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Short Report

Tetraploidy in a 26-month-old girl (cytogenetic and molecular studies)

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Liveborn infants with tetraploidy are very rare in human pregnancies and usually die during the first days or months. Seven cases of liveborn infants with tetraploidy have previously been reported. Among them only two 92, XXXX infants survived for longer than 12 months. Here we report on the case of a 26-month-old girl with tetraploidy. The main clinical features of tetraploidy are facial dysmorphism, severely delayed growth and developmental delay.
On the basis of molecular studies we discuss the possible origin of the additional chromosome sets in our proband. To our knowledge, this infant is the first reported case of tetraploidy who lived up to 26 months.

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Tetraploidy, or four times the haploid number, is rare in human pregnancies. The majority of these pregnancies end in spontaneous abortion during the first trimester and therefore, liveborn infants with non-mosaic tetraploidy are very rare, and usually die during the first days or months after birth. To the best of our knowledge, five cases of liveborn infants with non-mosaic tetraploidy have previously been reported (1–5). Among them, only two infants had 92, XXXX karyotype (2,3) and one of them survived until the age of 22 months (3). We present a case of 26-month-old girl with tetraploidy, and, based on molecular studies, we discuss the possible origin of the additional chromosome sets in this patient.

Patients and methods

Case report

The female probant was the first child of healthy unrelated parents. There was no family history of congenital anomalies or chromosomal disorders. She was born at term with a birth weight of 1800 g and an Apgar score of 3.

The patient was referred for clinical and cytogenetic evaluation at the age of 11 months because of growth retardation and generalized muscle hypotonia. On clinical examination body weight was 4850 g and head circumference 41.5 cm. The head was brachicephalic and microcephalic. Facial dysmorphism was present with small and hollow eyeball without eyelashes and poor eyebrows, hypertelorism, high forehead and bird-like nose, micrognathia and scanty cartilage of auricles. The mouth was fish-like, with thin lips and a hypoplastic mandibula. There was a short neck and thin limbs with long fingers and syndactyly of third and fourth fingers on the right foot. Congenital heart disease (Tetralogy of Fallot) was also present in this patient (Fig. 1).

The proband was hospitalized for the second time at the age of 26 months because of cyanosis and fever. Generalized hypotonia was present: she was unable to sit and walk, had non-purposeful

movements of the hands and legs, and facial dysmorphism was significant.

Cytogenetic and fluorescent *in situ* hybridization (FISH) analyzes

Preparation of chromosomes from peripheral blood and skin fibroblasts cultures, using G banding, were performed by routine methods.FISH (6) with centromeric probes for chromosomes 1 (puC/1.77), 13/21 (pL1.26), 18 (pL1.84) and X (DXZα) were used as an additional method to banding techniques.

Late replication pattern analysis

In order to investigate late replication patterns of X chromosomes, the continous BrdU labeling during the final six hours of culture was used (7).

Molecular analysis

In order to determine the parental origin of teraploidy, we performed analysis of polymorphic DNA markers in DNA samples of the proband and both parents. Five highly polymorphic microsatellite markers for chromosomes 7 (D7S486), 14 (D14S53), 17 (D17S806), 18 (D18S53) and 21 (D21S270) were analyzed by Polymerase chain reaction (PCR) (8) method.

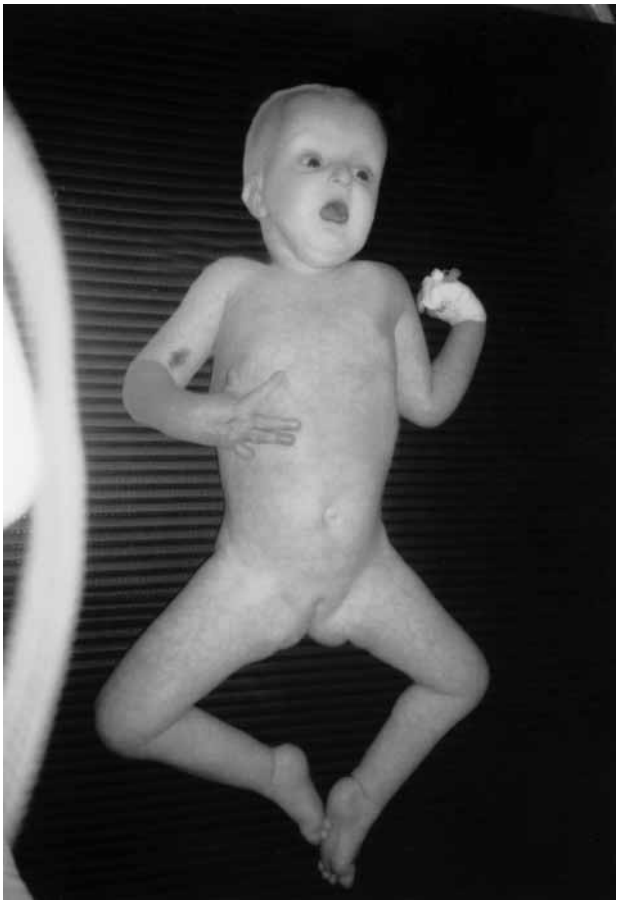

Fig. 1. Full body view of patient.

Results

A cytogenetic investigation performed on peripheral blood lymphocytes and skin fibroblasts showed the presence of a 92,XXXX karyotype (Fig. 2a). FISH method using centromeric probes for chromosomes 1, 13/21, 18 and X, confirmed tetraploidy. The result obtained with centromeric probe for chromosome 1 is presented in Fig. 2b. Parental chromosomes were normal. X inactivation studies showed the presence of two late replicating X chromosomes in 78% of analyzed cells (Fig. 2c).

In order to determine the origin of the additional chromosome sets in the proband, highly polymorphic microsatellite markers for chromosomes 7, 14, 17, 18 and 21 were used. Five different, informative markers were used to avoid bias due to potential recombination events in the meiotic division of the mother. For all tested microsatellites the child has showed four alleles, two maternal and two paternal, clearly indicating that two haploid sets of chromosomes originated from the mother and two from the father (Fig. 2d).

Discussion

The clinical manifestations observed in our proband are similar to previously reported cases of tetraploidy (1–5), except for the lack of myelomeningocele that is described in most patients with the same chromosomal anomaly. The longer survival period after the birth of our proband with 92, XXXX karyotype, in both lymphocytes and fibroblasts, may be related to the possible presence of mosaicism for cells with normal and tetraploid karyotype in other tissues (9, 10).

The results of DNA typing suggest that constitutive tetraploidy in our case cannot be the result of zygotic failure to complete the first division, in which case the proband could not have more than 2 alleles. By analogy with triploidy, mostly due to double fertilization of the egg (11), we might anticipate that ours was a case of a triple fertilization of the egg, but this possibility was also ruled out because the child would have only one allele from the mother and two alleles from father (of the three sperms, at least two have alleles which coincide).

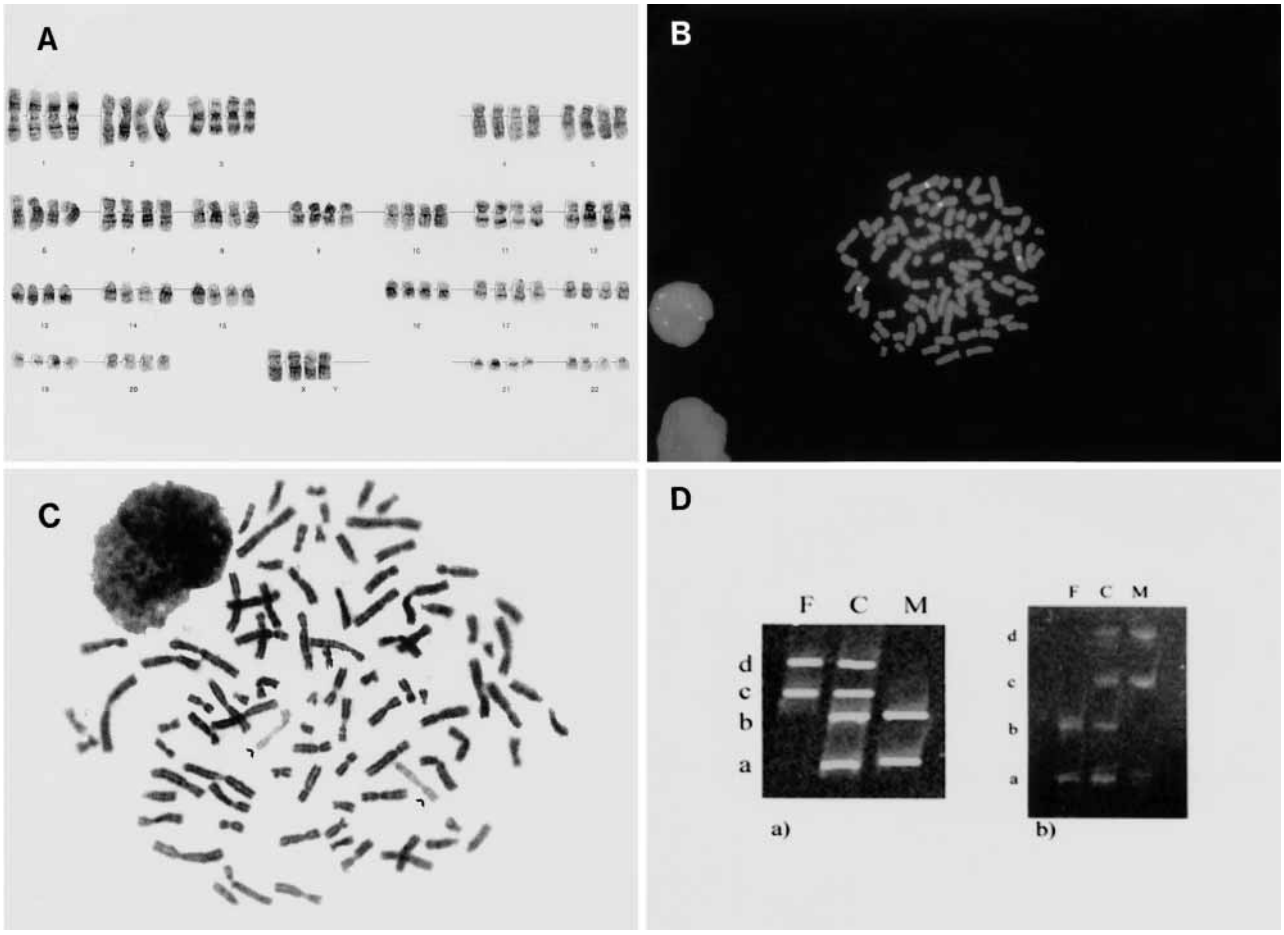

Fig. 2. (a) Karyotype of the patient with tetraploidy; *(b)* FISH with centromeric probe for chromosome 1 (note 4 signals); *(c)* Late replication pattern (RBG - R band by BrdU using Giemsa) in patient with tetraploidy. Arrows indicate two late replicating X chromosomes; *(d)* PCR amplified microsatellite markers: a) D14S53; b) D21S270. F: father; C: child; M: mother; a,b,c,d: four different alleles

Interestingly, the proband possessed two maternal and two paternal alleles. Diploid sperm could be excluded, because, if there were a failure in the father's first meiotic division, it would produce a 46,XY sperm (the proband is 92,XXXX). If second meiotic division failed, an XX sperm would be obtained, but the sperm would carry identical alleles on both sets of chromosomes, which is not found in our case. It can be concluded that two normal, haploid sperm participated in fertilization. Regarding the maternal sets of chromosomes, there are two possibilities. An abnormal first meiotic division could occur and produce a diploid egg, later fertilized with two haploid sperm. The second possibility is that two normal eggs were fertilized with two normal sperm and instead of developing into twins, the two zygotes fused giving one tetraploid organism.

In conclusion, it is important to realize, that although quite rare, tetraploid individuals can be liveborn, living at least till 26 months. This information is also relevant for pre-natal diagnosis and genetic counselling.

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