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PATIENT INFORMATION

Unique ID	[Unique ID]	**Physician**	[Physician]	**Sample type**	DBS
3billion ID	[3billion ID]	**Department**	Pediatrics	**Collected on**	yyyy-mm-dd
DOB* / Sex	yyyy-mm-dd / Female	**Institution**	[Institution]	**Ordered on**	yyyy-mm-dd
Ethnicity	Latino/Admixed American			**Accessioned on**	yyyy-mm-dd

* (YYYY-MM-DD)

CLINICAL INFORMATION

Symptoms	Intellectual disability, Atrial septal defect, Cryptorchidism

RESULT SUMMARY

Primary findings	Variant reported	**Additional findings**	No variant reported
Secondary findings	No variant reported		

PRIMARY FINDINGS

INCONCLUSIVE

A heterozygous variant of uncertain significance was identified in *PTPN11*. *PTPN11* is associated with autosomal dominant 'Noonan syndrome 1 (OMIM: 163950)'. Currently available evidence is insufficient to classify the variant as pathogenic or likely pathogenic. Clinical correlation may provide further evidence to reclassify the variant. Parental testing is also recommended to check if the variant is de novo or inherited.

Noonan syndrome 1 (OMIM: 163950)

Gene	Variant		Classification
PTPN11	Genomic Position	12-112453317-G-A (GRCh38)	VUS
	cDNA	NM_002834.5:c.455G>A	
	Protein	NP_002825.3:p.Arg152His	
	Zygosity	Heterozygous	
	Inheritance	Unknown	

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PRIMARY FINDINGS INTERPRETATION

PTPN11 NM_002834.5:c.455G>A (NP_002825.3:p.Arg152His)	
Population Data	The variant is observed at an extremely low frequency in the gnomAD v4.1.0 dataset (total allele frequency: 0.002%).
Predicted Consequence / Location	Missense changes are a common disease-causing mechanism.
Segregation Data	None
Computation and Functional Data	In silico tool predictions suggest damaging effect of the variant on gene or gene product [REVEL: 0.73 (>=0.6, sensitivity 0.68 and specificity 0.92); 3Cnet: 0.79 (>=0.6, sensitivity 0.72 and precision 0.9)].
Previously Reported Variant Data	Same nucleotide change resulting in same amino acid change has been previously reported to be associated with PTPN11 related disorder (PMID: 32164556). However, the evidence of pathogenicity is insufficient at this time.
Disease Association	Noonan syndrome 1 (OMIM: 163950)
Validation	Not performed as the variant was considered high-quality
Variant Classification	VUS

ADDITIONAL FINDINGS

No additional variants were identified, including variants of uncertain significance (VUSs) that could not be reported as primary findings due to limited evidence of pathogenicity, even though they may explain the patient's symptoms; pathogenic, likely pathogenic variants or VUSs that may partially explain the patient's symptoms, regardless of whether they fit the mode of inheritance; or variants associated with the family history provided by the healthcare provider, regardless of the patient's current symptoms.

SECONDARY FINDINGS

No clinically significant variant was identified in the 84 medically actionable secondary finding genelist recommended to be reported by the American College of Medical Genetics and Genomics (ACMG). However, there is a possibility of missing the disease-causing variant due to the test limitations (see below Recommendations #2, #3, and #5).

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RESOURCES

- Online Mendelian Inheritance in Man®:This report contains information from the Online Mendelian Inheritance in Man® (OMIM®) database, which has been obtained under a license from Johns Hopkins University. This report does not represent the entire, unmodified OMIM® database, which is available in its entirety at http://omim.org/downloads.
- gnomAD (genome Aggregation Database): gnomad.broadinstitute.org
- ClinVar (National Center for Biotechnology Information ClinVar Database): ncbi.nlm.nih.gov/clinvar
- HGVS (Human Genome Variation Society): varnomen.hgvs.org
- HGMD (The Human Gene Mutation Database) Professional
- MITOMAP (A human mitochondrial genome database): https://www.mitomap.org/MITOMAP

REFERENCES

1. Richards S et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24. PMID: 25741868.
2. Erin R et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med. 2020 Feb;22(2):245-257
3. Elizabeth M et al. Specifications of the ACMG/AMP standards and guidelines for mitochondrial DNA variant interpretation. Hum Mutat. 2020 Dec;41(12):2028-2057.
4. Seo GH et al. Diagnostic yield and clinical utility of whole exome sequencing using an automated variant prioritization system, EVIDENCE. Clin Genet. 2020 Dec;98(6):562-570. PMID: 901917.
5. Lee, K., Abul-Husn, N.S., Amendola, L.M. et al. ACMG SF v3.3 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. 2025 un 23;27(8):101454 PMID 40568962.
6. Dhong-Gun Won et al. 3Cnet: pathogenicity prediction of human variants using multitask learning with evolutionary constraints. Bioinformatics. 2021 Jul 16;btab529. PMID: 34270679
7. Ryan Poplin, Pi-Chuan Chang, David Alexander et al. A universal SNP and small-indel variant caller using deep neural networks. Nat Biotechnol. 2018 Nov;36(10):983-987. PMID: 30247488
8. Egor Dolzhenko, Adam English, Harriet Dashnow et al. Characterization and visualization of tandem repeats at genome scale Nat Biotechnol. 2024 Oct;42(10):1606-1614. PMID: 38168995
9. Xiao Chen, Daniel Baker, Egor Dolzhenko et al, Genome-wide profiling of highly similar paralogous genes using HiFi sequencing (https://doi.org/10.1101/2024.04.19.590294)
10. Quinodoz M, Peter VG, Bedoni N, et al. AutoMap is a high performance homozygosity mapping tool using next-generation sequencing data. Nat Commun. 2021 Jan 22;12(1):518. PMID: 33483490

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NOTES

1. Results summary: Results are categorized into positive, inconclusive, and negative. A variant in a known disease gene that would fit the patient's phenotype is reported.

Category	Explanation
Positive	• AD or XL disease: one heterozygous or hemizygous P/LP variant is identified in a known disease gene. • AR disease: one homozygous P/LP variant or two P/LP (potential) compound heterozygous variants are identified in a known disease gene.
Inconclusive	• AD or XL disease: one heterozygous or hemizygous VUS is identified in a known disease gene. • AR disease: At least two heterozygous or one homozygous VUS are identified in a known disease gene. • AR disease: One heterozygous P/LP variant is identified in a known disease gene. • A P/LP variant(s) are identified in a GUS that has sufficient evidence of being a disease gene.
Negative	• No clinically significant variant that would fit the patient's phenotype well is identified.

Abbreviation: AD; autosomal dominant, AR; autosomal recessive, XL; X-linked, P; Pathogenic, LP; likely pathogenic, VUS; variant of uncertain significance, GUS; gene of uncertain significance.

2. Variant Classification: A variant is classified according to the ACMG guideline (PMID 25741868) using the type of evidence including population data, computational and predictive data, functional data, segregation data, de novo data, and allelic data.

RECOMMENDATIONS

1. Genetic counseling is warranted to review the test results and interpretation.
2. This test can detect single nucleotide variants and small insertions/deletions (<50 bp), copy number variants (CNVs), structural variants (SVs) including inversions and translocations, and mitochondrial genome variants with high accuracy in most of the genomic regions. If low level mosaicism variants on autosomes and sex chromosomes are suspected, it is recommended to perform other tests specifically designed to detect these types of variants. Intronic variants in regions other than coding exons, epigenetic factors, or variants in regulatory regions may not be interpretable.
3. The test results are based on the clinical information and family history provided in the test order. If the information provided is incorrect or insufficient, the test may not yield reliable results. If the test results have weak clinical correlations, additional testing may be required at the discretion of your medical provider. Genome sequencing test or Sanger sequencing test on the biological parents or other family members is recommended to confirm segregation of the variant(s).
4. Variant interpretation is based on currently available scientific and medical information that were publicly available at the time the results were reported. Therefore, the referenced data may not be current at the time of genetic counseling.
5. In case of a negative result with no significant variants reported, it does not rule-out the possibility of having a genetic condition. As new clinical/scientific information becomes available, variant classification may change and a new diagnosis can emerge. In case a reanalysis is requested, newly available information is reflected in the reanalysis. and a reanalysis report is generated. The medical provider may also add new phenotypic information on the patient.

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METHODS

HiFi long-read sequencing data was generated using the PacBio Revio system at DNA LINK. Primary and secondary sequencing data analyses were performed at DNA LINK to generate BAM and VCF files (see below for further detail). Variant analysis and interpretation were performed at 3billion starting from the vcf file. In total, [[totalYield]] bases of sequence were generated and uniquely aligned to the Genome Reference Consortium Human Build [[build]] (GRCh[[build]]) and Revised Cambridge Reference Sequence (rCRS) of the mitochondrial genome, generating [[meanDepth]] mean depth-of-coverage across the entire genome. Approximately [[cov10x]]% of the genome was covered at a depth of ≥10x. Despite the insufficient coverage across [[cov10xBases]]% of the bases (see below for details), these metrics are consistent with high quality genome sequencing data and deemed adequate for analysis. In total, [[snp]] single nucleotide variants (SNV) and [[indel]] small insertions and deletions (INDEL) were identified. Variants were called using DeepVariant (Nat Biotechnol. 2018 Nov;36(10):983-987) for single nucleotide variants and small insertion/deletions (SNVs/INDELs), PBSV for structural variants (SV) including copy number variants (CNVs), Tandem Repeat Genotyping Tool (Nat Biotechnol. 2024 Oct;42(10):1606-1614) for repeat expansion variants, and Paraphase (https://doi.org/10.1101/2024.04.19.590294) for gene copy number and phased variants in the paralogous regions or segmental duplications. Regions of homozygosity (ROH) were detected with AutoMap v1.2 (Nat Commun. 2021;12:518). Finally, variant annotation was performed using Variant Effect Predictor (VEP) v104.2 (Genome Biology 2016;17:122). Sequencing data analysis and variant interpretation were performed using 3billion's proprietary system, EVIDENCE v4.1 (Clin Genet. 2020;98:562-570). Variants were prioritized based on the guideline recommended by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) (Genet Med. 2015;17:405-424, Genet Med. 2020:22:245-257, and Hum Mutat. 2020;41:2028-2057) in the context of the patient's phenotype, relevant family history and previous test results provided by the ordering physician. Only variants deemed clinically significant and relevant to the patient's clinical indications at the time of variant interpretation are reported. The raw data files including VCF files and/or annotated small variant lists are available upon request.

ADDITIONAL MEMO

Ginecoid lipoid distribution, microorchidea, normal male karyotype (46, XY), high sexual horomone-binding globulin 23.7nmol/L (normal range 72-220nmom/L), high Bioavailable testosterone 26.5 (0.2 - 3.4), high Free testosterone 2.35pg (0.15-0.6pg), HbA1c: 6.1%

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DISCLAIMER

This research service was developed by 3billion for the purpose of analyzing and interpreting single nucleotide variants, small insertions and deletions, and structural variants from the long-read HiFi genome sequencing data generated by PacBio Revio system. The interpretation depends on the quality of the sequencing data. However, because the sequencing data is generated from an external laboratory, 3billion does not take responsibility for the quality of the sequencing data provided. This report may not be copied or reproduced, except in its totality.

ACCREDITATIONS AND CERTIFICATIONS

CAP License #	CLIA ID #
8750906, AU-ID# 2052626	99D2274041

This case has been comprehensively reviewed by our clinical team of physicians, geneticists and informaticists.
Report electronically signed by:

Go Hun Seo, M.D, Ph.D.
Chief Medical Officer & Laboratory Director