SHORT REPORTS

Second polar body incorporation into a blastomere results in 46,XX/69,XXX mixoploidy

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Abstract
A case of 46,XX/69,XXX mixoploidy is described. The patient had a normal 46,XX diploid karyotype in lymphocytes but a triploid 69,XXX cell line in most of her fibroblasts. In order to learn more about the underlying mechanism resulting in mixoploidy, we studied short tandem repeat polymorphisms (STRPs) in lymphocyte DNA of the patient's parents and in both lymphocyte and fibroblast DNA of the proband. The findings showed maternal origin of the supernumerary chromosome complement and are best explained by second polar body incorporation into a blastomere. (J Med Genet 1993;30:597-600)

Triploty is common in man and occurs in approximately 1% of all conceptions.1 Most triploid pregnancies result in early spontaneous abortion. A small proportion survives to term, only to die in the neonatal period. Investigations of chromosomal heteromorphisms in triploid abortuses and parental lymphocytes suggest paternal origin of the additional haploid set in 62 to 77%, and maternal origin in 23 to 38%.2,3 Paternal origin (diandry) may occur through fertilisation of a haploid ovum by two haploid spermatozoa (dispermy) or by a diploid sperm. Conversely, maternal origin (digyny) may be explained by a haploid sperm fertilising either a diploid ovum or two fused haploid ova.

In contrast to the relatively common occurrence of a complete triploidy, diploid–triploid mosaicism (mixoploidy) is exceedingly rare.4–10 In mixoploidy, lymphocytes usually have a normal diploid chromosome complement, while the triploid cell line is detectable in other tissues, such as fibroblasts.11 Mixoploid conceptions are generally much more viable than complete triploid ones owing to less severe developmental anomalies. Of the more than 20 cases of mixoploidy published, the oldest was 21 years of age.12 The origin of mixoploidy is not understood. According to Niebuhr,13 several mechanisms are possible including errors at the time of fertilisation, at the cleavage divisions, and at a later cell division of the early embryo. In addition, chimera formation of diploid and triploid zygotes may result in mixoploidy.

Here we describe mixoploidy in a newborn triplet female. In order to learn more about the underlying mechanism resulting in mixoploidy, we studied the parental origin of the extra haploid chromosome complement using highly informative short tandem repeat polymorphisms (STRPs).

Case report
The proband was the third of female triplets born at 35 weeks of gestation weighing 1580 g to a 31 year old gravida 2 para 1 white woman. The pregnancy was induced by clomiphene because of a previous history of infertility. Serial prenatal ultrasonography showed cerebral ventriculomegaly in this fetus, but no other abnormalities. Each triplet had a separate gestational sac and placenta. Delivery was performed by a caesarian section. The family history was unremarkable. The two other triplets were not dysmorphic.

The proband was small for gestational age and manifested several dysmorphic features. Craniofacial findings included a broad forehead, frontal alopecia, small anterior and posterior fontanelles, a short upturned nose with depressed nasal bridge, incomplete helical folds, and micrognathia (fig 1A). The upper extremities showed bilateral camptodactyly with digitalised thumbs, incomplete palmar creases, and cutaneous syndactyly of the left third and fourth fingers (fig 1B). The big toes were short bilaterally, and a leg length discrepancy of 1 cm was present. The infant was diffusely hypotonic.

Echocardiography showed no abnormalities. Abdominal ultrasonography showed prominent renal lobar architecture bilaterally, but no hydronephrosis. Cranial ultrasonography indicated agenesia of the corpus callosum and mild bilateral ventriculomegaly, findings confirmed by cranial CT. An electroencephalogram was normal, as was an ophthalmological examination. Brainstem auditory evoked responses showed moderate bilateral sensorineural hearing loss.

During infancy, the infant developed intracranial myoclonic seizures requiring anticonvulsant therapy. Her development progressed initially, but regressed globally after the onset of seizures. She developed precocious puberty with the onset of menarche at the age of 6 months, associated with raised FH and LH levels. Repeat brainstem auditory evoked responses showed moderate sensorineural hear-
Priming loss on the right and normal left responses. At 9 months magnetic resonance imaging of the brain showed absence of the septum pellucidum, but an intact corpus callosum. At 10 months a barium swallow study showed malrotation of the small bowel and a Ladd's operation was performed.

Materials and methods

CYTOGENETICS
Chromosomes from both lymphocytes and skin fibroblasts were prepared and trypsin-Giemsa banded according to standard techniques.14

ANALYSIS OF SHORT TANDEM REPEAT POLYMORPHISMS (STRP)
Genomic DNA was extracted from peripheral white blood cells of the patient and her parents and from fibroblasts of the patient according to Aldridge et al.15 DNA was amplified in the polymerase chain reaction (PCR) using primers flanking short tandem repeat polymorphisms (STRPs).14 Each PCR reaction contained 20 ng genomic DNA, 10 mmol/l Tris, pH 8.3, 1.5 mmol/l MgCl2, 50 mmol/l KCl, = 0.01% gelatin (Sigma), 200 μmol/l each dGTP, dATP, dCTP, 2.5 μmol/l dCTP, 0.7 μCi 32P-dCTP, 10 ng of each primer, and 0.15 U AmpliTaq polymerase (Perkin Elmer Cetus) in a final volume of 10 μl. The amplification products were separated on a denaturing sequencing gel. Autoradiography was performed at room temperature with x-ray film (X-O-Mat, Kodak) for 12 to 36 hours.

PRIMERS
The chromosome specific primers used for amplification of STRPs are summarised in the table and in the report of the NIH/CEPH Collaborative Mapping Group.17 The results obtained at one STRP locus per chromosome are given.

Results
Cytogenetic analysis showed a normal diploid 46,XX, karyotype in all peripheral lymphocytes of the proband (50 metaphases analysed), but a triploid 69,XXX cell line in the majority of skin fibroblasts (46/50 metaphases analysed).

STRPs at various loci on the 22 autosomes and the X chromosome were analysed in DNA from peripheral blood of the patient's parents and from the diploid white blood cells and the triploid fibroblasts of the patient. For most chromosomes at least one informative marker was found. Results obtained at various loci on chromosomes 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 15, 16, 17, 19, and 21 showed maternal origin of the extra chromosome in the patient's triploid fibroblasts (table). Examples of STRPs at loci on chromosomes 3 (D3S240, marker Mfd 30), 16 (D16S266, marker Mfd 62), and 19 (D19S246, marker Mfd 232) are given in fig 2. Although PCR of STRPs is not quantitative,

Alleles of informative polymorphic STRP markers in peripheral blood DNA of the patient's father and mother, and in blood and fibroblast DNA of the patient.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>STRP marker</th>
<th>Locus</th>
<th>Father</th>
<th>Mother</th>
<th>Patient (blood)</th>
<th>Patient (fibroblasts)</th>
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<td>1</td>
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<td>96/102</td>
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<td>85/95</td>
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<td>170</td>
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*Uninformative markers. NT = no suitable markers could be tested.
Figure 2 STRPs detected by marker MFD30 at locus DSS240 (A), by marker MFD62 at locus D16S266 (B), and by marker MFD232 at locus D19S246 (C) in peripheral blood DNA of the patient's father (lane 1), her mother (lane 2), and in peripheral blood, 46,XX, (lane 3) and fibroblast DNA, 69,XXX, (lane 4) of the patient.

double dosage of alleles could be deduced at several loci (table).

No informative STRPs were found for chromosomes 8, 13, 22, and the X. In these cases, it was not possible to infer which allele was present in double dosage in the triploid cell line. Therefore, only two alleles are listed at these loci in triploid DNA (table). The results, however, are consistent with maternal origin of the supernumerary chromosome. Finally, no polymorphic markers could be studied for chromosomes 14, 18, and 20.

Discussion
In the present case, a triploid 69,XXX cell line was detected in fibroblasts, but not in the patient's lymphocytes, which were exclusively diploid. This finding is typical of triploidy: most cases are diagnosed only after chromosome analysis of fibroblasts. The phenotype of the patient is also characteristic of triploidy.19,20 The infant displayed typical craniofacial features, such as malformed ears, hypertelorism, a depressed nasal bridge, a broad forehead, and micrognathia, in addition to other manifestations, such as growth retardation, syndactyly, and limb length discrepancy. Seizure disorders, psychomotor retardation, and hearing loss are also well described in triploidy patients. Precocious puberty and absence of the septum pellucidum, however, have not been previously reported.

The fact that our patient was a product of a clomiphene induced pregnancy appears to be a pure coincidence. Clomiphene induced ovulation has not been associated with an increased rate of spontaneous abortions or congenital malformations.20,21 Recently, spontaneous abortuses from an in vitro fertilisation (IVF) programme were analysed for cytogenetic abnormalities.22 IVF programmes frequently use clomiphene as a component of their stimulation protocols. This study failed to show any increased incidence of chromosomal aberrations, compared to natural conceptions.

Various hypothetical mechanisms have been suggested to explain the occurrence of triploidy23 including (1) postzygotic maldivision of a triploid or diploid zygote, (2) incorporation of the second polar body in one of the blastomeric cells, (3) fertilisation of a diploid ovum with incorporation of the second diploid polar body, (4) fertilisation of the first polar body and ovum by individual spermatozoa with suppression of one second polar body, (5) dispermy or diploid sperm fertilisation with incorporation of an independently fertilised second polar body, and (6) chimerism originating from diploid and triploid zygotes.

The first two hypothetical mechanisms leading to triploidy may involve a single aberrant event, whereas the remaining four mechanisms require the occurrence of two sequential, independent errors, which would intuitively make them less likely.

Studies of cytogenetic heteromorphisms in triploid conceptuses and parental lymphocytes have yielded data showing that dispermy accounts for the majority of cases.14 No studies have yet been able to determine the relative frequencies of errors in meiosis I or II, or in early oogenesis or spermatogenesis in the triploid population.

In the present case, paternal origin is conclusively excluded by the STRP findings of a maternally derived supernumerary chromosome in all 16 informative chromosomes. STRP analysis also excludes chimerism as a mechanism in our patient. No qualitative allele differences were detected between the patient's diploid and triploid cell lines, a finding expected if both cell lines had originated from two different zygotes. Postzygotic maldivision of a maternally derived triploid zygote is also unlikely, given the STRP findings, as we would have expected loss to involve both maternal and paternal chromosomes randomly. Moreover, studies of tumours with conversions from triploid to diploid cell lines, and vice versa, have frequently shown incomplete conversion, which was not seen here cytogenetically.23-25

Of the theoretical mechanisms of triploidy, our data best support the incorporation of the second polar body into one of the diploid blastomeric cells. By this mechanism, the triploid cell line shows a uniformly maternally derived supernumerary haploid set, and the diploid line shows normal biparental chromosome origin. The STRP analysis shows this exact pattern.

In conclusion, maternal origin of the extra chromosome set was shown by STRP analysis in a case of 46,XX/69,XXX triploidy. Incorporation of the second polar body into a blastomere nucleus of the developing embryo best explains the DNA findings.

We thank Barbara Pober for her clinical insight in this case and Mike Bishop for his cytogenetic assistance.

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