PRELIMINARY REPORT

The HudsonAlpha CLINICAL SERVICES LAB

601 Genome Way, Room 3001, Huntsville, AL 35806

Phone: (256) 327-9670 Fax: (256) 327-9760

www.clinicallab.org

Patient Name	John Doe	**Ordering Physician**	Dr. Gene	**Specimen Type**	Blood
Date of Birth	01/01/2021	**Date of Report**	01/11/2021	**Test Type**	WGS
Race/Ethnicity	African American	**Sample Collection Date**	01/04/2021	**Accession #**	123456
Gender	Male	**Sample Received Date**	01/05/2021	**Provider Accession ID**	ABCDEF
		Test Request Date	01/05/2021		

Clinical Indications

The patient is a ten-day-old African American male with a history of congenital hypotonia, areflexia, ventilator dependency, multiple fractures, decreased bone density, mildly dysmorphic features, and low APGAR scores at birth. We were requested to perform STAT trio whole genome sequencing on samples from the patient, mother, and father.

Results Summary of Primary Findings

The following variant(s) were identified in gene(s) related to the indication for testing.

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification[1]
RYR1, NM_000540.2	Interior Intron, Canonical 5' Splice Site	chr19:38500727G>C (GRCh38) c.7444+1G>C	Heterozygous, Paternal	Congenital RYR1-Associated Myopathy (Laforgia, 2018)	Autosomal Recessive	Pathogenic
RYR1, NM_000540.2	Interior Intron, Non-Canonical 3' Splice Site	chr19:38523211C>G (GRCh38) c.10348-6C>G	Heterozygous, Maternal	Congenital RYR1-Associated Myopathy (Laforgia, 2018)	Autosomal Recessive	Pathogenic

[1] Based on ACMG guidelines (Richards, 2015)

Interpretation and Recommendation Summary for Primary Findings

The patient is compound heterozygous for two potentially clinically significant variants in RYR1. Pathogenic variants in this gene are associated with congenital RYR1-associated myopathy. Congenital RYR1-associated myopathy overlaps with this patient's reported presentation, suggesting that this individual has this disease. One variant was inherited from each parent. Orthogonal confirmation of the variants is pending.

Congenital RYR1-associated myopathy can present with variable severity (Laforgia, 2018; Helbling, 2019; Alkhunaizi, 2019; Bharucha-Goebel, 2013).

These results should be interpreted in the context of the patient's medical evaluation, family history, and racial/ethnic background. Genetic counseling is recommended to discuss the implications of this report. Please note that variant classification and/or interpretation, as well as our knowledge of the genome, will change over time as more information becomes available. Therefore, reanalysis of the genome at regular intervals should be considered.

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Other Variants of Medical Significance (Secondary Findings)

The following variant(s) were identified in gene(s) not related to the indication for testing.

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification[1]
No secondary or incidental findings requested.						

[1] Based on ACMG guidelines (Richards, 2015)

Interpretation and Recommendation Summary for Secondary Findings

These results should be interpreted in the context of the patient's medical evaluation, family history, and racial/ethnic background. Genetic counseling is recommended to discuss the implications of this report. Please note that variant classification and/or interpretation, as well as our knowledge of the genome, will change over time as more information becomes available. Therefore, reanalysis of the genome at regular intervals should be considered.

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Detailed Description of Primary Findings

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification
RYR1, NM_000540.2	Interior Intron, Canonical 5' Splice Site	chr19:38500727G>C (GRCh38) c.7444+1G>C	Heterozygous, Paternal	Congenital RYR1-Associated Myopathy (Laforgia, 2018)	Autosomal Recessive	Pathogenic

Genomic Position	Variant Frequency
chr19:38500727G>C (GRCh38)	Not identified in large population studies

Variant Interpretation and Disease Information

The patient is compound heterozygous for two potentially clinically significant variants in RYR1. Pathogenic variants in this gene are associated with congenital RYR1-associated myopathy.

The c.7444+1G>C variant in the RYR1 gene has not been published, to our knowledge. The c.7444+1G>C is a canonical splice site variant, which is predicted to result in loss of function in the RYR1 gene where loss of function is a known mechanism of congenital RYR1-associated myopathy. The Genome Aggregation Database (gnomAD) reports that the c.7444+1G>C variant was not observed; consistent with the expected frequency of a pathogenic variant in congenital RYR1-associated myopathy. The c.7444+1G>C variant was observed to be in trans with a pathogenic variant based on segregation analysis in the family. In summary, this collective evidence supports c.7444+1G>C as a pathogenic variant for congenital RYR1-associated myopathy. The variant was inherited from the patient's father.

Congenital RYR1-associated myopathy arises from the presence of a pathogenic variant in each copy of RYR1. Two potentially clinically significant variants were detected in RYR1. Segregation studies indicate that these variants are on different copies of RYR1.

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification
RYR1, NM_000540.2	Interior Intron, Non-Canonical 3' Splice Site	chr19:38523211C>G (GRCh38) c.10348-6C>G	Heterozygous, Maternal	Congenital RYR1-Associated Myopathy (Laforgia, 2018)	Autosomal Recessive	Pathogenic

Genomic Position	Variant Frequency
chr19:38523211C>G (GRCh38)	4/31396 of chromosomes in gnomAD Genome 5/251458 of chromosomes in gnomAD Exome

Variant Interpretation and Disease Information

The patient is compound heterozygous for two potentially clinically significant variants in RYR1. Pathogenic variants in this gene are associated with congenital RYR1-associated myopathy.

The c.10348-6C>G variant in the RYR1 gene has been previously reported in patients with congenital RYR1-associated myopathy (Bevilacqua, 2011; ClinVar Variation ID 132994; Monnier, 2008; Wilmshurst, 2010). The c.10348-6C>G variant has been shown to damage the gene or gene-product based on a well-established functional assay, splicing assay (Monnier, 2008). The c.10348-6C>G variant has been observed in multiple, unrelated, affected individuals with congenital RYR1-associated myopathy (Bevilacqua, 2011; Monnier, 2008; Wilmshurst, 2010). The Genome Aggregation Database (gnomAD) reports that the c.10348-6C>G variant was observed in 4/31396 and 5/251458 alleles for genome and exome data, respectively; consistent with the expected frequency of a pathogenic variant in congenital RYR1-associated myopathy. The c.10348-6C>G variant was observed to be in trans with a pathogenic variant based on segregation analysis in the family. The c.10348-6C>G variant has been reported as pathogenic by a reputable source (ClinVar Variation ID 132994). In summary, this collective evidence supports c.10348-6C>G as a pathogenic variant for congenital RYR1-associated myopathy. The variant was inherited from the patient's mother.

Congenital RYR1-associated myopathy arises from the presence of a pathogenic variant in each copy of RYR1. Two potentially clinically significant variants were detected in RYR1. Segregation studies indicate that these variants are on different copies of RYR1.

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Detailed Description of Secondary Findings

No secondary or incidental findings requested.

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Alkhunaizi E, Shuster S, Shannon P, et al. Homozygous/compound heterozygote RYR1 gene variants: Expanding the clinical spectrum. Am J Med Genet A. 2019;179(3):386-396.

Bevilacqua JA, Monnier N, Bitoun M, et al. Recessive RYR1 mutations cause unusual congenital myopathy with prominent nuclear internalization and large areas of myofibrillar disorganization. Neuropathol Appl Neurobiol. 2011;37(3):271-84.

Bharucha-Goebel DX, Santi M, Medne L, et al. Severe congenital RYR1-associated myopathy: the expanding clinicopathologic and genetic spectrum. Neurology. 2013;80(17):1584-9.

Helbling DC, Mendoza D, McCarrier J, et al. Severe Neonatal RYR1 Myopathy With Pathological Features of Congenital Muscular Dystrophy. J Neuropathol Exp Neurol. 2019;78(3):283-287.

Laforgia N, Capozza M, De Cosmo L, et al. A Rare Case of Severe Congenital RYR1-Associated Myopathy. Case Rep Genet. 2018;2018:6184185.

Monnier N, Marty I, Faure J, et al. Null mutations causing depletion of the type 1 ryanodine receptor (RYR1) are commonly associated with recessive structural congenital myopathies with cores. Hum Mutat. 2008;29(5):670-8.

Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.

Wilmshurst JM, Lillis S, Zhou H, et al. RYR1 mutations are a common cause of congenital myopathies with central nuclei. Ann Neurol. 2010;68(5):717-26.

https://www.ncbi.nlm.nih.gov/clinvar/variation/132994

Sequencing and Analysis Methodology and Limitations

Whole Genome Sequencing was performed by HudsonAlpha Clinical Services Lab, LLC, an Alabama limited liability company (“CSL”), on the provided sample(s) using the Illumina NovaSeq 6000 sequencing platform. DNA was measured for integrity via gel electrophoresis and appropriate concentration via fluorescence concentration determination. The DNA was then sonicated to a specific fragment size and prepared as a paired-end library with ligation of Illumina flowcell-specific adapter sequences and a unique barcode. The prepared library was then quality checked for adequate yield through fluorescence methods and quantitative polymerase chain reaction (PCR), as well as for appropriate library size and profile using bioanalysis. Libraries were clustered onto Illumina NovaSeq 6000 flowcells and sequenced using standard Illumina reagents and protocols.

After sequencing, reads were generated using Illumina’s bcl2fastq and data were aligned to the human reference GRCh38. Quality metrics were evaluated at each stage of sample preparation and analysis to ensure quality data. Fragments mapping to multiple regions of the reference genome were removed from analysis, as were fragments having a low quality score. Duplicate fragments were removed from analysis. Variant calling then proceeded using unique, quality mappings.

Sequence variants were loaded into a custom software analysis application called Codicem for interpretation. Within Codicem all sequence variants were annotated with relevant reference information from established data sources to

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provide support for the interpretations set forth in this test report. A listing of data sources is available upon request. Observed variants of interest were confirmed using an orthogonal sequencing technology (Sanger (dideoxy) sequencing). Certain individually validated loci are not confirmed by Sanger (dideoxy) sequencing. This list is available upon request.

The ability of this test to identify abnormal variants (the analytical sensitivity) is dependent on the presence of the variants in the sequencing data provided to Codicem for evaluation. Statistical analysis shows that a minimum coverage of seven reads at a locus is required to reliably call heterozygous variants with a power equal to or greater than 99%. This depth of coverage is expected to yield an error rate comparable to the error rate that occurs in Sanger dideoxy terminator sequencing.

Regions of the human genome exist that are unable to be resolved by current technology, or are duplications of other regions which make accurate alignment difficult. Regions of the genome not fully known or where alignment is not yet possible are not reported by this test. Using this technology, it is only possible to sequence 90% to 95% of the human reference genome. The process uses an over-sequencing approach to achieve an average depth of coverage to support accurate variant calling over most regions of the genome. Analysis of the sequencing depth of all known coding regions of the genome is available upon request. A gene with insufficient coverage may harbor variants that are not detected by this test. A gene may also appear to have inadequate coverage when there is a deletion or insertion in the individual's gene sequence compared to the reference sequence.

This genome sequence test is designed to evaluate single nucleotide variants and small insertions or deletions within the human genome. This test is limited in its ability to detect mosaicism and chimerism. This technology is limited in its ability to accurately identify variants occurring in regions with high sequence identity to other regions of the genome (e.g. paralogous genes and pseudogenes). Certain types of sequence variation are difficult to identify using this test and have not been validated to be reliably detected for current clinical use. These include insertions, deletions, copy number variations, long repeat sequences, triplet repeat expansions, structural chromosomal rearrangements, polyploidy, aneuploidy, repetitive regions such as mono-, di- and trinucleotide repeats, GC rich regions, intronic variants outside of canonical splice-sites, and epigenetic effects.

The clinical sensitivity of this test is affected by the genetic disorder sought in the patient. As WGS is used in the diagnostic evaluation of many different disorders, it is not possible to assign a single clinical sensitivity.

Primary/Secondary Bioinformatics Pipeline Version: DRAGEN Bio-IT platform (version 3.5.7)

Data Store Version: 0.21.1

Codicem Version (Annotation): 5.0.1

Codicem Version (Report): 5.0.1

Interpretation Limitations

The interpretations set forth in this report assume that all information provided to the CSL, including any family relationships and all information stated on the sample submission or test requisition form, are accurate and fully answered. Because of the limitation of today's sequencing technology and scientific knowledge, a genetic abnormality may still exist even if a variant is not included in this report. If specific clinical disorders are suspected, specific evaluation of known genes by alternate test methods should be considered. False positive, negative or misleading results may occur for reasons that include genetic variants, blood transfusions, bone marrow transplantation, erroneous representation of family relationship, or contamination of a specimen. However, the CSL

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has standard and effective procedures in place to protect against such technical and operational problems.

The interpretations set forth in this report are based only upon current scientific knowledge and technology. Each gene sequence is interpreted independently of all other gene sequences. This test attempts to use current scientific knowledge to identify possible genetic variants; however, current scientific knowledge about the function of variants, genes, and other portions of the genome, and the ways in which genetic disorders are inherited, is incomplete. For example, variants in different genes may sometimes interact to cause disease, and variants may modify the phenotype of a monogenic disease. It cannot be excluded that pathogenic variants were missed due to limitations inherent in the sequence analysis method used in this test.

These results should be interpreted in the context of an individual's medical evaluation, family history, and racial/ethnic background. The data, interpretations and results of this test are not intended to recommend or discourage any specific treatment plan, product or course of action in an individual's medical care.

The clinical implications of some variants may be uncertain at the time of analysis. As clinical testing and research continue, some variants which are presently identified as "variants of uncertain significance" in this report may later be identified as pathogenic or non-pathogenic variations based on advances in medical knowledge and new discoveries. Variants of uncertain significance have uncertain effects on gene function, have not been previously reported, or have been reported with inadequate or conflicting evidence regarding pathogenicity or clinical relevance. As such, please note that variant classification and/or interpretation may change over time if and when more information becomes available. Therefore, it is possible that re-interpretation of these test results after any scientific advancement related to a variant of uncertain significance could lead to new information about a medical condition or clinical disorder an individual may be experiencing. Such re-interpretation must be requested by an individual's health care provider, and will involve additional costs.

The identification of some variants, including those associated with disease, is limited by the current state of knowledge in the genomics field and the annotations of variants in currently available public and private databases.

Whole genome sequencing is often not able to identify the cause of an individual's medical issues, provide information about prognosis, disease severity or help guide medical screening or treatment. If these test results did not identify a genetic cause for a medical condition, it is still possible a genetically-determined, medical condition could exist. Therefore, these results should be supplemented with genetic counseling regarding the possible genetic findings and potential implications of the genetic information contained herein.

Variants found in the individual that are benign or likely benign, as identified in the medical literature based on ACMG criteria, are not generally reported but may be available upon request.

This test was developed, and the associated orthologous methods and performance characteristics were determined by, the CSL. It has not been cleared or approved by the U.S. Food and Drug Administration. To date, the U.S. Food and Drug Administration has determined that such clearance or approval is not necessary. These tests are used for clinical purposes, and therefore validation was done as required under the requirements of the Clinical Laboratory Improvement Act of 1988. These test results should not be regarded as investigational or for research. The chance of a false negative or a false positive result due to laboratory error cannot be completely excluded.

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CAP #8051488

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Signatures

David Bick, MD, Laboratory Director, Clinical Services Laboratory — Electronically signed by David Bick on 01/11/2021