Rady Children's Institute
Genomic Medicine

Clinical Genome Center
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CLIA ID# 05D2129627
CAP ID# 9487427

RCIGM Genome Report

PATIENT NAME	[redacted]	ORDERING PHYSICIAN	[redacted]	RCIGM CASE ID	[redacted]
SEX	[redacted]			MOTHER ID	[redacted]
DATE OF BIRTH	[redacted]	ORDERING PROVIDER	[redacted]	FATHER ID	[redacted]
MRN	[redacted]	HOSPITAL	[redacted]	REPORT DATE	06/07/2024
INDICATION FOR TESTING	Suspected Genetic Condition	SPECIMEN	Blood		
TEST TYPE	Trio Whole Genome Sequencing	COLLECTED	04/19/2024		
		RECEIVED	04/23/2024		

PATIENT PHENOTYPE

Neurodevelopmental delay; Esotropia; Hypermetropia; Amblyopia; Attention deficit hyperactivity disorder; Anxiety; Snoring; Insomnia; Feeding difficulties; Parasomnia; Hypermagnesemia; Failure to thrive; Carious teeth; Dental crowding; Constipation; Hand muscle weakness; Stuttering; Astigmatism; Eczematoid dermatitis; Nystagmus

TEST RESULT: PRIMARY FINDING IDENTIFIED

Sequence Variants

REPORT CATEGORY	GENE	VARIANT	CONDITION	ZYGOSITY (INHERITANCE)†	VARIANT CLASSIFICATION
VARIANTS RELATED TO PATIENT PHENOTYPE	SMARCA4	c.2524C>T p.Arg842Trp	SMARCA4-RELATED DISORDERS	Heterozygous (De novo)	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
Confirmed	SMARCA4 (ENST00000429416)	SMARCA4-RELATED DISORDERS	19:11130285	c.2524C>T p.Arg842Trp	Heterozygous (De novo)	Pathogenic

Variant Information

A heterozygous c.2524C>T (p.Arg842Trp) missense variant in the SMARCA4 gene was detected in this individual. The SMARCA4 gene is constrained against variation (Z-score= 8.81 and pLI = 1), and missense variants are a common mechanism of disease (HGMD, ClinVar database; PMID: 23556151). The c.2524C>T (p.Arg842Trp) 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 been previously reported as a de novo change in a patient with a neurodevelopmental disorder; additional clinical information is unavailable (PMID: 27479843, 35468861). This variant has been reported in the ClinVar database (Variation ID: 2866140). The c.2524C>T (p.Arg842Trp) variant is absent from the gnomAD v4 population database and thus is presumed to be rare. This result was confirmed by orthogonal testing. Analysis of the parental samples was negative for the variant, indicating this variant likely occurred as a de novo event. However, low-level parental mosaicism cannot be excluded. Based on the available evidence, c.2524C>T (p.Arg842Trp) is classified as a

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Pathogenic.

Gene Information: The SMARCA4 gene is located on chromosome 19p13.2 and SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, Member 4, which is involved in transcriptional activation of genes normally repressed by chromatin (PMID: 24658004).

Disease Association: Autosomal dominant SMARCA4-related disorders including Coffin-Siris syndrome 4 (CSS4; MIM: #614609) and SMARCA4-related rhabdoid tumor predisposition syndrome (RTPS; MIM: #613325).

Disease Summary:

- Coffin-Siris syndrome is characterized by aplasia or hypoplasia of the distal phalanx of the digits, developmental or cognitive delay, distinctive facial features, hypotonia, hirsutism/hypertrichosis, and sparse scalp hair.
- CSS4 is associated with mild to moderate growth impairment, feeding difficulties, less coarseness in facial features, and significant behavioral challenges.
- Penetrance for Coffin-Siris syndrome appears to be complete (PMID: 23556151).
- SMARCA4-related RTPS is characterized by the onset, from infancy to young adulthood, of various poorly differentiated tumors often arising in the central nervous system, ovary, kidney, and/or other extracranial sites (PMID: 24752781, 20137775, 21566516, 29215836).
- Penetrance for SMARCA4-related RTPS appears to be incomplete (PMID: 29215836).
- Pathogenic SMARCA4 variants in individuals with CSS4 are expected to exert a dominant-negative or gain-of-abnormal-function effect (PMID: 23556151, 25168959); whereas reported loss-of-function SMARCA4 variants are associated with RTPS (PMID: 29215836, 25060813). However, the literature also describes that there is variability with the mechanism of disease and resulting phenotype (PMID: 32686290, 28608987).

Disease Resources: GeneReviews provides additional clinical details and management recommendations for Coffin-Siris syndrome and rhabdoid tumor predisposition syndrome (PMID: 23556151, 29215836).

PREVIOUS GENETIC TESTING

Clinical records provided indicated that the following variant was detected by previous testing: a gain at chromosome 15q13.3. This copy number variant was also detected by this WGS test but was not considered reportable as primary finding for one or more of the following reasons: variant is inherited from a reportedly unaffected parent or has a greater than expected population frequency; gene is associated with autosomal recessive inheritance and no additional reportable variants were identified; reported patient phenotype did not overlap the established characteristics of the disorder; variant type is not a previously reported mechanism of disease; variant was classified as a variant of uncertain significance leaning likely benign; variant was classified as likely benign or benign.

REFERENCES

Adam MP, Feldman J, Mirzaa GM, Pagon RA, et al. None. 1993. Coffin-Siris Syndrome (PMID: 23556151)

Adam MP, Feldman J, Mirzaa GM, Pagon RA, et al. None. 1993. Rhabdoid Tumor Predisposition Syndrome (PMID: 29215836)

Errichiello E, Mustafa N, Vetro A, Notarangelo LD, et al. The Journal of pathology. 2017, Sep. SMARCA4 inactivating mutations cause concomitant Coffin-Siris syndrome, microphthalmia and small-cell carcinoma of the ovary hypercalcaemic type. (PMID: 28608987)

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Foulkes WD, Clarke BA, Hasselblatt M, Majewski J, et al. The Journal of pathology. 2014, Jul. No small surprise - small cell carcinoma of the ovary, hypercalcaemic type, is a malignant rhabdoid tumour. (PMID: 24752781)

Hamanaka K, Miyake N, Mizuguchi T, Miyatake S, et al. Genome medicine. 2022, Apr 26. Large-scale discovery of novel neurodevelopmental disorder-related genes through a unified analysis of single-nucleotide and copy number variants. (PMID: 35468861)

Hasselblatt M, Gesk S, Oyen F, Rossi S, et al. The American journal of surgical pathology. 2011, Jun. Nonsense mutation and inactivation of SMARCA4 (BRG1) in an atypical teratoid/rhabdoid tumor showing retained SMARCB1 (INI1) expression. (PMID: 21566516)

Hasselblatt M, Nagel I, Oyen F, Bartelheim K, et al. Acta neuropathologica. 2014, Sep. SMARCA4-mutated atypical teratoid/rhabdoid tumors are associated with inherited germline alterations and poor prognosis. (PMID: 25060813)

Jelinic P, Mueller JJ, Olvera N, Dao F, et al. Nature genetics. 2014, May. Recurrent SMARCA4 mutations in small cell carcinoma of the ovary. (PMID: 24658004)

Kosho T, Okamoto N. American journal of medical genetics. Part C, Seminars in medical genetics. 2014, Sep. Genotype-phenotype correlation of Coffin-Siris syndrome caused by mutations in SMARCB1, SMARCA4, SMARCE1, and ARID1A. (PMID: 25168959)

Lelieveld SH, Reijnders MR, Pfundt R, Yntema HG, et al. Nature neuroscience. 2016, Sep. Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. (PMID: 27479843)

Li D, Ahrens-Nicklas RC, Baker J, Bhambhani V, et al. American journal of medical genetics. Part A. 2020, Sep. The variability of SMARCA4-related Coffin-Siris syndrome: Do nonsense candidate variants add to milder phenotypes? (PMID: 32686290)

Schneppenheim R, Frühwald MC, Gesk S, Hasselblatt M, et al. American journal of human genetics. 2010, Feb 12. Germline nonsense mutation and somatic inactivation of SMARCA4/BRG1 in a family with rhabdoid tumor predisposition syndrome. (PMID: 20137775)

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

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. 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, and repeat expansions in PHOX2B and DMPK. 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. Repeat expansion calling for PHOX2B and DMPK only are performed using Expansion Hunter (v.4.0.2) in DRAGEN 3.10.4. Repeat expansions in PHOX2B may also be detected by the DRAGEN 3.10.4 SNVs & Indels analysis pipeline.

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 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 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.

Orthogonal confirmation of the PHOX2B and DMPK expansion variants is performed at an external CAP/CLIA laboratory. Final test results reporting may be affected by turnaround times of CAP/CLIA laboratory.

Reporting Categories

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

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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 Test Limitation section of the report).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).

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.

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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. 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. 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 Amended Exome Report

PATIENT NAME	[redacted]	ORDERING PROVIDER	[redacted]	RCIGM CASE ID	[redacted]
SEX	[redacted]	HOSPITAL	[redacted]	MOTHER ID	[redacted]
DATE OF BIRTH	[redacted]			FATHER ID	[redacted]
MRN	[redacted]	SPECIMEN	Blood	PREVIOUS APPROVAL DATE	06/10/2024
INDICATION FOR TESTING	Suspected Genetic Condition	COLLECTED	04/25/2024	REPORT DATE	07/03/2024
TEST TYPE	Trio Whole Exome Sequencing	RECEIVED	04/26/2024		

PATIENT PHENOTYPE

Atrial septal defect; Renal hypoplasia/aplasia; Feeding difficulties; Macrocephaly; Failure to thrive; High myopia; Global developmental delay; Hearing abnormality; Hemangioma; Torticollis; Bundle branch block; Pectus excavatum; Dilatation of the sinus of Valsalva; Elevated circulating hepatic transaminase concentration

TEST RESULT: PRIMARY FINDING IDENTIFIED

Amended: This report has been amended to include the patient's "Elevated circulating hepatic transaminase concentration" phenotype. No additional reportable variants, based on the RCIGM reporting guidelines, were identified.

Sequence Variants

REPORT CATEGORY	GENE	VARIANT	CONDITION	ZYGOSITY (INHERITANCE)†	VARIANT CLASSIFICATION
VARIANTS RELATED TO PATIENT PHENOTYPE	KDM2B	c.1841G>C p.Arg614Pro	KDM2B-RELATED NEURODEVELOPMENTAL DISORDER	Heterozygous (De novo)	Likely 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	KDM2B (ENST00000377071)	KDM2B-RELATED NEURODEVELOPMENTAL DISORDER	12:121891041	c.1841G>C p.Arg614Pro	Heterozygous (De novo)	Likely pathogenic

Variant Information

A heterozygous c.1841G>C (p.Arg614Pro) variant in the KDM2B gene was detected in this individual. The KDM2B gene is highly constrained (Z-score= 4.63 and pLI = 1), which suggests it is intolerant to variation. The c.1841G>C (p.Arg614Pro) variant affects a highly conserved amino acid; however, in silico tools used to predict the effect of this variant on protein function yield discordant results. This variant has not been previously reported or functionally characterized in the literature to our knowledge. The c.1841G>C (p.Arg614Pro) variant is absent from the gnomAD v4 population database and thus is presumed to be rare. Analysis of the parental samples was negative for the variant, indicating this variant

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likely occurred as a de novo event. However, low-level parental mosaicism cannot be excluded. Based on the available evidence, c.1841G>C (p.Arg614Pro) is classified as Likely Pathogenic.

Gene Information: The KDM2B gene is located on chromosome 12q24.31 and encodes Lysine Demethylase 2B, one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which functions as an epigenetic regulator in phosphorylation-dependent ubiquitination. Mouse models also suggest an important role during development (PMID: 15520277, 16362057, 17994099, 26237645, 36322151).

Disease Association(s): Autosomal dominant KDM2B-related neurodevelopmental disorder (PMID: 36322151)

Disease Summary:

- KDM2B-related neurodevelopmental disorder is characterized by speech delay, developmental delay, learning difficulties, and/or intellectual disability.
- Additional features may include behavioral differences (e.g. autism or attention deficit disorder/attention deficit hyperactivity disorder), congenital organ anomalies mainly of the heart, eyes, and urogenital system, and subtle facial dysmorphism (PMID: 36322151).
- Both loss-of-function and missense variants in the CxxC-domain were reported to present with a specific KDM2B-associated subepisignature detectable in peripheral blood (PMID: 36322151).
- Most pathogenic variants are of de novo origin, however, inheritance from mildly affected parents have be described (PMID: 36322151).

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Jiang Y, Qian X, Shen J, Wang Y, et al. Nature cell biology. 2015, Sep. Local generation of fumarate promotes DNA repair through inhibition of histone H3 demethylation. (PMID: 26237645)

Jin J, Cardozo T, Lovering RC, Elledge SJ, et al. Genes & development. 2004, Nov 01. Systematic analysis and nomenclature of mammalian F-box proteins. (PMID: 15520277)

Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, et al. Nature. 2006, Feb 16. Histone demethylation by a family of JmjC domain-containing proteins. (PMID: 16362057)

van Jaarsveld RH, Reilly J, Cornips MC, Hadders MA, et al. Genetics in medicine : official journal of the American College of Medical Genetics. 2023, Jan. Delineation of a KDM2B-related neurodevelopmental disorder and its associated DNA methylation signature. (PMID: 36322151)

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.

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- 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. 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, and repeat expansions in PHOX2B and DMPK. 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. Repeat expansion calling for PHOX2B and DMPK only are performed using Expansion Hunter (v.4.0.2) in DRAGEN 3.10.4. Repeat expansions in PHOX2B may also be detected by the DRAGEN 3.10.4 SNVs & Indels analysis pipeline.

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 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 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.

Orthogonal confirmation of the PHOX2B and DMPK expansion variants is performed at an external CAP/CLIA laboratory. Final test results reporting may be affected by turnaround times of CAP/CLIA laboratory.

Reporting Categories

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

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CLIA ID# 05D2129627
CAP ID# 9487427

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.

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 Test Limitation section of the report).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. Incidentally ascertained variants are reported if: 1) the variant is classified as pathogenic or likely pathogenic (note: classification requirements vary by gene); 2) the detected variant/s are in alignment with the known inheritance pattern of the genetic condition; 3) the gene and associated condition is shown to be medically actionable as established by RCIGM policy or the gene falls within the ACMG list of secondary findings genes.

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. 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. 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.

Lucia Guidugli, PhD, FACMG, CGMB
Clinical Laboratory Director

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RCIGM Genome Report

PATIENT NAME	Baby Boy	ORDERING PHYSICIAN	Dr. Jane Doe	RCIGM CASE ID	CSXXXX_INXXXX
SEX	Male	ORDERING PROVIDER	Dr. John Doe	REPORT DATE	02/11/2023
DATE OF BIRTH	01/21/2023	HOSPITAL	Children's Hospital		
MRN	H0000000	SPECIMEN	Dried blood spot		
INDICATION FOR TESTING	Research Subject IRB#	COLLECTED	01/27/2023		
TEST TYPE	Solo Whole Genome Sequencing	RECEIVED	01/30/2023		

PATIENT PHENOTYPE

Hydronephrosis; Encephalopathy; Patent ductus arteriosus; Patent foramen ovale; Anemia; Poor suck; Hypotension; Neonatal respiratory distress; Tachypnea; Hypokalemia; Abnormal renal collecting system morphology; Intraventricular hemorrhage; Elevated circulating 17-hydroxyprogesterone

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

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. 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, and repeat expansions in PHOX2B and DMPK. Alignment and variant calling are performed using the Illumina

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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. Repeat expansion calling for PHOX2B and DMPK only are performed using Expansion Hunter (v.4.0.2) in DRAGEN 3.10.4. Repeat expansions in PHOX2B may also be detected by the DRAGEN 3.10.4 SNVs & Indels analysis pipeline.

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 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 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.

Orthogonal confirmation of the PHOX2B and DMPK expansion variants is performed at an external CAP/CLIA laboratory. Final test results reporting may be affected by turnaround times of CAP/CLIA laboratory.

Reporting Categories

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

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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.

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Clinical Laboratory Director

Katarzyna (Kasia) Ellsworth, PhD, FACMG
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RCIGM Corrected Genome Report

PATIENT NAME	Patient Genetics	ORDERING PHYSICIAN	Dr. Jane Doe	RCIGM CASE ID	CSXXXX_INXXXX
SEX	Female			MOTHER ID	CSXXXX_INXXXX
DATE OF BIRTH	01/01/2018	HOSPITAL	Rady Children's Hospital San Diego	FATHER ID	CSXXXX_INXXXX
INDICATION FOR TESTING	External Approved IRB	SPECIMEN	Blood	PREVIOUS APPROVAL DATE	01/28/2021
		COLLECTED	01/04/2021		
TEST TYPE	Trio Whole Genome Sequencing	RECEIVED	01/05/2021	REPORT DATE	01/10/2022

PATIENT PHENOTYPE

Autism; Global developmental delay; Cranial asymmetry; Torticollis; Hemangioma; Recurrent upper respiratory tract infections; Impacted cerumen

TEST RESULT: VARIANTS REPORTED

Corrected (01/10/2022): This report corrects the classification of the previously reported deletion in the TBX1 gene due to the available evidence being insufficient to determine the role of this deletion in disease. The corrected classification of the variant is now reflected in the results table and in the variant description:

The 57 bp deletion at 22q11.2 (chr22:19748521-19748578) in TBX1 is classified as a **VARIANT OF UNCERTAIN SIGNIFICANCE**, previously reported as Pathogenic.

Copy Number Variants

REPORT CATEGORY	VARIANT	CONDITION	SIZE	EVENT / ZYGOSITY (INHERITANCE)	VARIANT CLASSIFICATION
VARIANTS POSSIBLY RELATED TO PATIENT PHENOTYPE	chr15:23681224-28563349, dup(15)(q11.2q13.1)	CHROMOSOME 15q DUPLICATION SYNDROME AND RELATED DISORDERS	4.88 MB	Duplication / Heterozygous (maternal)	Pathogenic
VARIANTS POSSIBLY RELATED TO PATIENT PHENOTYPE	chr22:19748521-19748578 (TBX1 c.146_202del57, p.Arg49_Pro67del)	TBX1-RELATED DISORDERS	57 bp	Deletion / Heterozygous (paternal)	Uncertain significance

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

VARIANTS POSSIBLY RELATED TO PATIENT PHENOTYPE

CONFIRMATION STATUS	VARIANT	CONDITION	SIZE	GENES	EVENT / ZYGOSITY (INHERITANCE)	CLASSIFICATION
Not Required	chr15:23681224-28563349, dup(15)(q11.2q13.1)	CHROMOSOME 15q DUPLICATION SYNDROME AND RELATED DISORDERS	4.88 MB	24 Genes	Duplication / Heterozygous (maternal)	Pathogenic
Not Required	chr22:19748521-19748578 (TBX1 c.146_202del57, p.Arg49_Pro67del)	TBX1-RELATED DISORDERS	57 bp	TBX1	Deletion / Heterozygous (paternal)	Uncertain significance

Variant 1 Information (15q11.2q13.3 Duplication)

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A duplication of approximately 4.88 Mb located at 15q11.2q13.3 (chr15:23681224-28563349) was detected in this individual. This duplication encompasses 119 genes of which 9 have been reported in OMIM with a disease association (PMID: 19344873). Duplications involving 15q11.2q13.3 are associated with Chromosome 15q11-q13 Duplication Syndrome (dup15q) (MIM: #608636), a disorder characterized by hypotonia and motor delays, autism spectrum disorder (ASD), intellectual disability, epilepsy including infantile spasms, and psychiatric disorders, such as schizophrenia (PMID: 27308687, 21324950). Dup15q is caused by presence of at least one extra maternally derived copy of the Prader-Willi/Angelman critical region (PWACR) within chromosome 15q11.2-q13.1. Evidence suggests a parent-of-origin effect, with maternally derived duplications being more frequently associated with abnormal phenotypes. Paternally derived duplications have been reported mostly in unaffected individuals (PMID: 18840528); however, some individuals with abnormal phenotypes, including autism (PMID: 23495136) and seizures (PMID: 23633446) have been identified. This variant has not been observed at a significant frequency in the Database of Genomic Variants and the gnomAD SV population database, 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, this duplication is classified as Pathogenic.

The list of genes overlapping this event and related phenotypic information for this region can be found using http://genome.ucsc.edu/cgi-bin/hgGateway and http://www.ncbi.nlm.nih.gov/omim using the chromosomal coordinates provided in this report.

The genomic coordinates of this deletion are assigned using GRCh37/hg19 as reference genome (see the method section of this report). The corresponding genomic coordinates on GRCh38/hg38 are chr15:23436077-28318203

Variant 2 Information (TBX1)

A heterozygous 57 bp deletion at 22q11.2 (chr22:19748521-19748578) in exon 3 of 9 in TBX1 was detected in this individual. This in-frame deletion variant leads to the loss of 19 amino acid residues but preserves the reading frame. This variant is also referred to as c.129_185del57 (p.Pro43_Pro61del) in the literature. This variant has been previously reported as a heterozygous change in a patient with non-syndromic Tetrology of Fallot and was inherited from the patient's unaffected mother (PMID: 20937753). Functional testing showed this variant decreased transcriptional activity of TBX1 and was less stable compared to wildtype (PMID: 20937753). This variant has been reported in the ClinVar database (Variation ID: 1014332). Analysis of the parental samples showed the mother is negative and the father is heterozygous for this variant. The frequency data for this variant in the population databases is unreliable due to insufficient coverage at this position in the gnomAD population database. Based on the available evidence, this variant is classified as Variant of Uncertain Significance.

Gene Information (TBX1)

The TBX1 gene encodes a transcription factor that is expressed in early embryonic development and is known to have an essential role in cardiac and pharyngeal development (PMID: 20301696). Larger (typically 1.5 to 3 MB) deletions in the 22q11.2 region encompassing the TBX1 gene are associated with chromosome 22q11.2 deletion syndrome, which is commonly referred to as DiGeorge syndrome (MIM: #188400) or Velocardiofacial syndrome (MIM: #192430). Individuals with chromosome 22q11.21 deletion syndrome have a range of phenotypic findings, including congenital heart defects, palatal abnormalities, feeding difficulties and dysphagia, characteristic facial features, learning difficulties, immune deficiency, hypocalcemia, and seizures (PMID:20301696, 27189754). Additional information for 22q11.21 deletion syndrome is summarized in GeneReviews (PMID: 20301696). TBX1 haploinsufficiency has been found to be responsible for major phenotypic features in this syndrome, predominantly the cardiac and cardiac outflow tract anomalies (PMID: 14585638, 15917203, 27326128). Heterozygous variants in TBX1 have been identified in some patients with clinical features of 22q11.2 syndrome who do not have the larger 22q11.2 deletion (PMID: 14585638, 11748311), and also found in patients with non-syndromic cardiac abnormalities (PMID: 29250159, 25860641, 11748311, 20937753, 24998776), and in two families with isolated hypoparathyroidism (PMID: 30137364). Reduced penetrance and variable expressivity of TBX1 variants has been observed (PMID: 30137364, 20937753, 17273972).

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The genomic coordinates of this deletion are assigned using GRCh37/hg19 as reference genome (see the method section of this report). The corresponding genomic coordinates on GRCh38/hg38 are chr22:19760998-19761055

REFERENCES

Adam MP, Ardinger HH, Pagon RA, Wallace SE, et al. None. 1993. 15q Duplication Syndrome and Related Disorders (PMID: 27308687)

Adam MP, Ardinger HH, Pagon RA, Wallace SE, et al. None. 1993. 22q11.2 Deletion Syndrome (PMID: 20301696)

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Ingason A, Kirov G, Giegling I, Hansen T, et al. The American journal of psychiatry. 2011, Apr. Maternally derived microduplications at 15q11-q13: implication of imprinted genes in psychotic illness. (PMID: 21324950)

Marini C, Cecconi A, Contini E, Pantaleo M, et al. American journal of medical genetics. Part A. 2013, Jun. Clinical and genetic study of a family with a paternally inherited 15q11-q13 duplication. (PMID: 23633446)

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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.
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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

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.

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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.

A phenotype-informed analysis is performed. However, phenotype-agnostic analysis is also performed. Incidentally ascertained medically-actionable findings are also reported unless the individual has opted- out of receiving incidental results. Likely pathogenic and pathogenic variants that may explain the patient's phenotype will be reported. Selected variants of uncertain significance may be reported as well. Reported variants are curated and classified in accordance with the American College of Medical Genetics and Genomics Guidelines (Richards et al. 2015, PMID: 25741868; Kearney et al. 2011, PMID 21681106; South et al. 2013, PMID 24071793; Riggs et al. 2019, PMID: 31690835).

Incidental findings may be reported if the patient and/or patient's family do not opt-out of receiving these results. Incidentally ascertained variants are reported if: 1) the variant is classified as pathogenic or likely pathogenic (note: classification requirements vary by gene); 2) the detected variant/s are in alignment with the known inheritance pattern of the genetic condition; 3) the gene and associated condition is shown to be medically actionable as established by RCIGM policy or the gene falls within the ACMG list of secondary findings genes.

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

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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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