Case Report

Thanatophoric Dysplasia: Roentgenographic Findings and Detection of a de Novo Mutation of FGFR3 Gene in a Thai Patient

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Thanatophoric dysplasia is the most common neonatal lethal skeletal dysplasia with an estimated incidence of 1 in 20,000 live births. This condition shares some similarity of radiological findings with other types of lethal skeletal dysplasias. Definite diagnosis is necessary for accurate medical and genetic counseling. The authors describe a male neonate who had characteristic features of thanatophoric dysplasia type I including severe shortening of limbs with redundant skin folds, large head, frontal bossing, depressed nasal bridge, and narrow thoracic cage with severe respiratory insufficiency. Postmortem radiographs revealed short ribs, flat vertebral bodies (platyspondyly), hypoplastic iliac bones, marked shortening of long bones including short and mild bowing of both femora, oval radiolucent area of proximal femur. Molecular analysis of Fibroblast Growth Factor Receptor 3 (FGFR3) gene identified a de novo *mutation, p.R248C, in exon 7.*

Keywords: *Thanatophoric dysplasia, Mutation, Fibroblast growth factor receptor 3 (*FGFR3*) gene*

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Thanatophoric Dysplasia type I (TDI, MIM 187600) is the most common form of Platyspondylic Lethal Skeletal Dysplasias (PLSD), which constitute a heterogeneous group of chondrodysplasia characterized by severe platyspondyly (flat vertebral bodies) and limb shortening[1]. TD was first described by Marateaux and Lamy[2]. The word "thanatophoros" (from Greek) means "death-bringing". Its estimated incidence is 1 in 20,000 live births. Almost all the cases are incompatible with life with a few exceptions[3-5]. For those who survive, the affected individuals are fertile and the phenotypes are transmitted in an autosomal dominant fashion[3].

Classical phenotypes of TDI include marked shortening of extremities with numerous skin folds, large head with flat nasal bridge, relatively normal trunk length, narrow thorax, and severe platyspondyly[1,6]. The other three types of PLSD are TD type II (TDII MIM 187610), TD San Diego type (MIM 270230) and TD Torrance-Luton type (MIM 151210)[1]. Radiological, and genetic findings of the four types are distinctive as summarized in Table 1[6,7].

TDI is caused by mutation of the Fibroblast Growth Factor Receptor 3 (FGFR3) gene that contains 19 exons, spanning 16.5 kb on 4p16.3[8]. The human *FGFR3* gene comprises 3 immunoglobulin-like domains, a transmembrane domain, and 2 intracytoplasmic tyrosine kinase domains[9]. All of the *FGFR3* mutations causing skeletal dysplasia are mostly segment-specific, with mutation in transmembrane segment leading to achondroplasia, in immunoglobulin-like domains for hypochondroplasia, and in tyrosine kinase domains for TD[8].

The authors present roentgenographic findings and demonstrate a *de novo* mutation in FGFR3 gene of a sporadic case of TDI.

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Case Report

The patient, a newly born male neonate, was the second child of a non-consanguineous Thai couple,

Table 1. Summary of distinctive features of the four types of platyspondylic lethal skeletal dysplasias (PLSD)

Type of PLSD	Distinctive radiologic phenotypes	Genetic defect
Thanatophoric dysplasia (TD) type I	long tubular bones particularly the femur: severely short and curved vertebral bodies: very flat craniosynostosis in 20% of the cases cloverleaf skull: uncommon and mild (2/58 cases)	mutations of *FGFR3* gene identified in most of the cases (91%)
TD type II	long tubular bones: severely short, but straight femur vertebral bodies: not as flat as in type I craniosynostosis: very common, in 93% of the cases cloverleaf skull: common and severe (53%)	mutations of *FGFR3* gene identified in most of the cases
PLSD (or TD) - San Diego type	long bones with metaphyseal spikes, better preserved growth plates	identical mutations of *FGFR3* as identified in TD type I
PLSD (or TD) - Torrance-Luton type	long bones with ragged metaphyses bowing of radius vertebral bodies: wafer-like apperance iliac bones: severe hypoplasia of lower iliac with medial bony projections	mutations of *COL2A1*, type II collagen gene

Adapted from reference[7]

a 32-year-old man and 31-year-old woman, both of whom were phenotypically normal. Family history was unremarkable. The patient was vaginally delivered at term following a pregnancy complicated by mild polyhydramnios. Disproportionate, severe dwarfism and respiratory insufficiency were noted at birth necessitating endotracheal intubation and ventilatory support that failed to save the patient. Postmortem examination revealed a birth weight of 2150 gm, length 38 cm, head circumference 35 cm, severe shortening of limbs with redundant skin folds, large head, frontal bossing, depressed nasal bridge, short and narrow thoracic cage, and a protuberant abdomen. No clover-leaf skull, cleft lip/palate, or other external malformation was observed. Postmortem radiographs disclosed severely short ribs, platyspondyly, hypoplastic iliac bones, marked shortening of long bones particularly both femora (Fig. 1A-E).

Genomic DNA was prepared from peripheral blood lymphocytes by use of the phenol-chloroform extraction[10]. PCR amplification of genomic DNA was performed in a total volume of 50 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 nM each of the four dNTP, 1.0 U of Taq polymerase (Boehringer Mannheim), and 0.5 mM of each PCR primer. The PCR conditions were as follows: initial denaturation at 95°C (5 min); 35 cycles of denaturation at 95°C (30 sec), annealing at 60°C (45 sec) and extension at 72°C (45 sec); and a final extension at 72°C (10 min). DMSO was also added to a final concentration of 10%. PCR using primer pairs, 5 - CGGCAGTGACGGTGGTGGTGA-3 (forward) and 5 - CCAAATCCTCACGCAACCC-3 (reverse) yielded a 341 basepair product containing the entire exon 7 and flanking intronic sequence of FGF3R[11]. Direct sequencing on the PCR amplicons was performed in an ABI 3100 DNA sequencer after purification with Wizard PCR prep kits, Promega, Madison, WI), and demonstrated a single nucleotide substitution from C to T at nucleotide 742 or c.742C > T (translation start site of NM_000531.3 is designated as +1) residing in the exon (Fig. 2), resulting in a change in codon 248 from arginine to cysteine, p.R248C. Analysis of the

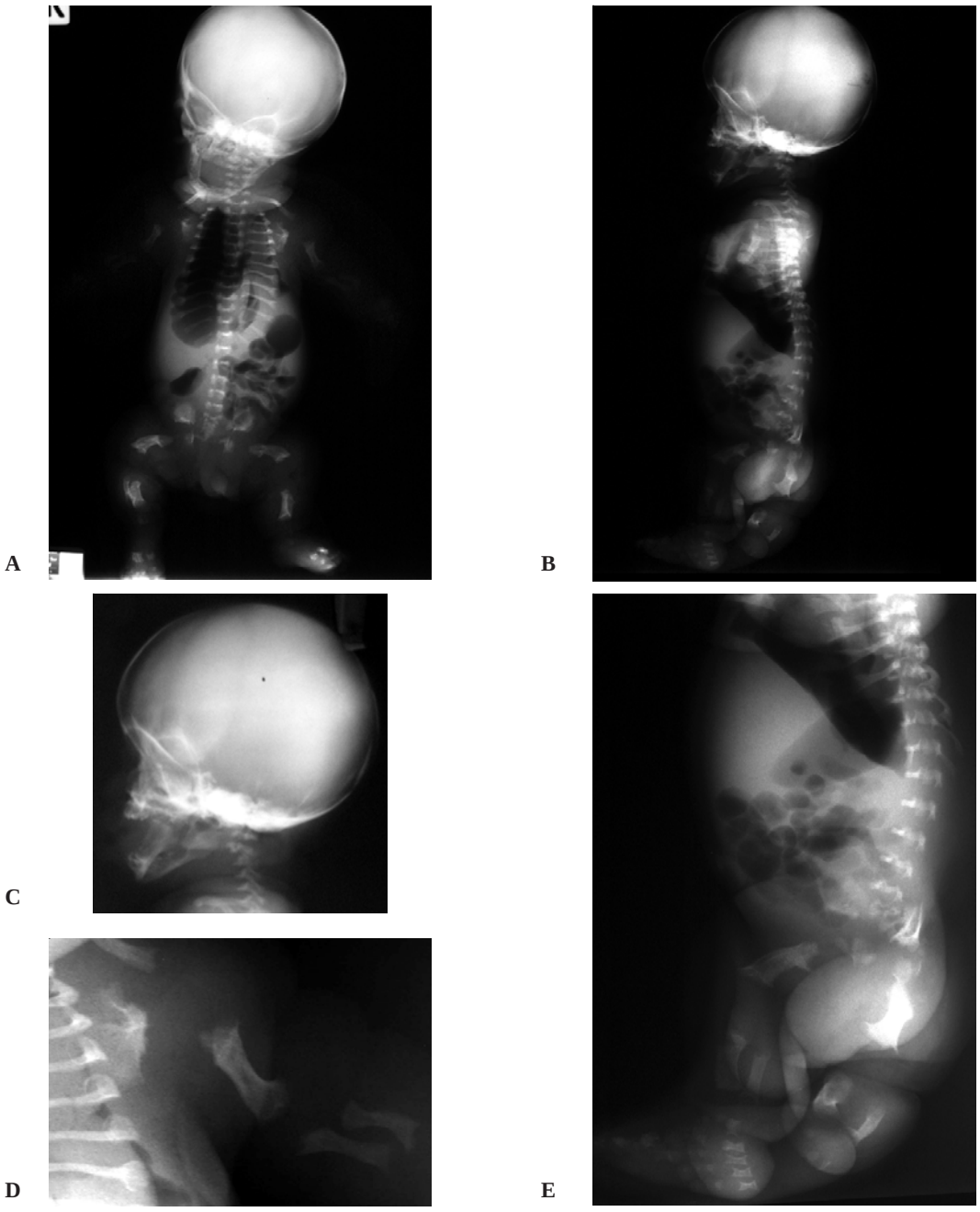

Fig. 1 Postmortem x-rays. A) and B) Babygram reveals bilateral pheumothorax with right sided tension pneumothorax secondary to vigorous resuscitation, disproportionately large cranium in relation to body length, narrowed thorax owing to short ribs. The scaplulae appear hypoplastic and deformed. The flat vertebral bodies are normal mineralized. There are marked shortening of the long tubular bones wild mild bowing of femur and humerus. The iliac bones are severely hypoplasia. C) Calvaria shows good mineralization, large fontanels, prominent forehead, depressed nasal bridge (arrows), small facial bone in relation to the calvaria with increased craniofacial ratio of 6:1 (normal ratio 4:1) D) The rib ends are wide and cupped (arrows). The humerus, and radius are short and bowing (arrows). E) The vertebral bodies are flat with a wide space between them. Phalanges and metatarsal bones are markedly short (arrows). The short metacarpals are not shown

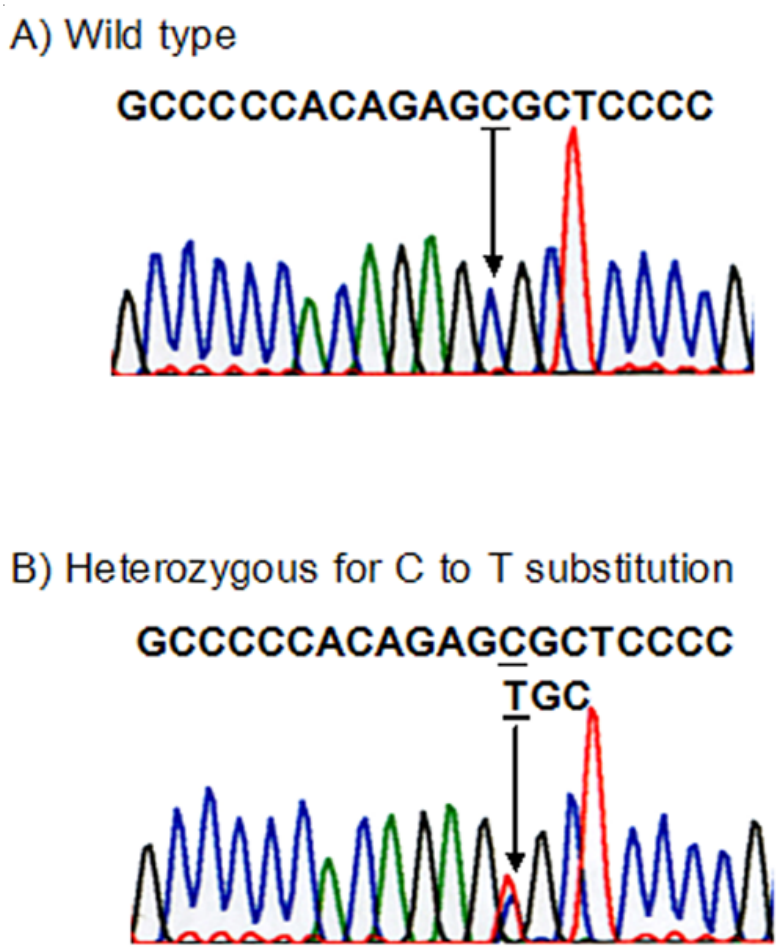

Fig. 2 Identification of p.R248C mutation in the patient. A) Sequenogram of control DNA showing C at nucleotide 742. B) Sequenogram of the patient with heterozygous C to T mutation at nucleotide 742, leading to change from arginine (CTG) to cysteine (TTG). Genetic analysis did not reveal any mutation in the parents' DNA (data not shown)

parents' DNA did not reveal the mutant allele, indicating a *de novo* mutation in the patient. Mutation screening in control individuals was not performed because the p.R248C is previously known as the most common mutation underlying TDI in various populations.

Discussion

Fibroblast growth factors are involved in a variety of activities including mutagenesis, angiogenesis, and wound healing[1]. This protein binds to cell surface receptor that is a member of the tyrosine kinase Fibroblast Growth Factor Receptor (FGFR) family[1]. Mutation of FGFR3 was first characterized in patients with achondroplasia[12], and later identified as a cause of hypochondroplasia[13], TDI and TDII[8]. To date, about 50 mutations have been described in TDI[1,7].

Using a mouse model, Naski et al have shown that mutations in the FGFR3 gene impair tyrosine phosphorylation and cell proliferation in TD, achondrogenesis and hypochondroplasia[14]. Chen et al demonstrated that heterozygous mice harboring G369C mutation in mouse FGFR3, equivalent to G375C mutation in human FGFR3, causes dwarfism with features mimicking human achondroplasia. Accordingly, homozygous mice are more severely affected than heterozygous animals[15].

The present finding supports previous observation that the R248C is the most common (66%) genetic alteration described in TDI world-wide[1]. There has been a previous report of thanatophoric dysplasia from Thailand[16], however, this is the first time a mutation of *FGFR3* was characterized in a Thai TD patient.

Although the present finding is not novel, it addresses the significance of determining the type of lethal skeletal dysplasia, which can lead to precise genetic counseling, risk estimation, and feasibility of prenatal diagnosis.

Interestingly, the common mutations of TDI (p.R248C) and hypochondroplasia (p.N540K) were recently reported in two patients, one of each, with achondroplasia phenotype[17]. The reason leading to such a discrepancy between genotype and phenotype is unclear[17]. This may alert clinicians to an appropriate medical and genetic counseling by using a combination of both molecular and clinical data.

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ลักษณะผิดปกติทางเอกซเรย์และการกลายพันธุ์ของยีน *FGFR3* ในทารกไทย 1 ราย ที่เป็นโรคตะนาโทไฟลิกดิสเพลเซีย

ดวงฤดี วัฒนศิริชัยกุล, ดุษฎี เจริญพิภพ

ตะนาโทไฟลิกดิสเพลเซีย เป็นโรคเตี้ยแคระพันธุกรรมชนิดรุนแรงถึงตาย (lethal skeletal dysplasia) ชนิดหนึ่งและเป็นชนิดที่พบได้บ่อยที่สุด อุบัติการณ์ประมาณ 1 ใน 20,000 ทารกเกิดมีชีพ โรคนี้มีความคล้ายคลึงกับโรคเตี้ยแคระพันธุกรรมชนิดรุนแรงถึงตายชนิดอื่น จึงมีความจำเป็นที่แพทย์จะต้องให้การวินิจฉัยที่ถูกต้อง ทั้งนี้เพื่อนำไปสู่การให้คำแนะนำเรื่องโรค อัตรารอดชีวิต และการให้คำปรึกษาทางพันธุกรรมที่แม่นยำ ผู้นิพนธ์รายงานผู้ป่วยทารกแรกเกิดชาย 1 ราย ซึ่งมีลักษณะทางคลินิกและเอกซเรย์เข้าได้กับโรคตะนาโทไฟลิกดิสเพลเซีย คือ แขนขาสั้นมากและผิวหนังมีรอยย่นเป็นปล้อง ๆ ศีรษะโต หน้าผากโหนก ดั้งจมูกแบน ทรวงอกแคบ และทารกหายใจลำบากรุนแรง เอกซเรย์พบลักษณะกระดูกซี่โครงสั้น กระดูกส้นหลังแบน กระดูกสะโพกเล็กมาก กระดูกแขนขาสั้นมาก และมีลักษณะโค้ง โดยเฉพาะอย่างยิ่งส่วนของกระดูกขาส่วนต้นสั้นมากและโค้งงอเล็กน้อย และมีความโปร่งเพิ่มขึ้นที่ส่วนต้นของกระดูกฟีเมอร์ จากการวิเคราะห์ลำดับเบสของยีน fibroblast growth factor receptor 3 (FGFR3) พบการกลายพันธุ์ที่ทำให้เกิดการเปลี่ยนแปลงของกรดอะมิโนที่ตำแหน่ง 248 จากอาร์จินีนเป็นซิสเตอีน (p.R248C)