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Case Report

Rapid detection of K650E mutation in *FGFR3* using uncultured amniocytes in a pregnancy affected with fetal cloverleaf skull, occipital pseudoencephalocele, ventriculomegaly, straight short femurs, and thanatophoric dysplasia type II

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Abstract

Objective: To present the ultrasound and molecular genetic diagnosis of thanatophoric dysplasia type II (TD2).
Case Report: A 35-year-old, primigravid woman was referred to our institution for genetic counseling and amniocentesis at 19 weeks of gestation because of advanced maternal age and sonographic abnormalities in the fetus. The prenatal ultrasound showed short straight femurs, prominent forehead, narrow chest, skin edema, short limbs, and cloverleaf skull consistent with the diagnosis of TD2. Amniocentesis revealed a karyotype of 46,XX. DNA testing for the *FGFR3* gene using uncultured amniocytes revealed a heterozygous c.1948A>G, AAG>GAG transversion leading to a p.Lys650Glu(K650E) mutation in the *FGFR3* gene. A prenatal ultrasound at 21 weeks of gestation showed ventriculomegaly, cloverleaf skull, straight femurs, micromelia, narrow chest, and pseudoencephalocele with a bulging occipital bone mimicking encephalocele. The pregnancy was subsequently terminated, and a 480-g malformed fetus was delivered with macrocephaly, depressed nasal bridge, short upturned nasal tip, hypoplastic midface, frontal bossing, short digits, trident-shaped hands, short limbs, cloverleaf skull, narrow chest, brachydactyly, nuchal edema, and bulging occipital bone.
Conclusion: A prenatal diagnosis of cloverleaf skull, short limbs, straight femurs, and occipital pseudoencephalocele should include a differential diagnosis of TD2. A molecular analysis of *FGFR3* using uncultured amniocytes is useful for the rapid confirmation of TD2 at prenatal diagnosis.

Keywords: encephalocele; *FGFR3*; prenatal diagnosis; pseudoencephalocele; thanatophoric dysplasia type II

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Introduction

Thanatophoric dysplasia (TD) is one of the most common lethal skeletal dysplasias in fetuses and neonates, and has an estimated prevalence rate of 0.2–0.5/10,000 births [1]. TD has been subdivided into two types according to the clinical features [2–4]. TD type I (TD1) (OMIM 187600) is characterized by curved femurs, short limbs, and narrow chest with or without cloverleaf skull. TD type II (TD2) (OMIM 187601) is characterized by straight femurs, short limbs, narrow chest, and uniform presence of severe cloverleaf skull. Both TD1 and TD2 can be associated with additional findings of macrocephaly, distinctive facial features, redundant skin fold, brachydactyly, and hypotonia [5]. TD1 and TD2 are caused by gain-of-function mutations in the fibroblast growth factor receptor 3 (*FGFR3*) gene (OMIM 134934), which is mapped to 4p16.3 [6–11].

With the advent of prenatal ultrasound and molecular technology, TD1 and TD2 can be diagnosed prenatally [9,10,12–16]. Here, we present our experience with prenatal ultrasound and molecular genetic diagnosis of TD2 using uncultured amniocytes at amniocentesis in the 2nd trimester.

Case report

A 35-year-old, primigravid woman was referred to our institution for genetic counseling and amniocentesis at 19 weeks of gestation because of advanced maternal age and sonographic abnormalities in the fetus. Her husband was 37 years of age. She and her husband were both healthy and unrelated, and there was no family history of congenital malformations. A prenatal ultrasound at 18 weeks of gestation showed a fetus with a biparietal diameter of 4.2 cm (18.87 weeks), an abdominal circumference of 13.6 cm (19.05 weeks), a femur length of 1.82 cm (15.39 weeks), short straight femurs, a prominent forehead, a narrow chest, and skin edema (Fig. 1). A level II ultrasound at 19 weeks of gestation revealed short limbs, a narrow thoracic cage, and cloverleaf skull consistent with the diagnosis of TD2. Amniocentesis revealed a karyotype of 46,XX. The array comparative genomic hybridization analysis revealed no genomic imbalance. A DNA testing for the *FGFR3* gene using uncultured amniocytes revealed a heterozygous c.1948A>G, AAG>GAG transversion leading to a p.Lys650Glu(K650E) mutation in the *FGFR3* gene (Fig. 2). A prenatal ultrasound at 21 weeks of gestation showed a biparietal diameter and an abdominal circumference equivalent to 21 weeks but a short femur length of 1.6 cm equivalent to 14 weeks, ventriculomegaly, cloverleaf skull, straight femurs, micromelia, narrow chest, and pseudoencephalocele with a bulging occipital bone mimicking encephalocele (Fig. 3). The pregnancy was subsequently terminated, and a 480-g malformed fetus was delivered with macrocephaly, depressed nasal bridge, short upturned nasal tip, hypoplastic midface, frontal bossing, short digits, trident-shaped hands, short limbs, cloverleaf skull, narrow chest, brachydactyly, nuchal edema, and bulging occipital bone (Figs. 4–6). X-ray showed cloverleaf skull,

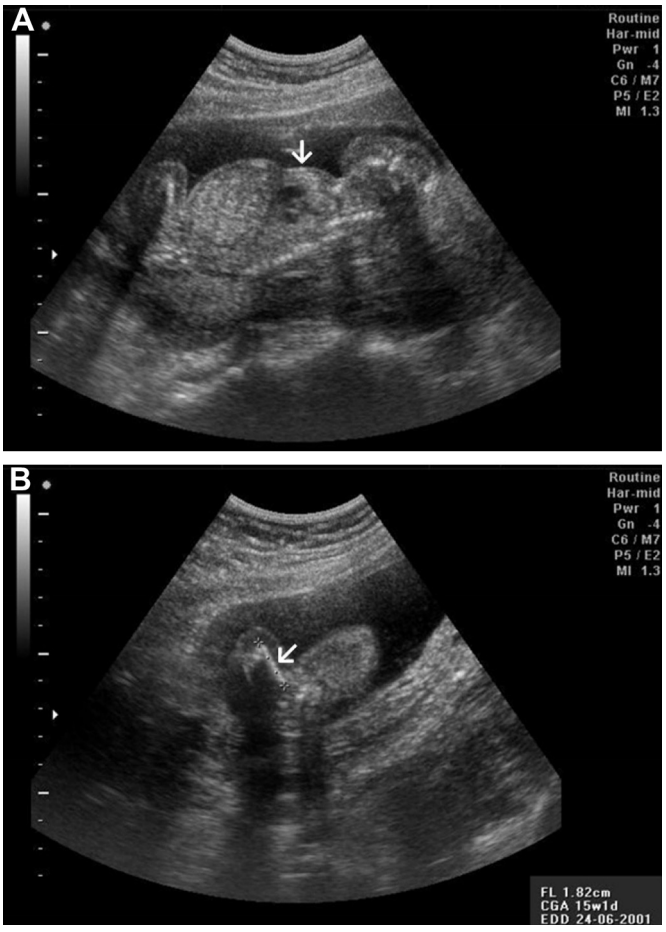

Fig. 1. Prenatal ultrasound at 18 weeks of gestation shows (A) a narrow thorax and (B) a straight femur.

pseudoencephalocele, small narrow thorax with horizontal ribs, platyspondyly, straight femurs, and micromelia (Fig. 7).

Discussion

The present case had a K650E mutation in *FGFR3* and was affected with TD2. In addition to TD1 and TD2, mutations of *FGFR3* have been associated with various skeletal dysplasias such as achondroplasia (OMIM 100800), hypochondroplasia

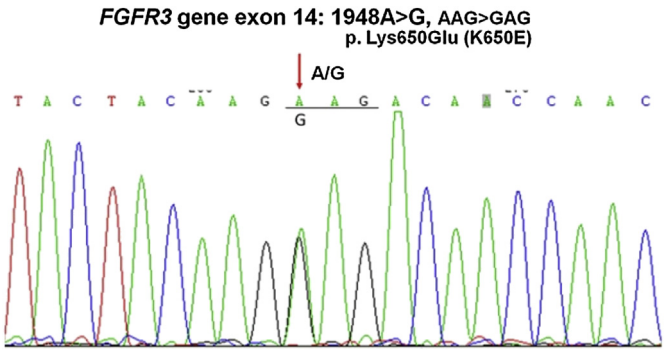

Fig. 2. DNA testing of *FGFR3* shows a heterozygous c.1948A>G, AAG>GAG transversion leading to a p.Lys650Glu(K650E) mutation, involving lysine-to-glutamine substitution at codon 650 of FGFR3.

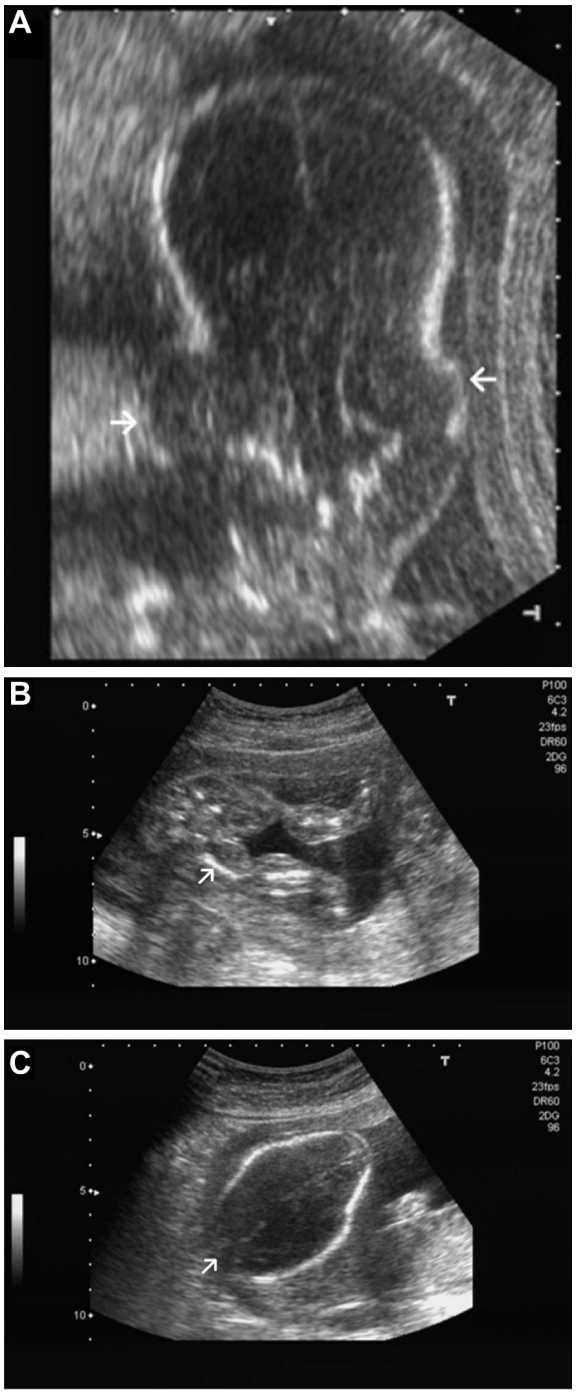

Fig. 3. Prenatal ultrasound at 21 weeks of gestation shows (A) cloverleaf skull with bulging parietal bone and temporal bones (arrows), (B) straight femur, and (C) occipital pseudoencephalocele.

(OMIM 146000), Muenke syndrome (OMIM 602849), Cruzon syndrome with acanthosis nigricans (OMIM 612247), lacrimo-auriculo-dento-digital syndrome (OMIM 149730), and CATSHL (camptodactyly, tall stature, scoliosis, and hearing loss) syndrome (OMIM 610474). FGFR3, a negative regulator of bone growth, regulates endochondral ossification by limiting osteogenesis [17]. Gain-of-function mutations in FGFR3 activate the negative growth control exerted by FGFR3 kinase [17], enhance osteoclastogenesis and bone resorption, inhibit bone marrow stromal cell proliferation, and promote osteogenic differentiation but block their mineralization [18]. TD1 has been associated with at least 13 distinct gain-of-function *FGFR3* mutations such as R248C (most common), S249C, G370C, S371C, Y373C, K650M, J807L, J807G, J807R(c.2419T>C), J807R(c.2419T>A), J807C(c2421A>T), J807C(c.2421A>C), and J807W [5]. TD2 has been associated with only one gain-of-function *FGFR3* mutation of K650E [5,8,10]. TD1 occurs more frequently than TD2. In a review of 91 cases of TD, Wilcox et al [10] reported 74 cases (81.3%) of TD1 and 17 cases (18.7%) of TD2. An advanced paternal age effect has been associated with *FGFR3* mutations in achondroplasia, hypochondroplasia, and TD [19]. The majority of TD patients have a *de novo FGFR3* mutation [5]. Hyland et al [20] reported a very unusual case of somatic and germline mosaicism for R248C mutation in *FGFR3* in a woman manifesting skeletal dysplasia distinct from TD and giving birth to a fetus with lethal TD.

Various hypotheses have been raised to explain the adverse effect of K650E mutation in *FGFR3* on skeletal development. Su et al [21] showed that TD2 FGFR3 activates the transcription factor Stat1 and uses Stat1 as a mediator of growth retardation in bone development. Bellus et al [22] found that FGFR3 Lys650 codon is located within the critical region of

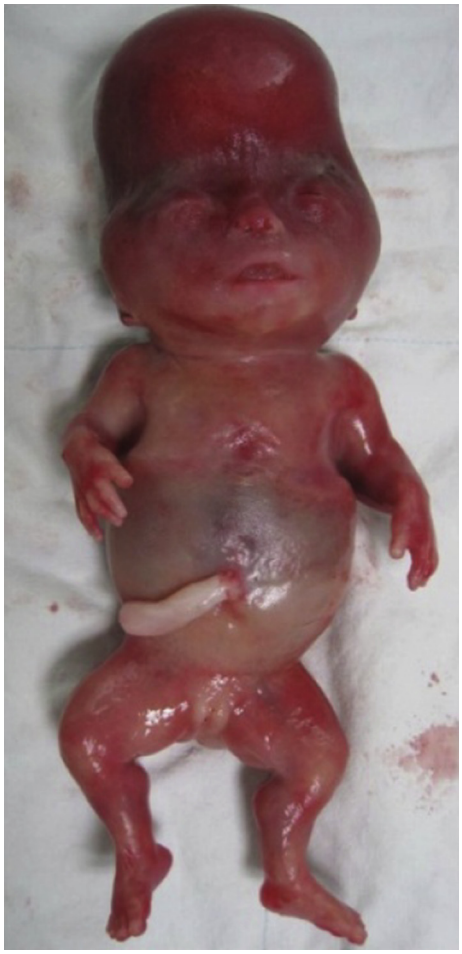

Fig. 4. Whole body view of the fetus at birth at 21 weeks of gestation.

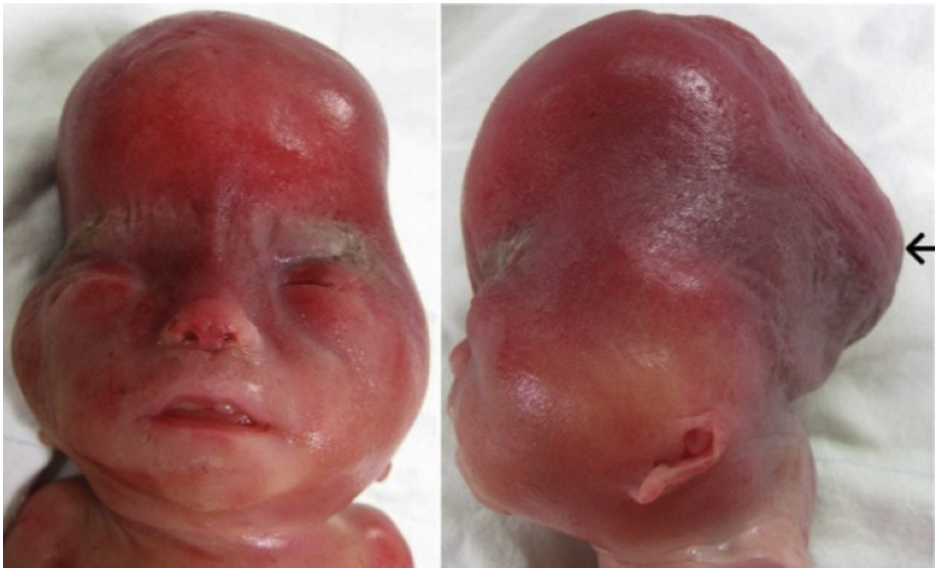

Fig. 5. Craniofacial appearance of the fetus at birth. The arrow indicates occipital pseudoencephalocele.

the tyrosine kinase-domain activation loop and two missense mutations of A1948G (Lys650Glu) and A1949T (Lys650Met), result in TD2, and SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans) and TD1, respectively, by constitutive activation of the FGFR3 tyrosine kinase. Lievens and Liboi [23] hypothesized that the K650E mutation in *FGFR3* interferes with the complete maturation of FGFR3, and the immature TD2-FGFR3 protein activates Stat1. Guo et al [24] provided evidence that the gain-of-function of *FGFR3* in TD2 is mediated by FGFR3's kinase activity and involves the constitutive induction and activation of Spry2, and activated Spry2 interferes with c-Cbl-mediated ubiquitination of FGFR3. Shung et al [25] suggested that the mutant FGFR3 in TD2 causes dysregulation of Sox9 and β-catenin levels and activity in growth plate chondrocytes.

The peculiar aspect of the present case is the association of cloverleaf skull with occipital pseudoencephalocele. Jap-A-Joe et al [26] first reported TD2 with cloverleaf skull and concomitant parietal meningoencephalocele in an anatomical specimen. Li et al [27] reported a prenatal diagnosis of TD2 with a large occipital encephalocele in a 2nd trimester fetus. Martínez-Frías et al [28] reported TD2 with encephalocele and holoprosencephaly in a newborn infant and suggested that the so-called encephalocele in association with TD2 is, in fact, pseudoencephalocele secondary to intracranial pressure generated by hydrocephalus and severe cranial skeletal dysplasia.

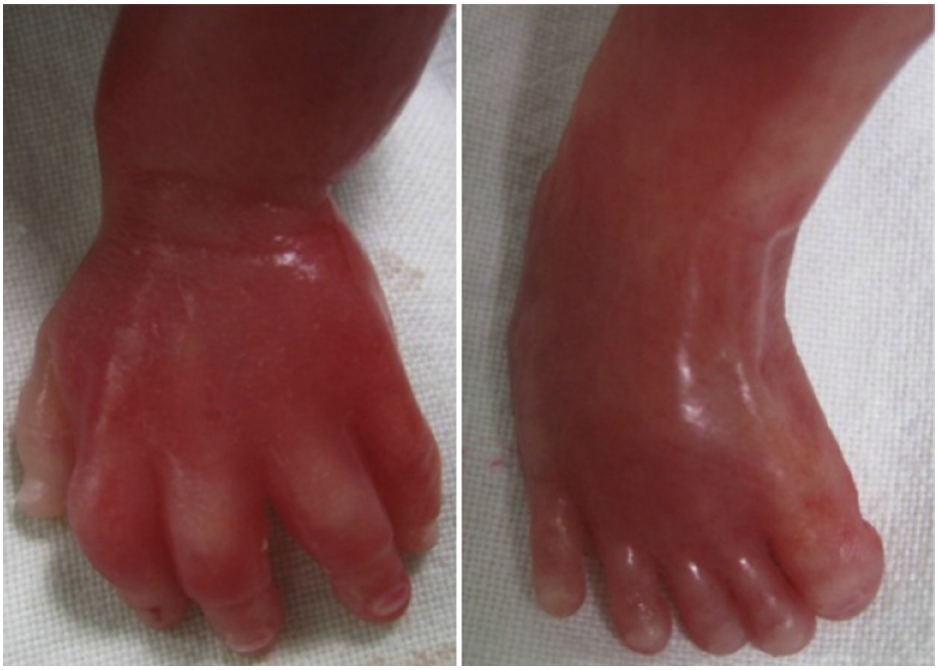

Fig. 6. Trident hands and brachydactyly of the digits.

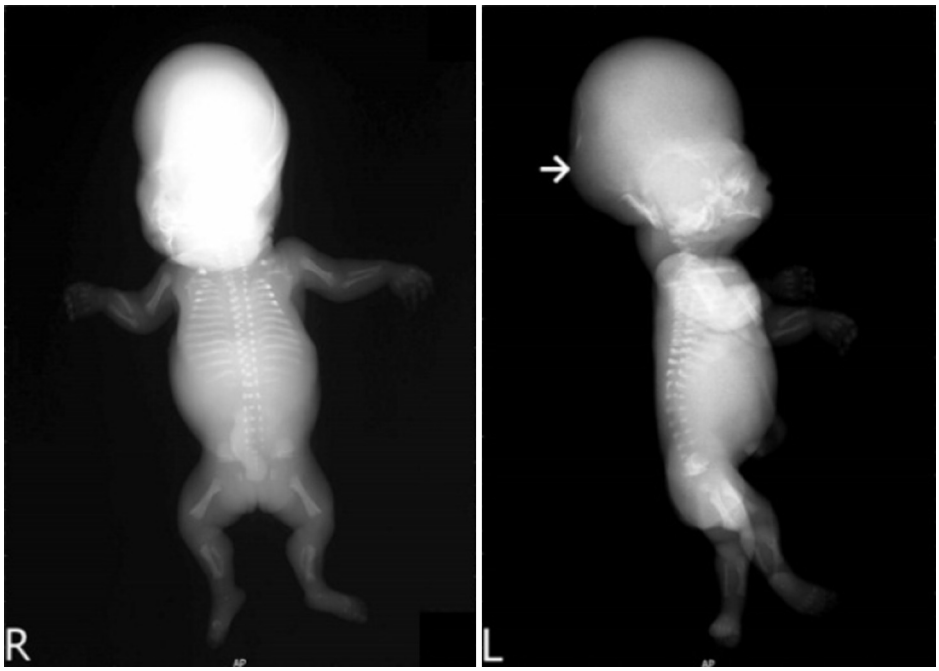

Fig. 7. X-ray of the fetus at 21 weeks of gestation. The arrow indicates occipital pseudoencephalocele.

With the sophisticated sonographic observations of cloverleaf skull, narrow chest, short limbs, straight femurs, and central nervous system abnormalities, a prenatal sonographic diagnosis of TD2 can be achieved in the 2nd trimester. Recently, it has been reported that a noninvasive prenatal diagnosis of TD2 using cell-free fetal DNA in maternal plasma by restriction enzyme analysis is possible [29]. However, rapid detection of the *FGFR3* mutation in suspicious TD2 on prenatal ultrasound using common mutation sequencing on uncultured amniocytes is easier and less expensive than noninvasive prenatal diagnosis using cell-free fetal DNA assays for TD. We suggest that molecular analysis of *FGFR3* using uncultured amniocytes is useful for the rapid confirmation of TD2 at prenatal diagnosis.

Acknowledgments

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