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**	Variant reported
Requested gene(s) findings	No variant reported	**Secondary findings**	Variant reported

PRIMARY FINDINGS

POSITIVE

A heterozygous likely pathogenic variant was identified in *NIPBL*. *NIPBL* is associated with autosomal dominant 'Cornelia de Lange syndrome 1 (OMIM: 122470)'. As this variant has never been reported in other patients, clinical correlation is recommended. Parental testing is also recommended to check if the variant is de novo or inherited.

Cornelia de Lange syndrome 1 (OMIM: 122470)

Gene	Variant		Classification
NIPBL	Genomic Position	5-36985791-AGG-A (GRCh38)	Likely pathogenic
	cDNA	NM_133433.4:c.2612_2613del	
	Protein	NP_597677.2:p.Arg871ThrfsTer2	
	Zygosity	Heterozygous	
	Inheritance	Unknown	

3B-GENOME, Proband

Clinical use

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

PRIMARY FINDINGS INTERPRETATION

NIPBL NM_133433.4:c.2612_2613del (NP_597677.2:p.Arg871ThrfsTer2)	
Population Data	The variant is not observed in the gnomAD v4.1.0 dataset.
Predicted Consequence / Location	Frameshift: predicted to result in a loss or disruption of normal protein function through nonsense-mediated decay (NMD) or protein truncation. Multiple pathogenic variants are reported downstream of the variant.
Segregation Data	None
Computation and Functional Data	None
Previously Reported Variant Data	None
Disease Association	Cornelia de Lange syndrome 1 (OMIM: 122470)
Validation	Not performed as the variant was considered high-quality
Variant Classification	Likely pathogenic

ADDITIONAL FINDINGS

The table(s) below provide a list of variants that require further clinical correlation and/or additional testing, such as segregation or functional analysis, to be proven clinically relevant to the patient's symptoms. These include variants of uncertain significance (VUS) 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 VUS 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.

Noonan syndrome 10 (OMIM: 616564, Autosomal dominant)

Gene	Variant	Consequence	Zygosity	In sillico	Allele frequency	Class
LZTR1	NM_006767 .4:c.295G>A (p.Asp99Asn)	Missense variant	Heterozygous	REVEL: 0.49, 3Cnet: 0.99 SpliceAI: 0.01	gnomAD: 1 / 1461814	VUS

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 gene requested by the provider.

3B-GENOME, Proband

Clinical use

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

SECONDARY FINDINGS

Breast-ovarian cancer, familial 2 (OMIM: 612555) is an autosomal dominant, multifactorial disorder. Individuals with pathogenic variants in *BRCA2* (OMIM: 600185) have an increased risk for developing breast cancer and ovarian cancer (includes fallopian tube and primary peritoneal cancers) and other cancers such as prostate cancer, pancreatic cancer, and melanoma to a lesser extent. Genetic counseling and clinical management are warranted.

Breast-ovarian cancer, familial, 2 (OMIM: 612555)

Gene	Variant		Classification
BRCA2	**Genomic Position**	13-32362596-A-T (GRCh38)	Pathogenic
	cDNA	cDNA: NM_000059.4:c.7879A>T	
	Protein	NP_000050.3:p.Ile2627Phe	
	Zygosity	Heterozygous	
	Inheritance	Unknown	

3B-GENOME, Proband

Clinical use

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

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: 32901917.
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. McKenna A, Hanna M, Banks E, Sivachenko A. et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010 Sep;20(9):1297–303. PMID: 20644199
8. Xiaoyu Chen, Ole Schulz-Trieglaff, Richard Shaw, et al. Manta v1.6.0: rapid detection of structural variants and indels for germline and cancer sequencing applications. Bioinformatics. 2016 Apr 15;32(8):1220-2. PMID: 26647377
9. Dolzhenko E, Deshpande V, Schlesinger F, et al. ExpansionHunter: a sequence-graph-based tool to analyze variation in short tandem repeat regions. Bioinformatics. 2019 Nov 15;35(22):4754–6. PMID: 31134279
10. Gardner EJ, Lam VK, Harris DN, et al. The Mobile Element Locator Tool (MELT): population-scale mobile element discovery and biology. Genome Res. 2017 Nov;27(11):1916–29. PMID: 28855259
11. 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

3B-GENOME, Proband

Clinical use

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

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 (<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 daily 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.

3B-GENOME, Proband

Clinical use

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

METHODS

Genomic DNA was extracted from DBS specimen using standard protocol. Exome capture was performed using xGen Exome Research Panel v2, supplemented with xGen human mtDNA panel and xGen Custom Hyb Panel v1 (Integrated DNA Technologies, Coralville, Iowa, USA). Sequencing was performed using NovaSeq X (Illumina, San Diego, CA, USA). In total, 11,323,631,778 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 161.27 mean depth-of-coverage within the 34,212,647 bases of the captured region, which is approximately 99.3% of the RefSeq protein coding region. Approximately 99.50% of the targeted bases were covered to a depth of ≥20x. Despite the insufficient coverage across 0.50% of the bases (see below for details), these metrics are consistent with high quality exome sequencing data and deemed adequate for analysis. Gene or exon level depth-of-coverage (DOC) information is available upon request. In total, 64,937 single nucleotide variants (SNV) and 11,758 small insertions and deletions (INDEL) were identified. Sequencing data analysis and variant interpretation were performed using 3billion's proprietary system, EVIDENCE v4.3 (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) and Manta v1.6.0 (Bioinformatics. 2016;32:1220-2) for calling CNV (copy number variants) based on paired-end information and 3bCNV v2.1, an internally developed tool, for calling CNV (copy number variants) 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 variants called with high quality scores, only low quality variants are 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%

3B-GENOME, Proband

Clinical use

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

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

3B-GENOME, Proband

Clinical use

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

APPENDIX. REQUESTED GENE(S) FINDINGS

The requested genes were covered: see below for the coverage information of each gene.

Gene	% bp >=20x	Gene	% bp >=20x
AVPR2	100.0	*FGFR3*	100.0