Blueprint Genetics

Whole Exome Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		0	Male	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID

SUMMARY OF RESULTS

TEST RESULTS

Analysis of variants in previously established disease genes

Patient is homozygous for *RAX* c.517delG, p.(Val173Serfs*30), which is classified as likely pathogenic.

Del/Dup (CNV) Analysis

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

GENETIC VARIANTS

VARIANT TABLE: Genetic alterations

GENE	POS	ID	INHERITANCE	CONSEQUENCE	TRANSCRIPT	DNA	PROTEIN	GENOTYPE	EXAC AF	EXAC AC/AN	CLASSIFICATION
RAX	18:56939618	rs768356659	AR	frameshift_variant	NM_013435.2	c.517delG	p.(Val173Serfs*30)	HOM	8.2492E-06	1/121224	Likely pathogenic

SEQUENCING PERFORMANCE METRICS

MEDIAN COVERAGE	MEAN COVERAGE	PERCENT >= 10X	PERCENT >= 15X	PERCENT >= 20X
229	247.26	99.6	99.5	99.5

TEST INFORMATION

Blueprint Genetics Whole Exome Plus Test (version 2, Feb 9, 2018) includes Whole Exome Sequence Analysis of single patient cases, coupled with Whole Exome Deletion/Duplication (CNV) Analysis. The test is targeting all protein coding exons, exon-intron boundaries (± 20 bps) and selected, clinically relevant non-coding variants. This test should be used to detect mutations such as single nucleotide substitutions, small insertions and deletions (INDELs) and large copy number variations (CNVs; deletions and duplications spanning one or more exons). The test should not be used for detection of repeat expansion disorders (e.g. FXR, SCAs, Huntington disease) 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 negative, exome variant data is 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 Methods). If over time other patients with similar phenotype and mutations in the same gene are identified, the variant may be reclassified as a likely cause of the disorder.
Blueprint Genetics Whole Exome Plus test includes also Blueprint Genetics Whole Exome Del/Dup (CNV) Analysis (version 2.0, updated Feb 8, 2018. Detection limit of the test varies through the genome depending on exon size, sequencing coverage and sequence content. Most of the single exon deletion events are detected and the resolution at five exon CNV level is >99%. Segmentally duplicated genomic regions may have reduced sensitivity. The exact boundaries of the copy number aberration cannot be determined with this test.

STATEMENT

CLINICAL HISTORY

Patient is a 4-month-old boy with with bilateral anophthalmia. Previous genetic test includes array, which was normal.

CLINICAL REPORT

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

The exome data of the patient was analyzed for variants following recessive inheritance pattern and for rare heterozygous variants.

This Blueprint Genetics Whole Exome Plus test identified a homozygous 1-bp deletion c.517delG, p.(Val173Serfs*30) in *RAX*.

There is one individual heterozygous for the *RAX* c.517delG, p.(Val173Serfs*30) variant in the Genome Aggregation Database (gnomAD; n>120,000 exomes and >15,000 genomes). This 1-bp deletion generates a frameshift leading to a premature stop codon at position 30 in a new reading frame (201 out of 346 aa) and is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay. To our knowledge, this variant has not been reported in the public mutation databases (HGMD, ClinVar) or described in the literature. However, homozygous and compound heterozygous variants in *RAX* have been reported in patients with anophthalmia / microphthalmia (HGMD Professional 2018.1).

The *RAX* gene (MIM *601881) encodes a homeobox-containing transcription factor that functions in eye development. The gene is expressed early in the eye primordia, and is required for retinal cell fate determination and also, regulates stem cell proliferation. Variants in *RAX* have been reported in patients with defects in ocular development, including microphthalmia, anophthalmia, and coloboma. Disease caused by pathogenic *RAX* variants is inherited in an autosomal recessive manner. Microphthalmia, anophthalmia and coloboma (MAC) represent a spectrum of structural eye malformations that result from developmental defects during ocular organogenesis at a rate of 1.9–3.5/10 000 live births. Deletion of *Rax* gene function in the mouse leads to non-viable anophthalmia (PMID: 9177348, 11105055). Voronina et al screened 75 patients with either unilateral or bilateral anophthalmia and/or microphthalmia and identified one patient with compound heterozygous variants p.(Gln147*) and p.(Arg192Gln) in *RAX* (PMID: 14662654). Since then, additional patients with *RAX* variants have been reported. For instance, Chassaing et al identified homozygous or compound heterozygous variants in *RAX* in 4 out of 150 (3%) patients with anophthalmia (PMID: 24033328). Two of the patients had bi-allelic truncating variants. Based on the data on published patients, the authors concluded that the ocular phenotype associated with recessive *RAX* mutations is often bilateral and severe. Neurological involvement (intellectual deficiency, autistic features) was observed in 43% (3/7) of the patients. Apart from the cerebral involvement, no other extra-ocular malformation was associated with *RAX* mutations. The HGMD Professional 2018.1 database reports 12 variants in *RAX* (classified as disease causing) in association with anophthalmia/microphthalmia/coloboma (6 missense, 4 truncating, 1 splice region, 1 gross deletion covering the whole gene). No individuals with homozygous truncating variant in *RAX* have been reported in the reference cohort of the gnomAD database (http://gnomad.broadinstitute.org/gene/ENSG00000134438), indicating that such changes are poorly tolerated.

Mutation nomenclature is based on GenBank accession NM_013435.2 (*RAX)* 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.

CONCLUSION

RAX c.517delG, p.(Val173Serfs*30) is classified as likely pathogenic considering the current evidence of the variant (established association between the gene and part of patient's phenotype, rarity in control populations, variant type (frameshift)). However, additional information is still needed to

confirm the pathogenicity of the variant, which could allow independent risk stratification based on this variant. Genetic counseling and family member testing are recommended. Disease caused by *RAX* variants is inherited in an autosomal recessive manner and the patient is homozygous for the variant, which is in line with autosomal recessive inheritance. If both parents are found to be carriers of this variant, each sibling of an affected individual has a 25% chance of being homozygous for the variant and thus affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being an unaffected non-carrier. BpG offers targeted variant testing for the family if requested.

SECONDARY FINDINGS

The patient was opted-in for an analysis of secondary findings, which are sequence variants unrelated to the indication for ordering the sequencing but of medical value for patient care. Whole Exome data of the patient was analysed for secondary findings in 59 genes according to recommendations of American College of Medical Genetics and Genomics (ACMG; PMID 27854360).

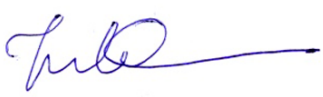
The analysis was negative for secondary findings.

CONFIRMATION

RAX c.517delG, p.(Val173Serfs*30) has been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	Mar 15, 2018
Sample received	Mar 15, 2018
Reported	May 09, 2018

On May 09, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

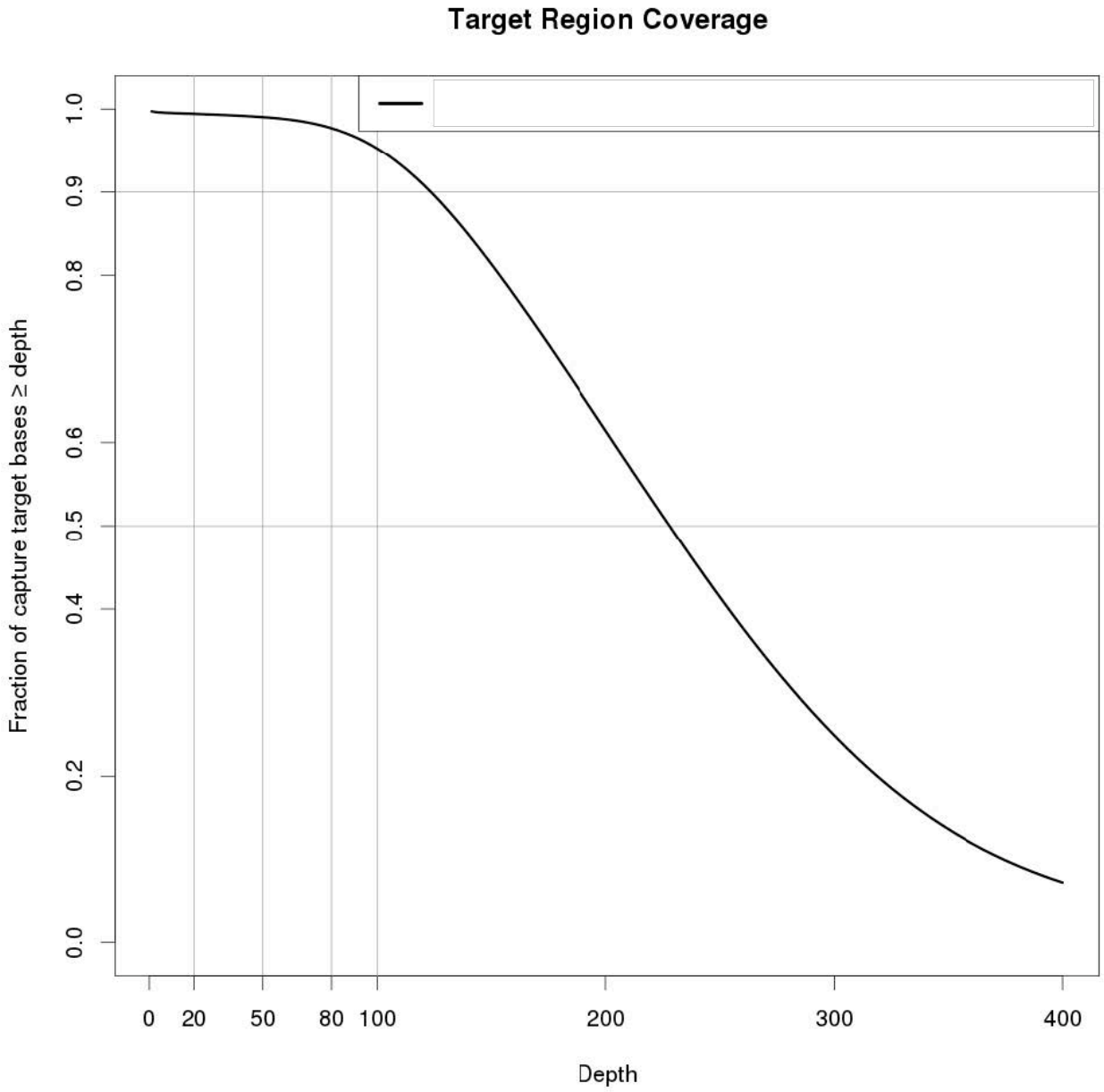

Sari Tuupanen, Ph.D.
Senior Geneticist

Juha Koskenvuo, MD, Ph.D.
Lab Director, Chief Medical Officer

Helena Kääriäinen, MD, Ph.D.
Clinical Consultant

APPENDIX 2: SEQUENCING COVERAGE

COVERAGE PLOT



APPENDIX 3: SUMMARY OF METHODS

WHOLE EXOME

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. Then, DNA fragments were 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). Amplified sequencing library was again purified using SPRI beads and hybridization-capture method was applied for enrichment of whole exome and selected non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). Enriched sequencing library was amplified by ligation-mediated PCR (LM-PCR) and purified using SPRI beads. 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 Illumina sequencing system. Paired-end sequencing (150 by 150 bases) was performed to yield the required number of reads (100M). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed to FASTQ format. The bioinformatics analysis began with quality control of raw sequence reads. Clean sequence reads of each sample was 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 sequencing depth and coverage for each individual were calculated based on the alignments.

Each exome batch was subjected to thorough quality control measures, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. The pathogenic potential of variants was predicted by taking into account the predicted consequence, biochemical properties of the codon change, the degree of evolutionary conservation as well as allelic frequencies from large population studies (1000 Genomes project, gnomAD) and mutation databases (HGMD, ClinVar) as well as an in-house variant database. The clinical association of genes was determined using our curated set of clinically associated genes included in Blueprint Genetics diagnostic panels, and in addition information from The Clinical Genomics Database and The Developmental Disorders Genotype-Phenotype Database (DD2GP) was used. The exome variant data was filtered based on all possible modes of inheritance of the disorder. 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. Variant(s) classified as pathogenic or likely pathogenic were confirmed using bi-directional Sanger sequencing with an exception – variant(s) fulfilling all three following criteria were not confirmed 1) high quality score (>500), 2) unambiguous IGV in-line with variant call and 3) previous confirmation at least three times at Blueprint Genetics. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing if quality score was below 500. 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:
1. Novel (absent in 1000 Genomes project, ESP, 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.
2. Rare (<1% MAF in 1000 Genomes project, ESP, 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, for criteria 1 and 2, 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 a patient 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).

This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.

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.

DEL/DUP (CNV) ANALYSIS

Analysis of copy number variations (CNVs; deletions and duplications involving one or more exons) was performed using whole exome sequencing data by 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. 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. Identified variants were annotated using data from our in-house curated and maintained database and public data including 1000 Genomes Project, ExAC and Database of Genomic Variants (DGV).

The clinical evaluation team assessed the pathogenicity of the identified CNVs by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases. Reported CNV(s) were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins is < 10. Reporting was done using an HGNC-approved gene nomenclature.