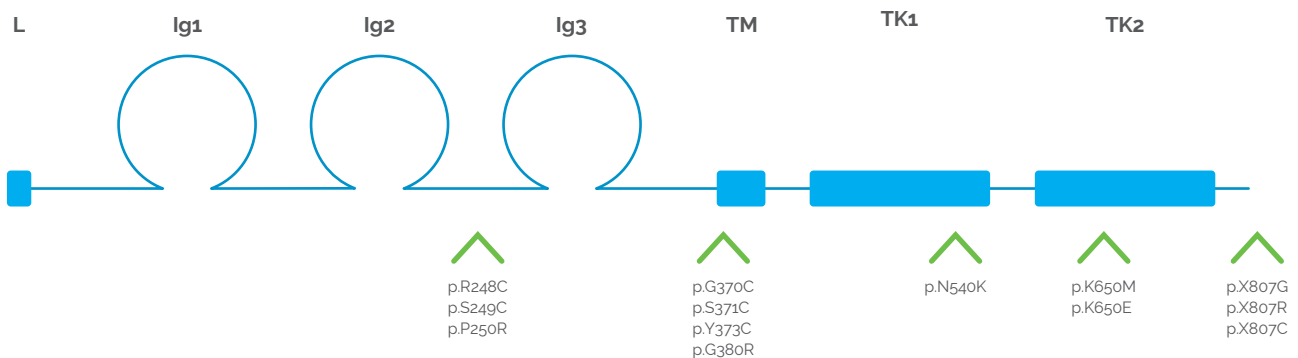


Skeletal dysplasias: *FGFR3* Hotspot Panel with reflex to full gene sequencing

The phenotypic variation of skeletal dysplasias points to a complex etiology for this class of disorders. However, some of the more common skeletal dysplasias have been shown to be a consequence of a limited number of mutations in the *fibroblast growth factor receptor 3* (*FGFR3*) gene. These skeletal dysplasias include

- Achondroplasia (ACH), the most common genetic form of dwarfism
- Thanatophoric dysplasia (TD1 and TD2), the most common form of sporadic, lethal skeletal dysplasia
- Hypochondroplasia (HCH), a milder skeletal dysplasia that resembles achondroplasia
- Non-syndromic coronal craniosynostosis



The figure above shows a schematic representation of the *FGFR3* gene and the relative positions of a variety of mutations known to result in skeletal dysplasias. The gene structure includes a putative leader sequence (L), a series of three immunoglobulin-like domains (Ig1, Ig2, Ig3), a transmembrane domain (TM), and segments of a tyrosine kinase domain (TK1 and TK2).

Figure 1: Known hot spots in the *FGFR3* gene

The skeletal dysplasias included on this panel have variable expressivity. Individuals with the same diagnosis may display differing features and severity of symptoms. A person can harbor a mutation from one of two sources:

- The mutation was inherited from an affected parent. All of the mutations on this panel are autosomal dominant. This means that a parent carrying a mutated gene has a 50% chance of passing it on to a biological child, regardless of gender
- The mutation was *de novo*

Mutations and molecular diagnostic rate

<i>FGFR3</i> -Related Disorders	Common Additional Phenotypic Traits	Tested Mutations: cDNA	Tested Mutations: Protein	Molecular Diagnostic Rate
Achondroplasia	Rhizomelic short stature, macrocephaly, frontal bossing, midface hypoplasia, kyphosis, hydrocephalus, foramen magnum stenosis	c.1138G>A or c.1138G>C	p.G380R	99%
Hypochondroplasia	Similar, but milder, features compared with achondroplasia	c.1620C>A or c.1620C>G	p.N540K	70%
Thanatophoric dysplasia type I	Narrow thorax, micromelic bone shortening, platyspondyly, depressed nasal bridge, curved femurs, lethal	c.742C>T	p.R248C	70-90%
		c.746C>G	p.S249C	
		c.1108G>T	p.G370C	
		c.1111A>T	p.S371C	
		c.1118A>G	p.Y373C	
		c.1949A>T	p.K650M	
		c.2419T>G	p.X807G	
		c.2419T>A	p.X807R	
Thanatophoric dysplasia type II	Narrow thorax, micromelic bone shortening, platyspondyly, depressed nasal bridge, cloverleaf skull, lethal	c.1948A>G	p.K650E	100%
SADDAN	Severe rhizomelic short stature, developmental delay, frontal bossing, midface hypoplasia, acanthosis nigricans	c.1949A>T	p.K650M	-
Craniosynostosis	Macrocephaly, midface hypoplasia, carpal-tarsal fusion, sensorineural hearing loss, developmental delay	c.749C>G	p.P250R	30%

Reflex to *FGFR3* full gene sequencing is available if the targeted mutation panel is negative. Mutations in other regions of the *FGFR3* gene, while less common, have been reported in association with skeletal/craniosynostosis disorders, including familial short stature, hypochondroplasia, lacrimo-auriculo-dento-digital (LADD) syndrome, Crouzon with acanthosis nigricans, and camptodactyly-tall stature-scoliosis-hearing (CATSHL) loss syndrome.* However, the molecular diagnostic rate for *FGFR3* mutations not included on the above panel is not currently available.

*CATSHL may be autosomal dominant or autosomal recessive.



Indication

- This panel has greatest utility as a prenatal test for fetuses that present with certain ultrasound findings after a normal chromosome analysis, such as shortened long bones, small ribcage, or a cloverleaf skull
- This test may be clinically useful for pediatric patients who display characteristic features, such as short stature or frontal bossing



Method

- Targeted genotyping is performed to look for the presence of specific mutations
- When ordered, reflexive full gene sequencing is performed by Sanger sequencing of all coding exons of the *FGFR3* gene (exons 2-18). All sequencing is bi-directional



Accuracy and sensitivity

- This test is approximately 99% accurate
- The technological and analytical test sensitivity for identifying alterations in the *FGFR3* gene by Sanger sequencing is >95%, as most reported mutations in the literature are point mutations or small indels



Please contact **800-298-6470** for additional information or questions about this test.

References: 1. Tavormina PL, et al. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor 3. *Nat Genet.* 1995;9(3):321-8.
2. Shiang R, et al. Mutations in the transmembrane domain of *FGFR3* cause the most common genetic form of dwarfism, achondroplasia. *Cell.* 1994;78(2):335-42.
3. Bellus GA, et al. A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. *Nat Genet.* 1995;10(3):357-9.
4. Passos-Bueno MR, et al. Clinical spectrum of fibroblast growth factor receptor mutations. *Hum Mutat.* 1999;14(2):115-25.



Specimen requirements

Prenatal

- Chorionic villus sampling: Minimum 4 mg in conical tube with transport media or cell culture media (available from our lab upon request)
- Amniocentesis: 10 mL in conical tube
- Cord blood: Minimum 2 mL in ACD-A or ACD-B tube (yellow top) or EDTA tube (lavender top)
- Cultures: 2 confluent T-25 flasks

For all prenatal specimens, 5-10 mL of maternal blood in an EDTA tube (lavender top) is also required for maternal cell contamination studies.

Newborn child

- Blood: 2 mL in ACD-A or ACD-B tube (yellow top) or EDTA tube (lavender top)
- Purified DNA: 2-5 ug at a concentration of 50 ng/ul
- Buccal swabs may also be requested from our lab

Pediatric or adult

- Blood: 2 ACD-A or ACD-B tubes (yellow top) or 2 EDTA tubes (lavender top)
- Saliva: Oragene kits may be requested from our lab

Please contact our lab for alternate specimen types.



Shipping requirements

- Store and ship specimens at room temperature or refrigerated
- Do not freeze specimens
- Please ship specimens to: 1428 Madison Ave, Atran Bldg, Rm 2-25 New York, NY 10029



Turnaround time

- 7-10 business days for genotyping
- Optional reflexive full gene sequencing is reported 2-3 weeks after the completion of the hotspot analysis

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