

Limb Defects

Sequencing Panel

Congenital Limb Defects (CLDs) are relatively common, complex disorders of upper and/or lower limb development. The incidence of congenital limb defects is estimated to be 1 in 1,500 - 1 in 3,000 in livebirths and represents the second most common congenital malformation after cardiac defects. Common clinical presentation includes polydactyly, syndactyly, limb reduction / long bone deficiencies, and multiple anomalies and/or generalized skeletal syndromes. Genetic syndromes including CLD may

be caused by germline mutations in genes involved in cell fate determination, regulation or patterning during embryogenesis. Phenotypes resulting from many of these can be quite variable. Genetic testing may be clinically useful in the postnatal period for individuals that display characteristic features or in the prenatal period for fetuses that present with certain ultrasound findings (polydactyly, limb reduction) after a normal chromosome analysis.

Genetics

Most of the genes in this panel cause disease in an autosomal dominant inheritance pattern. In these cases, a parent carrying the mutated gene has a 50% chance of passing it on to an offspring, regardless of gender. Many of these genes are not fully penetrant (implying that an individual may have a mutated gene but not display any of the signs/symptoms of the disorder). Additionally, these disorders have variable expressivity (individuals with the same diagnosis may display differing features and differing severity of symptoms).

A person can harbor a mutation from one of two sources:

- Either the person inherited this mutation from an affected (or unaffected, non-penetrant) parent
- The mutation is a "de novo" DNA change that occurred in the egg or sperm from which the affected individual developed

For diseases caused by mutations in *ROR2* and *WNT7A* genes, these predominately exhibit autosomal recessive inheritance. If a child is affected with one of these conditions, it is implied that each parent is a carrier of one mutated gene and the risk of having future affected offspring is 25% with each pregnancy. A person who is a carrier for an autosomal recessive condition does not display features of the disorder in question.

Our Limb Defects Sequencing Panel tests for the following disorders:

Limb Defects Disorders	Common Additional Phenotypic Traits	Gene(s): Inheritance Pattern*
Greig cephalopolysyndactyly syndrome (GCPS) Pallister-Hall syndrome Polydactyly, postaxial, types A1 and B Polydactyly, preaxial, type IV Hypothalamic hamartomas, somatic	Preaxial polydactyly or mixed pre- and postaxial polydactyly, true ocular hypertelorism, and macrocephaly	<i>GLI3</i> : AD
Townes-Brocks branchiootorenal-like syndrome Townes-Brocks syndrome	Imperforate anus, dysplastic ears (usually associated with sensorineural and/or conductive hearing impairment), and thumb malformations (triphalangeal thumbs, duplications of the thumb)	<i>SALL1</i> : AD
Duane-radial ray syndrome IVIC syndrome	Uni- or bilateral Duane anomaly and radial ray malformation	<i>SALL4</i> : AD
Holt-Oram syndrome	Upper-limb defects, congenital heart malformation, cardiac conduction disease	<i>TBX5</i> : AD
Brachydactyly, type B1 Robinow syndrome, autosomal recessive	Macrocephaly, Fetal Face syndrome, short stature with growth retardation	<i>ROR2</i> : AR
Brachydactyly, type D and type E Brachydactyly-syndactyly syndrome, Syndactyly, type V Synpolydactyly with foot anomalies and type I VACTERL association	Webbing of the 3rd and 4th fingers and 4th and 5th toes, with partial or complete digital duplication within the syndactylous web	<i>HOXD13</i> : AD
Fuhrmann syndrome Al-Awadi/Raas-Rothschild/Schinzel phocomelia syndrome (AARRS)	Limb aplasia/hypoplasia and joint dysplasia, short upper limbs, oligodactyly, absence of nails, severe reduction defects of lower limbs	<i>WNT7A</i> : AR
Preaxial polydactyly (PPD) Werner mesomelic syndrome Polysyndactyly	Hypo- or aplasia of the tibia, preaxial polydactyly (PPD) of the hands and feet and/or five-fingered hand with absence of thumbs.	<i>ZRS</i> (sonic hedgehog (SHH) cis-regulator): AD

* AR = autosomal recessive, AD = autosomal dominant



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Limb Defects Sequencing Panel

Testing Methods:

DNA is obtained from a blood (postnatal) sample or a prenatal specimen (direct or cultured Chorionic Villus Sampling or amniocytes). High-throughput, next generation sequencing is performed to examine

several genes at one time. All variants not determined to be benign will be subjected to Sanger sequencing for confirmation of the result.

Test Sensitivity and Limitations:

Technological and analytical test sensitivity of next generation sequencing used for this panel is 99% for detecting substitutions and 95% for detection small insertion and deletions. Larger genomic rearrangements and DNA insertions or deletions will likely be missed by this testing method. Untranslated regions (UTRs) and gene promoter regions are not tested. Patients with negative test results may still have a mutation in one of the genes on the panel that was not identified by this testing or may have a mutation(s) in a gene not included in this panel. For the majority of the genes on this panel, the clinical sensitivity of this assay cannot be estimated individually as each gene is a rare cause of limb defects. Approximately 5% of patients are expected to have positive results with this testing. A negative test does not exclude a genetic

cause for limb defect disorder. Most of the mutations reported thus far in these genes are single amino acid changes, splice site mutations, small deletions, small insertions, small indels and truncation of the protein. If a change is found that has not been reported before, it will be evaluated in the context of all findings, including what the predicted effect is on the protein. This technology may not detect all small insertion/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will only detect variants within the exons and the intron-exon boundaries of the target gene(s). Variants outside targeted regions will not be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic sequences.

Turnaround Time:

Results are reported to the referring physician within 3-4 weeks for prenatal cases and 4-8 weeks for postnatal cases from the receipt of the specimen.

Specimen & Shipping Requirements:

For postnatal cases: 2 yellow-top (ACD-A or ACD-B) or 2 lavender-top (EDTA), 5-10 ml tubes of blood from the patient and both of his/her parents are required.

For prenatal cases: 2 confluent T-25 flasks of cultured cells (originating from amniotic fluid or chorionic villi) or more than 4 mg of direct CVS tissue, or 15 ml of direct amniotic fluid (AF) as well as 2 lavender-top (EDTA), 5-10 ml tubes of blood from the pregnant patient and her partner are required.

Note: Parental blood samples are requested for confirmation studies necessary in some cases; maternal blood is also used for maternal cell contamination studies.

Tubes of blood, cultured cells, direct CVS, and direct AF should be kept and shipped refrigerated or at room temperature (please do NOT freeze).

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