Blueprint Genetics

FGFR3 single gene test Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for *FGFR3* c.1138G>A, p.(Gly380Arg), which is pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
FGFR3	NM_000142.4	c.1138G>A, p.(Gly380Arg)	HET	missense_variant	AD,AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs28931614	GRCh37/hg19	4:1806119	G/A		
					PHENOTYPE	
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	Achondroplasia, Camptodactyly, tall stature, and hearing loss (CATSHL) syndrome, Crouzon syndrome with acanthosis nigricans, Hypochondroplasia, Lacrimoauriculodentodigital syndrome, Muenke syndrome, SADDAN, Thanatophoric dysplasia type 1, Thanatophoric dysplasia type 2	
	0/250348	benign	tolerated	disease causing		

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
FGFR3 single gene test	1	18	3292	3292	422	100

TARGET REGION AND GENE LIST

The Blueprint Genetics FGFR3 single gene test (version 2, Oct 07, 2019) Plus Analysis includes sequence analysis and copy number variation analysis of the gene. This test targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in the SUMMARY OF THE TEST section). This test should be used to detect single nucleotide variants and small insertions deletions (INDELs) 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 detect balanced translocations or complex rearrangements, and it may not detect low-level mosaicism.

STATEMENT

CLINICAL HISTORY

Patient is an 18-year-old individual with a clinical suspicion of achondroplasia.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) *FGFR3* single gene test identified a heterozygous missense variant *FGFR3* c.1138G>A, p.(Gly380Arg).

FGFR3 c.1138G>A, p.(Gly380Arg)

This variant is absent 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 *FGFR3* c.1138G>A p.(Gly380Arg) variant is a well-known pathogenic variant that causes achondroplasia (PMID: 7913883, 28679403, 21739570, 21324899, 16475234, 26136890, 28253570, 8078586, 11556601, 11186940). More than 99% of cases of achondroplasia are caused by this variant or another *FGFR3* nucleotide substitution affecting the same position, c.1138G>C, both resulting in the same amino acid change, p.(Gly380Arg). In a study encompassing 324 cases from the International Skeletal Dysplasia Registry (ISDR), the *FGFR3* p.(Gly380Arg) amino acid change was found in approximately 90% of achondroplasia (ACH) cases, the majority due to the c.1138G>A substitution (PMID: 25614871). The *FGFR3* p.(Gly380Arg) amino acid change causes constitutive activation of fibroblast growth factor receptor 3 (FGFR3), which is a negative regulator of bone growth (PMID: 19716710). The variant has been detected by other laboratories in the context of clinical testing and submitted to ClinVar (variation ID 16327).

FGFR3

FGFR3 (MIM **134934*) encodes a member of the fibroblast growth factor receptor (FGFR) family, with its amino acid sequence highly conserved among divergent species. This membrane protein is a member of the receptor tyrosine kinase family of proteins that is involved in the regulation of skeletal growth and development. Three alternatively spliced transcript variants that encode different protein isoforms have been described. FGFR3 has three distinct domains: the ligand binding extracellular domain, the cytosolic kinase domain and the transmembrane domain (TMD). Pathogenic variants in *FGFR3* (MIM *134934) are associated with various clinically distinct autosomal dominant disorders, i.e. achondroplasia (MIM #100800), hypochondroplasia (MIM #146000), CATSHL syndrome (camptodactyly, tall stature, and hearing loss syndrome; MIM #610474), Crouzon syndrome with acanthosis nigricans (MIM #612247), LADD syndrome (lacrimoauriculodentodigital syndrome; MIM #149730), SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans; MIM #616482), thanatophoric dysplasia (MIM #187600, #187601), Muenke syndrome (MIM #602849), and isolated coronal synostosis (GeneReviews NBK1455).

Achondroplasia is the most common process resulting in disproportionate short stature caused by rhizomelic shortening of the limbs (GeneReviews NBK1152). Affected individuals have short arms and legs, a large head, and characteristic facial features with frontal bossing and midface retrusion (formerly known as midface hypoplasia), exaggerated lumbar lordosis, limitation of elbow extension and rotation, genu varum, brachydactyly, and trident appearance of the hands. Excess mobility of the knees, hips, and most other joints is common. In infancy, mild to moderate hypotonia is typical, and acquisition of developmental motor milestones is delayed and also shows unusual, aberrant patterns (PMID: *9213228*, *20081435*). Some infants with achondroplasia die in the first year of life from complications related to the craniocervical junction; population-based studies suggest that this excess risk of death may be as high as 7.5% (PMID: *3631079*). The risk appears to be secondary to central apnea associated with damage to respiratory control centers (PMID: *3415202*, *7887429*), and can be minimized by comprehensive evaluation of every infant with achondroplasia (PMID: *16140722*) and selective neurosurgical intervention. More than 99% of individuals with achondroplasia have one of the two pathogenic variants in *FGFR3;* two different substitutions at nucleotide 1138 both result in the amino acid change p.(Gly380Arg) (GeneReviews NBK1152).

The normal function of the FGFRs appears to be to restrain limb growth, as *FGFR3* knockout mice have elongated tails and hindlimbs (PMID: *8630492* and *8601314*). This phenomenon suggests that *FGFR* pathogenic variants are hypermorphic, causing the gene product to perform its normal function excessively. It has been suggested that newly created cysteine

residues in the extracellular domain of the protein play a key role in the severity of the disease in thanatophoric dysplasia; they may allow formation of disulfide-linked dimers and cause ligand-independent activation (PMID: *8845844*). The exact mechanism of the hypermorphic effect is different for different types of pathogenic variants that have been reported in the *FGFR*-related craniosynostosis syndromes (PMID: *9300656*). However, camptodactyly, tall stature, scoliosis, and hearing loss (CATSHL) syndrome is caused by a p.(Arg621His) pathogenic variant in the *FGFR3* tyrosine kinase domain that leads to decreased protein function, indicating that pathogenic variants in *FGFR3* can either hinder or promote bone growth (PMID: *17033969*).

There are currently 83 variants in *FGFR3* annotated as disease-causing (DM) in the HGMD Professional variant database (version 2021.3), of which the vast majority are missense variants [67 missense, 8 stop-loss, 7 small deletions/insertions/indels (of which 1 causes a frameshift), and 1 gross insertion].

Mutation nomenclature is based on GenBank accession NM_000142.4 (*FGFR3*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

FGFR3 c.1138G>A, p.(Gly380Arg) is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Disease caused by *FGFR3* c.1138G>A, p.(Gly380Arg) is expected to be inherited in an autosomal dominant manner. Any offspring of the patient are at 50% risk of inheriting the variant and of being affected. *FGFR3*-related disease may be caused by a de novo variant. Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

On MMM DD, YYYY the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

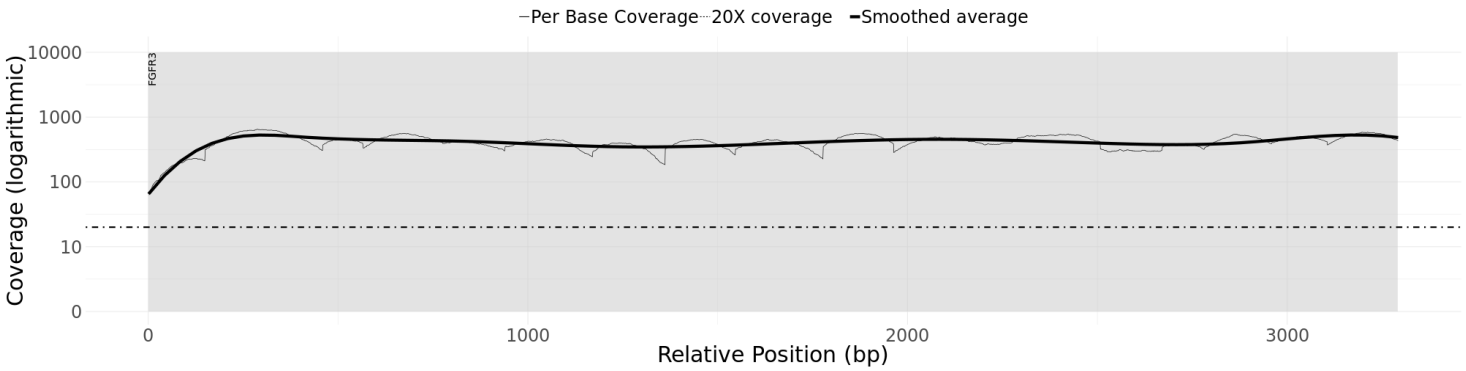
Johanna Huusko, Ph.D.

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

PLUS ANALYSIS

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 at Blueprint Genetics. 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.These steps were performed at Blueprint Genetics.
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. Bioinformatics and quality control processes were performed by Blueprint Genetics.
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. Likely benign and benign variants were not reported. The interpretation was performed at Blueprint Genetics.
Variant classification: 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. The classification and interpretation of the variant(s) identified reflect the current state of Blueprint Genetics' understanding at the time of this report. Variant classification and interpretation are subject to professional judgment, and may change for a variety of reasons, including but not limited to, updates in classification guidelines and availability of additional scientific and clinical information. This test result should be used in conjunction with the health care provider's clinical evaluation. Inquiry regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decision. For questions regarding variant classification updates, please contact us at support@blueprintgenetics.com
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 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.The confirmation of sequence alterations was performed at Blueprint Genetics.
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.The confirmation of copy number variants was performed at Blueprint Genetics.
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).

PERFORMING SITE:

BLUEPRINT GENETICS OY, KEILARANTA 16 A-B, 02150 ESPOO, FINLAND Laboratory Director: JUHA KOSKENVUO, MD, PhD, CLIA: 99D2092375

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AF = allele fraction (proportion of reads with mutated DNA / all reads)
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
MT = Mitochondria
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.