HHS Public Access

Author manuscript

Circ Genom Precis Med. Author manuscript; available in PMC 2024 October 01.

Published in final edited form as:

Circ Genom Precis Med. 2023 October ; 16(5): 421–430. doi:10.1161/CIRCGEN.123.004221.

DMD-Associated Dilated Cardiomyopathy: Genotypes, Phenotypes, and Phenocopies

Renee Johnson, PhD, MGC[1,2], **Robyn Otway, PhD**[1], **Ephrem Chin, BTech(Hon), MB(ASCP) QLC**[3,4], **Claire Horvat, PhD**[1], **Monique Ohanian, BMedSci(Hons)**[1], **Jon A.L. Wilcox, PhD**[5], **Zheng Su, BSc**[6], **Priscilla Prestes, MSc, PhD**[7], **Andrei Smolnikov, BAdvSci, BA**[6], **Magdalena Soka, BSc(Hons)**[1], **Guanglan Guo, PhD**[1], **Emma Rath, PhD**[1,2], **Samya Chakravorty, PhD**[3,8], **Lukasz Chrzanowski, MD**[9], **Christopher S. Hayward, MD**[1,2,10], **Anne M. Keogh, MD**[1,2,10], **Peter S. Macdonald, MBBS, PhD**[1,2,10], **Eleni Giannoulatou, DPhil**[1,2], **Alex C.Y. Chang, PhD**[11,12], **Emily C. Oates, MBBS, PhD**[6], **Fadi Charchar, PhD**[7], **Jonathan G. Seidman, PhD**[5,13], **Christine E. Seidman, MD**[5,14], **Madhuri Hegde, PhD**[3,4], **Diane Fatkin, MD**[1,2,10]

[1]Victor Chang Cardiac Rsrch Inst, Darlinghurst;

[2]School of Clinical Medicine, Faculty of Medicine & Health, UNSW Sydney, Kensington, NSW, Australia;

[3]Dept of Human Genetics, Emory Univ School of Medicine, Atlanta GA;

[4]PerkinElmer Genomics, PerkinElmer, Waltham;

[5]Dept of Genetics, Harvard Medical School, Boston, MA;

[6]School of Biotechnology & Biomolecular Sciences, Faculty of Science, UNSW Sydney, Kensington, NSW, Australia;

[7]Health Innovation & Transformation Ctr, Federation Univ Australia, Ballarat, Victoria, Australia;

[8]Biocon Bristol Myers Squibb Rsrch & Development Ctr (BBRC), Bangalore, India;

[9]Dept of Cardiology, Medical Univ of Lodz, Lodz, Poland;

[10]Cardiology Dept, St Vincent's Hospital, Darlinghurst, NSW, Australia;

[11]Dept of Cardiology & Shanghai Inst of Precision Medicine, Ninth People's Hospital, Shanghai Jiao Tong Univ School of Medicine, Shanghai, China;

[12]Baxter Laboratory for Stem Cell Biology, Dept of Microbiology & Immunology, Inst for Stem Cell Biology & Regenerative Medicine, Stanford Univ School of Medicine, Stanford, CA;

[13]Howard Hughes Medical Inst, Boston;

Correspondence: Diane Fatkin, MD, Victor Chang Cardiac Research Institute, 405 Liverpool St., Darlinghurst, NSW 2010, Australia, Tel: +61 2 9295 8618, Fax: +61 2 9295 8769, d.fatkin@victorchang.edu.au.

Disclosures: E.C. and M.H. are salaried employees of Perkin Elmer Inc. There are no disclosures for other authors.

Supplemental Material:
Supplemental Methods
Supplemental Tables I–XI
Supplemental Figures I–IV
References[38–85]

[14]Cardiovascular Division, Brigham and Women's Hospital, Boston MA

Abstract

Background: Variants in the *DMD* gene, that encodes the cytoskeletal protein, dystrophin, cause a severe form of dilated cardiomyopathy (DCM) associated with high rates of heart failure, heart transplantation, and ventricular arrhythmias. Improved early detection of individuals at risk is needed.

Methods: Genetic testing of 40 male probands with a potential X-linked genetic cause of primary DCM was undertaken using multi-gene panel sequencing, multiplex polymerase chain reaction, and array comparative genomic hybridization. Variant location was assessed with respect to dystrophin isoform patterns and exon usage. Telomere length was evaluated as a marker of myocardial dysfunction in left ventricular tissue and blood.

Results: Four pathogenic/likely pathogenic *DMD* variants were found in 5 probands (5/40: 12.5%). Only one rare variant was identified by gene panel testing with 3 additional multi-exon deletion/duplications found following targeted assays for structural variants. All of the pathogenic/likely pathogenic *DMD* variants involved dystrophin exons that had percent spliced-in scores >90, indicating high levels of constitutive expression in the human adult heart. 15 *DMD* variant-negative probands (15/40: 37.5%) had variants in autosomal genes including *TTN*, *BAG3*, *LMNA*, and *RBM20*. Myocardial telomere length was reduced in patients with DCM irrespective of genotype. No differences in blood telomere length were observed between genotype-positive family members with/without DCM and controls.

Conclusions: Primary genetic testing using multi-gene panels has a low yield and specific assays for structural variants are required if *DMD*-associated cardiomyopathy is suspected. Distinguishing X-linked etiologies of DCM from autosomal genes that show sex differences in clinical presentation is crucial for informed family management.

Keywords

X-linked dilated cardiomyopathy; *DMD* gene; dystrophin; titin; genetics; heart

X-linked dilated cardiomyopathy (DCM) was first described by Berko and Swift in 1987 as a rapidly progressive form of DCM affecting males in their late teens or early 20s, with milder and late-onset manifestations in carrier females.[1] It has been associated with variants in the *DMD* gene, that encodes the cytoskeletal scaffolding protein, dystrophin. However, knowledge of the genetic underpinnings of this disorder is incomplete since *DMD* variants have been identified in a minority (up to 14%) of males with suspected X-linked DCM.[2–4] Moreover, most studies have only undertaken limited screening of the *DMD* gene using hybridization techniques to detect large structural variants or focussed evaluation of mutation hotspots. X-linked DCM is often phenotypically indistinguishable from DCM due to other causes and there is increasing awareness that disease manifestations may not be restricted to young males.[5] These factors confound reliable differentiation between X-linked and autosomal dominant inheritance patterns, especially in sporadic cases and small families.

DMD mutations have been widely studied as a cause of Duchenne and Becker muscular dystrophies (MD), both of which are complicated by cardiac dysfunction. The varying severity of skeletal myopathy in Duchenne and Becker MD has been attributed to mutation impact (frameshift vs in-frame) and differences in the quantity and quality of dystrophin produced.[6] Why some *DMD* variants result in a primary cardiomyopathy (X-linked DCM) with absent or subclinical skeletal muscle involvement is unclear.

Restrepo-Cordoba and colleagues[5] recently evaluated 223 *DMD* variant carriers without severe skeletal myopathy and showed that 22% of individuals experienced end-stage heart failure or sudden cardiac death. These findings highlight the clinical importance of this disorder and the need for early recognition of genotype-positive cases. Here we report genetic analyses of a cohort of males with a potential X-linked cause of DCM using contemporary gene panel-based next generation sequencing in the first instance. We evaluated new bioinformatics methods to inform *DMD* variant interpretation based on dystrophin isoform expression and exon usage. We also investigated telomere length as a biomarker of cardiac dysfunction. Collectively our data highlight the spectrum of genetic variation underpinning *DMD*-associated DCM and the phenotypic overlap with forms of autosomal dominant DCM that show sex differences in clinical presentation. Our findings have implications for medical surveillance, risk stratification, and genetic counselling of probands and relatives.

Methods

An expanded Methods section is provided in the Supplemental Material. Study data and materials are available from the corresponding author upon reasonable request. All participants provided informed written consent and protocols were approved by the Human Research Ethics Committee of St Vincent's Hospital and the Stanford Institutional Review Board.

Results

Study subjects

174 probands with suspected heritable cardiomyopathies (60% males; aged 41± 15 years at diagnosis) were recruited for genetics research at a single site (Supplemental Methods). A subset of 40 male probands was selected for detailed evaluation of *DMD* variants. Selection criteria included: primary clinical presentation of DCM at <60 years of age, no identified acquired cause of DCM, and a family history at the time of study entry that was consistent with an X-linked etiology or indeterminate. Thirty-nine probands (98%) had self-reported European ancestry. Clinical features and genetic results for the 40 probands and their relatives are summarized in Supplemental Table III.

DMD Genotypes

Single nucleotide variants.—Multi-gene panel sequencing of proband DNA samples identified 22 protein-altering *DMD* variants, of which 3 were rare (MAF <0.1%; Supplemental Table IV; pedigrees shown in Figure 1). The latter included a splicing variant, c.31+1G>T, found in two probands, AI-IV-2 and DB-IV-6, that has been reported in several

kindreds with DCM.[4,7–9] Delving further into the family history, it was ascertained that Families AI and DB had an unrecognized distant common ancestor. The remaining 2 rare variants, p.S738L and p.M1576I, were both missense and classified as variants of uncertain significance (VUS). Four additional X-chromosome genes associated with DCM (*EMD*, *FHL1*, *LAMP2*, *TAZ*) were evaluated but none of the 13 variants identified in these genes met ACMG criteria for pathogenicity. Sequence data for the remaining 134 probands with suspected autosomal dominant disease were reviewed, with no additional *DMD* single nucleotide variants identified.

Structural variants—Using multiplex PCR to identify *DMD* structural variants we identified 1 deletion extending from exon 4 to exon 9 in ER-II-5 that was subsequently confirmed by aCGH (Supplemental Table V; pedigree shown in Figure 1). Two further exonic variants were found by aCGH. This included a duplication spanning exons 3 to 12 in EC-II-3, one of a set of previously-reported monozygotic triplets.[10] This was a *de novo* variant that was present in the other living affected triplet, EC-II-4, but absent in both unaffected parents and an older unaffected sibling. A deletion in the C-terminal region, involving exons 75 and 76, was identified in AJ-II-2. In addition to these 3 pathogenic/likely pathogenic (P/LP) structural variants, we found an exon 74 duplication in Q-II-3 that was classified as a VUS. Seven intronic variants were found in 10 probands, including duplications in introns 2, 62, 67 and deletions in introns 7, 29, 60, 64, all of which were VUS. Specific assays for structural variants were not performed in the 134 probands with suspected autosomal dominant disease. However, no variants of this type were evident in the 50 individuals (37%) who had undergone whole-genome sequencing.

DMD Variant Phenotypes

In the 5 families with P/LP *DMD* variants (total 22 individuals), there were 13 affected males (mean age 31 ± 18 years), many of whom had a rapidly progressive downhill course culminating in early heart transplantation (<25 years age, n=4), or premature death (n=5) (Supplemental Table III). Creatine kinase levels were elevated in a subset of males, and this had led to a diagnosis of atypical Becker MD in some cases. Three of the 7 adult genotype-positive females were affected, all of whom had relatively mild cardiac disease and absent skeletal muscle involvement. Two of these women had DCM onset aged >60 years, with the third being diagnosed with peripartum cardiomyopathy aged 34 years.

Reported DCM-Associated *DMD* Variants

To better understand the spectrum of *DMD* genetic variation associated with a primary clinical presentation of DCM, we undertook a literature review and identified 71 variants in 116 reported probands. Inclusion of variants in the present study yielded a total of 77 variants in 126 probands (Supplemental Table VI). Following re-curation of variant pathogenicity using ACMG criteria, only 59 (77%) variants were classified as P/LP: 36 large (>1 exon) variants, 21 small (<1 exon) variants, 2 intronic variants (Table 1). When compared to variants seen in Duchenne MD,[11] P/LP DCM-associated variants were less likely to be multi-exon structural variants (61% vs 79%) and more likely to be small deletions/insertions, splice-site or nonsense variants (36% vs 20%, Table 1). Of these, 35 (59%) variants (15 large, 20 small) were predicted to result in a shift of the reading frame.

39 (66%) P/LP variants were located in the 2 hotspots reported for Duchenne MD in exons 2–20 and exons 45–56, respectively (Figure 2;[12]). Outside of these hotspots, there were 9 variants (15%, all small) in the M promoter/exon 1 region, 10 variants (17%, 1 large, 9 small) in the proximal rod (exons 21–44) and 1 variant (large) in the C-terminus.

Derivation of Dystrophin PSI Scores and Impact of Variant Location

For genes that produce numerous isoforms via alternative splicing, percent spliced-in (PSI) scores represent the frequency in which individual exons are included across the range of transcripts, with variants in exons with high PSI scores having greater potential for functional effects.[13] To derive PSI scores for *DMD*, we first needed to characterize dystrophin isoforms in the heart. An analysis of *DMD* transcript promoters using bulk RNA sequencing data showed that Dp427m was the predominant transcript in human left ventricle (LV), right atrium (RA), and skeletal muscle, followed by the shorter C-terminal isoform, Dp71 (Figure 2, Supplemental Table VII). Nuclear sequencing of unaffected human heart tissue confirmed that most Dp427m transcripts occurred in ventricular (70%) or atrial (21%) cardiomyocytes (38% and 7% of the total assigned cell population, respectively), while 83% of Dp71 transcripts were located in pericytes (18% of the total assigned cell population; Supplemental Table VIII). In the GTEx data, *DMD* exons showed a high level of constitutive expression in adult heart, pediatric/fetal heart, and skeletal muscle, with the exception of exons 71 & 78, that had intermediate PSI values ($10 < PSI < 90$), with exon 71 uniquely showing significant differences between cardiac chambers: exon 71: LV, PSI=66, RA, PSI=60, $p=1.7\times10^{-11}$; exon 78: LV, PSI=75, RA, PSI=75, p=0.41 (Figure 2, Supplemental Table IX, Supplemental Figure I). All of the P/LP *DMD* variants involved exons with high (>90) PSI scores.

Phenocopies of *DMD*-Associated DCM

Sequencing data from the 40 study probands were also interrogated for other genetic causes of DCM. Fifteen probands in whom *DMD* variants were undetected had heterozygous P/LP variants in *TTN* (n=8), *BAG3* (n=2), *DES* (n=1), *LMNA* (n=1), *MYH7* (n=1), *RBM20* (n=1), and *SCGB* (n=1) (Supplemental Table X; pedigrees shown in Supplemental Figures II & III). This yield of autosomal gene variants (37.5%) was similar to that observed in the remaining 134 probands who were evaluated with the same gene set (25.4%, p=0.16; Supplemental Table XI). In these 15 families (total 67 individuals), the mean age at DCM diagnosis in affected males (32 ± 15 years, n=29; unknown age in 4 males) was significantly younger than in females (50 ± 17 years, n=19; p=0.0003) but equivalent to males with *DMD* variants (n=13; p=0.86). 25 family members (21 males) had heart transplantation (n=11), or premature death (n=14). The prevalence of these major adverse events (25/67 [37.3%] individuals) was also similar to the *DMD* variant group (9/22 [40.9%] individuals; p=0.80). Three affected females with *TTN* (n=2) and *BAG3* (n=1) variants respectively, had an accelerated disease onset due to peripartum cardiomyopathy, with two of these women needing heart transplantation and one dying suddenly. In Family CK, individuals with the *BAG3* nonsense variant also carried two common *DMD* VUS, p.E2910V and p.N2912D that together, have been shown to alter biophysical properties of the dystrophin rod, with potential phenotype-modifying effects (Supplemental Table IV).[14]

Telomere Length as a Biomarker of Disease Severity

Telomere shortening has been associated with accelerated cardiac dysfunction in Duchenne MD.[15] We investigated telomere length in LV tissues from 10 individuals undergoing heart transplantation who carried variants in *DMD* (Families AI & DB, n=3), *TTN* (Families AV & N, n=3) and *LMNA* (n=4). All samples showed a marked reduction in telomere length when compared to control hearts, with no genotype differences (ANOVA $p<0.0001$; Supplemental Figure IVA). Blood telomere length was also evaluated in 80 individuals from families with *DMD* and *TTN* variants. There were no differences in the T/S ratio between variant-positive individuals with DCM (G+P+, n=30), variant-positive individuals without DCM (G+P-, n=11), and variant-negative individuals without DCM (G-P-, n=39) groups (ANOVA $p=0.78$; Supplemental Figure IVB). These data suggest that: (i) telomere shortening in heart tissue is driven mainly by disease severity rather than by genotype, and (ii) assessment of telomere length in blood is unlikely to be a useful biomarker for early detection of myocardial disease.

Discussion

Recent data have highlighted DCM-associated *DMD* variants as an important cause of morbidity and mortality in males, thus making early recognition of genotype-positive cases a clinical imperative.[5] This requires a high level of clinical suspicion for a potential X-linked etiology, appropriate genetic testing and variant interpretation, and sensitive methods for detection and monitoring of myocardial dysfunction.

DMD-associated DCM is typically considered in young males with DCM ± raised creatine kinase levels, particularly in families where females are unaffected or have mild DCM in later life.[5] Accumulating evidence that DCM can arise in older males and females of any age blurs clear distinction between X-linked and autosomal dominant inheritance and raises a clinical conundrum. Reflecting this, we found that 5/40 (12.5%) of males with sporadic or possible X-linked DCM carried *DMD* variants, while an additional 15/40 (37.5%) probands had P/LP variants in autosomal genes, several of which have been reported to show sex differences in age at DCM diagnosis or disease severity (Table 2).[5,16–31] It is important to note that phenotypic manifestations are not determined solely by the underlying genetic variant and pinpointing a typical age at diagnosis within families may be confounded by extrinsic factors such as pregnancy, co-morbidities or lifestyle, that accelerate disease onset in individual cases. Phenotypic overlap may also occur with genes such as *DES* and *LMNA* in which DCM can be accompanied by skeletal myopathy (Table 2).

Historically, genetic testing for *DMD*-associated DCM focussed on evaluating large variants or sequencing known mutation hotspots rather than sequencing the whole gene. This biases against detection of small variants that may be deleterious. Contemporary genetic testing using multi-gene panel or exome sequencing introduces an opposite problem since large variants are generally unable to be assessed. Additional testing methods are required if an X-linked cause of DCM is suspected. Although much less common than in Duchenne MD, large variants comprise more than half of the P/LP variants reported in *DMD*-associated DCM (Table 1). We found only one P/LP *DMD* variant (c.31+1G>T) by sequencing, with 3 further P/LP variants identified by multiplex PCR and aCGH. In this context, whole-genome

sequencing provides an attractive first-line genetic testing method since large and small variants can be detected in the same dataset.[32] Following our re-curation of reported *DMD* variants, it was notable that none of the missense variants achieved P/LP status, and most were only classifiable as VUS due to insufficient clinical and functional data. The extent to which *DMD* missense variants might contribute to the burden of disease remains unresolved.

Truncating *TTN* variants (*TTN*tv) were the single most common cause of DCM, being present in 8 (20%) of our probands. Dystrophin and titin are both giant proteins that have key roles in cardiac structure and function. Experimental studies suggest that mutations in *DMD* and *TTN* share a number of pathophysiological mechanisms, including changes in force transmission, resistance to mechanical stress, cell signalling, myocardial energetics, and cell survival.[33,34] Titin transcripts show extensive alternative splicing and assessing PSI scores has become a cornerstone of clinical variant interpretation for *TTN*tv.[13] Here, for the first time, we derived PSI scores for cardiac dystrophin and found that unlike titin, most dystrophin exons are highly utilized across all transcripts. These findings indicate that exon PSI scores have limited application for prioritization of *DMD* variants.

It is unclear why *DMD* variants have tissue differences in phenotypic expression. Duchenne MD is characteristically associated with frameshift variants that abolish dystrophin expression while the relatively milder skeletal muscle phenotype of Becker MD is thought to result from persistent truncated protein associated with in-frame variants.[6] Here we find that two thirds of reported DCM-associated P/LP variants arise in exonic hotspots for Duchenne MD and more than half of all P/LP variants are predicted to be frame-shifting. The “reading frame rule” thus incompletely explains the severe cardiac dysfunction that can occur in both Duchenne and Becker MD or a primary DCM phenotype. An important cluster of 9 DCM variants (representing 15% of P/LP cases) was seen in the M promoter/first exon region. The c.31+1G>T variant abolishes the 5’ splice site of the large 1st intron at its junction with exon 1 and is an example of this variant type. Variants in this location have been associated with absent or very low levels of dystrophin expression in the heart, with the lack of overt skeletal muscle involvement attributed to selective up-regulation of brain and Purkinje isoforms.[6] Mechanisms for cardiac dysfunction associated with variants in other dystrophin regions remain to be elucidated but could involve perturbation of critical cardiac-specific protein interactions.

Emerging genetic correction strategies appear to ameliorate skeletal muscle dysfunction in Duchenne MD but their impact on cardiac function remains unproven.[35,36] Several drug therapies have also been used in animal models, including treatment with antioxidants to improve mitochondrial function and preserve telomere length.[15] Telomere shortening has been documented in hearts of patients with genetic cardiomyopathies[37] and was also present in heart tissues from affected individuals with *DMD, TTN,* and *LMNA* variants in our study. Although telomere shortening appears to be a nonspecific effect of DCM, these data suggest that protection of disease-related telomere erosion could be beneficial. Use of telomere length as a biomarker of disease progression is hampered by the lack of readily available serial myocardial tissue samples and inability of assessment of telomere length in blood to act as an informative surrogate.

There are several limitations of this study, including incomplete family member participation and small family sizes. The yield of *DMD* variants may be under-estimated due to insufficient supportive family segregation and functional data, particularly for the exon 74 duplication and intronic structural variants. Although we did not find *DMD* variants in any of the 134 probands in whom autosomal dominant disease was suspected at the time of study entry, structural variants were not evaluated in two-thirds of these cases. Collectively, however, our data suggest that the yield of *DMD* variants in unselected patients with familial DCM is low (<3%). These results highlight the need for a high index of clinical suspicion, given the significant consequences of P/LP *DMD* variants in variant carriers. Since our study subjects were predominantly European, further investigation is warranted to determine the applicability of our findings to other ancestry groups.

Our data provide fresh perspectives on the spectrum of variant types and phenotypic features of *DMD*-associated DCM and show how this disorder can mimic autosomal dominant forms of DCM and vice versa. If *DMD*-associated DCM is suspected, tailored genetic testing strategies are needed that include evaluation of structural variants. Accurate delineation of genetic causes of DCM is crucial for informed precision approaches to family management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Sources of Funding:

This work was supported by grants from the National Health and Medical Research Council of Australia (1123472: F.C; 1074386; D.F), NSW Health (D.F.), Shanghai Pujiang Program (19PJ1407000; A.C.Y.C.); The Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning (0900000024; A.C.Y.C.); Innovative Research Team of High-Level Local Universities in Shanghai (SHSMU-ZLCX20211700; A.C.Y.C.); the American Heart Association (13POST14480004, 18CDA34110411; A.C.Y.C.), National Heart, Lung, and Blood Institute or the National Institutes of Health (1R01HL080494,1R01HL084553; C.E.S. and J.G.S.), Howard Hughes Medical Institute (C.E.S).

Nonstandard Abbreviations and Acronyms

aCGH	array comparative genomic hybridization
ACMG	American College of Medical Genetics and Genomics
LP	likely pathogenic
MAF	minor allele frequency
MD	muscular dystrophy
P	pathogenic
PSI	percent spliced-in
Q-FISH	quantitative fluorescent *in situ* hybridization
VUS	variant of uncertain significance

References:

1. Berko BA, Swift M. X-linked dilated cardiomyopathy. N Engl J Med. 1987;316:1186–1191. [PubMed: 3574369]
2. Arbustini E, Diegoli M, Morbini P, Dal Bello B, Banchieri N, Pilotto A, Magani F, Grasso M, Narula J, Gavazzi A, et al. Prevalence and characteristics of dystrophin defects in adult male patients with dilated cardiomyopathy. J Am Coll Cardiol. 2000;35:1760–1768. [PubMed: 10841222]
3. Diegoli M, Grasso M, Favalli V, Serio A, Gambarin FI, Klersy C, Pasotti M, Agozzino E, Scelsi L, Ferlini A, et al. Diagnostic work-up and risk stratification in X-linked dilated cardiomyopathies caused by dystrophin defects. J Am Coll Cardiol. 2011;58:925–934. [PubMed: 21851881]
4. Feng J, Yan JY, Buzin CH, Sommer SS, Towbin JA. Comprehensive mutation scanning of the dystrophin gene in patients with nonsyndromic X-linked dilated cardiomyopathy. J Am Coll Cardiol. 2002;40:1120–1124. [PubMed: 12354438]
5. Restrepo-Cordoba MA, Wahbi K, Florian AR, Jimenez-Jaimez J, Politano L, Arad M, Climent-Paya V, Garcia-Alvarez A, Hansen RB, Larranaga-Moreira JM, et al. Prevalence and clinical outcomes of dystrophin-associated dilated cardiomyopathy without severe skeletal myopathy. Eur J Heart Fail. 2021;23:1276–1286. [PubMed: 34050592]
6. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. Lancet Neurol. 2003;2:731–740. [PubMed: 14636778]
7. Milasin J, Muntoni F, Severini GM, Bartoloni L, Vatta M, Krajinovic M, Mateddu A, Angelini C, Camerini F, Falaschi A, et al. A point mutation in the 5' splice site of the dystrophin gene first intron responsible for X-linked dilated cardiomyopathy. Hum Mol Genet. 1996;5:73–79. [PubMed: 8789442]
8. Nguyen TV, Tran Vu MT, Do TNP, Tran THN, Do TH, Nguyen TMH, Tran Huynh BN, Le LA, Nguyen Pham NT, Nguyen TDA, et al. Genetic determinants and genotype-phenotype correlations in Vietnamese patients with dilated cardiomyopathy. Circ J. 2021;85:1469–1478. [PubMed: 34011823]
9. Obler D, Wu BL, Lip V, Estrella E, Keck S, Haggan C, Semigran M, Smoot LB. Familial dilated cardiomyopathy secondary to dystrophin splice site mutation. J Card Fail. 2010;16:194–199. [PubMed: 20206892]
10. Chrzanowski L, Kasprzak JD, Trzos E, Wasikowski K, Drozdz J, Ryniewicz B, Krzeminska-Pakula M. Different expressions of X-linked cardiomyopathy in monozygotic triplets with Becker's dystrophy. Int J Cardiovasc Imaging. 2003;19:377–380. [PubMed: 14609186]
11. Bladen CL, Salgado D, Monges S, Foncuberta ME, Kekou K, Kosma K, Dawkins H, Lamont L, Roy AJ, Chamova T, et al. The TREAT-NMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. Hum Mutat. 2015;36:395–402. [PubMed: 25604253]
12. Liu W, Xie Y, Ma J, Luo X, Nie P, Zuo Z, Lahrmann U, Zhao Q, Zheng Y, Zhao Y, et al. IBS: an illustrator for the presentation and visualization of biological sequences. Bioinformatics. 2015;31:3359–3361. [PubMed: 26069263]
13. Roberts AM, Ware JS, Herman DS, Schafer S, Baksi J, Bick AG, Buchan RJ, Walsh R, John S, Wilkinson S, et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. Sci Transl Med. 2015;7:270ra276.
14. Legardinier S, Legrand B, Raguenes-Nicol C, Bondon A, Hardy S, Tascon C, Le Rumeur E, Hubert JF. A Two-amino acid mutation encountered in Duchenne muscular dystrophy decreases stability of the rod domain 23 (R23) spectrin-like repeat of dystrophin. J Biol Chem. 2009;284:8822–8832. [PubMed: 19158079]
15. Mourkioti F, Kustan J, Kraft P, Day JW, Zhao MM, Kost-Alimova M, Protopopov A, DePinho RA, Bernstein D, Meeker AK, et al. Role of telomere dysfunction in cardiac failure in Duchenne muscular dystrophy. Nat Cell Biol. 2013;15:895–904. [PubMed: 23831727]
16. Alsalem A, Zado ES, Deo R, Santangeli P, Garcia FC, Marchlinski FE, Hyman MC. Pathogenic variants in *EMD* are associated with an isolated cardiac emerinopathy. Heart Rhythm. 2022;19:S42.

17. Brambatti M, Caspi O, Maolo A, Koshi E, Greenberg B, Taylor MRG, Adler ED. Danon disease: Gender differences in presentation and outcomes. Int J Cardiol. 2019;286:92–98. [PubMed: 30857840]

18. Roberts AE, Nixon C, Steward CG, Gauvreau K, Maisenbacher M, Fletcher M, Geva J, Byrne BJ, Spencer CT. The Barth Syndrome Registry: distinguishing disease characteristics and growth data from a longitudinal study. Am J Med Genet A. 2012;158A:2726–2732. [PubMed: 23045169]

19. Dominguez F, Cuenca S, Bilinska Z, Toro R, Villard E, Barriales-Villa R, Ochoa JP, Asselbergs F, Sammani A, Franaszczyk M, et al. Dilated cardiomyopathy due to BLC2-associated athanogene 3 (BAG3) mutations. J Am Coll Cardiol. 2018;72:2471–2481. [PubMed: 30442290]

20. van Spaendonck-Zwarts KY, van Hessem L, Jongbloed JD, de Walle HE, Capetanaki Y, van der Kooi AJ, van Langen IM, van den Berg MP, van Tintelen JP. Desmin-related myopathy. Clin Genet. 2011;80:354–366. [PubMed: 20718792]

21. Bariani R, Cason M, Rigato I, Cipriani A, Celeghin R, De Gaspari M, Bueno Marinas M, Mattesi G, Pergola V, Rizzo S, et al. Clinical profile and long-term follow-up of a cohort of patients with desmoplakin cardiomyopathy. Heart Rhythm. 2022;19:1315–1324. [PubMed: 35470109]

22. Smith ED, Lakdawala NK, Papoutsidakis N, Aubert G, Mazzanti A, McCanta AC, Agarwal PP, Arscott P, Dellefave-Castillo LM, Vorovich EE, et al. Desmoplakin cardiomyopathy, a fibrotic and inflammatory form of cardiomyopathy distinct from typical dilated or arrhythmogenic right ventricular cardiomyopathy. Circulation. 2020;141:1872–1884. [PubMed: 32372669]

23. Wang W, Murray B, Tichnell C, Gilotra NA, Zimmerman SL, Gasperetti A, Scheel P, Tandri H, Calkins H, James CA. Clinical characteristics and risk stratification of desmoplakin cardiomyopathy. Europace. 2022;24:268–277. [PubMed: 34352074]

24. Gigli M, Stolfo D, Graw SL, Merlo M, Gregorio C, Nee Chen S, Dal Ferro M, Paldino MA, De Angelis G, Brun F, et al. Phenotypic expression, natural history, and risk stratification of cardiomyopathy caused by filamin C truncating variants. Circulation. 2021;144:1600–1611. [PubMed: 34587765]

25. Ortiz-Genga MF, Cuenca S, Dal Ferro M, Zorio E, Salgado-Aranda R, Climent V, Padron-Barthe L, Duro-Aguado I, Jimenez-Jaimez J, Hidalgo-Olivares VM, et al. Truncating *FLNC* mutations are associated with high-risk dilated and arrhythmogenic cardiomyopathies. J Am Coll Cardiol. 2016;68:2440–2451. [PubMed: 27908349]

26. Kumar S, Baldinger SH, Gandjbakhch E, Maury P, Sellal JM, Androulakis AF, Waintraub X, Charron P, Rollin A, Richard P, et al. Long-term arrhythmic and nonarrhythmic outcomes of lamin A/C mutation carriers. J Am Coll Cardiol. 2016;68:2299–2307. [PubMed: 27884249]

27. van Rijsingen IA, Nannenberg EA, Arbustini E, Elliott PM, Mogensen J, Hermans-van Ast JF, van der Kooi AJ, van Tintelen JP, van den Berg MP, Grasso M, et al. Gender-specific differences in major cardiac events and mortality in lamin A/C mutation carriers. Eur J Heart Fail. 2013;15:376–384. [PubMed: 23183350]

28. Hey TM, Rasmussen TB, Madsen T, Aagaard MM, Harbo M, Molgaard H, Moller JE, Eiskjaer H, Mogensen J. Pathogenic *RBM20* variants are associated with a severe disease expression in male patients with dilated cardiomyopathy. Circ Heart Fail. 2019;12:e005700. [PubMed: 30871348]

29. Parikh VN, Caleshu C, Reuter C, Lazzeroni LC, Ingles J, Garcia J, McCaleb K, Adesiyun T, Sedaghat-Hamedani F, Kumar S, et al. Regional variation in *RBM20* causes a highly penetrant arrhythmogenic cardiomyopathy. Circ Heart Fail. 2019;12:e005371. [PubMed: 30871351]

30. Akhtar MM, Lorenzini M, Cicerchia M, Ochoa JP, Hey TM, Sabater Molina M, Restrepo-Cordoba MA, Dal Ferro M, Stolfo D, Johnson R, et al. Clinical phenotypes and prognosis of dilated cardiomyopathy caused by truncating variants in the *TTN* gene. Circ Heart Fail. 2020;13:e006832. [PubMed: 32964742]

31. Tayal U, Newsome S, Buchan R, Whiffin N, Halliday B, Lota A, Roberts A, Baksi AJ, Voges I, Midwinter W, et al. Phenotype and clinical outcomes of titin cardiomyopathy. J Am Coll Cardiol. 2017;70:2264–2274. [PubMed: 29073955]

32. Minoche AE, Horvat C, Johnson R, Gayevskiy V, Morton SU, Drew AP, Woo K, Statham AL, Lundie B, Bagnall RD, et al. Genome sequencing as a first-line genetic test in familial dilated cardiomyopathy. Genet Med. 2019;21:650–662. [PubMed: 29961767]

33. Gowran A, Brioschi M, Rovina D, Chiesa M, Piacentini L, Mallia S, Banfi C, Pompilio G, Santoro R. Multiomic Approaches to uncover the complexities of dystrophin-associated cardiomyopathy. Int J Mol Sci. 2021;22. [PubMed: 35008458]
34. Loescher CM, Hobbach AJ, Linke WA. Titin (TTN): from molecule to modifications, mechanics and medical significance. Cardiovasc Res. 2022;118:2908–2918.
35. Johnston JR, McNally EM. Genetic correction strategies for Duchenne muscular dystrophy and their impact on the heart. Prog Pediatr Cardiol. 2021;63:101460. [PubMed: 34898968]
36. Kamdar F, Garry DJ. Dystrophin-deficient cardiomyopathy. J Am Coll Cardiol. 2016;67:2533–2546. [PubMed: 27230049]
37. Chang ACY, Chang ACH, Kirillova A, Sasagawa K, Su W, Weber G, Lin J, Termglinchan V, Karakikes I, Seeger T, et al. Telomere shortening is a hallmark of genetic cardiomyopathies. Proc Natl Acad Sci U S A. 2018;115:9276–9281. [PubMed: 30150400]
38. Ip E, Chapman G, Winlaw D, Dunwoodie SL, Giannoulatou E. VPOT: A customizable variant prioritization ordering tool for annotated variants. Genomics Proteomics Bioinformatics. 2019;17:540–545. [PubMed: 31765830]
39. Morales A, Kinnamon DD, Jordan E, Platt J, Vatta M, Dorschner MO, Starkey CA, Mead JO, Ai T, Burke W, et al. Variant interpretation for dilated cardiomyopathy: Refinement of the American College of Medical Genetics and Genomics/ClinGen Guidelines for the DCM Precision Medicine Study. Circ Genom Precis Med. 2020;13:e002480. [PubMed: 32160020]
40. Abbs S, Yau SC, Clark S, Mathew CG, Bobrow M. A convenient multiplex PCR system for the detection of dystrophin gene deletions: a comparative analysis with cDNA hybridisation shows mistypings by both methods. J Med Genet. 1991;28:304–311. [PubMed: 1865467]
41. Hegde MR, Chin EL, Mulle JG, Okou DT, Warren ST, Zwick ME. Microarray-based mutation detection in the dystrophin gene. Hum Mutat. 2008;29:1091–1099. [PubMed: 18663755]
42. Cardoso-Moreira M, Halbert J, Valloton D, Velten B, Chen C, Shao Y, Liechti A, Ascencao K, Rummel C, Ovchinnikova S, et al. Gene expression across mammalian organ development. Nature. 2019;571:505–509. [PubMed: 31243369]
43. Consortium GT. The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science. 2020;369:1318–1330. [PubMed: 32913098]
44. Reichart D, Lindberg EL, Maatz H, Miranda AMA, Viveiros A, Shvetsov N, Gartner A, Nadelmann ER, Lee M, Kanemaru K, et al. Pathogenic variants damage cell composition and single cell transcription in cardiomyopathies. Science. 2022;377:eabo1984.
45. Schafer S, Miao K, Benson CC, Heinig M, Cook SA, Hubner N. Alternative splicing signatures in RNA-seq Data: percent spliced in (PSI). Curr Protoc Hum Genet. 2015;87:11.16.1–11.16.14.
46. Chang AC, Ong SG, LaGory EL, Kraft PE, Giaccia AJ, Wu JC, Blau HM. Telomere shortening and metabolic compromise underlie dystrophic cardiomyopathy. Proc Natl Acad Sci U S A. 2016;113:13120–13125. [PubMed: 27799523]
47. Meeker AK, Hicks JL, Gabrielson E, Strauss WM, De Marzo AM, Argani P. Telomere shortening occurs in subsets of normal breast epithelium as well as in situ and invasive carcinoma. Am J Pathol. 2004;164:925–935. [PubMed: 14982846]
48. Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ, West of Scotland Coronary Prevention Study G. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet. 2007;369:107–114. [PubMed: 17223473]
49. Piko H, Vancso V, Nagy B, Ban Z, Herczegfalvi A, Karcagi V. Dystrophin gene analysis in Hungarian Duchenne/Becker muscular dystrophy families - detection of carrier status in symptomatic and asymptomatic female relatives. Neuromuscul Disord. 2009;19:108–112. [PubMed: 19084397]
50. Chen WJ, Lin QF, Zhang QJ, He J, Liu XY, Lin MT, Murong SX, Liou CW, Wang N. Molecular analysis of the dystrophin gene in 407 Chinese patients with Duchenne/Becker muscular dystrophy by the combination of multiplex ligation-dependent probe amplification and Sanger sequencing. Clin Chim Acta. 2013;423:35–38. [PubMed: 23588064]

51. Deepha S, Vengalil S, Preethish-Kumar V, Polavarapu K, Nalini A, Gayathri N, Purushottam M. MLPA identification of dystrophin mutations and in silico evaluation of the predicted protein in dystrophinopathy cases from India. BMC Med Genet. 2017;18:67. [PubMed: 28610567]
52. Tuffery-Giraud S, Beroud C, Leturcq F, Yaou RB, Hamroun D, Michel-Calemard L, Moizard MP, Bernard R, Cossee M, Boisseau P, et al. Genotype-phenotype analysis in 2,405 patients with a dystrophinopathy using the UMD-DMD database: a model of nationwide knowledgebase. Hum Mutat. 2009;30:934–945. [PubMed: 19367636]
53. Polavarapu K, Preethish-Kumar V, Sekar D, Vengalil S, Nashi S, Mahajan NP, Thomas PT, Sadasivan A, Warrier M, Gupta A, et al. Mutation pattern in 606 Duchenne muscular dystrophy children with a comparison between familial and non-familial forms: a study in an Indian large single-center cohort. J Neurol. 2019;266:2177–2185. [PubMed: 31139960]
54. Bastianutto C, Bestard JA, Lahnakoski K, Broere D, De Visser M, Zaccolo M, Pozzan T, Ferlini A, Muntoni F, Patarnello T, et al. Dystrophin muscle enhancer 1 is implicated in the activation of non-muscle isoforms in the skeletal muscle of patients with X-linked dilated cardiomyopathy. Hum Mol Genet. 2001;10:2627–2635. [PubMed: 11726549]
55. Muntoni F, Cau M, Ganau A, Congiu R, Arvedi G, Mateddu A, Marrosu MG, Cianchetti C, Realdi G, Cao A, et al. Brief report: deletion of the dystrophin muscle-promoter region associated with X-linked dilated cardiomyopathy. N Engl J Med. 1993;329:921–925. [PubMed: 8361506]
56. Muntoni F, Melis MA, Ganau A, Dubowitz V. Transcription of the dystrophin gene in normal tissues and in skeletal muscle of a family with X-linked dilated cardiomyopathy. Am J Hum Genet. 1995;56:151–157. [PubMed: 7825571]
57. Yoshida K, Ikeda S, Nakamura A, Kagoshima M, Takeda S, Shoji S, Yanagisawa N. Molecular analysis of the Duchenne muscular dystrophy gene in patients with Becker muscular dystrophy presenting with dilated cardiomyopathy. Muscle Nerve. 1993;16:1161–1166. [PubMed: 8413368]
58. Yoshida K, Nakamura A, Yazaki M, Ikeda S, Takeda S. Insertional mutation by transposable element, L1, in the *DMD* gene results in X-linked dilated cardiomyopathy. Hum Mol Genet. 1998;7:1129–1132. [PubMed: 9618170]
59. Papa AA, D'Ambrosio P, Petillo R, Palladino A, Politano L. Heart transplantation in patients with dystrophinopathic cardiomyopathy: Review of the literature and personal series. Intractable Rare Dis Res. 2017;6:95–101. [PubMed: 28580208]
60. Chan S, Ho R, Lo I, Kan A, Lun K. X-linked dilated cardiomyopathy with mutation in the 5′ splice site intron 1 of dystrophin gene with utrophin upregulation. J Pediatr Neurol. 2018;16:29–34.
61. Haas J, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, Feng Z, Muller S, Kayvanpour E, Vogel B, et al. Atlas of the clinical genetics of human dilated cardiomyopathy. Eur Heart J. 2015;36:1123–1135a. [PubMed: 25163546]
62. Tang J, Song X, Ji G, Wu H, Sun S, Lu S, Li Y, Zhang C, Zhang H. A novel DMD splicing mutation found in a family responsible for X-linked dilated cardiomyopathy with hyper-CKemia. Medicine (Baltimore). 2018;97:e11074. [PubMed: 29901616]
63. Wang Z, Lin L, Yuan Y, Song S. Three novel splicing mutations at 5' terminal of *DMD* gene corresponding to different phenotypes. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2019;36:666–671. [PubMed: 31302907]
64. Kimura S, Ikezawa M, Ozasa S, Ito K, Ueno H, Yoshioka K, Ijiri S, Nomura K, Nakamura K, Matuskura M, et al. Novel mutation in splicing donor of dystrophin gene first exon in a patient with dilated cardiomyopathy but no clinical signs of skeletal myopathy. J Child Neurol. 2007;22:901–906. [PubMed: 17715288]
65. Feng J, Yan J, Buzin CH, Towbin JA, Sommer SS. Mutations in the dystrophin gene are associated with sporadic dilated cardiomyopathy. Mol Genet Metab. 2002;77:119–126. [PubMed: 12359139]
66. Bies RD, Maeda M, Roberds SL, Holder E, Bohlmeyer T, Young JB, Campbell KP. A 5' dystrophin duplication mutation causes membrane deficiency of alpha-dystroglycan in a family with X-linked cardiomyopathy. J Mol Cell Cardiol. 1997;29:3175–3188. [PubMed: 9441825]
67. Oldfors A, Eriksson BO, Kyllerman M, Martinsson T, Wahlstrom J. Dilated cardiomyopathy and the dystrophin gene: an illustrated review. Br Heart J. 1994;72:344–348. [PubMed: 7833192]
68. Ortiz-Lopez R, Li H, Su J, Goytia V, Towbin JA. Evidence for a dystrophin missense mutation as a cause of X-linked dilated cardiomyopathy. Circulation. 1997;95:2434–2440. [PubMed: 9170407]

69. Sato T, Wakita M, Matsushima M, Sato T. A novel deletion of the dystrophin gene in a patient without muscle-related symptoms. Eur Heart J. 2021;42:1638. [PubMed: 33188597]
70. Ferlini A, Galie N, Merlini L, Sewry C, Branzi A, Muntoni F. A novel Alu-like element rearranged in the dystrophin gene causes a splicing mutation in a family with X-linked dilated cardiomyopathy. Am J Hum Genet. 1998;63:436–446. [PubMed: 9683584]
71. van Spaendonck-Zwarts KY, van Rijsingen IA, van den Berg MP, Lekanne Deprez RH, Post JG, van Mil AM, Asselbergs FW, Christiaans I, van Langen IM, Wilde AA, et al. Genetic analysis in 418 index patients with idiopathic dilated cardiomyopathy: overview of 10 years' experience. Eur J Heart Fail. 2013;15:628–636. [PubMed: 23349452]
72. Chamberlain RC, Smith EC, Campbell MJ. Novel rod domain duplication in dystrophin resulting in X-Linked dilated cardiomyopathy. Pediatr Neurol. 2015;53:439–441. [PubMed: 26294044]
73. Franz WM, Muller M, Muller OJ, Herrmann R, Rothmann T, Cremer M, Cohn RD, Voit T, Katus HA. Association of nonsense mutation of dystrophin gene with disruption of sarcoglycan complex in X-linked dilated cardiomyopathy. Lancet. 2000;355:1781–1785. [PubMed: 10832829]
74. Franz WM, Cremer M, Herrmann R, Grunig E, Fogel W, Scheffold T, Goebel HH, Kircheisen R, Kubler W, Voit T, et al. X-linked dilated cardiomyopathy. Novel mutation of the dystrophin gene. Ann N Y Acad Sci. 1995;752:470–491. [PubMed: 7755293]
75. Giudicessi JR, Maleszewski JJ, Tester DJ, Ackerman MJ. Prevalence and potential genetic determinants of young sudden unexplained death victims with suspected arrhythmogenic mitral valve prolapse syndrome. Heart Rhythm O2. 2021;2:431–438. [PubMed: 34667957]
76. Juan-Mateu J, Paradas C, Olive M, Verdura E, Rivas E, Gonzalez-Quereda L, Rodriguez MJ, Baiget M, Gallano P. Isolated cardiomyopathy caused by a *DMD* nonsense mutation in somatic mosaicism: genetic normalization in skeletal muscle. Clin Genet. 2012;82:574–578. [PubMed: 22092019]
77. Piccolo G, Azan G, Tonin P, Arbustini E, Gavazzi A, Banfi P, Mora M, Morandi L, Tedeschi S. Dilated cardiomyopathy requiring cardiac transplantation as initial manifestation of Xp21 Becker type muscular dystrophy. Neuromuscul Disord. 1994;4:143–146. [PubMed: 8012195]
78. Shimizu M, Ino H, Yasuda T, Fujino N, Uchiyama K, Mabuchi T, Konno T, Kaneda T, Fujita T, Masuta E, et al. Gene mutations in adult Japanese patients with dilated cardiomyopathy. Circ J. 2005;69:150–153. [PubMed: 15671604]
79. Papa AA, Gallinoro E, Palladino A, Golino P. Beneficial effects of one-month sacubitril/valsartan treatment in a patient affected by end-stage dystrophinopathic cardiomyopathy. Acta Myol. 2020;39:136–140. [PubMed: 33305170]
80. Nakamura A, Yoshida K, Fukushima K, Ueda H, Urasawa N, Koyama J, Yazaki Y, Yazaki M, Sakai T, Haruta S, et al. Follow-up of three patients with a large in-frame deletion of exons 45–55 in the Duchenne muscular dystrophy (DMD) gene. J Clin Neurosci. 2008;15:757–763. [PubMed: 18261911]
81. Tasaki N, Yoshida K, Haruta SI, Kouno H, Ichinose H, Fujimoto Y, Urasawa N, Kawakami T, Taniguchi M, Kurushima S, et al. X-linked dilated cardiomyopathy with a large hot-spot deletion in the dystrophin gene. Intern Med. 2001;40:1215–1221. [PubMed: 11813847]
82. Muntoni F, Di Lenarda A, Porcu M, Sinagra G, Mateddu A, Marrosu G, Ferlini A, Cau M, Milasin J, Melis MA, et al. Dystrophin gene abnormalities in two patients with idiopathic dilated cardiomyopathy. Heart. 1997;78:608–612. [PubMed: 9470882]
83. Ribeiro J, Rebelo O, Fernandez-Marmiesse A, Negrao L. Novel mosaic mutation in the dystrophin gene causing distal asymmetric muscle weakness of the upper limbs and dilated cardiomyopathy. Acta Myol. 2018;37:117–120. [PubMed: 30057996]
84. Liu XP, Feng YB, Zeng Y, Fan Q, Gao R, Wang HJ, Gao JL, Li YL, Su P, He RX. Screening of pathogenic genes in a Chinese familial dilated cardiomyopathy pedigree from Inner Mongolia. Zhonghua Xin Xue Guan Bing Za Zhi. 2019;47:197–203. [PubMed: 30897878]
85. Scheiper S, Ramos-Luis E, Blanco-Verea A, Niess C, Beckmann BM, Schmidt U, Kettner M, Geisen C, Verhoff MA, Brion M, et al. Sudden unexpected death in the young - Value of massive parallel sequencing in postmortem genetic analyses. Forensic Sci Int. 2018;293:70–76. [PubMed: 30415094]

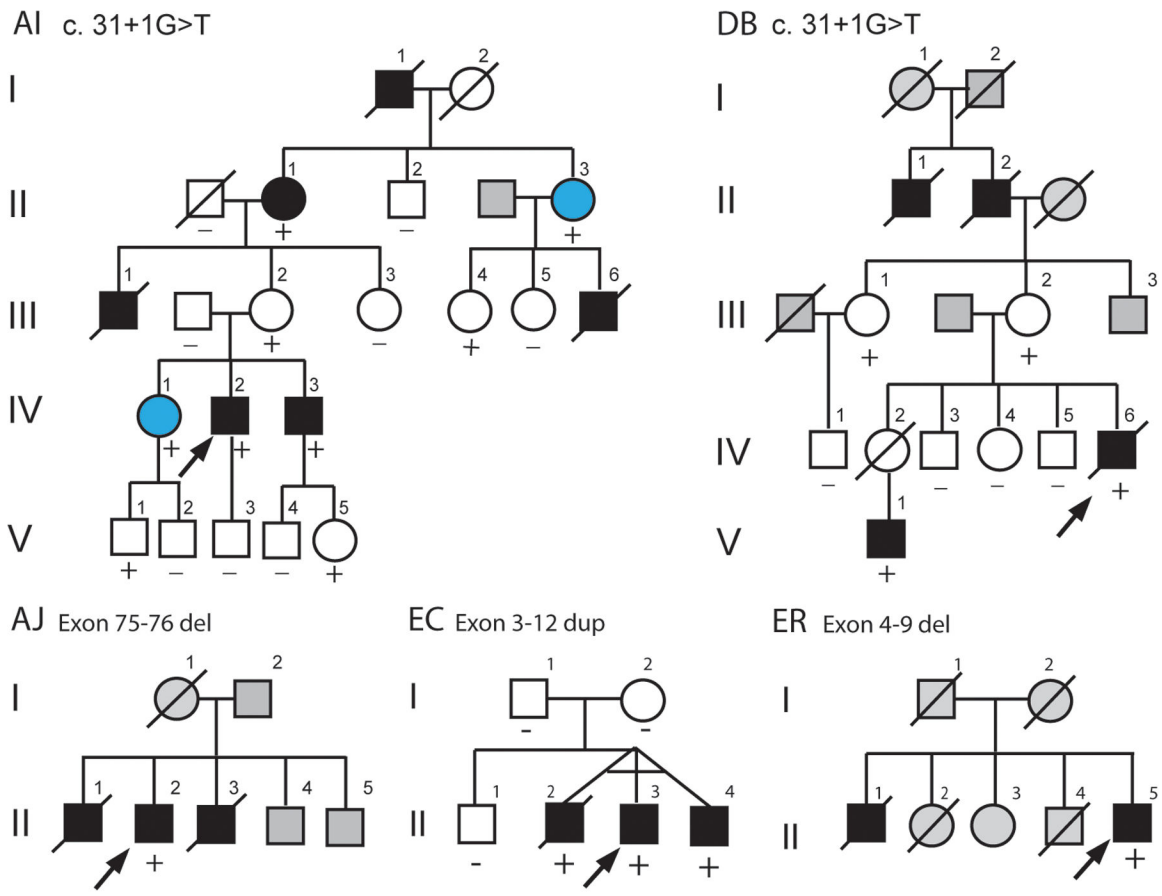

Figure 1.

Pedigrees for families with P/LP *DMD* variants. Phenotypes denoted as: affected (solid symbols: black = affected at time of study entry; blue = unaffected at time of study entry), unaffected (open symbols) or unknown (gray symbols), deceased (diagonal line); probands are indicated by arrows. The presence (+) or absence (−) of *DMD* variants are shown.

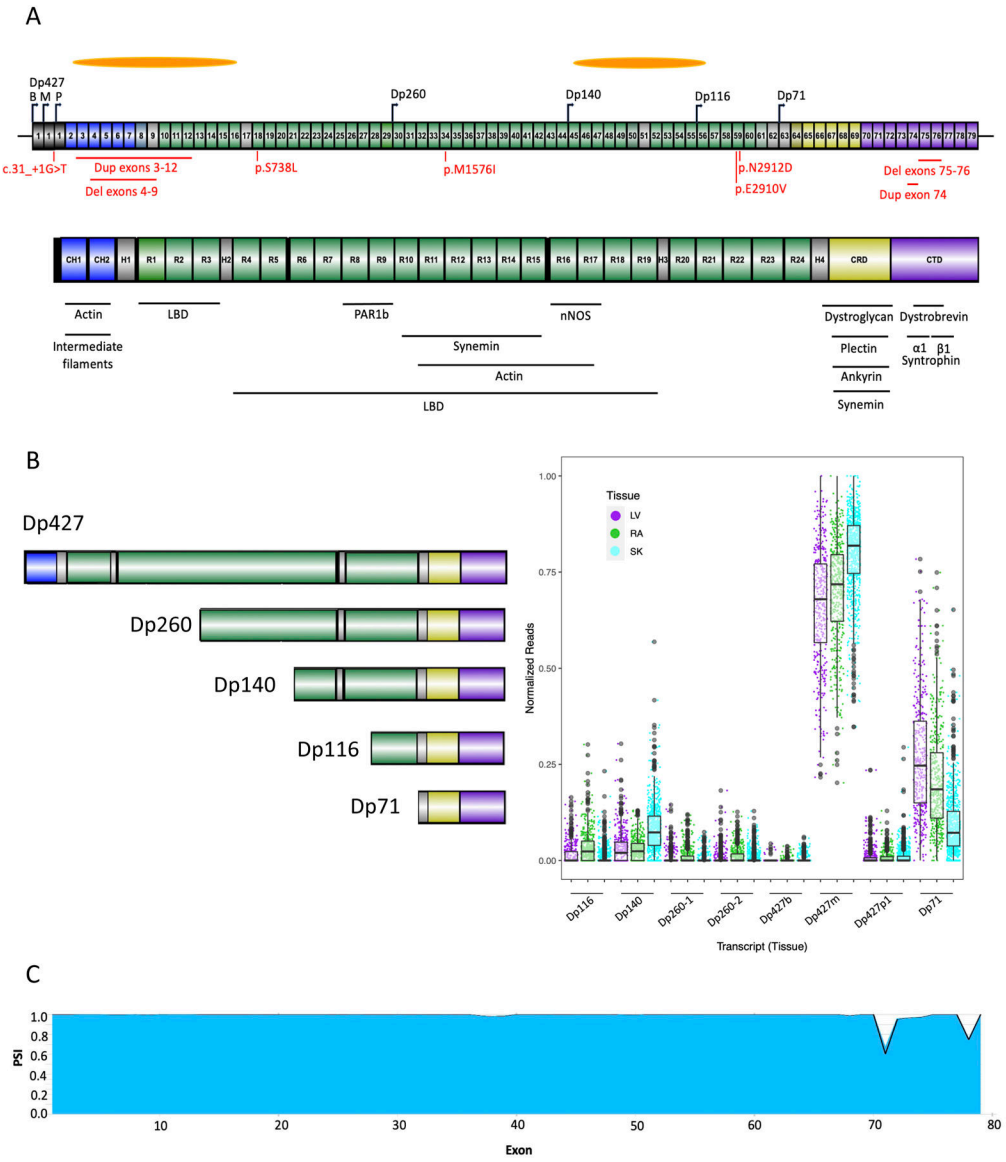

Figure 2.

Human full-length dystrophin and its isoforms. (**A**) Schematic of the 79 exons of the *DMD* gene (not to scale) and corresponding protein structural domains (to scale) including two calponin-homology domains (CH1 & CH2), four hinges (H1 to H4), central rod comprised of 24 spectrin repeats (R1 to R24), cysteine-rich domain (CRD) encompassing a WW domain, two EF-hands and a ZZ domain, and carboxy-terminal domain (CTD). Arrows indicate the multiple intronic *DMD* promoters. Full-length dystrophin protein (Dp427) is generated from three tissue-specific promoters mainly expressed in brain, muscle and Purkinje cells (B, M, and P), with each promoter driving a transcript that utilizes a unique first exon. Four internal promoters give rise to shorter dystrophin isoforms (Dp260, Dp140, Dp116 and Dp71) adjacent to exons 30, 45, 56, and 63, respectively. Locations of mutational hotspots for Duchenne MD (orange) and *DMD* variants identified in this study (red) are shown. Below protein schematic, dystrophin binding partners (black lines); lipid binding

domain (LBD), partitioning-defective 1b (PAR1b), neuronal nitric oxide synthase (nNOS). (**B**) Graphical representation of *DMD* isoform composition (left) and expression (right) in human left ventricle (LV), right atrium (RA) and skeletal muscle (SK). (**C**) Graphical representation of percent spliced-in (PSI) scores derived from human adult LV and RA. Panel A was created using Illustrator of Biological Sequences.[12]

Table 1.

Distribution of variant types in Duchenne MD and *DMD*-associated DCM.

Variant type	Duchenne MD*		*DMD*-associated DCM#			
	No. variants (n=7,149)	%, (All variants)	No. variants (n=77)	%, (All variants)	P/LP variants (n=59)	%, (P/LP variants)
Large variants (involving ≥1 exon)						
Deletions	4,894	68.5%	34	44.2%	32	54.2%
Duplications	784	11%	8	10.4%	4	6.8%
Small variants (involving <1 exon)						
Deletions	358	5%	3	3.9%	2	3.4%
Insertions	132	1.8%	2	2.6%	2	3.4%
Splice sites (<10bp from the exon)	199	2.8%	11	14.3%	9	15.3%
Nonsense variants	726	10.2%	8	10.4%	8	13.6%
Missense variants	30	0.4%	8	10.4%	0	0%
Intronic variants (outside canonical splice sites)						
Mid-intronic variants	22	0.3%	3	3.9%	2	3.4%

*Data sourced from TREAT-NMD DMD Global database.[11]

#Variants listed in Supplemental Table VI (literature + present study)

Table 2.

Genetic causes of DCM that can show sex differences in disease severity with/without skeletal muscle involvement.

Gene	Age at DCM diagnosis	ESHF/HTx	MVA/SCD	AA	CD	Notes	Ref.
X chromosome genes							
DMD	Adulthood, younger onset in males.	++ (M), + (F)	+ (M), + (F)	+ (M), + (F)	+ (M), + (F)	Phenotypic overlap with XLDCM/atypical Becker MD; exertional myalgia ± elevated CK levels may be present, especially in males.	5
EMD	Adulthood, younger onset in males.	+ (M)	+ (M)	++ (M)	++ (M)	Usually associated with Emery-Dreifuss MD but may have primary cardiac presentation (e.g. DCM, LVNC, SSS, AA, CHB, SCD ± TE stroke). Females less frequently affected.	16
LAMP2	Adolescence/adulthood, younger onset in males.	++ (M), ++ (F)	++ (M), + (F)	++ (M), ++ (F)	+++ (M), ++ + (F)	Danon disease: Males usually present with triad of HCM, SKM, cognitive impairment; females mainly cardiac features with HCM or DCM. Adverse events occur at earlier age in males.	17
TAZ	Childhood, mostly males.	++ (M)	++ (M)	NA	NA	Barth syndrome: Rare infantile-onset mitochondrial disorder with DCM, LVNC, mitochondrial myopathy, neutropenia. Females rarely affected.	18
Autosomal genes							
BAG3	Adulthood, variable sex effects.	++	+	+	+	DCM frequently caused by truncating variants; more ESHF-related adverse events in males. Missense variants associated with HCM, RCM, myofibrillar SKM. Males may present earlier or at similar ages to females.	19
DES	Adulthood, similar in males and females.	+	+	+	+++	Typically causes SKM ± cardiac involvement, but isolated DCM can occur. DCM more common in males. Also associated with RCM, HCM, LVNC, ARVC.	20
DSP	Adulthood, similar in males and females.	+	++	+	+	ACM with LV/RV/biV predominance. Males variably show more RV involvement, MVA, ESHF. Females often show higher disease prevalence, more LV involvement.	21–23
FLNC	Adulthood, similar in males and females.	++	++	+	++	ACM with LV/RV/biV predominance. DCM usually caused by truncating variants, mild CK elevation infrequently present; males variably show more adverse events. Missense variants associated with HCM, RCM, myofibrillar SKM.	24,25
LMNA	Adulthood, similar in males and females.	++ (M>F)	++ (M>F)	+++ (M/F)	+++ (M/F)	Commonly causes primary DCM; increased ESHF, MVA, and death in males. Overlap syndromes can occur with DCM ± SKM/other features, or Emery-Dreifuss MD/Limb-girdle MD ± DCM.	26,27
RBM20	Adulthood, younger onset in males.	++ (M), + (F)	++ (M/F)	++	+	Lower LVEF at diagnosis, higher HTx rates in males. SKM not reported.	28,29
TTN	Adulthood, younger onset in males.	+	+	++	++	Truncating variants associated with primary DCM; lower LVEF at diagnosis, more adverse events, reduced survival in males. Truncating variants also associated with a range of SKM disorders ± DCM.	30,31

AA, atrial arrhythmias; ACM, arrhythmogenic cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; biV, biventricular; CD, conduction-system defects; CHB, complete heart block; CK, creatine kinase; DCM, dilated cardiomyopathy; ESHF, end-stage heart failure; F, females; HCM, hypertrophic cardiomyopathy; HTx, heart transplant; LV, left ventricle; LVEF, LV ejection fraction; LVNC, LV non-compaction; M, males; MD, muscular dystrophy; MVA, malignant ventricular arrhythmias; NA, not available; RCM, restrictive cardiomyopathy; RV, right ventricle; SCD, sudden cardiac death; SKM, skeletal myopathy; SSS, sick sinus syndrome; TE, thromboembolic; XLDCM, X-linked DCM. Frequency of events denoted as: +++, >50%; ++, 11–50%; +, ≤10%