Prenatal Skeletal Dysplasia Panel

Panel Gene List: AGPS, ALPL, ARSE, BMP1, CEP120, COL11A1, COL11A2, COL1A1, COL1A2, COL2A1, COMP, CRTAP, DLL3, DYNC2H1, EBP, EVC, EVC2, FGFR1, FGFR2, FGFR3, FKBP10, FLNA, FLNB, GNPAT, HSPG2, IFITM5, IFT172, INPPL1, KIAA0586, LBR, LEPRE1, LIFR, NEK1, PEX7, PLOD2, POR, PPIB, RUNX2, SERPINH1, SLC26A2, SLC35D1, SOX9, TMEM38B, TRIP11, TRPV4, TTC21B, WDR34, WDR35

Clinical Features:

Skeletal dysplasias are a highly variable group of disorders affecting the bone and cartilage of the skeletal system, which are estimated to occur in 2.4 to 4.5 per 10,000 births and 20 per 10,000 stillbirths.[1,2,3] They are characterized by generalized structural abnormalities of bone and cartilage growth and modeling caused by a disturbance in bone growth beginning in the early stages of fetal development and evolving throughout life.[2] There are over 450 currently recognized skeletal dysplasias, which are divided into 40 categories based on molecular, biochemical and radiographic criteria.[1,2,3] Although each disorder presents with its own clinical findings, as a group, these conditions are characterized by anomalies of bone shape, size and density, which manifest as abnormalities of the limbs, chest, or skull. These conditions have variable etiologies including, chromosomal abnormalities or single-gene pathogenic variants as well as environmental factors such as teratogen exposure and autoimmune response.[1,2,3]

Prenatal Ultrasound Findings:

Skeletal dysplasias are commonly identified in the prenatal period by the presence of shortened long bones or other abnormal skeletal findings such as narrow thorax, polydactyly, frontal bossing, or poor mineralization of the calvarium on ultrasound.[4-7] Other imaging methods, such as 3D ultrasound, MRI and CT scan, can be used to evaluate and diagnose a skeletal dysplasia in utero. Due to genetic heterogeneity and overlapping phenotypes, the specific fetal skeletal dysplasia cannot be determined accurately with imaging alone. When available, molecular, genetic and/or biochemical testing can aid in determining the precise diagnosis after the differential has been established by imaging.

While there are a large number of different skeletal dysplasias, certain disorders are more common than others. A brief overview of some of the more common fetal skeletal dysplasias is given below.

FGFR3-related skeletal dysplasias / Achondroplasia / Thanatophoric Dysplasia (FGFR3)

FGFR3-related skeletal dysplasias refer to four distinct disorders caused by pathogenic variants in the *FGFR3* gene. The most common of these is achondroplasia (ACH), which is nonlethal and the most common condition associated with disproportionate short stature or dwarfism.[8,9] Prenatally, this disorder often presents in the third trimester and is associated with rhizomelic micromelia, macrocephaly with frontal bossing and midface hypoplasia. Mild limb bowing, brachydactyly, increased space between the third and fourth digits, and a depressed nasal bridge are also common.8,9 Hypochondroplasia (HCH) has a similar, but milder, phenotype to that of ACH and presents with micromelia, short stature and lumbar lordosis.[8,9] The prevalence of HCH is estimated to be 1 in 50,000 births, and together ACH and HCD are estimated to account for 20% of all cases of skeletal dysplasia in live births.[8] Thanatophoric dysplasia (TD) is the most common lethal skeletal dysplasia and has an incidence estimated to be between 1 in 17,000 and 1 in 50,000 births.[8] This disorder is characterized by disproportionate dwarfism with very short extremities, normal trunk length, very narrow thorax, macrocephaly, depressed nasal bridge, prominent forehead with protruding eyes, brachydactyly, platyspondyly, and normal bone mineralization without fractures. Severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) is a very severe form of achondroplasia caused by a rare pathogenic variant in the *FGFR3* gene.[8,9]

Osteogenesis Imperfecta (OI)
(BMP1, COL1A1, COL1A2, CRTAP, FKBP10, IFITM5, LEPRE1, PPIB, SERPINH1, TMEM38B)
Osteogenesis Imperfecta (OI) is characterized by bone fragility and consequent susceptibility to bone fractures. The severity of OI can range from severe perinatal lethal to asymptomatic with mild predisposition to fractures and a normal lifespan.[10,11] Other common characteristics include dentinogenesis imperfecta, blue sclerae, short stature and hearing loss in adulthood.[11] The most lethal form of OI is type II, which is characterized by compressible thin calvaria, severe micromelia and bowing of long bones with multiple fractures and a narrow thorax.[11] Together, all types of OI have a combined prevalence of between 1 in 15,000 and 1 in 30,000 births with about 90% of cases caused by pathogenic variants in either *COL1A1* or *COL1A2*.[10,11]

Achondrogenesis
(COL2A1, SLC26A2, TRIP11)
Achondrogenesis is a severe skeletal dysplasia classified into three types: type IA, type IB, and type II and characterized by a lack of ossification of the vertebral bodies as well as extreme micromelia, a barrel-shaped short trunk, and short ribs.[12] The most common Type II accounts for approximately 80% of cases of achondrogenesis and is due to de novo dominant pathogenic variants in the *COL2A1* gene.[12] Type 1A is due to pathogenic variants in the *SLC26A2* (*DTDST*) gene, and type IB is due to pathogenic variants in the *TRIP11* gene.[12] All three types are usually lethal in the perinatal period.

Chondrodysplasia Punctata

(AGPS, ARSE, EBP, GNPAT, PEX7)
Chondrodysplasia Punctata is a group of disorders characterized by chondrodysplasia punctata (stippled epiphyses). The most common form, rhizomelic chondrodysplasia punctata type 1 (RCDP1), is caused by pathogenic variants in the *PEX7* gene and is a peroxisome biogenesis disorder characterized by proximal shortening of the humerus and femur, punctate calcifications in cartilage with epiphyseal and metaphyseal abnormalities, congenital cataracts, low birth weight, length, and head circumference, severe postnatal growth deficiency, profound intellectual disability and seizures.[13] Less common disorders result from pathogenic variants in the *GNPAT* gene causing RCDP2, *AGPS* gene pathogenic variants causing RCDP3, *ARSE* pathogenic variants causing X-linked chondrodysplasia punctata 1 (CDPX1) and *EBP* pathogenic variants causing X-linked chondrodysplasia punctata 2 (CDPX2). These related disorders have similar punctate cartilaginous changes with variable limb shortening and/or asymmetry, short stature, intellectual disability, cataracts, and skin changes.[14]

Short-rib thoracic dysplasias (SRTDs)
(CEP120, DYNC2H1, EVC, EVC2, IFT172, KIAA0586, NEK1, WDR34, WDR35)

Short-rib thoracic dysplasias (SRTDs) are characterized by a constricted thoracic cage, short ribs, shortened tubular bones, and a 'trident' appearance of the acetabular roof (lateral surface of the hip bone). They are autosomal recessive and lethal. All are a part of a group of skeletal ciliopathies caused by problems with cilia and all involve bone abnormalities. Short-rib thoracic dysplasia-6 with or without polydactyly (SRTD6) is caused by pathogenic variants in the *NEK1* gene and short-rib thoracic dysplasia-3 with or without polydactyly (SRTD3) is caused by pathogenic variants in the *DYNC2H1* gene. Ellis-van Creveld syndrome (EVC) is an autosomal recessive condition additionally characterized by disproportionate short stature, congenital heart disease (most commonly ASD), postaxial polydactyly, dysplastic nails and teeth, and retinal degeneration. This disorder, caused by pathogenic variants in *EVC* and *EVC2*, may present prenatally with narrow thorax, shortening of the long bones, polydactyly and cardiac defects.[15]

Campomelic dysplasia (CD)
(SOX9)
Campomelic dysplasia (CD) is a rare, often lethal skeletal dysplasia characterized by angular bowing and shortening of the long bones, severe respiratory distress, and XY sex reversal. It is caused by chromosome abnormalities or pathogenic variants affecting expression of the *SOX9* gene.[16] Approximately 75% of patients with CD with a 46, XY karyotype exhibit partial or complete sex reversal, ranging from ambiguous genitalia to normal female external genitalia.[17] In addition to bowing of the long bones, skeletal features of CD include club feet, a bell-shaped and underdeveloped thorax, eleven pairs of ribs, and hypoplastic scalpulae. Other variable features include micrognathia and Pierre-Robin malformation. Many infants die shortly after

birth from respiratory compromise; however, those who survive the neonatal period can develop hearing loss, developmental delay, short stature and progressive kyphoscoliosis.[16,18]

See the full list of genes and their related conditions in the table below.

Genetics: Many severe skeletal dysplasias are due to single-gene disorders inherited in an autosomal dominant manner and are often sporadic pathogenic variants. Autosomal recessive and X-linked inheritance patterns are also observed.[1,2,3]

Test Methods: Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the genes tested are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be >99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size.

Additionally, genotype analysis of maternal and fetal DNA for several polymorphic markers to test for maternal cell contamination will be performed. Therefore, in all prenatal cases a maternal sample should accompany the fetal sample

Clinical Sensitivity:
Skeletal dysplasias are a genetically heterogeneous group of disorders with a wide pathogenic variant spectrum. The sensitivity of sequence analysis of this panel in prenatal cases ascertained based on fetal ultrasound abnormalities is currently unknown, and the clinical sensitivity depends on the clinical phenotype of the patient.

Gene	Inheritance	Disease Associations relevant to this panel
AGPS	AR	Rhizomelic chondrodysplasia punctata type
ALPL	AD, AR	Hypophosphatasia
ARSE	XLR	Chondrodysplasia punctata
BMP1	AR	Osteogenesis imperfecta, type XIII
CEP120	AR	Short-rib thoracic dysplasia 13 with or without polydactyly
COL11A1	AD, AR	Fibrochondrogenesis Stickler syndrome
COL11A2	AD, AR	Fibrochondrogenesis Stickler syndrome
COL1A1	AD	Osteogenesis imperfecta, types I, II, III & IV
COL1A2	AD	Osteogenesis imperfecta, types II, III & IV
COL2A1	AD	Achondrogenesis, type II (ACH2) Hypochondrogenesis Spondyloepiphyseal dysplasia (SED) with metatarsal shortening (also called Czech dysplasia) Spondyloepiphyseal dysplasia (SED) congenita Spondyloepiphyseal dysplasia (SED) (Namaqualand type) Spondyloepimetaphyseal (SMED) (Strudwick type) Otospondylomegaepiphyseal dysplasia Spondyloperipheral dysplasia Platyspondylic skeletal dysplasia (Torrance type) Kniest dysplasia
COMP	AD	Pseudoachondroplasia Multiple epiphyseal dysplasia
CRTAP	AR	Osteogenesis Imperfecta, type VII
DLL3	AR	Spondylocostal dysostosis type 1
DYNC2H1	AR	Asphyxiating thoracic dystrophy
EBP	XLD	Chondrodysplasia punctata
EVC	AR	Ellis-Van Creveld Syndrome
EVC2	AR	Ellis-van Creveld syndrome
FGFR1	AD	Pfeiffer syndrome Osteoglophonic dysplasia

Gene	Inheritance	Disease Associations relevant to this panel
FGFR2	AD	Bent bone dysplasia Antley-Bixler syndrome
FGFR3	AD	Achondroplasia Hypochondroplasia Thanatophoric dysplasia, type I / II
FKBP10	AR	Osteogenesis imperfecta, type XI Bruck syndrome
FLNA	XLR, XLD	Otopalatodigital syndrome Frontometaphyseal dysplasia Melnick-Needles syndrome
FLNB	AD, AR	Atelosteogenesis, type I / III (AOI / AOIII) Boomerang dysplasia (BD) Larsen syndrome Spondylocarpotarsal synostosis syndrome (SCT)
GNPAT	AR	Rhizomelic chondrodysplasia punctata type 2
HSPG2	AR	Dyssegmental dysplasia, Silverman-Handmaker type Schwartz-Jampel syndrome, type 1
IFITM5	AD	Osteogenesis imperfecta type V
IFT172	AR	Short-rib thoracic dysplasia 10 with or without polydactyly
INPPL1	AR	Opsismodysplasia
KIAA0586	AR	Short-rib thoracic dysplasia 14 with polydactyly
LBR	AR	Greenberg skeletal dysplasia
LEPRE1	AR	Osteogenesis imperfecta type VIII
LIFR	AR	Stuve-Wiedemann syndrome
NEK1	AR	Short rib-polydactyly syndrome, Majewski type Asphyxiating thoracic dystrophy
PEX7	AR	Rhizomelic chondrodysplasia punctata type 1
PLOD2	AR	Bruck syndrome 2
POR	AR	Antley-Bixler syndrome
PPIB	AR	Osteogenesis imperfecta, type IX
RUNX2	AD	Cleidocranial dysplasia

Gene	Inheritance	Disease Associations relevant to this panel
SERPINH1	AR	Osteogenesis imperfecta, type X
SLC26A2 (DTDST)	AR	Achondrogenesis type 1B Atelosteogenesis type II Diastrophic dysplasia
SLC35D1	AR	Schneckenbecken dysplasia
SOX9	AD	Campomelic dysplasia
TMEM38B	AR	Osteogenesis imperfecta, type XIV
TRIP11	AR	Achondrogenesis, type IA
TRPV4	AD	Metatropic dysplasia
TTC21B	AR	Short-rib thoracic dysplasia 4 with or without polydactyly
WDR34	AR	Short-rib thoracic dysplasia 11 with or without polydactyly
WDR35	AR	Short-rib thoracic dysplasia 7 with or without polydactyly

AD = Autosomal Dominant, AR = Autosomal Recessive, XLD = X-linked Dominant, XLR = X-linked Recessive

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