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Diagnosis of chromosomal abnormalities in a patient with thanatophoric dysplasia (TD) type I: The first report describing an important association between cytogenetic findings and TD

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Summary

Background: Thanatophoric dysplasia (TD) is the most lethal and most severe type of dysplasia. It has distinct features, the most important of which is short tubular bones and short ribs with platyspondyly, allowing a precise radiologic and prenatal ultrasonographic diagnosis. It has been reported to be caused by mutations in the FGFR3 gene, but exactly how cytogenetic abnormalities might lead to TD is unclear.

Case Report: We report a case of TD with different prenatal sonographic features compatible with the classification of type I. In the result of cytogenetic examination, we found *de novo* CAs in 28% of cells analyzed from the affected infant; 75% of the abnormalities were numerical, and of those, 25% were structural aberrations; 21% of cells revealed predominantly numerical aberrations. Monosomy 18, 21 and 22 was observed in 4% of cells, monosomy 20 in 2%, and monosomy 7, 8, 14, 17 and 19 in 1%. Structural changes were observed in 7% of cells. Conclusions: It appears that these chromosomes may be preferentially involved in and important for TD development.

key words: thanatophoric dysplasia • type I • chromosomal aberrations • monosomy

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Background

TD was first described in 1967 by Maroteaux et al. [1]. It is one of the most common forms of platyspondylic lethal skeletal dysplasias, and is a sporadic neonatal chondrodysplasia causing severe shortening of the limbs with macrocephaly, narrow thorax and short ribs. TD is inherited in an autosomal dominant manner; the majority of probands have a *de novo* mutation. The 2 clinical subtypes have been recently ascribed to mutations in the fibroblast growth factor receptor 3 (*FGFR3*) gene [2,3] which also accounts for achondroplasia [4,5] and hypochondroplasia [6]. TD type I (TD1) is characterized by short limbs, a narrow thoracic cage and curved femora, with or without a cloverleaf skull; whereas TD type II (TD2) is characterized by short limbs, a narrow thoracic cage, straight femora and a cloverleaf skull (12–14). Although lethal TD1 and TD2 cases are sporadic, in an epidemiological analysis TD was associated with increased paternal age and achondroplasia [7,8], suggesting that TD may be caused by autosomal dominant mutations. This was further confirmed by molecular genetics studies. But, exactly how cytogenetic findings might lead to TD is unclear, and there was no evidence of linkage to the cytogenetics in the patients affected with TD. Cytogenetic analysis is an important step in understanding the genetic background of TD, and may provide a valuable clue to the identification of target *loci* and aid in the successful search for major genes. Here, we report the first case of TD in which an apparent *de novo* cytogenetic finding was present in the affected infant. This case is the first report of a TD type I confirmed by both clinical and cytogenetic findings in Turkey.

Case Report

The patient was born by caesarean section at the gestational age of 38 weeks in the obstetrics and gynecology clinic of Adana Numune Training and Research Hospital, Adana, Turkey. The patient was the fifth living baby from the fifth gestation of the mother. The patient's father was 40 years old and the mother was 32 years old; they were second degree consanguineous. It was learned that there was no follow-up of the mother during pregnancy. Other siblings were healthy. Apgar score at the first minute was 1. The newborn infant was resuscitated for post-natal respiratory distress, cyanosis and bradycardia. Post-resuscitation fifth minute Apgar score was 3. The newborn infant was intubated because of the absence of adequate spontaneous respiration. We detected that temperature was 36°C, heart pulse was 100 beats per minute, birth weight was 2900 gr, length was 42 cm and head circumference was 37 cm. Other physical examination findings of the newborn infant were a large cranium, a large anterior fontanel, a depressed nasal bridge and a small face. The baby was relatively macrocephalic. We detected atypical facial findings of the newborn infant (Figure 1) – low-set ears, flat face, short neck and small mouth. The frontal and mandibular protrusions were evident. The lower and upper extremities were short and the thorax was narrow. There were coarse crackles in the lungs. Heart rhythm was tachycardic. There was no organomegaly. No additional sounds and no murmur were heard. Newborn reflexes were absent. According to the first examination, we thought that the patient had achondroplasia, but we were decided on thanatophoric dysplasia due to the narrowness of the patient's rib cage.

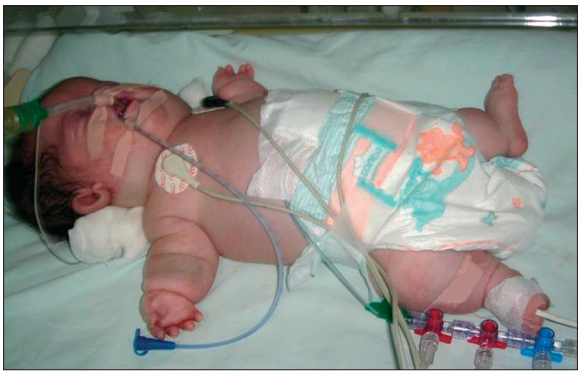

Figure 1. The whole body view of new-born infant with very short limbs, narrow chest, exaggerated the protuberance of abdomen and large head.

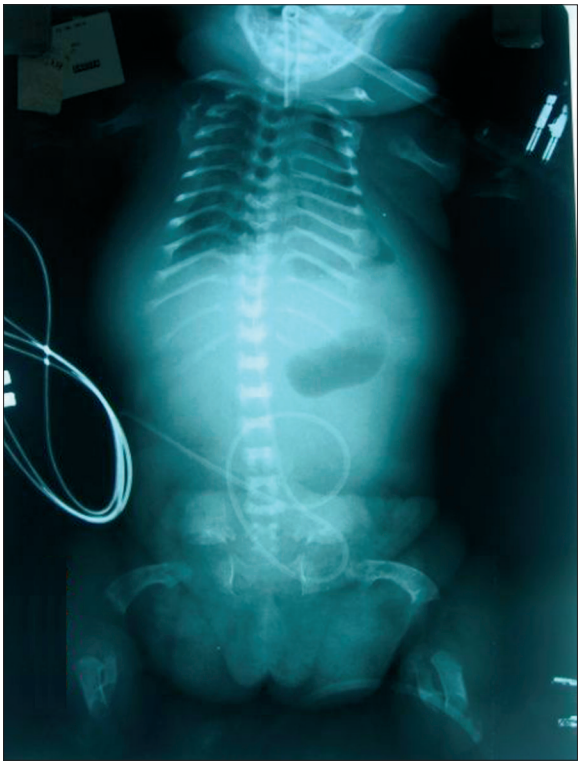

Figure 2. Anteroposterior radiograph of new-born infant with medial acetabula spurs, hypo plastic iliac bones, bowed femora with rounded protrusion of proximal femur, hypo plastic thorax and wafer-thin vertebral bodies.

Skeletal radiography showed the presence of medial acetabula spurs, hypoplastic iliac bones, bowed femora with rounded protrusion of the proximal femur, hypoplastic thorax and wafer-thin vertebral bodies (Figure 2). Our case was diagnosed as type 1 thanatophoric dysplasia because of the bowed and short femur, whereas patients with type 2 thanatophoric dysplasia have longer and flatter femurs. Ultrasonographic views of the abdomen and cranium were performed to search for additional anomalies, but these were normal. We found no pathology in routine blood tests and biochemical analysis. The newborn infant was placed in a mechanical ventilator and died after 33 days of hospitalization.

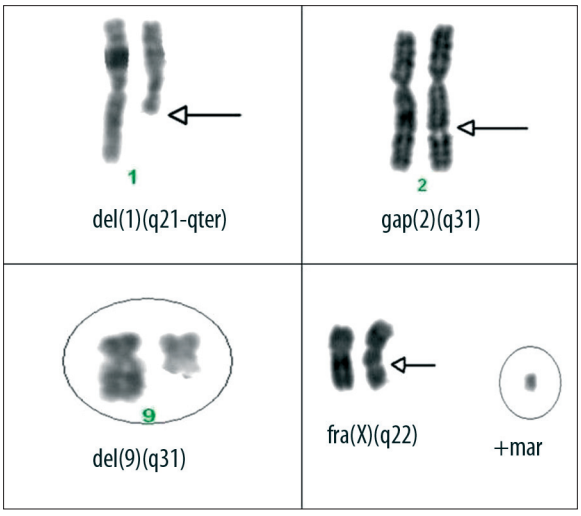

Figure 3. Partial metaphase figures showing chromosomal abnormalities.

Cytogenetic analysis

Peripheral blood was taken from the subject (patient) for culture studies. Each sample was examined for cytogenetic anomalies in our genetics laboratory of the Department of Medical Biology, Faculty of Medicine, Çukurova University, Adana, Turkey. A 0.3 ml sample of blood was incubated at 37°C for 72 h in 2 media (RPMI-1640). Standard cytogenetic techniques were used for harvesting and slide preparation (Figure 3). The slides were first stained only with Giemsa before the examination in order to avoid missing any gaps. For detailed analysis of the fragile sites, some slides were prepared by GTG banding, and 100 metaphases were scored. The classification of fragile sites was done according to the nomenclature established in human gene mapping HGM11.

Discussion

We observed a significantly greater number of single-cell numerical aberrations in 100 cells (Table 1). Chromosomal aberrations (numerical and structural abnormalities) (CAs) were found in 28 (28%) abnormal cells of 100 cells analyzed. A total of 18 cells revealed predominantly numerical aberrations. Of the abnormalities, 75% were numerical aberrations, and only 25% were structural (Table 1). Numerical aberrations consisted of aneuploidies (monosomies) in various chromosomes (20%). Monosomy 18x4, 20x2, 21x4, 22x4, 7, 8, 17, 14, 19, +p? and +mar were found to be the numerical abnormalities. Interestingly, monosomy 18, 21 and 22 was observed the 4% in all cells; monosomy 20 in 2 cells (2%); and monosomy 7, 8, 14, 17, 19, and trisomy +p? and +mar in 1 cell (1%). Structural changes were observed in 7% of 100 cells. Structural aberrations usually consisted of deletion, fragility and gaps in chromosomes 1, 2, 9 and X in 1 cell. The gap(2q31) were observed in 4 cells (8%), fra(Xq22), del(1)(q21-qter) and del(9)(q13-qter) in 1 cell.

Skeletal dysplasias are a heterogeneous group of conditions associated with abnormalities in size, shape and density of the skeleton [9]. Most are heritable and many have elaborate patterns of genetic transmission. TD results from dominant

Table 1. Cytogenetics results of patient.

Karyotypes
46,XX,gap(2)(q31) (3/100); 46,XX,del(1)(q21-qter) (1/100); 46, XX,-19,+p?, del(9)(q13) (1/100); 47,XX,+mar, fra(X)(q22) (1/100); 44,XX,-14,-22 (1/100); 44,XX,-18,-21 (1/100); 45,XX,-7 (1/100); 45,XX,-8 (1/100); 45,XX,-17 (1/100); 45,XX, -18 (2/100); 45,XX, -20 (2/100); 45,XX,-21 (3/100); 45,XX,-22 (3/100); 45,X (1/100)

new mutations and is thought to have an incidence of approximately 1 in 37,000 live births [7], although many clinicians suspect that its true incidence is greater. In a typically lethal disorder such as TD, it is necessary to provide appropriate and knowledgeable counseling where survivability is unlikely but conceivable.

Wilcox et al. [10] described phenotypic variability among patients of TD with the same mutation in FGFR3 and suggested that the variable presence of radiological and histological findings in each TD might be due to genetic, environmental and stochastic factors, but we have not performed direct sequencing analysis of the FGFR3 gene. The identification of CAs may provide important leads in the search for the chromosomal location of major genes influencing TD. We report the first case of TD in which an apparent *de novo* CAs was present in 28% of cells analyzed of the affected infant; 21% of cells revealed predominantly numerical aberrations. Numerical aberrations consisted of aneuploidies in various chromosomes (monosomy 18, 20, 21, 22, 7, 8, 17, 14, 19, and trisomy +p?, +mar). Interestingly, monosomy 18, 21 and 22 was observed in approximately 20% of cells. Structural changes were observed in 7% of cells. This case could have been diagnosed as an unclassifiable TD (like skeletal dysplasia) if chromosome analysis were not performed.

An increased risk for mental retardation and congenital anomalies is known to be directly related to the presence of a *de novo* unbalanced structural rearrangement. Aneuploidy refers to losses and/or gains of individual chromosomes from the normal chromosome set. The resulting gene dosage imbalance has a noticeable effect on the phenotype. Autosomal monosomies are lethal and usually not compatible with normal extra-uterine life. At the same time, the chromosome loss might be a hallmark of existing disease, even subclinically, but aberrations of the other chromosomes could be more important in the progression of the disease. The crucial role of chromosomal imbalance in abnormal early human development is well established. Approximately, 50–60% of first-trimester spontaneous abortions have karyotype abnormalities – mainly numerical chromosomal changes. In the present study, we reported the monosomy of chromosomes 18, 21 and 22 as the most frequent genetic alterations in the affected infant, occurring in approximately 20% of cells, but it has not been previously reported that these monosomies contributed to the pathogenesis of TD. It appears that together these monosomies may be preferentially involved and important for our case. Undoubtedly, further studies are necessary to understand the role of these chromosome changes in TD.

In the present study, monosomy 18 is the most common cytogenetic abnormality in the affected infant. Monosomy

18p refers to a chromosomal disorder resulting from the absence of all or part of the short arm of chromosome 18. It was reported in 1963 by the French geneticist Jean de Grouchy [11,12] and was the first example of a partial monosomy compatible with life. The incidence is estimated to be about 1: 50,000 of live-born infants. In the commonest form of the disorder, the dysmorphic syndrome is very moderate and non-specific. Clinical features typically include mild-to-moderate mental retardation, short stature, round face with short protruding philtrum, palpebral ptosis and large ears with detached pinnae. Various skeletal deformities such as scoliosis and/or kyphosis, coxa vara, dislocation of the hip and feet deformities have been reported. In males, genital hypoplasia with small penis and cryptorchidism is occasionally observed. Cardiac malformations appeared to be relatively uncommon, observed in about 10% of patients, with situs abnormalities in some cases [13]. Various other malformations have been rarely or occasionally reported, often for deletion 18p secondary to an unbalanced translocation with a concomitant partial trisomy.

Many autosomal monosomies are presumed to end in arrested growth in the first few mitoses, prior even to the time of implantation, with possibly some proceeding to the stage of occult abortion. The single exception may be monosomy 21, although this has been questioned, with most earlier reports of monosomy 21 recently re-interpreted as being due to an unbalanced translocation involving chromosome 21. A fetus with the combination of TD and monosomy 21 has not been reported previously. Monosomy 21 mosaicism or full monosomy 21 is another very uncommon chromosomal abnormality in live-born infants. Only 5 patients have been described, of which 2 were mosaic [14–16]. On the other hand, a fetus with the combination of TD and trisomy 21 has recently been reported [17], and there have been 5 cases reported with both trisomy 21 and achondroplasia [18,19]. Chen et al. [18] reported craniofacial features typical of Down syndrome, but had skeletal findings characteristic of achondroplasia. Likewise, the fetus described here exhibited the craniofacial gestalt consistent with trisomy 21, including a flat facial profile with low placement of deformed small ears, epicanthic folds, and blepharophimosis, as well as loose folds on the posterior neck. Macrocephaly was observed as in TD, while frontal bossing was absent, as in trisomy 21. The short nose with depressed nasal bridge and midface hypoplasia in the present fetus are manifestations seen in both TD and trisomy 21. The body proportion and radiological findings in the present fetus were characteristic of TD. We have reported here an unusual case of TD associated with monosomy 21 mosaicism (4%). The same dysmorphic facial features and the multiple malformations in our case remarkably resemble cases of monosomy 21 described in the literature. As exemplified in this report, the possibility of concurrence of common disorders should always be considered.

Chromosome 22 contains a considerable number of uncharacterized disease genes (eg, familial schizophrenia susceptibility, glioblastoma and other types of astrocytoma, ependymoma, meningioma, schwannomatosis, pheochromocytoma, breast and colon cancer) [20]. In particular, imbalances of chromosomes that are recurrently involved in familial transmission from a normal mother to affected children will pose specific problems for genetic counselling, as illustrated by the monosomy 22. Here, we also often observed the monosomy 22 mosaicism (4%) in the infant. Monosomy of a chromosome 22 compatible with survival occurs rarely and there have been more than 100 cases with partial monosomy 22q [21–23]. Features reported in the literature include: significant delay in motor and mental development, hypotonia, large ears/low set ears, hyperextensible joints, cutaneous syndactyly, short neck, failure to thrive, epicanthal folds, hypertelorism, flat nasal bridge, club foot, hip dysplasia, cardiac anomalies, humoral immunodeficiency and gastroschisis. Saugier-Veber et al. [24] detected a 22q11 deletion in a patient with moderate MR, obesity, and facial dysmorphism. A significant delay in motor and mental development was observed by almost all. These suggest that our case had loss of chromosomes 21 and 22; this unstable chromosome is considered to have important candidate loci for TD.

Conclusions

The CAs in our patient is the first described in TD. There is a potential association between autosomal monosomies and TD phenotype in our case. It seems that monosomy 18, 21 and 22 mosaicisms are particularly likely to hold keys to the understanding of TD pathogenesis, and are also interesting candidates in the search for the gene *loci* of TD. These findings, therefore, may represent the initial step in identifying the gene responsible for this condition, and providing information to determine whether TD type 1 is genetically distinct entities, with overlapping features.

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