Blueprint Genetics

LGMD and Congenital Muscular Dystrophy Panel

Test code: NE0801

Is ideal for patients with a clinical suspicion of congenital muscular dystrophy or limb-girdle muscular dystrophy. The genes on this panel are included on the Comprehensive Muscular Dystrophy / Myopathy Panel.

Limb-girdle muscular dystrophy (LGMD) is inherited in an autosomal dominant or recessive manner. More than 50 loci have been associated to different forms of LGMD, making accurate diagnosis and genetic counseling a challenge. The *DMD* gene causing Duchenne muscular dystrophy and Becker muscular dystrophy is also included to the panel. We offer two panels for other muscular dystrophies: Emery Dreifuss Muscular Dystrophy Panel and Collagen Type VI-Related Disorders Panel. For differential diagnostics, also Comprehensive Muscular Dystrophy / Myopathy Panel including all of these genes is availabe.

About LGMD and Congenital Muscular Dystrophy

Limb-girdle Muscular Dystrophies (LGMD) are a group of disorders with atrophy and weakness of proximal limb girdle muscles, typically sparing the heart and bulbar muscles. However, cardiac and respiratory impairment may be observed in certain forms of LGMD. In congenital muscular dystrophy (CMD), the muscle weakness typically presents shortly after birth to early infancy. The clinical severity, age of onset, and disease progression are highly variable among the different forms of LGMD/CMD. Phenotypes overlap both within CMD subtypes and among the congenital muscular dystrophies, congenital myopathies, and limb-girdle muscular dystrophies. LGMDs are inherited in an autosomal dominant or recessive manner. More than 50 loci have been associated to different forms of LGMD, making accurate diagnosis and genetic counseling a challenge. Genetic testing aids tremendously in these areas.

Availability

Results in 3-4 weeks

Gene set description

Genes in the LGMD and Congenital Muscular Dystrophy Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
ANO5	Gnathodiaphyseal dysplasia, LGMD2L and distal MMD3 muscular dystrophies	AD/AR	64	121
B3GALNT2	Muscular dystrophy-dystroglycanopathy	AR	18	14
BICD2	Childhood-onset proximal spinal muscular atrophy with contractures	AD	12	28
CAPN3	Muscular dystrophy, limb-girdle, Eosinophilic myositis	AR	184	437
CAV3	Creatine phosphokinase, elevated serum, Hypertrophic cardiomyopathy (HCM), Long QT syndrome, Muscular dystrophy, limb-girdle, type IC, Myopathy, distal, Tateyama type, Rippling muscle disease 2	AD/AR	23	50
CHKB	Muscular dystrophy, congenital, megaconial	AR	11	27
COL4A1	Schizencephaly, Anterior segment dysgenesis with cerebral involvement, Retinal artery tortuosity, Porencephaly, Angiopathy, hereditary, with nephropathy, aneurysms, and muscle cramps, Brain small vessel disease	AD	58	107
COL4A2	Hemorrhage, intracerebral	AD	14	12

CRYAB	Cataract, myofibrillar myopathy and cardiomyopathy, Congenital cataract and cardiomyopathy, Dilated cardiomyopathy (DCM), Myopathy, myofibrillar, Cataract 16, multiple types, Myopathy, myofibrillar, fatal infantile hypertonic, alpha-B crystallin-related	AD	14	28
DES	Dilated cardiomyopathy (DCM), Myopathy, myofibrillar, Scapuloperoneal syndrome, neurogenic, Kaeser type	AD/AR	64	124
DMD	Becker muscular dystrophy, Duchenne muscular dystrophy, Dilated cardiomyopathy (DCM)	XL	832	3915
DNAJB6	Muscular dystrophy, limb-girdle	AD	11	17
DYSF	Miyoshi muscular dystrophy, Muscular dystrophy, limb-girdle, Myopathy, distal, with anterior tibial onset	AR	244	529
FKRP	Muscular dystrophy-dystroglycanopathy	AR	66	140
FKTN	Muscular dystrophy-dystroglycanopathy, Dilated cardiomyopathy (DCM), Muscular dystrophy-dystroglycanopathy (limb-girdle)	AD/AR	45	58
GAA	Glycogen storage disease	AR	193	573
GMPPB	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), Limb-girdle muscular dystrophy-dystroglycanopathy	AR	19	41
GOLGA2	Microcephaly, seizures, and developmental delay	AR		2
INPP5K	Muscular dystrophy, congenital, with cataracts and intellectual disability (MDCCAID)	AR	8	10
ISPD	Muscular dystrophy-dystroglycanopathy	AR	38	53
ITGA7	Muscular dystrophy, congenital, due to integrin alpha-7 deficiency	AR	16	8
LAMA2	Muscular dystrophy, congenital merosin-deficient	AR	199	301
LARGE	Muscular dystrophy-dystroglycanopathy	AR	19	27
LIMS2	Muscular dystrophy, limb-girdle	AR	2	3
LMNA	Heart-hand syndrome, Slovenian, Limb-girdle muscular dystrophy, Muscular dystrophy, congenital, LMNA-related, Lipodystrophy (Dunnigan), Emery-Dreiffus muscular dystrophy, Malouf syndrome, Dilated cardiomyopathy (DCM), Mandibuloacral dysplasia type A, Progeria Hutchinson-Gilford type	AD/AR	250	564
MAP3K20	Centronuclear myopathy	AR	5	7
MEGF10	Myopathy, early-onset, areflexia, respiratory distress, and dysphagia	AR	20	19
MSTO1	Myopathy, mitochondrial, and ataxia	AD/AR	7	8
MYH7	Hypertrophic cardiomyopathy (HCM), Myopathy, myosin storage, Myopathy, distal, Dilated cardiomyopathy (DCM)	AD	305	986
MYO18B	Klippel-Feil syndrome 4, autosomal recessive, with myopathy and facial dysmorphism	AR	2	4
MYOT	Myopathy, myofibrillar, Muscular dystrophy, limb-girdle, 1A, Myopathy, spheroid body	AD	6	16

PNPLA2	Neutral lipid storage disease with myopathy	AR	13	35
POGLUT1	Dowling-Degos disease 4, Muscular dystrophy, limb-girdle, type 2Z	AD	6	13
POMGNT1	Muscular dystrophy-dystroglycanopathy	AR	96	88
POMGNT2	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 8	AR	6	9
POMK	Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies, type A, 12, Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies, type C, 12, Muscle-eye brain disease, Walker-Warburg syndrome	AR	6	8
POMT1	Muscular dystrophy-dystroglycanopathy	AR	47	96
POMT2	Muscular dystrophy-dystroglycanopathy	AR	45	73
PYROXD1	Myopathy, myofibrillar 8	AR	5	6
RYR1	Central core disease, Malignant hyperthermia, Minicore myopathy with external ophthalmoplegia, Centronuclear myopathy, Minicore myopathy, Multicore myopathy	AD/AR	241	666
SELENON	Muscular dystrophy, rigid spine, Myopathy, congenital, with fiber-disproportion	AR	38	63
SGCA	Muscular dystrophy, limb-girdle	AR	60	100
SGCB	Muscular dystrophy, limb-girdle	AR	37	64
SGCD	Muscular dystrophy, limb-girdle, Dilated cardiomyopathy (DCM)	AR	21	27
SGCG	Muscular dystrophy, limb-girdle	AR	33	63
SMCHD1	Facioscapulohumeral muscular dystrophy, Facioscapulohumeral muscular dystrophy, type 2	AD	51	79
SPEG	Centronuclear myopathy 5	AR	5	11
SPTBN4	Myopathy, congenital, with neuropathy and deafness	AR	6	7
SYNE1	Spinocerebellar ataxia, autosomal recessive 8	AD/AR	83	136
TCAP	Muscular dystrophy, limb-girdle, Hypertrophic cardiomyopathy (HCM), Dilated cardiomyopathy (DCM)	AD/AR	12	28
TNPO3	Muscular dystrophy, limb-girdle	AD	3	5
TOR1AIP1	Muscular dystrophy with progressive weakness, distal contractures and rigid spine	AD/AR	3	5
TRAPPC11	Limb-girdle muscular dystrophy	AR	13	17
TRIM32	Bardet-Biedl syndrome, Muscular dystrophy, limb-girdle	AR	13	16
TTN*	Dilated cardiomyopathy (DCM), Tibial muscular dystrophy, Limb-girdle muscular dystrophy, Hereditary myopathy with early respiratory failure, Myopathy, early-onset, with fatal cardiomyopathy (Salih myopathy), Muscular dystrophy, limb-girdle, type 2J	AD	818	327

VMA21	Myopathy, X-linked, with excessive autophagy	XL	9	11

*Some regions of the gene are duplicated in the genome. Read more.

The gene has suboptimal coverage (means <90% of the gene's target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel as they are not sufficiently covered with high quality sequence reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#)

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), mitochondrial (mi), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Mitomap databases.

Non-coding disease causing variants covered by the panel

Gene	Genomic location HG19	HGVS	RefSeq	RS-number
CAPN3	Chr15:42678352	c.380-13T>A	NM_000070.2	
CAPN3	Chr15:42695919	c.1746-20C>T	NM_000070.2	
CAPN3	Chr15:42697047	c.-188G>C	NM_173089.1	
CAPN3	Chr15:42702715	c.2184+21G>A	NM_000070.2	rs763572829
CAPN3	Chr15:42702770	c.2185-16A>G	NM_000070.2	
COL4A1	Chr13:110802675	c.*35C>A	NM_001845.4	
COL4A1	Chr13:110802678	c.*32G>A/T	NM_001845.4	
COL4A1	Chr13:110802679	c.*31G>T	NM_001845.4	
DMD	ChrX:31165653	c.10554-18C>G	NM_004006.2	
DMD	ChrX:31200680	c.9974+175T>A	NM_004006.2	
DMD	ChrX:31224814	c.9564-30A>T	NM_004006.2	
DMD	ChrX:31225211	c.9564-427T>G	NM_004006.2	
DMD	ChrX:31226400	c.9563+1215A>G	NM_004006.2	
DMD	ChrX:31229031	c.9362-1215A>G	NM_004006.2	
DMD	ChrX:31241047	c.9361+117A>G	NM_004006.2	
DMD	ChrX:31279293	c.9225-160A>G	NM_004006.2	
DMD	ChrX:31279418	c.9225-285A>G	NM_004006.2	
DMD	ChrX:31279420	c.9225-287C>A	NM_004006.2	
DMD	ChrX:31279780	c.9225-647A>G	NM_004006.2	rs398124091

DMD	ChrX:31279781	c.9225-648A>G	NM_004006.2	rs398124084
DMD	ChrX:31332523	c.9224+9192C>A	NM_004006.2	
DMD	ChrX:31382270	c.9085-15519G>T	NM_004006.2	
DMD	ChrX:31613687	c.8217+32103G>T	NM_004006.2	
DMD	ChrX:31627738	c.8217+18052A>G	NM_004006.2	
DMD	ChrX:31697714	c.7661-11T>C	NM_004006.2	
DMD	ChrX:31897527	c.6913-4037T>G	NM_004006.2	
DMD	ChrX:31983146	c.6614+3310G>T	NM_004006.2	rs797045526
DMD	ChrX:32274692	c.6290+30954C>T	NM_004006.2	
DMD	ChrX:32305833	c.6118-15A>G	NM_004006.2	
DMD	ChrX:32360414	c.5740-15G>T	NM_004006.2	
DMD	ChrX:32366860	c.5326-215T>G	NM_004006.2	
DMD	ChrX:32379144	c.5325+1743_5325+1760delTATTAAAAAATGGGTAGA	NM_004006.2	
DMD	ChrX:32398808	c.4675-11A>G	NM_004006.2	
DMD	ChrX:32460274	c.3787-843C>A	NM_004006.2	
DMD	ChrX:32470726	c.3603+2053G>C	NM_004006.2	
DMD	ChrX:32479316	c.3432+2240A>G	NM_004006.2	
DMD	ChrX:32479520	c.3432+2036A>G	NM_004006.2	
DMD	ChrX:32669100	c.961-5831C>T	NM_004006.2	rs398124099
DMD	ChrX:32669194	c.961-5925A>C	NM_004006.2	
DMD	ChrX:32716130	c.832-15A>G	NM_004006.2	rs72470513
DMD	ChrX:32756908	c.650-39498A>G	NM_004006.2	
DMD	ChrX:32827744	c.531-16T>A/G	NM_004006.2	
DMD	ChrX:32827744	c.531-16T>A	NM_004006.2	
DMD	ChrX:32827744	c.531-16T>G	NM_004006.2	
DMD	ChrX:32841967	c.265-463A>G	NM_004006.2	
DMD	ChrX:33032666	c.93+5590T>A	NM_004006.2	
DMD	ChrX:33192452	c.31+36947G>A	NM_004006.2	
DMD	ChrX:33229483	c.-54T>A	NM_004006.2	
DYSF	Chr2:71817308	c.3443-33A>G	NM_003494.3	rs786205083
DYSF	Chr2:71840553	c.4410+13T>G	NM_003494.3	

DYSF	Chr2:71889030	c.4886+1249G>T	NM_003494.3	
DYSF	Chr2:71900503	c.5668-824C>T	NM_003494.3	
DYSF	Chr2:71913729	c.*107T>A	NM_003494.3	rs11903223
FKRP	Chr19:47249328	c.-272G>A	NM_024301.4	
FKTN	Chr9:108368857	c.648-1243G>T	NM_006731.2	
GAA	Chr17:78078341	c.-32-13T>G	NM_000152.3	rs386834236
GAA	Chr17:78078341	c.-32-13T>A	NM_000152.3	
GAA	Chr17:78078351	c.-32-3C>A/G	NM_000152.3	
GAA	Chr17:78078352	c.-32-2A>G	NM_000152.3	
GAA	Chr17:78078353	c.-32-1G>C	NM_000152.3	
GAA	Chr17:78078369	c.-17C>T	NM_000152.3	
GAA	Chr17:78082266	c.1076-22T>G	NM_000152.3	rs762260678
GAA	Chr17:78090422	c.2190-345A>G	NM_000152.3	
GAA	Chr17:78092432	c.2647-20T>G	NM_000152.3	
GMPPB	Chr3:49761246	c.-87C>T	NM_013334.3	rs780961444
LAMA2	Chr6:129633984	c.3175-22G>A	NM_000426.3	rs777129293
LAMA2	Chr6:129636608	c.3556-13T>A	NM_000426.3	rs775278003
LAMA2	Chr6:129714172	c.5235-18G>A	NM_000426.3	rs188365084
LAMA2	Chr6:129835506	c.8989-12C>G	NM_000426.3	rs144860334
LMNA	Chr1:156100609	c.513+45T>G	NM_170707.3	
LMNA	Chr1:156105681	c.937-11C>G	NM_170707.3	rs267607645
LMNA	Chr1:156107037	c.1608+14G>A	NM_170707.3	
LMNA	Chr1:156107433	c.1609-12T>G	NM_170707.3	rs267607582
POMT1	Chr9:134379574	c.-30-2A>G	NM_007171.3	
POMT2	Chr14:77751989	c.1333-14G>A	NM_013382.5	
RYR1	Chr19:38997317	c.8692+131G>A	NM_000540.2	
RYR1	Chr19:39074134	c.14647-1449A>G	NM_000540.2	rs193922886
SELENON	Chr1:26143316	c.*1107T>C	NM_020451.2	
SGCA	Chr17:48246419	c.585-31_585-23delTCTGCTGAC	NM_000023.2	
SGCA	Chr17:48246421	c.585-31_585-24delTCTGCTGA	NM_000023.2	
SGCA	Chr17:48247492	c.748-12_748-11delCTinsAA	NM_000023.2	

SGCG	Chr13:23755086	c.-127_-121delACAGTTG	NM_000231.2	rs1422849467
SGCG	Chr13:23755215	c.-1+1G>T	NM_000231.2	
SMCHD1	Chr18:2701019	c.1647+103A>G	NM_015295.2	
SMCHD1	Chr18:2705677	c.1843-15A>G	NM_015295.2	
SMCHD1	Chr18:2743740	c.3634-19A>G	NM_015295.2	
SYNE1	Chr6:152640163	c.16237-13C>G	NM_182961.3	
SYNE1	Chr6:152643033	c.15918-12A>G	NM_182961.3	rs606231134
VMA21	ChrX:150572076	c.54-27A>C/T	NM_001017980.3	
VMA21	ChrX:150572076	c.54-27A>C	NM_001017980.3	rs878854352
VMA21	ChrX:150572076	c.54-27A>T	NM_001017980.3	
VMA21	ChrX:150572082	c.54-16_54-8delGTTTACTTT	NM_001017980.3	rs878854357

Test Strengths

The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Some of the panels include the whole mitochondrial genome (please see the Panel Content section)
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- Our publicly available analytic validation demonstrating complete details of test performance
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see 'Non-coding disease causing variants covered by this panel' in the Panel Content section)
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

Test Limitations

The following exons are not included in the panel as they are not sufficiently covered with high quality sequence reads: *B3GALNT2* (NM_001277155:2), *SELENON* (NM_020451:3). Genes with suboptimal coverage in our assay are marked with number sign (#) and genes with partial, or whole gene, segmental duplications in the human genome are marked with an asterisk (*) if they overlap with the UCSC pseudogene regions. Gene is considered to have suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with mapping quality score (MQ>20) reads. The technology may have limited sensitivity to detect variants in genes marked with these symbols (please see the Panel content table above).

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
- Some of the panels include the whole mitochondrial genome but not all (please see the Panel Content section)

- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see above Panel Content / non-coding variants covered by the panel).

This test may not reliably detect the following:

- Low level mosaicism in nuclear genes (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Low level heteroplasmy in mtDNA (>90% are detected at 5% level)
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments
- Some disease causing variants present in mtDNA are not detectable from blood, thus post-mitotic tissue such as skeletal muscle may be required for establishing molecular diagnosis.

The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics.

For additional information, please refer to the Test performance section and see our Analytic Validation.

Test performance

Our panels are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection performance table for details regarding our ability to detect different types of alterations (Table).

Assays have been validated for various sample types including EDTA-blood, isolated DNA (excluding from formalin fixed paraffin embedded tissue), saliva and dry blood spots (filter cards). These sample types were selected in order to maximize the likelihood for high-quality DNA yield. The diagnostic yield varies depending on the assay used, referring healthcare professional, hospital and country. Plus analysis increases the likelihood of finding a genetic diagnosis for your patient, as large deletions and duplications cannot be detected using sequence analysis alone. Blueprint Genetics' Plus Analysis is a combination of both sequencing and deletion/duplication (copy number variant (CNV)) analysis.

Performance of Blueprint Genetics high-quality, clinical grade NGS sequencing assay for panels.

	Sensitivity % (TP/(TP+FN)	Specificity %
Single nucleotide variants	99.89% (99,153/99,266)	>99.9999%
Insertions, deletions and indels by sequence analysis		
1-10 bps	99.2% (7,745/7,806)	>99.9999%
11-50 bps	99.13% (2,524/2,546)	>99.9999%
Copy number variants (exon level dels/dups)		
1 exon level deletion (heterozygous)	100% (20/20)	NA
1 exon level deletion (homozygous)	100% (5/5)	NA
1 exon level deletion (het or homo)	100% (25/25)	NA
2-7 exon level deletion (het or homo)	100% (44/44)	NA
1-9 exon level duplication (het or homo)	75% (6/8)	NA

Simulated CNV detection		
5 exons level deletion/duplication	98.7%	100.00%
Size range (0.1-47 Mb)	100% (25/25)	
The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics		
Mean sequencing depth	143X	
Nucleotides with >20x sequencing coverage (%)	99.86%	

Performance of Blueprint Genetics Mitochondrial Sequencing Assay.

		Specificity
ANALYTIC VALIDATION (NA samples; n=4)		
Single nucleotide variants		
Heteroplasmic (45-100%)	100.0% (50/50)	100.0%
Heteroplasmic (35-45%)	100.0% (87/87)	100.0%
Heteroplasmic (25-35%)	100.0% (73/73)	100.0%
Heteroplasmic (15-25%)	100.0% (77/77)	100.0%
Heteroplasmic (10-15%)	100.0% (74/74)	100.0%
Heteroplasmic (5-10%)	100.0% (3/3)	100.0%
Heteroplasmic (<5%)	50.0% (2/4)	100.0%
CLINICAL VALIDATION (n=76 samples)		
All types		
Single nucleotide variants n=2084 SNVs		
Heteroplasmic (45-100%)	100.0% (1940/1940)	100.0%
Heteroplasmic (35-45%)	100.0% (4/4)	100.0%
Heteroplasmic (25-35%)	100.0% (3/3)	100.0%
Heteroplasmic (15-25%)	100.0% (3/3)	100.0%
Heteroplasmic (10-15%)	100.0% (9/9)	100.0%
Heteroplasmic (5-10%)	92.9%(12/13)	99.98%
Heteroplasmic (<5%)	88.7% (47/53)	99.93%

Insertions and deletions by sequence analysis n=42 indels		
Heteroplasmic (45-100%) 1-10bp	100.0% (32/32)	100.0%
Heteroplasmic (5-45%) 1-10bp	100.0% (3/3)	100.0%
Heteroplasmic (<5%) 1-10bp	100.0% (5/5)	>0.9999
SIMULATION DATA /(mitomap mutations)		
Insertions, and deletions 1-24 bps by sequence analysis; n=17		
Homoplasmic (100%) 1-24bp	100.0% (17/17)	99.98%
Heteroplasmic (50%)	100.0% (17/17)	99.99%
Heteroplasmic (25%)	100.0% (17/17)	100.0%
Heteroplasmic (20%)	100.0% (17/17)	100.0%
Heteroplasmic (15%)	100.0% (17/17)	100.0%
Heteroplasmic (10%)	94.1% (16/17)	100.0%
Heteroplasmic (5%)	94.1% (16/17)	100.0%
Copy number variants (separate artifical mutations; n=1500)		
Homoplasmic (100%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (50%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (30%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (20%) 500 bp, 1kb, 5 kb	99.7%	100.0%
Heteroplasmic (10%) 500 bp, 1kb, 5 kb	99.0%	100.0%
The performance presented above reached by following coverage metrics at assay level (n=66)		
	Mean of medians	Median of medians
Mean sequencing depth MQ0 (clinical)	18224X	17366X
Nucleotides with >1000x MQ0 sequencing coverage (%) (clinical)	100%	
rho zero cell line (=no mtDNA), mean sequencing depth	12X	

Bioinformatics

The target region for each gene includes coding exons and ±20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding and regulatory variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the 'Test limitations" section above. The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases including, but not limited, to 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation

software to make the process effective and efficient. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, ordering providers have access to the details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with <20X sequencing depth if applicable. This reflects our mission to build fully transparent diagnostics where ordering providers can easily visualize the crucial details of the analysis process.

Clinical interpretation

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, our PhD molecular geneticists, medical geneticists and clinical consultants prepare the clinical statement together by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the Blueprint Genetics Variant Classification Schemes based on the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Our experience with tens of thousands of clinical cases analyzed at our laboratory allowed us to further develop the industry standard.

The final step in the analysis is orthogonal confirmation. Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) are confirmed using bi-directional Sanger sequencing when they do not meet our stringent NGS quality metrics for a true positive call. Reported heterozygous and homo/hemizygous copy number variations with a size <10 and <3 target exons are confirmed by orthogonal methods such as qPCR if the specific CNV has been seen and confirmed less than three times at Blueprint Genetics.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information (genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene's variation in population cohorts and detailed information about related phenotypes. We also provide links to the references, abstracts and variant databases used to help ordering providers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification. We do not recommend using variants of uncertain significance (VUS) for family member risk stratification or patient management. Genetic counseling is recommended.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Our internal database and our understanding of variants and related phenotypes increases with every case analyzed. Our laboratory is therefore well-positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.

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ICD codes

Commonly used ICD-10 codes when ordering the LGMD and Congenital Muscular Dystrophy Panel

ICD-10	Disease
G71.0	Limb-girdle muscular dystrophy
G71.2	Congenital muscular dystrophy

Accepted sample types

- EDTA blood, min. 1 ml
- Purified DNA, min. 3µg*
- Saliva (Oragene DNA OG-500 kit)

Label the sample tube with your patient's name, date of birth and the date of sample collection.

Note that we do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue.

Resources

- Cure CMD
- GeneReviews - Becker/Duchenne Muscular Dystrophy
- GeneReviews - Congenital Muscular Dystrophy
- GeneReviews - Fukuyama Congenital Muscular Dystrophy
- GeneReviews - Limb-Girdle Muscular Dystrophy
- LGMD-Info
- LGMD2I Research Fund
- NORD - Congenital Muscular Dystrophy
- NORD - Fukuyama Congenital Muscular Dystrophy
- NORD - Limb-Girdle Muscular Dystrophy
- NORD - Walker-Warburg Syndrome
- Thompson R. & Straub V, 2016. Limb-girdle muscular dystrophies - international collaborations for translational research. Nat Rev Neurol. 2016 May;12(5):294-309.