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EVERYGENE CARDIOMYOPATHY PANEL (50 GENES):

The Everygene Cardiomyopathy Panel includes 50 genes associated with hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC) and is performed by next generation sequencing.

Testing strategy

This genetic test is intended for individuals with a personal or family history of inherited cardiomyopathies, a group of genetically heterogeneous cardiac diseases with a substantial genetic component and association with sudden cardiac death. Familial inheritance typically follows an autosomal dominant pattern, though all other forms of inheritance exist. The predominant forms are hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), followed by arrhythmogenic cardiomyopathy (ARVC). These conditions are relatively common, collectively affecting an estimated 1 in 250 to 1 in 500 individuals, making them among the most common genetic heart disorders.

Despite their prevalence and the associated risk of sudden cardiac death, access to genetic testing remains limited—especially for individuals who are uninsured or those who face financial barriers to accessing available testing. This test is also designed to support individuals who encounter challenges to accessing care through traditional healthcare channels.

The initial DNA extraction and sequencing component of this test was performed by the Broad Clinical Laboratories (BCL; 27 Blue Sky Drive, Burlington, MA 01803; CLIA#22D2055652), and interpretive algorithms, clinical reports and Sanger confirmation when applicable, were generated by the Laboratory for Molecular Medicine at Partners Healthcare Personalized Medicine (LMM, 65 Landsdowne St, Cambridge, MA 02139; 617-768-8500; CLIA#22D1005307).

Gene information

Gene	Protein	OMIM#	Locus	Reported Associated Disorder(s); (cardiomyopathy)^	Inheritance*
ACTC1	Actin, Alpha, Cardiac Muscle	102540	15q14	HCM, DCM	AD
ACTN2	Actinin, Alpha-2	102573	1q43	HCM, DCM	AD
ALPK3	Alpha Kinase 3	617608	15q25.3	HCM	AD/AR
BAG3	Bag Cochaperone 3	603883	10q26.11	DCM	AD
BRAF	B-RAF Protooncogene, Serine/Threonine Kinase	164757	7q34	RASopathy (HCM and other cardiac issues)	AD

CSRP3	Cysteine- And Glycine-Rich Protein 3	600824	11p15.1	HCM	AD/AR (Semi-dominant)
DES	Desmin	125660	2q35	DCM, ARVC	AD
DSC2	Desmocollin 2	125645	18q12.1	ARVC	AD
DSG2	Desmoglein 2	125671	18q12.1	ARVC	AD
DSP	Desmoplakin	125647	6p24.3	*DSP*-related cardiocutaneous syndrome (Arrhythmogenic cardiomyopathy/DCM-like, Carvajal syndrome)	AD/AR
EMD	Emerin	300384	Xq28	Emery-Dreifuss muscular dystrophy (HCM)	XL
FHL1	Four-And-A-Half Lim Domains 1	300163	Xq26.3	Emery-Dreifuss muscular dystrophy (HCM/DCM)	XL
FLNC	Filamin C	102565	7q32.1	DCM	AD
GAA	Glucosidase, Alpha, Acid	606800	17q25.3	Glycogen storage disease II (HCM)	AR
GLA	Galactosidase, Alpha	300644	Xq22.1	Fabry disease (HCM and other cardiac issues including LVH)	XL
HRAS	HRAS Protooncogene, GTPase	190020	11p15.5	RASopathy (HCM and other cardiac issues)	AD
JPH2	Junctophilin 2	605267	20q13.12	HCM; DCM	AD; AD/AR (Semi-dominant)
JUP	Junction Plakoglobin	173325	17q21.2	ARVC; Naxos disease (ARVC)	AD AR
KRAS	KRAS Protooncogene, GTPase	190070	12p12.1	RASopathy (HCM and other cardiac issues)	AD
LAMP2	Lysosome-Associated Membrane Protein 2	309060	Xq24	Danon disease (HCM)	XL
LMNA	Lamin A/C	150330	1q22	DCM; Laminopathies with cardiac involvement (e.g. muscular dystrophies)	AD
LZTR1	Leucine Zipper-Like Transcriptional Regulator 1	600574	22q11.21	RASopathy (HCM and other cardiac issues)	AD/AR
MAP2K1	Mitogen-Activated Protein Kinase Kinase 1	176872	15q22.31	RASopathy (HCM and other cardiac issues)	AD
MAP2K2	Mitogen-Activated Protein Kinase Kinase 2	601263	19p13.3	RASopathy (HCM and other cardiac issues)	AD
MYBPC3	Myosin-Binding Protein C, Cardiac	600958	11p11.2	HCM	AD
MYH7	Myosin, Heavy Chain 7, Cardiac Muscle, Beta	160760	14q11.2	HCM, DCM	AD
MYL2	Myosin, Light Chain 2, Regulatory, Cardiac, Slow	160781	12q24.11	HCM	AD
MYL3	Myosin, Light Chain 3, Alkali, Ventricular, Skeletal, Slow	160790	3p21.31	HCM	AD
NEXN	Nexilin F-Actin-Binding Protein	613121	1p31.1	DCM	AD
NRAS	NRAS Protooncogene, GTPase	164790	1p13.2	RASopathy (HCM and other cardiac issues)	AD
PKP2	Plakophilin 2	602861	12p11.21	ARVC	AD
PLN	Phospholamban	172405	6q22.31	HCM, DCM, ARVC	AD

PRKAG2	Protein Kinase, AMP-Activated, Noncatalytic, Gamma-2	602743	7q36.1	*PRKAG2* – related cardiomyopathy (Glycogen storage disease of the heart; HCM and other cardiac issues)	AD
PTPN11	Protein-Tyrosine Phosphatase, Nonreceptor-Type, 11	176876	12q24.13	RASopathy (HCM and other cardiac issues)	AD
RAF1	RAF1 Protooncogene, Serine/Threonine Kinase	164760	3p25.2	RASopathy (HCM and other cardiac issues)	AD
RBM20	RNA-Binding Motif Protein 20	613171	10q25.2	DCM	AD
RIT1	RIC-Like Protein Without Caax Motif 1	609591	1q22	RASopathy (HCM and other cardiac issues)	AD
RRAS2	Related RAS Viral Oncogene Homolog 2	600098	11p15.2	RASopathy (HCM and other cardiac issues)	AD
SCN5A	Sodium Channel, Voltage-Gated, Type V, Alpha Subunit	600163	3p22.2	*SCN5A* – channelopathy (DCM and other conduction disorders; Brugada and Long QT syndrome)	AD
SOS1	SOS RAS/RAC Guanine Nucleotide Exchange Factor 1	182530	2p22.1	RASopathy (HCM and other cardiac issues)	AD
SOS2	SOS RAS/RAC Guanine Nucleotide Exchange Factor 2	601247	14q21.3	RASopathy (HCM and other cardiac issues)	AD
TAFAZZIN	Tafazzin, Phospholipid-Lysophospholipid Transacylase	300394	Xq28	Barth Syndrome (DCM)	XL
TMEM43	Transmembrane Protein 43	612048	3p25.1	ARVC	AD
TNNC1	Troponin C, Slow	191040	3p21.1	HCM; DCM	AD
TNNI3	Troponin I, Cardiac	191044	19q13.42	HCM; DCM	AD
TNNT2	Troponin T2, Cardiac	191045	1q32.1	HCM; DCM	AD
TPM1	Tropomyosin 1	191010	15q22.2	HCM; DCM	AD
TTN	Titin	188840	2q31	DCM	AD
TTR	Transthyretin	176300	18q12.1	Transthyretin amyloidosis (HCM)	AD
VCL	Vinculin	193065	10q22.2	DCM	AD

^ HCM = Hypertrophic cardiomyopathy; DCM = Dilated cardiomyopathy; ARVC = Arrhythmogenic right ventricular cardiomyopathy; LVH = Left ventricular hypertrophy

*AD = Autosomal dominant; AR = Autosomal recessive; XL = X-Linked

Methodology:

Cardiomyopathy panel:

This panel includes 50 genes: *ACTC1* (NM_005159.5), *ACTN2* (NM_001103.4), *ALPK3* (NM_020778.5), *BAG3* (NM_004281.4), *BRAF* (exons 1-18: NM_004333.6, exon 10A: NM_001374258.1), *CSRP3* (NM_003476.5), *DES* (NM_001927.4), *DSC2* (NM_024422.6), *DSG2* (NM_001943.5), *DSP* (NM_004415.4), *EMD* (NM_000117.3), *FHL1* (exons1-6: NM_001159699.2, exon 7A: NM_001159702.3), *FLNC* (NM_001458.5), *GAA* (NM_000152.5), *GLA* (NM_000169.3), *HRAS* (exons 2-5: NM_005343.4, exon 5A: NM_176795.5), *JPH2* (NM_020433.5), *JUP* (NM_002230.4), *KRAS* (exons 2-5: NM_004985.5, exon 5A: NM_033360.4), *LAMP2* (NM_002294.3), *LMNA* (exons 1-12: NM_170707.4, exon 10A: NM_005572.4), *LZTR1* (NM_006767.4), *MAP2K1* (NM_002755.4), *MAP2K2* (NM_030662.4), *MYBPC3* (NM_000256.3), *MYH7* (NM_000257.4), *MYL2* (

NM_000432.4), *MYL3* (NM_000258.3), *NEXN* (NM_144573.4), *NRAS* (NM_002524.5), *PKP2* (NM_001005242.3), *PLN* (NM_002667.5), *PRKAG2* (NM_016203.4), *PTPN11* (NM_002834.5), *RAF1* (NM_002880.4), *RBM20* (NM_001134363.3), *RIT1* (NM_006912.6), *RRAS2* (NM_012250.6), *SCN5A* (exons 2-28: NM_000335.5, exons 6A and 18A: NM_001099404.2), *SOS1* (NM_005633.4), *SOS2* (NM_006939.4), *TAFAZZIN* (NM_000116.5), *TMEM43* (NM_024334.3), *TNNC1* (NM_003280.3), *TNNI3* (NM_000363.5), *TNNT2* (NM_001276345.2), *TPM1* (NM_001018005.2), *TTN* (NM_001267550.2, except for exons 147,149,158-201 and 212-216 which are not present in any biological isoform), *TTR* (NM_000371.4), and *VCL* (NM_014000.3). Except when otherwise noted, sequencing is limited to coding regions and splice sites (+/- 15bp) of these genes. For additional information on reference sequences and exon coverage, please email lmm@mgb.org.

Genome sequencing and variant calling (from BCL validation document): The clinical Blended Genome Exome (cBGE) laboratory workflow utilized the New England Biolabs NEBNext Ultra II FS DNA Library Preparation it and NEBNext Multiplex unique dual index adapter oligos set 1-4, which transformed genomic DNA extracted from blood or saliva into DNA libraries that are compatible with Illumina sequencing platforms. During the library construction workflow, gDNA was enzymatically fragmented, adapter-ligated, and barcoded to create a single PCR-free whole genome. Following PCR-free generation, an aliquot from the PCR-free whole genome library was taken through PCR amplification and exome selection. The PCR-free genome and whole exome were then rejoined and blended together pre-sequencing, to deliver and optimal coverage balance and combined sequencing output. Library fragments were sequenced (2x150 base paired-end) using Sequencing-By-Synthesis (SBS) chemistry on the Illumina NovaSeq X Plus sequencer. Sequencing data were aligned to the GRCh38 assembly after discarding low-quality sequences. Illumina's DRAGEN (Dynamic Read Analysis for GENomics) platform was used for demultiplexing, read mapping, genome alignment, read sorting, duplicate marking, and variant calling. The DRAGEN pipeline generated the quality metrics that met the following thresholds: ≥ 90% of exome bases at or greater than 20X, genome mean coverage ≥ 1X, and mean target coverage ≥ 60X. Other quality metrics that are reviewed are percent contamination (≤ 2.5%), percent mapped (≥ 75%), and percent exome callability (≥ 95%). The DRAGEN pipeline also generated a CRAM file (compressed BAM file), and a hard-filtered VCF (Variant Call Format) file that contains SNPs/indels (single nucleotide variants and small insertions/deletions) with a target region of +/- 16 base pairs into the introns.

Filtration Strategy and variant interpretation:
Variants in the 50 genes described above are subsequently filtered to identify:
(1) variants classified as disease causing mutations in public databases (ClinVar and the Human Gene Mutation Database (HGMD)) that have a minor allele frequency <5.0% in the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/,); and
(2) nonsense, frameshift, and +/-1,2 splice-site variants in disease associated genes with a minor allele frequency ≤1% in gnomAD. The evidence for phenotype-causality is then evaluated for each variant identified from the filtering strategies listed above and variants are classified based on ACMG/AMP criteria (Richards et al. 2015) with ClinGen rule specifications (https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/). Variants are reported using the GRCh38 human genome reference sequence and according to HGVS nomenclature (http://varnomen.hgvs.org/).

Limitations and additional comments: Specific types of genetic variation, such as short tandem repeats (including triplet repeat expansions), structural variation, and large copy number events are currently not detected by this assay. Additionally, while ≥ 90% of the exome is guaranteed to be covered at 20X (with >90% of the exome typically covered at 20X), there are certain challenging regions of the genome for which the assay may fail to accurately detect variation, such as regions of high homology, regions that are highly repetitive, regions with low coverage, and regions with poor mapping quality or base quality. No orthogonal assay is used for fill-in for low coverage regions or bases. The sequencing data is limited to the cell-type (material) source used for DNA extraction. Additionally, not all variants identified have been analyzed. Variant classification may change over time if more information becomes available.

Only those variants with evidence for causing or contributing to disease are reported. Variants of uncertain significance are not reported but may be included in some instances. Variants meeting predetermined quality thresholds are not orthogonally confirmed. All other reported variants are confirmed via Sanger sequencing or another orthogonal technology. Please contact the laboratory for additional information.

Analytic Sensitivity and Technical Positive Predictive Value:

Variant Class	Analytic Sensitivity *	Technical positive predictive value *
Single nucleotide variant	99.31% (95% CI = 99.22 – 99.40%)	99.87% (95% CI = 99.85 – 99.89%)
Insertions and Deletions	96.93% (95% CI = 96.64 – 97.21%)	97.28% (95% CI = 96.88 – 97.67%)

* Within covered regions
CI = Confidence interval

There is demonstrated reduced detection for larger indels, especially in low complexity regions with corresponding low sequence coverage and in regions with high homology.

Clinical sensitivity

Variable, depending on disease:

- HCM: 30 – >60%; familial HCM on higher end of the range[1,2]
- DCM: 10 – 40%; familial DCM on higher end of the range[2]
- ARVC: 10 – >50% [2,3]

The detection rate for the other cardiomyopathies is unknown.

References

1. Ireland CG, Ho CY. 2024. Genetic Testing in Hypertrophic Cardiomyopathy. Am J Cardiol. Feb 1;212S:S4-S13. PMID: 38368035.

2. Hershberger RE, Givertz MM, Ho CY, Judge DP, Kantor PF, McBride KL, Morales A, Taylor MRG, Vatta M, Ware SM. 2018. Genetic Evaluation of Cardiomyopathy-A Heart Failure Society of America Practice Guideline. J Card Fail. May;24(5):281-302. PMID: 29567486.

3. Pinamonti B, Brun F, Mestroni L, Sinagra G. 2014. Arrhythmogenic right ventricular cardiomyopathy: From genetics to diagnostic and therapeutic challenges. World J Cardiol. Dec 26;6(12):1234-44. PMID: 25548613