Client: Example Client ABC123
123 Test Drive
Salt Lake City, UT 84108
UNITED STATES

Physician: Doctor, Example

Patient: Patient, Example

DOB	5/2/1982
Gender:	Female
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Skeletal Dysplasia Panel, Sequencing and Deletion/Duplication, Fetal

ARUP test code 2012010

Maternal Contamination Study Fetal Spec	Fetal Cells According to information provided to ARUP, this pregnancy is the result of an egg donation. Therefore, non-maternity was detected, as expected. Single fetal genotype present; no maternal cells present. Fetal and maternal samples were tested using STR markers to rule out maternal cell contamination.
Maternal Contam Study, Maternal Spec	Whole Blood
Skeletal Dysplasia Panel Specimen, Fetal	Cultured Amnio
Skeletal Dysplasia Panel Interp, Fetal	Positive RESULT One pathogenic variant was detected in the FGFR3 gene. One variant of uncertain significance was detected in the COL11A2 gene. PATHOGENIC VARIANT Gene: FGFR3 (NM_000142.5) Nucleic Acid Change: c.1118A>G; Heterozygous Amino Acid Alteration: p.Tyr373Cys Inheritance: Autosomal dominant VARIANT OF UNCERTAIN SIGNIFICANCE Gene: COL11A2 (NM_080680.3) Nucleic Acid Change: c.3932A>G; Heterozygous Amino Acid Alteration: p.Asn1311Ser Inheritance: Autosomal dominant/ Autosomal recessive INTERPRETATION According to information available to ARUP, this fetus (now deceased) is suspected to have had thanatophoric dysplasia. There were noted to be abnormal ribs/small chest, bowed bones, and shortening of the bones of the arms/legs. Of note, an anonymous egg donor was reported to have been used to achieve the pregnancy. One pathogenic variant, c.1118A>G; p.Tyr373Cys, was detected in the FGFR3 gene by massively parallel sequencing in this prenatal sample. Pathogenic FGFR3 variants are inherited in an autosomal dominant manner, and are associated with

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thanatophoric dysplasia (TD) (MIM: 187600 and 187601), achondroplasia (MIM: 100800) and hypochondroplasia (MIM: 146000, OMIM(R)). This result is consistent with a diagnosis of thanatophoric dysplasia in this fetus.

One variant of uncertain clinical significance, c.3932A>G; p.Asn1311Ser, was detected in the COL11A2 gene by massively parallel sequencing in this prenatal sample. Pathogenic variants in COL11A2 are associated with autosomal dominant deafness 13 (MIM: 601868), autosomal recessive deafness 53 (MIM: 609706), autosomal dominant and recessive otospondylomegaepiphyseal dysplasia (MIM: 184840 and 215150), and autosomal dominant and recessive fibrochondrogenesis 2 (MIM: 614524).. However, it is uncertain whether this variant is disease-associated or benign.

Please refer to the background information included in this report for a list of the genes analyzed, methodology, and limitations of this test.

Evidence for variant classification:
The FGFR3 c.1118A>G; p.Tyr373Cys variant (rs121913485) is reported in the literature in multiple individuals affected with thanatophoric dwarfism (Brodie 1999, Rousseau 1996), and it was identified in a cohort of fetal skeletal dysplasia patients (Carass 2014). This variant is reported in ClinVar (Variation ID: 16342), and it is also absent from the Genome Aggregation Database, indicating it is not a common polymorphism. Carass et al. found the p.Tyr373Cys variant to be de novo in a male fetus with features consistent with lethal skeletal dysplasia. Furthermore, a mouse model of the p.Tyr373Cys variant displays features similar to achondroplasia (Di Rocco 2014, Lorget 2012) and in vitro studies performed on the p.Tyr373Cys variant in human chondrocytes show decreased proliferation (Krejci 2008). The tyrosine at codon 373 is moderately conserved, and computational analyses predict that this variant is deleterious (REVEL: 0.800). Based on available information, this variant is considered to be pathogenic.

The COL11A2 c.3932A>G; p.Asn1311Ser variant (rs727504460), to our knowledge, is not reported in the medical literature but is reported in ClinVar (Variation ID: 178812). This variant is found in the Ashkenazi Jewish population with an allele frequency of 0.25% (26/10,272 alleles) in the Genome Aggregation Database (v2.1.1). Computational analyses are uncertain whether this variant is neutral or deleterious (REVEL: 0.269). Due to limited information, the clinical significance of this variant is uncertain at this time.

RECOMMENDATIONS
Medical screening and management should rely on clinical findings and family history. Genetic consultation is recommended. At-risk family members should be offered testing for the identified pathogenic FGFR3 variant (Familial Targeted Sequencing, ARUP test code 3005867). Because paternal somatic or germline mosaicism for the identified FGFR3 pathogenic variant cannot be excluded, this individual's parents should be offered prenatal diagnosis in future pregnancies. Surveillance of the literature for new information concerning the uncertain variant is recommended.

COMMENTS
Likely benign and benign variants are not reported.
Variants in the following region(s) may not be detected by NGS with sufficient confidence in this sample due to technical limitations: None

REFERENCES

Brodie SG et al. Platyspondylic lethal skeletal dysplasia, San Diego type, is caused by FGFR3 mutations. Am J Med Genet. 1999 Jun 11;84(5):476-80. PMID: 10360402.

Carss et al. Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound. Hum Mol Genet. 2014; 23(12):3269-3277. PMID: 24476948.
Di Rocco et al. FGFR3 mutation causes abnormal membranous ossification in achondroplasia. Hum Mol Genet. 2014; 23(11):2914-2925. PMID: 24419316.
Krejci et al. Analysis of STAT1 Activation by Six FGFR3 Mutants Associated with Skeletal Dysplasia Undermines Dominant Role of STAT1 in FGFR3 Signaling in Cartilage. PLoS One. 2008;3(12):e3961. PMID: 19088846.
Lorget F et al. Evaluation of the therapeutic potential of a CNP analog in a Fgfr3 mouse model recapitulating achondroplasia. Am J Hum Genet. 2012 Dec 7;91(6):1108-14. PMID: 23200862.
Rousseau et al. Missense FGFR3 mutations create cysteine residues in thanatophoric dwarfism type I (TD1). Hum Mol Genet. 1996; 5(4):509-512. PMID: 8845844.

This result has been reviewed and approved by [redacted]
BACKGROUND INFORMATION: Skeletal Dysplasia Panel, Sequencing and Deletion/Duplication, Fetal
CHARACTERISTICS: Skeletal dysplasias are a heterogeneous group of more than 400 disorders characterized by abnormal growth of cartilage or bone. Clinical features may include shortening, bowing, fracturing, thinning, thickening, or under mineralization of the bones; abnormal ribs; small chest circumference; and extra fingers or toes. Some disorders may be detectable prenatally while others are not identified until birth or later childhood.

EPIDEMIOLOGY: Collective incidence of 1 in 5000

CAUSE: Pathogenic germline variants in genes associated with cartilage and bone growth.

INHERITANCE: Contingent on etiology; autosomal recessive, autosomal dominant, and X-linked inheritance, depending on the causative gene

CLINICAL SENSITIVITY: Dependent on the specific skeletal dysplasia; 99 percent for achondroplasia and thanatophoric dysplasia; greater than 95 percent for COL1A1/2 osteogenesis imperfecta; greater than 90 percent for achondrogenesis type 1B, diastrophic dysplasia, and campomelic dysplasia.

GENES TESTED: AGPS, ALPL, ARSL, CANT1, CCN6, CILK1, COL1A1, COL1A2,* COL2A1, COL10A1, COL11A1, COL11A2, COMP, CRTAP, DDR2, DLL3, DYM,* DYNC2H1, EBP, EVC,* EVC2, FGFR1,* FGFR2, FGFR3, FKBP10, FLNA, FLNB, GDF5, GNPAT, HSPG2, IFT80, INPPL1, LBR, LIFR, NEK1,* NPR2, P3H1, PCNT, PEX7, POR,* PPIB, PTH1R, RUNX2, SERPINH1, SLC26A2, SLC35D1, SMARCAL1, SOX9, TRIP11, TRPV4, TTC21B, WDR19, WDR35
*One or more exons are not covered by sequencing and/or deletion/duplication analysis for the indicated gene; see limitations section below.

METHODOLOGY: Probe hybridization-based capture of all coding exons and exon-intron junctions of the targeted genes, followed by massively parallel sequencing. Sanger sequencing was performed as necessary to fill in regions of low coverage and to confirm reported variants that do not meet acceptable quality metrics. A proprietary bioinformatic algorithm was used to detect large (single exon-level or larger) deletions or duplications in the indicated genes. Large deletions/duplications confirmed using an orthogonal exon-level microarray. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY: The analytical sensitivity is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions (indels) from 1-10 base pairs

in size. Indels greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced. Deletions of 2 exons or larger are detected with sensitivity greater than 97 percent; single exon deletions are detected with 62 percent sensitivity. Duplications of 3 exons or larger are detected at greater than 83 percent sensitivity. Specificity is greater than 99.9 percent for all variant classes.

LIMITATIONS: A negative result does not exclude diagnosis of a skeletal dysplasia. This test only detects variants within the coding regions and intron-exon boundaries of the targeted genes. Variants in the chr17:70,119,704-70,119,743 region of SOX9 exon 3 may not be detected. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Regulatory region variants and deep intronic variants will not be identified, including deletions/duplications in the upstream regulatory region of SOX9. Precise breakpoints for large deletions or duplications are not determined in this assay and single exon deletions/duplications may not be detected based on the breakpoints of the rearrangement. The actual breakpoints for the deletion or duplication may extend beyond or be within the exon(s) reported. This test is not intended to detect duplications of 2 or fewer exons in size, though these may be identified. Single exon deletions are reported but called at a lower sensitivity. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations caused by the presence of pseudogenes, repetitive, or homologous regions. This test is not intended to detect low-level mosaic or somatic variants, gene conversion events, complex inversions, translocations, mitochondrial DNA (mtDNA) mutations, or repeat expansions. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Noncoding transcripts were not analyzed.

The following regions are not sequenced due to technical limitations of the assay:
EVC(NM_153717) exon(s) 1

Single exon deletions/duplications will not be called for the following exons:
COL1A2(NM_000089) 3; EVC (NM_153717) 1; EVC(NM_001306090) 1; EVC(NM_001306092) 1; FGFR1(NM_001354367) 18; FGFR1(NM_001354369) 18; FGFR1(NM_001354370) 17; DYM(NM_001353212) 14; DYM(NM_001353213) 14; DYM(NM_001353214) 14; DYM(NM_001353215) 14; DYM(NM_001374428) 15; DYM(NM_001374429) 14; DYM(NM_001374430) 14 18; DYM(NM_001374431) 14; DYM(NM_001374432) 13; DYM(NM_001374433) 17; DYM(NM_001374441) 9; NEK1(NM_001374422) 17; NEK1(NM_001374423) 16; POR (NM_001382655) 3

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the US Food and Drug Administration. This test was performed in a CLIA certified laboratory and is intended for clinical purposes.

Counseling and informed consent are recommended for genetic testing. Consent forms are available online.

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Maternal Contamination Study Fetal Spec	24-318-401538	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Maternal Contam Study, Maternal Spec	24-318-401538	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Skeletal Dysplasia Panel Specimen, Fetal	24-318-401538	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Skeletal Dysplasia Panel Interp, Fetal	24-318-401538	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

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