Limb Abnormalities and Reduction Defects Panel

Panel Gene List:

ANKRD11, ARHGAP31, ARID1A, ARID1B, BHLHA9, BMP2, BMPR1B, BTRC, CC2D2A, CDH3, CEP290, CHSY1, DLL4, DLX5, DOCK6, DPCD, DVL1, DVL3, DYNC1I1, EOGT, ESCO2, FBXW4, FGF10, FGF16, FGFR1, FGFR2, FGFR3, GDF5, GLI3, GNAS, HDAC4, HDAC8, HOXD13, IHH, KIF7, KMT2A, LBX1, LMBR1 (including ZRS regulatory region), LRP4, MGP, MKS1, MYCN, NIPBL, NOG, NOTCH1, NSDHL, PHF6, PIGV, POLL, PTHLH, RAD21, RBM8A, RBPJ, RECQL4, ROR2, RPGRIP1L, SALL1, SALL4, SHH, SMARCA2, SMARCA4, SMARCB1, SMARCE1, SMC1A, SMC3, SOX11, SOX9, TBX15, TBX3, TBX5, THPO, TP63, WNT10B, WNT3, WNT5A, WNT7A and deletion/duplication coverage for 10q24

Any gene not assessed in its entirety by sequencing and copy-number assessment is addressed in the Test Methods section.

Clinical Features

Limb abnormalities and reduction defects are a broad category of genetically heterogeneous syndromic and non-syndromic skeletal disorders. Pathogenic variants in the tested genes cause a variety of limb malformations and reduction defects that range from complete absence of all four limbs to mild phalangeal abnormalities.

Syndromic Limb Abnormalities

Adams-Oliver Syndrome

(ARHGAP31, DLL4, DOCK6, EOGT, NOTCH1, and RBPJ genes)
Adams-Oliver syndrome (AOS) is estimated to occur in approximately 4 per 1,000,000 live births. Affected individuals present with aplasia cutis congenita of the scalp and terminal transverse limb defects primarily affecting the lower extremities more severely than upper. Observed limb defects range from unilateral or bilateral short distal phalanges to complete absence of toes, fingers, feet or hands. Less common clinical features include cardiovascular (23%), neurological (~30% in autosomal recessive kindreds), renal (<5%), and ophthalmologic abnormalities (<10%), as well as cutis marmorata telangiectatica congenita (~20%) (PMID: 27077170). Autosomal dominant Adams-Oliver syndrome is associated with pathogenic variants in the ARHGAP31, DLL4, NOTCH1, and RBPJ genes (PMID: 27077170, 21565291, 29924900, 26299364, 25132448, 25963545, 22883147). Autosomal recessive Adams-Oliver syndrome is associated with the DOCK6 and EOGT genes (PMID: 27077170, 25824905).

Coffin-Siris Syndrome

(ARID1A, ARID1B, PHF6, SMARCA2, SMARCA4, SMARCB1, SMARCE1, and SOX11 genes)
Coffin-Siris syndrome (CSS) is classically characterized by aplasia or hypoplasia of the distal phalanx or the nail of the fifth digit, developmental delays, dysmorphic facial features, hypotonia, hirsutism/hypertrichosis, and sparse scalp hair. Congenital cardiac anomalies or renal and genitourinary malformations have been observed in ~35% of cases. Other common findings include feeding difficulties, poor growth, ophthalmologic abnormalities, and hearing impairment. CSS is most commonly caused by de novo pathogenic variants in one of eight genes: ARID1A, ARID1B, PHF6, SMARCA2, SMARCA4, SMARCB1, SMARCE1, and SOX11 (PMID: 23556151, 22426308, 24886874, 23906836). However, individuals with *SMARCA2* or *PHF6* may have phenotypes more

consistent with Nicolaides-Baraitser syndrome or Borjeson-Forssman-Lehmann syndrome, respectively (PMID: 23556151, 22366787, 23906836).

Cornelia de Lange Syndrome

(ANKRD11, HDAC8, KMT2A, NIPBL, RAD21, SMC1A and SMC3 genes)

Cornelia de Lange syndrome (CdLS) is a pan-ethnic disorder characterized by pre- and postnatal growth retardation and various congenital anomalies. Distinct craniofacial dysmorphisms include microbrachycephaly, synophrys, long eyelashes, long philtrum, thin upper lip, downturned mouth, and small upturned nasal tip. Limb anomalies range from oligodactyly and small hands to absence of the forearm. Gastrointestinal disorders and hirsutism are also common. Intellectual disability varies greatly, with an average IQ of 53 (PMID: 8291521). Less common features include psychomotor retardation, high arched palate with cleft, autism-like behavior, self-injurious behaviors, speech impairment, sensorineural hearing loss, and ophthalmological, genito-urinary (cryptorchidism) and heart anomalies (PMID: 8291521). CdLS is estimated to occur in 1 in 10,000 to 1 in 100,000 individuals and presents in mild to severe forms with variable expressivity (PMID: 20301283). Pathogenic variants in six genes: ANKRD11, HDAC8, KMT2A, NIPBL, RAD21, SMC1A, and SMC3 have been identified in patients with clinical features of CdLS (PMID: 20301283, 25574841, 24038889, 25652421).

Robinow Syndrome

(DVL1, DVL3, ROR2, and WNT5A genes)

Robinow syndrome is characterized by distinct dysmorphic craniofacial features resembling a fetal face, skeletal features including short stature, acromesomelic or mesomelic limb shortening predominantly affecting the upper libs as well as brachydactyly, and genital abnormalities in males: micropenis/webbed penis, hypoplastic scrotum, cryptorchidism; in females: hypoplastic clitoris and labia majora. Growth retardation, dental abnormalities, bilobed tongue, prenatal macrocephaly, and postnatal microcephaly have also been reported as common features. Less common findings include renal abnormalities, radial head dislocation, vertebral anomalies, nail dysplasia, cardiac defects, cleft lip/palate and rarely cognitive delay. Milder autosomal dominant Robinow results from pathogenic variants in *DVL1*, *DVL3* or *WNT5A* (PMID: 25577943, 26924530), while autosomal recessive Robinow syndrome is caused by variants in the ROR2 gene (PMID: 20301418). A variant of Robinow syndrome associated with osteosclerosis, normal stature, persistent macrocephaly, increased bone mineral density and hearing loss is also associated with heterozygous variants in *DVL1* (PMID: 25577943).

Ciliopathies

(CC2D2A, CEP290, KIF7, MKS1, and RPGRIP1L genes)

Ciliopathies comprise a group of disorders associated with abnormal formation or function of the cilia. Ciliary dysfunction can manifest as a constellation of features that include primary retinal degeneration, renal disease and cerebral anomalies. Skeletal dysplasia and polydactyly are common features in some ciliopathies, specifically those caused by pathogenic variants in the CC2D2A, CEP290, KIF7, MKS1, and RPGRIP1L genes (PMID: 21210154).

Other syndromic limb abnormalities with limited genetic heterogeneity include Wolff-Parkinson-White syndrome (BMP2 gene) (PMID: 18812404), Cousin syndrome (TBX15 gene) (PMID: 24039145, 19068278), Al-Awadi-Raas-Rothschild syndrome (WNT7A gene) (PMID: 23727605, 27638328), Feingold syndrome (MYCN gene)(PMID: 18470948, 18671284), Roberts syndrome (ESCO2 gene) (PMID: 20301332), Townes-Brocks

syndrome (SALL1 gene) (PMID: 20301618), Duane-radial ray syndrome (SALL4 gene) (PMID: 20301547), Holt-Oram syndrome (TBX5 gene) (PMID: 20301290), Lacrimo-auriculo-dental-digital syndrome (FGF10, FGFR2, FGFR3) (PMID: 16630169, 16501574, 27323706), Cenani-Lenz syndrome (LRP4) (PMID: 20381006), Keutel syndrome (MGP) (PMID: 24458983), CHILD syndrome (NSDHL) (PMID: 15689440), Mabry syndrome (PIGV) (PMID: 27177984), Rothmund-Thomson/RAPADILINO syndrome (RECQL4) (PMID: 20301383, 20301415), Ulnar-mammary syndrome (TBX3) (PMID: 28145909), multiple syndromes associated with pathogenic variants in the TP63 gene (PMID: 20556892), and Thrombocytopenia with Absent Radii (TAR) syndrome (RBM8A gene) (PMID: 22366785).

Non-Syndromic limb abnormalities

Ectrodactyly or split hand/split foot malformations (SHSF) are unique to BHLHA9, CDH3, DLX5, DYNC1I1, FGFR1, TP63, and WNT10B genes. Non-syndromic or isolated SHSF malformations caused primarily by sequence variants involve the TP63, CDH3, and FGFR1 genes (PMID: 20556892, 22140374, 18199584, 25394172). Sequence variants as well as small copy number variants in *WNT10B* and *DLX5*, inherited in an autosomal recessive manner, have been reported to cause non-syndromic SHSF in multiple unrelated individuals (PMID: 21554266, 22121204). Isolated split-hand/foot malformations may be associated with copy number variations at 17p13.3 involving the BHLHA9 gene (PMID: 23790188, 25466284), at 7q21.3 involving the DYNC1I1 gene (PMID: 25231166), and at chromosome 10q24 (PMID: 28539665). Rare chromosomal abnormalities involving 2q31, 3q27, 17q25, Xq26 and other loci have also been reported (PMID: 28539665, 26749485).

Other non-syndromic limb abnormalities and reduction defects include conditions such as non-syndromic syndactylies and polydactylies caused by pathogenic variants in LRP4, HOXD13, and GLI3 genes (PMID: 22333904, 20672375, 22428873, 19429598). Syndromic and non-syndromic brachydactyly is seen in patients with pathogenic variants in BMPR1B, CHSY1, GDF5, HDAC4, IHH, NOG, PTHLH, and SOX9 genes (PMID: 25776145, 25758993, 21129728, 25092592, 25820810, 20691407, 24715439, 19277064, 27508084, 26640227, 20301724). Other limb abnormalities include metacarpal 4/5 fusion, reported to be caused by pathogenic variants in the FGF16 gene (PMID: 25333065), Madelung deformity associated with pathogenic variants in the GNAS gene (PMID: 21910239), tetra-amelia caused by pathogenic variants in the WNT3 gene (PMID: 14872406), and congenital transverse limb defects caused by pathogenic variants in the THPO gene (PMID: 22453305).

Pre-axial polydactyly and triphalangeal thumbs can be seen in patients with pathogenic variants in the SHH gene or its regulatory element, the ZRS region of the LMBR1 gene (PMID: 12837695).

Because of the significant clinical overlap and phenotypic heterogeneity of disorders causing limb abnormalities and reduction defects, it can be difficult to make a clinical diagnosis. Additionally, variants in a single gene may be associated with a broad spectrum of clinical presentations (clinical heterogeneity). Therefore, testing of multiple genes is very useful in helping to establish the etiology of syndromic and non-syndromic limb malformations and reduction defects.

Inheritance Pattern/Genetics

Autosomal dominant (AD), autosomal recessive (AR), and X-linked inheritance (XL).

Clinical Sensitivity

Limb abnormalities and reduction defects are a genetically heterogeneous group of disorders with a wide variant spectrum. The clinical sensitivity of this test depends in part on the patient's clinical presentation and is expected to be highest for individuals with a clearly defined phenotype and/or family history. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate.

Test Methods

Genomic DNA is extracted directly from the submitted specimen or, if applicable, from cultured fibroblasts. The DNA is enriched for the complete coding regions and splice junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next-generation sequencing. 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. Alternative sequencing detection methods are used to analyze or confirm regions with inadequate sequence data by NGS. Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Exon-level oligo array CGH (ExonArrayDx) is performed for most, if not all, of the coding exons of the requested genes. Exon-level array CGH can detect deletions and duplications greater than 250 bp. Data analysis is performed using gene-specific filtering. Probe sequences and locations are based on human genome build GRCh37/UCSC hg19. Confirmation of copy number changes is performed by MLPA, qPCR, or repeat array CGH analysis. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known. 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. Available evidence for variant classification may change over time and variant(s) may be reclassified according to the ACMG/AMP Standards and Guidelines (PMID: 25741868), which may lead to issuing a revised report.

The following gene-specific information applies: Sequencing analysis of BTRC, DPCD, DYNC1I1, FBXW4, LBX1, and POLL genes is not performed. Gene-specific exclusions for exon-level deletion/duplication testing for this panel are: BHLHA9, MYCN, RECQL4, and SHH genes, no copy number testing; FGF10, FGFR3, KIF7, and WNT3 genes, only whole gene deletions or duplications may be detected. For the LMBR1 gene, the intronic ZRS region, which is a regulatory element for SHH gene expression, is included. Copy number analysis for 10q24 (chr10:103,113,290-103,317,578) is also performed.

Disclaimer:

Genetic testing using the methods applied at GeneDx is expected to be highly accurate. However, normal findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable by this test.

Unless otherwise indicated, the methods used cannot reliably detect deletions of 20bp to 500bp in size, or insertions of 10bp to 500bp in size. Sequencing cannot detect low-level mosaicism. Copy number assessment methods cannot reliably detect mosaicism and cannot identify balanced chromosome aberrations. Regions of certain genes have inherent sequence properties (for example: repeat, homology, or

pseudogene regions, high GC content, rare polymorphisms) that yield suboptimal data, potentially impairing accuracy of the results.

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