Blueprint Genetics

Familial Variant Testing

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

INDIVIDUAL

NAME **DOB** **AGE** **GENDER** **ORDER ID**
3

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE** **CUSTOMER SAMPLE ID**

TEST INFO

NUMBER OF VARIANTS
1

INDEX ORDER ID
11111

SUMMARY OF RESULTS

This individual is heterozygous for *USH2A* c.11864G>A, p.(Trp3955*), which is classified as pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
USH2A	NM_206933.2	c.11864G>A, p.(Trp3955*)	HET	stop_gained	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs111033364	GRCh37/hg19	1:215901574	C/T		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	33/282556	N/A	N/A	disease causing	Retinitis pigmentosa 39, Usher syndrome, type 2A	

STATEMENT

CLINICAL HISTORY

This individual is a 3-year-old child who is considered to be healthy and unaffected. This individual is the index patient's (ID_11111) full sibling. Previous genetic testing has identified USH2A c.11864G>A p.(Trp3955*) variant in the family. Targeted testing of the variant was requested.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Familial Variant Testing identified a heterozygous nonsense variant *USH2A* c.11864G>A, p.(Trp3955*).

USH2A c.11864G>A, p.(Trp3955*)

There are 31 individuals heterozygous and 1 individual homozygous for this variant in gnomAD, a large reference population database (n>120,000 exomes and >15,000 genomes) which aims to exclude individuals with severe pediatric disease. The variant generates a premature stop codon in exon 61 (of total 72 exons) and is predicted to lead to loss of normal protein function, either through protein truncation or nonsense-mediated mRNA decay. *USH2A* c.11864G>A, p.(Trp3955*) has been reported in the literature and in clinical testing in multiple patients with Usher syndrome type 2 (USH2), and also in some patients with retinitis pigmentosa (RP) (ClinVar variation ID: 2357, LOVD DB-ID USH2A_00159). It was originally described by van Wijk et al, (PMID: 15015129), and has subsequently been reported in many studies in a compound heterozygous or homozygous state (PMID: 22135276, 25575603, 26927203, 27460420). In the study by Bonnet et al, the p.(Trp3955*) variant was identified in 22% of the USH2 patients, and it was as common as the c.2299delG variant (PMID: 27460420).

USH2A

The *USH2A* gene (OMIM *608400) encodes a protein called usherin. Usherin is a large glycoprotein that is found in basement membranes specifically in retinal photoreceptors and developing cochlear hair cells (PMID: 17360538). It is a single-pass type I membrane protein with extracellular (aa 32−5042), helical (5043−5063), and cytoplasmic (5064−5202) domains (Uniprot - O75445). Pathogenic variants in the *USH2A* gene have been associated with autosomal recessive Usher syndrome type II (USH2; OMIM #276901) and autosomal recessive retinitis pigmentosa (RP; OMIM #613809). In addition, pseudodominant inheritance has been described in families with *USH2A*-related disease (PMID: 26310143, 23737954). USH2 is characterized by congenital, moderate to severe hearing impairment, onset of RP in the first or second decade of life and no vestibular impairment (GeneReviews: NBK1341). USH2 appears to be the most common clinical form of Usher syndrome, accounting for more than 50% of all patients. Pathogenic variants in the *USH2A* gene have been found in approximately 75% of patients with USH2 (PMID: 9624053, 18273898).

In a cohort of 118 Scandinavian USH2 patients, c.2299delG was found to account for 30.6% of all disease alleles detected, whereas missense variant p.(Asn346His) accounted for 7.1% (PMID: 18273898). Spectrum of the 57 disease-causing variants identified in this study was as follows: 23 nonsense, 23 missense, 14 indels leading to frameshift, one splice-site variant, and one in-frame deletion. Patients from 10 families in the study remained heterozygous upon extensive *USH2A* mutation screening, suggesting that there might be large deletions in this gene that remain undetected by conventional PCR-based methods. Consistent with this, several large deletions that span one or several exons of the *USH2A* gene have been reported in probands affected with USH2 (PMID: 16098008, 17405132, 18273898, 22135276, ClinVar Variation ID: 178657, 178656). Recently, Pierrache et al demonstrated that the presence of two truncating *USH2A* variants is restricted to Usher syndrome phenotype, whereas the presence of at least one “eye-specific” variant together with another *USH2A* variant leads to nonsyndromic RP (PMID: 26927203).

HGMD lists over 1100 disease-associated variants, of which the majority have been associated with Usher syndrome and includes 99 gross deletions and 13 gross duplications at the single- or multi-exon level (HGMD Professional 2020.2). ClinVar lists over 300 *USH2A* variants identified in clinical testing and classified as pathogenic, of which those associated with USH2 are mainly

truncating variants (Last accessed: July 2020).

Mutation nomenclature is based on GenBank accession NM_206933.2 (*USH2A*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

A heterozygous *USH2A* c.11864G>A, p.(Trp3955*) variant was detected in this individual. Disease caused by *USH2A* variants is inherited in an autosomal recessive manner. Thus, this individual is a carrier of the variant. Genetic counselling is recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

On November 1, 2020 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

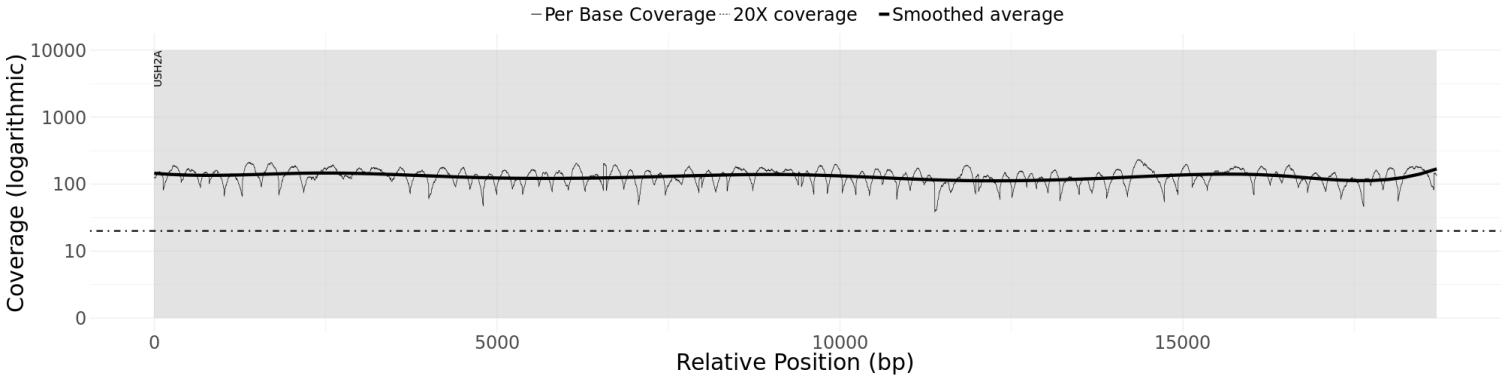
Nelli Paasikoski, MSc

Geneticist

Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.



APPENDIX 5: SUMMARY OF THE TEST

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based method. DNA quality and quantity were assessed using electrophoretic methods. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the final output.
Bioinformatics and quality control: Base called raw sequencing data was transformed into FASTQ format using Illumina's software (bcl2fastq). Sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon) for nDNA. Variant data for was annotated using a collection of tools (VcfAnno and VEP) with a variety of public variant databases including but not limited to gnomAD, ClinVar and HGMD. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures including assessments for contamination and sample mix-up. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. The expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content.
Interpretation: The clinical interpretation team assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Variant classification: Our variant classification follows the Blueprint Genetics Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report.
Databases: The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited to, the gnomAD, ClinVar, HGMD Professional and Alamut Visual. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as Database of Genomic Variants and DECIPHER. For interpretation of mtDNA variants specific databases including e.g. Mitomap, HmtVar and 1000G were used.
Confirmation: Additional confirmation has been used when the variant quality score (QS) has not met the internal threshold for a true positive/negative call.
Analytic validation: The detection performance of this panel is expected to be in the same range as our high-quality, clinical grade NGS sequencing assay used to generate the panel data (nuclear DNA: sensitivity for SNVs 99.89%, indels 1-50 bps 99.2%, one-exon deletion 100% and five exons CNV 98.7%, and specificity >99.9% for most variant types). It does not detect very low level mosaicism as a variant with minor allele fraction of 14.6% can be detected in 90% of the cases. Detection performance for mtDNA variants (analytic and clinical validation): sensitivity for SNVs and INDELs 100.0% (10-100% heteroplasmy level), 94.7% (5-10% heteroplasmy level), 87.3% (<5% heteroplasmy level) and for gross deletions 100.0%. Specificity is >99.9% for all.
Test restrictions: A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be

undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.
Technical limitations: This test does not detect the following: complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see the list of non-coding variants covered by the test). Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics. Laboratory error is also possible. Please see the Analytic validation above.
Regulation and accreditations: This test was developed and its performance characteristics determined by Blueprint Genetics (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation (excluding mtDNA testing and digital PCR confirmation).

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
CNV = Copy Number Variation eg, one exon or multiexon deletion or duplication
gnomAD = genome Aggregation Database (reference population database; >138,600 individuals)
gnomAD AC/AN = allele count/allele number in the genome Aggregation Database (gnomAD)
HEM = hemizygous
HET = heterozygous
HOM = homozygous
ID = rsID in dbSNP
MutationTaster = *in silico* prediction tools used to evaluate the significance of identified amino acid changes.
NGS = Next Generation Sequencing
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
OMIM = Online Mendelian Inheritance in Man®
PolyPhen = *in silico* prediction tool used to evaluate the significance of amino acid changes.
POS = genomic position of the variant in the format of chromosome:position
SIFT = *in silico* prediction tool used to evaluate the significance of amino acid changes.
WT = wild type (normal allele)