

## Genetic Testing for Duchenne, Becker, Facioscapulohumeral, and Limb-Girdle Muscular Dystrophies

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### I. Policy Description

Muscular dystrophies, genetic conditions characterized by progressive muscle atrophy, can be caused by several genetic mutations. Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are due to mutations in the dystrophin gene on the X chromosome. Facioscapulohumeral muscular dystrophy (FSHD) occurs due to a contraction of the polymorphic macrosatellite repeat D4Z4 on chromosome 4q35.

Duchenne muscular dystrophy (DMD), the more severe of the dystrophin-related muscular dystrophies, typically presents in males during their toddler years. Patients with DMD rarely survive beyond their thirties due to respiratory insufficiency or cardiomyopathy. Unlike DMD, Becker muscular dystrophy (BMD) is less severe and typically presents in the teen years or adulthood; moreover, patients with BMD usually survive beyond thirty years of age.

In Facioscapulohumeral muscular dystrophy (FSHD), an autosomal dominant disorder, the contraction of the macrosatellite repeat D4Z4 results in an inappropriate expression of the *DUX4* (double homeobox protein 4) gene, which alters chromatin structure. FSHD has also been linked to DNA hypomethylation. Symptoms include muscle weakness in the face, arms, legs, abdomen, and scapula with a variable age of onset; however, 90% of patients are affected by the age of 20. Disease progression is slower than DMD with a normal or near-normal life span.

The limb-girdle muscular dystrophies (LGMDs) are a group of approximately 30 rare hereditary progressive neuromuscular disorders characterized by predominantly proximal distribution of weakness in the pelvic and shoulder girdles. LGMDs result from mutations in genes required for normal muscle function and vary in severity, phenotype, pathology, and age of onset (Darras, 2018c). A newer definition of LGMD has been proposed as “a genetically inherited condition that primarily affects skeletal muscle leading to progressive, predominantly proximal muscle weakness at presentation caused by a loss of muscle fibres. To be considered a form of limb girdle muscular dystrophy the condition must be described in at least 2 unrelated families with affected individuals achieving independent walking, must have an elevated serum creatine kinase activity, must demonstrate degenerative changes on muscle imaging over the course of the disease, and have dystrophic changes on muscle histology, ultimately leading to end-stage pathology for the most affected muscles.”

### II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request

- 1) Genetic testing for *DMD/BMD* gene mutations **MEETS COVERAGE CRITERIA** under the following conditions:
  - a) In individuals with signs and symptoms of a dystrophinopathy in order to confirm the diagnosis and direct treatment, OR
  - b) For at-risk female relatives: (at-risk females are defined as first- and second-degree female relatives and include the proband's mother, female siblings of the proband, female offspring of the proband, the proband's maternal grandmother, maternal aunts and their offspring)
    - i) To confirm or exclude the need for cardiac surveillance, OR
    - ii) For preconception testing to determine the likelihood of an affected offspring in a woman considering a pregnancy.
- 2) Genetic testing for facioscapulohumeral muscular dystrophy **MEETS COVERAGE CRITERIA** to confirm a diagnosis in a patient with clinical signs of the disease.
- 3) Genetic testing for mutations associated with limb-girdle muscular dystrophy (LGMD) to confirm a diagnosis of LGMD **MEETS COVERAGE CRITERIA** when signs and symptoms of LGMD are present but a definitive diagnosis cannot be made without genetic testing, , and when the results of testing may lead to changes in clinical management that improve outcomes (e.g., confirming or excluding the need for cardiac surveillance).
- 4) Genetic testing for mutations associated with limb-girdle muscular dystrophy (LGMD) in the reproductive setting **MEETS COVERAGE CRITERIA** when:
  - a) There is a diagnosis of LGMD in one or both of the parents, AND
  - b) Results of testing will allow informed reproductive decision making.
- 5) Genetic testing for mutations associated with LGMD **MEETS COVERAGE CRITERIA** in an asymptomatic individual to determine future risk of disease when both the following criteria (A & B) are met:
  - a) The individual has:
    - i) A close relative (i.e., first- or second-degree relative) with a known mutation consistent with LGMD; OR
    - ii) A close relative (i.e., first- or second-degree relative) diagnosed with LGMD whose genetic status is unavailable.
  - b) Results of testing will lead to changes in clinical management (e.g., confirming or excluding the need for cardiac surveillance).

*The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.*

- 6) Genetic testing for *DMD/BMD* gene mutations **DOES NOT MEET COVERAGE CRITERIA** in all other situations.
- 7) Genetic testing for facioscapulohumeral muscular dystrophy **DOES NOT MEET COVERAGE CRITERIA** for all other indications.

- 8) Genetic testing for mutations associated with LGMD **DOES NOT MEET COVERAGE CRITERIA** in all other situations.

### III. Scientific Background

#### *Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD)*

Dystrophinopathies are due to mutations in the *DMD* (Dystrophin) gene located on the X-chromosome inherited in a recessive pattern. All hemizygous males will exhibit the characteristic phenotype whereas heterozygous females for a pathogenic mutation may exhibit a range of clinical manifestations. If skeletal muscles are affected, dystrophinopathies are classified as either DMD or BMD; however, if cardiac muscle is primarily affected, it is characterized as *DMD-associated dilated cardiomyopathy* (DCM).

DMD, the more severe phenotype of the skeletal muscle dystrophinopathies, typically presents in males before the age of five with progressive, symmetric muscle weakness and calf hypertrophy. Affected males are typically wheelchair-dependent before their teens, and the individual rarely survives beyond their thirties due to respiratory complications and heart failure. BMD is often less severe and manifests later in affected individuals at 30 years of age. Most of these individuals remain ambulatory into adulthood, with some reported to remain ambulatory as late as their sixties. Although skeletal muscle deterioration progresses more slowly in BMD, cardiomyopathy is the most common cause of death in these patients, shortening the life expectancy to the mid-forties. Many BMD patients receive heart transplants within 5 years after diagnosis of cardiomyopathy. The prevalence of DMD in the United States has been estimated between 1.3 and 2.1 cases per 10,000 live male births, though the number differed by race/ethnicity in a recent study.

DMD or BMD should be suspected in males with elevated serum creatine phosphokinase (CK) levels (>10-times of normal and >5-times of normal, respectively) and clinical symptoms of dystrophinopathy. As permeability of the sarcolemma increases due to muscle damage, creatine phosphokinase is released beyond the normal range, which can be used as a diagnostic value. In DMD, serum CK peaks by age 2 years, with levels reaching 10 to 20 times the upper limit of normal. As the disease progresses, dystrophic muscle fibers begin to decay and the rate of CK release decreases (Kim et al., 2017). Hemizygous female carriers can also exhibit elevated serum CK levels, which are 2-10 times that of the normal range. A normal CK level, however, makes DMD and BMD unlikely and alternative diagnoses should be considered in these cases.

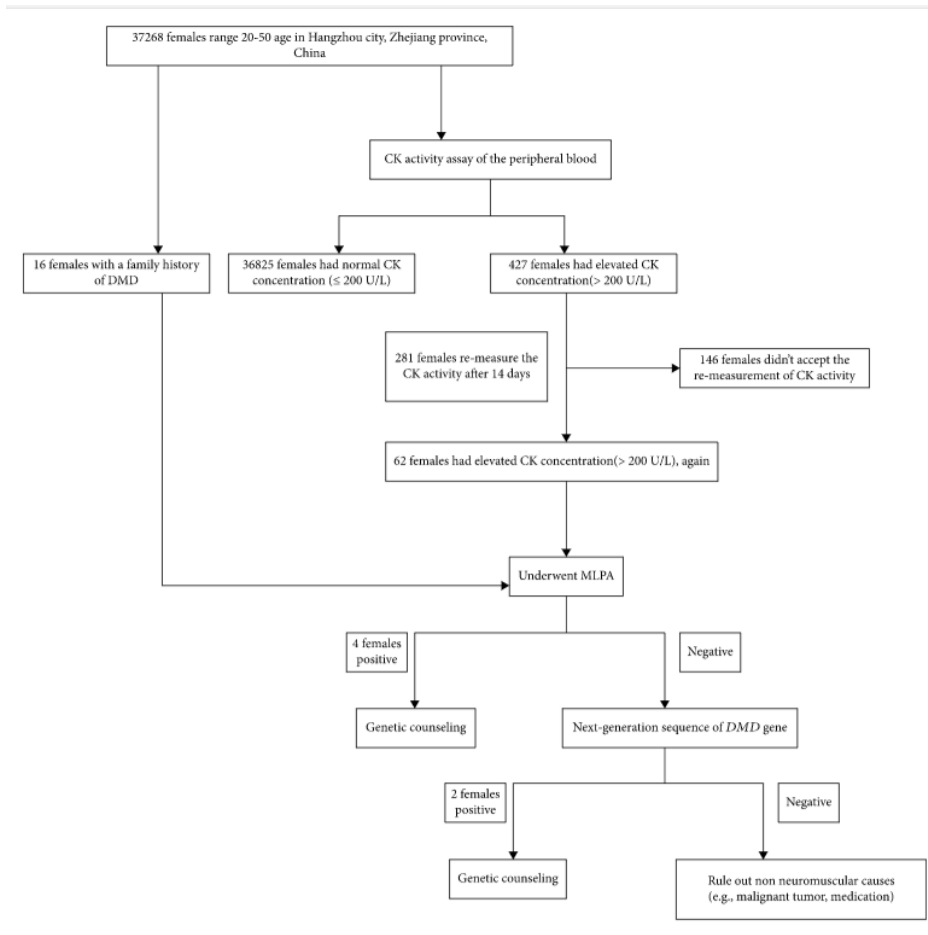
A variety of genetic testing methodologies are available to assist in the diagnosis of DMD/BMD. In general, it is advisable to try larger deletion/duplication genetic testing first and, if negative, move on to next generation exome or genome sequence analysis, which can detect "small" mutations [like single nucleotide polymorphisms (SNPs)] as well as micro deletion and duplications. Multiplex ligation-dependent probe amplification (MLPA) has been used successfully to detect duplication or deletion mutations that cause DMD/BMD. It has also been reported that up to 98% of disease-causing deletions could be detected with multiplex polymerase chain reaction (PCR). Quantitative PCR, long-range PCR, and chromosomal microarray (CMA) may also be considered; however, CMA is not recommended as a primary confirmatory assay for dystrophinopathies as the sensitivity of the assay may not be sufficient to detect all exon-level DMD deletions and duplications.

#### *Clinical Validity and Utility of DMD/BMD Genetic Testing*

A recent Chinese study of 146 at-risk pregnancies in 131 DMD families report a 99% mutation detection rate using “a prenatal diagnosis algorithm for dystrophinopathies that combines multiplex ligation-dependent probe amplification (MLPA), quantitative PCR, sequencing and linkage analyses.” This data also shows that 51.1% of the probands had de novo exon deletions. Recombination of the *DMD* gene occurred in 9 of the 146 pregnancies. The authors conclude that “the present results demonstrate the importance of considering maternal germline mosaicism in the genetic assessment. Prenatal diagnosis should be suggested to the parent with a DMD proband whether carrier testing found the causative mutation in the mother’s blood or not.” The reported accuracy rate of this multiplex/quantitative PCR-based method is considerably higher than the reported accuracy rate (>70%) of a real-time PCR assay of the *DMD* gene.

MLPA-based genetic testing of dystrophinopathies has been reported in many studies with varying degrees of sensitivity. A large study of 1053 Chinese DMD/BMD patients using MLPA testing reported identifying 70.56% of the probands whereas a smaller study of 121 individuals (both male and female) reports confirmation of only 63% of patients and symptomatic females. A third study of using an algorithm of mPCR and MLPA on 150 male patients reported a 75% confirmation rate (Murugan, Chandramohan, & Lakshmi, 2010). Another study using MLPA concluded that “the reading-frame rule held in 90% to 94% of children, which is consistent with reports from other parts of the world. However, testing by MLPA is a limitation, and advanced sequencing methods including analysis of the structure of mutant dystrophin is needed for more-accurate assessments of the genotype-phenotype correlation.”

In a study by Han and colleagues (2020), 37,268 females of reproductive age were recruited in Hangzhou, China, to identify definite DMD carriers prior to or early in pregnancy. Participants were screened for high CK activity (>200 U/L), a typical finding in 50-70% of asymptomatic heterozygous female DMD carriers, and DMD family history. The screening process narrowed down the number of participants for DMD genetic sequencing performed with Multiplex Ligation-Dependent Probe Amplification (MLPA) to detect gross deletions and duplications and Next-Generation Sequencing (NGS) based on Multiplex PCR to detect the small point mutations, insertions, and deletions. From the 37,268 participants, 427 females were identified to have CK levels above 200 U/L and 16 females with a previous family history of DMD. 427 females with high CK levels were asked to repeat CK testing, to which 281 females agreed, and 62 females showed to have sustained elevated serum CK levels. The 16 participants with DMD family history and 62 females with sustained CK levels >200 U/L were asked to undergo *DMD* genetic testing. “MLPA and NGS of the *DMD* gene identified 6 definite DMD carriers with clear pathogenic variants (3 of the 16 subjects with positive family history and 3 of the 62 subjects with negative family history) ... 4 cases had deletions and/or duplications of *DMD* gene, mostly located in a hotspot mutation region (exons 44-55), one case carried a previously reported pathogenic missense variant, and a novel deleterious frameshift pathogenic variant was found in one case.” Results of the screening for DMD carriers are summarized in the figure below.



Confirmed carriers of DMD pathogenic variant were provided professional genetic counseling and several reproductive choices such as preimplantation genetic diagnosis, prenatal genetic testing through chorionic villus, amniotic fluid, or umbilical cord blood sampling during different gestation weeks as needed. The authors state that current carrier screening for DMD, which is provided for females with a family history of DMD, misses the many spontaneous cases resulting from unsuspected variants. Therefore, the authors suggest that “Carrier screening before or early in pregnancy will allow carrier females to receive genetic counseling and be informed of fertility choices and recurrent risk. Moreover, carrier screening will help carriers prepare for the possibility of manifesting DMD-related symptoms later on in life... Furthermore, female relatives of positive carriers should be recommended for genetic testing in order to evaluate their carrier status.”

### *Facioscapulohumeral Muscular Dystrophy (FSHD)*

FSHD is the third most common type of muscular dystrophy, with an estimated prevalence of 4 to 12 per 100,000 population (Darras, 2018b). It is an autosomal dominant genetic disorder that is caused by a deletion of the macrosatellite repeat regions D4Z4 of the *DUX4* gene in the sub-telomeric region of chromosome 4q. In healthy individuals, the *DUX4* gene is epigenetically silenced in somatic tissues; however, contraction of the D4Z4 repeats allows for inefficient chromatin silencing due to abnormal chromatin structure, resulting in inappropriate somatic expression. Unaffected individuals have a variable number of D4Z4 repeats, ranging from 11 to more than 100, whereas FSHD1 patients have only 1 – 10 repeats on one of the copies of chromosome 4. The *DUX4* protein is usually only expressed in the

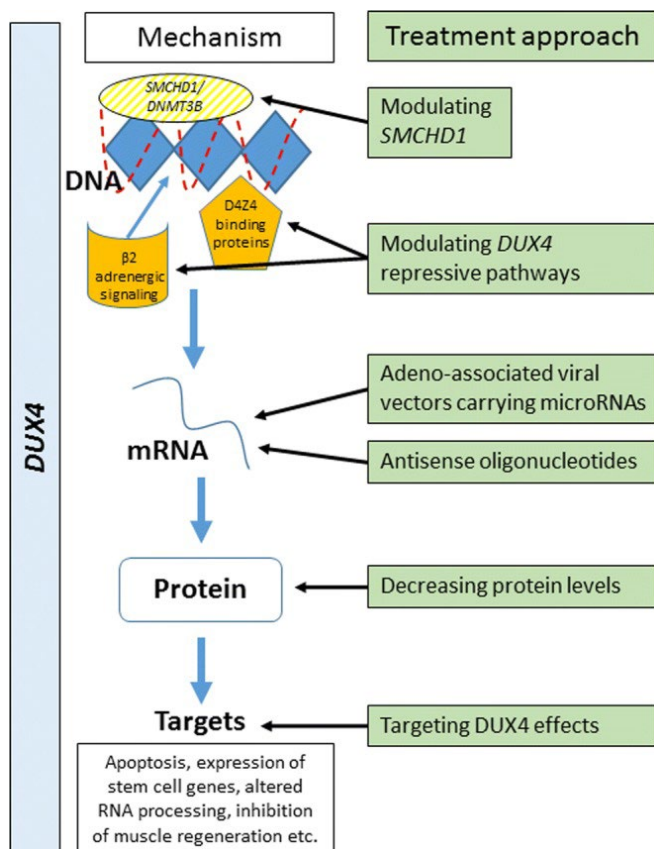
germline as a DNA-binding protein with presumed transcription factor activity. Its toxicity in somatic cells is unknown. Two forms of FSHD have been classified—FSHD1, the major form due to a major contraction of the D4Z4 macrosatellite repeat sequences, and FSHD2, a minor form with a normal number of D4Z4 repeats but abnormal D4Z4 chromatin structure. FSHD2 patients have a disease status that cannot be confirmed by using the standard molecular diagnostic testing used in FSHD1 patients. Further, 85% of patients with FSHD2 have mutations in the *SMCHD1* gene on chromosome 18, which encodes for a chromatin modifier believed to be involved in maintaining the D4Z4 chromatin structure. Pathogenic variants of *DNMT3B* and *LRIF1* genes are less common causes of FSHD2. Despite the different genetic causes, the phenotypes of FSHD1 and FSHD2 patients are often clinically indistinguishable.

FSHD patients exhibit a progressive muscular dystrophy with variability of affected muscles between patients. Generally, muscles of the face, arms, legs, shoulders, and abdomen can be affected. Serum-based diagnostic testing for FSHD has been elusive. A cross-sectional study by Petek and colleagues, using high-throughput proteomics, shows that the levels of creatine kinase MM and MB isoforms, carbonic anhydrase III, and troponin I type 2 were elevated at least 1.5-fold in affected individuals and correlated with the severity and state of disease. Because of the variability of FSHD, genetic testing is still “the preferred diagnostic choice.”

Methylation of the D4Z4 regions also plays a role in disease expression and progression. A study by Mul and colleagues researched the clinical variability of FSHD1 patients for a possible linkage between the severity of disease, the repeat array size of D4Z4, and D4Z4 methylation. Unsurprisingly, unaffected gene carriers had both a higher number of array repeats and higher methylation levels. One interesting result is that the location of the affected body region did show a correlation between disease severity and DNA modification. “The D4Z4 repeat array size and D4Z4 methylation contribute to variability in disease severity and penetrance, but other disease modifying factors must be involved as well, such as polyadenylation of the *DUX4* transcript. Polyadenylated *DUX4* transcripts are not degraded and lead to the development of FSHD by a toxic gain of function mechanism. The larger effect of the D4Z4 repeat array on facial muscle involvement suggests that these muscles are more sensitive to the influence of the FSHD1 locus itself, whereas leg muscle involvement seems highly dependent on modifying factors.”

Inappropriate expression of *DUX4* in somatic tissue is ultimately responsible for the pathogenesis of FSHD; hence, recent efforts to treat the disorder have targeted the expression of this gene and/or its protein product, as summarized in the table below.





### Clinical Validity and Utility of FSHD Genetic Testing

The data on the clinical validity and utility of FSHD genetic testing is limited. The American Academy of Neurology and the American Association of Neuromuscular & Electrodiagnostic Medicine released joint guidelines stating the following: “our systematic review identified 9 Class III studies from specialty clinics that, together, demonstrate that the finding of a D4Z4 contraction on chromosome 4q35 likely has a sensitivity of 93% and a specificity of 98% for diagnosis of clinically defined FSHD.” A 2010 study of more than 800 individuals, however, question the criteria for the molecular diagnosis of FSHD. In this study, 3% of asymptomatic, healthy individuals reported a reduced number of D4Z4 repeats, varying 4 to 8 units on chromosome 4; further, almost one-half of probands had a normal copy number of D4Z4 repeats. These “results suggest that the genetic basis of FSHD, which is remarkably heterogeneous, should be revisited because this has important implications for genetic counseling and prenatal diagnosis of at-risk families.”

The most common method of molecular diagnosis for FSHD is pulsed-field gel electrophoresis with Southern Blotting to discriminate between chromosome 4 and chromosome 10 D4Z4 arrays. A study by Dai and colleagues describes a novel method to characterize the D4Z4 repeat numbers in FSHD by using a single molecule optical mapping platform that helps detect structural variants. With Bionano genome mapping, the number of D4Z4 repeats with 4qA allelic configuration and the levels of postzygotic mosaicism were determined. In the study, the primary cohort consisting of five patients had a confirmed diagnosis of FSHD based on Southern blot analysis. The second cohort consisted of eight patients that were suspected to have FSHD, but the patients did not have a prior molecular diagnosis. Optical mapping was performed, and Southern blot was used as a validation assay. In both cohorts, the results obtained from optical mapping have 100% concordance with the results from Southern blot

analysis. Although optical mapping is a novel method that can improve accuracy and reliability of FSHD molecular testing, the authors also discuss the disadvantages of the optical mapping platform. Optical mapping is an expensive and time-consuming tool compared to Southern blot, which works reliably for diagnosing patients with 1-10 D4Z4 repeats in a cost-effective manner. Despite the disadvantages, the authors believe that costs for optical mapping will decrease and the tool will be incorporated into the clinical setting.

The genetic diagnosis of FSHD can be confounded by false positive or false negative results. A common test for FSHD uses a p13E-11 DNA probe to confirm a specific deletion that is indicative of the disorder. However, a false negative may occur if the patient has a deletion that affects the diagnostic probe's region of recognition. A "false" negative result may also occur with this test for patients with FSHD2, since they may not possess the characteristic deletion that is present in typical FSHD1 cases. If clinical suspicion of FSHD exists in patients with a negative test result, then further genetic testing that includes the *SMCHD1*, *DNMT3B*, and/or *LRIF1* genes may be warranted to confirm FSHD2. Genetic testing should also include haplotype analysis, since a false positive result may occur "if the contracted D4Z4 array is located on the non-permissive 4qB haplotype."

#### *Limb-Girdle Muscular Dystrophies (LGMDs)*

. Together, the group of disorders that constitute LGMD occur with an estimated minimum prevalence between 10 and 23 per 100,000. The LGMDs vary widely in their genetics and clinical features, ranging from mild forms allowing patients to maintain a fairly normal life to severe deterioration of proximal limb muscles with significant physical weakness and shortened life-span. Approximately 30 LGMDs are recognized currently, most of which are inherited in an autosomal recessive pattern. The table below displays the recognized LGMDs according to the updated classification system:

#### **Nomenclature, genes, and protein products of limb-girdle muscular dystrophies**

New nomenclature	Old nomenclature	Gene	Protein product
<b>Autosomal dominant</b>			
LGMD D1	LGMD1D	<i>DNAJB6</i>	DnaJ heat shock protein family (Hsp40) member B6
LGMD D2	LGMD1F	<i>TNPO3</i>	Transportin 3



LGMD D3	LGMD1G	<i>HNRNPDL</i>	Heterogeneous nuclear ribonucleoprotein D like protein
LGMD D4	LGMD1I	<i>CAPN3</i>	Calpain 3
LGMD D5		<i>COL6A1</i>	Collagen type VI alpha 1 chain
<b>Autosomal recessive</b>			
LGMD R1	LGMD2A	<i>CAPN3</i>	Calpain 3
LGMD R2	LGMD2B	<i>DYSF</i>	Dysferlin
LGMD R3	LGMD2D	<i>SGCA</i>	Sarcoglycan alpha
LGMD R4	LGMD2E	<i>SGCB</i>	Sarcoglycan beta
LGMD R5	LGMD2C	<i>SGCG</i>	Sarcoglycan gamma
LGMD R6	LGMD2F	<i>SGCD</i>	Sarcoglycan delta
LGMD R7	LGMD2G	<i>TCAP</i>	Telethonin
LGMD R8	LGMD2H	<i>TRIM32</i>	Tripartite motif-containing 32
LGMD R9	LGMD2I	<i>FKRP</i>	Fukutin-related protein
LGMD R10	LGMD2J	<i>TTN</i>	Titin
LGMD R11	LGMD2K	<i>POMT1</i>	Protein O-mannosyltransferase 1
LGMD R12	LGMD2L	<i>ANO5</i>	Anoctamin 5
LGMD R13	LGMD2M	<i>FCMD</i>	Fukutin

LGMD R14	LGMD2N	<i>POMT2</i>	Protein O-mannosyltransferase 2
LGMD R15	LGMD2O	<i>POMGNT1</i>	Protein O-linked mannose <i>N</i> -acetylglucosaminyltransferase 1 (beta 1,2-)
LGMD R16	LGMD2P	<i>DAG1</i>	Dystroglycan 1
LGMD R17	LGMD2Q	<i>PLEC1</i>	Plectin
LGMD R18	LGMD2S	<i>TRAPPC11</i>	Trafficking protein particle complex 11
LGMD R19	LGMD2T	<i>GMPPB</i>	GDP-mannose pyrophosphorylase B
LGMD R20	LGMD2U	<i>CRPPA</i>	CDP-L-ribitol pyrophosphorylase A
LGMD R21	LGMD2Z	<i>POGLUT1</i>	Protein O-glucosyltransferase 1
LGMD R22		<i>COL6A1/2/3</i>	Collagen VI subunits A1, A2, or A3
LGMD R23		<i>LAMA2</i>	Laminin subunit alpha 2

LGMD R24		<i>POMGNT2</i>	Protein O-linked mannose <i>N</i> -acetylglucosaminyltransferase 2 (beta 1,4-)
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The autosomal recessive LGMD R1 is considered the most common form of LGMD worldwide, making up an estimated 15 to 40 percent of all cases of LGMD.

Dilated cardiomyopathy (DCM) is the most common cardiac phenotype associated with LGMD, and its prevalence varies in the different subgroups of patients. The risk of developing DCM is particularly high in the LGMD1B patient subgroup, such that clinicians might consider preventive interventions due to the high risk of sudden death.

#### *Clinical Validity and Utility of LGMD Genetic Testing*

Based on published literature, the clinical validity of genetic testing for LGMD is difficult to ascertain; however, broad genetic testing is becoming the standard for LGMD diagnosis for patients suspected of having LGMD. Testing should be performed by way of an LGMD or neuromuscular gene panel that looks for alterations in genes known to be involved in LGMD or other dystrophies and myopathies. If such a panel is inconclusive, broader testing by way of whole exome sequencing or whole genome sequence with NGS may be used. Unless clinical findings suggest a particular subtype of LGMD, whole exome or whole genome testing may increase the likelihood of an accurate diagnosis as compared to single-gene sequencing.

The yield of genetic testing in patients with signs and symptoms of LGMD varies depending on the mutation and population characteristics. Some studies conclude that the clinical validity is reasonably high. According to Norwood et al. (2007), “DNA analysis directed to provide confirmation of mutation in the affected gene(s) is the gold standard of diagnosis, and necessary to be able to offer carrier or pre-symptomatic testing to other family members.” Other researchers have attempted to utilize whole-exome sequencing to diagnose LGMD in 100 individuals with familial LGMD; however, a diagnostic success rate of only 45% was achieved.

Monies et al. (2016) screened 50 random genetically unstudied families with LGMD via a gene panel incorporating 759 OMIM (Online Mendelian Inheritance in Man) genes associated with neurological disorders. OMIM is a catalogue of human genes and genetic disorders that provides comprehensive information based on the periodical biomedical literature. The panel “identified the mutation in 76 % of families (38/50; 11 novel). A total of 34 families had mutations in LGMD-related genes with four others having variants not typically associated with LGMD. The majority of cases had recessive inheritance with homoallelic pathogenic variants (97.4 %, 37/38), as expected considering the high rate of consanguinity in the study population.” The authors concluded that the “neurological panel achieved a high clinical sensitivity (76 %) and is an effective first-line laboratory test in patients with LGMD and other myopathies. This sensitive, cost-effective, and rapid assay significantly assists clinical practice especially in these phenotypically and genetically heterogeneous disorders. Moreover, the application of the

American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) guidelines applied in the classification of variant pathogenicity provides a clear interpretation for physicians on the relevance of such findings.”

Harris et al. (2017) performed whole exome sequencing (WES) on 104 patients with LGMD in which standard gene testing had not yet yielded a diagnosis, and 91 patients using sequential gene by gene testing. They found that “patients selected for WES had undergone more extensive prior testing than those undergoing standard genetic testing, and on average had had eight genes screened already. In this extensively investigated cohort WES identified the genetic diagnosis in 28 families (28/75, 37%), including the identification of the novel gene *ZAK* and two unpublished genes. WES of a single affected individual with sporadic disease yielded a diagnosis in 13/38 (34%) of cases. In comparison, conventional gene by gene testing provided a genetic diagnosis in 28/84 (33%) families.” The authors concluded that “WES was able to overcome many limitations of standard testing and achieved a higher rate of diagnosis than standard testing even in this cohort of extensively investigated patients. Earlier application of WES is therefore likely to yield an even higher diagnostic rate. We obtained a high diagnosis rate in simplex cases and therefore such individuals should be included in exome or genome sequencing projects. Disease due to somatic mosaicism may be increasingly recognized due to the increased sensitivity of next generation sequencing techniques to detect low level mosaicism.” A similar study by Reddy and colleagues reported 40% of the LGMD families tested “had novel and previously reported pathogenic mutations, primarily in LGMD genes, and also in genes for Duchenne muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital myopathy, myofibrillar myopathy, inclusion body myopathy and Pompe disease.”

#### IV. Guidelines and Recommendations

##### **Best Practice Guidelines on Molecular Diagnostics in Duchenne/Becker Muscular Dystrophies Workshop Report (Abbs et al., 2010)**

The international workshop comprised of scientists from Europe, the US, India, and Australia was organized and sponsored by the European Neuro-Muscular Centre, the European Molecular Genetics Quality Network, TREAT-NMD, and Euro-Gentest. The flow chart for the diagnostic work-up of a dystrophinopathy they recommend is shown below in Figure 1.

S. Abbs et al. / *Neuromuscular Disorders* 20 (2010) 422–427

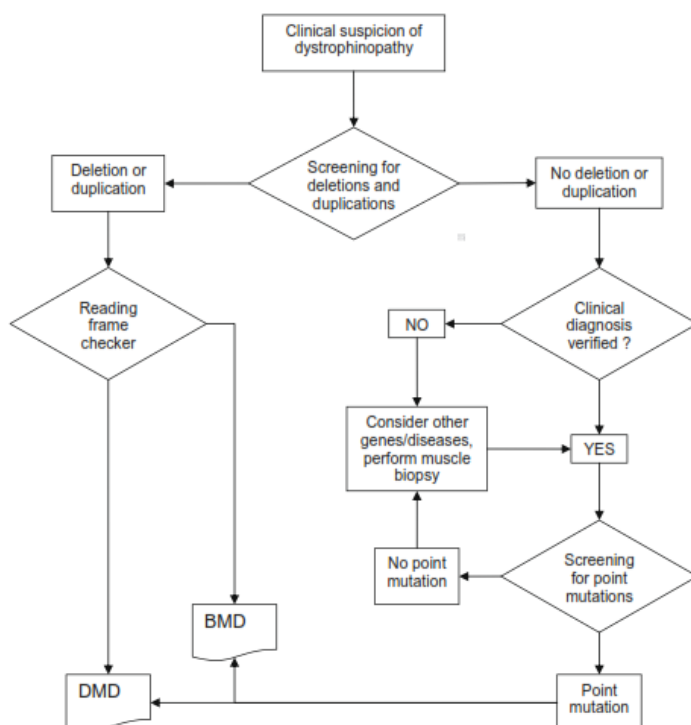


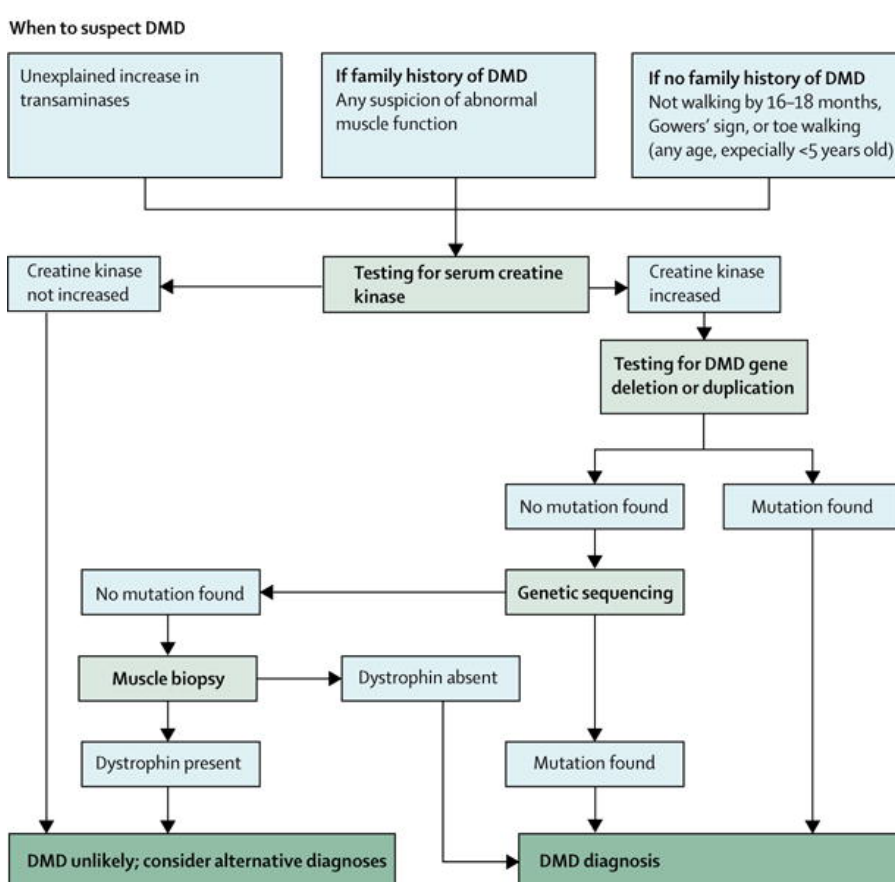
Fig. 1. Flow chart for the diagnostic work-up of a dystrophinopathy.

Recommendations for testing are as follows: if there is a clinical suspicion of a dystrophinopathy, first screen for deletions and duplications. If no deletion or duplication is detected, but the clinical diagnosis is verified, screening for point mutations should be performed.

### The DMD Care Considerations Working Group (Birnkrant et al., 2018; Bushby et al., 2010)

The CDC selected 84 clinicians to comprise the DMD Care Considerations Working Group to develop recommendations regarding all aspects of DMD care, including the diagnosis and genetic testing of muscular dystrophy. They state the following: “Testing for a DMD mutation in a blood sample is always necessary even if DMD is first confirmed by the absence of dystrophin protein expression on muscle biopsy. The results of genetic testing provide the clinical information required for genetic counselling, prenatal diagnosis, and consideration for future mutation-specific therapies... If analysis by one or more of these techniques leads to the identification and full characterisation of a dystrophin mutation, then no further testing is required. If deletion/ duplication testing is negative, then dystrophin gene sequencing should be done to look for point mutations or small deletions/insertions. Full characterisation of the mutation (deletion endpoints or exact position of any point mutation) is required to allow correlation of the predicted effect of the mutation on the reading frame of the gene, which is the major determinant of the phenotypic variability seen in dystrophinopathy, as well as to determine eligibility for the mutation-specific treatments currently in trials.”

More recently, an update of the 2010 DMD Care considerations was published to further improve patient care. A diagnostic flow chart for DMD was provided and can be found below.



**Most commonly observed early signs and symptoms in patients with DMD**

Motor	Non-motor
<ul style="list-style-type: none"> <li>• Abnormal gait</li> <li>• Calf pseudohypertrophy</li> <li>• Inability to jump</li> <li>• Decreased endurance</li> <li>• Decreased head control when pulled to sit</li> <li>• Difficulty climbing stairs</li> <li>• Flat feet</li> <li>• Frequent falling or clumsiness</li> <li>• Gowers' sign on rising from floor</li> <li>• Gross motor delay</li> <li>• Hypotonia</li> <li>• Inability to keep up with peers</li> <li>• Loss of motor skills</li> <li>• Muscle pain or cramping</li> <li>• Toe walking</li> <li>• Difficulty running or climbing</li> </ul>	<ul style="list-style-type: none"> <li>• Behavioural issues</li> <li>• Cognitive delay</li> <li>• Failure to thrive or poor weight gain</li> <li>• Learning and attentional issues</li> <li>• Speech delay or articulation difficulties</li> </ul>

The DMD Care Considerations Working Group has stated that “Because approximately 70% of individuals with DMD have a single-exon or multi-exon deletion or duplication in the dystrophin gene, dystrophin gene deletion and duplication testing is usually the first confirmatory test. Testing is best done by multiplex ligation-dependent probe amplification (MLPA) or comparative genomic hybridisation



array, since use of multiplex PCR can only identify deletions... If deletion or duplication testing is negative, genetic sequencing should be done to screen for the remaining types of mutations that are attributed to DMD (approximately 25–30%). Finally, if genetic testing does not confirm a clinical diagnosis of DMD, then a muscle biopsy sample should be tested for the presence of dystrophin protein by immunohistochemistry of tissue cryosections or by western blot of a muscle protein extract (Birnkrant et al., 2018).” Also stated is that “Family members of an individual with DMD should receive genetic counselling to establish who is at risk of being a carrier. Carrier testing is recommended for female relatives of a boy or man who has been genetically confirmed to have DMD.”

**American Academy of Neurology (AAN) and the American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM) (P. B. Kang et al., 2015; Narayanaswami et al., 2014) (reaffirmed in 2021)**

The Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular and Electrodiagnostic Medicine published recommendations that “Targeted genetic testing often identifies causative mutations in the classic CMD subtypes. Genetic diagnoses are beneficial to the patient, as they often enable physicians to provide more accurate prognoses and facilitate genetic counseling and family-planning discussions, and may enable patients to become more aware of future clinical trials for which they may be eligible... when available and feasible, physicians might order targeted genetic testing for specific CMD subtypes that have well-characterized molecular causes” and “In individuals with CMD who either do not have a mutation identified in one of the commonly associated genes or have a phenotype whose genetic origins have not been well characterized, physicians might order whole-exome or whole-genome sequencing when those technologies become more accessible and affordable for routine clinical use.”

In 2014, the American Academy of Neurology and the Practices Issues Review Panel of the American Association of Neuromuscular and Electrodiagnostic Medicine issued evidenced-based guidelines for the diagnosis and treatment of limb-girdle and distal dystrophies, who made the following recommendations:

For the diagnosis of LGMD:

- For patients with suspected muscular dystrophy, clinicians should use a clinical approach to guide genetic diagnosis based on the clinical phenotype, including the pattern of muscle involvement, inheritance pattern, age at onset and associated manifestations (e.g., early contractures, cardiac or respiratory involvement) (Level B recommendation).
- In patients with suspected muscular dystrophy in whom initial clinically directed genetic testing does not provide a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, whole-exome sequencing, whole genome screening, or next-generation sequencing to identify the genetic abnormality (Level C recommendation).

The AAN Guidelines state: “Diagnosis assists in defining the long-term prognosis, since some dystrophies are more rapidly progressive, involve the cardiorespiratory systems more frequently, or are associated with other disorders. The identification of these dystrophies through genetic testing will not only inform long-term prognosis but will also assist in directing care more efficiently (e.g., more frequent

cardiorespiratory monitoring and prophylactic treatments such as pacer/defibrillator placement for those disorders known to be associated with cardiac involvement). Precise identification of the disorder also eliminates the need for repeated testing for an acquired, treatable disorder such as an inflammatory myopathy, because some dystrophies have inflammation on muscle biopsy, making diagnosis difficult on the basis of routine biopsy findings. In addition, the temptation to try immunosuppressive agents repeatedly, looking for a therapeutic response, is not unusual when there is no diagnosis and the patient is worsening. This exposes patients to potentially serious side effects of immunosuppressive medications. Patients on immunosuppressants need regular monitoring, adding logistical difficulties to a population that may have significantly impaired mobility. Health care costs are increased by repeated investigations, immunosuppressive treatments, and laboratory monitoring. Although establishing a genetic diagnosis is expensive on the front end, the costs of continued investigation for other causes and the risks and expenses associated with empiric trials of immunosuppressants make a strong case for establishing a genetic diagnosis, which often provides patients a sense of closure. Establishing a genetic diagnosis is crucial for genetic counseling to inform decision-making about having children and for screening of offspring. Treatment of cardiomyopathy, arrhythmias, and ventilatory failure prolongs life and improves quality of life in patients with other neuromuscular diseases.”

#### **American Academy of Neurology (AAN) (Tawil et al., 2015) (reaffirmed in 2021 (AAN, 2018))**

The American Academy of Neurology published evidence-based guidelines which found that “the finding of a D4Z4 contraction on chromosome 4q35 likely has a sensitivity of 93% and a specificity of 98% for diagnosis of clinically defined FSHD.” They recommend that “Clinicians should obtain genetic confirmation of FSHD1 in patients with atypical presentations and no first-degree relatives with genetic confirmation of the disease [Level B].” Concerning the use of genetics as a predictor of severity in FSHD, they recommend, “Large D4Z4 deletion sizes (contracted D4Z4 allele of 10–20 kb) should alert the clinician that the patient is more likely to develop more significant disability and at an earlier age. Patients with large deletions are also more likely to develop symptomatic extramuscular manifestations [Level B].”

#### **International Standard of Care Committee for Congenital Muscular Dystrophy (C. H. Wang et al., 2010)**

As a part of the guidelines concerning newly diagnosed patients, the International Standard of Care Committee for Congenital Muscular Dystrophy recommends that “if a genetic diagnosis is known, the recurrence risk and impact on future family planning should be discussed. Even if the exact genetic defect is not known, recurrence risk can sometimes be discussed using a common genetic model that is often associated with the diagnosis.”

#### **171st European Neuromuscular Centre International Workshop on Standards of Care and Management of Facioscapulohumeral Muscular Dystrophy (FSHD)**

In a report from the 171st European Neuromuscular Centre International Workshop Standards of Care and Management of FSHD held in January 2010, it is stated that “when a physician concludes facioscapulohumeral syndrome based on clinical findings, the odds are in favor of FSHD, and genetic testing is the preferred diagnostic choice.”

## **Evidence-Based Consensus and Systematic Review on Reducing the Time to Diagnosis of Duchenne Muscular Dystrophy Joint Report of the EPNS, MDA, PPMD, TREAT-NMD, and DPPI**

According to this evidence-based report endorsed by the European Paediatric Neurology Society (EPNS), the Muscular Dystrophy Association (MDA), Duchenne Parent Project Italy (DPPI), Parent Project Muscular Dystrophy (PPMD), and TREAT-NMD, “Genetic testing is crucial for obtaining a complete diagnosis of DMD, and should be considered the gold standard”; accepted DMD symptoms are listed and include “calf hypertrophy (pseudohypertrophy); delayed walking; difficulty climbing/descending stairs; difficulty rising from the floor; difficulty running/walking; elevated serum CK levels (including elevated ALT and AST); a family history of DMD; frequent falls; Gowers' sign; male sex; and muscle weakness;” additional recommendations include that “patients with signs and symptoms of DMD and elevated serum CK levels should be referred for genetic testing to either a clinical geneticist or a neuromuscular specialist.” In regard to other family members, Aartsma-Rus et al. (2019) recommends that “After a patient receives a complete genetic diagnosis of DMD, it is mandatory that carrier testing of the mother and other at-risk female family members be offered with appropriate pre- and post-genetic counseling.”

### **U.S. Preventive Services Task Force (USPSTF)**

No U.S. Preventive Services Task Force recommendations for genetic testing for muscular dystrophy have been identified. A search for “muscular dystrophy” on the USPSTF website turned up 0 results on October 2<sup>nd</sup>, 2020.

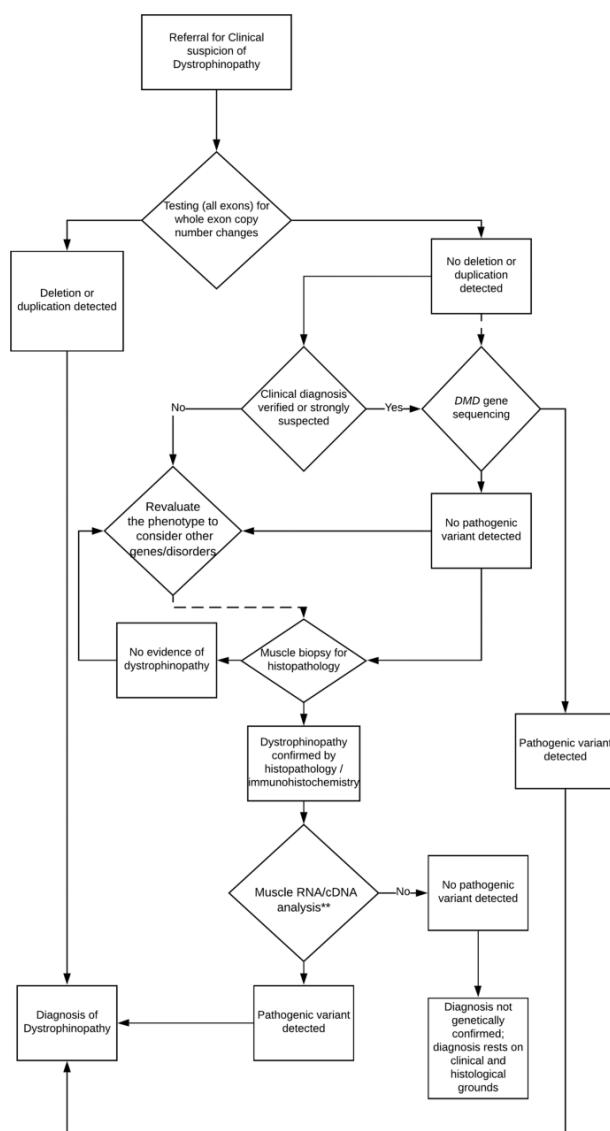
### **European Molecular Genetics Quality Network (EMQN) Best Practice Guidelines on molecular diagnostics in the Duchenne/Becker muscular dystrophies**

The EMQN summarizes current diagnostic methods to analyze the *DMD* gene and categorizes these methods according to specificity of the tests into Level 1 (copy number variations detection), Level 2 (small variants detection), and Level 3 (RNA Analysis). 78% of the pathogenic *DMD* variants is due to whole-exon deletions and duplications; therefore, detection of the relative copy number of all exons within the *DMD* gene (copy number variations) is the first level of diagnostic tests offered. Several quantitative tests can be used to detect these copy number variations (CNVs). The most reliable method is multi-plex ligation-dependent probe amplification (MLPA), which detects the number of deletions or duplications to the exon level. MLPA is more reliable when there are multiple exons involved, but less if there is a single exon deletion. Therefore, real-time PCR, multi-plex PCR, Sanger sequencing, or microsatellite marker analysis must follow to confirm single exon deletions. Comparative genomic hybridization (CGH) is another method which provides a slightly higher detection rate than MLPA by use of oligonucleotide probes to interrogate copy number across the entire 2.2 Mb genomic region of the *DMD* gene. The last method is next generation sequencing (NGS), which can detect single nucleotide variants (SNVs) and copy number variations (CNVs); however, NGS is not routinely used to detect CNVs because it is not sensitive enough to pick up on all types of CNVs. Level 2 testing detects missense, nonsense, small insertions and deletions, and indel and splicing variants through either Sanger sequencing or NGS. NGS is less time consuming as it allows for many targets to be sequenced with deep sequence coverage in multiple patients at a time and enhances detection of low-level somatic mosaicism in patients' or probands' mothers; however, Sanger sequencing is still the standard method used for known familial variant testing. Level 3 testing, such as muscle cDNA analysis, is used when a patient

presents with clinical symptoms of a dystrophinopathy, but no CNVs or small variants were detected from Level 1 and Level 2 diagnostic techniques.

A flowchart for the recommended molecular diagnostic algorithm for dystrophinopathy is shown in the figure above (Fratter et al., 2020). When dystrophinopathy is suspected due to clinical symptoms, high serum creatine kinase levels, and a possible family history, molecular testing to detect copy number variations (CNVs) is recommended. If a pathogenic deletion and duplication is not detected, the next step is to sequence the coding region of the entire *DMD* gene. If a pathogenic variant is not detected from these two tests, a muscle biopsy, dystrophin gene analysis via immunohistochemistry, or muscle cDNA sequencing may be performed. If the patient presents with a positive muscle biopsy test and positive symptoms of a dystrophinopathy, but no pathogenic variant is detected through genetic testing, the patient should still be diagnosed with a dystrophinopathy.

The EMQN also provides guidelines on genetic testing for females affected by dystrophinopathies. Although dystrophinopathies predominantly affect males, females could have a pathogenic *DMD* variant



\*\*This may be followed by whole-*DMD* gene sequencing (i.e. including introns; e.g. by whole-genome sequencing) if required to try to identify the genomic DNA variant underlying aberrant splicing.

and present with a milder phenotype most likely due to a skewed X-inactivation. In this case, the diagnostic method would be identical to the one presented in the flowchart: evaluation of clinical symptoms, CNV analysis and *DMD* gene sequencing followed by muscle biopsy, dystrophin gene analysis, and muscle cDNA sequencing as needed. If a female presents with the complete *DMD* phenotype, karyotyping is recommended due to possible chromosomal aberrations or autosomal translocations that could cause 100% skewed X-inactivation.

Females who are carriers of dystrophinopathies are usually clinically asymptomatic but may develop some symptoms during their lifetime or pass it down to their children. Therefore, carrier testing is important for family planning. If the familial pathogenic variant is known, then two complementary tests should be performed (MLPA and Sanger Sequencing or MLPA and microsatellite analysis). High density array CGH can also be used, which is a single test that interrogates multiple loci within the deletion or duplication. If the familial pathogenic variant is not known and an affected male in the family is not available, then female carriers who are at risk of being carriers should be offered CNV analysis and *DMD* gene sequencing followed by muscle biopsy, dystrophin gene analysis, and muscle cDNA sequencing as needed. Measuring serum creatine kinase levels may also be helpful.

EMQN also comments on prenatal diagnosis of dystrophinopathies. Since it is not possible to predict whether a female will present symptoms of a dystrophinopathy, prenatal diagnosis should only be performed on pregnancies with a male fetus unless there is documented familial reoccurrence of complete skewed X inactivation. Prenatal testing is performed on placental biopsy through chorionic villus sampling (CVS) within 11-12 weeks of gestation or amniocentesis within 15-17 weeks of gestation. CVS is the preferred method as it provides higher DNA quality and greater safety for the pregnancy. The obtained DNA from placental biopsy can be analyzed through next generation sequencing (NGS) or CNV detection. For non-invasive prenatal diagnostic options, relative haplotype dosage analysis (RHDO) may be performed. "RHDO analysis examines SNPs in the cell-free DNA from a maternal blood sample and shows whether the male foetus has inherited the high risk or low risk haplotype across the *DMD* gene."

## V. State and Federal Regulations, as applicable

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: <http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx>. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

A search of the FDA Device database on 10/02/2020 for "genotyping tests" yielded 15 results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.



## VI. Important Reminder

The purpose of this Medical Policy is to provide a guide to coverage. This Medical Policy is not intended to dictate to providers how to practice medicine. Nothing in this Medical Policy is intended to discourage or prohibit providing other medical advice or treatment deemed appropriate by the treating physician.

Benefit determinations are subject to applicable member contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control.

This Medical Policy has been developed through consideration of the medical necessity criteria under Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4), or for QUEST Integration members under Hawaii Administrative Rules (HAR 1700.1-42), generally accepted standards of medical practice and review of medical literature and government approval status. HMSA has determined that services not covered under this Medical Policy will not be medically necessary under Hawaii law in most cases. If a treating physician disagrees with HMSA's determination as to medical necessity in a given case, the physician may request that HMSA reconsider the application of the medical necessity criteria to the case at issue in light of any supporting documentation.

Genetic testing is covered for level 1 or 2A recommendations of the National Comprehensive Cancer Network (NCCN and in accordance with Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, the Hawaii Administrative Rules (HAR 1700.1-42).

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## VIII. Policy History

Policy approved by Medical Directors	9/20/2022
Policy approved at UMC	12/16/2022
Policy effective	6/1/2023
Updated Lines of Business	12/18/2023