Blueprint Genetics

Albinism Panel (Ophthalmology) Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		14	Female	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
Blood		

SUMMARY OF RESULTS

TEST RESULTS

Patient is homozygous for *TYR* c.832C>T, p.(Arg278*), which is pathogenic.

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
TYR	11:88924382	NM_000372.4	c.832C>T, p.(Arg278*)	stop_gained	HOM	**Pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs62645904	49/276772	N/A	N/A	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Albinism oculocutaneous		AR	-	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Albinism Panel (Ophthalmology)	23	324	65977	65977	172	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Albinism Panel (version 3, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: AP3B1, BLOC1S3, BLOC1S6, C10ORF11, DTNBP1, GPR143, HPS1*, HPS3*, HPS4, HPS5, HPS6, LYST*, MC1R, MITF, MLPH, MYO5A, OCA2, RAB27A, SLC24A5, SLC38A8, SLC45A2, TYR* and TYRP1. This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in Appendix 5). This panel 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 panel 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.

*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/
The sensitivity to detect variants may be limited in genes marked with an asterisk (*).

STATEMENT

CLINICAL HISTORY

Patient is a 14-year-old girl with skin and hair hypopigmentation, nystagmus, and decreased visual acuity. Family History: suspicion of albinism in patient´s younger sister.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Albinism Panel identified a homozygous nonsense variant c.832C>T, p.(Arg278*) in *TYR*.

There are 49 individuals heterozygous for the p.(Arg278*) variant in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). Database curators have made every effort to exclude individuals with severe pediatric diseases from these cohorts. The variant generates a premature stop codon at position 278 out of 529 amino acids. It is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay. The *TYR* c.832C>T, p.(Arg278*) variant is a well-recognized pathogenic mutation listed in several public databases. (ClinVar ID 99583, HGMD, Albinism Database - TYR). The exact number of patients with this mutation is unclear, but is has been reported at least twice in clinical testing (ClinVar ID 99583, classified as pathogenic). In the literature, the p.(Arg278*) variant has been reported in association with OCA1 in several patient as homozygous or compound heterozygous together with another *TYR* variant (PMID: 7902671, 13680365, 16907708, 18701257).

TYR

Oculocutaneous albinism (OCA) is characterized by reduction or absence of melanin in the skin, hair, and eyes (PMID: 17980020). OCA patients show symptoms such as reduced skin and hair pigmentation and consequent photosensitivity, high risk of skin cancer, and reduced visual acuity and nystagmus. The non-syndromic OCA is inherited in an autosomal recessive manner, and is mainly due to mutations in four genes: *TYR* (OCA1), *OCA2* (OCA2), *TYRP1* (OCA3) and *SLC45A2* (OCA4). (GeneReviews NBK1510)

The *TYR* (MIM *606933) gene encodes Tyrosinase, which participates in the catalysis of the conversion of tyrosine to melanin. Mutations in *TYR* cause autosomal recessive oculocutaneous albinism, types 1A (OCA1A) and 1B (OCA1B) (MIMs #203100 and #606952). Oculocutaneous albinism is a genetically heterogeneous congenital disorder characterized by decreased or absent pigmentation in the hair, skin, and eyes and consequent photosensitivity, high risk of skin cancer, and reduced visual acuity and nystagmus. Oculocutaneous albinism caused by mutations in the *TYR* gene, is divided clinically into two types: type IA is characterized by complete lack of tyrosinase activity due to production of an inactive enzyme, and type IB is characterized by reduced activity of tyrosinase. In a study on a cohort of 321 albino patients, the frequencies of mutations in the major causative oculocutaneous albinism genes were: *TYR* (44%), *OCA2* (17%), *TYRP1* (1%), *SLC45A2* (7%) and *SLC24A5* (<0.5%) (PMID 27734839). An additional 5% of patients had *GPR143* mutations, which are causative of an X-linked Ocular albinism type I (MIM #300500). A second reliable mutation was not detected for 19% of cases and in 7% of the patients no mutation was detected. OCA1 is the most common subtype found in Caucasians and accounts for about 50% of albinism cases worldwide (PMID: 18463683, 18821858). Prevalence of OCA1 is 1:40,000 (ORPHA79431). Most individuals with OCA1 are compound heterozygotes with different paternal and maternal *TYR* mutations (GeneReviews: NBK1166). ClinVar reports > 50 pathogenic or likely pathogenic variants detected in clinical testing in the *TYR* gene, including missense (70%), and truncating (frameshift, nonsense and splice site variants) (30%). The HGMD professional mutation database lists over 400 variants in *TYR* in association with OCA (mainly OCA1), of which the majority are missense variants (70%) and the remaining 30% are truncating variants.

Mutation nomenclature is based on GenBank accession NM_000372.4 (*TYR*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

TYR c.832C>T, p.(Arg278*) is classified as pathogenic, considering the current literature and the well-established role as a disease- causing variant. Disease caused by *TYR* variants is inherited in an autosomal recessive manner. The patient is homozygous for the variant, which is in line with autosomal recessive inheritance. If both parents are found to be carriers of the variant, each sibling of an affected individual has a 25% chance of being homozygous for the variant and thus being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being an unaffected non-carrier. Genetic counseling and family member testing are recommended. BpG offers targeted variant testing for the family if requested.

CONFIRMATION

TYR c.832C>T, p.(Arg278*) has been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	Jul 31, 2018
Sample received	Sep 14, 2018
Reported	Oct 05, 2018

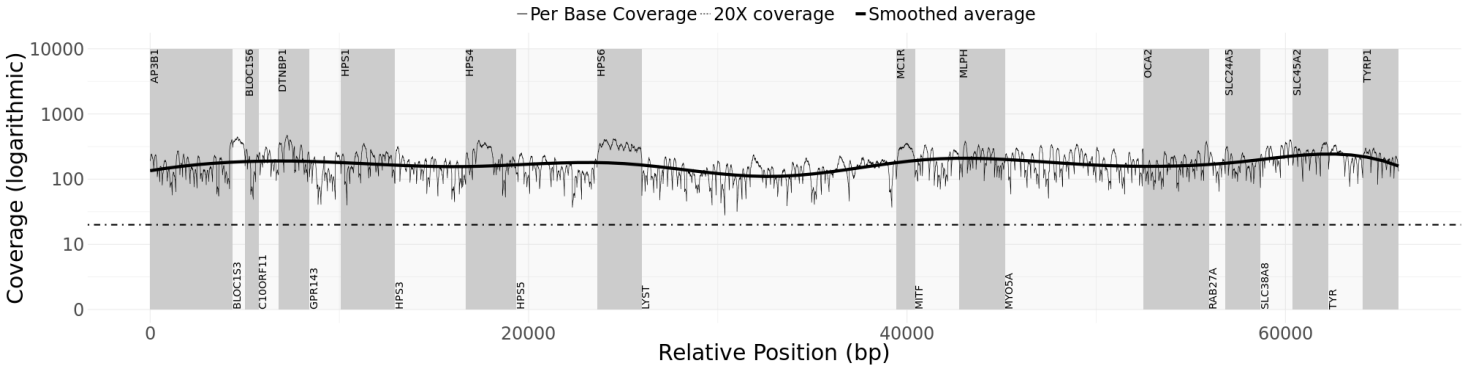
On Oct 05, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Milja Kaare, Ph.D.

Senior Geneticist

Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer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20X coverage
Smoothed average
Coverage (logarithmic)
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1000
100
10
0
AP3B1
BLOC1S3
BLOC1S6
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DTNBP1
GPR143
HPS1
HPS3
HPS4
HPS5
HPS6
LYST
MC1R
MITF
MLPH
MYO5A
OCA2
RAB27A
SLC24A5
SLC38A8
SLC45A2
TYR
TYRP1
0
20000
40000
60000
Relative Position (bp)

APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: Total genomic DNA was extracted from the biological sample using a spin column 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 and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.
Bioinformatics and quality control: The bioinformatics analysis began with quality control of raw sequence reads. Clean 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). The panel content was sliced from high-quality exome sequencing data acquired as presented above. The sequencing depth and coverage for the tested sample was calculated based on the alignments. 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 as well, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. 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, which processes aligned sequence reads. 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: Our variant classification follows the 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. 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 the 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.
Confirmation: Pathogenic and likely pathogenic variants that established a molecular diagnosis were confirmed with bi-directional Sanger sequencing unless all of the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call, 2) an unambiguous manual curation of the variant region using IGV was concordant with the variant call and 3) previous Sanger confirmation of the same variant has been performed at least three times in our laboratory. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing only if the QS was below our internally defined score for a true positive call. CNVs (Dels/Dups) were confirmed using a quantitative-PCR assay if they covered less than 10 target exons or were not confirmed at least three times previously at our laboratory.
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%, indels 1-50 bps 99.07%, one-exon deletion 92.3% and two exons CNV 100%, and specificity >99.9% for most variant types). 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.
Regulation and accreditations: This test has not been cleared or approved by the FDA. 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.

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_000273.2(GPR143):c.885+748G>A
NM_000273.2(GPR143):c.659-131T>G
NM_032383.3(HPS3):c.2888-1612G>A
NM_000275.2(OCA2):c.1045-15T>G
NM_000372.4(TYR):c.1037-18T>G

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
CNV = Copy Number Variation e.g. 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.

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.