

# CAP TODAY

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## Male or female? Integrated molecular and cytogenetic testing resolves discordant prenatal results

CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from ARUP Laboratories/University of Utah. If you would like to submit a case report, please send an email to the AMP at [amp@amp.org](mailto:amp@amp.org). For more information about the AMP and all previously published case reports, visit [www.amp.org](http://www.amp.org).



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In this case report, we aim to elucidate the importance of collaborative molecular and cytogenetic testing to offer a diagnosis for complex prenatal situations, such as chimerism, defined by the presence of two genetically distinct cell lines derived from two or more zygotes.

**Case.** A pregnant 35-year-old had two consecutive low-risk female prenatal cell-free DNA (cfDNA) screens, which conflicted with the 24-week ultrasound showing a singleton male fetus. Amniocentesis was performed at 24 weeks, six days to resolve the sex discrepancy, with concurrent fluorescence in situ hybridization, microarray, and associated short tandem repeat (STR) analysis of direct amniotic fluid. There was no history of genetic abnormalities, and the testing indication was "sex nonconcordance between cfDNA and ultrasound."

FISH aneuploidy panel was ordered to enumerate DNA markers for

autosomal chromosomes (13, 18, 21) and sex chromosomes (X, Y) to access fetal gender and common aneuploidies. Microarray, a technique based on polynucleotide probe hybridization to patient genomic DNA, was employed to evaluate genomewide unbalanced copy number change, including aneuploidy. Microarrays also include polymorphic probes that are used to detect genomic admixture or regions of homozygosity. The prenatal microarray is typically linked with maternal cell contamination (MCC) testing, which is an STR-based PCR assay to detect the presence of maternal alleles in the fetal sample.

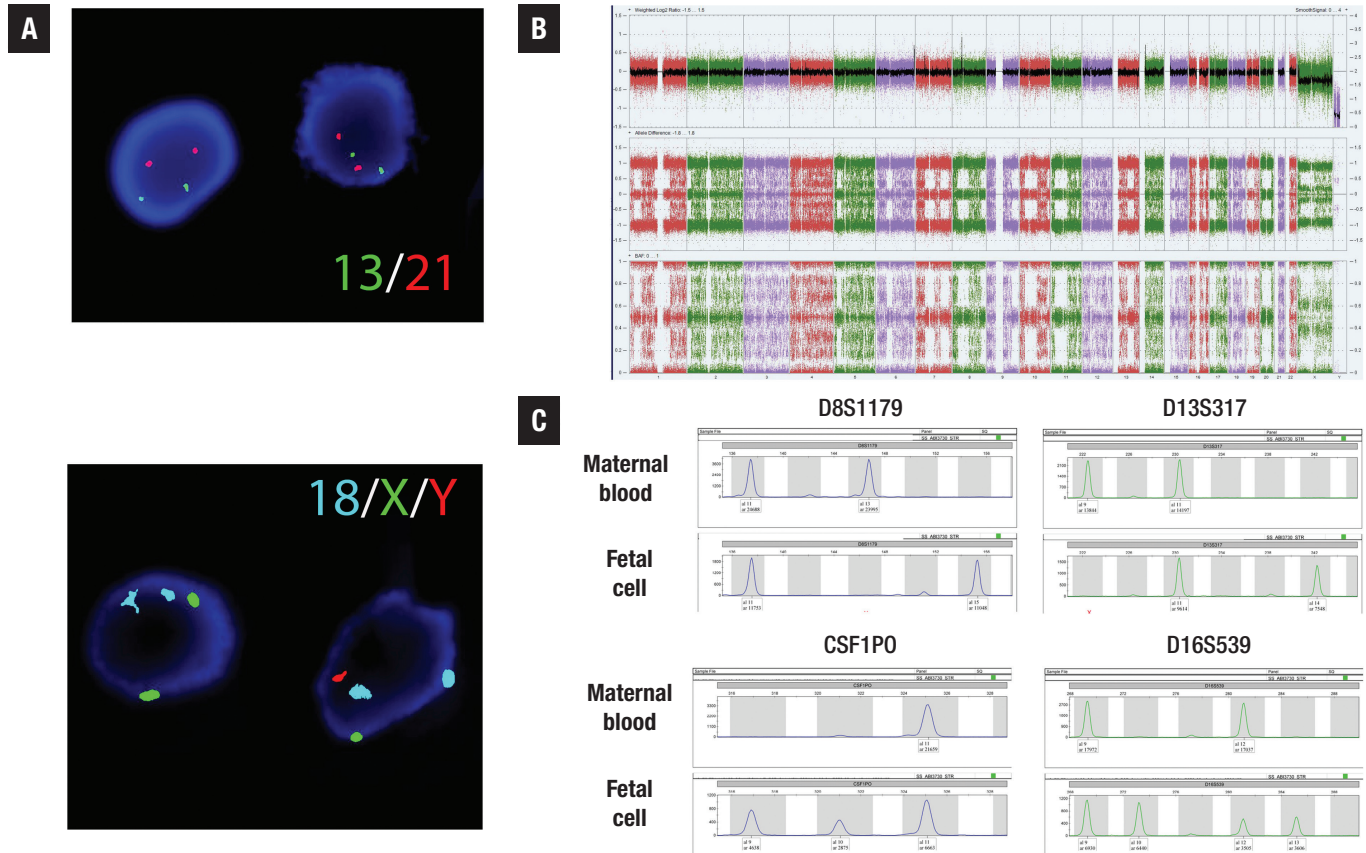
Interphase aneuploidy FISH showed normal results for chromosomes 13, 18, and 21, and indeterminate results for the sex chromosomes with a mixture of 60 percent XX and 40 percent XY cells (Fig. 1A, next page). Given the XX/XY admixture, the testing team contacted the provider and confirmed that this was a singleton fetus throughout the pregnancy. Therefore, the possibility of a vanishing twin was reasonably ruled out.

Microarray analysis of direct amniocytes showed normal autosomal copy number (2n), and 1.6 and 0.4 copies for the X and Y chromosomes, respectively (Fig. 1B). Allele differ-

ence and B-allele frequency data revealed a mixture of genotypes with alternating three tracks and seven tracks within a chromosome, similar to the pattern of dizygotic twins and inconsistent with MCC. Concurrent STR analyses of amniocytes and maternal blood independently ruled out MCC. Focusing on the STR pattern, the two to four alleles present in the fetus were consistent with dizygotic twin admixture, which would result in any number of alleles from one to four (Fig. 1C). Using D8S1179 and D13S317 as examples, MCC was ruled out by an absence of one maternal allele in the fetal genotype. In contrast, D16S539 showed that the fetus had more than two alleles, with two of the four being nonmaternal alleles. Integrating the molecular and cytogenetic information in a singleton pregnancy, these admixture results are consistent with an XX/XY tetragametic chimeric pregnancy.

Tetragametic chimerism results from a fusion between two individually fertilized eggs by two individual sperm. With copy number remaining at 2n, there are two individual diploid cell lines with two sets of maternal chromosomes and two sets of paternal chromosomes, resulting in up to four different haplotypes. The number of allele tracks depends on the

Fig. 1



number of haplotypes, generally formulated as “haplotype number + 1.” As schematically illustrated in **Fig. 2A** (next page), for regions with identical haplotypes between the two fusing zygotes (1 maternal + 1 paternal), three tracks will be present, as in a wild-type result. If the four haplotypes are different from each other (2 maternal + 2 paternal), there will be five tracks (4+1). So, how were seven tracks formed, and what drives the abrupt allele pattern changes within a chromosome?

The simple answer is, during meiosis 1, homologous chromosomes cross over and exchange genetic material. As shown from this simplified illustration (**Fig. 2B**), crossing over during maternal and paternal gametogenesis generates alternating haplotype patterns along the length of each chromosome. For instance, if the STR marker or array probe queries the presence of two alleles A and B at

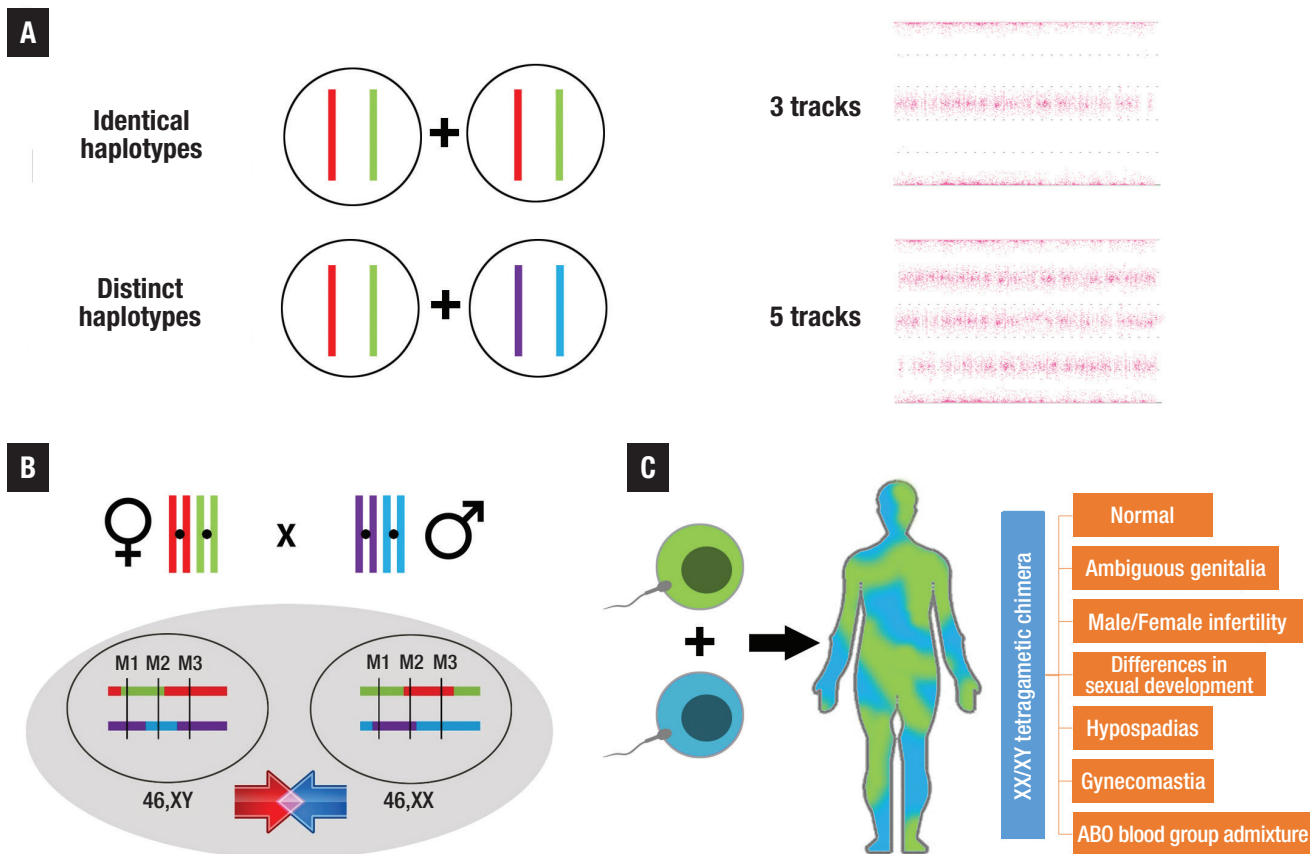
the M1 position covering two identical haplotypes, this would result in three tracks (AAAA, AABB, BBBB). Likewise, there are four alleles at the M2 locus, resulting in n+1 equating to five tracks (AAAA, AAAB, AABB, ABBB, BBBB). However, these outcomes assume the mix ratio is 50:50 for the fused embryos. When the mix ratio deviates from 50:50, seven tracks will emerge due to the ratio and its impact on allele tracks. The allelic data are consistent with a 60:40 admixture, which is compatible with the 60 percent XX cells and 40 percent XY cells observed by FISH. Notably, compared with microarray and STR analysis, FISH provides the only single cell data with cellular insight. However, this integrated molecular and cytogenetic testing approach, along with full clinical information, was essential to rule out MCC and appreciate the chimeric admixture.

From a genetic counseling per-

spective, the clinical outcome of XX/XY chimerism is hard to predict in the prenatal setting and can be variable depending on tissue distribution and tissue specific mixture.<sup>1</sup> In the current case, XY-bearing cells likely led to the development of male genitalia, resulting in an apparently male fetus by ultrasound. There is significant phenotypic variability among XX/XY chimeric individuals, ranging from phenotypically normal male or female to reproductive phenotypes such as infertility and differences in sexual development (**Fig. 2C**). Our microarray report concluded “mixed female/male genomes (XX/XY chimerism) with normal copy number” and recommended repeating FISH or microarray for blood, buccal tissue, and/or skin biopsy to assess the tissue distribution in the newborn after delivery.

In summary, contradictory cfDNA and ultrasound results were resolved

Fig. 2



by a combination of molecular and cytogenetic techniques, emphasizing the importance of full clinical and genetic information in interpreting test results. For the purpose of reporting standardization, the International System for Human Cytogenomic Nomenclature offers standard nomenclature that is employed internationally to describe cytogenetic abnormalities. We used the following ISCN description in our final report:  $\text{chi arr}(X,1-22) \times 2[0.6]/(X,Y) \times 1,(1-22) \times 2[0.4]$ . □

1. Madan K. Natural human chimeras: a review. *Eur J Med Genet.* 2020;63(9):103971.

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## Test yourself

Here are three questions taken from the case report. Answers are online now at [www.amp.org/casereports](http://www.amp.org/casereports) and will be published next month in CAP TODAY.

- Which of the following is true about XX/XY tetragametic chimera?
  - Autosomal copy number is usually  $2n$ .
  - It is a type of molar pregnancy.
  - It always results in infertility.
  - cfDNA is able to detect it with high sensitivity.
- In terms of microarray allele track pattern, the difference between XX/XY tetragametic chimera and maternal cell contamination is:
  - Maternal cell contamination shows three tracks and XXXY shows seven tracks.
  - They typically result in different copy number ratios.
  - XX/XY shows alternating allele tracks, while maternal cell contamination shows a non-alternating pattern.
  - These two situations are indistinguishable by microarray.
- When is the XX/XY fusion most likely to occur?
  - During female gametogenesis, in the ovary.
  - At the zygote stage, in a fallopian tube.
  - At the blastocyst stage, before implantation.
  - In the first trimester, in the uterus.