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PATIENT REPORT

Patient Age/Sex: Male

Specimen Collected: 27-Feb-23 07:15

Rapid Whole Genome Sequencing **|Received: 27-Feb-23 07:16** **Report/Verified: 27-Feb-23 07:34**

Procedure	Result	Units	Reference Interval
RWGS NGS Int	Positive f1 i1		

Result Footnote

f1: RWGS NGS Int
TEST PERFORMED
Rapid Whole Genome Sequencing
Samples tested: Proband and both parents

RESULT
Primary findings: Positive; one de novo pathogenic variant was detected in the RIT1 gene.
Secondary findings: Negative

KEY CLINICAL FINDINGS
Low-set ears, sacral dimple, hypotonia, premature birth, respiratory failure, hyperechogenic kidneys, redundant neck skin, abdominal wall muscle weakness, flat face

HPO terms used: HP:0000369 (low-set ears), HP:0000960 (sacral dimple), HP:0001252 (hypotonia), HP:0001622 (premature birth), HP:0002878 (respiratory failure), HP:0004719 (hyperechogenic kidneys), HP:0005989 (redundant neck skin), HP:0009023 (abdominal wall muscle weakness), HP:0012368 (flat face).

INTERPRETATION
One de novo pathogenic variant was identified in the RIT1 gene. Pathogenic germline variants in RIT1 typically occur de novo and are associated with autosomal dominant Noonan syndrome 8 (MIM: 615355).

DE NOVO PATHOGENIC VARIANT
Gene: RIT1 (NM_006912.6)
OMIM disease: Noonan syndrome 8 (MIM: 615355)
Inheritance pattern: Autosomal dominant
Variant: c.170C>G; p.Ala57Gly - heterozygous
Chr1(GRCh37):g.155874589
Frequency: Not in gnomAD
Computational prediction programs: Uncertain (REVEL: 0.62)

Pathogenic variants in RIT1 are associated with Noonan syndrome 8. The identified c.170C>G; p.Ala57Gly was not detected in the parental samples. This variant is reported in the literature in multiple individuals with Noonan syndrome, including instances of de novo inheritance (Aoki, 2013; Bertola, 2014; Chen, 2014; Iglesias, 2014; Jin, 2017; Koenighofer, 2016). Functional analyses show that the p.Ala57Gly variant increases the nucleotide exchange rate in the RIT1 protein and activates the ERK1/2 pathway (Aoki, 2013; Chen, 2014; Fang, 2016; Koenighofer, 2016). Mouse and zebrafish models with this variant recapitulate a Noonan syndrome phenotype (Koenighofer, 2016; Takahara, 2019). This variant is also reported in ClinVar (Variation ID: 60506). Based on available information, this variant is considered to be pathogenic.

No secondary pathogenic variants were detected in the v3.1 list of genes that the American College of Medical Genetics and Genomics (ACMG) recommends reporting in all individuals undergoing genome sequencing (Miller, 2022). A list of ACMG genes is included in the additional technical information. These genes are evaluated only to the extent that standard genome sequencing allows. Single pathogenic variants in autosomal recessive ACMG genes are not reported.

RECOMMENDATIONS
Genetic consultation is indicated, including a discussion of medical screening and management. Although the identified RIT1 variant is presumed to be de novo and recurrence risk is thought to be low, this

Result Footnote

f1: RWGS NGS Int
patient's parents should be offered the option of prenatal diagnosis for the identified variant in future pregnancies (Familial Targeted Sequencing, Fetal, ARUP test 3005869).

NOTES
99.1% of bases in the targeted genome were covered by more than 20 sequencing reads. Intergenic variants, deep intronic variants not predicted to alter splicing, large deletions/duplications, and chromosomal rearrangements are not analyzed by this method; therefore, additional variants in the reported genes have not been excluded. Refer to background for details of limitations. Unless otherwise specified, confirmation by Sanger sequencing was not performed for variants with acceptable quality metrics.

REFERENCES
Aoki Y, et al. Gain-of-function mutations in RIT1 cause Noonan syndrome, a RAS/MAPK pathway syndrome. Am J Hum Genet. 2013;93(1):173-180. PMID: 23791108.

Bertola DR, et al. Further evidence of the importance of RIT1 in Noonan syndrome. Am J Med Genet A. 2014;164A(11):2952-2957. PMID: 25124994.

Chen PC, et al. Next-generation sequencing identifies rare variants associated with Noonan syndrome. Proc Natl Acad Sci U S A. 2014;111(31):11473-11478. PMID: 25049390.

Fang Z, et al. Biochemical classification of disease-associated mutants of RAS-like protein expressed in many tissues (RIT1). J Biol Chem. 2016;291(30):15641-15652. PMID: 27226556.

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Jin SC, et al. Contribution of rare inherited and de novo variants in 2,871 congenital heart disease probands. Nat Genet. 2017;49(11):1593-1601. PMID: 28991257.

Koenighofer M, et al. Mutations in RIT1 cause Noonan syndrome - additional functional evidence and expanding the clinical phenotype. Clin Genet. 2016;89(3):359-366. PMID: 25959749.

Miller DT, et al. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2022;24(7):1407-1414. PMID: 35802134.

Takahara S, et al. New Noonan syndrome model mice with RIT1 mutation exhibit cardiac hypertrophy and susceptibility to beta-adrenergic stimulation-induced cardiac fibrosis. EBioMedicine. 2019;42:43-53. PMID: 30898653.

Test Information

i1: RWGS NGS Int
BACKGROUND INFORMATION: Rapid Whole Genome Sequencing

CHARACTERISTICS: The purpose of rapid genome sequencing is to determine the patient's diagnosis when a genetic condition is suspected in acute clinical scenarios. The analyzed genome includes exons from all known human genes and all intronic variants suspected of influencing splicing. Parental samples are required for interpretation of results.

CLINICAL SENSITIVITY: Varies based on clinical symptoms, family history, inheritance pattern, and previous clinical evaluations

Test Information

i1: RWGS NGS Int

METHODOLOGY: Genomic DNA is extracted from whole blood, prepared into libraries, then sequenced by massively parallel sequencing (MPS; also known as next generation sequencing [NGS]). Variant calling is performed using a custom bioinformatics pipeline that includes phenotype-based scores. Human genome build 19 (Hg 19) is used for data analysis.

ANALYTICAL SENSITIVITY: The analytical sensitivity of this test is 98.6 percent for single nucleotide variants (SNVs). Analytical sensitivity is 97.4 percent for insertions/duplications /deletions ranging in size from 1-15 bp, and 92.0 percent for those 16-50 bp in size.

LIMITATIONS OF ANALYSIS: A negative result does not exclude all genetic diagnoses. The human genome is not able to be completely analyzed as some genes have not been identified while others, due to technical limitations, cannot be sequenced or interpreted. Variants in intergenic or deep intronic regions will only be evaluated if an effect on gene expression is predicted via annotation software. Mode of inheritance, reduced penetrance, and genetic heterogeneity can reduce clinical sensitivity. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions. This assay is not designed to detect low-level somatic variants associated with disease. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Please see Additional Technical Information located at http://ltd.aruplab.com/Tests/Pub/3005935 for more information.

LIMITATIONS FOR REPORTING AND INTERPRETATION: Only variants in genes suspected to be causative of the patient's symptoms are reported, with the exception of secondary pathogenic findings, if elected. Incorrect reporting of biological relationships among family members may affect result interpretation. Mode of inheritance, reduced penetrance, and genetic heterogeneity can reduce clinical sensitivity. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the U.S. Food and Drug Administration. This test was performed in a CLIA-certified laboratory and is intended for clinical purposes.

Counseling and informed consent are recommended for genetic testing. Consent forms are available online.