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

Gx™ Deafness Gene Panel Testing Report

Patient Information

Patient Name	xxxx yyyy
Date of Birth	mm dd, yyyy
Age	#
Sex	xx
Ethnicity	xx

Provider Information

Provider	Children's Hospital of xxx
Provider ID	xxxxxxxxxxxxxx
Physician	Jean Smith

Specimen

Accession ID	17######
Sample ID	COtGx####
Specimen Type	Blood
Collection Date	mmdd, yyyy
Report Date	mm dd yyyy

Patient Results: Likely Pathogenic Variant(s) Detected

Variant Summary

Variant Identified	Type	Genotype	dbSNP ID	Phenotype	Classification
GATA3 NM_001002295.1: c.681delC p.E228fs*38	Deletion	Heterozygous		hearing loss	Likely Pathogenic

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	dbSNP ID	Genotype	Assessment
GATA3	3	c.681delC	p.E228fs*38		Het	Likely Pathogenic

GATA3 belongs to a family of zinc-finger transcription factors that are involved in vertebrate embryonic development. GATA3 gene is essential in the embryonic development of the parathyroids, auditory system and kidneys [PMID: 10935639]. It is haplo-insufficient.

Evidence for Pathogenicity

- PVS1 - Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon) in a gene where loss of function (LOF) is a known mechanism of disease (Very Strong)
- PM2 - Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or gnomAD [In these sources of population frequency data, this variant's frequency is 0% or <= 0.001%] (Moderate)
- A similar variant causes hearing loss (Very strong)

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Additional Comments

GATA3 is haplo-insufficient, or mutations in one copy of the gene leads to an abnormal or disease condition. Large deletions, frame shift deletion of 49 bp from codon 156, in frame deletion of codons 316-319, a single non-sense mutation R277X, or other mutations in one copy of the GATA3 gene, leads to hypoparathyroidism, sensorineural deafness, renal anomaly (HDR) syndrome. The manifestation of renal abnormality is variable. Selected relevant publications are included at the end of this report. The GATA3 gene mutation detected in the patient, c.681delC (p.E228fs*38), is a frame shift, nonsense mutation that likely has a similar, if not stronger, effect to a known pathogenic mutation, R277X.

There are several mitochondria variants detected that could be classified as variants of unknown significance (VUS). There is not enough information at this time to support a more definitive classification of these VUSs. Note that Mitochondria DNA is maternally inherited, and likely do not contribute critically to paternally inherited conditions. The mitochondrial VUSs are: m.####C>T (p.T###I); m.####A>G (p.T###A); m.####A>G (p.T###A); m.####A>G (p.###2A).

Followup Recommendations

Follow up with your physicians for clinical correlation, family member testing, and updated information on hearing loss testing.

Genes Tested

Targeted regions for “Deafness Gene Panel” include the exonic regions of the following 167 genes: ACTB, ACTG1, AIFM1, ATP6V1B1, ATP6V1B2, BCS1L, BSND, BTD, CABP2, CATSPER2, CCDC50, CDC14A, CDH23, CEACAM16, CEMIP, CIB2, CLDN14, CLRN1, COCH, COL11A1, COL11A2, COL2A1, COL4A3, COL4A4, COL4A5, Col4A6, COL9A2, COL9A3, CRYM, DCDC2, DFNA5, DIABLO, DIAPH1, DSPP, ECE1,EDN3, EDNRA, EDNRB, ELMOD3, ERCC2, ERCC3, ESPN, ESRRB, EYA1, EYA4, FAS, FGF3, FGFR3, USH2A, FOXI1, GATA3, GIPC3, GJA1, GJB1, GJB2, GJB3, GJB4, GJB6, GPR98, GPSM2, GRHL2, GRXCR1, GRXCR2, GSTP1, HAL, HGF, HOMER2, ILDR1, JAG1, KARS, KCNE1, KCNJ10, KCNQ1, KCNQ4, KITLG, LHFPL5, LHX3, LOXHD1, LRTOMT, MARVELD2, MCM2, MET, MIR182, MIR183, miR96, MITF, MSRB3, MTAP, MT-CO1, MT-RNR1, MT-TD, MT-TE, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TM, MT-TQ, MT-TS1, MT-TS2, MYH14, MYH9, MYO15A, MYO1A, MYO1C, MYO1F, MYO3A, MYO6, MYO7A, NDP, NF2, NR2F1, OSBPL2, OTOA, OTOF, OTOG, OTOGL, OTOR, P2RX2, PAX3, PAX3, PCDH15, PDZD7, PEX7, PHYH, PJVK, PMP22, PNPT1, POU3F4, POU4F3, PRPS1, PTPRQ, RDX, RIPOR2, S1PR2, SANS, SERPINB6, SIX1, SIX5, SLC17A8, SLC26A4, SLC26A5, SLC4A11, SMPX, SNAI2, SOX10, SOX2, SPINK5, STRC, SYNE4, TBC1D24, TBL1X, TCF21, TECTA, TFCP2, TIMM8A, TJP2, TMC1, TMIE, TMPRSS3, TMPRSS5, TNC, TPRN, TRIOBP, USH1C, USH2A, WFS1, WHRN.

Methods and Limitations

Sample Processing and Sequencing and Variant Detection - This gene panel focuses on the coding exonic regions of genes. Genomic targets were identified based on information in the Human Gene Mutation Database (HGMD), the Online Mendelian Inheritance in Man (OMIM) catalog, GeneTests.org, Illumina TruSight sequencing panels, 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 a minimum average coverage depth of 100X was required.

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 for this proband. 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 in the patient 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. The current technology targets the coding exonic regions of the targeted 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.

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, GX 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 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

Van Esch H, Groenen P, Nesbit MA, Schuffenhauer S, Lichtner P, Vanderlinden G, Harding B, Beetz R, Bilous RW, Holdaway I, Shaw NJ, Fryns JP, Van de Ven W, Thakker RV, Devriendt K (2000) GATA3 haplo-insufficiency causes human HDR syndrome. Nature 2000 Jul 27;406(6794):419-22 https://www.ncbi.nlm.nih.gov/pubmed/10935639?dopt=Abstract PubMed PMID: 10935639

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Selected Citations

Wang L, Lin QF, Wang HY, Guan J, Lan L, Xie LY, Yu L, Yang J, Zhao C, Liang JL, Zhou HL, Yang HM, Xiong WP, Zhang QJ, Wang DY, Wang QJ (2017) Clinical Auditory Phenotypes Associated with GATA3 Gene Mutations in Familial Hypoparathyroidism-deafness-renal Dysplasia Syndrome. Chin Med J (Engl) 2017 Mar 20;130(6):703-709 https://www.ncbi.nlm.nih.gov/pubmed/28303854?dopt=Abstract PubMed PMID: 28303854