Thanatophoric dysplasia

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Abstract
*Thanatophoric Dysplasia (TD) is a severe skeletal disorder that is lethal in the neonatal period. Two clinically defined TD subtypes have been classified: type I (TDI), characterized by micromelia with bowed femurs and, occasionally, by the presence of cloverleaf skull deformity of varying severity and type II (TDII), characterized by micromelia with straight femurs and a moderate to severe cloverleaf skull deformity. TD is caused by specific autosomal dominant mutations in the gene that codifies for the Fibroblast Growth Factor Receptor 3 (*FGFR3). *The mutations constitutively activate the tyrosine kinase activity of the receptor. As normally* FGFR3 *is a negative regulator of bone growth, the gain-of-function mutations associated to TD allow the activated receptor to send negative signals within the cells of the cartilage (chondrocytes), thus leading to the generalized disorganization of endochondral ossification at the bone growth plate.*
The estimated birth incidence is approximately 1/20,000 to 1/50,000 TDI being more frequent than TDII. Most individuals with TD die within the first few hours or days of life by respiratory insufficiency secondary to reduced thoracic capacity or compression of the brainstem. Currently, specific therapeutic regiments other than sustenance of symptoms do not exist. Prenatal diagnosis is available, both by ultrasonography and by molecular studies.

Keywords
Thanatophoric dysplasia, dwarfism, Fibroblast Growth Factor Receptor 3 (*FGFR3*), Tyrosine Kinase, endochondral ossification

Disease names and synonyms
Thanatophoric dysplasia, Thanatophoric dwarfism

Definition
Thanatophoric Dysplasia (TD) is the most common skeletal dysplasia to be lethal in the neonatal period. TD is characterized by severe shortening of the limbs, a narrow thorax, macrocephaly, and a normal trunk length. TD is divided into 2 clinically defined subtypes: TDI and TDII. TDI is characterized by bowed femurs; affected children have sometimes a cloverleaf skull. TDII always presents with

a cloverleaf skull and the neonates have straight femurs.
Other common features to both TD include prominent platyspondyly of the vertebrae, a small foramen magnum with a severe risk for brain stem compression and redundant skin folds along the limbs.

Diagnosis criteria
Diagnosis can be performed according to the following criteria:

Prenatal ultrasound examination
- growth deficiency recognizable by 20 weeks gestation
- well-ossified spine and skull
- platyspondyly
- ventriculomegaly
- narrow chest cavity with short ribs
- polyhydramnios
- bowed femurs (for TDI)
- cloverleaf skull (kleeblattschaedel) for TDII; sometimes in TDI.

For references see: Loong, 1987; Sawai *et al.*1999; De Biasio *et al.* 2000; Chen *et al.* 2001. The identification of a severe skeletal dysplasia in the second trimester is usually straightforward, but establishing a specific diagnosis like TD can be rather difficult (Sawai *et al.* 1999, Parilla *et al.* 2003).

Postnatal physical examination
- macrocephaly
- large anterior fontanel
- frontal bossing, flat facies with low nasal bridge, proptosis
- marked shortening of the limbs (micromelia)
- trident hand with brachydactyly
- redundant skin folds
- narrow, bell-shaped thorax with short ribs and protuberant abdomen
- relatively normal trunk length
- generalized hypotonia.

For references see: Tavormina *et al.* 1995; Lemyre *et al.* 1999; Passos-Bueno *et al.* 1999; Sawai *et al.* 1999; De Biasio *et al.* 2000.

Radiographs
- rhizomelic shortening of the long bones
- irregular metaphyses of the long bones
- platyspondyly
- small foramen magnum with brain stem compression
- cental nervous system (CNS) abnormalities including temporal lobe malformations, hydrocephaly, brainstem hypoplasia, neuronal migration abnormalities
- bowed femurs (TDI).

For references see: Wilcox *et al.* 1998; Lemyre *et al.* 1999; Gorlin *et al.* 2001.

Histopathology
- disorganized chondrocytes columns
- poor cellular proliferation
- lateral overgrowth of the metaphyses
- mesenchymal cells extending inward forming a fibrous band at the periphery of the metaphyses
- increased vascularity of the resting cartilage

For references see: Tavormina *et al.* 1995; Lemyre *et al.* 1999; Wilcox *et al.* 1998.

Molecular genetic testing
FGFR3 mutants are the cause of both TDI and TDII.
For TDI a series of missense mutations have been identified: R248C*, Y373C*, S249C, G370C, S371C (Rousseau *et al.* 1996; Passos-Bueno *et al.* 1999). The most common mutants (*) account for 60-80% of TDI (of note all mutations create new, unpaired cysteine residues in the *FGFR3*). Furthermore, stop codon mutations (X807L, X807G, X807R, X807C, X807W) have been identified (Rousseau *et al.* 1995; Rousseau *et al.* 1996).
For TDII a single point mutation in the *FGFR3* gene (K650E) has been identified in all TDII analyzed (Rousseau *et al.* 1996; Gorlin 1997; Bellus *et al.* 2000).

Differential diagnosis
The differential diagnosis includes a number of well-delineated skeletal dysplasias (Passos Buenos *et al.* 1999, De Biasio *et al.* 2000, Gorlin *et al.* 2001, Lee *et al.* 2002, Neumann *et al.* 2003):
Asphyxiating thoracic dysplasia (Jeune syndrome), a rare autosomal recessive chondrodysplasia that often leads to death in infancy because of a severely constricted thoracic cage and respiratory insufficiency.
Homozygous achondroplasia has a similar clinical presentation and should be a part of the differential diagnosis when both parents have achondroplasia.
Achondrogenesis, type IA, type IB and type II, Schneckenbecken dysplasia.
SADDAN (Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans).
Short-rib polydactyly syndromes (especially the Saldino-Noonan type).
Metatropic dysplasia.

Osteogenesis Imperfecta (OI) type II that is the perinatal lethal form. OI type II has mutations in either *COL1A1* or *COL1A2* genes and is inherited in an autosomal dominant manner.
Campomelic syndrome. This is an entity characterized by congenital bowing and angulations of long bones, together with other skeletal and extra skeletal defects. It is caused by mutation in the *SOX9* gene.
Rhizomelic chondrodysplasia punctata also shows micromelia but this is almost exclusively rhizomelic. Radiologically the stippling is characteristic; it is caused by mutations in the *PEX7* gene, which encodes the peroxisomal type 2 targeting signal (PTS2) receptor (Marzioch 1994).
Hypophosphatasia.
The group of platyspondylic lethal skeletal dysplasia, such as Torrance-Luton type (Gorlin *et al.* 2001). The latter was originally grouped with TD however, it is not caused by mutations in the *FGFR3* (Neumann *et al.* 2003).
Dyssegmental dysplasia, Silverman-Handmaker type (DDSH). DDSH is caused by mutations in the heparan sulfate proteoglycan (*HSPG*) gene.

Frequency
TD has an incidence of approximately 1/20,000 to 1/50,000 live births (Orioli *et al.* 1986; Wilcox *et al.* 1998; Sawai *et al.* 1999, Bartner et al 2000; Chen *et al.* 2001).

Etiology
The long bones elongation is governed by a process known as endochondral ossification, a tightly regulated developmental process that occurs in the epiphyseal growth plate (Caplan and Pechak 1987). Chondrocytes of the growth plate are arranged in columns that sequentially and synchronously progress through proliferative, pre-hypertrophic and hypertrophic stages. *FGFR3* has been identified as a negative regulator of endochondral growth since the targeted disruption of the mouse *fgfr3* gene causes a skeletal overgrowth (Colvin *et al.* 1996; Deng *et al.* 1996). *FGFR3* belongs to the receptor tyrosine kinase family (RTKs). *FGFR3* is a glycosylated transmembrane protein (Keegan *et al.* 1991, Lievens and Liboi 2003) that upon binding with FGF ligands and heparin dimerizes and undergoes interchain autophosphorylation of key tyrosine residues, thus transmitting signals into the cells (Basilico and Moscatelli 1992; Plotnikov *et al.* 1999). The TD mutations in *FGFR3* are gain-of-function mutations that produce a constitutively active receptor capable of initiating intracellular signal pathways in the absence of ligand binding.
TDI - All reported mutations cause constitutive *FGFR3* activation through the creation of new, unpaired cysteine residues that induce ligand-independent dimerization (Rousseau *et al.* 1996; Cohen 2002) or the creation of an elongated protein through destruction of the native stop codon (Rousseau *et al.* 1995; Rousseau *et al.* 1996). It has been recently proposed that mutated *FGFR3* induces premature exit of proliferative cells from the cell cycle and their differentiation into pre-hypertrophic chondrocytes thus ascribing to the defective differentiation of chondrociytes the main cause of long bone growth defects in TDI (Legai-Mallet *et al.* 2004).
TDII - A single *FGFR3* mutation (K650E) has been identified in all cases of TDII (Rousseau *et al.* 1996; Bellus *et al.* 2000). The K650E mutation is located within a critical region of the tyrosine kinase domain activation loop. Indeed, amino acid substitution at this codon results in strong autophosphorylation of multiple tyrosine residues within the intracellular domain (Webster *et al.* 1997). In *in vitro* studies and in mice carrying the TDII mutation it was shown that the signal transduction and activator of transcription STAT1 is activated and translocated into the nucleus (Iwata *et al.* 2000). This, together with activation of the cell cycle inhibitor $p21^{waf1}$ was proposed as the molecular mechanism responsible for the TDII pathology (Su *et al.* 1997). Furthermore, ligand-independent activation of the STAT signalling pathway was demonstrated in cultured TD cells and confirmed by immunodetection of activated STAT1 associated to apoptosis of chondrocytes in TD fetus (Li *et al.* 1999; Legai-Mallet *et al.* 1998). More recently, *in vitro* studies with chondrocytic cells (RCS) and human epithelial cells (HEK293) show that the TDII mutation hampers the complete maturation of *FGFR3* leading the immature phosphorylated *FGFR3* forms to signal from the Endoplasmic Reticulum, which fails to be degraded (Lievens and Liboi 2003). Consequently, it was proposed that the defect in down regulation of the highly activated receptor results in the increased signalling capacity

from intracellular compartments that may determine the severity of the disease. This has been associated to the high level of *FGFR3* tyrosine kinase activity caused by the K650E substitution (Lievens *et al.* 2004).

Clinical description

Prenatal diagnosis allows determining both TDI and TDII. Most affected individuals die of respiratory insufficiency within the first hours of life. Some die after a few days of life. Respiratory insufficiency may be secondary to a small chest cavity and lung hypoplasia, compression of the brain stem by the small foramen magnum or a combination of these (Baker *et al.* 1997). Rare long-term survival (a 4.7 year male and a 3.7 year female) has been reported (MacDonald *et al.* 1989). Both had birth length and weight below the third percentile. In both, growth plateaued after 10 months of age. Clinical profile includes micromelia, redundant skin folds, hydrocephalus and a small foramen magnum. Furthermore, a 9 year-old male with TDI (R248 mutation) with extensive acanthosis nigricans and a severe developmental delay with no language has been reported (Baker *et al.* 1997).

A 47-year-old female with TDI (R248C mutation) presents asymmetrical limb length, bilateral congenital hip dislocation, focal areas of bone bowing and an S-shaped humerus, extensive acanthosis nigricans, redundant skin folds along the length of the limbs and a flexion deformities of the knees and elbows (Hyland *et al.* 2003).

No strong genotype-phenotype correlation for *FGFR3* mutations causing TD exists.

Genetic counseling

Thanatophoric dysplasia type I and thanatophoric dysplasia type II are caused by *de novo* autosomal dominant mutations in *FGFR3*. Recurrence risk is not significantly increased over that of the general population. Germline mosaicism in healthy parents, although not previously reported, remains a theoretical possibility. Prenatal diagnosis is clinically available, and is reliable both through sonography and through molecular studies.

Diagnostic methods

Prenatal diagnosis is performed by analysis of DNA (*FGFR3* sequences) extracted from fetal cells obtained by amniocentesis usually performed at 15-18 weeks gestation or chorionic villus sampling at about 10-12 weeks gestation.

Routine prenatal ultrasound examination may identify skeletal alterations associated to TD such as cloverleaf skull, very short extremities, and a small thorax.

Management

Most individuals with TD die within the first few hours or days of life by respiratory insufficiency secondary to reduced thoracic capacity or compression of the brainstem. Management concerns are limited to extreme life support measures for the newborn.

In the rare cases of long-term survival, the management consists in treatment of manifestations:

- respiratory support (tracheostomy, ventilation)
- medication to control seizures
- shunt placement when hydrocephaly is identified
- suboccipital decompression for relief of craniocervical junction constriction
- hearing aids when hearing loss is identified
- orthopedic evaluation upon the development of joint contractures or joint hypermobility.

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