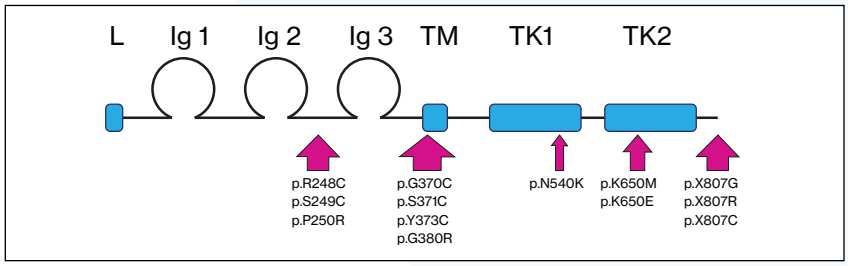
Skeletal Dysplasias: *FGFR3* Hotspot Panel with Reflex to Full Gene Sequencing

The phenotypic variation of skeletal dysplasias points to a complex etiology for this class of disorders. Some of the more common skeletal dysplasias, however, 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 which resembles achondroplasia
- Non-syndromic coronal craniosynostosis

Performing genetic testing can be clinically useful postnatally for individuals that display characteristic features such as short stature or frontal bossing; however, this panel has greater utility prenatally for fetuses that present with certain ultrasound findings (shortened long bones, small ribcage, cloverleaf skull) after a normal chromosome analysis.

The figure below 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).



Testing Methods, Sensitivity And Limitations:

DNA is obtained from a blood (postnatal) sample or a prenatal specimen (direct or cultured chorionic villus sampling or amniocytes). Targeted genotyping is then performed to look for the presence of specific mutations. This testing is approximately 99% accurate (see table on next page for specific Molecular Diagnostic Rates). 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. The technological and analytical test sensitivity by Sanger sequencing for identifying alterations in the *FGFR3* gene is >95% as most reported mutations in the literature are point mutations or small indels.

Turnaround Time:

For hotspot targeted genotyping analysis, results are reported to the referring physician within 5-7 days from the receipt of the specimen. Optional reflexive full gene sequencing is reported 2-3 weeks after the completion of the hotspot analysis.

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 10 ml of direct amniotic fluid (AF) as well as 1 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).

Genetics:

All of the mutations on 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. 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 parent
- The mutation was a "*de novo*" DNA change that occurred in the egg or sperm from which the affected individual developed

Our FGFR3 hotspot panel tests for the following mutations:

***FGFR3* -related Disorders**	**Common Additional Phenotypic Traits**	**Tested Mutations**		**Molecular Diagnostic Rate**
		cDNA	**Protein**	
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 than 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	
		c.2421A>T	p.X807C	
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	N/A
Craniosynostosis	Macrocephaly, midface hypoplasia, carpal-tarsal fusion, sensorineural hearing loss, developmental delay	c.749C>G	p.P250R	30%

References:

i. *Tavormina PL et al. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. Nat Genet. 1995 Mar;9(3):321-8.*

ii. *Shiang R et al. Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. Cell. 1994 Jul 29;78(2):335-42.*

iii. *Bellus GA et al. A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. Nat Genet. 1995 Jul;10(3):357-9.*

iv. *Passos-Bueno MR et al. Clinical spectrum of fibroblast growth factor receptor mutations. Hum Mutat. 1999;14(2):115-25.*

sema4

Mail: One Gustave L. Levy Place, Box 1497
Specimens: 1428 Madison Ave, Atran Bldg, Rm 2-25
New York, NY 10029

T: 800-298-6470
F: 212-241-0139
www.sema4genomics.com

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