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Implications of chimerism for cfDNA/NIPT prenatal screening

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I. Introduction

Noninvasive prenatal testing (NIPT) for fetal aneuploidies by massively parallel sequencing has emerged as a powerful tool in management of high-risk pregnancies. It is important that patients receive pre-test counseling regarding limitations. Chimerism is defined as the coexistence of more than one cell line in an individual, originating from multiple zygotes. Mosaicism represents single zygote origin, but differing co-segregating cell lines, such as 45,X/46,XY, etc. Testing such as variable number tandem repeat (VNTR) can clarify chimera vs. mosaic.

Chimerism: artificial (transplant), tetragametic (two zygote fusion), or feto-fetal transfusion/"twin chimerism" (with co-twin demise), either fetal or maternal in origin, can confound NIPT data, most often by falsely predicting male fetal sex when fetus is phenotypically female. We previously presented false male NIPT results for recipients of a male derived bone marrow or organ transplant[1]. Here, we discuss four select examples of chimerism discerned after NIPT screening, three of which had false male fetal sex prediction.

II. Methods

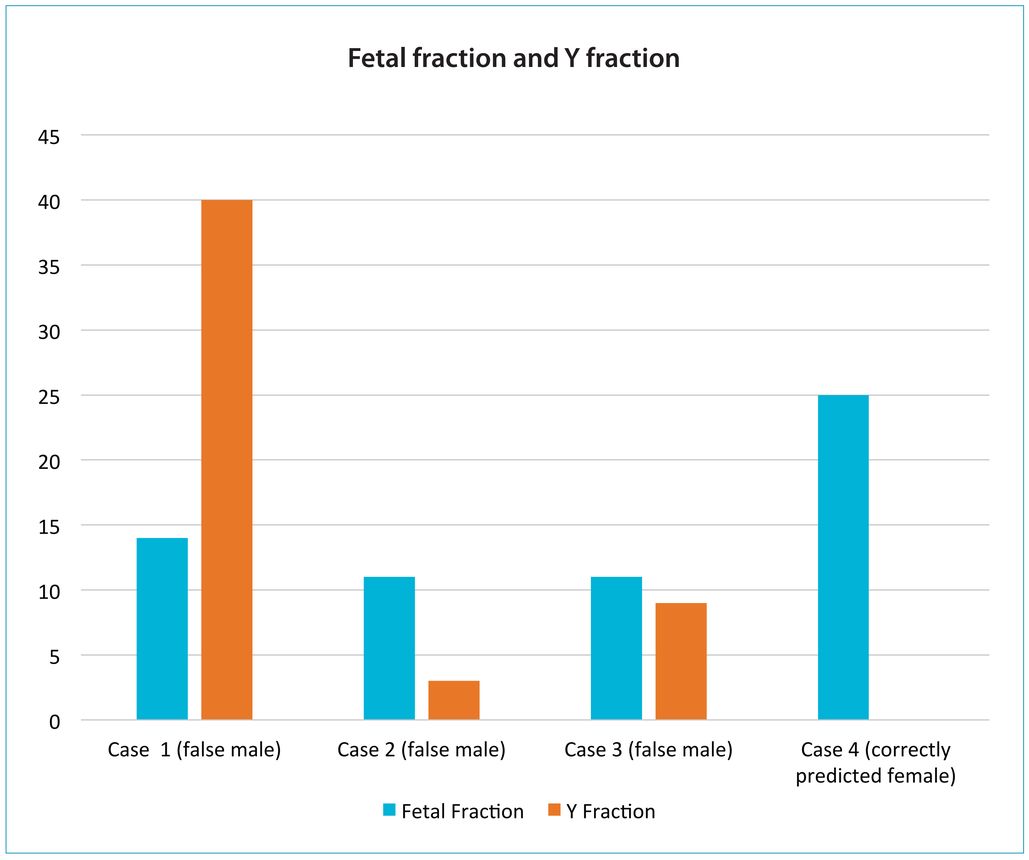
Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® 21 PLUS or MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing (MPS) as described by Jensen et al and Lefkowitz et al.[2,3]

III. Results

Table 1. Likely chimera diagnosis cases

Case	NIPT Result	Fetal Sex by Ultrasound	Diagnostic Testing	Diagnostic Test Results	Proposed Biologic Mechanism
1	Negative male	Female	Patient (maternal) blood karyotype Amnio array	Patient chimera: 46,XY[16]/46,XX[4] Fetal: Normal female microarray (1-22,X)x2	Tetragametic or (vanished) twin feto-fetal chimerism in patient, not fetus
2	Negative male; Redraw due to sex discrepancy. Redraw result: negative male	Female	Amniocentesis	Fetal chimera: 46,XY[25%]/46,XX[75%]	Tetragametic or (vanished) twin feto-fetal chimerism
3	Negative male; Redraw due to sex discrepancy. Redraw result: negative male	Female	Newborn blood karyotype Buccal karyotype ISH for SRY gene	Fetal chimera likely Blood 46,XX[>90%] Buccal: 46,XY[37.5%] FISH: SRY: 4/100 FISH: Y centromere: 2/100	Tetragametic chimerism likely, mosaicism cannot be ruled out
4	Negative female	Not reported	Amniocentesis	Female with likely chimera of a diploid homozygotic parental cell line and biparental line arr (1-22, X)x2/ (1-22,X) x2 hmz	Tetragametic chimerism

Figure 1. Y fetal fraction by case



IV. Conclusions

Cases 1-3: (**Table 1**) False male result and subsequent diagnosis of chimerism. Fetal sex is predicted by presence/absence of Y chromosome signal. With this MPS platform, male calls will have Y fraction generally equivalent to overall fetal fraction. In cases of maternal Y contribution, Y signal can be markedly increased above fetal fraction (**Figure 1**). Fetal sex discrepancies between ultrasound and NIPT are a rare but known limitation of all NIPT methodologies, often explained by a co-twin loss/second sac, fetal sex reversal syndromes, maternal or fetal chromosome abnormalities, history of transplant, and now rarely fetal or maternal chimerism.

Case 4: A rare likely chimera of a diploid homozygous parental cell line and a biparental cell line.

While it is unlikely that aneuploidy risk assessment would be impacted, NIPT screening is inherently bound by the cell line(s) represented in placental trophoblast. Appropriate pre-test counseling including potential discovery of fetal or maternal genomic abnormalities that could have major, minor, or no clinical significance is essential. Prenatal screening requires a multifaceted approach to uncover the whole story. These cases are evidence that chimerism is a rare but documented explanation of NIPT discrepancies.

V. References

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