otogenetics corporation®

Patient: 1234####-COtGx####
CLIA ID#: 11D2066426
First Last, MD, Laboratory Director

Gx™ Cardiomyopathy Gene Testing Report

Patient Information

Patient Name	First Last
Date of Birth	Mot dd, yyyy
Age	##
Sex	F
Ethnicity	xxxxxx

Provider Information

Provider	xxx Clinic
Provider ID	##############
Physician	First Last

Specimen

Accession ID	####1234
Sample ID	COtGx####
Specimen Type	Saliva
Collection Date	Mot1d, 2017
Report Date	Mot d, 2017

Patient Results: Positive - Pathogenic Variant(s) Detected

Variant Summary

Variant Identified	Type	Genotype	dbSNP ID	Phenotype	Classification
MYL2 NM_000432.3: c.172C>T p.R58*	SNV	Heterozygous	rs756671869	familial hypertrophic cardiomyopathy	Pathogenic

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	dbSNP ID	Genotype	Assessment
MYL2	4	c.172C>T	p.R58*	rs756671869	Het	Pathogenic

The MYL2 gene encodes the regulatory slow light chain of cardiac myosin. This protein is associated with the beta (or slow) heavy chain subunit of cardiac myosin, and is important in the regulation of myosin ATPase activity in smooth muscle. Calcium triggers the phosphorylation of the regulatory light chain that triggers contraction. Heterozygous MYL2 gene mutations have been reported in patients with mid-left ventricular chamber type hypertrophic cardiomyopathy [PMID:8673105]. MYL2 mutations have also been associated with familial and classic forms of hypertrophic cardiomyopathy [PMID:9535554].

Additional Comments

A pathogenic variant was detected in MYL2 gene. The variant in MYL2, c.172C>T p.R58*, is a nonsense mutation that is damaging to the function of MYL2. This mutant human MYL2 protein (inferred germline substitution p.R58*, result of c.172C>T [heterozygous]) is observed with childhood-onset dominant hypertrophic cardiomyopathy in human.

Followup Recommendations

Follow up with healthcare providers for clinical correlation of personal and family clinical history, family member and reproductive partner testing, and updated genetic testing information.

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Genes Tested

Targeted regions for “Cardiomyopathy Gene Testing” includes the exonic regions of the following genes: ABCC9, ACTC1, ACTN2, BMPR2, CAV3, DES, GLA, LAMA4, LAMP2, LDB3, LMNA, MT-TD, MT-TG, MT-TH, MT-TI, MT-TL1, MT-L2, MT-TM, MT-TQ, MT-TS1, MT-TS2, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYPN, PLN, PRKAG2, PSEN1, PSEN2, RBM20, SCN5A, SGCD, TAZ, TCAP, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTR, VCL

Methods and Limitations

Sample Processing and Sequencing and Variant Detection - This gene panel focuses on the coding exonic regions of genes. Target genes were identified based on clinical genetic data and recommendations from profession societies. Genomic targets were identified based on information in the HGMD, the Online Mendelian Inheritance in Man (OMIM) catalog, GeneTests.org, and other commercially available sequencing panels. Combining data from these sources ensured that genes currently identified in clinical research settings as pathogenic were included in the panel. Standard Operation Procedures were used to process the samples. Genomic DNA was extracted from clinical samples (saliva, blood, swab, or as specified in the report), library preparation via Illumina protocols, capture based enrichment of a targeted region was performed by solution-based hybridization which enriches for coding regions of targeted genes with specific probes. Multiple quality control steps were performed for sample and derivative quality evaluation. Sequencing was performed using Illumina Sequencer(s), and an average coverage depth of 100X was designated.

Variant call Format (VCF) Generation - VCF file was generated using either the Sentieon analysis pipeline or the Best Practices from GATK pipeline on DNAnexus platform. Reference genome used is UCSC hg19. Additional quality filters, including quality score of 20 and a minimum coverage (DP) of 8x, were applied to generate the VCF subjected to QIAGEN Clinical Insight interpretation for reporting as described below.

Limitations - Absence of a primary diagnostic finding identified by this test does not exclude the possibility of a genetic basis for the clinical condition or a carrier status. Variants in the intronic, UTR and promoter regions and other copy number variants are not intended to be detected by this assay.

Specifically, detection of abnormal variants depends on the presence of these sequence variants in the targeted region that was sequenced. It is possible that the gene region where a disease causing mutation exists was not captured using the current technologies of this test and therefore was not detected.

This sequence test is designed to evaluate single nucleotide variants, 1-3 nucleotide variants and small insertions and deletions (<10 nucleotides) within the targeted region. CNV kit analysis was applied to assess large deletions and duplications of selected genes. The current technology targets the coding exonic regions of the genes and not the 5’ or 3’ untranslated regions, promoter or splice sites of these genes. Thus, a variant in these non-coding exonic regions will not be sequenced at high depth, and may not be identified in this test. Coverage within the target region may also influence the identification of variants. Testing for CGG repeats in FMR1 gene may also be included.

Additionally, certain types of genetic abnormalities are difficult to identify in sequencing data and have not been validated for clinical use including but not limited to insertions, deletions, copy number alterations, long repetitive sequences, triplet repeat expansions, chromosomal rearrangements, polyploidy, repetitive regions including mono-, di- and tri-nucleotide repeats, GC rich regions, intronic variants outside the splice-site and epigenetic effects.

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It is possible that a particular genetic abnormality may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the function of all genes in the human genome and the impact of variants in those genes. Clinical correlation and periodic review of scientific and medical literature is recommended to determine whether Variants of Unknown Significance may be consistent with the patient's or carrier's phenotype.

Validation is recommended for exons with depth of coverage <8x. We recommend that variants of interest which do not meet the coverage minimum be confirmed clinically before treatment is undertaken. Validation of variants reported is also recommended.

Access to sequencing data, intermediate data files, and detailed analysis tools applied is available upon request.

QIAGEN Clinical Insight - Interpret software was used in sequence analysis and interpretation. The application was internally designed and developed by QIAGEN. All analyses were based on: QIAGEN Clinical Insight-Interpret (5.0.20170906), Ingenuity Knowledge Base (Narnia 170831.002), CADD (v1.3), CentoMD (-), EVS (ESP6500SI-V2), Allele Frequency Community (2017-07-03), JASPAR (2013-11), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), gnomAD (2.0.1), Clinical Trials (Narnia 170831.002), BSIFT (2016-02-23), TCGA (2013-09-05), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), Clinvar (2017-06-01), DGV (2016-05-15), COSMIC (v81), ExAC (0.3.1), HGMD (2017.2), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (150), TargetScan (6.2), SIFT4G (2016-02-23). Weekly updates to Ingenuity Knowledge Base for clinical trials recruitment status and new findings from recent articles. Variants are reported according to HGVS nomenclature and were classified following ACMG guidelines. Information on therapeutic agents and clinical trials were obtained from publicly available information. Variants, therapies, and trials listed in this report are not ranked in order of potential clinical significance or predicted efficacy for this patient.

Laboratory Statement

This Laboratory Developed Test for Next-Generation sequencing of genomic DNA was developed and its performance characteristics established by Otogenetics Corporation, Atlanta, GA. This laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing and has validated the test's accuracy according to CAP proficiency testing. This test has not been cleared nor approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. CLIA number – 11D2066426.

Selected Citations

Berge KE, Leren TP (2013) Genetics of hypertrophic cardiomyopathy in Norway. Clin Genet. 2014 Oct;86(4):355-60. Epub 2013 Oct 23 https://www.ncbi.nlm.nih.gov/pubmed/24111713?dopt=Abstract PubMed PMID: 24111713

Flavigny J, Richard P, Isnard R, Carrier L, Charron P, Bonne G, Forissier JF, Desnos M, Dubourg O, Komajda M, Schwartz K, Hainque B (1998) Identification of two novel mutations in the ventricular regulatory myosin light chain gene (MYL2) associated with familial and classical forms of hypertrophic cardiomyopathy. J Mol Med (Berl). 1998 Mar;76(3-4):208-14 https://www.ncbi.nlm.nih.gov/pubmed/9535554?dopt=Abstract PubMed PMID: 9535554

Poetter K, Jiang H, Hassanzadeh S, Master SR, Chang A, Dalakas MC, Rayment I, Sellers JR, Fananapazir L, Epstein ND (1996) Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. Nat Genet. 1996 May;13(1):63-9 https://www.ncbi.nlm.nih.gov/pubmed/8673105?dopt=Abstract PubMed PMID: 8673105

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