Blueprint Genetics

Osteopetrosis and Dense Bone Dysplasia Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**
11 Female

CUSTOMER SAMPLE ID

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE**
Blood

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for two variants in *CTSK:* c.721C>T, p.(Arg241*) and c.436G>C, p.(Gly146Arg), both of which are classified as 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**
CTSK	1:150772083	NM_000396.3	c.721C>T, p.(Arg241*)	stop_gained	HET	**Pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs74315303	17/246248	N/A	N/A	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Pycnodysostosis		AR	-	
GENE	**POS**	**TRANSCRIPT**	**NOMENCLATURE**	**CONSEQUENCE**	**GENOTYPE**	**CLASSIFICATION**
CTSK	1:150776679	NM_000396.3	c.436G>C, p.(Gly146Arg)	missense_variant	HET	**Pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs74315302	5/246126	probably damaging	deleterious	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Pycnodysostosis		AR	-	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Osteopetrosis and Dense Bone Dysplasia Panel	25	594	64995	64977	323	99.9

TARGET REGION AND GENE LIST

The Blueprint Genetics Osteopetrosis and Dense Bone Dysplasia Panel (version 2, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: AMER1, ANKH, CA2, CLCN7, COL1A1, CTSK, DLX3, FAM20C, GJA1*, LEMD3, LRP4, LRP5*, OSTM1, PTDSS1, PTH1R, SLC29A3, SLCO2A1, SNX10, SOST, TCIRG1, TGFB1, TNFRSF11A, TNFRSF11B, TNFSF11 and TYROBP. 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 11-year-old girl with clinical diagnosis of pyknodysostosis. She has the typical complications associated with the condition, short stature, frequent fracture risk, small nasal passages small palate with crowded teeth, lack of closure of fontanelles, and learning disabilities.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Osteopetrosis and Dense Bone Dysplasia Panel identified two heterozygous variants in *CTSK:* a nonsense variant c.721C>T, p.(Arg241*) and a a missense variant c.436G>C, p.(Gly146Arg). Due to the large genomic distance between these variants, we were unable to determine whether they occur in the same or in different alleles.

There are 17 individuals heterozygous for *CTSK* c.721C>T, p.(Arg241*) 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 this reference population database. The variant causes a premature stop codon and is thus predicted to cause loss of normal protein function either through protein truncation (240 out of 329 aa) or nonsense-mediated mRNA decay. Laffranchi et al (2010) reported this variant in an almost 10-year old Caucasian boy with pycnodysostosis (PMID: 21217630). Hou et al (1999) found c.721C>T, p.(Arg241*) variant in a Spanish and Portuguese families (PMID: 10074491), and it has also been reported in a Mexican-American family (PMID: 8703060), and in an extended Mexican kindred in which all affected individuals were homozygous for the variant (PMID: 8938428).

There are 5 individuals heterozygous for *CTSK* c.436G>C, p.(Gly146Arg) in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). *In silico* tools PolyPhen, SIFT, and MutationTaster predict the variant as deleterious. *CTSK* c.436G>C, p.(Gly146Arg) has been found as homozygous in an Algerian boy with pycnodysostosis. Additionally, it was reported in two affected Moroccan Arab siblings, and in an American Hispanic patient with c.721C>T, p.(Arg241*) (PMID: 21217630). Expression studies showed that Gly146 had no detectable mature protein or enzyme activity (PMID: 10074491). The three-dimensional structure of cathepsin K shows that Gly146 lies deep within the active-site cleft, so the substitution of the large, charged Arg residue was expected to have significant adverse effects upon this protein. *CTSK* c.436G>C, p.(Gly146Arg) has also been found in clinical testing and classified as pathogenic (ClinVarID: 8421).

CTSK

CTSK (MIM *601105) Cathepsin K, a lysosomal cysteine protease critical for bone remodeling by osteoclasts, is the deficient enzyme causing pycnodysostosis (MIM #265800). Pycnodysostosis an autosomal recessive osteosclerotic skeletal dysplasia (PMID: 8703060). The features of pycnodysostosis are deformity of the skull (including wide sutures), maxilla and phalanges (acroosteolysis), osteosclerosis, and fragility of bone. Affected individuals usually have small stature (adult height <150 cm), frontal bossing, wormian bones, a persistent (open) anterior fontanel (PMID: 10997431). The bones are generally sclerotic and prone to fractures. In some patients the clinical presentation can resemble IAO (PMID: 24269275). Dental abnormalities such as decayed, poorly located or abnormally shaped (pointed or conical) teeth and delayed tooth eruption may be observed. Nails are sometimes irregular and cracked. Very rarely, the disease is associated with anemia, hepatosplenomegaly, hematologic alterations, respiratory distress and sleep apnea. The disease is discovered at variable ages, ranging from 9 months to 50 years. The condition is most often diagnosed in childhood, but sometimes the condition is not detected until adulthood, usually as a result of a fracture or a routine examination. Pycnodysostosis is very rare, the exact prevalence is unknown but it is less than 1/100,000 (Orphanet). More than 50 mutations have been reported in patients with pycnodysostosis, majority being missense variants (64%), but also nonsense (11%), splicing variants (6%), small deletions/insertions (17%) and one gross deletion have been reported (HGMD 2018.1).

Mutation nomenclature is based on GenBank accession NM_000396.3 (*CTSK*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

CTSK c.436G>C, p.(Gly146Arg) and *CTSK* c.721C>T, p.(Arg241*) are classified as pathogenic, considering the current literature and their well-established role as disease-causing variants. Disease caused by *CTSK* variants is inherited in an autosomal recessive manner. Testing of parental samples is needed to determine whether the variants occur in *cis* (the same copy of the gene) or in *trans* (different copies of the gene). Compound heterozygosity of the identified variants (variants in *trans*) would explain the patient's disease. If the parents of the affected individual carry one pathogenic variant, then each sibling of the affected individual has a 25% chance of being compound heterozygous and affected, a 50% chance of being an asymptomatic carrier of one of the variants, 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

CTSK c.721C>T, p.(Arg241*) and *CTSK* c.436G>C, p.(Gly146Arg) have been confirmed with bi-directional Sanger sequencing.

STEP	DATE

STEP	DATE
Order date	May 04, 2017
Sample received	Apr 03, 2018
Reported	May 16, 2018

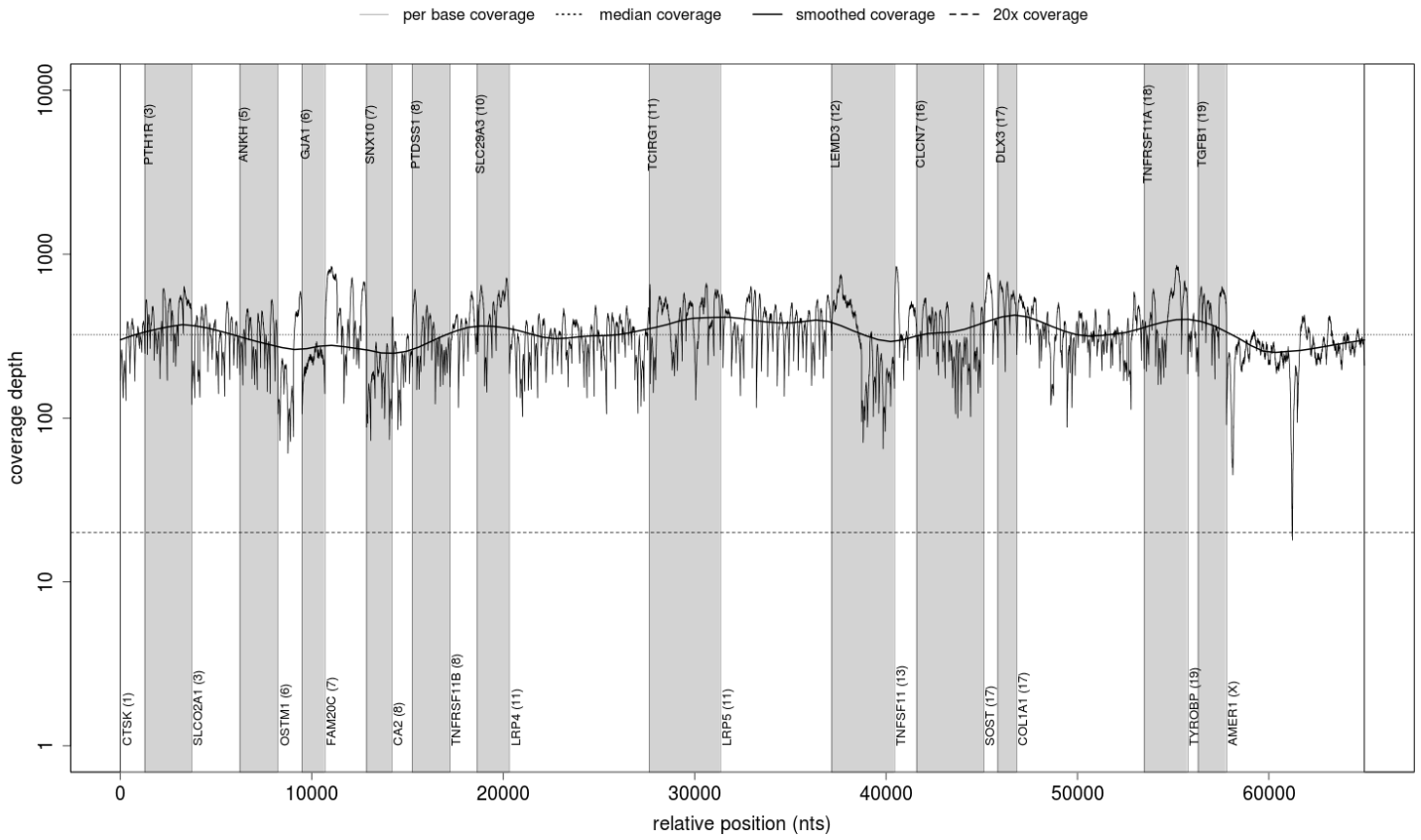
On May 16, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Tiia Kangas-Kontio, Ph.D., CLG
Senior Geneticist

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

Jennifer Schleit, Ph.D. FACMG
Senior Geneticist

Matti Hero, MD, Ph.D.
Clinical Consultant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median coverage
smoothed coverage
20x coverage
coverage depth
10000
1000
100
10
1
PTH1R (3)
ANKH (5)
GJA1 (6)
SNX10 (7)
PTDSS1 (8)
SLC29A3 (10)
TCIRG1 (11)
LEMD3 (12)
CLCN7 (16)
DLX3 (17)
TNFRSF11A (18)
TGFB1 (19)
CTSK (1)
SLCO2A1 (3)
OSTM1 (6)
FAM20C (7)
CA2 (8)
TNFRSF11B (8)
LRP4 (11)
LRP5 (11)
TNFSF11 (13)
SOST (17)
COL1A1 (17)
TYROBP (19)
AMER1 (X)
0
10000
20000
30000
40000
50000
60000
relative position (nts)

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_054027.4(ANKH):c.-11C>T
NM_001287.5(CLCN7):c.916+57A>T
NM_000088.3(COL1A1):c.2451+94G>T
NM_000088.3(COL1A1):c.2451+77C>T
NM_000088.3(COL1A1):c.2343+31T>A
NM_000088.3(COL1A1):c.1354-12G>A
NM_000088.3(COL1A1):c.904-14G>A
NM_000316.2(PTH1R):c.1049+29C>T
NM_006019.3(TCIRG1):c.-5+1G>C/T
NM_006019.3(TCIRG1):c.1887+132T>C
NM_006019.3(TCIRG1):c.1887+142T>A
NM_006019.3(TCIRG1):c.1887+146G>A
NM_006019.3(TCIRG1):c.1887+149C>T

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.