GENETIC TESTING RESULTS
Mitochondrial Genome Testing

bionano laboratories

Patient Name: SAMPLE	**Provider Name:** SAMPLE	**Sample Type:** Buccal
Patient Date of Birth: SAMPLE	**Provider Phone:** SAMPLE	**Sample Collected:** SAMPLE
Patient Sex: SAMPLE	**Provider Fax:** SAMPLE	**Sample Received:** SAMPLE
Patient ICD-10 code(s): SAMPLE	**Provider Institution:** SAMPLE	**Order Date:** SAMPLE
Accession Number: SAMPLE		**Report Date:** SAMPLE

Test Result Summary: Pathogenic (SAMPLE)

Mitochondrial DNA sequencing with deletion and duplication analysis identified one sequence variant. The variant in the *MT-RNR1* gene is clinically significant and is consistent with mitochondrial nonsyndromic hearing loss.

Mitochondrial DNA Sequencing and Deletion Analysis Results

Gene (Transcript)	Alteration	Percent Heteroplasmy	Classification	Associated Syndrome
MT-RNR1 (chrM:1555)	m.1555A>G	100%	Pathogenic	Mitochondrial nonsyndromic hearing loss

Clinical Interpretation and Discussion

The Mitochondrial DNA sequencing with deletion and duplication analysis test identified one sequence variant. The sequence variant reported in the *MT-RNR1* gene is clinically significant (pathogenic) and is consistent with mitochondrial nonsyndromic hearing loss. Below is a summary of the current evidence about this finding as well as details about genetic counseling options available to the family.

All sequence variants reported are classified based on the American College of Medical Genetics (ACMG) standards and guidelines (PMID: 25741868).

MT-RNR1 gene, m.1555A>G, pathogenic

A pathogenic sequence variant in the *MT-RNR1* gene was found. This sequence variant is consistent with mitochondrial nonsyndromic hearing loss and is expected to result in an increased risk of the associated symptoms.

The *MT-RNR1* gene is located in the mitochondrial DNA (mtDNA). Mitochondrial DNA is separate from the DNA located in the nucleus. It is unique as there are multiple mitochondria in a single cell so there are multiple copies of mtDNA. The *MT-RNR1* variant is present in 100% of the mtDNA tested in the sample provided. Because the variant is present in all the mtDNA tested, it is considered homoplasmic. When the variant is only present in a portion of the mtDNA tested, it is considered heteroplasmic. The portion of the sample a mtDNA variant is present in can differ between tissue types (such as blood, muscle, or skin) in a single individual. It is unknown at what proportions this variant is present in other tissues, as buccal was the only tissue tested. The proportion of mtDNA with a sequence variant must exceed a critical threshold level before a cell expresses a biochemical abnormality of the mitochondrial respiratory chain (PMID: 9239539).

SAMPLE

Genetic variants affecting the *MT-RNR1* gene cause mitochondrial nonsyndromic hearing loss. Individuals with the m.1555A>G variant develop hearing loss within a few days to weeks following any amount of aminoglycoside antibiotic exposure; however, individuals have also been reported to develop hearing loss even without aminoglycoside exposure (PMIDs: 20301595, 20100600, 20111055). The hearing loss is bilateral, severe to profound, and sensorineural in nature. Approximately, 40% of individuals not exposed to aminoglycosides still develop hearing loss by 30 years of age. Reduced penetrance, meaning not all individuals with the variant develop symptoms, has been documented in several families (PMID: 27654872). Clinical correlation is recommended.

Typically, no other health or developmental concerns are known to be associated with this variant. However, there are a couple reports in the medical literature of families with additional symptoms other than hearing loss.

- There is one large Filipino family reported with the 1555A>G variant who not only had hearing loss but also premature greying, depigmented patches, and spinal anomalies (PMID: 10661905). It is unknown if this variant was the cause of these other features as this association has only been reported in one family.
- Additionally, there is another report of a 35-year-old woman with restrictive cardiomyopathy starting in early adulthood; her brother was also affected as was one of her daughters who had transient valvular heart disease in early childhood (PMID: 9915970). Further, the woman's mother and grandmother both died suddenly in their 30s of cardiac failure. The 1555A>G variant was heteroplasmic in all these relatives.

It is unknown if this individual would have an increased risk for any of these symptoms as these are only two reports. Additional studies are needed before any conclusions can be made about this variant's role in other symptoms aside from hearing loss.

Although not determined from this testing, mtDNA variants are typically inherited from the mother as mitochondria are transmitted from the egg rather than from sperm cells. Mothers with mtDNA variants may or may not have symptoms of mitochondrial disease. All children of a female with a homoplasmic mtDNA variant will inherit the mtDNA variant, although the heteroplasmy (percentage of mtDNA that harbors the variant) varies and consequently, the presence and severity of symptoms also vary. Alternatively, it is also possible this variant is *de novo* (not inherited).

This variant was found in 74 of 56,401 (0.13%) individuals (63 homoplasmic, 0.11%; 11 heteroplasmic, 0.02%) in gnomAD, a database of individuals without severe genetic conditions (PMID: 32461654). This variant has been reported in 80 of 56,910 (0.14%) individuals in MITOMAP, a database of mtDNA variants (PMID: 25489354). It was also found in 297 of 195,983 (0.15%) individuals (246 homoplasmic, 0.13%; 51 heteroplasmic, 0.03%) in the Helix database, which contains genetic data from the general population (doi:10.1101/798264). IF APPLICABLE: This variant has been reported as pathogenic by five submitters in ClinVar, a database that curates genetic information from individuals with clinical features (Variation ID: 9628; PMID: 26582918), and has been reviewed by an expert panel. This variant is a well-established as pathogenic and seen with varying prevalence worldwide (PMID: 20301595).

Summary
This result alone may not provide a genetic diagnosis; however, follow up testing or evaluations may be recommended. Comparison of this individual's symptoms with symptoms of the conditions in this report may be useful in determining concern or follow up evaluations. Evaluation with a medical geneticist or discussion with a genetic counselor may be considered to determine if additional testing or assessments would be useful.

References

The references in this report can be found by searching the PubMed IDs (PMID) from the PubMed home page: http://www.ncbi.nlm.nih.gov/pubmed/

For more information on how to use PubMed, see the following tutorial:
http://www.nlm.nih.gov/bsd/disted/pubmedtutorial/cover.html

Genetic Counseling and Family Resources

Mitochondrial DNA sequencing with deletion and duplication analysis was completed. This test looks for genetic changes (variants) in the DNA located in the mitochondria. Some types of genetic changes may be responsible for a person's developmental and/or medical symptoms. Since everyone has genetic changes, the genetic community is continuously learning what variants in which genes cause symptoms and which variants do not (called benign variants).

Mitochondrial DNA and Heteroplasmy

Mitochondria are structures in a cell often described as the "powerhouse" of a cell because they provide most of the energy needed for a cell to function. Mitochondria are unique because they have their own DNA that is separate from the DNA in the nucleus of the cell. This mitochondrial DNA (mtDNA) in conjunction with nuclear DNA provides important instructions for how to make energy. This is why a separate test that looks specifically at the mtDNA is needed if a mitochondrial condition is suspected. Mitochondrial DNA results are often complex and challenging to interpret due to a phenomenon known as heteroplasmy. Each cell has multiple mitochondria which means they have multiple copies of mtDNA. The number of mitochondria a cell has depends on how much energy the cell needs to function. For example, a muscle or nerve cell has many mitochondria because it requires a lot of energy, while a skin cell has fewer mitochondria because it requires less energy. Sometimes these mtDNA copies are different from each other, meaning some mtDNA will have genetic variants while others do not within the same cell. Further, mtDNA variants can differ between different tissues in a single individual (such as blood, muscle, or skin tissue). The mixture of normal mtDNA and variant mtDNA is called heteroplasmy. The amount (percentage) of heteroplasmy and the type of tissues harboring the mtDNA variant determines the severity and type of symptoms an individual may have. This means individuals with the same mitochondrial variant can have different symptoms.

Unlike nuclear DNA, which is inherited from both parents, mitochondrial DNA is only inherited from the mother. This is because mitochondria are only present in the egg cell and not the sperm cell. Mothers may or may not have symptoms of the mitochondrial condition depending on the level of heteroplasmy. Importantly, if a mother has a variant in the mtDNA, all of her children will inherit that variant. However, the amount of heteroplasmy will vary in each child and consequently, each child may or may not be affected or may have different symptoms from others in the family.

You can schedule a time to speak with a Bionano Laboratories genetic counselor about these test results by calling 801-931-6191. A genetic counselor can help review what these results mean for an individual and family members, background information on genetics, and discuss additional resources or next steps that may be helpful. Additionally, the genetic counselor may review medical, developmental, and family history of the person tested. This will help the genetic counselor better answer questions about how a specific result may impact the family. To best prepare for a genetic counseling session, it may be helpful for a family to create a list of questions. Additionally, it may be helpful to review some information on basic genetic concepts such as genes and genetic changes. The following resource(s) are a good place to start for information about a variety of genetic topics:

MedlinePlus: This website can be used to find information about genetic topics, genes, specific genetic conditions, and broad topics like autism spectrum disorder. It also provides links to other websites for more in-depth information about genetic conditions, patient support and advocacy resources, and relevant clinical trials. https://medlineplus.gov/genetics

United Mitochondrial Disease Foundation https://www.umdf.org/

Hands & Voices https://handsandvoices.org/

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National Association of the Deaf https://www.nad.org/

Genes Tested

The entire mitochondrial DNA genome, including the Control Region, 37 genes and 16,569 nucleotides MT-ATP6, MT-ATP8, MT-CO1, MT-CO2, MT-CO3, MT-CYB, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6, MT-RNR1, MT-RNR2, MT-TA, MT-TC, MT-TD, MT-TE, MT-TF, MT-TG, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TM, MT-TN, MT-TP, MT-TQ, MT-TR, MT-TS1, MT-TS2, MT-TT, MT-TV, MT-TW, MT-TY

Bionano Laboratories' Clinical Interpretation Process

Variants identified by Mitochondrial DNA sequencing with deletion and duplication analysis are analyzed with a comprehensive interpretation process. Guidelines from the American College of Medical Genetics and Genomics (ACMG) are applied to variants reported (PMID: 25741868). The interpretation process involves collaboration between experts including laboratory directors, variant analysts, and certified genetic counselors. This team completes an up-to-date review of the medical literature, patient databases (PMIDs: 26582918, 12754702) and control datasets (PMIDs: 24174537, 32461654). This process provides comprehensive information about the current evidence for genes and specific sequence variants within them. The relevant evidence from this interpretation process is summarized in each report.

Methodology and Limitations

METHODS: Genomic DNA was isolated from the submitted specimen indicated above (if cellular material was submitted). Mitochondrial DNA was amplified by PCR using four sets of overlapping primers so that all 16,569 nucleotides were covered. Prepared DNA libraries from the amplified mitochondrial DNA were then sequenced using a Next Generation Sequencing technology. The minimum sequencing coverage for every nucleotide in the mitochondrial DNA is 1000X; the mean coverage over the entire mitochondrial DNA in this specimen is at least 10,000X. Sequences were aligned to the Revised Cambridge Reference Sequence (rCRS) for mitochondrial DNA. Variants were interpreted manually using locus specific databases, literature searches, and other molecular biological principles. Only variants classified as pathogenic, likely pathogenic, or unknown significance which are thought to be related to the patient's phenotype or test indication are reported. Bioinformatics: Fulgent Germline Pipeline v2019.1 was used to generate variant calls for this test.

LIMITATIONS: All laboratory tests have limitations. These results assume that the specimen received in the laboratory belongs to the named individual and that no mix-up or co-mingling of specimens has occurred. Positive results do not imply that there are no other pathogenic alterations in the patient's genome, and negative results do not rule out a genetic cause for the indication for testing. This assay assumes that any stated familial relationships are accurate. This assay has been validated for the detection of mitochondrial DNA heteroplasmy at a minimum level of 2-5%. Official gene names change over time. Fulgent uses the most up to date gene names based on HUGO Gene Nomenclature Committee (https://www.genenames.org) recommendations. If the gene name on report does not match that of ordered gene, please contact the laboratory and details can be provided. Result interpretation assumes that the human reference sequences are correct at the queried loci. Result interpretation is based on the collected information available at the time of reporting; additional information may exist in the future which will not be represented. All sequencing technologies have limitations. There are technical limitations on the ability of DNA sequencing to detect small insertions and deletions. Our laboratory uses a sensitive detection algorithm, however these types of alterations are not detected as reliably as single nucleotide variants. Rarely, due to systematic chemical or computational issues, or human error, DNA variants may be missed.

Disclaimer: This test was developed and its performance characteristics determined by Fulgent Genetics CAP #8042697 CLIA #05D2043189; 4978 Santa Anita Ave., Suite 205, Temple City, CA 91780. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. Since genetic variation, as well as systematic and technical factors, can affect the accuracy of testing, the results of testing should always be interpreted in the context of clinical and familial data. It is recommended that patients receive appropriate genetic counseling to explain the implications of the test result, including its residual risks, uncertainties and reproductive or medical options. Fulgent CLIA Laboratory Director: Hanlin Gao, M.D.

Lineagen, Inc.'s (DBA Bionano Laboratories) reporting service is certified under CLIA (CLIA# 46D2042721).
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