Genetic Patterns of Selected Muscular Dystrophies in the Muscular Dystrophy Surveillance, Tracking, and Research Network

Peter B. Kang, MD, Magali Jorand-Fletcher, MPH, Wanfang Zhang, MS, Suzanne W. McDermott, PhD, Reba Berry, RN, Chelsea Chambers, MS, CGC, Kristen N. Wong, MS, CGC, Yara Mohamed, MD, Shiny Thomas, MBBS, MPH, Y Swamy Venkatesh, MD, Christina Westfield, BSN, Nedra Whitehead, MS, PhD, and Nicholas E. Johnson, MD, for the Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STARnet)

Correspondence
Dr. Kang
pkang@umn.edu

Abstract

Background and Objectives

To report the genetic etiologies of Emery-Dreifuss muscular dystrophy (EDMD), limb-girdle muscular dystrophy (LGMD), congenital muscular dystrophy (CMD), and distal muscular dystrophy (DD) in 6 geographically defined areas of the United States.

Methods

This was a cross-sectional, population-based study in which we studied the genes and variants associated with muscular dystrophy in individuals who were diagnosed with and received care for EDMD, LGMD, CMD, and DD from January 1, 2008, through December 31, 2016, in the 6 areas of the United States covered by the Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STAR*net*). Variants of unknown significance (VUSs) from the original genetic test reports were reanalyzed for changes in interpretation.

Results

Among 243 individuals with definite or probable muscular dystrophy, LGMD was the most common diagnosis (138 cases), followed by CMD (62 cases), DD (22 cases), and EDMD (21 cases). There was a higher proportion of male individuals compared with female individuals, which persisted after excluding X-linked genes (*EMD*) and autosomal genes reported to have skewed gender ratios (*ANOS*, *CAV3*, and *LMNA*). The most common associated genes were *FKRP*, *CAPN3*, *ANOS*, and *DYSF*. Reanalysis yielded more definitive variant interpretations for 60 of 144 VUSs, with a mean interval between the original clinical genetic test of 8.11 years for all 144 VUSs and 8.62 years for the 60 reclassified variants. Ten individuals were found to have monoallelic pathogenic variants in genes known to be primarily recessive.

Discussion

This study is distinct for being an examination of 4 types of muscular dystrophies in selected geographic areas of the United States. The striking proportion of resolved VUSs demonstrates the value of periodic re-examinations of these variants. Such re-examinations will resolve some genetic diagnostic ambiguities before initiating repeat testing or more invasive diagnostic procedures such as muscle biopsy. The presence of monoallelic pathogenic variants in recessive genes in our cohort indicates that some individuals with muscular dystrophy continue to face

Glossary

BMD = Becker muscular dystrophy; **CMD** = congenital muscular dystrophy; **DD** = distal muscular dystrophy; **DMD** = Duchenne muscular dystrophy; **EDMD** = Emery-Dreifuss muscular dystrophy; **LGMD** = limb-girdle muscular dystrophy; **VUS** = variants of unknown significance.

incomplete genetic diagnoses; further refinements in genetic knowledge and diagnostic approaches will optimize diagnostic information for these individuals.

Introduction

Four classic but less common forms of muscular dystrophy are Emery-Dreifuss muscular dystrophy (EDMD), limb-girdle muscular dystrophy (LGMD), congenital muscular dystrophy (CMD), and distal muscular dystrophy (DD). These disorders share some overlapping associated genes and some phenotypic features. A number of epidemiologic studies that include various combinations of these muscular dystrophies have been published over the years, ranging from broad-based reports[1] and a genetic database mining study of LGMD[2] to focused population studies on individual genes or even individual variants.[3-8] Several geographically defined population-based studies have been conducted for one or more of these muscular dystrophies, primarily outside the United States.[9-14] Variants of unknown significance (VUSs) often complicate the interpretation of genetic test reports, either when they are the primary findings or when they are secondary findings in addition to pathogenic or likely pathogenic variants. The presence of such VUSs often leads to ambiguous conclusions from genetic test reports.

The Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STARnet) has reported general sociodemographic and clinical characteristics of individuals with these MDs in specific regions of the United States[15,16]; however, MD STARnet has not previously analyzed detailed patterns of genes and variants associated with the 4 less common MD types. In this report, we characterize such patterns of genetic test results for the 4 MDs under investigation. Our primary analysis is followed by a review of information in publicly available databases that can be used to inform interpretation and classification of VUSs.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Study activities were conducted under protocols that were approved by the institutional review board and/or public health authority for surveillance at each MD STARnet site. These activities qualified for waivers of consent at each site.

Study Population and Data Sources (Standard MD STARnet Methodology)

Individuals with EDMD, LGMD, CMD, and DD were identified through MD STARnet surveillance using previously described methods.[15,17] MD STARnet is a multisite, population-based muscular dystrophy surveillance system in the United States that currently identifies individuals who were diagnosed with one of 8 muscular dystrophies (Becker [BMD], CMD, DD, Duchenne [DMD], EDMD, facioscapulohumeral [FSHD], LGMD, and myotonic [DM]). Cohort eligibility for case abstraction included meeting the following criteria from January 1, 2008, to December 31, 2016: clinical diagnosis of an eligible MD and receipt of clinical care and residency in Colorado (CO), Iowa (IA), South Carolina (SC), the Piedmont region of North Carolina (NC), a 21-county area in Western New York State (wNY), or Utah (UT/NV) (Figure).

Case Abstraction (Standard MD STARnet Methodology)

Potential cases were identified by *International Classification of Disease* codes (ICD-9-CM: 359.0, 359.1, 359.21; ICD-10: G71.0, G71.1)[18] from clinic and administrative data and screened for cohort eligibility. Data abstraction of medical records for eligible cases began in 2016 by trained abstractors who reviewed and abstracted medical records for clinical data during the follow-up period. Collected data included demographic characteristics, medical history (including earliest signs and symptoms), diagnostic testing (including genetic testing, muscle biopsy immunostaining, skin biopsy immunostaining, diagnostic Western blot, and/or diagnostic MRI), clinical care, and family history of muscular dystrophy.

Case Review (Standard MD STARnet Methodology)

Abstracted clinical data for each eligible case were reviewed by a panel of MD STARnet neuromuscular physicians who assigned clinical MD type and a case classification of definite, probable, possible, asymptomatic, or not an eligible MD (eAppendix 1, links.lww.com/NXG/A649). MD type was assigned by the panel based on defined patterns of clinical signs and symptoms for each type, as well as diagnostic findings. A case was categorized as definite if there were documented clinical symptoms referable to one of the MD types, a genetic report of DNA analysis with the identification of pathogenic findings in the patient or a family history of

Figure Analytic Flowchart
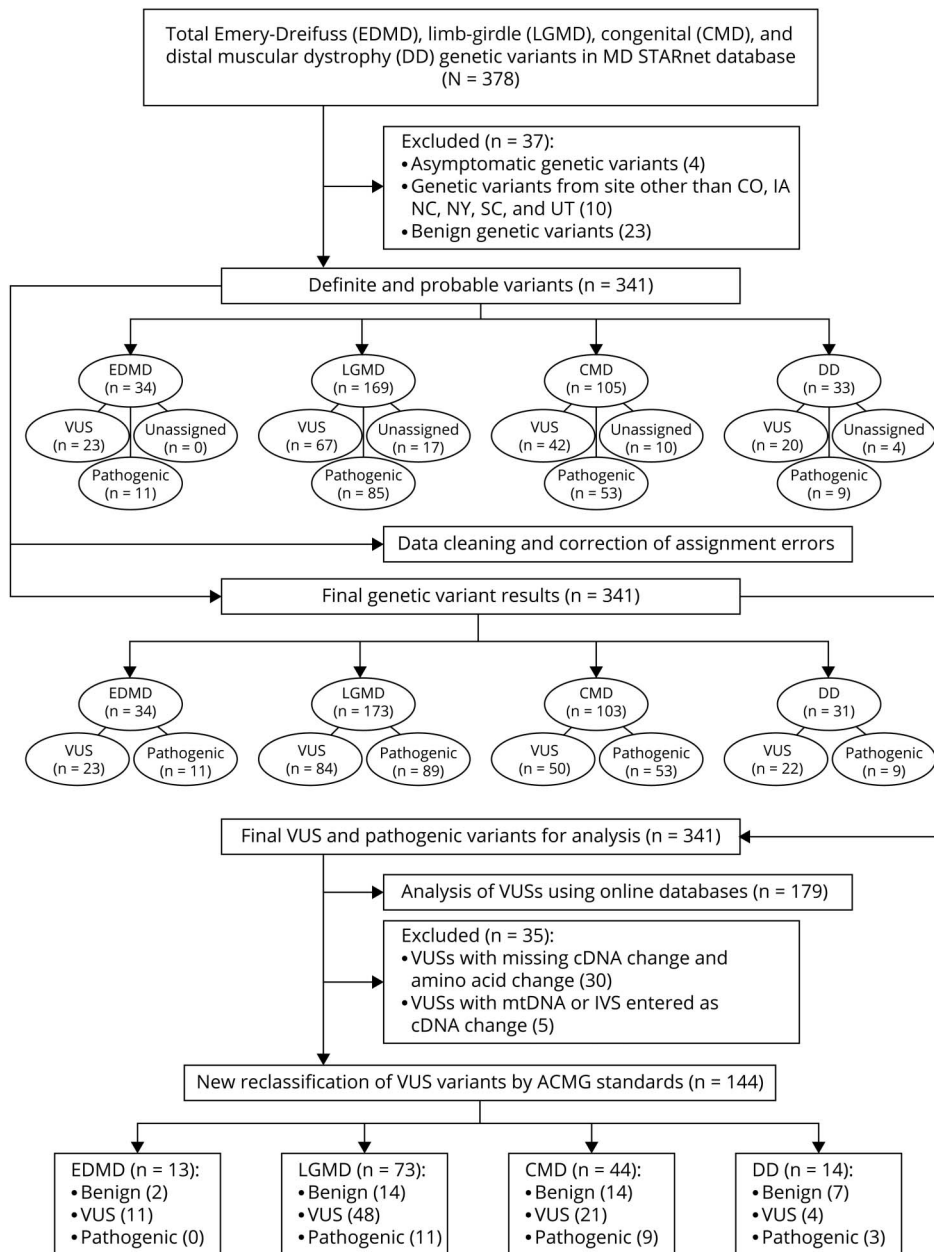
Flowchart of the analytic process from the total initial cohort to final genetic analysis of variants, including exclusions.

genetically confirmed case status in a family member showing a recognizable inheritance pattern, and other confirmatory testing. Probable cases were defined by documented clinical symptoms referable to an MD type and supported by family history and laboratory results referable to one of the selected MDs, but without meeting the criteria for a definite case. Asymptomatic cases were those who had positive genetic test results for an associated gene but showed no signs or symptoms of muscular dystrophy.

Data Pooling

A pooled, analytic data set was created and included clinical data from each MD STAR*net* site for individuals classified as having definite, probable, and asymptomatic diagnoses of EDMD, LGMD, CMD, and DD. From the pooled data, we excluded asymptomatic individuals, individuals whose genetic tests showed benign genes, individuals whose abstracted genetic test results lacked sufficient details for analysis, and individuals who resided in Nevada and were ascertained under UT authority (cases residing in UT were included in the analysis), the latter due to a small, unrepresentative subgroup (Figure). The asymptomatic cases were excluded because the clinical diagnosis assignment could not be confirmed.

Variables

We studied the ages when the diagnosis was confirmed by genetic testing or the first abnormal neuromuscular diagnostic test (serum creatine kinase [CK], EMG, and/or muscle

biopsy). Racial and ethnic classifications were recorded when available. Family history status was defined as yes, no, and unknown.

Statistical Analysis

Frequencies and proportions were calculated for categorical variables; mean and SD or range were used for continuous variables. The distribution of age in years when 50% (25%, 75%) of the study group had each outcome was estimated using the Kaplan-Meier estimator. A minimum of 10 individuals per table cell were required to report numbers and percentages for demographic data to avoid potentially compromising patient privacy, including racial and ethnic identities.

Pathogenicity of Variants and Reanalysis of VUSs

The lists of pathogenic variants and VUSs were obtained from the pooled MD STAR*net* database. The information in these fields was supplemented by additional variants identified in a free text "description" field of the database. Duplicate entries were deleted, and typographical errors were corrected. The original variant classification categories in the database were pathogenic, VUS, normal, and unknown, with "normal" corresponding to the currently accepted variant classification "benign" and "unknown" corresponding to a variant classification that could not be confirmed based on the abstracted information. In light of the possibility that some of the VUSs and unassigned ("unknown") variants may be subject to reinterpretation, we included the unassigned variants in the general category of VUS and re-evaluated this combined variant list, with each variant assessed by a pair of authors who determined the current ACMG classification[19] and Revel score[20] (franklin.genoox.com), along with ClinVar[21] interpretation and gnomAD[22] allele frequencies. When a pair of authors did not agree on the current ACMG classification of a particular variant, they reviewed their findings with each other to reach consensus, consulting with the lead author (P.B.K.) when needed. Using information from these online databases, we then determined whether a VUS would still be classified as a VUS or be reclassified for the purposes of this analysis as pathogenic, likely pathogenic, likely benign, or benign. For those variants that could be reclassified for this analysis, we determined whether the change would either alter the original overall interpretation of the primary genetic test finding or eliminate a secondary finding. A secondary finding is defined as a VUS that is noted in a genetic test report in the presence of a pathogenic or likely pathogenic variant in a different gene. We also recorded the intervals between the original clinical genetic test date and the date of completion of our reanalysis (December 13, 2022) and calculated mean and median intervals for each diagnosis (EDMD, LGMD, CMD, and DD).

Monoallelic Pathogenic Variants in Genes With Recessive Inheritance ("Single Hits")

We reviewed all pathogenic variants of muscular dystrophy–associated genes that are known to have recessive or primarily recessive patterns of inheritance and noted when one pathogenic allele was unaccompanied by a second pathogenic allele for the same gene ("single hit").

Missing Variants

At the time of abstraction, pathogenic variant information from genetic test reports was entered into the MD STAR*net* database. However, some variants did not have nucleotide or amino acid positions entered, rendering them impossible to characterize or analyze further. In some cases where a VUS test result was missing cDNA or amino acid change information in the structured part of the MD STAR*net* database, this information was found in the free text "description" field of the database. In those cases, study authors filled in the missing data manually.

Data Availability

Owing to privacy concerns, data from MD STAR*net* are not publicly available. Researchers interested in MD STAR*net* data can contact MDSTARnet@cdc.gov.

Results

Demographics

We first examined key demographic data among the 243 individuals in our cohort to characterize basic information (Table 1). 64.6% of our cohort was male and 35.4% was female. The unexpectedly higher proportion of male individuals compared with female individuals was present in all 4 diagnostic categories, despite the presence of only one X-linked gene (*EMD*) among the commonly associated genes. Regarding other aspects of our cohort, 44.4% had no known family history of the disease in question. The mean age at diagnosis was 27.2 years, and the median age at diagnosis was 22.1 years, and as noted above, the diagnosis had to be made in the 2008–2016 period for inclusion. The mean age at the last abstracted clinic visit was 37 years, and the median age at the last clinic visit was 35 years. These mean and median ages were younger in the CMD group (20.7 and 18 years, respectively) compared with the other groups.

Category Distributions and Genetic Findings

As 4 major categories of muscular dystrophy were represented in our cohort, we examined the distribution of individuals among these categories. LGMD was the most common clinical diagnosis, followed by CMD, with DD and EDMD being the least common and nearly equivalent to each other numerically (Table 2). Overall, 60.1% of the cohort had a definite classification, with LGMD having the lowest proportion (Table 2). As originally characterized by the clinical genetic test reports, the associated genes with the highest overall occurrence of pathogenic variants, excluding VUSs, in the cohort were *FKRP*, *CAPN3*, *ANO5*, and *COL6A1* (eTable 1, links.lww.com/NXG/A650), all of which are most commonly found in LGMD, except *COL6A1*, which is typically found in CMD (eTable 2). In EDMD, the genes with the most frequent pathogenic variants were *EMD* and *LMNA*. In CMD, the genes with the most frequent pathogenic variants were

Table 1 Demographic Features of 243 Individuals With EDMD, LGMD, CMD, and DD, With Comparisons Among the 4 Muscular Dystrophy Categories for Each Set of Variables

	Total (N = 243)	p Value[a]
Sites, n (%)		0.0628
Colorado	34 (14.0)	
Iowa	45 (18.5)	
North Carolina (Piedmont region)	28 (11.5)	
New York (Western 21 counties)	54 (22.2)	
South Carolina	32 (13.2)	
Utah	50 (20.6)	
No known family history, n (%)		0.0786
No	135 (55.6)	
Yes	108 (44.4)	
Sex, n (%)		0.0777
Female	86 (35.4)	
Male	157 (64.6)	
Race[b], n (%)		0.00571
White	189 (77.8)	
Others/Unknown	54 (22.2)	
Ethnicity, n (%)		0.0939
Hispanic	17 (7.0)	
Non-Hispanic	211 (86.8)	
Unknown	15 (6.2)	
Age at first diagnosis		<0.001
Mean (SD)	27.2 (20.7)	
Median [Min, Max]	22.1 [0, 79.1]	
Projected age on June 15, 2022		<0.001
Mean (SD)	37.0 (20.5)	
Median [Min, Max]	35.0 [6.00, 90.0]	

Abbreviations: CMD = congenital muscular dystrophy; DD = distal muscular dystrophy; EDMD = Emery-Dreifuss muscular dystrophy; LGMD = limb-girdle muscular dystrophy.
[a] p-values are for the association between categorical variables and the 4 categories of muscular dystrophy, examined with a χ^2 test, and for the association between continuous variables and the 4 categories of muscular dystrophy, examined with an ANOVA.
[b] Others/Unknown category includes American Indian or Alaska Native, Black or African American, Asian, Multiple, Other and Unknown. Owing to small subgroup sizes, individual categories are not enumerated.

COL6A1, LAMA2, COL6A3, COL6A2, and FKRP. For DD, the genes with the most frequent pathogenic variants were DYSF and GNE. Most of the variants were pathogenic for CMD and LGMD, whereas VUSs were proportionately more frequent for EDMD and DD (eTable 3). The proportions of male and female individuals were analyzed in aggregate, both before and after exclusion of the X-linked gene EMD and autosomal genes previously found in a higher proportion of male individuals (ANO5, CAV3, and LMNA)[23] (eTable 4).

Pathogenicity of Variants and Reanalysis of VUSs and Unassigned Variants

Given the high number of VUSs that appear in clinical genetic testing, we asked whether genetic findings could be refined by reanalysis using online databases. Before data cleaning, we identified 162 pathogenic variants, 169 VUSs, and 10 unassigned variants. After data cleaning and review of the initial abstraction results, we identified 162 pathogenic variants and 179 VUSs for a total of 341 variants (Figure). Among the 179 VUS results reviewed, 35 were classified as having missing data with the following breakdown: 26 were missing cDNA change or amino acid change information, 4 had unverifiable information, 4 only listed intervening sequence information, and 1 had mtDNA information, yielding 144 VUSs with adequate information for analysis (eTable 5, links.lww.com/NXG/A650). Our review of those 144 VUSs using currently accepted standard classification systems and databases yielded reclassification of 23 variants to pathogenic or likely pathogenic and 37 variants to benign or likely benign. Eighty-four VUSs remained unchanged (Table 3). The reclassifications changed the interpretations of primary genetic test findings for 35 variants and eliminated secondary findings for 23 variants (Table 4 and eTables 6 and 7). Of note, there were individuals with multiple VUSs, thus the number of individuals with reinterpretations of primarily genetic findings was 28 and the number of individuals with elimination of secondary findings was 18 (Table 4). The mean intervals between the original clinical genetic test report and the time of VUS reanalysis was 8.11 years for all 144 VUSs analyzed (Table 5) and 8.62 years for the 60 reclassified VUSs (Table 6).

Monoallelic Pathogenic Variants in Recessive Genes ("Single Hits")

The problem of VUSs is often accompanied by the dilemma presented by single pathogenic variants identified in genes that are known to be recessive, leaving diagnostic uncertainty. We identified 10 individuals with such monoallelic pathogenic variants in genes with recessive or primarily recessive patterns of inheritance ("single hits"). These individuals have ambiguous genetic diagnoses despite the presence of pathogenic variants. These single hits were most common for LGMD (eTable 8, links.lww.com/NXG/A650), and after removing individuals with duplicate pathogenic genes, the most common gene in which this phenomenon was observed was FKRP (eTable 9). For all 10 affected symptomatic individuals, there were no other findings in the original genetic test report to indicate the presence of alternative genetic diagnoses.

Cases With Pathogenic Variants in Multiple Genes

The possibility of digenic Mendelian inheritance as an explanation for some genetically unsolved cases of muscular

Table 2 Clinical Classification of the 243 Individuals With Certainty of Diagnoses

Case status	EDMD (N = 21)	LGMD (N = 138)	CMD (N = 62)	DD (N = 22)	Total (N = 243)
Definite, n (%)	14 (66.7)	67 (48.6)	52 (83.9)	13 (59.1)	146 (60.1)
Probable, n (%)	7 (33.3)	71 (51.4)	10 (16.1)	9 (40.9)	97 (39.9)

Abbreviations: CMD = congenital muscular dystrophy; DD = distal muscular dystrophy; EDMD = Emery-Dreifuss muscular dystrophy; LGMD = limb-girdle muscular dystrophy.

dystrophy has been raised. In our cohort, 3 individuals were found to have pathogenic or likely pathogenic alleles in 2 different genes: (1) *DNAJB6* c.271T>G (p.F91V) paired with *GAA* c.546G>A (p.T183 =); (2) *TTN* c.70493dupA paired with *FKRP* c.826C>A (p.L2761); and (3) *ANO5* c.191dupA paired with *TTN* c.85692_85696delAGCTT.

Discussion

Prior geographically defined studies of EDMD, LGMD, CMD, and DD in the United States consist principally of 2 MD STAR*net* reports that did not include the genetic analysis presented here.[15,16] In other countries, epidemiologic studies focusing on LGMD have been published from Austria,[9] Chile,[10] Italy,[11,23] the Netherlands,[12] and Spain[13] and CMD from Italy.[14] These and other studies from around the world that covered these diagnoses provide valuable information but had differences in scope from our study because they did not include genetic subtype information,[24] were broad-based general studies of muscle diseases or neuromuscular disorders,[25-35] or focused on genetic subsets of one of these muscular dystrophies.[36-41] Our findings are consistent with prior studies for the relative frequency of the 4 MD types and the common genes identified in our cohort.

The skewed sex ratio is striking and cannot be explained by expected genetic distributions, given that only one major gene, *EMD*, is X-linked.[42] There are several autosomal genes that have previously been associated with male-predominant ratios, including *ANO5*, *CAV3*, and *LMNA*.[23] A study of EDMD, LGMD, CMD, and DD from the prior MD STAR*net* cycle only detected a skewed male/female ratio in EDMD.[16]

Although the available information does not enable us to draw definitive conclusions about the origins of this sex distribution, it is plausible that female individuals affected by these categories of muscular dystrophy were diagnosed at lower rates or sought specialty care less often than affected male individuals during this more recent surveillance period. Milder manifestations in female individuals could account for either explanation. As the most widely known muscular dystrophy, Duchenne muscular dystrophy (DMD) is X-linked and almost exclusively affects male individuals; there may be a misperception that muscular dystrophy of all kinds does not tend to affect female individuals. It is thus important that outreach efforts for the medical community and for the general public emphasize that both female and male individuals can be affected by many of the subtypes of muscular dystrophy.

The excess of individuals in our cohort with probable rather than definite diagnoses indicates that a gap in confirmatory genetic diagnosis persists in these categories of muscular dystrophy. Of note, the case definitions (eAppendix 1, links. lww.com/NXG/A649) classify affected individuals with a family history of genetic confirmation as definite cases. The percentage of probable cases is highest for LGMD, similar to the high unsolved rates for this type of muscular dystrophy found on both clinical genetic testing and research-based genomic analyses.[43-45] This may in part be due to uneven access to genetic testing in some populations.

The common occurrence of VUSs in clinical genetic test reports and the unexpectedly high rate of reclassification on reanalysis of these VUSs in this study indicate that further advances are needed in genetic diagnostic technology and

Table 3 Clinical MD Types and ACMG Classifications for VUSs

ACMG classification	EDMD (N = 13)	LGMD (N = 73)	CMD (N = 44)	DD (N = 14)	Total (N = 144)
Benign, n (%)	1 (7.7)	5 (6.8)	11 (25.0)	6 (42.9)	23 (16.0)
Likely benign, n (%)	1 (7.7)	9 (12.3)	3 (6.8)	1 (7.1)	14 (9.7)
VUS, n (%)	11 (84.6)	48 (65.8)	21 (47.7)	4 (28.6)	84 (58.3)
Likely pathogenic, n (%)	0 0.00	4 (5.5)	5 (11.4)	1 (7.1)	10 (6.9)
Pathogenic, n (%)	0 (0.0)	7 (9.6)	4 (9.1)	2 (14.3)	13 (9.0)

Abbreviations: CMD = congenital muscular dystrophy; DD = distal muscular dystrophy; EDMD = Emery-Dreifuss muscular dystrophy; LGMD = limb-girdle muscular dystrophy.

Table 4 Changes in Interpretation and Elimination of Secondary Findings for VUSs by Clinical MD Type

By variants

	EDMD (N = 23)	LGMD (N = 84)	CMD (N = 50)	DD (N = 22)	Total (N = 179)
Change in genetic diagnosis, n (%)					
No	22 (95.7)	69 (82.1)	39 (78.0)	14 (63.6)	144 (80.4)
Yes	1 (4.3)	15 (17.9)	11 (22.0)	8 (36.4)	35 (19.6)
Eliminated secondary finding, n (%)					
No	22 (95.7)	74 (88.1)	40 (80.0)	20 (90.9)	156 (87.2)
Yes	1 (4.3)	10 (11.9)	10 (20.0)	2 (9.1)	23 (12.8)

By individuals

	EDMD (N = 11)	LGMD (N = 49)	CMD (N = 29)	DD (N = 8)	Total (N = 97)
Change in genetic diagnosis, n (%)					
No	10 (90.9)	36 (73.5)	18 (62.1)	5 (62.5)	69 (71.1)
Yes	1 (9.1)	13 (26.5)	11 (37.9)	3 (37.5)	28 (28.9)
Eliminated secondary finding, n (%)					
No	10 (90.9)	40 (81.6)	22 (75.9)	7 (87.5)	79 (81.4)
Yes	1 (9.1)	9 (18.4)	7 (24.1)	1 (12.5)	18 (18.6)

Abbreviations: CMD = congenital muscular dystrophy; DD = distal muscular dystrophy; EDMD = Emery-Dreifuss muscular dystrophy; LGMD = limb-girdle muscular dystrophy.

interpretation to improve the accuracy and detection rate of genetic testing. At the very least, a basic scan of VUSs identified on clinical genetic testing using online databases is warranted when the original genetic test is more than a few years old. In light of the frequent posting of new online resources for variant interpretation, we recommend consulting with a neuromuscular neurologist, geneticist, or genetic counselor with expertise in these resources to determine which ones to use at a given time.

Cases in which a monoallelic pathogenic variant ("single hit") is unaccompanied by a pathogenic variant in the same gene on the other allele, for genes known to have recessive or primarily recessive inheritance, are frustrating for the patients and clinicians involved because it leaves the genetic diagnosis without a full resolution. Approaches that promise to improve the genetic diagnosis of muscular dystrophies include transcriptome analysis (RNAseq), computational reanalysis to detect more subtle changes such as splice variants, and long

Table 5 Time Intervals Between Clinical Genetic Tests and Reanalysis of All 144 VUSs That Were Reanalyzed (y)

	CMD (N = 44)	DD (N = 14)	EDMD (N = 13)	LGMD (N = 73)	Total (N = 144)
Intervals					
Mean (SD)	8.22 (1.99)	7.00 (0)	7.14 (1.21)	8.31 (2.18)	8.11 (2.00)
Median [Min, Max]	8.00 [6.00, 13.0]	7.00 [7.00, 7.00]	7.00 [6.00, 9.00]	7.50 [6.00, 14.0]	7.00 [6.00, 14.0]
Missing	17 (38.6%)	11 (78.6%)	6 (46.2%)	37 (50.7%)	71 (49.3%)
ACMG classification, n (%)					
Benign	11 (25.0)	6 (42.9)	1 (7.7)	5 (6.8)	23 (16.0)
Likely benign	3 (6.8)	1 (7.1)	1 (7.7)	9 (12.3)	14 (9.7)
Likely pathogenic	5 (11.4)	1 (7.1)	0 (0)	4 (5.5)	10 (6.9)
Pathogenic	4 (9.1)	2 (14.3)	0 (0)	7 (9.6)	13 (9.0)
VUS	21 (47.7)	4 (28.6)	11 (84.6)	48 (65.8)	84 (58.3)

The date used for reanalysis was December 13, 2022, the date when the VUS analysis was completed.

Table 6 Time Intervals Between Clinical Genetic Tests and Reanalysis of 60 VUSs That Were Reclassified (y)

	CMD (N = 23)	DD (N = 10)	EDMD (N = 2)	LGMD (N = 25)	Total (N = 60)
Lag years					
Mean (SD)	8.29 (1.86)	7.00 (NA)	NA (NA)	9.18 (2.79)	8.62 (2.28)
Median [Min, Max]	8.00 [6.00, 13.0]	7.00 [7.00, 7.00]	NA [NA, NA]	8.00 [6.00, 14.0]	8.00 [6.00, 14.0]
Missing, n (%)	9 (39.1)	9 (90.0)	2 (100)	14 (56.0)	34 (56.7)
ACMG classification, n (%)					
Benign	11 (47.8)	6 (60.0)	1 (50.0)	5 (20.0)	23 (38.3)
Likely benign	3 (13.0)	1 (10.0)	1 (50.0)	9 (36.0)	14 (23.3)
Likely pathogenic	5 (21.7)	1 (10.0)	0 (0)	4 (16.0)	10 (16.7)
Pathogenic	4 (17.4)	2 (20.0)	0 (0)	7 (28.0)	13 (21.7)

The date used for reanalysis was December 13, 2022, the date when the VUS analysis was completed.

read sequencing. Long read sequencing in particular holds promise to find the "second hits" for those individuals with monoallelic pathogenic variants in genes with recessive inheritance.[46]

It has been postulated that there may be rare cases in which variants at 2 different loci may together cause disease. For muscular dystrophy, this is best documented for facioscapulohumeral muscular dystrophy type 2, caused by variants in *SMCHD1* paired with a D4Z4 allele harboring a polyadenylation signal.[47,48] There are sparse reports of compound pathogenic variants in different genes potentially causing muscular dystrophy, including *SCGB* paired with *SCGD*[49] and *COL6A1* paired with *COL6A2*.[50] We found only 3 cases of potential digenic inheritance in our cohort; more extensive studies are required to determine whether both variants are necessary and sufficient to cause disease in these circumstances.

Our study has some limitations. The subgroups for EDMD and DD were small, although the presence of expected common genes in those subgroups indicates that they were to some extent representative of broader populations with these disease categories. The absence of *ANO5* in the DD group was likely because of the small cohort size. The numbers for some genetic subtypes did not meet the MD STAR*net* reporting threshold of at least 10 cases. Thus, we were not able to present details of the distributions of certain variables within these subtypes such as sex ratios. As genetic testing was performed at different times at different diagnostic facilities, variant interpretation practices likely varied throughout the cohort, although all clinical genetic diagnostic test facilities in the United States are required to qualify for and maintain Clinical Laboratory Improvement Amendment certification, providing some standardization in variant interpretation practices over time. Beyond variant reanalysis, reanalysis of raw sequence data and the use of newer technologies such as nanopore whole-genome long read sequencing[46] and whole-

transcriptome sequencing (RNAseq), as well as review of muscle imaging studies, could yield additional meaningful diagnostic information. However, MD STAR*net* does not collect raw sequence data, genomic DNA samples, specimens from muscle biopsies, or images from muscle ultrasound and MRI studies; thus those types of investigations are beyond the scope of this study.

EDMD, LGMD, CMD, and DD collectively comprise a significant portion of the muscular dystrophy population. Their genetic heterogeneity, compared with more common muscular dystrophies such as dystrophinopathies (DMD and BMD), facioscapulohumeral muscular dystrophy (FSHD), and myotonic dystrophy (DM1 and DM2), leads to distinct challenges in diagnosis, prognosis, and management. Our findings indicate that periodic reanalysis of VUSs using publicly available databases will at times yield new information. It will be important to continue characterizing these MDs to optimize genetic diagnosis, clinical management, and research studies that will help lead to novel therapies. Encouragingly, investigational therapies are already undergoing human clinical trials for some of these muscular dystrophies, providing a great deal of hope for the future.

Acknowledgment

Study Funding

Disease Control and Prevention (CDC). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. The Iowa site was additionally supported by DD001247. Partial support for all data sets with the Utah Population Database (UPDB) was provided by the University of Utah Huntsman Cancer Institute and the Huntsman Cancer Institute Cancer Center Support Grant, P30 CA042014, from the National Cancer Institute.

Disclosure

Publication History

Appendix Authors

Name	Location	Contribution
Peter B. Kang, MD	Paul & Sheila Wellstone Muscular Dystrophy Center, Department of Neurology, and Institute for Translational Neuroscience, University of Minnesota, Minneapolis	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
Magali Jorand-Fletcher, MPH	Department of Pediatrics, University of Florida College of Medicine, Gainesville	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Wanfang Zhang, MS	Department of Epidemiology and Biostatistics, University of South Carolina, Columbia	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data

Appendix *(continued)*

Name	Location	Contribution
Suzanne W. McDermott, PhD	Department of Environmental, Occupational, and Geospatial Health Sciences, Graduate School of Public Health and Health Policy, City University of New York	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
Reba Berry, RN	Division of Population Health Surveillance, Bureau of Maternal and Child Health, South Carolina Department of Health and Environmental Control, Columbia	Analysis or interpretation of data
Chelsea Chambers, MS, CGC	Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond	Analysis or interpretation of data
Kristen N. Wong, MS, CGC	Department of Pediatrics, University of Utah, Salt Lake City	Analysis or interpretation of data
Yara Mohamed, MD	Department of Pediatrics, University of Florida College of Medicine, Gainesville	Analysis or interpretation of data
Shiny Thomas, MBBS, MPH	New York State Department of Health, Albany	Analysis or interpretation of data
Y. Swamy Venkatesh, MD	Department of Neurology, University of South Carolina, Columbia	Analysis or interpretation of data
Christina Westfield, BSN	Division of Population Health Surveillance, Bureau of Maternal and Child Health, South Carolina Department of Health and Environmental Control, Columbia	Analysis or interpretation of data
Nedra Whitehead, MS, PhD	RTI International, Research Triangle Park, NC	Analysis or interpretation of data
Nicholas E. Johnson, MD	Department of Neurology, Virginia Commonwealth University, Richmond	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data

References

1. Mah JK, Korngut L, Fiest KM, et al. A systematic review and meta-analysis on the epidemiology of the muscular dystrophies. *Can J Neurol Sci.* 2016;43(1):163-177. doi:10.1017/cjn.2015.311
2. Liu W, Pajusalu S, Lake NJ, et al. Estimating prevalence for limb-girdle muscular dystrophy based on public sequencing databases. *Genet Med.* 2019;21(11):2512-2520. doi:10.1038/s41436-019-0544-8
3. Polavarapu K, Mathur A, Joshi A, et al. A founder mutation in the GMPPB gene [c.1000G > A (p.Asp334Asn)] causes a mild form of limb-girdle muscular dystrophy/congenital myasthenic syndrome (LGMD/CMS) in South Indian patients. *Neurogenetics.* 2021;22(4):271-285. doi:10.1007/s10048-021-00658-1
4. Pantoja-Melendez CA, Miranda-Duarte A, Roque-Ramirez B, Zenteno JC. Epidemiological and molecular characterization of a mexican population isolate with high prevalence of limb-girdle muscular dystrophy type 2A due to a novel calpain-3 mutation. *PLoS One.* 2017;12(1):e0170280. doi:10.1371/journal.pone.0170280
5. Tétreault M, Srour M, Allyson J, et al. Founder mutation for α-sarcoglycan-LGMD2D in a Magdalen Islands Acadian cluster. *Can J Neurol Sci.* 2011;38(5):747-752. doi:10.1017/s0317167100054135
6. Frosk P, Greenberg CR, Tennese AAP, et al. The most common mutation in FKRP causing limb girdle muscular dystrophy type 2I (LGMD2I) may have occurred only once and is present in Hutterites and other populations. *Hum Mutat.* 2005;25(1):38-44. doi:10.1002/humu.20110

7. Merlini L, Kaplan JC, Navarro C, et al. Homogeneous phenotype of the gypsy limb-girdle MD with the gamma-sarcoglycan C283Y mutation. *Neurology*. 2000;54(5):1075-1079. doi:10.1212/wnl.54.5.1075

8. Al-Zaidy SA, Malik V, Kneile K, et al. A slowly progressive form of limb-girdle muscular dystrophy type 2C associated with founder mutation in the SGCG gene in Puerto Rican Hispanics. *Mol Genet Genomic Med*. 2015;3(2):92-98. doi:10.1002/mgg3.125

9. Krenn M, Tomschik M, Wagner M, et al. Clinico-genetic spectrum of limb-girdle muscular weakness in Austria: a multicentre cohort study. *Eur J Neurol*. 2022;29(6):1815-1824. doi:10.1111/ene.15306

10. Cerino M, González-Hormazábal P, Abaji M, et al. Genetic profile of patients with limb-girdle muscle weakness in the chilean population. *Genes (Basel)*. 2022;13(6):1076. doi:10.3390/genes13061076

11. Guglieri M, Magri F, D'Angelo MG, et al. Clinical, molecular, and protein correlations in a large sample of genetically diagnosed Italian limb girdle muscular dystrophy patients. *Hum Mutat*. 2008;29(2):258-266. doi:10.1002/humu.20642

12. van der Kooi AJ, Frankhuizen WS, Barth PG, et al. Limb-girdle muscular dystrophy in The Netherlands: gene defect identified in half the families. *Neurology*. 2007;68(24):2125-2128. doi:10.1212/01.wnl.0000264853.40735.3b

13. Urtasun M, Sáenz A, Roudaut C, et al. Limb-girdle muscular dystrophy in Guipúzcoa (Basque Country, Spain). *Brain*. 1998;121(Pt 9):1735-1747. doi:10.1093/brain/121.9.1735

14. Mostacciuolo ML, Miorin M, Martinello F, Angelini C, Perini P, Trevisan CP. Genetic epidemiology of congenital muscular dystrophy in a sample from north-east Italy. *Hum Genet*. 1996;97(3):277-279. doi:10.1007/BF02185752

15. Do TN, Street N, Donnelly J, et al. Muscular dystrophy surveillance, tracking, and research network pilot: population-based surveillance of major muscular dystrophies at four U.S. sites, 2007-2011. *Birth Defects Res*. 2018;110(19):1404-1411. doi:10.1002/bdr2.1371

16. Wallace B, Smith KT, Thomas S, et al. Characterization of individuals with selected muscular dystrophies from the expanded pilot of the Muscular Dystrophy Surveillance, Tracking and Research Network (MD STARnet) in the United States. *Birth Defects Res*. 2021;113(7):560-569. doi:10.1002/bdr2.1764

17. Miller LA, Romitti PA, Cunniff C, et al. The muscular dystrophy surveillance tracking and research network (MD STARnet): surveillance methodology. *Birth Defects Res A Clin Mol Teratol*. 2006;76(11):793-797. doi:10.1002/bdra.20279

18. World Health Organization, ed. *International Statistical Classification of Diseases and Related Health Problems*, 10th revision, 2nd ed. World Health Organization; 2004.

19. Richards S, Aziz N, Bale S, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-425. doi:10.1038/gim.2015.30

20. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. *Am J Hum Genet*. 2016;99(4):877-885. doi:10.1016/j.ajhg.2016.08.016

21. Landrum MJ, Chitipiralla S, Brown GR, et al. ClinVar: improvements to accessing data. *Nucleic Acids Res*. 2020;48(D1):D835–D844. doi:10.1093/nar/gkz972

22. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434-443. doi:10.1038/s41586-020-2308-7

23. Magri F, Nigro V, Angelini C, et al. The Italian limb girdle muscular dystrophy registry: relative frequency, clinical features, and differential diagnosis. *Muscle Nerve*. 2017;55(1):55-68. doi:10.1002/mus.25192

24. Deenen JCW, van Doorn PA, Faber CG, et al. The epidemiology of neuromuscular disorders: age at onset and gender in The Netherlands. *Neuromuscul Disord*. 2016;26(7):447-452. doi:10.1016/j.nmd.2016.04.011

25. Norwood FLM, Harling C, Chinnery PF, Eagle M, Bushby K, Straub V. Prevalence of genetic muscle disease in Northern England: in-depth analysis of a muscle clinic population. *Brain*. 2009;132(Pt 11):3175-3186. doi:10.1093/brain/awp236

26. Megarbane A, Bizzari S, Deepthi A, et al. A 20-year clinical and genetic neuromuscular cohort analysis in Lebanon: an international effort. *J Neuromuscul Dis*. 2022;9(1):193-210. doi:10.3233/JND-210652

27. Müller KI, Ghelue MV, Lund I, Jonsrud C, Arntzen KA. The prevalence of hereditary neuromuscular disorders in Northern Norway. *Brain Behav*. 2021;11(1):e01948. doi:10.1002/brb3.1948

28. Pagola-Lorz I, Vicente E, Ibáñez B, et al. Epidemiological study and genetic characterization of inherited muscle diseases in a northern Spanish region. *Orphanet J Rare Dis*. 2019;14(1):276. doi:10.1186/s13023-019-1227-x

29. Lefter S, Hardiman O, Ryan AM. A population-based epidemiologic study of adult neuromuscular disease in the Republic of Ireland. *Neurology*. 2017;88(3):304-313. doi:10.1212/WNL.0000000000003504

30. Chung B, Wong V, Ip P. Prevalence of neuromuscular diseases in Chinese children: a study in southern China. *J Child Neurol*. 2003;18(3):217-219. doi:10.1177/08830738030180030201

31. El-Tallawy HN, Khedr EM, Qayed MH, Helliwell TR, Kamel NF. Epidemiological study of muscular disorders in Assiut, Egypt. *Neuroepidemiology*. 2005;25(4):205-211. doi:10.1159/000088674

32. Hughes MI, Hicks EM, Nevin NC, Patterson VH. The prevalence of inherited neuromuscular disease in Northern Ireland. *Neuromuscul Disord*. 1996;6(1):69-73. doi:10.1016/0960-8966(94)00017-4

33. Nakagawa M, Nakahara K, Yoshidome H, et al. Epidemiology of progressive muscular dystrophy in Okinawa, Japan. Classification with molecular biological techniques. *Neuroepidemiology*. 1991;10(4):185-191. doi:10.1159/000110268

34. Darin N, Tulinius M. Neuromuscular disorders in childhood: a descriptive epidemiological study from western Sweden. *Neuromuscul Disord*. 2000;10(1):1-9. doi:10.1016/s0960-8966(99)00055-3

35. Theadom A, Rodrigues M, Poke G, et al. A nationwide, population-based prevalence study of genetic muscle disorders. *Neuroepidemiology*. 2019;52(3-4):128-135. doi:10.1159/000494115

36. Guimarães-Costa R, Fernández-Eulate G, Wahbi K, et al. Clinical correlations and long-term follow-up in 100 patients with sarcoglycanopathies. *Eur J Neurol*. 2021;28(2):660-669. doi:10.1111/ene.14592

37. Alonso-Pérez J, González-Quereda L, Bello L, et al. New genotype-phenotype correlations in a large European cohort of patients with sarcoglycanopathy. *Brain*. 2020;143(9):2696-2708. doi:10.1093/brain/awaa228

38. Bardhan M, Anjanappa RM, Polavarapu K, et al. Clinical, genetic profile and disease progression of sarcoglycanopathies in a large cohort from India: high prevalence of SGCB c.544A>C. *Neurogenetics*. 2022;23(3):187-202. doi:10.1007/s10048-022-00690-9

39. Fanin M, Duggan DJ, Mostacciuolo ML, et al. Genetic epidemiology of muscular dystrophies resulting from sarcoglycan gene mutations. *J Med Genet*. 1997;34(12):973-977. doi:10.1136/jmg.34.12.973

40. Fanin M, Nascimbeni AC, Fulizio L, Angelini C. The frequency of limb girdle muscular dystrophy 2A in northeastern Italy. *Neuromuscul Disord*. 2005;15(3):218-224. doi:10.1016/j.nmd.2004.11.003

41. Stensland E, Lindal S, Jonsrud C, et al. Prevalence, mutation spectrum and phenotypic variability in Norwegian patients with Limb Girdle Muscular Dystrophy 2I. *Neuromuscul Disord*. 2011;21(1):41-46. doi:10.1016/j.nmd.2010.08.008

42. Heller SA, Shih R, Kalra R, Kang PB. Emery-Dreifuss muscular dystrophy. *Muscle Nerve*. 2020;61(4):436-448. doi:10.1002/mus.26782

43. Ghaoui R, Cooper ST, Lek M, et al. Use of whole-exome sequencing for diagnosis of limb-girdle muscular dystrophy: outcomes and lessons learned. *JAMA Neurol*. 2015;72(12):1424-1432. doi:10.1001/jamaneurol.2015.2274

44. Reddy HM, Cho KA, Lek M, et al. The sensitivity of exome sequencing in identifying pathogenic mutations for LGMD in the United States. *J Hum Genet*. 2017;62(2):243-252. doi:10.1038/jhg.2016.116

45. Saha M, Reddy HM, Salih MA, et al. Impact of PYROXD1 deficiency on cellular respiration and correlations with genetic analyses of limb-girdle muscular dystrophy in Saudi Arabia and Sudan. *Physiol Genomics*. 2018;50(11):929-939. doi:10.1152/physiolgenomics.00036.2018

46. Bruels CC, Littel HR, Daugherty AL, et al. Diagnostic capabilities of nanopore long-read sequencing in muscular dystrophy. *Ann Clin Transl Neurol*. 2022;9(8):1302-1309. doi:10.1002/acn3.51612

47. Lemmers RJLF, Tawil R, Petek LM, et al. Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. *Nat Genet*. 2012;44(12):1370-1374. doi:10.1038/ng.2454

48. Mitsuhashi S, Boyden SE, Estrella EA, et al. Exome sequencing identifies a novel SMCHD1 mutation in facioscapulohumeral muscular dystrophy 2. *Neuromuscul Disord*. 2013;23(12):975-980. doi:10.1016/j.nmd.2013.08.009

49. Trabelsi M, Kavian N, Daoud F, et al. Revised spectrum of mutations in sarcoglycanopathies. *Eur J Hum Genet*. 2008;16(7):793-803. doi:10.1038/ejhg.2008.9

50. Nadeau A, Kinali M, Main M, et al. Natural history of Ullrich congenital muscular dystrophy. *Neurology*. 2009;73(1):25-31. doi:10.1212/WNL.0b013e3181aae851