

Prenatal Limb Abnormalities Panel Sequence Analysis and Deletion/Duplication Testing of 5 Genes

Panel Gene List: NIPBL, SALL1, SALL4, TBX5, TP63 (TP73L)

Clinical Features:

Cornelia de Lange syndrome (CdLS) is a pan-ethnic disorder characterized by distinct craniofacial dysmorphisms including 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 forearm. Gastrointestinal disorders and hirsutism are common. Intellectual disability varies greatly, with an average IQ of 53.⁹ 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.⁹

Townes-Brocks syndrome is a rare multiple malformation syndrome characterized by anal, limb, ear, and renal anomalies. Intelligence is normal in most affected individuals. Diagnostic features include ano-rectal abnormalities (imperforate or anteriorly placed anus, anal stenosis, prominent midline perineal raphe); abnormalities of the hands and feet (preaxial polydactyly, triphalangeal thumbs, bifid thumbs and toes, finger and toe syndactyly); external ear malformations (preauricular tags or pits, “lop” or “satyr” ear, microtia, abnormal helix) with hearing loss (sensorineural, conductive or mixed); and renal anomalies leading to impaired renal function or renal failure (unilateral or bilateral hypoplastic or dysplastic kidneys, multicystic kidneys, renal agenesis, posterior urethral valves, vesico-ureteral reflex). Other, less common features are cardiac defects, mental retardation, eye, genitourinary and vertebral abnormalities, hypothyroidism, umbilical hernia, and gastroesophageal reflux.¹⁰

Duane-Radial Ray syndrome (DRRS) is characterized by the Duane eye anomaly and radial ray malformations of the limbs. The Duane anomaly is a congenital disorder of eye movement defined by the limited or absent ability to move the eye outward (abduction) and/or inward (adduction). Radial ray malformations observed in this syndrome can include triphalangeal thumbs, preaxial polydactyly, hypoplasia/aplasia of the thumbs, hypoplasia/aplasia of the radii, and shortening and radial deviation of the forearms. Acro-Renal-Ocular syndrome (AROS), which is allelic to DRRS, presents with radial ray malformations and Duane anomaly, along with other features such as ocular coloboma and renal abnormalities (renal hypoplasia horseshoe kidney, vesico-utereral reflux, bladder diverticular, ectopia, and mild malrotation).

Holt-Oram syndrome is a malformation syndrome characterized by upper limb abnormalities and heart defects. Affected individuals may present in infancy with obvious limb malformations and/or signs of cardiac failure secondary to cardiac malformations and/or cardiac conduction disease. Although the condition is considered to be fully penetrant, subtle limb involvement may not become clinically apparent without radiographic studies. The spectrum of limb defects ranges from severe (phocomelia) to mild (slight carpal bone abnormalities), the most common limb anomalies being either triphalangeal (finger-like) or absent thumbs. Upper limb deformities are usually bilateral and are frequently asymmetrical. Cardiac abnormalities occur in approximately 75% of patients with HOS (95% of familial cases).

Ectrodactyly-Ectodermal Dysplasia-Cleft Lip/Palate (EEC) consists of limb malformations, ectodermal dysplasia, and cleft lip and palate (in ~40% of patients; isolated cleft lip or palate is rare). The disorder shows variable expressivity and reduced penetrance. The ectodermal dysplasia in EEC is characterized by hypohidrosis, hypotrichosis, and anodontia. The limb anomalies include ectrodactyly (in 2/3 of patients), split-hand/split-foot, or polysyndactyly. Associated findings may include lacrimal-duct abnormalities, urinary tract anomalies, dysmorphic facies, and developmental delay.

Split Hand-Split Foot Malformation (SHFM) is characterized by limb malformation involving the central rays of the autopod and presenting with syndactyly, median clefts of the hands and feet, aplasia or hypoplasia of the phalanges, metacarpals, and metatarsals.

Limb-Mammary syndrome (LMS) includes hand/foot anomalies and hypoplasia/aplasia of the mammary gland and nipple. Less frequent findings include lacrimal-duct problems, ectodermal dysplasia (hypohidrosis (~ 30% of cases), hypodontia, nail dysplasia), cleft palate, and bifid uvula.

ADULT syndrome is clinically similar to LMS in that both have mammary gland hypoplasia. However, orofacial clefting has not been observed in affected patients, while nails, skin, and teeth are affected in almost all cases. Hypohidrosis is seen rarely.

Inheritance Pattern/Genetics:

Autosomal dominant inheritance, albeit most occur sporadic. Familial cases, as well as somatic and gonadal mosaicism, have been described in NIPBL.¹¹⁻¹⁶

Test Methods:

Using genomic DNA obtained from prenatal specimens, the coding exons and flanking splice junctions of 5 genes are enriched using a proprietary targeted capture method developed by GeneDx. The products are sequenced on an Illumina instrument using paired-end reads. The sequence data is aligned to reference sequences based on human genome build

GRCh37/UCSC hg19. Sanger sequencing is used to compensate for low coverage and refractory amplifications. Concurrently, targeted array CGH analysis with exon-level resolution is performed to evaluate for a deletion or duplication of one or more exons of the 5 genes included on the panel. The presence of any potentially disease associated sequence variant(s) or copy number variant(s) is confirmed by dideoxy DNA sequence analysis or quantitative PCR, respectively, or by other methods as appropriate. This panel includes sequencing of the coding regions and canonical splice junctions of 5 genes involved in prenatal limb abnormalities: NIPBL, SALL1, SALL4, TBX5, and TP63 (exons 5-8, 13, and 14 only) as well as comprehensive targeted array CGH analysis with exon-level resolution. 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:

Limb abnormalities are a genetically heterogeneous group of disorders with a wide variant spectrum. The clinical sensitivity of sequence and deletion/duplication analysis of the 5 genes included in this panel depends on the clinical phenotype of the patient. One report of NIPBL sequence analysis on 12 prenatal samples suspicious for CdLS found pathogenic variants in 9 of the 12 cases,¹¹ suggesting a high sensitivity of sequence analysis in prenatal CdLS cases ascertained based on fetal ultrasound abnormalities

The technical sensitivity of gene sequencing in prenatal cases ascertained based on fetal ultrasound abnormalities is estimated to be greater than 99%. It will not detect deletions, insertions, or rearrangements greater than or equal to ten base pairs. The deletion/duplication testing can detect deletions or duplications encompassing one or more exons, including changes as small as 500bp.

Gene	Inheritance	Disease Associations
NIPBL	Autosomal dominant; Familial cases, as well as somatic and gonadal mosaicism, have been described in NIPBL. ¹¹⁻¹⁶	In-utero growth restriction (IUGR), characteristic facial profile, and/or increased nuchal translucency (NT) accompanied by abnormalities of the limbs and extremities ranging in severity from small hands or flexed forearms to complete absence of limbs ¹⁻³ .
SALL1	Autosomal dominant	Renal malformations and/or congenital heart defects accompanied by abnormalities of the limbs and extremities, such as preaxial polydactyly and hypoplastic thumbs without radial shortening. ⁴

SALL4	Autosomal dominant	Renal malformations and/or congenital heart defects accompanied by abnormalities of the limbs and extremities, such as preaxial polydactyly, hypoplastic thumbs, and other radial ray malformations ⁵
TBX5	Autosomal dominant	Congenital heart defects and/or upper-extremity malformations including hypoplastic thumbs and other radial ray malformations ^{6,7} .
TP63	Autosomal dominant	Cleft/lip palate and/or abnormalities of the limbs or extremities including split-hand/foot malformation, oligodactyly, and syndactyly ⁸ .

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