Prenatal Skeletal Dysplasia Panel

Disorder also known as: Osteochondrodysplasias

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.[1-3] 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.[3-6] There are over 450 currently recognized skeletal dysplasias, which are divided into 40 categories based on molecular, biochemical and radiographic criteria.[1-3] 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.[8] 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.[3]

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:

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.[17] 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.[17]

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.[5,7] 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.[3,5-7] ACH is estimated to occur in 1 in 10,000 to 1 in 40,000 births with more than 250,000 affected individuals worldwide.[5,7] Hypochondroplasia (HCH) has a similar, but milder, phenotype to that of ACH and presents with micromelia, short stature and lumbar lordosis.[5,7] 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.[5] 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.[3,5] 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.[3-6] 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.[5,7]

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.[1,4,8] Other common characteristics include dentinogenesis imperfecta, blue sclerae, short stature and hearing loss in adulthood.[8] 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.[3] 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.[4, 8]

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.[3,6] 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.[3,6] Type 1A is due to pathogenic variants in the DTDST (SLC26A2) gene, and type IB is due to pathogenic variants in the TRIP11 gene.[3,10] All three types are usually lethal in the perinatal period.[6]

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.[18,19] 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.[20]

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 located on chromosome 17q24.3-q25.1. 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.[11] 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.[12]

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 in an ultrasound.[3,5] Other imaging methods, such as 3D ultrasound, MRI and CT scan, can be used to evaluate and diagnose a skeletal dysplasia in utero.[3] Due to genetic heterogeneity and overlapping phenotypes, the specific fetal skeletal dysplasia cannot be determined accurately with imaging alone.[3-5] When available, molecular, genetic and/or biochemical testing can aid in determining the precise diagnosis after the differential has been established by imaging.

Inheritance Pattern/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.[1,3,9]

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

Test 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 of analysis of the 23 genes included in the Prenatal Skeletal Dysplasias Panel depends on the clinical phenotype of the patient. Specific information about the sensitivity of each gene in selected populations is included in the attached clinical sensitivity table.

Gene	Inheritance	Disease Associations	Diagnostic Yield for Disorder
AGPS	Autosomal recessive	Rhizomelic chondrodysplasia punctata type 3	Unknown
ALPL			
ARSE	X-Linked recessive	Chondrodysplasia punctata	60-75% for sequence variants, multi-exonic and whole-gene deletions in affected males[1]
COL1A1	Autosomal	Osteogenesis imperfecta, types I, II, III & IV	>95% [2]

Gene	Inheritance	Disease Associations	Diagnostic Yield for Disorder
	dominant		
COL1A2	Autosomal dominant, Autosomal recessive	Osteogenesis imperfecta, types II, III & IV	>95% [2]
COL2A1	Autosomal dominant	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	>75% of COL2A1-related disorders[3]
CRTAP	Autosomal resessive	Osteogenesis Imperfecta, type IIB and VII	
DLL3	Autosomal recessive	Spondylocostal dysostosis type 1	
DYNC2H1	Autosomal recessive	Asphyxiating thoracic dystrophy 3	Unknown
EBP	X-Linked dominant	Chondrodysplasia punctata	~70%[4]
EVC	Autosomal recessive	Ellis-Van Creveld Syndrome	Unknown
EVC2	Autosomal recessive	Ellis-van Creveld syndrome	~90% in affected females[5]
FGFR2	Autosomal dominant	Bent bone dysplasia	~74%[6]
FGFR3	Autosomal dominant	Achondroplasia Thanatophoric dysplasia, type I / II Hypocondroplasia	~26%[6]

Gene	Inheritance	Disease Associations	Diagnostic Yield for Disorder
FLNB	Autosomal dominant	Atelosteogenesis, type I / III (AOI / AOIII) Boomerang dysplasia (BD) Larsen syndrome Spondylocarpotarsal synostosis syndrome (SCT) *Autosomal recessive	Unknown
GNPAT	Autosomal recessive	Rhizomelic chondrodysplasia punctata type 2	>99% [7,8,9]
IFITM5	Autosomal dominant	Osteogenesis imperfecta type 5	
LEPRE1	Autosomal recessive	Osteogenesis imperfecta type VIII	
NEK1	Autosomal recessive	Short rib-polydactyly syndrome, Majewski type Asphyxiating thoracic dystrophy 1	~97% in patients with a radiographic diagnosis[10]
PEX7	Autosomal recessive	Rhizomelic chondrodysplasia punctata type 1	
PPIB	Autosomal recessive	Osteogenesis imperfecta, type IX	Unknown
SLC26A2 (DTDST)	Autosomal recessive	Achondrogenesis type 1B Atelosteogenesis type II Diastrophic dysplasia	Unknown
SOX9	Autosomal dominant	Campomelic dysplasia	Unknown
TRIP11	Autosomal recessive	Achondrogenesis, type IA	Unknown

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