Thanatophoric dysplasia: A review

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Thanatophoric dysplasia is a well-known cause of potentially lethal short-limbed dwarfism in the newborn. The diagnosis is usually made by the recognition of characteristic radiological changes and confirmed at autopsy by demonstration of specific morphological and histological changes in the brain. This review is based upon the author's personal experience and archived data of 19 cases and concerns the clinical and radiographic manifestations, autopsy findings, molecular pathogenesis and the approach to antenatal diagnosis.

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Thanatophoric dysplasia (TD) is the most common neonatal lethal skeletal dysplasia, and it is regularly encountered in the context of antenatal diagnosis and autopsy. This review of the disorder is based upon personal experience and data concerning 19 cases archived in the Division of Pathological Anatomy, University of Cape Town (UCT).

Professor Peter Beighton had a special interest in genetic bone disorders and following his appointment at UCT in 1972 he established a special clinic for affected persons at the Princess Alice Orthopaedic Hospital. He also undertook diagnostic screening at facilities for physically handicapped persons throughout South Africa. These activities led to the publication of his early monographs[1-3] and to his involvement in the International Classification of Osteochondrodysplasias.[4] After mandatory retirement in 1999, he extended his interest in skeletal dysplasias to neonates, stillbirths and fetuses and assisted me with my research in this field. Together we have co-authored 11 articles concerning the most severe and lethal skeletal dysplasias. In early intrauterine life, the radiological features may be poorly developed and the pathological changes in the bones more difficult to determine than in a full-term pregnancy. Information from prenatal ultrasonic imaging from 12 weeks' gestation onwards is now available and it is invaluable in the early detection and diagnosis of a specific skeletal dysplasia in order to facilitate appropriate obstetrical and genetic management.

The incidence of type 1 TD (TD1) is variously quoted as 1 in 20 000 - 40 000 stillborn and liveborn infants (MIM 187600)[5] or 1 in 33 000 - 47 000 live births[6]]. The condition was initially termed thanatophoric dwarfism in 1967,[7] and a decade later the name was changed to thanatophoric dysplasia. The name was derived from the Greek word *thanatos* meaning 'death' and *phoros* meaning 'provoking'.

Manifestations

TD is traditionally divided into two forms on a basis of the radiographic appearances – TD1 with curved femora and usually a normal skull and type 2 (TD2) with straight femora and frequently a trilobal clover-leaf skull.

Phenotypically, there is marked symmetrical shortening of the limbs with redundant skin folds, shortening of hands and feet, macrocephaly with frontal bossing, a depressed nasal bridge, small chest, large protuberant abdomen, and a trunk of relatively normal length (Fig. 1). The radiological features of TD1 are characteristic when the infant is delivered at term – macrocephaly, symmetrical micromelic shortening of long bones, metaphyseal cupping of the proximal femora, telephone-receiver shaped femora, hypoplastic or small scapulae, platyspondyly with H-shaped vertebrae in the anterior-posterior view, a narrow thorax with short ribs, and characteristic triradiate acetabulum with short sacrosciatic notches. Interpediculate narrowing of the spinal canal evident on lateral views results in damage to the spinal cord in rare survivors. In TD2, the skull has a marked anterior depression forming the trilobal clover-leaf skull.[8]

Fig. 1. Immature fetus with TD1 with macrocephaly, frontal bossing, depressed nasal bridge, small chest, protuberant abdomen and short symmetrical bowed limbs.

Bone radiographic morphometry shows that longitudinal bone growth is not equally inhibited across the growth plate, resulting in the lateral spurs at the metaphyseal growth plates. The platyspondyly is due to a reduced height of the anterior ossification centres of the vertebral bodies, while laterally there is no restriction in growth. Interplay between mechanical factors and the effects of the underlying mutation are additional factors in this process.[9]

Autopsy findings

At postmortem, the head is macrocephalic due to macroencephaly and symmetrical deep grooves are present on the basal surfaces of the temporal lobes of the brain. Histologically, the ends of the long bones show markedly disordered growth plates. The normal growth plate has three zones – the resting zone with disordered chondrocytes and abundant matrix, the proliferative zone, and the hypertrophic zone where the chondrocytes die. Below the hypertrophic zone is the zone of provisional calcification, then the primary and secondary spongiosum. In TD1, the resting zone is uninvolved. The proliferative and hypertrophic zones show variable disorganisation with complete absence in some areas and

focal columns in others. A typical horizontal band extends from the perichondrial fibrous tissue at the periphery into the growth plate and there is transformation of this mesenchymal tissue into irregular, poorly orientated bony trabeculae. The cartilaginous bars are reduced in number and are thin and distorted. Perichondrial ossification extends above the level of endochondral ossification. The value of histological assessment of the growth plate in the diagnosis of TD1 has been known since 1979 (Fig. 2).[10]

The ultrasonographic, radiological and histological features in fetuses and newborns are well documented.[11] Young fetuses pose a special challenge to diagnosis as diagnostically characteristic radiological features may be absent. Ultrasound shows severely shortened and bowed long bones, a narrow thorax filled by the heart, a protuberant abdomen and bossing of the skull. In young fetuses, the ultrasonographic diagnosis may not be specific. Nevertheless, detection of severe shortening of limbs with bowing and severe thoracic hypoplasia suggest a lethal skeletal dysplasia with pulmonary hypoplasia, which is usually a sufficient indication for termination of pregnancy.

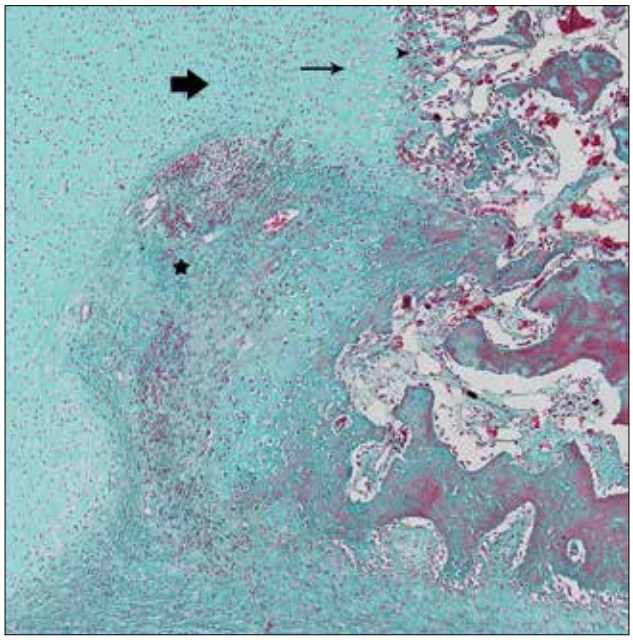

Fig. 2. Masson's trichrome stain shows histology of the markedly disordered growth plate with a horizontal band extending into the growth plate indicated by a star; the short arrow shows the proliferative zone, the long arrow shows the short chondrocyte columns – the arrowhead shows the disordered metaphysis.

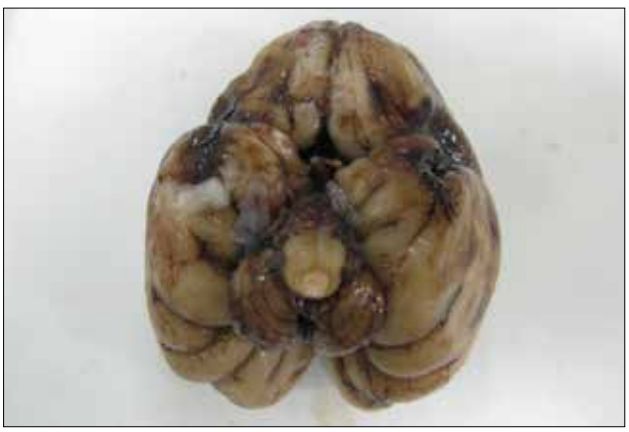

Fig. 3. Inferior surface of the brain with the basal surfaces of the temporal lobes showing deep symmetric grooves diagnostic of TD1.

The central nervous system abnormality is not secondary to the bone changes in the skull, such as synostosis which occur late in gestation, but due to the underlying *FGFR3* mutation. Fibroblast growth factors (FGFs) 1 - 4 are expressed in the developing brain. Using a mouse model, it has been shown that *FGFR3* is involved in area patterning, progenitor proliferation and a reduction in apoptosis within the brain. The most typical neuropathological changes are megaloencephaly, dysplasia of the hippocampus, rudimentary dentate nucleus, polymicrogyria, temporal lobe hyperplasia, 1 - 5 abnormal deep transverse sulci on the basal surface, subependymal neuronal heterotopia and subarachnoid neuroglial heterotopia (Fig. 3).[12] Temporal lobe ventriculomegaly is common, but severe hydrocephalus occurs only in 18% of cases due to a small foramen magnum. The cerebellum may be hypoplastic or normal. The diagnostic basal transverse sulci are seen at 20 weeks' gestation when the brain normally has a smooth surface. The abnormal sulcation on the medial aspects of both temporal and occipital lobes has been identified on antenatal sonography at 21 weeks and confirmed on antenatal MRI, postnatal MRI and at postmortem.[13]

Molecular pathogenesis

The inception of the molecular age brought major changes to the former categorisation of skeletal dysplasias which has now been supplemented or replaced by a molecular classification.[14] TD1 now falls under the broad group of the Fibroblast Growth Factor Receptor 3 (*FGFR3*) family [*FGFR3*, OMIM 134934]. The first activating or gain-in-function mutation in *FGFR3* receptor tyrosine kinase that binds fibroblast growth factors was discovered in 1994 in achondroplasia.[15,16] Other conditions associated with mutations in this gene include TD2. Numerous somatic mutations that cause diverse conditions such as specific skeletal dysplasias, skin disorders and malignancies have also been identified.

The *FGFR3* gene encodes a protein, predominantly in bone-producing cells, which straddles the cell membrane with the inner end within the cell. The outer end projects from the surface and growth factors attach to it, resulting in its activation and action on bone. The gene regulates skeletal development by degradation of the bone morphogenetic protein (BMP) type 1 receptor. It has a negative effect on chondrogenesis/endochondral bone growth via inhibition of BMPR1a, which is required for chondrocyte differentiation.[17]

TD1 may result from a single amino acid substitution in either the extracellular or intracellular domains of the protein. Examples involving the extracellular domain include p.R248C (c.742C>T); p.Y373C (c.1118A>G).[18,19] Stop codon mutations have been reported: *p.X807G (c.2419T>G)*; *p.x807R (c.2419T>A)*; *p.X807C (c.2421A>T)* and these mutations result in elongation of the protein.[20]

Both *K650M* and *K650E* mutations in the *FGFR3* gene show abnormal cellular location to the endoplasmic reticulum[21] and they activate downstream signalling intermediates such as ERKMAP kinase.[22] These two mutations can be recognised by light microscopy as large cytoplasmic inclusion bodies that stain positively with antibodies to *FGFR3* protein.[23] Other mutants such as *R248C* and *Y373C* signal from the cell membrane and cause severe skeletal dysplasia.[24]

Numerous studies have involved investigations of mutations in TD1. The largest series consisted of 91 cases of TD1 and TD2 with *FGFR3* mutations identified in the International Skeletal Dysplasia Registry (ISDR).[25] The most common mutation was *R248C*, occurring in 50% of cases of TD1, followed by *Y373C* in

20%. These cases had more severe radiological manifestations than TD1 with *R248C*, but there was phenotypic overlap. Nineteen cases with TD2 from the ISDR had the *K650E* mutation and showed better preservation of the growth plate compared with TD1. Two mutations in the *FGFR3* gene result in virtually all cases of achondroplasia, which is allelic with TD2. The *N540K* mutation alone results in the less severe phenotype of hypochondrodyplasia.

In 2014, Xue, *et al.*[26] reported an update from the ISDR in which mutation analysis involved sequencing of the entire coding region in 324 cases, including achondroplasia and hypochondroplasia. This extensive series showed that 90% of TD1 mutations were either *pArg248Cys* (66%) or *pTyr373Cys*. The third most common was a stop codon mutation *pX807* and the fourth was *pSer249Cys*. The mutation *pGlu370Cys* accounted for 2.3% and *pLys650Met* for 1.2%. This information is extremely useful when designing and costing commercial tests for TD1.

Mutations cause activation of *FGFR3* by different mechanisms. Extracellular FGF ligands form dimers. The TD1 mutation *Y373C* forms covalent bound dimers between cysteine residues near the juxtamembrane domain. Amino acid substitutions in the intracellular domain, such as *K650M* in TD 1/SADDAM or *K650E* in TD2, mimic conformational changes resulting in dimerisation and autophosphorylation.[27]

Low levels of activity require FGF ligand for activation: high levels of activity, as occurs with *R248C* and *Y373C* mutations, result in spontaneous dimerisation and are independent of ligand. Different amino acid substitutions cause differing degrees of activation of *FGFR3,* which translates into varying levels of chondrocyte inhibition. The most severe inhibition will result in the most severe degree of skeletal dysplasia.

In the absence of a mutation, the inhibition of chondrocytes via *FGFR3* can be regarded as senescence, which can be experimentally reversed. Elsewhere in the body this senescence represents a natural barrier against tumour development. In embryogenesis, newly formed chondrocytes trigger strong initiation of *FGFR3* expression as required for differentiation.

FGFR3 is a physiological negative regulator of bone growth. In humans, loss of function of *FGFR3* causes an autosomal recessive syndrome CATSHL, which is characterised by camptodactyly, hearing loss and skeletal overgrowth.[28] Somatic mutations identical to those associated with TD1 can cause skin overgrowth or cancer, demonstrating that the TD mutation can act as an oncogene.[29] The growth inhibitory role of *FGFR3* in cartilage is unique in comparison to its aberrant signalling in other tissues.[30]

Aberrant signalling in the RAS/ERK pathway due to *FRGF3* results in skeletal dysplasias. The signaling interferes with cyclin D3 complexes in the G1 phase of the cell cycle. There is an increase in cell cycle inhibitors that inhibit kinase activities. *FGFR3* activation causes increased cell cycle inhibitors that prolong ERK activation only in chondrocytes so that the pathology is limited to the growth plate, as seen in skeletal dysplasias.

Animal studies in mice have produced an equivalent model to TD1 by introducing the *S365C* mutation into exon 10 of mouse *FGFR3*. However, the mutant mouse has severe dwarfism but no pulmonary hypoplasia, and the condition is therefore non-lethal. Other mouse models include short stature and a dome-shaped skull when the *K6434E* mutation is introduced into *FGFR3*. This mouse has a small thoracic cage and lethality. Mutation *G369C* provides an animal model for achondrogenesis. Regular subcutaneous injections of parathyroid hormone (PTH) permit a larger thorax to develop, thereby preventing pulmonary hypoplasia and causing an increase in length of long bones and a round head in the mouse models.[31] For these reasons, mouse models can be used for testing potential therapies.

Antenatal diagnosis in TD

Early first-trimester prenatal diagnosis of skeletal dysplasia requires expertise. At 12 weeks, there may be subtle features such as frontal bossing, rarely an increase in nuchal translucency and short limbs. Early in the second trimester, a small thorax and short, bowed long bones may be demonstrated particularly by 2D and 3D ultrasound imaging. Ultrasound alone provides a specific antenatal diagnosis in 1/3 - 2/3 of cases.[32] The size of the thorax alone does not always indicate lethality, but parameters such as thoracic circumference and the thoracic-to-abdominal ratios may be more helpful.[33]

If the necessary facilities are available, mutational analysis during pregnancy is useful when TD is suspected.[34] The *FGFR3* gene consists of 19 exons and 18 introns spanning 16.5kb[35] and screening for the most frequently occurring mutations is cost effective.

Genotyping of mutations can be performed by high-resolution melting analysis. *R248C* is the most common mutation. Exon 7 was analysed in 10 samples with TD1 and 30 controls using melting curve analysis with a high-resolution melting instrument. This mutation was present in all 10 samples, giving a sensitivity and specificity of 100%.[36]

Other strategies for molecular testing use hot-spot exons 7, 10, 15 and 19 analysis with 80 - 90% sensibility. If hot spot exons are negative, then a search for rare mutations using sequential analysis of *FGFR3* gene is performed.[37] The nucleotide sequence of *FGFR3* is highly conserved between man and mouse.

Future prospects

In the context of the *FGFR3* group of disorders, postnatal treatment of achondroplasia is becoming a possibility in 2015 in First-World countries, raising hope for therapeutic intervention in certain skeletal dysplasias. Nevertheless, therapy for TD remains highly unlikely at the present time.

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