

Test Specific Guidelines

Limb-Girdle Muscular Dystrophy Genetic Testing

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Introduction

Genetic testing for limb-girdle muscular dystrophy is addressed by this guideline.

Procedures Addressed

The inclusion of any procedure code in this table is provided for informational purposes and is not a guarantee of coverage nor an indication that prior authorization is required.

<u>Procedure addressed by this guideline</u>	<u>Procedure code</u>
<u>LGMD Gene Analysis</u>	<u>81400</u>
	<u>81401</u>
	<u>81402</u>
	<u>81403</u>
	<u>81404</u>
	<u>81405</u>
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<u>LGMD Known Familial Mutation Analysis</u>	<u>81403</u>
<u>LGMD Multigene Panel</u>	<u>81479</u>
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What Is Limb-Girdle Muscular Dystrophy?

Definition

Limb-girdle muscular dystrophy (LGMD) is a rare, inherited, heterogeneous group

of over 30 myopathies with predominant involvement of the proximal musculature.¹ They are typically progressive myopathies characterized by weakness and atrophy of muscle without primary involvement of the nervous system or neurogenic atrophy. The LGMDs are classified into groups, based on inheritance pattern. Historically, these were denoted as LGMD1 (autosomal dominant) and LGMD2 (autosomal recessive). In 2018, the European Neuromuscular Centre published new nomenclature with the types of LGMD denoted as LGMD D (autosomal dominant) and LGMD R (autosomal recessive) with the subtype denoted with a numeral to categorize the order of discovery, and inclusion of the affected protein, if known. 'LGMD unclassified' refers to individuals with symptoms consistent with LGMD but with negative genetic testing.²

Prevalence

Autosomal recessive LGMD is more common, with an overall prevalence of about 1/15,000.³ Dominant forms are comparatively rare, representing 10% of LGMD cases.³ The prevalence of specific LGMD subtypes may differ in certain populations:¹

LGMD R5 (previously known as LGMD2C) is more common in Roma and Tunisian populations,¹

LGMD R1 (previously known as LGMD2A) is more common in Southern European, Eastern European, and British populations⁴, and

LGMD R9 (previously known as LGMD2I) is more common in Northern European populations⁴.

Symptoms

Signs and symptoms typically begin anytime between childhood and adulthood depending on the subtype but are generally not congenital. Symptoms can include the following:

Upper and lower limb weakness, proximal greater than distal weakness

Gait weakness

Foot drop

Cramps

Exercise intolerance

LGMDs are most often non-syndromic and usually limited to skeletal muscle, but not always. For example, certain subtypes involve cardiac and respiratory muscles. The clinical course can range from mild, with relatively normal activity and life span, to severe with rapid onset and progression of disease.³

The muscle atrophy in LGMD is greatest at the shoulder girdle (scapulohumeral) and pelvic girdle (pelvifemoral), although it may progress distally. Bulbar muscles (including facial muscles and oropharyngeal muscles innervated by cranial nerves VII-XII) are relatively spared depending on the subtype of LGMD. This general pattern of girdle muscle weakness as well as onset, progression, and distribution help classify LGMD and its genetic subtypes.

Cause

There are more than 30 genes implicated in LGMD subtypes, which manifest in overlapping and variable clinical presentations.³ The genes identified so far encode muscle proteins within the sarcomere-sarcolemma-sarcoplasm-extracellular-matrix network.⁵

Inheritance

LGMD inheritance is typically autosomal with updated LGMD subtype nomenclature reflecting autosomal dominant inheritance (LGMD D with subtypes designated by a numeral), and autosomal recessive inheritance (LGMD R with subtypes designated by a numeral). This autosomal inheritance pattern helps distinguish LGMD from the more common X-linked dystrophies (Duchenne, Becker and Emery-Dreifuss).^{2,6}

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

Diagnosis

The diagnosis of muscular dystrophies is typically based on clinical phenotype and inheritance pattern.⁵ Although classification schema are becoming more reliant on molecular test results, the 2014 American Academy of Neurology guidelines for LGMD still recommend genetic testing that is directed by clinical assessment.¹

The phenotype must be more consistent with LGMD than other myopathies

Muscle weakness in the proximal limbs and limb girdle (i.e., scapular winging)

Myopathic and not neuropathic symptoms

Sparing of extra-ocular muscles (although eye anomalies are seen in some severe allelic disorders)³

Onset is not congenital

Course is progressive

Biochemical/histological investigation should suggest muscle damage (although findings can be non-specific)⁴

Creatine kinase can be elevated or normal

EMG typically shows myopathic rather than neuropathic changes

Muscle biopsy shows “dystrophic” changes” (degeneration / regeneration of fibers), and immunohistochemical staining may reveal aberrant or absent muscle specific proteins.

Dystrophinopathy and inflammatory myopathy should be excluded

Identification of pathogenic variants in an LGMD-associated gene can confirm a clinical diagnosis of LGMD

Given the expanding number of loci involved in LGMD subtypes, a negative molecular test result does not rule out LGMD. There are more than 50 loci implicated in LGMD subtypes.

When a specific LGMD subtype is clinically favored over another, genetic testing specific to that subgroup is supported over large panels. However, given the number of loci, and phenotypic overlap among the limb girdle muscular dystrophies, panel testing grouped by inheritance pattern is acceptable.

Large deletions in autosomal LGMD related genes are infrequently reported. Therefore, deletion/duplication analysis is done as second tier testing or first tier in some cases to help rule out X linked dystrophies if they are a part of the differential.

Management

There is no cure for LGMD. Treatment is symptom driven and includes weight control, physical therapy, surgery, use of respiratory aids, and cardiology monitoring.¹

Survival

LGMDs have a broad range of severity. Many are life shortening and debilitating.³

Test Information

Introduction

Testing for LGMD disease may include known familial mutation analysis, next generation sequencing, deletion/duplication analysis, and/or multigene panel testing.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Next Generation Sequencing Assay

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in patient management, and/or minimize the chance of finding variants of uncertain clinical significance.

Guidelines and Evidence

Introduction

The following section includes relevant guidelines and evidence pertaining to Limb-girdle muscular dystrophy testing.

American Academy of Neurology and American Association of Neuromuscular and Electrodiagnostic Medicine

The Guideline Development Subcommittee of the American Academy of Neurology (AAN, 2014) and the Practice Issues Review Panel of the American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM, 2014) issued recommendations for the approach to genetic testing in LGMD:¹

Clinically directed genetic testing is recommended (See Table e-2 for reference of clinical features suggestive of LGMD subtypes).

Clinicians should use a clinical phenotype, inheritance pattern, and associated manifestations to guide genetic diagnosis (Level 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 sequencing, or next-generation sequencing to identify the genetic abnormality (Level C)."

Selected Relevant Publications

Studies evaluating diagnostic yield from small and large panels found both number and composition of genes sequenced have a sizeable impact. A 3-fold greater diagnostic pickup rate was seen when the LGMD panel was increased from 11 genes to a more comprehensive panel containing 41 genes (15 - 46%).⁷

Sequencing of 18 LGMD related genes in 35 individuals suspected of having a muscular dystrophy (unknown genetic diagnosis, high CK values and dystrophic changes on muscle biopsy, DMD ruled out prior to study inclusion) was reported.⁸ Pathogenic variants confirmed a LGMD-related molecular etiology in 20 individuals (57.1%). The study population was ascertained through the neurology clinic at the University of Seoul, Korea. Information regarding consanguinity was not stated in the report and may not have been specifically queried in the study.⁸

While some panels are getting so large as to overlap with WES, a comprehensive panel approach has been suggested to be similar or superior to WES.^{7,9,10} One study analyzed 50 families with an LGMD type distribution of muscle weakness.⁹ They showed that after large LGMD panel testing as a first line diagnostic, follow-up WES did not yield further diagnosis. On the other hand, smaller panels would have missed several LGMD related genes.⁹ Weaknesses of this study includes the specialized population investigated and the small sample size, albeit somewhat

large for this rare disease. The population was suspected to be highly consanguineous (in Saudi Arabia) which authors suggest led in part to their 76% diagnostic yield. The authors also analyzed cost, and, despite the large panel size (759 OMIM genes), the actual cost of sequencing with batching was around \$150.00 per sample. This study did not include deletion/duplication analysis. Follow-up analysis after negative large panel testing was carried out with only a small cohort of nine people. Also, the size of the large sequencing panel used approximates the size of the interpretive gene set that a bioinformatician would look at when analyzing results from WES with a myopathic proband.⁹ A large gene panel may also increase the risk of incidental findings or variants of uncertain clinical significance.

A US study of 4656 individuals with clinically suspected LGMD (no prior molecular testing) underwent genetic testing via a 35-gene NGS panel (included LGMD or LGMD-like genes).¹¹ A molecular diagnosis was established in 27% (N=1259). There was a high prevalence of individuals with pathogenic variants in more than one LGMD gene (N=31), raising the question of possible synergistic heterozygosity/digenic/multigenic contribution to disease presentation/progression.

A group in Australia performed exome sequencing (ES) on 60 families with LGMDs and achieved a diagnostic success rate of 45%.¹² All patients had normal dystrophin immunohistochemistry results. In 14 of the 60 families, pathogenic variants were identified in genes typically associated with other forms of inherited myopathy, highlighting the diagnostic challenge with overlapping clinical presentation among individuals with features of LGMD. An international study including 1001 undiagnosed patients from Europe and the Middle East performed exome sequencing, evaluating 429 genes involved in muscle conditions.¹³ In this cohort of patients with limb-girdle weakness, they identified pathogenic or likely pathogenic variants in 87 genes, with a diagnostic yield of 52% of patients.

A US study of 55 families affected by LGMD demonstrated pathogenic variants in 22 families using exome sequencing.⁵ Most of the probands had clinical muscle biopsies, and none of the muscle biopsies led to a genetic diagnosis prior to enrollment. "Among the pathogenic mutations identified in our cohort, six were found in loci not traditionally classified as being associated with LMGD (e.g., DMD, GAA, SMCHD1, VCP, FLNC, and the D4Z4 region of 4q35)", suggesting that gene panels include a broad array of muscle disease genes, beyond just LGMD, particularly given the decreasing use of muscle biopsy in clinical settings.⁵

Given the degree of phenotypic overlap among LGMD subtypes, atypical presentations of non-LGMD myopathies, and variable expressivity of LGMD, panel testing may be superior to a candidate gene approach when multiple LGMD subtypes are being considered.

Criteria

Introduction

Requests for LGMD testing are reviewed using the following clinical criteria.

LGMD Known Familial Mutation Analysis

Genetic Counseling:

Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

Previous Genetic Testing:

No previous genetic testing that would detect the familial mutation, AND

Diagnostic Testing for Symptomatic Individuals:

Known family mutation(s) in LGMD subtype related gene in 1st or 2nd degree biologic relative, OR

Presymptomatic Testing for Asymptomatic Individuals:

Age 18 years or older, and

At increased risk of developing an LGMD phenotype, and

Known family mutation(s) in LGMD subtype related gene in 1st or 2nd degree biologic relative, AND

Rendering laboratory is a qualified provider of services per the Health Plan policy.

LGMD Single Gene Analysis

Genetic Counseling:

Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

Previous Genetic Testing:

No redundant previous LGMD related gene sequencing, and

No known LGMD related gene mutation in family, AND

Diagnostic Testing for Symptomatic Individuals:

Member displays clinical features of LGMD by the following

Muscle weakness and atrophy not secondary to a neurogenic cause in a limb-girdle distribution, and

Member does not have a congenital myopathy, and

EMG does not show evidence of a nerve etiology as the primary cause, OR

Member has had a muscle biopsy and results are consistent with the LGMD subtype for which testing is being requested, AND

Inheritance pattern is consistent with the LGMD subtype for which testing is being requested, AND

The results of the test will directly impact the diagnostic and treatment options that are recommended for the individual, AND

Rendering laboratory is a qualified provider of services per the Health Plan policy.

LGMD Multi-Gene Diagnostic Panels

Genetic Counseling:

Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

Previous Genetic Testing:

No known molecular cause of LGMD (single disease-causing mutation in dominant forms or biallelic disease-causing mutations in recessive forms) in family, and

No mutations or one mutation associated with recessive form of LGMD detected by single gene analysis or different mutation panel than being requested, AND

Diagnostic Testing for Symptomatic Individuals:

Muscle weakness and atrophy not secondary to a neurogenic cause in a limb-girdle distribution, and

Member does not have a congenital myopathy, and

EMG does not show evidence of a nerve etiology as the primary cause, and

Muscle biopsy, if available, shows dystrophic changes (degeneration / regeneration of fibers), and immunohistochemical staining may reveal aberrant or absent muscle specific proteins, AND

Inheritance pattern not suggestive of Duchenne muscular dystrophy or other X-linked muscular dystrophies, AND

The results of the test will directly impact the diagnostic and treatment options that are recommended for the individual, AND

Rendering laboratory is a qualified provider of services per the Health Plan policy

Billing and Reimbursement Considerations:

For a panel to be considered for reimbursement, it must be limited to LGMD-associated genes. Broad neuromuscular panels are not reimbursable.

If the inheritance pattern in the family is evident based on pedigree analysis, a panel specific to the inheritance pattern will be reimbursable; however, panels of all LGMD genes will not.

If a muscle biopsy has been performed with IHC staining, only genes associated with findings will be reimbursable.

When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, the laboratory will be redirected to the appropriate panel code(s).

References

Introduction

This guideline cites the following references.

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