Rady Children's Institute Genomic Medicine

Clinical Genome Center
7910 Frost Street, Suite 240
San Diego, CA 92123

RCIGM_rWGS@rchsd.org
P: 858 / 966-8127
F: 858 / 966-8092

CLIA ID# 05D2129627
CAP ID# 9487427

RCIGM Genome Report

PATIENT NAME	Patient Genetics	ORDERING PHYSICIAN	Dr. John Doe	RCIGM CASE ID	CSXXXX_INXXXX
SEX	Female	HOSPITAL	Rady Children's Hospital San Diego	MOTHER ID	CSXXXX_INXXXX
DATE OF BIRTH	01/01/2022	SPECIMEN	Blood	FATHER ID	CSXXXX_INXXXX
INDICATION FOR TESTING	Suspected Genetic Condition	COLLECTED	01/24/2022	PRELIMINARY REPORT DATE	01/26/2022
TEST TYPE	Trio Whole Genome Sequencing	RECEIVED	01/25/2022	REPORT DATE	01/31/2022

PATIENT PHENOTYPE

Seizure; Lethargy; Hypertonia; Small for gestational age; Patent foramen ovale; Weight loss; Thrombocytopenia; Anemia; Metabolic acidosis; Dehydration; Subarachnoid hemorrhage; EEG abnormality; Increased CSF lactate; Hyponatremia; Lactic acidosis; Abdominal distention; Concern for molybdenum cofactor deficiency; Respiratory failure requiring assisted ventilation; Feeding difficulties; Meconium stained amniotic fluid; Sepsis; Brain imaging abnormality

TEST RESULT: PRIMARY FINDINGS IDENTIFIED

Sequence Variants

REPORT CATEGORY	GENE	VARIANT	CONDITION	ZYGOSITY (INHERITANCE)	VARIANT CLASSIFICATION
VARIANTS RELATED TO PATIENT PHENOTYPE	CFTR	c.1521_1523del p.Phe508del	CYSTIC FIBROSIS	Heterozygous (maternal)	Pathogenic
VARIANTS RELATED TO PATIENT PHENOTYPE	CFTR	c.1585-1G>A	CYSTIC FIBROSIS	Heterozygous (paternal)	Pathogenic

*Details on the variant(s) and gene(s) are located in the subsequent sections of the report

VARIANTS RELATED TO PATIENT PHENOTYPE

CONFIRMATION STATUS	GENE (TRANSCRIPT)	CONDITION	GENOMIC COORDINATES	VARIANT	ZYGOSITY (INHERITANCE)	CLASSIFICATION
Not Required	CFTR (ENST00000003084)	CYSTIC FIBROSIS	7:117199644	c.1521_1523del p.Phe508del	Heterozygous (maternal)	Pathogenic
Not Required	CFTR (ENST00000003084)	CYSTIC FIBROSIS	7:117227792	c.1585-1G>A	Heterozygous (paternal)	Pathogenic

Variant 1 Information

A heterozygous c.1521_1523del (p.Phe508del) variant in CFTR was detected in this individual. This variant has been previously reported as the most common pathogenic variant in the CFTR gene, accounting for an estimated 30%-80% of pathogenic variants in cystic fibrosis (CF) (PMID: 20301428). This variant has been reported in the ClinVar database (Variation ID: 7105). Functional studies have demonstrated that this variant disrupts the normal function of the protein (PMID: 2475911). It is present in the heterozygous state in the gnomAD population database at a frequency of 0.72% (2027/282630) and thus is presumed to be rare. Analysis of the parental samples showed the mother is heterozygous and the father is negative for this variant. Based on the available evidence, the c.1521_1523del (p.Phe508del) variant is classified as Pathogenic.

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Variant 2 Information

A heterozygous c.1585-1G>A variant in CFTR was detected in this individual. This variant has also been reported in the literature as c.1717-1G>A. This variant affects the canonical splice acceptor site of intron 10 and is therefore predicted to interfere with splicing and result in loss of normal protein function through either protein truncation or nonsense-mediated mRNA decay (NMD). This variant has been previously reported as a common pathogenic variant in individuals with cystic fibrosis (CF) (PMID: 2236053, 23974870, 20301428). This variant has been reported in the ClinVar database (Variation ID: 7112). Functional studies have demonstrated that this variant results in aberrant transcription of the CFTR gene (PMID: 25066652). It is present in the heterozygous state in the gnomAD population database at a frequency of 0.0071% (20/282220) and thus is presumed to be rare. Analysis of the parental samples showed the mother is negative and the father is heterozygous for this variant. Based on the available evidence, the c.1585-1G>A variant is classified as Pathogenic.

Gene Information

The CFTR gene is located on chromosome 7q31.2 and encodes a protein called the cystic fibrosis transmembrane conductance regulator (CFTR). This protein functions as a channel that transports chloride ions into and out of cells, which is necessary for the production of mucus in the lining of the airways, digestive system, reproductive system, and other organs and tissues (PMID: 16554808). Pathogenic variation in the CFTR gene is associated with CFTR-Related Disorders (MIM: *602421), including autosomal recessive Cystic Fibrosis (CF) (MIM: #219700) and autosomal recessive Congenital Absence of the Vas Deferens (CAVD) (MIM: #277180). CF is a multisystem disorder affecting epithelia of the respiratory tract, exocrine pancreas, intestine, hepatobiliary system, and exocrine sweat glands (PMID: 20301428). CF is classically described as a triad of chronic obstructive pulmonary disease, exocrine pancreatic insufficiency, and elevation of sodium and chloride concentration in sweat. Almost all males with CF are infertile due to congenital bilateral absence of the vas deferens. CAVD is characterized by male infertility as a result of hypoplasia or aplasia of the vas deferens and seminal vesicles may occur either bilaterally or unilaterally. CAVD can occur alone or as a sign of cystic fibrosis (PMID: 20301428, 27189798). Additional information and clinical management of Cystic Fibrosis and Congenital Absence of the Vas Deferens has been reviewed in GeneReviews (PMID: 20301428).

REFERENCES

Adam MP, Ardinger HH, Pagon RA, Wallace SE, et al. None. 1993. Cystic Fibrosis and Congenital Absence of the Vas Deferens (PMID: 20301428)

Gadsby DC, Vergani P, Csanády L. Nature. 2006, Mar 23. The ABC protein turned chloride channel whose failure causes cystic fibrosis. (PMID: 16554808)

Kerem BS, Zielenski J, Markiewicz D, Bozon D, et al. Proceedings of the National Academy of Sciences of the United States of America. 1990, Nov. Identification of mutations in regions corresponding to the two putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. (PMID: 2236053)

Ratjen F, Bell SC, Rowe SM, Goss CH, et al. Nature reviews. Disease primers. 2015, 05 14. Cystic fibrosis. (PMID: 27189798)

Riordan JR, Rommens JM, Kerem B, Alon N, et al. Science (New York, N.Y.). 1989, Sep 08. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. (PMID: 2475911)

Sharma N, Sosnay PR, Ramalho AS, Douville C, et al. Human mutation. 2014, Oct. Experimental assessment of splicing variants using expression minigenes and comparison with in silico predictions. (PMID: 25066652)

Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, et al. Nature genetics. 2013, Oct. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. (PMID: 23974870)

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RECOMMENDATIONS

- Clinical correlation is recommended.
- Clinical molecular testing should be interpreted in the context of the patient's clinical presentation and the prior probability of the clinical signs and symptoms being associated with known single gene disorders (i.e. defects in the identified gene).
- Genetic counseling is recommended to assess the specific implications of these results relative to an individual's clinical context
- Additional testing may be appropriate to evaluate for other types of variants not evaluated by this test.
- As knowledge increases, periodic re-evaluation of the clinical implications of variants is appropriate. Please contact RCIGM_rWGS@rchsd.org for questions about the RCIGM re-analysis policy.
- Mitochondrial DNA disorders can be sporadic or maternally inherited. If the reported mtDNA variant is found in the mother, the testing of appropriate matrilineal relatives is recommended.
- If there is a strong clinical suspicion of mitochondrial disease, additional testing of different tissue types may be warranted.

METHODOLOGY

Sequence via next generation sequencing (NGS) technology is generated from genomic DNA. PCR-free library preparation is performed prior to whole genome sequencing (WGS). An average genomic coverage of at least 35x, and/or at least 90% of OMIM genes will achieve 100% of coding base coverage of >10x for each proband. This ensures robust and uniform genome coverage. Alignment and variant calling are performed using the Illumina DRAGEN pipeline using the official reference build 37.1 (hg19). Copy number variation (CNV) calling is performed using a combination of CNV callers. Interpretation of CNVs is focused on variants that overlap or have a boundary that lies within 1 kb of an exon in all coding genes. The current version of this test assesses single nucleotide variants (SNVs), small deletions and insertions, larger deletions and duplications, the mitochondrial genome, and SMN1 and SMN2 copy number analysis.

Orthogonal Confirmation Policy

Reported sequencing variants are confirmed by Sanger sequencing, but may not be confirmed using orthogonal technologies if the following criteria are met: 1) the coverage at the variant's position is ≥ 20x; 2) the allelic balance for heterozygous calls is between 0.3-0.7; 3) the allelic balance is 0 (wild type allele as reference) for homozygous and hemizygous calls; 4) no systematic sequencing errors or local alignment problems are observed; 5) the call is not located in difficult sequence context (highly homologous and repetitive regions); 6) the call is not a complex insertion/deletion call resulting from nearby variants that may be difficult to align. If specific protocols require orthogonal confirmations of all reported variants, confirmations will be performed. If the case is ordered as proband-only and parental samples are available, targeted inheritance studies will be conducted for selected variants of interest.

Reported copy number variants are confirmed using orthogonal technologies including Multiplex ligtation-dependent probe amplification (MLPA), but may not be confirmed using orthogonal technologies if the following criteria are met: 1) the deletion or duplication event contains robust coverage and/or NGS read support; 2) no systematic sequencing errors or local alignment problems are observed; 3) the call is not located in difficult sequence context (highly homologous, repetitive regions, or segmental duplication regions. If specific protocols require orthogonal confirmations of all reported variants, confirmations will be performed. If the case is ordered as proband-only and parental samples are available, targeted inheritance studies will be conducted for selected variants of interest.

Reporting Categories

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Variants related to patient's phenotype – findings with strong variant pathogenicity evidence and strong evidence that the reported gene-disease association overlaps with the patient's phenotype

Variants possibly related to patient's phenotype - findings that are suggestive of a diagnosis but lacks either conclusive variant pathogenicity evidence or lack conclusive gene-disease association evidence

Variants in genes of uncertain significance – findings in genes that lack strong or supporting evidence for association with human disease

Variants in the mitochondrial genome – findings located within the mitochondrial genome

Incidental findings - findings in genes that do not overlap with the patient's reported phenotype, but may be medically actionable for the patient or tested family members

Test Specifications

The sensitivity and specificity for SNVs (single nucleotide variants) and small insertions and deletions up to 50 base pairs is greater than 99%. The analytical validity of SNVs was assessed using reference samples provided through the Genome in a Bottle (Zook et al. 2019, PMID: 30936564).

The sensitivity for larger deletions and duplications from WGS is estimated to be greater than 85%, although reliable reference data for these types of events are not well established. Deletions and duplications from 1 Kb to whole chromosomal abnormalities are detected with this test.

This test is validated for copy number analysis of exons 7 and 8 of the SMN1 and SMN2 genes. Over 95% of pathogenic variation for SMA involves biallelic loss of exon 7 of the SMN1 gene (Prior et al. 2010, PMID: 20057317). Other variation within the SMN1 gene is not detected with this assay. Results will only be reported in the proband if 0 copies of SMN1 are detected. Parental carrier status will be reported for affected patients if samples are available. Results are orthogonally confirmed by Multiplex Ligation-dependent Probe Amplification (MLPA). Whole genome sequencing is unable to determine the phase of SMN1 variants in the absence of parental testing. Therefore, in the absence of phasing, this assay does not exclude the possibility than an individual harbors two copies of SMN1 on the same allele and no copies on the other allele (silent carriers), two pathogenic sequence variants on the same SMN1 allele, two pathogenic sequence variants on opposite SMN1 alleles, and one pathogenic sequence variant and the loss of exon 8 on the opposite SMN1 allele.

Non-PCR amplified whole-genome sequencing (WGS) provides a stable, at least ~1,000-fold average, coverage across the entire mitochondrial genome (mtDNA). This test can detect SNVs, small insertions and deletions, as well as large deletions in the mtDNA. For mtDNA SNVs, variants with a heteroplasmy lower than 1% may not be detected. Variants that are classified as pathogenic or likely pathogenic that overlap with the patient's phenotype, with levels of heteroplasmy of >5% will be reported. However, suspicious variants of uncertain clinical significance will only be reported if heteroplasmy levels are >20%. If a patient is identified in having a SNV with heteroplasmy of >20%, Sanger sequencing will be performed for sequence confirmation. Variants with heteroplasmy levels between 1% - 20% will not be confirmed. Variants are considered to be rare if present in asymptomatic adults in fewer than 5 families in mtDB and the RCIGM internal database, combined. The revised Cambridge Reference sequence is used as a reference (rCRS NC_012920). Interpretations are made with the assumption that any information provided on family relationship is accurate.

Both phenotype-informed and phenotype-agnostic analyses are performed. Likely pathogenic and pathogenic variants that may explain the patient's phenotype will be reported as related/possibly related to the patient phenotype. Selected variants of uncertain significance may be reported as well. Should an incidental finding be revealed during genomic analysis for proband, and proband and parents have opted-in to receive incidental findings, it will be included on the proband's report. Parents do not have the option to opt-in for incidental findings if proband has opted-out. Reported variants are curated and classified in accordance with the American College of Medical Genetics and Genomics Guidelines (PMID: 25741868, 21681106, 24071793, 31690835).

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Incidental findings may be reported if the patient and/or patient's family do not opt-out of receiving these results. RCIGM's internal incidental finding policy includes the following: 1) variant must be classified as pathogenic per ACMG guidelines and in alignment with the known inheritance pattern of the genetic condition; 2) the variant is located in a gene with a well-established gene-disease relationship; 3) the gene and associated condition is shown to be medically actionable as established by RCIGM policy and in consultation with the RCIGM clinical team. Pathogenic variants included within the ACMG list of 59 secondary findings genes that are identified will be reported as well as other findings that meet the above criteria.

LIMITATIONS

Full coverage of the genome is not currently possible due to technically challenging repetitive elements and duplicated regions within the genome. Thus, not all regions of the genome are sequenced and/or uniquely aligned to the reference genome. Mosaic variant detection is limited using whole genome sequencing. Non-diagnostic findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable with the current version of this test. False negative results may occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. The chance of false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. This test is set up to evaluate the potential contribution of rare disease causing variants in known disease genes. It is not designed to evaluate for common variants in genes that might contribute to disease risk or for disorders that have a multigenic inheritance. Based on current knowledge, potential disease causing variants may not always be recognized at the time of testing.

REGULATORY DISCLOSURES

This test was developed and its performance characteristics determined by the Rady Children's Hospital and the Rady Children's Institute for Genomic Medicine. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. RCIGM has established and verified the test's accuracy and precision as outlined in the requirements of CLIA '88. The test is used for clinical purposes. It should not be regarded as investigational or for research. The Rady Children's Institute for Genomic Medicine is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing.

Lucia Guidugli, PhD, FACMG, CGMB
Clinical Laboratory Director

Katarzyna (Kasia) Ellsworth, PhD, FACMG
Senior Director of Clinical Operations

Test results reviewed and approved by:
Kasia Ellsworth, PhD, FACMG
Senior Director of Clinical Operations
RCIGM_rWGS@rchsd.org