3B-GENOME, Proband

Clinical use

PATIENT INFORMATION

Unique ID	[Unique ID]	**Physician**	[Physician name]	**Sample type**	DBS
3billion ID	EPH23-XXXX	**Department**	Pediatrics	**Collected on**	2023-08-25
DOB / Sex	2016-08-08 / Male	**Institution**	[Institution name]	**Ordered on**	2023-08-25
Ethnicity	Latino/Admixed American			**Accessioned on**	2023-08-28

CLINICAL INFORMATION

Symptoms	HP:0000047 Hypospadias, HP:0000252 Microcephaly, HP:0001249 Intellectual disability, HP:0012758Neurodevelopmental delay

RESULT SUMMARY

NEGATIVE

No clinically significant variant relevant to the patient's phenotype was identified.

RESULTS INTERPRETATION

No clinically significant variant relevant to the patient's phenotype was identified. However, the possibility of missing the disease-causing variant due to technical limitations and/or genotype-phenotype knowledge limitation cannot be excluded (see below Recommendations #2, #3, and #5). If requested, an automated daily reanalysis will be performed and any updated results will be provided to the medical provider (see below Recommendation #5).

SECONDARY FINDINGS

No clinically significant variant was identified in the 81 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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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.
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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
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8. Xiaoyu Chen, Ole Schulz-Trieglaff, Richard Shaw, et al. Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications. Bioinformatics. 2016 Apr 15;32(8):1220-2. PMID: 26647377
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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 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 (<30%) 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. 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. To be compliant with Korean Bioethics and Safety Act Article 53 (Provision and Discarding of Materials for Testing), 3billion discards all specimens after the initial testing and cannot confirm the newly identified variant(s) from reanalysis by Sanger sequencing unless a new specimen is provided by the patient.

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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 6000 (Illumina, San Diego, CA, USA). In total, [X] 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 [X] mean depth-of-coverage across the entire genome. Approximately [X]% of the genome ([X]% of the autosomes) was covered at a depth of ≥20x. Despite the insufficient coverage across [X]% 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, [X] single nucleotide variants (SNV) and [X] small insertions and deletions (INDEL) were identified. Sequencing data analysis and variant interpretation were performed using 3billion's proprietary system, EVIDENCE v4.1 (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), Manta (Bioinformatics. 2016;32:1220-2) for structural variant calling including CNV (copy number variants) based on paired-end information, and 3bCNV v23.0818, an internally developed tool, for calling CNV including aneuploidy based on the DOC information. It also incorporates ExpansionHunter v5.0.0 for calling repeat expansion variants (Bioinformatics. 2019;35:4754–6), MELT v2.2.2 (Genome Res. 2017;27:1916–29) for calling mobile element insertion, AutoMap v1.2 (Nat Commun. 2021;12:518) for detecting regions of homozygosity (ROH), and Variant Effect Predictor v104.2 (VEP, Ensembl, Genome Biology 2016;17:122) 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. All SNVs, all INDELs and 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.

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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. This test is intended for clinical purposes and should not be regarded as investigational or for research. This laboratory is certified under the Clinical 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). 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