otogenetics® corporation

Otogenetics® Whole Exome Sequencing

Physician Name: XXXXXXXXXX	Report Date: *mm-dd-yyyy*
Patient: T_SRVY1 Name: XXXXXX DOB: *mm-dd-yyyy* Gender: Ethnicity: XXXX	Hospital / Clinic: XXXXXXX Specimen type: Saliva/Blood Date of Sample Collection: *mm-dd-yyyy*
Prepared by:	**Date:**
(signature)	*mm-dd-yyyy*

TEST ADMINISTERED:

Otogenetics® Whole Exome Sequencing (WES). Clinical grade WES is one of the most comprehensive tools for detection of rare disease causing and associated variants in an individual's DNA. Whole Exome includes all the expressed and majority of the transcribed regions in human genome, and it is believed to cover over 85% of known and to be discovered disease causing genetic variants. Purification of genomic DNA, sample preparation, enrichment for exonic regions, Illumina HiSeq next generation sequencing, variant filtration described in Appendix A, clinical interpretation.

CLINICAL INDICATION AND NOTES

Information provided indicates that this individual might have a condition caused be germline genetic variant(s).

Patient information

Clinical indication and reported phenotypes that were considered for generating this report:

Name	Description	Suspected Mode of Inheritance
Muscle breakdown	dietary therapy has failed	
elevated CK levels	25,000 - 30,000	
motor delay		
CPTII	under consideration for diagnosis, experimental oil therapy or Duchenne therapy.	

TEST RESULTS SUMMARY

Primary findings of Genetic Testing

These genetic findings have been identified by Variantyx as the most relevant to the reported phenotype(s). These variants are deemed to be potentially pathogenic as determined by a combination of curated databases of disease association, and predicted severity of the mutations. Variants were validated using another sequencing technology (Sanger sequencing) as indicated below.

Substitutions and other small (<50 bp) genetic variants:

Variant name	Type	Zygosity	Associated phenotype/ MOI	Pop. Freq.	Sanger Validation
DMD c.2463delG p.Trp821fs chrX ENST00000378677	Exonic, Frameshift	Homozygous alternate (1,39)	[Gene]:Duchenne muscular dystrophy;Becker muscular dystrophy;Dilated cardiomyopathy 3B	0.0000	YES

Zygosity/MOI: Zygosity (ref allele coverage, alt allele coverage) Mode of Inheritance

DMD p.Trp821fs
This variant is a Frameshift mutation in the DMD gene. Zygosity state is Homozygous alternate.
It is a variant with a 0.0000 maximal allele frequency in the population databases available for review.
This variant has not been previously reported in peer reviewed clinical literature, however other variants in this gene were reported to be associated with phenotype(s) listed in the Associated phenotype field in the table above. Calculated severity score is 1.00000 on a scale of 0.0 – 1.0. According to HGMD/ClinVar, the DMD gene is associated with Muscular dystrophy, Duchenne; Muscular dystrophy, Duchenne/Becker; Muscular dystrophy, Becker; Muscular dystrophy; Muscular dystrophy, intermediate, and other diseases.
Additional gene description (Uniprot): Anchors the extracellular matrix to the cytoskeleton via F-actin. Ligand for dystroglycan. Component of the dystrophin-associated glycoprotein complex which accumulates at the neuromuscular junction (NMJ) and at a variety of synapses in the peripheral and central nervous systems and has a structural function in stabilizing the sarcolemma. Also implicated in signaling events and synaptic transmission.The gene is considered essential and its tolerance score (RVIS) is in the top 11.28%.
Additional comments:

Follow up Recommendations

Recommendation for follow up actions based on this screening process include:

- Follow up with physicians/medical geneticists for updated genetic risk information. Future findings may provide new clinical interpretation of certain variants.

Test Statistics

Sample Identifier (Barcode)	XXXXX
Patient ID	XXXXXX
Amounts of DNA read	1,404,696,081
Targeted capture region	2,862,223,786
Total number of mutations compared to HG19	6,760,068
Bases in targeted exons with < 8X coverage	28,339,938
HGMD or ClinVar annotated locations < 8X coverage	509
Average coverage	57
Median coverage	56

Appendix A

Test Details - Methods and Limitations

General information

This genetic test report is based on analysis of raw data of Whole Exome Sequencing performed by Otogenetics. The report is intended for clinical diagnostics use. The sequencing protocol performed on the samples is of clinical grade, it is CLIA/CAP compliant, and is certified for diagnosis use. The primary purpose of this report is to communicate variants with strong evidence supporting their association to the reported phenotype(s). Incidental germline findings that do not correlate with the provided phenotype(s) are included in this report in the Incidental Findings section, if elected to be included in the report by the patient or legal guardian. Not all detected variants have been analyzed, and not all regions of the genome have been adequately sequenced. These results should be interpreted in the context of the patient's medical evaluation, family history, and genealogy. Please note that variant classification and/or interpretation may change over time as more information becomes available. Sanger validation status, when applicable, is indicated per variant in the tables above.

Sequencing and Variant Detection

Genomic DNA was extracted from clinical sample (saliva or blood), 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 the Illumina HiSeq 2500, with 100-125 bp reads, sequence QC metrics were required, and a

minimum average coverage depth of 100X was required. Sequencing reads were aligned to the reference genome (UCSC hg19) by BWA-MEM and variants were called using GATK 3.6. The minimum sequence depth for all targeted exons was evaluated; further validation is recommended for exons with depth of coverage <10x. We recommend that variants of interest which do not meet the coverage minimum be confirmed clinically before treatment is undertaken.

Variant Analysis and Report Generation

Reported variants were filtered to include those present in the exonic regions and adjacent splice sites. Resulting variants were analyzed and reported using the Variantyx Genomic Intelligence platform. To maintain most up-to-date annotations, the Variantyx database is updated quarterly. As a result, variant classification and/or interpretation may change over time as more information becomes available.
The following databases and tools are included in Variantyx Genomic Intelligence platform:

1. Disease association: HGMD Professional (http://www.hgmd.cf.ac.uk/), Genome Trax (http://www.biobase-international.com/product/genome-trax), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), OMIM (http://www.omim.org/), Orphanet (www.orpha.net/), GeneTests (https://www.genetests.org/).
2. Population frequencies: dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), ensemble (www.ensembl.org/), 1000 Genomes Project (www.1000genomes.org/), ExAC (http://exac.broadinstitute.org/), NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) and the Variantyx allele frequency database (http://variantyx.com/).
3. Severity predict ion: SIFT, MutationAssessor, Mutation Taster, GWAVA, PolyPhen2, FATHMM, Silva, LRT.
4. Conservation prediction: SiPhy, GERP++, PhyloP and PhastCons.
5. Gene Essentiality: According to published work 10.1371/journal.pgen.1003484
6. Gene tolerance: RVIS score, according to published work 10.1371/journal.pgen.1003709

Secondary/Incidental Sequence Variant(s) based on ACMG guidelines are not included in this report.

Not all mutations compared to the reference sequence have been listed on this report. Mutations were identified using the filters described below. These mutations were further reviewed by a medical geneticist, and only variations of clinical significance (primary findings) are included in this report.

Filters included:
Description of the filters used in preparation of this report can be for at the following url:
http://www.otogenetics.com/inherited-cancer-testing

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. The current technology targets the coding exonic regions of the 39 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.

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.

CLIA Statement

Otogenetics: 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.

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