3B-GENOME, Proband

Clinical use

Unique ID: [Unique ID]
3billion ID: [3billion ID]

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
Requested gene(s) 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-mid
	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.

REQUESTED GENE FINDINGS

No clinically significant variant was identified in the requested gene. See the appendix on the last page for the coverage information of the genes requested by the provider.

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

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NOTES

1. Results summary: Results are categorized into positive, inconclusive and negative

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 (<20%) mosaicism variants on autosomes and sex chromosomes are suspected, it is recommended to perform other tests specifically designed to detect these types of variants. Variants in regions of high sequence homology, such as pseudogenes, may be difficult to detect. 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. Whole genome sequencing test or Sanger sequencing test on the biological parents or other family members is recommended to confirm segregation of the variant(s). For structural variants (SVs), including copy number variants (CNVs), only variants for which the exact breakpoint has been identified can be tested by Sanger sequencing. Low heteroplasmic (<20%) level mitochondrial variants cannot be tested by Sanger sequencing.
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. If the patient has consented to reanalysis, 3billion will perform an automated reanalysis using the most recent information and send an updated report to the ordering physician if the results change. The provider may also add new phenotypic information about the patient. To comply with the Korean Bioethics and Safety Act 53 and the operational procedures set forth by 3billion, Inc., specimens will be retained for a maximum period of six months and disposed of without prior notice thereafter. Consequently, any genetic variants identified and reported through reanalysis will not be subject to confirmation by Sanger sequencing unless the patient provides a new specimen.
6. Patient consent for reanalysis is renewed on a 10-year cycle. After 10 years, when a physician renews the patient's consent for reanalysis, the reanalysis period will be extended for another 10 years.
7. Variants reported outside of Primary and/or Secondary Findings are not confirmed by Sanger sequencing. Therefore, only variants meeting strict quality criteria are reported.

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METHODS

Genomic DNA was extracted from DBS specimen using standard protocol. Library was prepared using TruSeq DNA PCR-Free kit, and sequencing was performed using NovaSeq X (Illumina, San Diego, CA, USA). In total, 129,429,129,749 bases of sequence were generated and uniquely aligned to the Genome Reference Consortium Human Build 38 (GRCh38) and Revised Cambridge Reference Sequence (rCRS) of the mitochondrial genome, generating 38.34 mean depth-of-coverage across the entire genome. Approximately 95.76% of the genome (98.48% of the autosomes) was covered at a depth of ≥20x. Despite the insufficient coverage across 4.24% of the bases (see below for details), these metrics are consistent with high quality genome sequencing data and deemed adequate for analysis. Gene or exon level depth-of-coverage (DOC) information is available upon request. In total, 3,952,778 single nucleotide variants (SNV) and 956,370 small insertions and deletions (INDEL) were identified. Sequencing data analysis and variant interpretation were performed using 3billion's proprietary system, EVIDENCE v4.2 (Clin Genet. 2020;98:562-570). EVIDENCE incorporates bioinformatics pipeline for calling SNV/INDEL based on the GATK best practices (GATK v4.4.0, Genome Res. 2010;20:1297-303), Manta v1.6.0 (Bioinformatics. 2016;32:1220-2) for structural variant calling including CNV (copy number variants) based on paired-end information, and 3bCNV v2.1, an internally developed tool, for calling CNV including aneuploidy based on the DOC information. It also incorporates Mutect2 v4.4.0 (Genome Res.2010;20:1297–303) for calling lower level heteroplasmic SNV/INDEL in the mitochondrial genome, ExpansionHunter v5.0.0 (Bioinformatics.2019;35:4754–6) for calling repeat expansion variants, MELT v2.2.2 (Genome Res. 2017;27:1916–29) for calling mobile element insertion variants, AutoMap v1.2 (Nat Commun. 2021;12:518) for detecting regions of homozygosity (ROH). Variant Effect Predictor v104.2 (VEP, Ensembl, Genome Biology 2016;17:122) is used for variant annotation. 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. Based on internal studies validating the accuracy of the SNVs and INDELs called with high quality scores, only low quality variants are confirmed by Sanger sequencing. SVs with breakpoints identified were attempted to be confirmed by Sanger sequencing. The raw data files including FASTQ files, 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 test was developed by 3billion in the purpose of identifying single nucleotide variants, small insertions and deletions, and structural variants from the whole genome. Repeat expansion detection is possible for the following 45 genes. Repeat expansion number may be underestimated for the starred (*) gene with compromised sensitivity (*AFF2*, AR, ARX, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS*, ATXN10*, BEAN1*, C9ORF72, CACNA1A, CNBP, COMP, DAB1, DIP2B*, DMPK*, FGF14, FMR1*, FOXL2, FXN, GIPC1*, GLS*, HOXD13, HTT, JPH3, LRP12*, MARCHF6*, NOP56, NOTCH2NLC, NUTM2B-AS1*, PABPN1, PHOX2B, PPP2R2B, PRDM12, RAPGEF2*, RFC1*, RILPL1*, SAMD12*, STARD7*, TBP, TCF4, XYLT1*, ZIC2*). Only SNV/INDEL (>10% heteroplasmic level) are called within the mitochondrial genome. This laboratory is certified under the College of American Pathologists (CAP#:8750906) and Clinical Laboratory Improvement Amendments (CLIA#: 99D2274041) as qualified to perform high complexity clinical laboratory testing. Assay validation and clinical validation were performed following the Korea Institute of Genetic Testing Evaluation, the American College of Medical Genetics and Genomics (ACMG) Technical Standards and Guidelines Section G (https://www.acmg.net/PDFLibrary/Standards-Guidelines-Clinical-Molecular-Genetics.pdf) and the CAP Next Generation Sequencing (NGS) Worksheets (Santani A et al. J Mol Diagn. 2019 May;21(3):369-374; https://www. cap.org/member-resources/precision-medicine/next-generation-sequencing-ngs-worksheets). If low level mosaicism or variants within regions that are incompletely sequenced due to technical difficulties with amplification, sequencing, and alignment are suspected, it is recommended to perform appropriate testing that is designed to detect this type of variant. 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

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APPENDIX. REQUESTED GENE(S) FINDINGS

The 10 requested genes were covered well except for *TPRN, MAFA, PMS2*: see below for the coverage information of about the genes (95%>=10x). (The gene(s) marked N/A are pseudogenes or non-coding genes without clinical relevance.)

Gene	% bp >=10x	Gene	% bp >=10x	Gene	% bp >=10x
TPRN	94.0	*MAFA*	94.0	*PMS2*	94.0