Whole Exome Sequencing and Deletion/Duplication Trio Analysis

Patient Name:
Date of Birth:
Gender:
Accession ID:
Cross Reference: Not provided

Specimen Type:
Receive Date:
Collection Date:
Report Date:

Client Name:
Hospital/Institution:
Mailing Address:
Phone Number:
Fax Number:

Test Performed: Whole Exome Sequencing and Deletion/Duplication Analysis, Trio
Reason for Referral: Clinical features of disease

Likely pathogenic sequence variant(s) in gene related to reported phenotype detected.

No reportable copy number variants (CNV) related to phenotype detected.

Correlation with clinical profile and family history is required.

A laboratory report provided to us indicates that maternal cell contamination (MCC) studies did not show evidence of maternal cell contamination in the fetal sample reported to be the source of the DNA provided to us for testing.

Relevant Findings and Interpretation

Phenotypic terms applied: Non-immune ascites, pleural effusion, generalized skin edema, polyhydramnios, hydrops fetalis

Sequence variants related to phenotype:

Classification	Gene	Exon/Intron	DNA Change	Protein Change	Zygosity	Inheritance	Associated Disease
Likely Pathogenic	RAF1	7	c.779C>T	p.Thr260Ile	Heterozygous	Autosomal Dominant	Cardiomyopathy, dilated, 1NN; LEOPARD syndrome 2; Noonan syndrome 5

RAF1 **c.779C>T (p.Thr260Ile) - Likely Pathogenic.** The c.779C>T (p.Thr260Ile) missense variant results in the substitution of the threonine codon at amino acid position 260 with an isoleucine codon. This variant has been reported in an individual with hypertrophic cardiomyopathy (PMID: 17603483). This variant has not been observed in the general population (11/27/19 PMID: 27535533). *In silico* analyses imply a potentially deleterious effect to the protein function (PolyPhen-2, SIFT, MutationTaster). The c.779C>T (p.Thr260Ile) *RAF1* variant is classified as likely pathogenic. Clinical and biochemical correlation is required.

This variant was **NOT** detected in either parent.

Findings Unrelated To Phenotype

Diagnostic findings in genes defined as highly penetrant and medically actionable by ACMG (PMID: 27854360):

No pathogenic variants detected.

Diagnostic findings in other disease-causing genes not related to indications for testing:

No pathogenic variants detected.

Carrier status for autosomal recessive conditions:

Not requested.

Pharmacogenetic variants (only selected CPIC Class 1A alleles with clinical utility are evaluated):

Not requested.

Recommendations

The detection of a likely pathogenic variant in the *RAF1* gene is consistent with a diagnosis of disease in this individual; however, these results must be interpreted in the context of this individual's clinical and biochemical profile. Genetic counseling is recommended.

For more information, please contact the laboratory at 1-866-354-2910.

Identified data will be stored at PerkinElmer Genomics.

Notes

Variants related to phenotype are reported based on analysis of clinical information provided by the ordering provider. There may be variants of uncertain significance present in the sample with partial overlap to some of the given phenotypic information which were not determined to be relevant enough for reporting. A list of all variants identified in this individual is available upon request. The analyzed region of genes includes the coding exons and 10bp of flanking intronic region on both sides of each exon. Select pathogenic deep intronic sites may also be targeted. In some cases, due to the complexity of the sequence, not all variants in the flanking intronic sequence are able to be analyzed. Variants are evaluated by their reported frequency in databases such as the Genome Aggregation Database (gnomAD), Human Gene Mutation Database (HGMD), and ClinVar. Variants that have a population frequency greater than expected given the prevalence of the disease in the general population are considered to be benign variants. Benign and likely benign variants are not reported. Silent variants and intronic variants beyond +/-3 are not reported unless known or suspected to be pathogenic. The interpretation of variants is based on our current understanding of the genes involved. These interpretations may change over time as more information about the gene(s) and this individual's clinical phenotype becomes available. Raw sequencing data is available upon request.

Variant Statistics:

Gene	Transcript	DNA Change	Protein Change	Genomic Location	Coverage	Alternate Allele Fraction	dbSNP rsID
RAF1	NM_002880.3	c.779C>T	p.Thr260Ile	Chr3:12645690-12645690	128	53.1%	rs869025501

Data Quality Statistics:

% Fully Covered Disease Causative Gene Target Bases	99.2
% Fully Covered Disease Causative Gene Exons	99.1
Average Coverage per Target Base	145.9

Methods and Limitations

Whole exome sequencing is performed on genomic DNA using the Agilent v6CREv2 targeted sequence capture method to enrich for the exome. Direct sequencing of the amplified captured regions was performed using 2X100bp reads on Illumina next generation sequencing (NGS) systems. A base is considered to have sufficient coverage at 20X and an exon is considered fully covered if all coding bases plus three nucleotides of

flanking sequence on either side are covered at 20X or more. Low coverage regions, if any, are limited to ~1% or less of the nucleotides included in this panel unless a pathogenic variant explaining the phenotype is discovered. A list of these regions is available upon request. Alignment to the human reference genome (hg19) is performed and annotated variants are identified in the targeted region. Variants are called at a minimum coverage of 8X and an alternate allele frequency of 20% or higher. Single nucleotide variants (SNVs) meeting internal quality assessment guidelines are confirmed by Sanger sequence analysis for records after results are reported. Indels and SNVs may be confirmed by Sanger sequence analysis before reporting at director discretion. This assay cannot detect variants in regions of the exome that are not covered, such as deep intronic, promoter, and enhancer regions, areas containing large numbers of tandem repeats, and variants in mitochondrial DNA. Copy number variation (CNV) analysis is designed to detect deletions and duplications of three exons or more; in some instances, due to the size of the exons or other factors, not all CNVs may be analyzed. Only CNVs related to phenotype are reported. This assay is not designed to detect mosaicism; possible cases of mosaicism may be investigated at the discretion of the laboratory director. Primary data analysis is performed using Illumina DRAGEN Bio-IT Platform v.2.03. Secondary and tertiary data analysis is performed using PerkinElmer's internal ODIN v.1.01 software for SNVs and Biodiscovery's NxClinical v.4.3 or Illumina DRAGEN Bio-IT Platform v.2.03 for CNV and absence of heterozygosity (AOH).

Possible sources of testing error include rare genetic variants that interfere with analysis, sample misidentification, and other sources. Pursuant to the requirements of CLIA '88, this test was developed and its performance validated by PerkinElmer Genomics. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes.

Director Signature(s)

- XXXXXXXXX, FACMG– Clinical Laboratory Director, PerkinElmer Genomics Laboratory