BlueCross BlueShield of Alabama

Name of Policy:
Genetic Testing for Limb-Girdle Muscular Dystrophies

Policy #: 641
Category: Laboratory
Latest Review Date: May 2018
Policy Grade: B

Background/Definitions:

As a general rule, benefits are payable under Blue Cross and Blue Shield of Alabama health plans only in cases of medical necessity and only if services or supplies are not investigational, provided the customer group contracts have such coverage.

The following Association Technology Evaluation Criteria must be met for a service/supply to be considered for coverage:

1. *The technology must have final approval from the appropriate government regulatory bodies;*
2. *The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes;*
3. *The technology must improve the net health outcome;*
4. *The technology must be as beneficial as any established alternatives;*
5. *The improvement must be attainable outside the investigational setting.*

Medical Necessity means that health care services (e.g., procedures, treatments, supplies, devices, equipment, facilities or drugs) that a physician, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury or disease or its symptoms, and that are:

1. *In accordance with generally accepted standards of medical practice; and*
2. *Clinically appropriate in terms of type, frequency, extent, site and duration and considered effective for the patient's illness, injury or disease; and*
3. *Not primarily for the convenience of the patient, physician or other health care provider; and*
4. *Not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient's illness, injury or disease.*

Description of Procedure or Service:

The limb-girdle muscular dystrophies (LGMDs) are a genetically heterogeneous group of muscular dystrophies characterized by predominantly proximal muscle weakness (pelvic and shoulder girdles). A large number of genetic variants have been associated with LGMD.

Muscular Dystrophies

Muscular dystrophies are a group of inherited disorders characterized by progressive weakness and degeneration of skeletal muscle, cardiac muscle, or both, which may be association with respiratory muscle involvement or dysphagia and dysarthria. Muscular dystrophies are associated with a wide spectrum of phenotypes, which may range from rapidly progressive weakness leading to death in the second or third decade of life to clinically asymptomatic disease with elevated CK levels. Muscular dystrophies have been classified on the basis of clinical presentation and genetic etiology. The most common are the dystrophinopathies, Duchenne (DMD) and Becker (BMD) muscular dystrophies, which are characterized by pathogenic variants in the dystrophin gene. Other muscular dystrophies are characterized by the location of onset of clinical weakness and include the LGMDs, facioscapulohumeral muscular dystrophy, oculopharyngeal muscular dystrophy, distal muscular dystrophy, and humeroperoneal muscular dystrophy (also known as Emery-Dreifuss muscular dystrophy). The congenital muscular dystrophy is a genetically heterogeneous group of disorders, which historically included infants with hypotonia and weakness at birth and findings of muscular dystrophy on biopsy. Finally, myotonic dystrophy is a multisystem disorder characterized by skeletal muscle weakness and myotonia in association with cardiac abnormalities, cognitive impairment, endocrinopathies, and dysphagia.

Limb-Girdle Muscular Dystrophies

The term *limb-girdle muscular dystrophy* is a clinical descriptor for a group of muscular dystrophies characterized by predominantly proximal muscle weakness (pelvic and shoulder girdles) which may be included in the differential diagnosis of DMD and BMD. Onset can be in childhood or adulthood. The degree of disability depends on the location and degree of weakness. Some LGMD subtypes are characterized by only mild, slowly progressive weakness, while others are associated with early-onset, severe disease with loss of ambulation. LGMDs may be associated with cardiac dysfunction, cardiomyopathy (dilated or hypertrophic), respiratory depression, and dysphagia or dysarthria. Of particular note is the risk of cardiac complications, which is a feature of many but not all LGMDs. Most patients have an elevated CK.

LGMDs have an estimated prevalence ranging from 2.27 to 4 per 100,000 in the general population, constituting the fourth most prevalent muscular dystrophy type after the dystrophinopathies (DMD and BMD), facioscapulohumeral muscular dystrophy, and myotonic dystrophy. The prevalence of specific types increases in populations with founder pathogenic variants (e.g., Finland, Brazil).

Genetic Basis and Clinical Correlation

As the genetic basis of the LGMDs has been elucidated, it has been recognized that there is tremendous heterogeneity in genetic variants that cause the LGMD phenotype. LGMDs were initially classified based on a clinical and locus-based system. As of 2015, at least nine

autosomal dominant types (designated LGMD1A through LGMD1H) and at least 23 autosomal recessive types (designated LGMD2A through LGMD2W) have been identified. Subtypes vary in inheritance, pathophysiology, age of onset, and severity. Table 1 provides a summary of involved gene and protein, clinical characteristics (if known), and proportion of all cases represented by a specific genotype (if known).

Table 1: Summary of Genetic Basis of LGMD

LGMD Type	Involved Gene	Involved Protein	Age at Onset	Rate of Progression	Cardiac Involvement	Percent AR LGMD Cases
Autosomal Dominant						
1A	*MYOT*	Myotilin	Adulthood	Slow	Yes	
1Ba	*LMNA*	Lamin A/C	Adolescence or variable	Slow	Yes	
1Ca	*CAV3*	Caveolin-3	Variable	Slow	Yes	
1D	*DNAJB6*	DNAJ/Hsp40 homolog	Adulthood	Slow	No	
1E	*DES*	Desmin	Adulthood	Slow	Yes	
1F	*TNPO3*	Transportin3	Variable	Slow	No	
1G	*HNRPDL*	Heterogeneous nuclear ribonucleoprotein D-like protein	Adulthood	Slow	No	
1H			Variable	Slow	No	
Autosomal Recessive						
2A	*CAPN3*	Calpain 3	Adolescence to adulthood	Moderate	Rare	≈10% to ≈40%
2B	*DYSF*	Dysferlin	Adolescence to adulthood	Slow	Yes	≈5% to ≈25%
2C	*SGCG*	γ-sarcoglycan	Early childhood	Rapid	Yes	68% with childhood onset; ≈10% with adult onset
2D	*SGCA*	α-sarcoglycan	Early childhood	Rapid	Yes	
2E	*SGCB*	β-sarcoglycan	Early childhood	Rapid	Yes	
2F	*SGCD*	δ-sarcoglycan	Early childhood	Rapid	Yes	
2G	*TCAP*	Telethonin	Adolescence	Slow	Yes	3%
2H	*TRIM32*	Tripartite motif containing 32	Adulthood	Slow	No	
2I	*FKRP*	Fukutin-related protein	<10 to >40 y Late childhood or variable	Moderate	Yes	6%
2J	*TTN*	Titin	Young adulthood	Rapid	No	
2K	*POMT1*	Protein-O-mannosyltransferase 1	Childhood	Slow	No	
2L	*ANO5*	Anoctamin-5	Variable	Slow	No	25% in U.K.
2M	*FKTN*	Fukutin	Early childhood	Slow moderate	Yes	
2N	*POMT2*	Protein-O-mannosyltransferase 2	Early childhood	Slow moderate	Rare	

2O	*POMGnT1*	Protein O-linked mannose beta1, 2-Nacetyl-glucosaminyl-transferase	Late childhood	Moderate	No	
2P	*DAG1*	Dystroglycan	Early childhood	Moderate	No	
2Q	*PLEC1*	Plectin	Early childhood	Slow	No	
2R	*DES*	Desmin	Young adulthood		Yes (AV conduction block)	
2S	*TRAPPC11*	Transport protein particle complex 11	Young adulthood	Slow	No	
2T	*GMPPB*	GDP-mannose pyrophosphorylase B	Early childhood to young adulthood		Yes	
2U	*ISPD*	Isoprenoid synthase domain containing	Variable	Moderate rapid	Yes	
2V	*GAA*	Glucosidase, α-1	Variable	Variable	Yes	
2W	*LIMS2*	Lim and senescent cell antigen-like domains 2	Childhood		Yes	

AD: autosomal dominant; AR: autosomal recessive; AV: atrioventricular; LGMD: limb-girdle muscular dystrophy. a Rare recessive cases have been described for IB and IC.
(Adapted From Norwood et al (2007), Mahmood et al (2014), Nigro et al (2011), Nigro et al (2011), Nigro et al (2014), Pegoraro & Huffman (2000)

The prevalence of various variants and LGMD subtypes can differ widely by country, but the autosomal recessive forms are generally more common. Pathogenic variants in *CAPN3* represent 20% to 40% of LGMD cases, and LGMD2A is the most frequent LGMD in most countries. *DYSF* pathogenic variants leading to LGMD2B are the second most common LGMD in many, but not all, areas (15-25%). Sarcoglycanopathies constitute about 10% to 15% of all LGMDs, but 68% of the severe forms.

In an evaluation of 370 patients with suspected LGMD enrolled in a registry from six U.S. university centers, 312 of whom muscle biopsy test results were available, Moore et al reported the distribution of LGMD subtypes based on muscle biopsy results as follows: 12% LGMD2A; 18% LGMD2B; 15% LGMD2C-2F; and 1.5% LGMD1C.

Clinical Variability
Other than presentation with proximal muscle weakness, the LGMD subtypes can have considerable clinical variability in terms of weakness severity and associated clinical conditions. The sarcoglycanopathies (LGMD2C-2F) cause a clinical picture similar to that of the intermediate forms of DMD and BMD, with risk of cardiomyopathy in all forms of the disease.

Of particular clinical importance is that fact that while most, but not all, LGMD subtypes are associated with an increased risk of cardiomyopathy, arrhythmia, or both, the risk of cardiac disorders is variable across subtypes. LGMD1A, LGMD1B, LGMB2C-K, and LGMD2M-P have all been associated with cardiac involvement. Sarcoglycan variants tend to be associated with severe cardiomyopathy. Similarly, the LGMD subtypes of LGMD2I and 2C-2F are at higher risk of respiratory failure.

Many of the genes associated with LGMD subtypes have allelic disorders, both with neuromuscular disorder phenotypes and clinically unrelated phenotypes. Variants in the lamin A/C proteins, which are caused by splice-site variants in the LMNA gene, are associated with several different neuromuscular disorder phenotypes, including Emery-Dreifuss muscular dystrophy, a clinical syndrome characterized by childhood-onset elbow, posterior cervical, and ankle contractures and progressive humeroperoneal weakness, autosomal dominant LGMD (LGMD1B), and congenital muscular dystrophy. All forms have been associated with cardiac involvement, including atrial and ventricular arrhythmias and dilated cardiomyopathy.

Clinical Diagnosis
A diagnosis of LGMD is suspected in patients who have myopathy in the proximal musculature in the shoulder and pelvic girdles, but the distribution of weakness and the degree of involvement of distal muscles is variable, particularly early in the disease course. Certain LGMD subtypes may be suspected on the basis of family history, patterns of weakness, CK level, and associated clinical findings. However, there is considerable clinical heterogeneity and overlap across the LGMD subtypes.

Without genetic testing, diagnostic evaluation can typically lead to a general diagnosis of a LGMD, with limited ability to determine the subcategory. Most cases of LGMD will have elevated CK levels, with some variation in the degree of elevation based on subtype. Muscle imaging with computed tomography (CT) or magnetic resonance imaging (MRI) may be obtained to assess areas of involvement and guide muscle biopsy. MRI or CT may be used to evaluate patterns of muscle involvement. At least for calpainopathy (LGMD2A) and dysferlinopathy (LGMD2B), MRI may show particular patterns distinct from other neuromuscular disorders, including hyaline body myopathy and myotonic dystrophy. In 1 study that evaluated muscle CT in 118 patients with LGMD and 32 controls, there was generally poor overall interobserver agreement (ƙ=0.27, and low sensitivity (40%) and specificity (58%) for LGMD.

Electromyography (EMG) has limited value in LGMD, although it may have clinical utility if there is clinical concern for type III spinal muscular atrophy. EMG typically shows myopathic changes with small polyphasic potentials.

Muscle biopsy may be used in suspected LGMD to rule out other, treatable causes of weakness (in some cases), and to attempt to identify a LGMD subtype. All LGMD subtypes are characterized on muscle biopsy by dystrophic features, with degeneration and regeneration of muscle fibers, variation in fiber size, fiber splitting, increased numbers of central nuclei, and endomysial fibrosis. Certain subtypes, particularly in dysferlin deficiency (LGMD2B) may show inflammatory infiltrates, which may lead to an inaccurate diagnosis of polymyositis.

Following standard histologic analysis, immunohistochemistry and immunoblotting are typically used to evaluate myocyte protein components, which may include sarcolemma-related proteins (e.g., α-dystroglycan, sarcoglycans, dysferlin, caveolin-3), cytoplasmic proteins (e.g., calpain-3, desmin), or nuclear proteins (e.g., lamin A/C). Characteristic findings on muscle biopsy immunostaining or immunoblotting can be seen for calpainopathy (LGMD2A),

sarcoglycanopathies (LGMD2C-2F), dysferlinopathy (LGMD2B), and O-linked glycosylation defects (dystroglycanopathies; LGMD2I, LGMD2K, LGMD2M, LGMD2O, LGMD2N). However, muscle biopsy is imperfect: secondary deficiencies in protein expression can be seen in some LGMD. In the Moore et al study previously described, 9% of all muscle biopsy samples had reduced expression of more than one protein tested. In some types of variants, muscle immunohistochemistry results may be misleading because the variant leads to normal protein amounts but abnormal function. For example, Western blot analysis for calpain 3, with loss of all calpain 3 bands, may be diagnostic of LGMD2A, but the test is specific but not sensitive, because some LGMD2A patients may retain normal amounts of nonfunctional protein.

A blood-based dysferlin protein assay, which evaluates dysferlin levels in peripheral blood CD14+ monocytes, has been evaluated in a sample of 77 individuals with suspected dysferlinopathy. However, the test is not yet in widespread use.

Treatment
At present, no therapies have been clearly shown to slow the progression of muscle weakness for the LGMDs. Treatment is focused on supportive care to improve muscle strength, slow decline in strength, preserve ambulation, and treat and prevent musculoskeletal complications that may result from skeletal muscle weakness, such as contractures or scoliosis. Clinical management guidelines are available from the American Academy of Neurology and Association of Neuromuscular & Electrodiagnostic Medicine (see Practice Guidelines and Position Statements section).

Monitoring for Complications
Different genetic variants associated with clinical LGMD are associated with different rates of complications and the speed and extent of disease progression.

Monitoring for respiratory depression and cardiac dysfunction is indicated for LGMD subtypes that are associated with respiratory or cardiac involvement, because patients are often asymptomatic until they have significant organ involvement. When respiratory depression is present, patients may be candidates for invasive or noninvasive mechanical ventilation. Treatments for cardiac dysfunction potentially include medical or device-based therapies for heart failure or conduction abnormalities.

Patients may need monitoring and treatment for swallowing dysfunction, if it is present, along with physical and occupation therapy and bracing for management of weakness.

Investigational Therapies
A number of therapies are under investigation for LGMD. Glucocorticoids have been reported to have some benefit in certain subtypes (LGMD2D, LGMD2I, LGMD2L). However, 1 small (N=25) randomized, double-blind, placebo controlled trial of the glucocorticoid deflazacort in patients with genetically confirmed LGMD2B (dysferlinopathy) showed no benefit and a trend toward worsening strength associated with deflazacort therapy. Autologous bone marrow transplant has been investigated for LGMD, but is not in general clinical use. Adeno-associated virus-mediated gene transfer to the extensor digitorum brevis muscle has been investigated in

LGMD2D, and in a phase 1 trial in LGMD2C. Exon-skipping therapies have been investigated as a treatment for dysferlin gene variants (LGMD2B) given the gene's large size.

Molecular Diagnosis of LGMDs

Because most variants leading to LGMD are single nucleotide variants, the primary method of variant detection is gene sequencing using Sanger sequencing or NGS methods. In cases in which an LGMD is suspected but gene sequencing is normal, deletion/duplication analysis through targeted comparative genomic hybridization (CGH) or multiplex ligation-dependent probe amplification (MLPA) may also be obtained.

A number of laboratories offer panels of tests for LGMD that rely on either Sanger sequencing or NGS. The following list is not exhaustive.

- GeneDx (Limb-Girdle Muscular Dystrophy Panel; Gaithersburg, MD: NGS, with reporting only on panel genes, with concurrent targeted array CGH analysis to evaluate for deletions/duplications for most genes (exceptions, *GMPPB* and *TNPO3*). Multiplex polymerase chain reaction (PCR) assay is performed to assess the presence of the 3' untranslated region insertion in the *FKTN* gene. All reported sequence variants are confirmed by conventional di-deoxy DNA sequence analysis, quantitative PCR, MLPA, repeat PCR analysis, or another appropriate method.
- Prevention Genetics offers several LGMD tests. These include an autosomal dominant LGMD Sanger sequencing panel, which includes *MYOT, LMNA, DNAJB6,* and *CAV3* sequencing either individually or as a panel, followed by array-CGH for deletions/duplications. The company also offers an autosomal recessive LGMD Sanger sequencing panel, which includes sequencing of *SGCG, SGCA, SGCB, SGCD, TRIM32, CAPN3, DYSF, FKRP, TTN, TCAP, GMPPB, ANO5,* and *TRAPPC11*, either individually or as a panel, followed by array-CGH for deletions/duplications. In addition, Prevention Genetics offers two NGS panels for LGMD, which involve NGS followed by array-CGH if variant analysis is negative. Additional Sanger sequencing is performed for any regions not captured or with insufficient number of sequence reads. All pathogenic, undocumented and questionable variant calls are confirmed by Sanger sequencing.
- Counsyl offers a Family Prep Screen, which includes testing for multiple diseases that may require early intervention or cause shortened life or intellectual disability and is designed to be used for carrier testing in reproductive planning. Testing for LGMD2D and LGMD2E may be added to the panel. Testing is conducted by NGS, without evaluation for large duplications or deletions
- Centogene (Rostock, Germany) offers an NGS panel for LGMD, which includes sequencing of the included variants (with hot spot testing for TTN), followed by deletion/duplication testing by MLPA (if ordered), with whole exome sequencing if no variants are identified.
- Athena Diagnostics offers NGS testing for *FKRP, LMNA, DYSF, CAV3, CAPN3* (NGS followed by dosage analysis), along with a NGS panel, with deletion/duplication testing for *SGCA, SGCG*, and *CAPN3*.

Variants included in some of the currently available NGS testing panels are summarized in Table 2.

Table 2: LGMD Variants Included in Commercial NGS Test Panels

Gene	GeneDx	Prevention Genetics		Centogene	Athena Diagnostics[b]
Autosomal Dominant			**Autosomal Recessive**		
MYOT	X	X		X	X
LMNA	X	X		X	X
CAV3	X	X		X	X
DNAJB6	X	X		X	X
DES	X	X			X
TNPO3	X	X			
HNRPDL					
CAPN3	X		X	X	X
DYSF	X		X	X	X
SGCG	X		X	X	X
SGCA	X		X	X	X
SGCB	X		X	X	X
SGCD	X		X	X	X
TCAP	X		X	X	X
TRIM32	X		X	X	X
FKRP	X		X	X	X
TTN	X		X	X	X
POMT1	X			X	X
ANO5	X		X	X	X
FKTN	X			X	X
POMT2	X			X	X
POMGnT1	X			X	X
DAG1				X	X
PLEC1				X	X
DES			X		
TRAPPC11			X		X
GMPPB	X		X		
ISPD			X		
GAA					
LIMS2				X	

LGMD: limb-girdle muscular dystrophy; NGS: next-generation sequencing.
a This panel also includes testing for *SMCHD1*, which is associated with facioscapulohumeral muscular dystrophy
b This panel also includes testing for *PNPLA2*, which is associated with neutral lipid storage disease with myopathy, and TOR1AIP1.

Policy:

Genetic testing for genes associated with limb-girdle muscular dystrophy (LGMD) to confirm a diagnosis of LGMD meets Blue Cross and Blue Shield of Alabama's medical criteria for coverage **when signs and symptoms of LGMD are present but a definitive diagnosis cannot be made without genetic testing**, **AND** when at least **ONE** of the following criteria are met:

- Results of testing may lead to changes in clinical management that improve outcomes (e.g., confirming or excluding the need for cardiac surveillance); **OR**
- Genetic testing will allow the affected patient to avoid invasive testing, including muscle biopsy;

Targeted genetic testing for a known familial variant associated with LGMD meets Blue Cross and Blue Shield of Alabama's medical criteria for coverage in **an asymptomatic individual to determine future risk of disease** when **BOTH** of the following criteria are met:

- The individual has a close relative (i.e., first- or second-degree) with a **known** familial variant consistent with LGMD, **AND**
- Results of testing will lead to changes in clinical management (e.g., confirming or excluding the need for cardiac surveillance).

Targeted genetic testing for genes associated with LGMD meets Blue Cross and Blue Shield of Alabama's medical criteria for coverage in **an asymptomatic individual to determine future risk of disease** when **BOTH** of the following criteria are met:

- The individual has a close relative (i.e., first- or second-degree) diagnosed with LGMD whose **genetic status is unavailable**, **AND**
- Results of testing will lead to changes in clinical management (e.g., confirming or excluding the need for cardiac surveillance).

Genetic testing for gene mutations associated with LGMD does not meet Blue Cross and Blue Shield of Alabama medical criteria for coverage in all other situations and/or when utilizing expanded panels of tests and is considered **investigational**.

Blue Cross and Blue Shield of Alabama does not approve or deny procedures, services, testing, or equipment for our members. Our decisions concern coverage only. The decision of whether or not to have a certain test, treatment or procedure is one made between the physician and his/her patient. Blue Cross and Blue Shield of Alabama administers benefits based on the member's contract and corporate medical policies. Physicians should always exercise their best medical judgment in providing the care they feel is most appropriate for their patients. Needed care should not be delayed or refused because of a coverage determination.

Key Points:

The most recent literature review was performed through February 05, 2018.

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Testing Individuals with Signs or Symptoms of LGMD

Clinical Context and Test Purpose

The purpose of genetic testing of individuals with suspected LGMD is to establish the diagnosis of LGMD, direct treatment and monitoring based on a genetic diagnosis. Changes in management may include discontinuation of routine cardiac and/or respiratory surveillance in the absence of a specific genetic diagnosis with specific complications, avoidance of therapies not known to be efficacious for LGMD, potential avoidance of invasive testing, and informing the reproductive decision making.

The question addressed in this evidence review is: In individuals with suspected LGMD, does use of genetic testing result eliminate or reduce the need for a muscle biopsy, need for cardiac and/or respiratory surveillance and lead to improvements in health outcomes?

The following PICOTS were used to select literature to inform this review.

Patients

The relevant population of interest includes individuals with signs or symptoms of LGMD.

Interventions

Genetic testing of genes associated with LGMD.

Comparators

The following practice is currently being used: standard diagnostic workup without genetic testing.

Outcomes

The potential beneficial outcome of primary interest would be reductions in muscle biopsies to confirm diagnosis of LGMD and if changes in management are initiated based on confirming a genetic diagnosis of LGMD.

Potential harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to inappropriate initiation of treatments or psychological harm

after receiving positive test results. False-negative test results can lead to lack of cardiac and/or respiratory surveillance.

Timing
The time frame for outcomes measures varies from short-term changes in disease status or changes in cardiac and/or respiratory surveillance to long-term changes in outcomes.

Setting
Patients suspected of LGMD are actively managed by neurology. Genetic testing is utilized to confirm a diagnosis of LGMD. Referral for genetic counseling is important for explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Clinical Validity
Clinical validity refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values).

For limb-girdle muscular dystrophy (LGMD), clinical validity may refer to the overall yield of testing for any LGMD-associated variant in patients with clinically suspected disease, or the yield of testing for specific variants. The genetic test is generally considered the criterion standard for determining a specific LGMD subtype.

Simplifying Test Terms
There are 3 core characteristics for assessing a medical test. Whether imaging, laboratory, or other, all medical tests must be:

- Technically reliable
- Clinically valid
- Clinically useful.

Because different specialties may use different terms for the same concept, we are highlighting the core characteristics. The core characteristics also apply to different uses of tests, such as diagnosis, prognosis, and monitoring treatment.

Diagnostic tests detect presence or absence of a condition. Surveillance and treatment monitoring are essentially diagnostic tests over a time frame. Surveillance to see whether a condition develops or progresses is a type of detection. Treatment monitoring is also a type of detection because the purpose is to see if treatment is associated with the disappearance, regression, or progression of the condition.

Prognostic tests predict the risk of developing a condition in the future. Tests to predict response to therapy are also prognostic. Response to therapy is a type of condition and can be either a beneficial response or adverse response. The term predictive test is often used to refer to response to therapy. To simplify terms, we use prognostic to refer both to predicting a future condition and to predicting a response to therapy.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and

unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).
For LGMD, clinical validity may refer to the overall yield of testing for any LGMD-associated variant in patients with the clinically suspected disease, or the testing yield for specific variants. The genetic test is generally considered the criterion standard for determining a specific LGMD subtype

Unselected LGMD Populations
One potential role for genetic testing in LGMD is among patients with clinically suspected LGMD, but who do not necessarily have results of a muscle biopsy available.

In 2014, the American Academy of Neurology (AAN) published an evidence-based guideline on the diagnosis and treatment of limb-girdle and distal dystrophies, which included a systematic review of studies that addressed the yield of genetic testing for LGMD in patients who present with suspected MD. The types of studies available, and the study size and population included (if described), are summarized in Table 3.

Table 3: Genetic Testing Yield in Patients With Suspected LGMD

LGMD Type	Involved Protein	Evidence Summary	Population	Summary of Variant Detection Frequency
LGMD1A	Myotilin	1 Class I study	1105 patients with genetic muscle disorders; 68 with LGMD	No myotilin variants among patients with LGMD
		3 Class III studies	Not described	<1% to 1.7%
LGMD1B	Lamin A/C	1 Class I study	1105 patients with genetic muscle disorders; 68 with LGMD	8.8% of all muscle disorder cases
		9 Class III studies	Patients with clinical LGMD	0.9%-4%
LGMD1C	Caveolin-3	3 Class III studies	Not described	1.3%-2.6%
LGMD2A	Calpain-3	2 Class I studies	1105 patients with genetic muscle disorders; 68 with LGMD	26.5% of all LGMD cases
			84 patients with unknown MD	46.4%
		19 Class III studies	Not described	6%-57%; majority of series reporting 18.5%-35%
LGMD2B	Dysferlin	1 Class I study	1105 patients with genetic muscle disorders; 68 with LGMD	5.9% of LGMD cases

		11 Class III studies	Not described	0.6%-33% of LGMD
LGMD2C	ɣ-sarcoglycan	2 Class I studies	1105 patients with genetic muscle disorders; 68 with LGMD	5.9% of all muscle disorder cases
			204 patients with dystrophy on muscle biopsy and normal dystrophin	2%
		16 Class III studies	Not described	1.3%-13.2%
	α-sarcoglycan	2 Class I studies	1105 patients with genetic muscle disorders; 68 with LGMD	0.07 per 100,000
			204 patients with dystrophy on muscle biopsy and normal dystrophin	3.4%
		14 Class III studies	Not described	3.3%-15%
	β-sarcoglycan	2 Class I studies	1105 patients with genetic muscle disorders; 68 with LGMD	2.9% of all muscle disorder cases
			204 patients with dystrophy on muscle biopsy and normal dystrophin	1%
		13 Class III studies	Not described	0%-23%
	δ-sarcoglycan	2 Class I studies	1105 patients with genetic muscle disorders; 68 with LGMD	None
			204 patients with dystrophy on muscle biopsy and normal dystrophin	None
		12 Class III studies	Not described	0%-14%
LGMD2G	Telethonin	2 Class III studies	63 patients with myofibrillar myopathy	None
			140 patients with LGMD from 40 families	4.2%
LGMD2I	Fukutin-related protein	1 Class I study	1105 patients with genetic muscle disorders; 68 with LGMD	19.1% of LGMD cases
		1 Class II study	102 patients with persistent hyper-CK-emia	5.1%
		12 Class III studies	Not described	4%-30%
LGMD2J	Titin	1 Class III study	25 families and 25	16% of familial

			sporadic cases; primarily distal myopathy	cases; none in sporadic cases
LGMD2K	POMT1	1 Class III study	92 patients with evidence of dystroglycanopathy on muscle biopsy and negative *FKRP* variant testing	8%
LGMD2L	Anoctamin-5	2 Class III studies	64 patients with LGMD or Miyoshi myopathy without dysferlin variants	31.3%
			101 patients with undetermined LGMD, distal myopathy, or elevated CK	24.8%
LGMD2M	Fukutin	1 Class III study	92 patients with evidence of dystroglycanopathy on muscle biopsy and negative *FKRP* variant testing	6.5%
LGMD2N	POMT2	1 Class III study	92 patients with evidence of dystroglycanopathy on muscle biopsy and negative *FKRP* variant testing	9.7%
LGMD2O	POMGNT1	1 Class III study	92 patients with evidence of dystroglycanopathy on muscle biopsy and negative *FKRP* variant testing	7.6%

CK: creatine kinase; LGMD: limb-girdle muscular dystrophy; MD: muscular dystrophy. (Adapted From Narayanaswami et al)
a Class I studies include statistical, population-based samples of patients studied at a uniform point in time (usually early) during the course of the condition, with all patients undergoing the intervention of interest, and with outcomes determined in an evaluation that is masked to patients' clinical presentations. Class II studies are similar to class I, but the patient population is a non-referral-clinic-based sample, and most, not all, patients undergo the intervention of interest. Class III studies include samples of patients studied during the course of the condition, some of whom undergo the intervention of interest, and in whom the outcome is determined by someone other than the treating physician.

The studies included in the AAN systematic review of the prevalence of various variants in various populations were heterogeneous in terms of the patient population used. Some of the representative studies are described in more detail next.

In 2009, Norwood et al reported the prevalence of genetic variants in a large, single-clinic population of patients with genetic muscle disorders (included in the AAN systematic review). The population included 1105 cases with a variety of inherited muscle diseases diagnosed and treated by at a single neuromuscular clinic, which was considered to be the only neuromuscular disorders referral center for northern England. Of the total patient population, 75.7% (n=836) had

a confirmed genetic diagnosis. Myotonic dystrophy was the most commonly represented single diagnosis, representing 28.1% of the total sample, while 22.9% had a dystrophinopathy. Sixty-eight patients had a clinical diagnosis of LGMD, of whom 43 (6.15%) had positive genetic testing for a gene known to be associated with LGMD. Of patients with a clinical diagnosis of LGMD, 72.1% had positive genetic testing, most commonly for LGMD2A (26.5%; 95% confidence interval [CI], 16.0% to 37.0%).

Variable Gene Expression

For some LGMD subtypes, there is variable expressivity for a given gene variant, which has been characterized in several retrospective analyses of clinical features for patients with a specific gene variant. Maggi et al (2014) conducted a retrospective cohort study to characterize the clinical phenotypes of myopathic patients (n=78) and non-myopathic patients with LMNA variants (n=78). Of the 78 myopathic patients, 37 (47%) had an LGMD phenotype (LGMD1B), 18 (23%) had congenital muscular dystrophy, 17 (22%) had autosomal dominant Emery-Dreifuss muscular dystrophy, and 6 (8%) had an atypical myopathy. Of the myopathic patients, 54 (69.2%) had cardiac involvement, and 41 (52.6%) underwent implantation of an implantable cardioverter defibrillator (ICD). Among 30 family members without myopathy but with LMNA variants, 20 (66.7%) had cardiac involvement, and 35% underwent ICD implantation. Among all patients, frameshift variants were associated with a higher risk of heart involvement.

Sarkozy et al (2013) evaluated the prevalence of *ANO5* variants and associated clinical features among 205 patients without a genetic diagnosis but with a clinical suspicion of *ANO5* variant, or LGMD2L, who were evaluated a single European center. A clinical suspicion of *ANO5* variant (anoctaminopathy) could be based on clinical examination, muscle assessment, and clinical evaluations including CK analysis, electromyography, muscle magnetic resonance imaging (MRI), and/or muscle biopsy. *ANO5* gene sequence variants were identified in 90 unrelated individuals (44%) and 5 affected relatives. Sixty-one percent of variants were a c.191dupA variant, which is a founder variant found in most British and German LGMD2L patients. Age of onset was variable, ranging from teens to late 70s, with lower-limb predominance of symptoms. Three individuals with *ANO5* variants had very mild clinical disease, and 1 patient was asymptomatic, but no specific genotype-phenotype correlations were demonstrated.

Panel Testing

Ghosh et al (2012) described the yield of a LGMD panel, which included testing for genes associated with lamin A/C (LGMD1B), caveolin-3 (LGMD1C), calpain-3 (LGMD2A), dysferlin (LGMD2B), the sarcoglycans (LGMD2C-2F), and Fukutin-related protein (LGMD2I), among 27 patients with a clinical suspicion of LGMD seen at a single center. Ten patients (37%) had positive testing, most commonly for LGMD2A (n=4). The yield of testing was higher among children (3/6 [50%] patients tested), although the sample was limited by a small number of children.

LGMD Patients with Muscle Biopsy Results

A smaller number of studies have evaluated the yield of genetic variant testing for LGMD in patients who are suspected of having one particular LGMD subtype on the basis of muscle biopsy.

In 2009, Fanin et al evaluated the yield of molecular diagnostics among 550 cases with specific LGMD-related phenotypes, including severe childhood-onset LGMD, adult-onset LGMD, distoproximal myopathy, and asymptomatic hyper-CK-emia, who had undergone muscle biopsy with multiple protein screening. Patients had all had exclusion of recent physical exercise or toxic or endocrinologic causes of myopathy before muscle biopsy. Dystrophinopathy was excluded in all cases. Muscle biopsy samples underwent a systematic evaluation of calpain-3 (for LGMD2A), dysferlin (for LGMD2B), and α-sarcoglycan (for LGMD2D) by immunoblotting and of caveolin-3 (for LGMD1C) by immunohistochemistry. Calpain-3 autolytic activity was also evaluated by a functional in vitro assay. Genetic testing of *DYSF*, *CAPN3*, sarcoglycans, *FKRP*, and *LMNA* was conducted single-strand conformational polymorphism (SSCP) or denaturing high performance liquid chromatography (DHPLC) analysis, which are older methods of gene variant analysis. Of the 550 cases with muscle biopsies, 122 had childhood-onset LGMD, 186 had adult-onset LGMD, 38 had distoproximal myopathy, and 204 had asymptomatic hyper-CK-emia. In the entire cohort, a molecular diagnosis (positive genetic testing) was made in 234 cases (42.5%), most commonly a calpain-3 variant, consistent with LGMD2A. Excluding patients with asymptomatic hyper-CK-emia, a molecular diagnosis was made in 205 cases (59.2% of 346 with LGMD phenotype). Patients with childhood-onset LGMD were more likely to have a molecular diagnosis (94/122 [77.0%]). Of the 226 patients with a protein abnormality on muscle biopsy, 193 (85.4%) had a genetic diagnosis.

In an earlier, smaller study, Guglieri et al (2008) reported results from molecular diagnostic testing on a series of 181 patients (155 families) with clinical signs of LGMD and muscle biopsy with dystrophic features. The yield of genetic testing varied by muscle biopsy protein (Western blotting and immunohistochemistry) findings: among 72 subjects with calpain-3 deficiency on protein testing, the variant detection rate was 61%, compared with 93.5% of the 31 subjects with dysferlin deficiency, 87% (for any sarcoglycan gene variant) of the 32 subjects with sarcoglycan deficiency, and 1005 of the 52 subjects with caveolin-3 deficiency. The frequency of LGMD subtypes was as follows: LGMD1C (caveolin-3) 1.3%; LGMD2A (calpain-3) 28.4%; LGMD2B (dysferlin) 18.7%; LGMD2C (□-sarcoglycan) 4.5%; LGMD2D (α-sarcoglycan) 8.4%; LGMD2E (β-sarcoglycan) 4.5%; LGMD2F (δ-sarcoglycan) 0.7%; LGMD2I (Fukutin-related protein) 6.4%; and undetermined 27.1%.

In another smaller study, Fanin et al (2009) reported rates of sarcoglycan gene variants among 18 subjects with muscular dystrophy and α-sarcoglycan deficiency on immunohistochemistry and immunoblotting of muscle biopsy samples. Pathogenic variants in one gene involved in the sarcoglycan complex were identified in 13 patients.

Krahn et al (2009) evaluated the yield of testing for *DYSF* variant in a cohort of 134 patients who had a clinical phenotype consistent with LGMD2B, loss or strong reduction of dysferlin protein expression on muscle biopsy Western blot and/or immunohistochemistry, or both. *DYSF* variants known to be associated with myopathy were detected in 89 (66%) patients. Bartoli et al (2014) reported results of whole exome sequencing in a follow up analysis of 37 patients who had negative targeted *DYSF* variant testing. In five (13.5%) cases, molecular diagnosis could be made directly by identification of compound heterozygous or homozygous variants previously associated with LGMD on whole exome sequencing, including two *CAPN3* variants, one *ANO5*

variant, one *GNE* variant, and one *DYSF* variant, with one additional case requiring additional Sanger sequencing for complete identification.

Section Summary: Clinically Valid
Estimates of the yield of genetic testing for variants associated with LGMD vary depending on the variants included and the characteristics of the patient populations tested. The true clinical sensitivity and specificity of genetic testing for LGMD variants in general cannot be determined, because there is no criterion standard test for diagnosing LGMD. Studies report a yield of genetic testing from 37% to greater than 70% in patients with clinically suspected LGMD. The criterion standard for diagnosing a LGMD subtype is the genetic test. The specificity of a positive LGMD genetic test result in predicting the clinical phenotype of LGMD is not well-defined. However, there is some evidence to support that some variants associated with LGMD predict the presence of cardiac complications.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

No randomized controlled trials were identified addressing the clinical utility of managing patients with genetic testing. In the absence of direct evidence of clinical utility, a chain of evidence must be assessed to determine the potential clinical utility of a test.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

The clinical utility of testing for variants associated with LGMD for an index case (a patient with clinically suspected LGMD) includes:

- Confirming the diagnosis of LGMD and initiating/directing treatment of the disease, including evaluation by a cardiologist/cardiac testing, respiratory function testing/monitoring, and prevention of secondary complications (e.g., through immunizations, physical therapy/bracing, fracture risk reduction).
- Avoidance of treatments that might be initiated for other neuromuscular disorders not known to be efficacious for LGMD, such as glucocorticoids for suspected dystrophinopathy or immunosuppressants for suspected myositis.
- Potential discontinuation of routine cardiac and respiratory surveillance in patients who have an identified variant not known to be associated with cardiac or respiratory dysfunction.

- Potential avoidance of invasive testing (e.g., muscle biopsy).
- Reproductive planning.

The clinical utility of testing for variants associated with LGMD for an at-risk family member (i.e., first- or second-degree relative of a proband) includes:

- Confirming or excluding the need for cardiac surveillance.
- Reproductive planning in individuals considering offspring who would alter reproductive decision making based on test results.

Management of Cardiac Complications
Similar to Duchenne and Becker muscular dystrophies, patients with LGMD are at higher risk of cardiac abnormalities, including dilated cardiomyopathy and various arrhythmias. Specific LGMD subtypes are more likely to be associated with cardiac disorders. Potential device-based therapies for patients at risk of arrhythmias include cardiac pacing and implantation of an implantable cardioverter-defibrillator. Guidelines from the American College of Cardiology/American Heart Association regarding the use of device-based therapy of cardiac rhythm abnormalities published in 2008 recommend that indications for a permanent pacemaker address the presence of MD. These guidelines recommend the consideration of implantation of a permanent pacemaker for patients with LGMD with any degree of atrioventricular (AV) block (Class IIb recommendation; level of evidence: B), or bifascicular block or any fascicular block (Class IIb recommendation; level of evidence: C), with or without symptoms, because there may be unpredictable progression of AV conduction disease.

Certain LGMD subtypes are more strongly associated with cardiac disorders than others. LGMD Types 2C-2F and 2I are associated with a primary dilated cardiomyopathy, with conduction disorders occurring as a secondary phenomenon. In contrast, some LGMD subtypes are recognized to not have associations with cardiomyopathy or conduction disorders. In these cases, recommendations from AAN indicate that routine cardiac surveillance in asymptomatic individuals is not required.

There is clinical utility for identifying a specific LGMD gene variant for patients presenting with signs/symptoms of LGMD to allow discontinuation of cardiac surveillance in patients who are found to have a variant not associated with cardiac disorders.

On the other hand, there may be clinical utility for testing of asymptomatic family members of a proband with an identified LGMD variant to determine cardiovascular risk. Patients with *LMNA* variants, regardless of whether they have an *LGMD1B* phenotype, are at risk for cardiac arrhythmias. Similarly, *FKTN* variants can be associated with dilated cardiomyopathy, with or without the presence of myopathy. Murakami et al reported a cases series of six patients from four families with compound heterozygous *FKTN* variants who presented with dilated cardiomyopathy and no or minimal myopathic symptoms.

Section Summary: Clinically Useful
In patients with clinically suspected LGMD, genetic testing is primarily to confirm a diagnosis, but may also have a prognostic role given the clinical variability across LGMD subtypes. For asymptomatic but at-risk family members, testing may also confirm a diagnosis or allow prediction of symptoms. No direct evidence exists of the impact of testing on outcomes. However, a chain of evidence suggests that the establishment of a specific genetic diagnosis has the potential to change clinical management.

Targeted Testing of Asymptomatic Individuals with Relatives with LGMD and a Known Familial Variant

Clinical Context and Test Purpose
The purpose of genetic testing an asymptomatic individual with first- and second-degree relatives with of asymptomatic relatives of patients with LGMD is to determine carrier or genetic status to confirm or exclude the need for cardiac surveillance and inform the reproductive planning process.

The question addressed in this evidence review is: In individuals with suspected LGMD, does use of genetic testing result lead to reductions in unnecessary cardiac surveillance and lead changes in reproductive planning?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest includes asymptomatic first- and second-degree relatives of a patient with LGMD and a known familial variant.

Interventions
Targeted familial variant testing.

Comparators
Standard diagnostic workup without genetic testing.

Outcomes
The potential beneficial outcome of primary interest would be confirming or excluding the need for cardiac surveillance based on LGMD subtype and changes in reproductive planning.

Setting
The time frame for outcomes measures varies from short-term changes in development of symptoms, disease status or changes in cardiac function to long-term improvements in outcomes or changes in reproductive decision making.

In asymptomatic individuals, evaluation may occur in pediatrics, primary care or neurology due to the variability in clinical presentation and age of onset. Genetic testing is utilized to confirm a genetic status of a known familial variant. If the known familial variant is detected, referral to cardiology is important to initiate cardiac surveillance if the specific LGMD subtype is associated with the development of cardiac symptoms. Referral for genetic counseling is

important for explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).
See the discussion of clinical validity in the Testing Individuals With Signs or Symptoms of LGMD section above.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

No randomized controlled trials were identified addressing the clinical utility of managing patients with genetic testing. In the absence of direct evidence of clinical utility, a chain of evidence must be assessed to determine the potential clinical utility of a test.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Genetic testing of asymptomatic individuals with family members with LGMD may have clinical utility in:

- Confirming or excluding the need for cardiac surveillance based on the presence or absences of a known familial variant.
- Informing the reproductive decision making process for preimplantation testing and/or prenatal (in utero) testing when a known familial variant is present in a parent. Preimplantation testing is addressed elsewhere (*see Preimplantation Genetic Testing Policy 593).*

Section Summary: Targeted Testing of Asymptomatic Individuals With Relatives With LGMD and a Known Familial Variant
For individuals who are asymptomatic with a first- or second-degree relative with LGMD and a known familial variant who are tested for targeted familial variants, the evidence is limited. Data

on the clinical validity for testing for a known familial variant are lacking but validity is expected to be high. Direct evidence on the clinical utility of LGMD-associated familial variant testing in asymptomatic relatives is lacking. However, the chain of evidence is strong, because determination of carrier status for an LGMD familial variant necessitates or eliminates the need for routine cardiac surveillance and can indicate the likelihood of an affected offspring in women considering children.

Testing of Asymptomatic Individuals with Relatives with LGMD and Unknown Genetic Status

Clinical Context and Test Purpose

The purpose of genetic testing of asymptomatic individuals with first- and second-degree who have LGMD and an unknown genetic status is to determine carrier or genetic status relatives of patients with LGMD is to determine carrier or genetic status to confirm or exclude the need for cardiac surveillance and inform the reproductive planning process.

The question addressed in this evidence review is: In individuals with suspected LGMD, does use of genetic testing result lead to reductions in unnecessary cardiac surveillance and lead changes in reproductive planning?

The following PICOTS were used to select literature to inform this review.

Patients

The relevant population of interest is asymptomatic patients with first- and second-degree relatives who have LGMD whose genetic status is unknown.

Interventions

Genetic testing for genes associated with LGMD.

Comparators

Standard diagnostic workup without genetic testing.

Outcomes

The potential beneficial outcome of primary interest would be confirming or excluding the need for cardiac surveillance based on LGMD subtype and changes in reproductive planning.

Timing

The time frame for outcomes measures varies from short-term changes in development of symptoms, disease status or changes in cardiac function to long-term improvements in outcomes or changes in reproductive decision making.

Setting

In asymptomatic individuals, evaluation may occur in pediatrics, primary care or neurology due to the variability in clinical presentation and age of onset. Genetic testing is utilized to confirm a genetic status of a pathogenic variant in a LGMD-associated gene. If the pathogenic variant in a LGMD-associated gene is detected, referral to cardiology is important to initiate cardiac surveillance if the specific LGMD subtype is associated with the development of cardiac

symptoms. Referral for genetic counseling is important for explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

See the discussion of clinical validity in the Testing Individuals with Signs or Symptoms of LGMD section above.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

No randomized controlled trials were identified addressing the clinical utility of managing patients with genetic testing. In the absence of direct evidence of clinical utility, a chain of evidence must be assessed to determine the potential clinical utility of a test.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Genetic testing of asymptomatic individuals with family members with LGMD may have clinical utility in:

- Confirming or excluding the need for cardiac surveillance based on the presence or absence of a pathogenic variant in a LGMD-associated gene.
- Informing the reproductive decision making process for preimplantation testing and/or prenatal (in utero) testing when a known familial variant is present in a parent. Preimplantation testing is addressed elsewhere (*see Preimplantation Genetic Testing Policy 593).*

Section Summary: Testing of Asymptomatic Individuals with Relatives with LGMD and unknown Genetic Status
For individuals who are asymptomatic and have a first- or second-degree relative with LGMD whose genetic status is unknown who are given genetic testing for LGMD-associated genes, the evidence is limited. Data for the clinical validity of testing for a known familial variant are lacking but validity is expected to be high. Direct evidence on the clinical utility of genetic testing for LGMD-associated genes in asymptomatic relatives is lacking. However, the chain of evidence is strong, because determination of carrier status for an LGMD pathogenic variant necessitates or eliminates the need for routine cardiac surveillance and can indicate the likelihood of an affected offspring in women considering children.

Summary of Evidence
For individuals who have signs or symptoms of a limb-girdle muscular dystrophy (LGMD) who receive genetic testing for LGMD-associated genes, the evidence includes systematic reviews, case series and genotype-phenotype correlations evaluating the clinical validity and yield of genetic testing. Relevant outcomes are overall survival, test accuracy and validity, changes in reproductive decision making, change in disease status, and morbid events. The true clinical sensitivity and specificity of genetic testing for LGMD in general cannot be determined. While the yield of genetic testing in patients with clinically suspected LGMD varies depending on the population characteristics (i.e., patients with only clinical symptoms vs patients with biopsy findings suggestive of LGMD), the available body of evidence suggests that the yield of testing is reasonably high. Genetic testing is generally considered the criterion standard for diagnosis of a specific LGMD subtypes. For patients with clinically suspected LGMD, there is clinical utility in genetic testing to confirm a diagnosis of LGMD and direct treatment and monitoring on the basis of a specific genetic diagnosis (including discontinuation of routine cardiac and/or respiratory surveillance if a specific genetic diagnosis not associated with these complications can be made), avoid therapies not known to be efficacious for LGMD, and potentially avoid invasive testing. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

For individuals who are asymptomatic with a first- or second-degree relative with a known LGMD pathogenic variant who receive targeted genetic testing for the known LGMD familial variant, the evidence is limited. Relevant outcomes are overall survival, test accuracy and validity, changes in reproductive decision making, change in disease status, and morbid events. Published data for the clinical validity for testing for a known familial variant are lacking, but is expected to be high. Direct evidence on the clinical utility of LGMD-associated familial variant testing in asymptomatic relatives is lacking. However, the chain of evidence is strong, because determination of carrier status for a LGMD familial variant necessitates or eliminates the need for routine cardiac surveillance. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are asymptomatic with family members with LGMD whose genetic status is unknown who receive genetic testing for LGMD-associated genes, the evidence is limited. Relevant outcomes are overall survival, test accuracy and validity, changes in reproductive decision making, change in disease status, and morbid events. Published data for the clinical validity for testing for a known familial variant are lacking, but is expected to be high. Direct

evidence on the clinical utility of genetic testing for LGMD-associated genes in asymptomatic relatives is lacking. However, the chain of evidence is strong, because determination of carrier status for a LGMD pathogenic variant necessitates or eliminates the need for routine cardiac surveillance. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

Practice Guidelines and Position Statements

Table 4. AAN and AANEM Guidelines for LGMDs

Recommendations	LOR
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)	B
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	C
Management of cardiac complications in LGMD	
Clinicians should refer newly diagnosed patients with (1) LGMD1A, LGMD1B, LGMD1D, LGMD1E, LGMD2C–K, LGMD2M–P or (2) muscular dystrophy without a specific genetic diagnosis for cardiology evaluation, including ECG and structural evaluation (echocardiography or cardiac MRI), even if they are asymptomatic from a cardiac standpoint, to guide appropriate management.	B
If ECG or structural cardiac evaluation (e.g., echocardiography) has abnormal results, or if the patient has episodes of syncope, near-syncope, or palpitations, clinicians should order rhythm evaluation (e.g., Holter monitor or event monitor) to guide appropriate management	B
Clinicians should refer muscular dystrophy patients with palpitations, symptomatic or asymptomatic tachycardia or arrhythmias, or signs and symptoms of cardiac failure for cardiology evaluation	B
It is not obligatory for clinicians to refer patients with LGMD2A, LGMD2B, and LGMD2L for cardiac evaluation unless they develop overt cardiac signs or symptoms	B
Management of respiratory complications in LGMD	
Clinicians should order pulmonary function testing (spirometry and maximal inspiratory/ expiratory force in the upright and, if normal, supine positions) or refer for pulmonary evaluation (to identify and treat respiratory insufficiency) in muscular dystrophy patients at the time of diagnosis, or if they develop pulmonary symptoms later in their course.	B
In patients with a known high risk of respiratory failure (e.g., those with LGMD2I), clinicians should obtain periodic pulmonary function testing (spirometry and maximal inspiratory/expiratory force in the upright position and, if normal, in the supine position) or evaluation by a pulmonologist to identify and treat respiratory insufficiency.	B
It is not obligatory for clinicians to refer patients with LGMD2B and LGMD2L for pulmonary evaluation unless they are symptomatic.	C
Clinicians should refer muscular dystrophy patients with excessive daytime somnolence, nonrestorative sleep (e.g., frequent nocturnal arousals, morning headaches, excessive daytime fatigue), or respiratory insufficiency based on pulmonary function tests for pulmonary or sleep medicine consultation for consideration of noninvasive ventilation to improve quality of life.	B

AAN: American Academy of Neurology; AANEM: American Association of Neuromuscular and Electrodiagnostic Medicine; ECG: electrocardiogram; LGMD: limb-girdle muscular dystrophies; LOR: level of recommendation.

U.S. Preventive Services Task Force Recommendations

Not applicable

Key Words:
Limb-Girdle Muscular Dystrophies, (LGMDs)

Approved by Governing Bodies:
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Tests from laboratories such as GeneDx, Prevention Genetics, Centogene, Counsyl, and Athena Diagnostics are offered under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

Benefit Application:
Coverage is subject to member's specific benefits. Group specific policy will supersede this policy when applicable.
ITS: Home Policy provisions apply.
FEP: Special benefit consideration may apply. Refer to member's benefit plan. FEP does not consider investigational if FDA approved and will be reviewed for medical necessity.

Current Coding:
CPT Codes:
There are no specific CPT codes for this testing. Several of these tests can be reported with Tier 2 CPT codes.

81400	Molecular pathology procedure, Level 1 (e.g., identification of single germline variant [e.g., SNP] by techniques such as restriction enzyme digestion or melt curve analysis) – includes *FKTN* retrotransposon insertion variant
81404	Molecular pathology procedure, Level 5 (e.g., analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis) - includes *CAV3, FKRP*, and *SGCG* duplication/deletion
81405	Molecular pathology procedure, Level 6 (e.g., analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis) - includes *DES, ISPD, MYOT, SGCA, SGCB*, *SGCD*, and full gene sequencing of *FKTN*
81406	Molecular pathology procedure, Level 7 (e.g., analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array

	analysis for neoplasia) - includes *ANO5, CAPN3, GAA, LMNA, POMGnT1, POMT1,* and *POMT2*
81408	Molecular pathology procedure, Level 9 (e.g., analysis of >50 exons in a single gene by DNA sequence analysis) ABCA4 (ATP-binding cassette, sub-family A [ABC1], member 4) (e.g., Stargardt disease, age-rel) - includes *DYSF*

Tests that are not specifically codified in the CPT codes would be reported with the unlisted molecular pathology code 81479.

References:

1. Ankala A, Nallamilli BR, Rufibach LE, et al. Diagnostic overview of blood-based dysferlin protein assay for dysferlinopathies. Muscle Nerve. Sep 2014; 50(3):333-339.
2. Bartoli M, Desvignes JP, Nicolas L, et al. Exome sequencing as a second-tier diagnostic approach for clinically suspected dysferlinopathy patients. Muscle Nerve. Dec 2014; 50(6):1007-1010.
3. Centogene. Factsheet: LGMD Panel. 2015; www.centogene.com/centogene/inc/testCatalogueDetail/factsheet/5030_Limb-girdle_muscular_dystrophy_panel_V1.pdf. Accessed April 29, 2015.
4. Epstein AE, DiMarco JP, Ellenbogen KA, et al. ACC/AHA/HRS 2008 Guidelines for Device-Based Therapy of Cardiac Rhythm AbnormalitiesA Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the ACC/AHA/NASPE 2002 Guideline Update for Implantation of Cardiac Pacemakers and Antiarrhythmia Devices) Developed in collaboration with the American Association for Thoracic Surgery and Society of Thoracic Surgeons. J Am Coll Cardiol. 2008; 51(21):e1-e62.
5. Fanin M, Duggan DJ, Mostacciuolo ML, et al. Genetic epidemiology of muscular dystrophies resulting from sarcoglycan gene mutations. J Med Genet. Dec 1997; 34(12):973-977.
6. Fanin M, Nascimbeni AC, Aurino S, et al. Frequency of LGMD gene mutations in Italian patients with distinct clinical phenotypes. Neurology. Apr 21 2009; 72(16):1432-1435.
7. Finsterer J, Stollberger C, Keller H. Arrhythmia-related workup in hereditary myopathies. J Electrocardiol. Jul-Aug 2012; 45(4):376-384.
8. GeneDx. Information Sheet on Limb Girdle Muscular Dystrophy Panel 2014; www.genedx.com/wp-content/uploads/2014/12/info_sheet_LGMD.pdf. Accessed April 29, 2015.
9. Ghosh PS, Zhou L. The diagnostic utility of a commercial limb-girdle muscular dystrophy gene test panel. J Clin Neuromuscul Dis. Dec 2012; 14(2):86-87.
10. Groh WJ. Arrhythmias in the muscular dystrophies. Heart Rhythm. Nov 2012; 9(11):1890-1895.
11. Guglieri M, Magri F, D'Angelo MG, et al. Clinical, molecular, and protein correlations in a large sample of genetically diagnosed Italian limb girdle muscular dystrophy patients. Hum Mutat. Feb 2008; 29(2):258-266.

12. Herson S, Hentati F, Rigolet A, et al. A phase I trial of adeno-associated virus serotype 1-gamma-sarcoglycan gene therapy for limb girdle muscular dystrophy type 2C. Brain. Feb 2012; 135(Pt 2):483-492.
13. Krahn M, Beroud C, Labelle V, et al. Analysis of the DYSF mutational spectrum in a large cohort of patients. Hum Mutat. Feb 2009; 30(2):E345-375.
14. Limb-Girdle Muscular Dystrophy Sequencing Panel; GTR test ID GTR000506322.3. GTR: Genetic Testing Registry www.ncbi.nlm.nih.gov/gtr/tests/506322/overview/. Accessed April 28, 2015.
15. Maggi L, D'Amico A, Pini A, et al. LMNA-associated myopathies: the Italian experience in a large cohort of patients. Neurology. Oct 28 2014; 83(18):1634-1644.
16. Mahmood OA, Jiang XM. Limb-girdle muscular dystrophies: where next after six decades from the first proposal (Review). Mol Med Rep. May 2014; 9(5):1515-1532.
17. Menezes MP, Waddell LB, Evesson FJ, et al. Importance and challenge of making an early diagnosis in LMNA-related muscular dystrophy. Neurology. Apr 17 2012; 78(16):1258-1263.
18. Moore SA, Shilling CJ, Westra S, et al. Limb-girdle muscular dystrophy in the United States. J Neuropathol Exp Neurol. Oct 2006; 65(10):995-1003.
19. Murakami T, Hayashi YK, Noguchi S, et al. Fukutin gene mutations cause dilated cardiomyopathy with minimal muscle weakness. Ann Neurol. Nov 2006; 60(5):597-602.
20. Narayanaswami P, Weiss M, Selcen D, et al. Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology. Oct 14 2014; 83(16):1453-1463.
21. Nigro V, Aurino S, Piluso G. Limb girdle muscular dystrophies: update on genetic diagnosis and therapeutic approaches. Curr Opin Neurol. Oct 2011; 24(5):429-436.
22. Nigro V, Savarese M. Genetic basis of limb-girdle muscular dystrophies: the 2014 update. Acta Myol. May 2014; 33(1):1-12.
23. Norwood F, de Visser M, Eymard B, et al. EFNS guideline on diagnosis and management of limb girdle muscular dystrophies. Eur J Neurol. Dec 2007; 14(12):1305-1312.
24. Norwood FL, Harling C, Chinnery PF, et al. Prevalence of genetic muscle disease in Northern England: in-depth analysis of a muscle clinic population. Brain. Nov 2009; 132(Pt 11):3175-3186.
25. Pegoraro E, Hoffman EP. Limb-Gridle Muscular Dystrophy Overview. In: Pagon RA, Adam MP, Ardinger HH, eds. GeneReviews. Seattle, WA: National Library of Medicine, NCBI Bookshelf; 2012.
26. Piluso G, Dionisi M, Del Vecchio Blanco F, et al. Motor chip: a comparative genomic hybridization microarray for copy-number mutations in 245 neuromuscular disorders. Clin Chem. Nov 2011; 57(11):1584-1596.
27. Prevention Genetics. Autosomal Dominant Limb Girdle Muscular Dystrophy (LGMD) Sanger Sequencing Panel. 2014; www.preventiongenetics.com/clinical-dna-testing/test/autosomal-dominant-limb-girdle-muscular-dystrophy-lgmd-sanger-sequencing-panel/1035/#method_1. Accessed April 29, 2015.
28. Prevention Genetics. Autosomal Recessive Limb Girdle Muscular Dystrophy (LGMD) Sanger Sequencing Panel. 2014; www.preventiongenetics.com/clinical-dna-

testing/test/autosomal-recessive-limb-girdle-muscular-dystrophy-lgmd-sanger-sequencing-panel/1050/. Accessed April 29, 2015.
29. Rocha CT, Hoffman EP. Limb-girdle and congenital muscular dystrophies: current diagnostics, management, and emerging technologies. Curr Neurol Neurosci Rep. Jul 2010; 10(4):267-276.
30. Sarkozy A, Hicks D, Hudson J, et al. ANO5 gene analysis in a large cohort of patients with anoctaminopathy: confirmation of male prevalence and high occurrence of the common exon 5 gene mutation. Hum Mutat. Aug 2013; 34(8):1111-1118.
31. Sharma A, Sane H, Badhe P, et al. A clinical study shows safety and efficacy of autologous bone marrow mononuclear cell therapy to improve quality of life in muscular dystrophy patients. Cell Transplant. 2013; 22 Suppl 1:S127-138.
32. Stramare R, Beltrame V, Dal Borgo R, et al. MRI in the assessment of muscular pathology: a comparison between limb-girdle muscular dystrophies, hyaline body myopathies and myotonic dystrophies. Radiol Med. Jun 2010; 115(4):585-599.
33. ten Dam L, van der Kooi AJ, van Wattingen M, et al. Reliability and accuracy of skeletal muscle imaging in limb-girdle muscular dystrophies. Neurology. Oct 16 2012; 79(16):1716-1723.
34. Walter MC, Reilich P, Thiele S, et al. Treatment of dysferlinopathy with deflazacort: a double-blind, placebo-controlled clinical trial. Orphanet J Rare Dis. 2013; 8:26.

Policy History:

Medical Policy Panel, April 2017
Medical Policy Group, June 2017 **(3):** Newly Adopted Policy
Medical Policy Administration Committee, June 2017
Available for comment June 16 through July 30, 2017
Medical Policy Panel, April 2018
Medical Policy Group, May 2018 **(6)**: Updates to Key Points.