3B-EXOME, Unaffected individual

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 / Male	**Institution**	[Institution]	**Ordered on**	yyyy-mm-dd
Ethnicity	Latino/Admixed American			**Accessioned on**	yyyy-mm-dd
Family history	Mother				

* (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	**Carriership Findings**	Variant reported
Requested gene(s) findings	No variant reported		

PRIMARY FINDINGS

DETECTED

A heterozygous pathogenic variant was identified in *GAA*. *GAA* is associated with autosomal recessive 'Glycogen storage disease II (OMIM: 232300)' and therefore this proband is a carrier. Family history is considered compatible with this disorder, but clinical correlation is recommended. Only clinically significant variants that can explain the provided phenotype were considered reportable for this test and therefore, the proband may be a carrier for additional diseases/conditions not related to the provided phenotype.

Glycogen storage disease II (OMIM: 232300)

Gene	Variant		Classification
GAA	Genomic Position	17-80117016-G-C (GRCh38)	Pathogenic
	cDNA	NM_000152.5:c.2238G>C	
	Protein	NP_000143.2:p.Trp746Cys	
	Zygosity	Heterozygous	
	Inheritance	Unknown	

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PRIMARY FINDINGS INTERPRETATION

GAA NM_000152.5:c.2238G>C (NP_000143.2:p.Trp746Cys)	
Population Data	The variant is observed at an extremely low frequency in the gnomAD v4.1.0 dataset (total allele frequency: 0.048%).
Predicted Consequence / Location	Missense variant
Segregation Data	None
Computation and Functional Data	Functional studies provide supporting evidence of the variant having a damaging effect on the gene or gene product (PMID: 21757382, 23430493, 7981676). In silico tool predictions suggest damaging effect of the variant on gene or gene product [REVEL: 0.90 (>=0.6, sensitivity 0.68 and specificity 0.92); 3Cnet: 0.99 (> 0.75, sensitivity 0.96 and precision 0.92)].
Previously Reported Variant Data	The same nucleotide change resulting in the same amino acid change has been previously reported as pathogenic/likely pathogenic with strong evidence (ClinVar ID: VCV000265160 / PMID: 18458862). The variant has been reported to be in trans with a pathogenic variant as either compound heterozygous or homozygous in at least 4 similarly affected unrelated individuals (PMID: 21232767, 25093132, 25526786). Different missense changes at the same codon (p.Trp746Arg, p.Trp746Gly, p.Trp746Ser) have been reported as pathogenic/likely pathogenic with strong evidence (ClinVar ID: VCV000188484, VCV000499293, VCV000556431 / PMID: 18425781, 20080426, 23430493).
Disease Association	Glycogen storage disease II (OMIM: 232300)
Validation	Not performed as the variant was considered high-quality
Variant Classification	Pathogenic

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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CARRIERSHIP FINDINGS

The table(s) below summarize heterozygous pathogenic or likely pathogenic variants associated with carrier status were detected within the 2037 genes analyzed, other than those reported in the Primary Findings section. Additional variants may remain undetected due to test limitations (see Recommendations #2, #7, #8, and #9).

Gene	Variant	Zygosity	Inheritance	In sillico	Allele frequency	Class	Disease
CFTR	NM_000492.4:c.1021_1022dup	Heterozygous	Unknown	REVEL: - 3Cnet: - SpliceAI: -	gnomAD: 1 / 1461814	Pathogenic	Cystic fibrosis

REQUESTED GENE FINDINGS

No clinically significant variant was identified in the requested gene. The 10 requested genes were covered well.

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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: 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

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NOTES

1. Results summary: Results are categorized into detected and not detected.

Category	Explanation
Detected	• The candidate variant (P/LP/VUS) is identified in a gene associated with family history in a known disease gene.
Not Detected	• No clinically significant variant is identified in a gene associated with family history.

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, small insertions/deletions (<50 bp), large (>=3 consecutive exons) copy number variants and level mitochondrial genome variants with high accuracy in most of the genomic regions. If low level (<20%) mosaicism variants on autosomes and sex chromosomes, small (<3 consecutive exons) copy number variants (CNVs), or structural variants (SVs) including inversions and translocations variants 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, epigenetic factors, or variants in regulatory regions called by being near coding exons 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 exome 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 level heteroplasmic (<20%) 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. 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.
6. Variants reported outside of Primary and/or Secondary Findings are not confirmed by Sanger sequencing. Therefore, only variants meeting strict quality criteria are reported.
7. Carrier Status Findings are limited to Pathogenic or Likely Pathogenic variants within a defined list of genes associated with Autosomal Recessive or X-linked disorders. For inversions and translocations, variants are reported only if a breakpoint is located within a listed gene. Autosomal Dominant, mitochondrial, mosaic variants, and VUS are excluded. This analysis is not a comprehensive carrier screening test and is not intended for diagnosis.

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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, 11,458,836,111 bases of sequence were generated and uniquely aligned to a modified version of the Genome Reference Consortium Human Build 38 (GRCh38), in which falsely duplicated regions of chromosome 21 were masked to N's based on the masking file developed in collaboration with the GRC (Genome Reference Consortium) (Nat Biotechnol. 2022;40:672-680) and Revised Cambridge Reference Sequence (rCRS) of the mitochondrial genome, generating 168.58 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 99.40% of the targeted bases were covered to a depth of ≥20x. Despite the insufficient coverage across 0.60% 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, 65,500 single nucleotide variants (SNV) and 12,080 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 v3.8, Genome Res. 2010;20:1297–303) and Manta (Bioinformatics. 2016;32:1220-2) for calling CNV (copy number variants) based on paired-end information and 3bCNV v23.0818, an internally developed tool, for calling CNV (copy number variants) including aneuploidy based on the DOC information. It also incorporates Mutect2 (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%

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DISCLAIMER

This test was developed by 3billion in the purpose of identifying single nucleotide variants (SNV), small insertions/deletions (INDEL, <50 bp), large (>=3 consecutive exons) copy number variants, mobile element insertion variants and repeat expansion variants within the targeted genomic regions. Repeat expansion detection is possible for the following 18 genes. Repeat expansion number may be underestimated for the starred (*) gene with compromised sensitivity ({italic::AR, ARX, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS*, CACNA1A, COMP, FOXL2, HOXD13, HTT, PABPN1, PHOX2B, PRDM12, TBP, 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 and 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). The test was performed following the standard operation procedure and quality control measures developed for the clinical testing. If other types of variants such as translocation, inversion, low-level mosaicism, low heteroplasmic level mitochondrial genome variants, and mitochondrial genome large deletion/duplication are suspected, it is recommended to perform appropriate testing that are designed to detect those types of variants. Also, there are certain exonic regions that are incompletely sequenced due to technical difficulties with amplification, sequencing and alignment. If variants within these regions are suspected, it is recommended to perform alternate testing that are designed to sequence those regions/genes adequately. 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