otogenetics® corporation

Patient: 12345678-COtGx00XX
CLIA ID#: 11D2066426
Larry Hung, MD, Laboratory Director

Gx™ Carrier Screen Testing Report

Patient Information		Provider Information		Specimen	
Patient Name		Provider		Accession ID	12345678
Date of Birth		Provider ID	- - - - -	Sample ID	COtGx00XX
Age		Physician		Specimen Type	Saliva
Sex				Collection Date	- -
Ethnicity				Report Date	
				Test Ordered	CF

Patient Results: Variant(s) of Unknown Significance (VUSs) Detected

Variant Summary

Variant Identified	Type	Genotype	dbSNP ID	Phenotype	Classification
CFTR NM_000492.3: c.1727G>C p.G576A	SNV	Heterozygous	rs1800098	Cystic Fibrosis	Uncertain Significance

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	dbSNP ID	Genotype	Assessment
CFTR	13	c.1727G>C	p.G576A	rs1800098	Het	Uncertain Significance

No description available

Additional Comments

This report is based on the analysis of CFTR gene included in the Carrier Screen. The patient is a carrier of a variant in CFTR that has been observed to be associated with CFTR-related disorders. The c.1727G>C (p.G576A) and c.2002C>T (p.R668C) variants have frequently been reported to be in cis and have also been associated with elevated sweat chloride levels and an increased risk of pancreatic insufficiency and pseudomonas infection. Chloride transport studies in cell based assays were inconclusive.6 Other studies suggest that these nucleotide changes may result in altered splicing. However, it has also been observed in health adult (homozygous). This variant has been recently classified as variant of unknown significance by reputable laboratories. CFTR-related disorders are inherited in an autosomal recessive manner. Autosomal recessive is one of the several ways that a trait, disorder, or disease can be passed down through families. An autosomal recessive disorder means two copies of an abnormal gene must be present in order for the disease or trait to develop. Traditionally, a carrier of a genetic mutation is defined as a person who inherits an altered form of a gene but shows no effects of that mutation. For autosomal recessive disorders such as CF, people with one faulty copy and one functional copy are called "carriers".
Cystic fibrosis (CF) is the most common life-limiting autosomal recessive disorder in the US population. CF occurs in all ethnic and racial populations, with the highest disease incidence in white-one in 2000–4000 live births and a disease prevalence of approximately 30,000 affected individuals in the US population; and with lower frequency in other ethnic and racial groups -one in 9200 Hispanic Americans; one in 10,900 Native Americans; one in 15,000 African Americans; one in 31,000 Asian

Americans). In the North American white population, the carrier (heterozygote) frequency is approximately one in 28. The carrier frequency is one in 29 among Ashkenazi Jews, and one in 60 among African Americans.

Followup Recommendations

Follow up with physicians for family member testing and for updated carrier screen information.

Genes Tested

Targeted regions for "Carrier Screen Testing" includes the exonic regions of the following genes: ABCC8, ABCD1, ABCD4, ACAD8, ACADM, ACADS, ACADSB, ACADVL, ACAT1, ACSF3, ACTA2, ACTC1, ADA, ADAMTS2, AGXT, AHCY, APC, APOB, ARG1, ASL, ASPA, ASS1, ATP7B, AUH, BCKDHA, BBS2, BCKDHB, BLM, BTD, CBS, COL3A1, COL4A3, CD320, CFTR, CLRN1, CPT1A, CPT2, CYP1B1, CYP21A2, DBT, DHCR7, DHDDS, DLD, DMD, DNAJC19, DSC2, DSG2, DSP, DUOX2, ETFA, ETFB, ETFDH, FAH, FANCC, FBN1, FKTN, G6PC, G6PD, GAA, GALC, GALE, GALK1, GALT, GBA, GCDH, GCH1, GJB2, GJB3, GJB6, GLA, GNMT, GRHPR, HADH, HADHA, HADHB, HBA1, HBA2, HBB, HCFC1, HEXA, HEXB, HLCS, HMGCL, HPD, HSD17B10, IDUA, IKBKAP, IL2RG, IVD, KCNH2, KCNQ1, MAT1A, MCCC1, MCCC2, MCEE, MCOLN1, MLYCD, MMAA, MMAB, MMACHC, MMADHC, MPL, MTHFR, MTR, MTRR, MTTP, MUT, MyBPC2, MYH11, MYH7, MYL2, MYL3, NEB, NPC1, NPC2, NPHS1, NPHS2, OPA3, OTC, PAH, PAX8, PC,PCBD1, PCCA, PCCB, PCDH15, PEX1, PEX2, PHGDH, PKHD1, PKP2, PMM2, POMGNT1, PRKAG2, RTE1, RYR1, RYR2, PTS, QDPR, SCN5A, SLC22A5, SLC25A13, SLC25A20, SLC26A4, SLC35A3, SLC5A5, SMN1, SMPD1, SUMF1, TAT, TAZ, TCN2, TG, TGFBR1, TGFBR2, THRA, THRB, TMEM216, TMEM43, TNNI3, TNNT2, TPM1, TPO, TSHB, TSHR, USH1b (Myo7a), USH1C

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 including ACMG and ACOG. 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.

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 (5.0.20170801), Ingenuity Knowledge Base (Narnia 170721.001), 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 170721.001), 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

El-Seedy A, Girodon E, Norez C, Pajaud J, Pasquet MC, de Becdelièvre A, Bienvenu T, des Georges M, Cabet F, Lalau G, Bieth E, Blayau M, Becq F, Kitzis A, Fanen P, Ladeveze V (2012) CFTR mutation combinations producing frequent complex alleles with different clinical and functional outcomes. Hum Mutat. 2012 Nov;33(11):1557-65. Epub 2012 Jul 2 https://www.ncbi.nlm.nih.gov/pubmed/22678879?dopt=Abstract PubMed PMID: 22678879