Blueprint Genetics

Whole Exome Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		5		

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
DNA		

SUMMARY OF RESULTS

TEST RESULTS

Analysis of whole exome sequence variants in previously established disease genes

The patient is compound heterozygous for *FOXI1* c.386C>T, p.(Ser129Leu) and *FOXI1* c.442C>G, p.(Gln148Glu). They are both classified as variants of uncertain significance (VUS).

Del/Dup (CNV) analysis

Negative

Analysis of candidate variants in genes not yet associated with disease

Negative

ADDITIONAL FINDINGS

The patient is heterozygous for *FLG* c.5198C>G, p.(Ser1733*), which is likely pathogenic.

The patient is heterozygous for *FLG* c.2838T>G, p.(Val946=), which is a variant of uncertain significance (VUS).

PRIMARY VARIANT TABLE: SEQUENCE ALTERATIONS IN ESTABLISHED DISEASE GENES

GENE	**TRANSCRIPT**	**NOMENCLATURE**	**GENOTYPE**	**CONSEQUENCE**	**INHERITANCE**	**CLASSIFICATION**
FOXI1	NM_012188.4	c.386C>T, p.(Ser129Leu)	HET	missense_variant	AR	**Variant of uncertain significance**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	5:169533347	C/T		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	0/0	probably damaging	deleterious	disease causing	Enlarged vestibular aqueduct, Pendred syndrome	
GENE	**TRANSCRIPT**	**NOMENCLATURE**	**GENOTYPE**	**CONSEQUENCE**	**INHERITANCE**	**CLASSIFICATION**
FOXI1	NM_012188.4	c.442C>G, p.(Gln148Glu)	HET	missense_variant	AR	**Variant of uncertain significance**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	5:169533403	C/G		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	98/282564	probably damaging	deleterious	disease causing	Enlarged vestibular aqueduct, Pendred syndrome	

Please see APPENDIX 2: Additional Findings
Please see APPENDIX 3: Secondary Findings

SEQUENCING PERFORMANCE METRICS

SAMPLE	MEDIAN COVERAGE	PERCENT >= 20X
Index	162	99.27

TEST INFORMATION

Blueprint Genetics Whole Exome Plus Test (version 2, Feb 9, 2018) consists of sequence analysis of all protein coding genes in the genome for the proband, coupled with Whole Exome Deletion/Duplication (CNV) Analysis. The test targets all protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in Appendix). This test should be used to detect single nucleotide variants and small insertions and deletions (INDELs) up to 220 bps and copy number variations defined as single exon or larger deletions and duplications. This test should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

Analysis of Whole Exome Plus Test is primarily focused on established disease genes that have been previously associated with genetic disorders. The genes with known clinical association include those curated by Blueprint Genetics (BpG) and included in BpG diagnostic panels (>2400 genes). These genes are supplemented with genes included in The Clinical Genomics Database (>3350 genes) and the Developmental Disorders Genotype-Phenotype Database (DD2GP) (>1640 genes). Total number of genes that are considered as clinically associated in the Whole Exome Plus analysis is >3750 (and the number is constantly updated).

If analysis of exome variants in previously established disease genes is inconclusive, exome variant data are also analyzed for variants that are not located within known clinically associated genes but have properties that make them candidates for potentially disease-causing variants (please see Appendix: Summary of the Test). If over time other patients with similar phenotype and variants in the same gene are identified, the variant may be reclassified as a likely cause of the disorder.

STATEMENT

CLINICAL HISTORY

Patient is a 5-year-old child with sensorineural hearing loss, cochlear implants, and enlarged vestibular aqueducts, dry skin, and delayed speech and language development.

There is no consent to report secondary findings for this individual.

CLINICAL REPORT

Whole-exome sequence analysis of variants in previously established disease genes

The exome data of the patient were analysed for rare heterozygous variants (potential *de novo* variants) and variants following recessive inheritance pattern.

Sequence analysis using the Blueprint Genetics (BpG) Whole Exome Plus identified a heterozygous missense variant *FOXI1* c.386C>T, p.(Ser129Leu) and a heterozygous missense variant *FOXI1* c.442C>G, p.(Gln148Glu). Our NGS data indicate that these variants are on different parental alleles (in *trans*) in this patient.

FOXI1 c.386C>T, p.(Ser129Leu)

This variant is absent in the Genome Aggregation Database control population cohorts (gnomAD, n>120,000 exomes and >15,000 genomes). Serine is highly conserved amino acid and there is a large physicochemical difference between serine and leucine. All *in silico* tools utilized predict this variant to be damaging to protein structure and function. To the best of our knowledge, this variant has not been described in the medical literature or reported in disease-related variation databases such as ClinVar or HGMD.

FOXI1 c.442C>G, p.(Gln148Glu)

There are 98 individuals heterozygous for this variant in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). No homozygotes were observed in the dataset. Database curators have made every effort to exclude individuals with severe pediatric diseases from these cohorts. Glutamine is highly conserved amino acid and there is a small physicochemical difference between glutamine and glutamic acid. All *in silico* tools utilized predict this variant to be damaging to protein structure and function. The variant has been identified in clinical testing (ClinVar ID: 290061).

FOXI1

FOXI1 gene (OMIM **601093*) encodes forkhead box I1 protein. This protein belongs to the forkhead family of winged helix transcription regulators. This activator is required for the development of normal hearing, sense of balance, and kidney function.

Recessive mutations in the *FOXI1* gene are a rare cause of enlarged vestibular aqueduct (EVA). Yang *et al*. found two patients with Pendred syndrome and four with EVA who had altogether five different variants in the *FOXI1* that compromise its ability to activate the transcription of *SLC26A4*, that is a known gene behind Pendred syndrome and EVA (PMID: *17503324*). Patients were heterozygous for the variants, except in one family, were the EVA phenotype segregated with a double-heterozygous variant in the *SLC26A4* and *FOXI1*. This finding of digenic inheritance was also supported by the double-heterozygous mouse mutant model that has a similar phenotype.

Variants in the *FOXI1* are rare causes of hearing loss. Recent large study with over 1100 sequentially accrued patients with hearing loss did not find any causative mutations in this gene (PMID: *26969326*). Only one variant of uncertain significance has been seen in clinical testing and reported in the ClinVar (ID: 252675). However, unfortunately no clinical information is provided on this submission. In the HGMD mutation database, there are seven variants reported associating with EVA or Pendred syndrome. Six of these are missense variants, while one is an inframe deletion.

Mutation nomenclature is based on GenBank accession NM_012188.4 (*FOXI1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

Upon request, filtered variant files and raw data files from the whole exome analysis can also be provided.

Analysis of candidate variants in genes not yet associated with disease

If analysis of exome variants in previously established disease genes is inconclusive, exome variant data are also analyzed for variants that are not located within known clinically associated genes but have properties that make them candidates for

potentially disease-causing variants (please refer to Appendix: Summary of the Test).

No candidate variants are reported in this patient.

CONCLUSION

FOXI1 c.386C>T, p.(Ser129Leu) and *FOXI1* c.442C>G, p.(Gln148Glu) are both classified as variants of uncertain significance (VUS), as there is insufficient evidence to evaluate their clinical relevance. These variants should not be used for clinical decision-making or risk evaluation in family members. Our NGS data indicates that these variants are on different parental alleles (in *trans*) in this patient. Management of the patient and family should be based on clinical evaluation and judgment. Genetic counseling is recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

On May 10, 2020 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Kirsi Alakurtti, Ph.D., CLG

Senior Geneticist

Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer

APPENDIX 2: ADDITIONAL FINDINGS

This table includes the following variants:

1. a pathogenic or likely pathogenic variant in a gene that causes an autosomal recessive disorder with a good match to the phenotype described in the patient but with no second rare, potentially disease-causing variant identified
2. variants related to patient's phenotype and potentially relevant to the patient's medical care (such as low-penetrance risk variants)

VARIANT TABLE: ADDITIONAL SEQUENCE ALTERATIONS

GENE FLG	**TRANSCRIPT** NM_002016.1	**NOMENCLATURE** c.5198C>G, p.(Ser1733*)	**GENOTYPE** HET	**CONSEQUENCE** stop_gained	**INHERITANCE** AD,AR	**CLASSIFICATION** **Likely pathogenic**
	ID	**ASSEMBLY** GRCh37/hg19	**POS** 1:152282164	**REF/ALT** G/C		
	gnomAD AC/AN 1/251474	**POLYPHEN** N/A	**SIFT** N/A	**MUTTASTER** disease causing	**PHENOTYPE** Icthyosis vulgaris	
GENE FLG	**TRANSCRIPT** NM_002016.1	**NOMENCLATURE** c.2838T>G, p.(Val946=)	**GENOTYPE** HET	**CONSEQUENCE** synonymous_variant	**INHERITANCE** AD,AR	**CLASSIFICATION** **Variant of uncertain significance**
	ID rs149967165	**ASSEMBLY** GRCh37/hg19	**POS** 1:152284524	**REF/ALT** A/C		
	gnomAD AC/AN 167/282858	**POLYPHEN** N/A	**SIFT** N/A	**MUTTASTER** N/A	**PHENOTYPE** Icthyosis vulgaris	

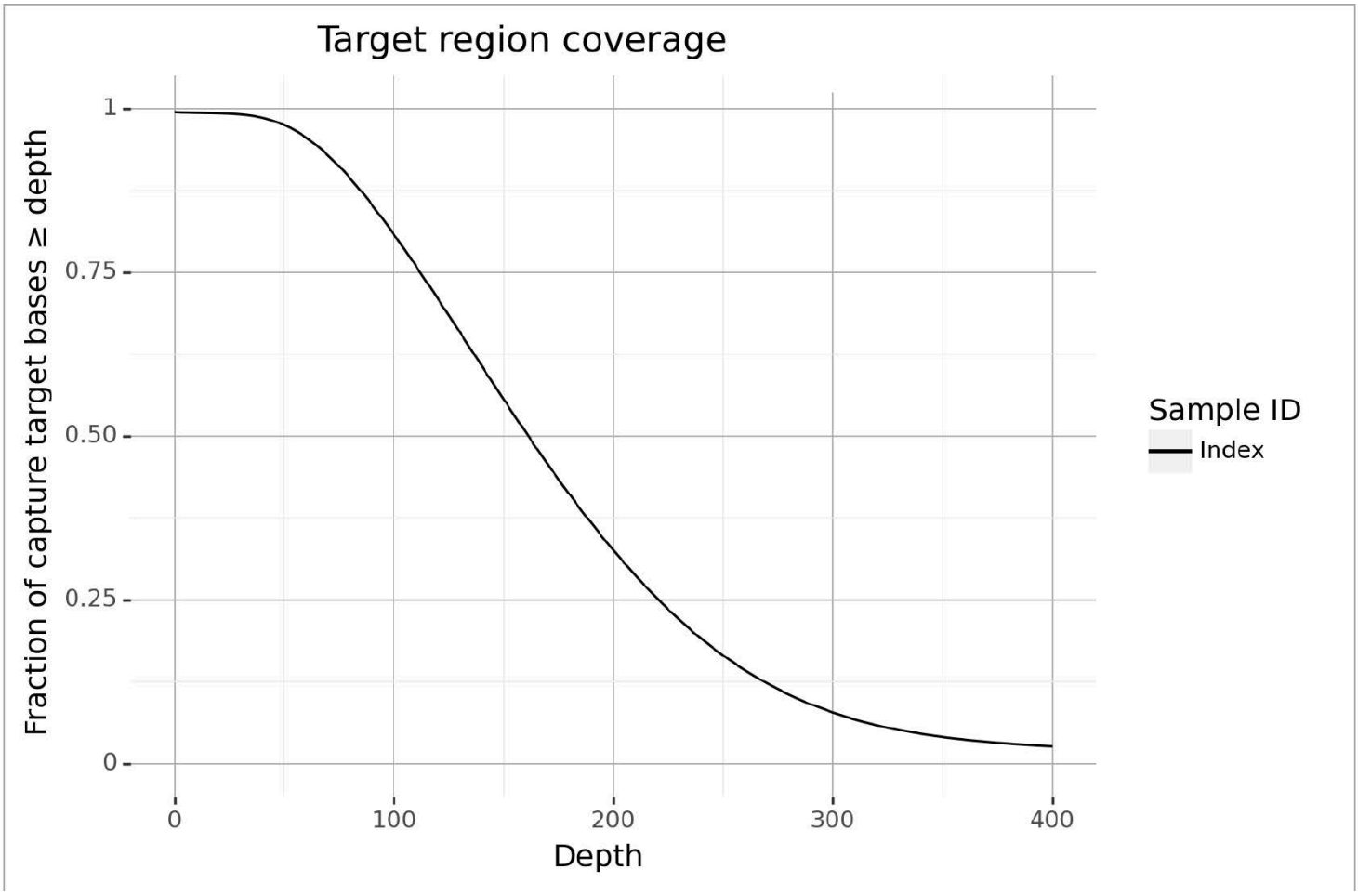
NOTES REGARDING ADDITIONAL FINDINGS

The patient is heterozygous for a likely pathogenic variant *FLG* c.5198C>G, p.(Ser1733*) and for a variant of uncertain significance *FLG* c.2838T>G, p.(Val946=). *In silico* splice prediction tool MaxEntScan predicts the *FLG* c.2838T>G substitution will create a cryptic donor splice site. The variant might therefore lead to abnormal splicing, although transcriptional analysis would be necessary to determine this variant's effect on splicing. Due to the large genomic distance between these variants, NGS-based methods cannot determine whether they occur on the same (in *cis*) or different (in *trans*) parental alleles. Pathogenic variants in *FLG* are considered to increase the risk of ichthyosis vulgaris and atopic dermatitis. Ichtyosis vulgaris caused by *FLG* mutations is inherited in a semidominant manner with high penetrance and more severe phenotype in *FLG*-null allele homozygotes or compound heterozygotes and reduced penetrance and milder phenotype in heterozygous individuals. Genetic counseling is recommended.

APPENDIX 3: SECONDARY FINDINGS

The index patient was not opted-in for analysis of secondary findings.

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

WHOLE EXOME

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 were adjusted to account for the effects of varying guanine and cytosine content.
Interpretation: Our variant classification follows the Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase the reproducibility of the variant classification and to improve the clinical validity of the report. Likely benign and benign variants were not reported. The pathogenicity potential of the identified variants were assessed by considering the predicted consequence, the biochemical properties of the codon change, the degree of evolutionary conservation as well as a number of reference population databases and mutation databases such as, but not limited, to the 1000 Genomes Project, gnomAD, ClinVar and HGMD. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster were used to assist with variant classification. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as 1000 Genomes Project, Database of Genomic Variants, ExAC, DECIPHER. The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data if needed. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
In addition to analysis of variants in previously established disease genes, variants in genes where disease association has not yet been established were considered as potentially disease-causing using the following scheme:

- For probands who were whole-exome sequenced with parents, all coding region de novo variants were considered as candidate variants.
- Novel (absent in gnomAD) heterozygous, truncating variants (nonsense, frameshift, canonical splice site variants) in genes predicted to be intolerant for loss-of-function variation based on ExAC variant data. Genes were determined as intolerant if probability of loss-of-function intolerance score pLI≥0.9 . The closer pLI is to one, the more LoF intolerant the gene appears to be. Genes with pLI≥0.9 are defined as an extremely LoF intolerant set of genes.

- Rare (<1% MAF in gnomAD), truncating homozygous or (predicted) compound heterozygous variants, or a combination of rare truncating and rare missense variant that is predicted deleterious by multiple *in silico* tools.

In addition, only variants in genes whose known expression pattern and function are considered relevant for the phenotype are included (e.g., variants in genes exclusively expressed in a muscular tissue are not considered as a candidate for a central nervous system disease). Candidate variants are not validated by Sanger sequencing, but their quality is inspected by visualization of sequence reads and evaluation of quality metrics, and only likely true variants are reported.

For proband and family members who were opted-in for analysis of secondary findings from the WES data, 59 clinically actionable genes were analyzed and reported for secondary findings according to recommendations by ACMG (PMID 27854360) with minor modifications aiming to increase the clarity of the classifications of the reportable variants (please see our website/clinical interpretation). Secondary findings are not analyzed or reported for deceased individuals or fetal samples.

Confirmation of sequence alterations: Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a true positive call. In addition, prenatal case with diagnostic findings were confirmed.
Confirmation of copy number variants: CNVs (Deletions/Duplications) were confirmed using a digital PCR assay if they covered less than 10 exons (heterozygous), less than 3 exons (homo/hemizygous) or were not confirmed at least three times previously at our laboratory. Furthermore, CNVs of any size were not confirmed when the breakpoints of the call could be determined.
Analytic validation: This laboratory-developed test has been independently validated by Blueprint Genetics. The sensitivity of this panel is expected to be in the same range as the validated whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.65%, and indels 1-50 bps 99.07%, one-exon deletion 92.3% and two exons CNV 100%, 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.
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.
Please refer to Appendix 8 of the report in Nucleus ordering and reporting portal for full list of non-coding variants included in the Whole Exome analysis.

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GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
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.
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.
Transcript = GenBank accession for reference sequence used for variant nomenclature