PerkinElmer
For the Better

PerkinElmer GENOMICS

Whole Genome Sequencing and Deletion/Duplication Analysis

Patient Name:
Date of Birth:
Gender:
Accession ID:
Cross Reference:

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

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

Test Performed: Whole Genome Sequencing and Deletion/Duplication Analysis, Proband Only
Reason for Referral: Clinical features of disease

Likely pathogenic sequence variant(s) and sequence variant(s) of uncertain significance in genes related to reported phenotype detected.

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

Likely pathogenic sequence variant(s) in gene for carrier status detected.

Correlation with clinical profile and family history is required.

Relevant Findings and Interpretation

Phenotypic terms applied: XXXXXX

Sequence variants related to phenotype:

Classification	Gene	Exon/ Intron	DNA Change	Protein Change	Zygosity	Inheritance	Associated Disease
Likely Pathogenic	*NR2F2*	1	c.103_109del	-	Heterozygous	Autosomal Dominant	Congenital heart defects, multiple types, 4
Uncertain Significance	*CBL*	3'UTR	c.*6886G>A	-	Heterozygous	Autosomal Dominant	Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia
Uncertain Significance	*FLNA*	20	c.2904C>A	p.Ser968Arg	Heterozygous	X-linked	FLNA - Related Disorder

Uncertain Significance	*LZTR1*	5'UTR	c.-975G>A	-	Heterozygous	Autosomal Dominant; Autosomal Recessive	Noonan syndrome 10; Noonan syndrome 2

***NR2F2* c.103_109del (-) - Likely Pathogenic.** The c.103_109del variant results in the deletion of seven nucleotides at positions c.103 through c. 109 of the *NR2F2* gene, causing a frameshift in the protein reading frame. This variant has been reported as *de novo* in an individual with a testicular disorder of sex development (TDSD) and congenital heart disease (PMID: 29478779). This variant has not been reported as a variant in the general population (12/4/19 PMID: 27535533). Loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay is expected. The c.103_109del *NR2F2* variant is classified as pathogenic. Clinical and biochemical correlation is required.

***CBL* c.*6886G>A (-) - Uncertain Significance.** The c.*6886G>A variant is a substitution of a G with an A in the 3' untranslated region of the *CBL* gene. To our knowledge, this variant has not been reported as causative of disease. This variant has been observed in five alleles in the general population (12/5/19, PMID: 27535533). It is possible for this change to alter gene expression; however, the effect of this change on expression cannot be directly tested at this time. There is currently insufficient evidence to determine the pathogenicity of this variant, therefore the c. *6886G>A *CBL* variant is classified as a variant of uncertain significance. Clinical and biochemical correlation is required.

***FLNA* c.2904C>A (p.Ser968Arg) - Uncertain Significance.** The c.2904C>A (p.Ser968Arg) missense variant results in the substitution of the serine codon at amino acid position 968 with an arginine codon. To our knowledge, this variant has not been reported in individuals with disease. This variant has not been observed in the general population (12/5/19 PMID: 27535533). *In silico* analyses are inconsistent regarding the effect to protein function (PolyPhen-2, SIFT, MutationTaster). There is currently insufficient evidence to determine the pathogenicity of this variant, therefore the c.2904C>A (p.Ser968Arg) *FLNA* variant is classified as a variant of uncertain significance. Clinical and biochemical correlation is required.

***LZTR1* c.-975G>A (-) - Uncertain Significance.** The c.-975G>A variant is a substitution of a G with an A in the 5' untranslated region of the *LZTR1* gene. This variant has not been reported in individuals with disease to our knowledge or as a variant in the general population (12/5/19, PMID: 27535533). It is possible for this change to alter gene expression; however, the effect of this change on expression cannot be directly tested at this time. There is currently insufficient evidence to determine the pathogenicity of this variant, therefore the c.-975G>A *LZTR1* variant is classified as a variant of uncertain significance. Clinical and biochemical correlation is required.

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:

Gene	OMIM	Disease	Inheritance	Exon/ Intron	DNA Change	Protein Change	Zygosity	Classification
ST3GAL5	604402	Salt and pepper developmental regression syndrome	Autosomal Recessive	4	c.353del	-	Heterozygous	Likely Pathogenic

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

Consultation with a physician to discuss relevant Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines is recommended.

Gene	Allele Haplotype	Phenotype
SLCO1B1	*1A/*15	Decreased Function

Recommendations

The detection of a likely pathogenic variant in the *NR2F2* gene may be 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.

If considered relevant to this individual's clinical presentation and/or family history, targeted testing of the parents of this individual for the *CBL, FLNA* and/or *LZTR1* variants of uncertain significance may help to interpret these results.

Targeted testing is available for family members at risk to carry the likely pathogenic variants identified in this individual. 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. 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. Only pathogenic and likely pathogenic mitochondrial variants related to disease are reported. 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
NR2F2	NM_021005.3	c.103_109del	-	Chr15:96875432-96875438	73	45.2%	.
ST3GAL5	NM_003896.3	c.353del	-	Chr2:86075293-86075293	41	43.9%	rs754643632
CBL	NM_005188.3	c.*6886G>A	-	Chr11:119177377-119177377	34	58.8%	rs542495083
FLNA	NM_001456.3	c.2904C>A	p.Ser968Arg	ChrX:153590078-153590078	57	50.9%	.
LZTR1	NM_006767.3	c.-975G>A	-	Chr22:21335686-21335686	52	50.0%	rs752742670
SLCO1B1	NM_006446.4	c.388A>G	p.Asn130Asp	Chr12:21329738-21329738	37	43.2%	rs2306283
SLCO1B1	NM_006446.4	c.521T>C	p.Val174Ala	Chr12:21331549-21331549	36	38.9%	rs4149056

Data Quality Statistics:

% Fully Covered Disease Causative Gene Target Bases	99.2
% Fully Covered Disease Causative Gene Exons	99.2
Average Coverage per Target Base	53.7

Methods and Limitations

Whole genome sequencing is performed on genomic DNA using 2X150bp reads on Illumina next generation sequencing (NGS) systems at a mean coverage of 30X in the target region. The target region includes coding exons and 10bp of flanking intronic sequence of the known protein-coding RefSeq genes. This sequencing provides >97% coverage of the 22,000 genes in the genome at >30x. 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. A list of low coverage 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 areas containing large numbers of tandem repeats. Mitochondrial DNA is sequenced and analyzed using the same pipeline. Genes and/or exons located in pseudogene regions are not covered in this assay. 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)