WGS Report Examples
RCIGM_WI_287
3.0
7/2/2025
7/2/2025

# RCIGM_WI_287 WGS Report Examples

## Copy of version 3.0 (approved and current)

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| **Last Approval or Periodic Review Completed** | 7/2/2025 |
| **Next Periodic Review Needed On or Before** | 7/2/2026 |
| **Effective Date** | 7/2/2025 |

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**Organization** Rady Children's Institute for Genomic Medicine

### Description

RCIGM website: Linked under the Ordering ToolBox page where it can be downloaded externally

https://www.medialabinc.net/dv/dl.aspx?d=2718608&dh=4f437&u=97047&uh=429eb

Report examples can include: Primary, Amended, Negative, Corrected

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### Approval and Periodic Review Signatures

| Type | Description | Date | Version | Performed By | Notes |
|---|---|---|---|---|---|
| Approval | Lab Director | 7/2/2025 | 3.0 | Lucia Guidugli (Clinical Laboratory Director) | |
| Approval | QA | 7/1/2025 | 3.0 | Fabian Flores<br>Fabian Flores (Project Manager) | |

Signatures from prior revisions are not listed.

### Version History

| Version | Status | Type | Date Added | Date Effective | Date Retired |
|---|---|---|---|---|---|
| 3.0 | Approved and Current | Major revision | 7/1/2025 | 7/2/2025 | Indefinite |

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CAP ID# 9487427

# RCIGM Exome Report

| PATIENT NAME | | ORDERING PROVIDER | | RCIGM CASE ID | |
|---|---|---|---|---|---|
| SEX | | HOSPITAL | | FATHER ID | |
| DATE OF BIRTH | | SPECIMEN | | MOTHER ID | |
| MRN | | COLLECTED | | REPORT DATE | |
| INDICATION FOR TESTING | | RECEIVED | | | |
| TEST TYPE | | | | | |

## PATIENT PHENOTYPE

Failure to thrive; Severe global developmental delay; Hypotonia; Abnormal brain morphology; Tapered finger; Vesicoureteral reflux; Poor appetite; Abnormality of body weight; Constipation; Abnormal gastrointestinal tract morphology; Vomiting; Diarrhea; Dehydration; Malnutrition; Delayed speech and language development; Hydronephrosis; Recurrent urinary tract infections; Neurogenic bladder; Dimple chin; Open mouth; Dysphagia; Pyelonephritis; Bladder trabeculation

## TEST RESULT: PRIMARY FINDING IDENTIFIED

*Note: Two or more regions of homozygosity greater than or equal to 10Mb were detected in this individual. These regions are located in chromosomes 6, 7, and 12.*

### Sequence Variants

| REPORT CATEGORY | GENE | VARIANT | CONDITION | ZYGOSITY (INHERITANCE)† | VARIANT CLASSIFICATION |
|---|---|---|---|---|---|
| VARIANTS RELATED TO PATIENT PHENOTYPE | *HACE1* | c.1589G>A p.Trp530Ter | HACE1-RELATED NEURODEVELOPMENTAL DISORDER | Homozygous (Maternal and Paternal) | Pathogenic |

*Details on the variant(s) and gene(s) are located in the subsequent sections of the report
†When parental samples are unavailable at the time of testing, inheritance of the variant(s) will not be noted on the report

## VARIANTS RELATED TO PATIENT PHENOTYPE

| CONFIRMATION STATUS | GENE (TRANSCRIPT) | CONDITION | GENOMIC COORDINATES | VARIANT | ZYGOSITY (INHERITANCE) | CLASSIFICATION |
|---|---|---|---|---|---|---|
| Not required | *HACE1* (NM_020771.4) | HACE1-RELATED NEURODEVELOPMENTAL DISORDER | 6:104777295 | c.1589G>A p.Trp530Ter | Homozygous (Maternal and Paternal) | Pathogenic |

### Variant Information

A homozygous c.1589G>A (p.Trp530Ter) variant in the HACE1 gene was detected in this individual. This nonsense variant found in exon 15 of 24 is predicted to result in loss of normal protein function through either protein truncation or nonsense-mediated mRNA decay. This variant has been previously reported as a homozygous change in a patient with delayed motor milestones, leg spasticity, arm and leg weakness, seizure, lack of speech development, and abnormal findings on brain MRI (PMID: 38301322). The c.1589G>A (p.Trp530Ter) variant is present in the latest version of the gnomAD population database at an allele frequency of 0.00006% (1/1611538) and absent in the homozygous state, thus is



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## VARIANTS RELATED TO PATIENT PHENOTYPE (CONT.)

presumed to be rare. Based on the available evidence, c.1589G>A (p.Trp530Ter) is classified as Pathogenic.

**Gene Information:** The HACE1 gene is located on chromosome 6q16.3 and encodes HECT Domain And Ankyrin Repeat Containing E3 Ubiquitin Protein Ligase 1, involved in regulating the activity of cellular GTPases (PMID: 26424145).

**Disease Association(s):** Autosomal recessive HACE1-related neurodevelopmental disorder (MIM: #616756)

**Disease Summary:**

- HACE1-related neurodevelopmental disorder is characterized by infantile-onset of hypotonia and slowly progressive lower limb spasticity followed by severe impairments in global development and significant impairment of motor function. Additional features may include intellectual disability, seizures, speech delay, ocular abnormalities, foot deformities, corpus callosum hypoplasia, and decreased white matter (PMID: 26437029, 26424145, 31321299, 38301322).
- Loss-of-function variants are commonly reported in individuals with HACE1-related neurodevelopmental disorder, and is considered the mechanism of disease (PMID: 26424145, 26437029).

## REFERENCES

Akawi N, McRae J, Ansari M, Balasubramanian M, et. al. Discovery of four recessive developmental disorders using probabilistic genotype and phenotype matching among 4,125 families. Nat Genet 2015 Nov (PMID 26437029)

Deng HX. HACE1, RAC1, and what else in the pathogenesis of SPPRS? Neurol Genet 2019 Jun (PMID 31321299)

Hollstein R, Parry DA, Nalbach L, Logan CV, et. al. HACE1 deficiency causes an autosomal recessive neurodevelopmental syndrome. J Med Genet 2015 Dec (PMID 26424145)

Kilic MA, Yildiz EP, Deniz A, Coskun O, et. al. A Retrospective Review of 18 Patients With Childhood-Onset Hereditary Spastic Paraplegia, Nine With Novel Variants. Pediatr Neurol 2024 Mar (PMID 38301322)

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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.

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## METHODOLOGY

Genomic DNA is sequenced via next-generation sequencing (NGS) technology. 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. The current version of this test assesses single nucleotide variants (SNVs), small deletions and insertions, larger deletions and duplications, the mitochondrial genome, SMN1 and SMN2 copy number analysis, repeat expansions in PHOX2B and DMPK. Additionally, this test assesses UPD15 on trio WGS (proband and biological parents). Alignment and variant calling are performed using the Illumina DRAGEN pipeline using the official reference build GRCh38 (hg38). 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. Repeat expansion calling for PHOX2B and DMPK only are performed using Expansion Hunter algorithm. Analysis for Uniparental Disomy (UPD) associated with imprinting disorders is performed using a RCIGM proprietary algorithm on trio WGS only.

### Orthogonal Confirmation Policy

Reported sequencing variants are confirmed by Sanger sequencing at RCIGM or at an external CAP/CLIA laboratory, 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 a 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.

Reported copy number variants are confirmed using orthogonal technologies including Multiplex ligation-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 a 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.

### Reporting Categories

Variants related to patient's phenotype – findings with strong evidence of variant pathogenicity 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.

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## METHODOLOGY (CONT.)

### 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.

The sensitivity for detecting GCN trinucleotide repeat expansions within the PHOX2B carboxy terminal polyalanine repeat region by Expansion Hunter on short-read NGS is >90% for expansions greater than 20 repeats. The sensitivity for detecting 3' UTR DMPK trinucleotide repeat expansion (CTG repeats) is >90% for expansions greater than 50 repeats. However, due to short-read NGS technology's limitations, repeat expansion size cannot be precisely determined and reported (see Limitations section). The sensitivity for detecting in trio genome sequencing (proband and biological parents) only clinically relevant UPD associated with imprinting disorders at chromosome 15 is >90% (see Limitations section). This validation does not include distinction between pure isodisomy, pure heterodisomy or mixed hetero/iso-disomy calls; complete (affecting the whole chromosome) or segmental (affecting a part of a chromosome) UPD calls. In addition, the test is not validated to distinguish between Identity By Descent (IBD) and UPD and to detect mosaic UPD calls. 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. If parental samples are available, only parental SMN1 copy number will be reported. 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. 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 has a SNV with heteroplasmy of >20%, Sanger sequencing will be performed for sequence confirmation. 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).

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 secondary findings genes that are identified will be reported as well as other findings that meet the above criteria.

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## 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. Repeat expansion detection is limited to the DMPK and PHOX2B genes. The exact number of repeats cannot be determined by the current methodology and therefore orthogonal confirmation for precise sizing may be required. UPD calling is limited to trio WGS on chr15 associated with imprinting disorders. 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. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. 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. Analytical portions of the testing process may have been performed at one of these locations: 1 – 30. 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.

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Clinical Laboratory Director

Katarzyna (Kasia) Ellsworth, PhD, FACMG
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Test results reviewed and approved by:
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# RCIGM Genome Report

| PATIENT NAME | | ORDERING PHYSICIAN | | RCIGM CASE ID | |
|---|---|---|---|---|---|
| SEX | | ORDERING PROVIDER | | FATHER ID | |
| DATE OF BIRTH | | HOSPITAL | | MOTHER ID | |
| MRN | | SPECIMEN | | REPORT DATE | |
| INDICATION FOR TESTING | | COLLECTED | | | |
| TEST TYPE | | RECEIVED | | | |

## PATIENT PHENOTYPE

Patent foramen ovale; Right atrial enlargement; Tricuspid regurgitation; Reduced systolic function; Left atrial enlargement; Mitral regurgitation; Pericardial effusion; Respiratory failure; Metabolic acidosis; Hypotension; Pallor; Hepatomegaly; Tachycardia; Holosystolic murmur; Abnormal pulmonary thoracic imaging finding; Cardiomegaly; Tachypnea; Weak cry; Hypoxemia; Hypotonia; Lethargy; Abnormality of movement; Abnormality of eye movement

**TEST RESULT: NO VARIANTS REPORTED**

## 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

Genomic DNA is sequenced via next-generation sequencing (NGS) technology. 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. The current version of this test assesses single nucleotide variants (SNVs), small deletions and insertions, larger deletions and duplications, the mitochondrial genome, SMN1 and SMN2 copy number analysis, repeat expansions in

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## METHODOLOGY (CONT.)

PHOX2B and DMPK. Additionally, this test assesses UPD15 on trio WGS (proband and biological parents). Alignment and variant calling are performed using the Illumina DRAGEN pipeline using the official reference build GRCh38 (hg38). 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. Repeat expansion calling for PHOX2B and DMPK only are performed using Expansion Hunter algorithm. Analysis for Uniparental Disomy (UPD) associated with imprinting disorders is performed using a RCIGM proprietary algorithm on trio WGS only.

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1) the coverage at the variant's position is ≥ 20x;

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### Reporting Categories

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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).

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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. 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 has a SNV with heteroplasmy of >20%, Sanger sequencing will be performed for sequence confirmation. 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).

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 secondary findings genes that are identified will be reported as well as other findings that meet the above criteria.

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## 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

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## LIMITATIONS (CONT.)

genome. Mosaic variant detection is limited using whole genome sequencing. Repeat expansion detection is limited to the DMPK and PHOX2B genes. The exact number of repeats cannot be determined by the current methodology and therefore orthogonal confirmation for precise sizing may be required. UPD calling is limited to trio WGS on chr15 associated with imprinting disorders. 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. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. 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. Analytical portions of the testing process may have been performed at one of these locations: 1 – 30. 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.

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Clinical Laboratory Director

Katarzyna (Kasia) Ellsworth, PhD, FACMG
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**CLIA ID# 05D2129627**
**CAP ID# 9487427**

## RCIGM Amended Report

| | | | | | |
|---|---|---|---|---|---|
| PATIENT NAME | | ORDERING PROVIDER | | RCIGM CASE ID | |
| SEX | | HOSPITAL | | PREVIOUS APPROVAL DATE | |
| DATE OF BIRTH | | SPECIMEN | | REPORT DATE | |
| INDICATION FOR TESTING | | COLLECTED | | | |
| TEST TYPE | | RECEIVED | | | |

## PATIENT PHENOTYPE

Hypoplastic left heart; Thrombocytopenia; Submucous cleft hard palate; Bilateral ptosis; Hypoplasia of the maxilla

## TEST RESULT: VARIANT REPORTED

**Amended:** This report was amended to update the variant classification of FLI1 c.852G>T (p.Trp284Cys). Recuration of the variant revealed a decrease in the strength of evidence supporting pathogenicity, warranting a reclassification.

The FLI1 c.852G>T (p.Trp284Cys) is now classified as **VARIANT OF UNCERTAIN SIGNIFICANCE**, previously classified as a Likely Pathogenic.

### Sequence Variants

| REPORT CATEGORY | GENE | VARIANT | CONDITION | ZYGOSITY (INHERITANCE)† | VARIANT CLASSIFICATION |
|---|---|---|---|---|---|
| VARIANTS POSSIBLY RELATED TO PATIENT PHENOTYPE | *FLI1* | c.852G>T p.Trp284Cys | BLEEDING DISORDER, PLATELET-TYPE, 21 | Heterozygous (Maternal) | Uncertain Significance |

*Details on the variant(s) and gene(s) are located in the subsequent sections of the report
†When parental samples are unavailable at the time of testing, inheritance of the variant(s) will not be noted on the report

## VARIANTS POSSIBLY RELATED TO PATIENT PHENOTYPE

| CONFIRMATION STATUS | GENE (TRANSCRIPT) | CONDITION | GENOMIC COORDINATES | VARIANT | ZYGOSITY (INHERITANCE) | CLASSIFICATION |
|---|---|---|---|---|---|---|
| Confirmed | *FLI1* (ENST00000527786) | BLEEDING DISORDER, PLATELET-TYPE, 21 | 11:128680376 | c.852G>T p.Trp284Cys | Heterozygous (Maternal) | Uncertain Significance |

### Variant Information

A heterozygous c.852G>T (p.Trp284Cys) variant in the FLI1 gene was detected in this individual. This region in the FLI1 gene is highly constrained (regional missense constraint = 0.21), which suggests it is intolerant to variation. The c.852G>T (p.Trp284Cys) variant affects a highly conserved amino acid and is predicted by multiple in silico tools to have a deleterious effect on protein function. This variant has not been previously reported or functionally characterized in the literature to our knowledge. This variant has been reported in the ClinVar database (Variation ID: 986337). The c.852G>T (p.Trp284Cys) variant is absent from the latest version of the gnomAD population database and thus is presumed to be rare. This result was confirmed by orthogonal testing. Based on the available evidence, c.852G>T (p.Trp284Cys) is classified as a Variant of Uncertain Significance.



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## VARIANTS POSSIBLY RELATED TO PATIENT PHENOTYPE (CONT.)

**Gene Information:** The FLI1 gene is located on chromosome 11q24.3 and encodes Fli-1 Proto-Oncogene, ETS Transcription Factor, which regulates gene expression during embryogenesis, vascular development, and megakaryopoiesis (PMID: 10981960).

**Disease Association(s):** Autosomal dominant platelet-type bleeding disorder-21 (BDPLT21) (MIM #617443)

**Disease Summary:**

- BDPLT21 is a hematologic disorder characterized by increased risk of bleeding resulting from a functional platelet defect. For people with BDPLT21, platelets are usually enlarged, have defective secretion responses to agonists, and have decreased or absent dense bodies (PMID: 24100448, 28255014). Clinical manifestations of BDPLT21 include excessive bleeding, thrombocytopenia, alopecia, eczema or psoriasis, and recurrent viral infections (PMID: 27450272).

## REFERENCES

Daly ME. Transcription factor defects causing platelet disorders. Blood Rev 2017 Jan (PMID 27450272)

Hart A, Melet F, Grossfeld P, Chien K, et. al. Fli-1 is required for murine vascular and megakaryocytic development and is hemizygously deleted in patients with thrombocytopenia. Immunity 2000 Aug (PMID 10981960)

Saultier P, Vidal L, Canault M, Bernot D, et. al. Macrothrombocytopenia and dense granule deficiency associated with FLI1 variants: ultrastructural and pathogenic features. Haematologica 2017 Jun (PMID 28255014)

Stockley J, Morgan NV, Bem D, Lowe GC, et. al. Enrichment of FLI1 and RUNX1 mutations in families with excessive bleeding and platelet dense granule secretion defects. Blood 2013 Dec 12 (PMID 24100448)

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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 (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 detected in this test.
- As knowledge increases, periodic re-evaluation of the clinical implications of variants is appropriate.

## 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 is obtained for each proband genome. Alignment and variant calling are performed using the Edico DRAGEN pipeline using the official reference build 37.1. The current version of this test assesses single nucleotide variants (SNVs), small deletions and insertions, and larger deletions and duplications. The sensitivity and specificity for SNVs (single nucleotide variants) and small insertions and deletions is greater than 99%. The sensitivity for larger deletions and duplications from WGS is estimated to be greater than 80%, although reliable reference data for these types of events are not well established. All likely pathogenic and pathogenic reported variants are confirmed using orthogonal technologies.



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## METHODOLOGY (CONT.)

Variants are curated and classified in accordance with the American College of Medical Genetics and Genomics Guidelines (Richards et al. 2015; PMID: 25741868).

## LIMITATIONS

Full coverage of the genome is not currently possible due to technical challenging repetitive elements and duplicated regions within the genome. Thus, not all regions of the genome are sequenced or uniquely aligned to the reference

genome. Certain genomic alterations may not be covered with the current version of this test. This test only interprets single nucleotide variants, small insertions and deletions, and larger deletions and duplications for the phenotypes

indicated. Thus, genomic alterations such as trinucleotide repeat expansions and translocations will not be analyzed with the current version of the test.

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

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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.

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