining for dystrophin in dystrophic-appearing biopsies from male patients of any age will often pay diagnostic dividends.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMD	Becker muscular dystrophy
CK	creatine kinase
DMD	Duchenne muscular dystrophy
LGMD	limb-girdle muscular dystrophy
UIHC	University of Iowa Hospitals and Clinics

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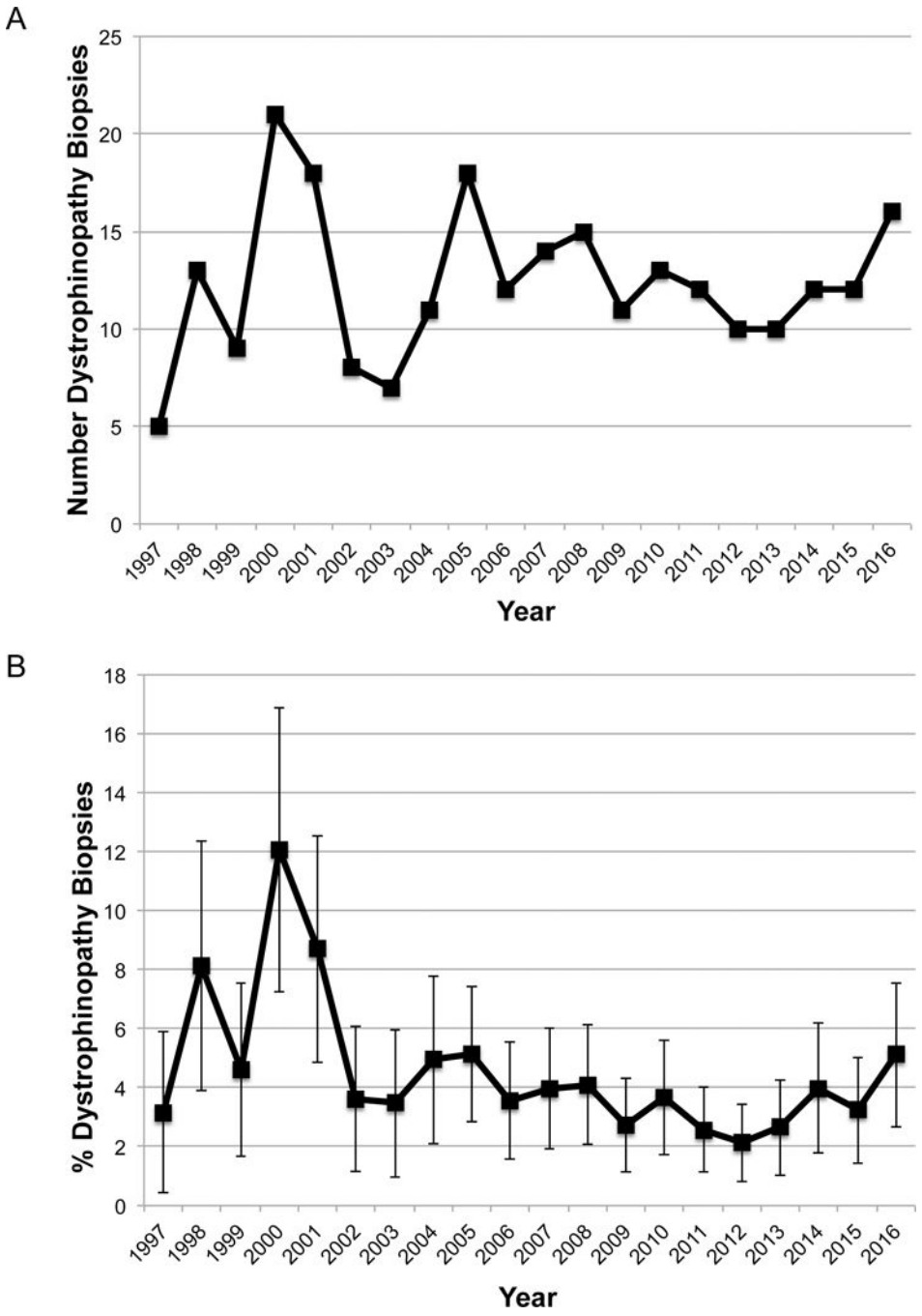

Figure 1.

A: The number of dystrophinopathy biopsies evaluated at UIHC per year since 1997. **B**: The percentage of dystrophinopathy muscle biopsies evaluated at UIHC per year since 1997, with error bars representing 95% confidence intervals.

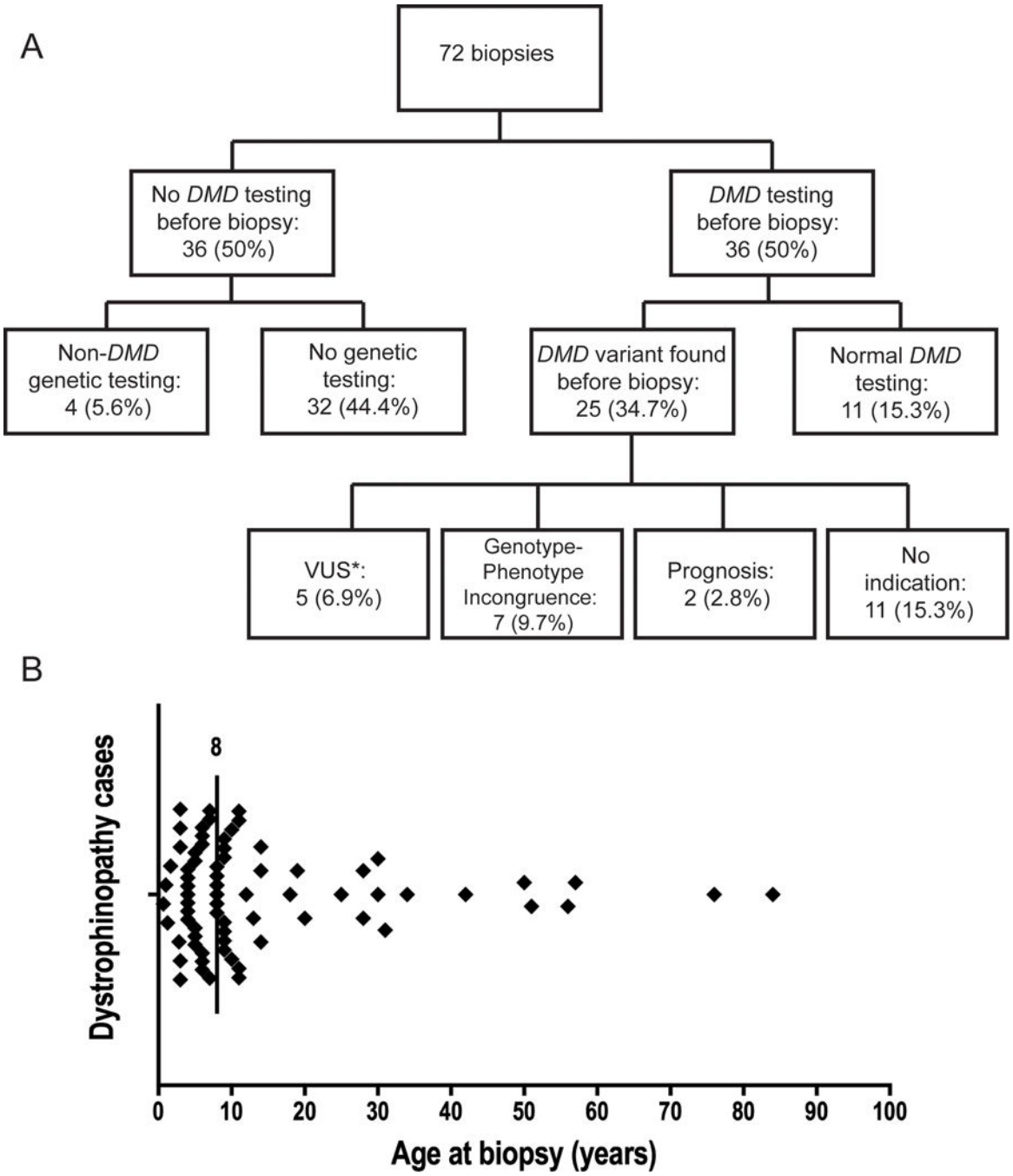

Figure 2.

A. Distribution of 72 dystrophinopathy biopsies diagnosed from 2011–2016. **B.** Distribution of the 72 patients' ages at biopsy. The vertical line is drawn at the median age at biopsy: 8 years.

*see supplemental table

DMD: dystrophin gene; VUS: variant of unknown significance

Table 1

Summary of atypical presentations of dystrophinopathy

Age at biopsy (years)	Sex	Genes tested	Variant(s) found	Comorbidities	Symptoms reported
3	F	*CAPN3*	None	–	Elevated CK, constipation, elevated “liver enzymes”
9	F	–	–	–	Elevated CK, family history MD, calf hypertrophy, muscle weakness, + Gowers’ maneuver
56	F	–	–	Peripheral neuropathy	Family history MD, myalgia, mildly elevated CK
0.67	M	–	–	Feeding disorder	Elevated CK, delayed motor development
1.25	M	*LAMA2*	None	Delayed myelination on MRI	Global developmental delay, FTT, muscle weakness, elevated CK
1	M	–	–	Elevated CK discovered during evaluation of GI illness	–
9	M	–	–	Non-ambulatory since age 5	Muscle weakness
9	M	–	–	Cerebral palsy, peripheral neuropathy	Elevated CK, global developmental delay, muscle weakness
13	M	Congenital muscular dystrophy panel, with *DMD* testing	Xp22.31 duplication, paternally inherited *COL6A* variant, and an *RYR2* variant	Microcephaly and congenital heart defects	Hypotonia, normal CK
25	M	LGMD panel	None	Scapular winging	Muscle weakness, calf hypertrophy, abnormal gait
30	M	–	–	Neuropathy	Clinical childhood MD diagnosis with new symptoms (cardiomyopathy, neuropathy, asthma)
57	M	–	–	–	Fatigue and muscle cramping
76	M	–	–	Difficulty swallowing, neuropathy	Elevated CK, muscle weakness, abnormal gait

F= female, M= male, CK= creatine kinase, MD= muscular dystrophy, LGMD= limb-girdle muscular dystrophy, FTT= failure to thrive, GI= gastrointestinal, “-“ indicates no information provided