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Thanatophoric Dysplasia Variant, San Diego Type in a Chinese Fetus, Caused by C746G Missense Mutation in *FGFR3* Gene

ACF **LAM**, TMF **TONG**, MHY **TANG**, S **LO**, CP **LEE**, E **LAU**, STS **LAM**

Abstract Thanatophoric dysplasia variant – San Diego type (TD-SD) is a variant of thanatophoric dysplasia (TD), sharing with TD subtype TD1, common mutations in the *FGFR3* gene. We describe the first local case of TD-SD with missense mutation C746G in *FGFR3* gene. Radiologically, major features consist of macrocephaly, wafer thin vertebral bodies, and metaphyseal flaring of tubular bones that differentiate from other types of thanatophoric dysplasia.

Key words *FGFR3* gene; Platyspondyly lethal skeletal dysplasia; San Diego type; Thanatophoric dysplasia

Introduction

The thanatophoric dysplasias (TDs) include the classic thanatophoric dysplasia (TD), and the so called TD variants; the "San Diego type" (TD-SD) and the "Torrance-Luton type". TD is one of the commonest neonatal lethal skeletal dysplasias,[1] and is subclassified into TD1 and TD2 on radiological features and specific fibroblast growth factor receptor 3 (*FGFR3*) mutation. Thanatophoric dysplasia occurs in approximately 1/20,000 to 1/50,000 births.[2] There is 100% penetrance of *FGFR3* mutations and no phenotype-genotype correlation for reported mutations.

The San Diego type (TD-SD) of TD has now been shown to be part of the same continuous spectrum of TD1 caused by mutations in *FGFR3* gene. Although not specific, in the past, TD-SD has been distinguished from the usual thanatophoric dysplasia by the presence of large inclusion bodies in the rough endoplasmic reticulum of hypertrophic chondrocytes. Radiologically, wafer thin vertebral bodies and metaphyseal flaring of tubular bones differentiate TD-SD from TD1; and macrocephaly differentiates TD-SD from Torrance-Luton types of TD. We here report a case of TD-SD diagnosed at 20 weeks of gestation, and molecular analysis confirmed a pathologenic mutation in the *FGFR3* gene.

Subject, Methods and Results

The fetus was the first conceptual product of a non-consanguineous couple with father aged 42 and mother aged 37. Father's height was 164 cm and mother's height was 152.4 cm. There was no past history of miscarriage for the couple, and no familial history of short stature. Amniocentesis was performed at 17 weeks of gestation because of advanced maternal age and antenatal ultrasound detected abnormality of short limbs and narrowed chest, the result of cytogenetics study was 46,XY. The couple elected to have termination of pregnancy at 20 weeks

Clinical Genetic Service, Department of Health, Hong Kong, China

ACF **LAM** （林傳發） *MRCPCH*
TMF **TONG** （唐鳴科） *MSc*
STS **LAM** （林德深） *FHKAM*

Prenatal Diagnostic and Counselling Centre, Tsan Yuk Hospital, Hong Kong, China

MHY **TANG**（唐海燕） *FHKAM(O&G)*
CP **LEE** （李之朋） *FHKAM(O&G)*
E **LAU** （劉嚴德光） *PhD*

Obstetrics Department, Pamela Youde Nethersole Eastern Hospital, 3 Lok Man Road, Chaiwan, Hong Kong, China

S **LO**（羅宇帆） *FHKAM(O&G)*

Correspondence to: Dr STS **LAM**

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gestation. Post-mortem examination revealed a hydropic fetus with disproportionate body proportion. There was macrocephaly, mid-face hypoplasia, short limbs, narrowed chest, protuberant abdomen, normal male genitalia. There was no clefting of lip or palate, both atria and ventricles showed normal anatomical connection. Examination of the Central Nervous System was unremarkable. Microscopic examination of bones (that included ribs, upper & lower limbs bones) showed disruption of endochondrial ossification with hypertrophic chondrocytes and arranged in disordered positions. Babygram (Figure 1) showed Macrocephaly without cloverleaf appearance, relative long trunk with short limbs, short ribs, bowing of femori in the shape of "telephone receivers", generalised metaphyseal irregularity with cupping deformity of tubular bones, generalised wafer thin vertebral bodies with ossification defect in cervical regions. Small irregular scapulae were seen, and hypoplastic ilia with narrowed sacral-sciatic notch and horizontal acetabular ridge noted. Accessory ossification centres in the ischia and ilia were present (common in TD and TD-SD).[3]

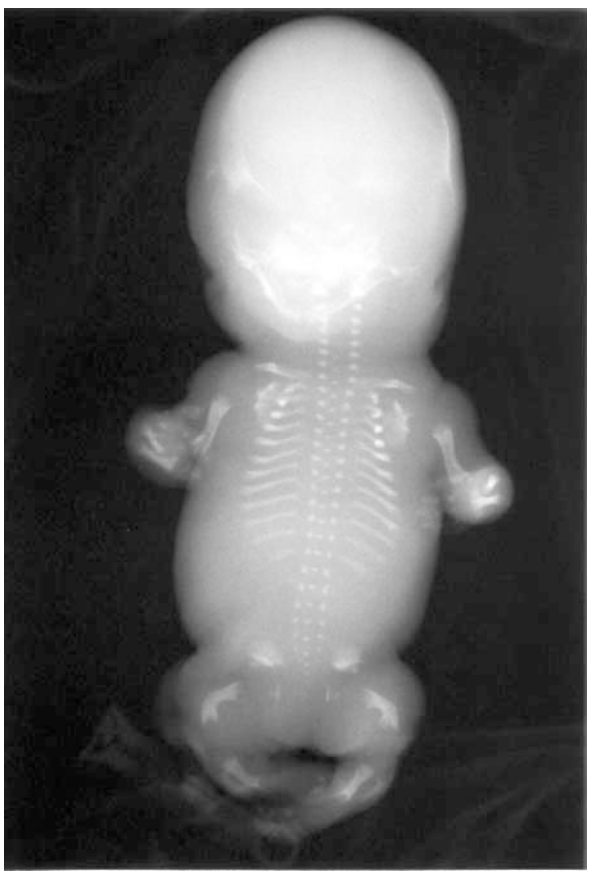

Figure 1 Babygram of affected fetus.

Placental villi were dissected from maternal tissue and used for DNA preparation.[4] Briefly, villi were added with lysis buffer (100 mM Tris.HCL pH8.5, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, 500 µg/ml Proteinase K) and incubated at 65˚C for 2 hours. Cold absolute ethanol were then added and the solution mixed by inverting the tube several times. DNA fiber clump was then lifted from the solution and rinse in 70% ethanol, air dried, and dissolved in T0.1E (10 mM Tris.HCL pH8.0, 0.1 mM EDTA).

FGFR3 fragment containing nt746 was amplified by polymerase chain reaction (PCR) for direct sequencing. The forward and the reverse primer were described by Tavormina et al.[5] After initial 4 min denaturing at 95˚C, PCR was cycled 10 times at 95˚C for 30 sec, at 50˚C for 30 sec, at 72˚C for 2.5 min and 20 times at 95˚C for 30 sec, at 50˚C for 30 sec, at 72˚C for 2.5 min with auto-extend by 5 sec per cycle in a 50 µl mixture containing 1 x GC-rich PCR reaction buffer, 0.1 mM each dNTP, 0.2 mM each primer, 15 µl of 5M GC-rich resolution solution and 2 units enzyme mix (Roche, Germany). PCR products were purified using the QIAquick PCR purification kit (Qiagen, Germany) and sequenced on both strands with BigDye terminator chemistry by the standard protocols (Applied Biosystems).[5]

Parental DNA was analysed by direct sequencing for the specific mutation identified in the proband.

A heterozygous c.746C>G transversion mutation (Figure 2) was found in the *FGFR3* gene. This is a de novo

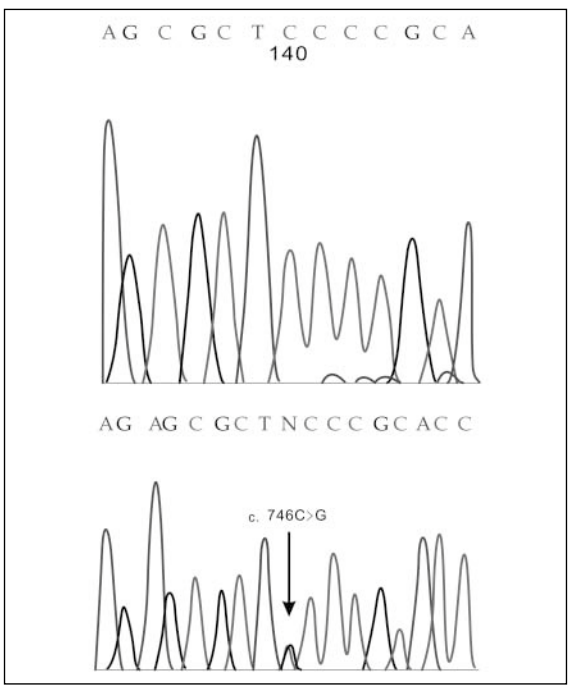

Figure 2 Chromatogram showing c.746 C>G mutation.

missense mutation that resulted in a change in amino acid code from Serine to Cysteine at codon 249. This mutation is not found in either parent.

Parental consent for publication that included photos and radiographs were kindly given and signed.

Discussion

The TDs is a group of genetically heterogeneous disorders that have similar clinical and radiological features. Thanatophoric dysplasia is one of the commonest forms of neonatal lethal skeletal dysplasia. Both type 1 (TD1) and type 2 (TD2) of thanatophoric dysplasia do not share common mutation in the *FGFR3* gene. The so called TD variant; the "San Diego type" has been re-classified and is now thought to be part of a continuous spectrum of TD1 sharing with it, common mutations in *FGFR3* gene.[6] In the old classification before year 2001, TD-SD was grouped under the "Platyspondyly lethal skeletal dysplasia" as a separate entity together with thanatophoric dysplasia and its two other closely resembled radiologically PLSD-subtypes, the "Torrance type" and the "Luton type". The "Torrance type" and the "Luton type" represent different ends in severity, and this "Torrance-Luton type" is now found to be part of the big family of type II collagenopathy.[7]

In the past, one of the distinguishing features of TD-SD from the usual thanatophoric dysplasia was by the presence of large inclusion bodies in the rough endoplasmic reticulum of hypertrophic chondrocytes that are seen in poor organisation of chondrocyte column microscopically. This has been shown to be an unreliable distinguishing feature between the TD subtypes despite its preponderance in the TD-SD type.[8] Since the material contained within the endoplasmic reticulum is the mutant *FGFR3* protein, the exact mechanism as to how the body handles the material in the inclusion bodies that leads to the variation in severity of the TD-SD phenotype remains to be answered.[9]

FGFR3 is the only gene known to cause TD1, TD2 and TD-SD. Mutation K650E has been identified in all cases of TD2. Reported mutations of TD1 responsible for at least more than 90% of cases include missense mutations (80%) (R248C, Y373C, S249C, G370C, S371C), and nonsense mutations (10%) (X807L, X807G, X807R, X807C, X807W). Brodie et al 1999 reported 17 cases of TD-SD. Seven had R248C, 2 had S249C, six had Y373C and two had nonsense mutations in *FGFR3*. The TD-SD reported here had S249C. As in most cases of thanatophoric dysplasia with de novo mutation in *FGFR3* gene, the parents of our proband were found to be unaffected.

FGFR3 contains three main components – an extracellular domain with three immunoglobulin-like domains, a transmembrane domain and two intracellular tyrosine kinase domains. Ligand binding occurs between IgII and IgIII.[10] Missense mutation S249C reported here is located between the peptides linking the second and third immunoglobulin extracellular domain of *FGFR3*.

FGFR3 is a proteoglycan that function as tyrosine kinase. Mutations cause loss of ligand-binding specificity. The normal function of *FGFR3* is to serve as a negative regulator of bone growth during ossification.[11] Mutations in *FGFR3* gene are gain-of-function mutations through the creation of new, unpaired cysteine residues that induce ligand-independent dimerization[11] and undergo phosphorylation of the tyrosine residues in the tyrosine kinase domain. This frees up intracellular binding sites and allows intracellular proteins to bind and initiate a signal cascade that influences protein activation or gene expression,[11] thus produces a constitutively active protein capable of initiating intracellular signal pathways in the absence of ligand binding.[11] This activation leads to premature maturation of the bones of the skeleton and cranium[11] and thus shortened bones. Since the same mutation has been known to cause both TD1 and TD-SD, the question remains whether one phenotype is only the early presentation of the other phenotype. Alternatively, whether other epigenetic factors may contribute to the differing phenotypes await future confirmation.

Brodie et al (1999) hypothesised that the TD-SD is the milder early presentation of TD1 because of better preserved growth plate microscopically and larger inclusion bodies in endoplasmic reticulum preventing mutant *FGFR3* reaching the cell surface and participate in cell signalling. However, with the wafer thin vertebral bodies, generalised metaphyseal irregularities in tubular bones in TD-SD; in contrast to the better ossified H shaped vertebral bodies, smoother metaphyses of tubular bones in TD1 may seem to suggest the reverse.

An advanced paternal age effect was reported by Lemyre et al in 1999.[12] The father of our proband was aged 42 at time of fetal conception. The significance of this is unknown at present. More collaborative epidemiological data analysis is needed to draw such conclusion.

TD1 and TD2 are diagnosed prenatally or in the immediate newborn period. Both subtypes are considered lethal skeletal dysplasias. For these, diagnosis is usually straight forward. For other TDs, diagnosis may not be so

easy because of atypical features. Correct diagnosis allows proper genetic counselling and prognostication. For "Torrance-Luton" types, survival to adulthood has been reported.[13] In addition, germline mosaicism for *FGFR3* mutation (R248C) was reported in an affected individual whose offspring had a lethal skeletal dysplasia suggestive of thanatophoric dysplasia.[14]

In developed countries, there is an emerging trend of starting a family at a later stage in life and having fewer children. Many pregnant women received prenatal ultrasound examinations. Skeletal dysplasias are increasingly being diagnosed in the prenatal period in women with no known family history. While the prediction of lethality was reasonably accurate based on prenatal ultrasound examination, the definitive specific diagnosis was only correctly made prenatally in 48% of the cases of lethal skeletal dysplasia.[15] It is often based on the prediction of lethality that a decision of termination of pregnancy is made, without definitive diagnosis of the type of skeletal dysplasia, which is important for genetic counselling. Therefore, definite diagnosis must be obtained by post-mortem examination of the fetus including radiological studies; and molecular analysis where indicated. This is crucial for post termination genetic counseling which should be offered to anyone who went through termination for suspected fetal skeletal dysplasia in current medical standard that demands good quality patient care.

Without proper genetic counselling, most families with neonatal lethal skeletal dysplasia would be too worried to have further pregnancy. Since the majority of cases occur sporadically, it is essential to counsel that the recurrence risk is low for only one affected fetus, and that the extended family members of the proband are not at increased risk. To relieve parental anxiety in such low risk couple, prenatal ultrasound examination may be offered in subsequent pregnancies to identify features suggestive of TD, such as macrocephaly, vertebral ossification defect, bowed femori, micromelia, and small thorax with protuberant abdomen. If indicated, amniocentesis may be offered, and diagnosis be made by molecular analysis.

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