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.
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Triploidy is common in man and occurs in approximately 1% of all conceptuses.[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%.[2-4] 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.[5-10] 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 conceptuses 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 agenesis 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 intractable 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-

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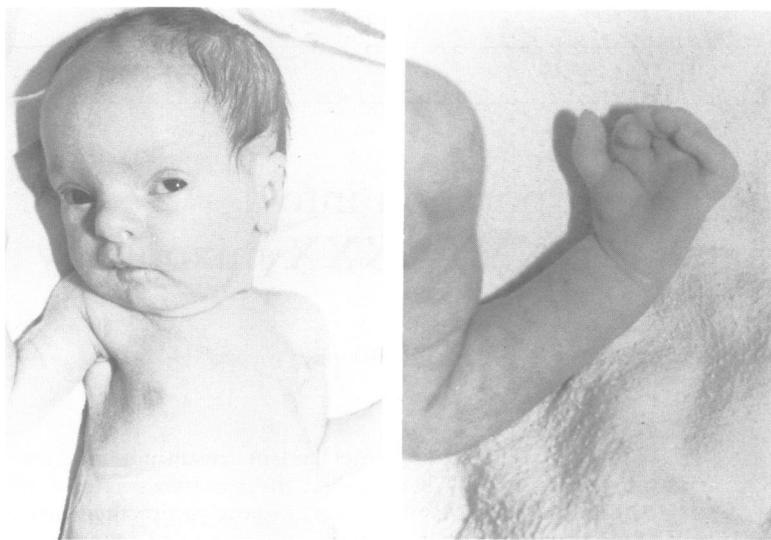
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Figure 1 (A) Proband at three weeks showing typical facial features of mixoploidy. (B) Camptodactyly and 3/4 finger syndactyly.

ing 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).[16] 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, dTTP, 2·5 μmol/l dCTP, 0·7 μCi α³²P-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.

Chromosome	STRP marker	Locus	Allele sizes (bp) in DNA from			
			Father	Mother	Patient (blood)	Patient (fibroblasts)
1	MFD 60	D1S322	102/118	96/102	102/118	96/102/118
2	MFD 68	JLIA	133/133	133/133	133/133	133/133/137
3	MFD 30	D3S240	85/85	85/95	85/85	85/85/95
4	MFD 146	D4S194	101/103	109/115	103/115	103/109/115
5	MFD 154	D5S211	194/202	194/204	194/202	194/202/204
6	MFD 47	D6S87	145/153	141/147	141/145	141/145/147
7	MFD 172	D7S472	122/124	120/122	120/124	120/122/124
8*	MFD 117	D8S165	146/144	146/142	144/142	144/142
9	MFD 189	D9S106	101/105	101/107	101/105	101/105/107
10	MFD 187	D10S169	113/99	113/107	113/113	113/113/107
11	MFD 166	D11S875	103/115	103/111	103/115	103/111/115
12	MFD 92	F8VWF	119/123	119/125	123/125	119/123/125
13*	MFD 44	D13S71	77/75	79/67	77/79	77/79
14	NT	—	—	—	—	—
15	MFD 81	D15S106	101/101	101/109	101/101	101/101/109
16	MFD 62	D16S266	96/96	96/100	96/96	96/96/100
17	MFD 152	D17S578	148/168	148/148	148/168	148/148/168
18	NT	—	—	—	—	—
19	MFD 232	D19S246	213/213	217/185	213/185	213/217/185
20	NT	—	—	—	—	—
21*	MFD 55	D21S156	77/89	77/101	77/89	77/89/101
22*	MFD 51	D22S257	133/133	125/137	133/125	133/125
X*	MFD 66	DXS453	170	166/170	166/170	166/170

*Uninformative markers. NT = no suitable markers could be tested.
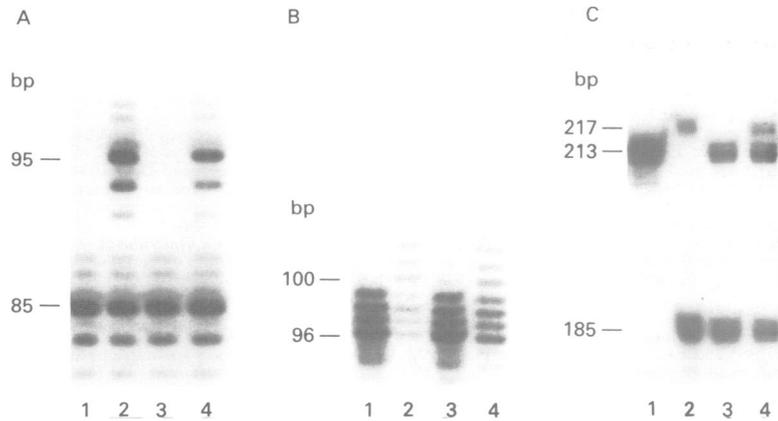
Figure 2   STRPs detected by marker MFD30 at locus D3S240 (A), by marker MFD62 at locus D16S266 (B), and by marker MFD232 at locus D19S246 (C) in peripheral blood DNA of the patient's father (lane 1), her mother (lane 2), and in peripheral blood, 46,XX, (lane 3) and fibroblast DNA, 69,XXX, (lane 4) of 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 mixoploidy: most cases are diagnosed only after chromosome analysis of fibroblasts. The phenotype of the patient is also characteristic of mixoploidy.[18][19] 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 mixoploid 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 conceptuses.

Various hypothetical mechanisms have been suggested to explain the occurrence of mixoploidy[13] including (1) postzygotic maldivision of a triploid or diploid zygote, (2) incorpora-

tion 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 mixoploidy 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.[1][3][4] 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 mixoploidy, 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 uniformally 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 mixoploidy. Incorporation of the second polar body into a blastomere nucleus of the developing embryo best explains the DNA findings.

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