

Brief Report

A TRUE HERMAPHRODITE CHIMERA RESULTING FROM EMBRYO AMALGAMATION AFTER IN VITRO FERTILIZATION

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HIGH rates of successful pregnancy after in vitro fertilization depend on placing more than one embryo into the mother, a practice resulting in a 30-to-35-fold increase in dizygotic-twin deliveries.¹ Increased frequencies of twin-associated anomalies might also therefore be expected. Chimerism, the presence in a single person of cells derived from two or more zygotes, is one such rare anomaly. It is usually ascertained through anomalous blood-grouping results or (for XX/XY chimeras) sex reversal or intersex.

We used DNA polymorphisms to investigate a 46,XX/46,XY hermaphrodite conceived by in vitro fertilization. We found not only that the child is a chimera, but also that he must have resulted from amalgamation of two embryos, each derived from an independent, separately fertilized ovum.

CASE REPORT

The mother was a 31-year-old woman with primary infertility. Hormonal and laparoscopic investigation indicated a normal pelvis and normal ovulation. Her partner, who was 41 years old, had had a child by another partner but was severely oligozoospermic. The woman was given buserelin and human menopausal gonadotropins, after which 18 oocytes were harvested, of which 15 were fertilized in vitro with anonymous donor sperm and maintained in separate dishes. Two days after insemination (the four-cell stage), three embryos were transferred to the woman. Ultrasonography 36 days after transfer showed a single fetus and sac. A 3.46-kg infant was delivered vaginally at term; he had a normal right testis and an undescended left testis, with otherwise normal male genitalia. At the age of six months, the left testis was palpable at the inguinal ring. Surgical exploration at the age of 15 months revealed a hernial sac containing an abnormal gonad and vas deferens. These structures were excised; they proved on histologic examination to be an ovary with a fallopian tube attached

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to a horn of uterus. Karyotyping of peripheral-blood lymphocytes then revealed two cell lines, one 46,XX and the other 46,XY.

At the age of 20 months, the infant's serum follicle-stimulating hormone and luteinizing hormone concentrations were normal for his age, both basally and in response to gonadotropin-releasing hormone. The basal serum testosterone concentration was normal (<20 ng per deciliter [<0.7 nmol per liter]) and rose normally to 180 ng per deciliter (6.3 nmol per liter) three days after a single intramuscular injection of 2000 IU of human chorionic gonadotropin. Ultrasonography at three years eight months revealed an apparently normal right testis in the scrotum and normal kidneys, bladder, and pelvic structures. At laparoscopy at four years four months, the right vas deferens and testicular vessels appeared to be normal; no female genital structures were seen. A skin biopsy was performed. Subsequently, the child has grown and developed normally, with height at the 90th percentile and weight at the 75th percentile. He has no neurodevelopmental abnormalities, and he attends a regular school.

METHODS

Informed consent for all genetic investigations and publication of the information was obtained from the child's parents. Separate samples of DNA were prepared from his peripheral blood and each of three flasks of fibroblasts cultured from his skin-biopsy specimen. Short tandem-repeat polymorphisms corresponding to anonymous loci were analyzed after amplification by the polymerase chain reaction with fluorescein-labeled primers, as described previously.² Primer sequences were obtained from the Genome Database or the Génethon linkage map.³

RESULTS

Demonstration of Chimerism by Analysis of DNA Polymorphisms

We first examined X-chromosome markers, because the results were less likely to be uninformative due to allele sharing between the patient's mother and father (who was not available for testing). For DXS3 (chromosome Xq21.3) and DXS451 (chromosome Xp22.1), the patient had three alleles (Fig. 1A and 1B). In each case, one was paternal, indicating (since the patient also has a Y chromosome) the involvement of two sperm. The presence of two other alleles indicates that two different maternal X chromosomes were present.

Comparison of the results for the different samples of fibroblast DNA (Fig. 1A and 1B) revealed a consistent peak height (mass) ratio between the paternal allele and one of the maternal alleles. These alleles (paternal 2 and maternal 1 for DXS3; paternal 1 and maternal 3 for DXS451) must therefore be the two in the XX cell line. In contrast, because of the differing proportions of XY and XX cells in each fibroblast culture, the height of the other maternal allele (XY cell line) varied independently (peak 3 for DXS3; peak 2 for DXS451). Fibroblast culture 3 thus contained mostly XY cells, and culture 2 almost entirely XX cells. Fibroblast culture 1 and the patient's blood had intermediate proportions of XX and XY cells.

The lack of paternal DNA made the results of studies of many autosomal markers inconclusive, but for D17S1178 (chromosome 17q11.2-q12) we found four distinguishable parental alleles. Again, the different samples showed constant height ratios be-

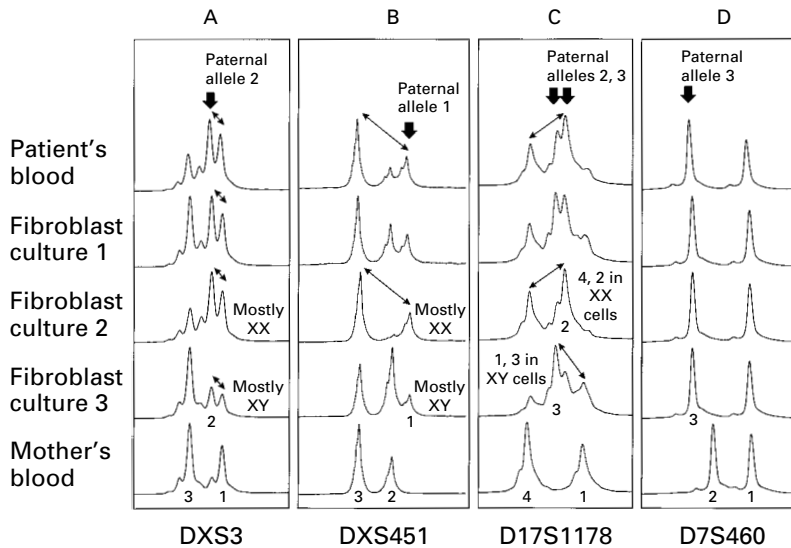


Figure 1. Demonstration of Chimerism through the Use of DNA Polymorphisms.

After amplification by the polymerase chain reaction, the fluorescein-labeled products were separated by electrophoresis with a Pharmacia ALF sequencer. For each marker, the patient's blood and skin fibroblasts (three cultures) and maternal blood were analyzed. The number 1 indicates the largest allele, and the number 4 the smallest. The broad solid arrows indicate paternal alleles. For DXS3 (Panel A) and DXS451 (Panel B), three alleles (two maternal and one paternal) are present in the patient's blood and fibroblasts. Since one of the patient's two cell lines has a paternal Y chromosome, there are four different sex chromosomes in the patient (diagnostic of chimerism). The thin arrows indicate pairs of peaks with constant height ratios, implying that these alleles are in the same cell line (see the Results section). In Panel C, four D17S1178 alleles are present. In Panel D, the absence of one maternal D7S460 allele (2) from the chimera DNA rules out the possibility of sample mixing.

tween pairs of peaks, indicating that alleles 4 (maternal) and 2 (paternal) were in the XX cell line, whereas 1 (maternal) and 3 (paternal) were in the XY cell line (Fig. 1C; compare the reciprocal patterns indicated for fibroblast cultures 2 and 3). The uninformative marker D7S460 indicates that inadvertent mixing of patient and maternal DNA did not occur; the prominent maternal allele 2 is completely absent from all samples from the patient (Fig. 1D). Therefore, the biallelic maternal contributions for other markers are genetic ones and not contamination artifacts.

Origin of Chimerism

The DNA results prove the involvement of two sperm but leave open several embryologic possibilities⁴: fertilization both of a mature ovum and its first polar body⁵; fertilization of an ovum and second polar body; or "complete" chimerism, the amalgamation of independent embryos, each derived from an independently ovulated and separately fertilized ovum. Discriminating among these mechanisms is important, since only the third could result from the transfer of multiple embryos after in vitro fertilization.

Because of recombination during the first meiotic division, distinguishing among these possibilities genetically requires analysis of polymorphisms near the chromosomal centromeres. Three patterns can be predicted. In the case of fertilization of the first po-

TABLE 1. PERICENTROMERIC-MARKER SEGREGATION IN DNA FROM THE PATIENT AND HIS MOTHER.

| MARKER | ESTIMATED DISTANCE FROM CENTROMERE* | | ALLELES† | | NO. OF MATERNAL ALLELES TRANSMITTED |
|-----------------|-------------------------------------|------|----------|------------|-------------------------------------|
| | Mb | cM | MOTHER | CHILD | |
| D3S1271 | 2.7 | 1.0 | 1, 2 | 1, 2 | |
| D4S2996 | 1.6 | <0.5 | 2, 3 | 1, 2 | One |
| D5S407 | <1 | 2.2 | 1, 2 | 1, 2 | |
| D6S430 | <1 | <0.5 | 1, 2 | 1 | One |
| D7S502 | 1.6 | <2.1 | 1, 2 | 1, 2 | |
| D8S532 | 2.0 | 0.5 | 2, 3 | 1, 2, 3 | |
| D9S1874 | 3.1 | <0.5 | 1, 3 | 1, 2, 3 | (Both)‡ |
| D11S4109 | 3.5 | 2.0 | 1, 4 | 1, 2, 3, 4 | Both |
| D12S87 | 1.9 | <0.5 | 1, 2 | 1, 2 | |
| D14S72 | <1 | 1.7 | 2, 2 | 1, 2 | |
| D18S1104 | <1 | 1.0 | 1, 2 | 1, 2 | |
| D19S49 | 8.3 | 2.8 | 1, 3 | 1, 2, 3 | (Both)‡ |
| D20S871 | <1 | 1.9 | 1, 4 | 2, 3, 4 | One |
| D21S258 | 1.7 | 5.6 | 1, 2 | 1, 2, 3 | |
| AR (Xq11.2-q12) | 4.1 | <0.5 | 1, 3 | 1, 2, 3 | Both |

*The estimated distances from the centromeres are from the Location Database⁶; physical distances of less than 1 megabase (Mb; 1 Mb = 1 million bp) and genetic distances (female meiosis) of less than 0.5 cM (equal to a 0.5 percent probability of recombination) are listed as <1 or <0.5.

†For each marker, the number 1 indicates the largest allele.

‡The parentheses indicate that the conclusion is based on the interpretation of allele peak heights shown in Figure 2.

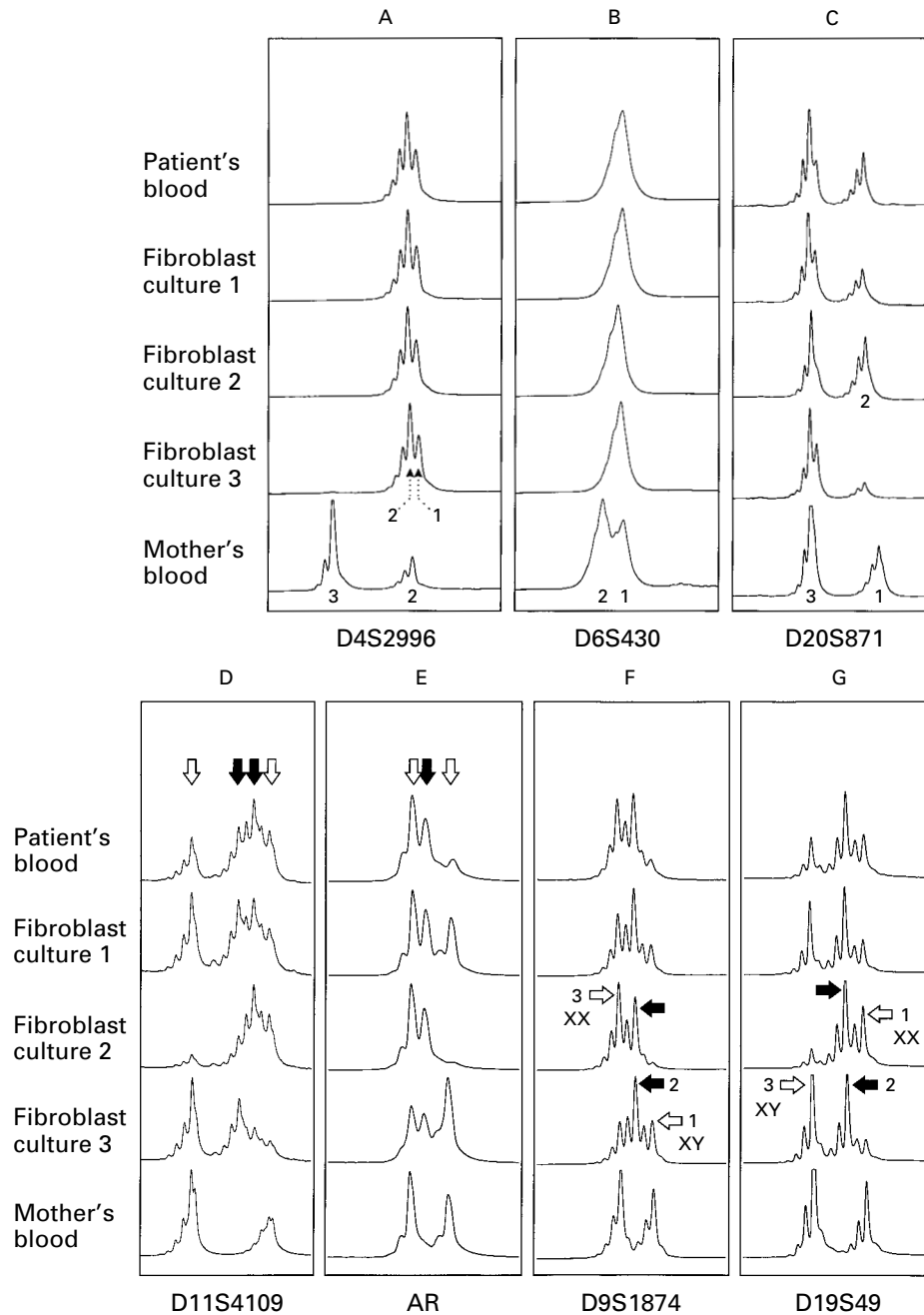


Figure 2. Segregation of Pericentromeric Markers in DNA from the Patient and His Mother.

Panels A, B, and C show markers for which only one of the two maternal alleles was transmitted. Panels D and E show markers for which transmission of both maternal alleles is clear. Panels F and G show markers for which transmission of both maternal alleles can be inferred. Although only three D9S1874 alleles are present, comparison of the peak heights in the different fibroblast cultures allows the following interpretation. Fibroblast culture 2 (known to contain almost all XX cells) has clearly received the maternal 3 allele (and allele 2 from the father). In fibroblast culture 3 (which contains mostly XY cells), allele 2 has by far the largest peak, implying that this allele was also transmitted by the father to the XY line. This means that allele 1 in fibroblast culture 3 must be maternal in origin. Thus, opposite maternal D9S1874 alleles have been transmitted to the two lines in the chimera. Similar deductions can be made for D19S49. Solid arrows indicate paternal origin of the indicated peak, and open arrows maternal origin.

lar body, the two cell lines in the chimera will inherit opposite maternal centromeres. For any informative marker near the centromere, therefore, both maternal alleles will be present in the chimera. After fertilization of the second polar body, in contrast, the two chimera cell lines will have the same maternal centromere. For any informative markers near the centromere, the chimera will inherit only one maternal allele. In the case of embryo amalgamation, the centromeres of each maternal chromosome pair will have segregated randomly and independently in each zygote. For markers close to some centromeres, the opposite maternal alleles will be transmitted to the two chimera cell lines, whereas for others, the same allele will be present in both cell lines.

We analyzed polymorphisms close to the centromeres of 15 different chromosomes (Table 1). Markers on three chromosomes (D4S2996, D6S430, and D20S871) (Fig. 2A, 2B, and 2C) demonstrated transmission of only one maternal allele, ruling out the first mechanism. In contrast, one autosomal marker (D11S4109) (Fig. 2D) and one X-linked marker (AR) (Fig. 2E) clearly showed the transmission of both maternal alleles, ruling out the second mechanism.

By comparing the peak heights of the samples with different proportions of XX and XY cells, we could also infer the transmission of both maternal alleles for two further markers — D9S1874 (Fig. 2F) and D19S49 (Fig. 2G). Thus, four pericentromeric markers reveal the transmission of both maternal alleles, and three the transmission of only one maternal allele (Table 1).

Though each marker has a small chance of error due to recombination with its centromere, the findings for at least three chromosomes are inconsistent with fertilization of either the first or the second polar body as a mechanism. They instead strongly suggest independent segregation of maternal centromeres in two separate meioses (the third mechanism). We conclude that two embryos, independently fertilized in vitro, fused, presumably after transfer into the mother, because ova or embryos were not cocultured during or after fertilization.

DISCUSSION

It is standard practice to replace more than one embryo after in vitro fertilization (preferably two embryos, because of high rates of the delivery of triplets after the placement of three or more^{7,8}). The resulting proportion of twin pregnancies in most in vitro fertilization programs is 20 to 25 percent.^{1,9} The perinatal or postnatal complications of these twin gestations are generally thought to be those of multiple conception and not specific for in vitro fertilization,^{9,10} although the discordance in birth weight between twins seems to be greater than in natural pregnancies.¹¹ The increase in the frequency of dizygotic

twins after in vitro fertilization by a factor of approximately 33 implies a similarly increased risk of rare twin-associated anomalies such as chimerism.

The natural incidence of chimerism is unknown. Phenotypes of XX/XY chimeras range from normal fertile males^{12,13} through males with hypospadias or ambiguous genitalia and hermaphroditism¹⁴⁻¹⁸ and fertile female hermaphrodites¹⁹ to phenotypically normal, fertile females.²⁰ This sparse literature is undoubtedly biased toward cases with sexual ambiguity or other gonadal problems, and many XX/XY chimeras may go unnoticed. Same-sex chimeras should be almost invariably phenotypically normal.

The observation of chimerism after in vitro fertilization should therefore be taken seriously. Not only does the great rarity of XX/XY chimerism suggest a causal link to the in vitro fertilization, but also its incidence could be higher than suspected.

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