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Application of mosaicism ratio from cell-free DNA (cfDNA) screening to multifetal gestations

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I. Introduction

When a cfDNA sample submitted for MaterniT® 21 PLUS testing is identified as having an overrepresentation of chromosome material suggestive of aneuploidy, a 'mosaicism ratio' (MR) is calculated. As previously described, the MR is derived by dividing the fetal fraction estimated for the aberrant chromosome or chromosomal segment over the fetal fraction estimated for all chromosomes.[1]

For singleton gestations MR is used to identify samples for which the results are suggestive of mosaicism, which may translate to a reduced positive predictive value.

For multifetal gestations, MR could be used predict: (1) whether one vs. multiple fetuses are affected with aneuploidy, and (2) the anticipated sex of the fetuses.

A series of cases that illustrate the potential clinical application of MR for multifetal gestations are presented here.

II. Description of Cases

The six cases presented in **Table 1** were submitted for MaterniT® 21 PLUS testing due to advanced maternal age and results were positive for trisomy 21 in at least one fetus. Karyotypes from CVS or amniocentesis were available to confirm the predicted cfDNA results in all cases. These particular cases were selected to demonstrate a variety of scenarios in which the MR metric may have clinical utility (i.e. predicting one vs. multiple fetuses with aneuploidy, and predicting number of male/female fetuses present).

For reference, **Figures 1 & 2** demonstrate "ideal" mosaicism ratios for various aneuploidy and fetal sex scenarios for multifetal gestations.

Sample 'traces' are shown in **Figures 3-6** to demonstrate how sequencing data would appear for various fetal aneuploidy and fetal sex scenarios for multifetal gestations.

Table 1. Six multifetal cases selected to demonstrate a variety of scenarios in which mosaicism ratio may have clinical utility

Multifetal status	Gestational age at time of draw	Indication for cfDNA screening	MaterniT®21 result	Chr21 FF	Overall sample FF	Chr21 MR	ChrY FF	ChrY MR	cfDNA suggested outcome	Prenatal diagnostic test	Karyotype result
Twins	11	AMA	Positive for trisomy 21 – Y present	16.68	14.22	1.17	7.5	0.53	1 male 1 female Both affected	CVS	47,XX,+21 47,XY,+21
Twins	10	AMA	Positive for trisomy 21 – Y present	5.29	9.76	0.54	5.4	0.55	1 male 1 female 1 affected	CVS	46,XX 47,XY,+21
Twins	11	AMA	Positive for trisomy 21 – Y absent	7.75	17.54	0.44	0.15	<0.01	Both female 1 affected	Amnio	46,XX 47,XX,+21
Twins	11	AMA	Positive for trisomy 21 – Y present	5.27	8.77	0.60	10.9	1.24	Both male 1 affected	CVS	46,XY 47,XY,+21
Triplets (with demise of one fetus at 12 weeks)	13	AMA	Positive for trisomy 21 – Y present	3.58	11.00	0.33	2.0	0.18	1 male 2 female 1 affected	CVS	46,XX 47,XY,+21
Triplets (with demise of one fetus)	10	AMA	Positive for trisomy 21 – Y absent	6.18	9.99	0.62	0	0.00	All 3 female 2 affected	CVS	46,XX 47,XX,+21 47,XX,+21

Figure 1. "Ideal" mosaicism ratios for various multifetal aneuploidy scenarios

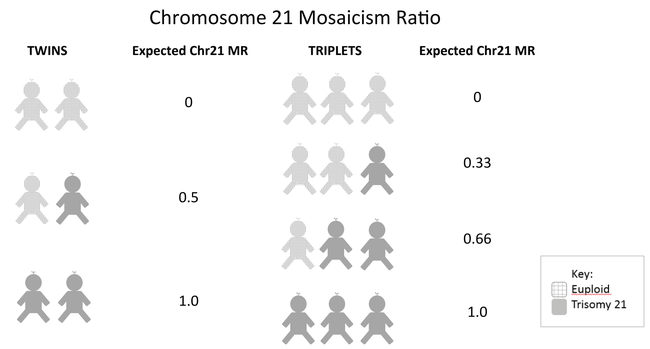


Figure 2. "Ideal" mosaicism ratios for various fetal sex scenarios in multifetal gestations

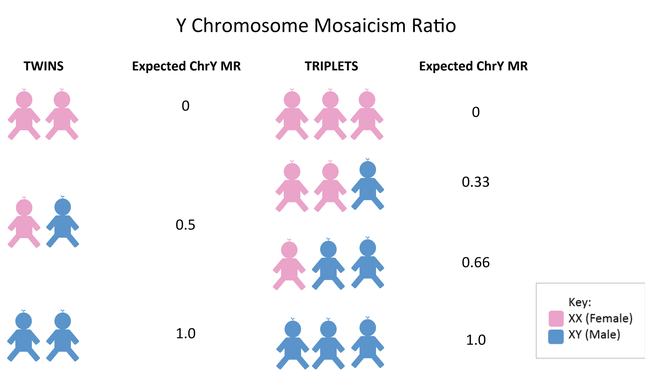


Figure 3. Example of sequencing data from a twin specimen suggestive of mixed-sex fetuses, one fetus with trisomy 21

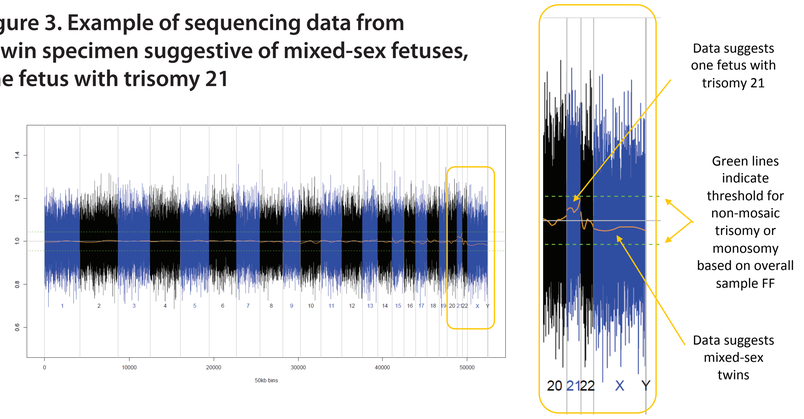


Figure 4. Example of sequencing data from a twin specimen suggestive of two euploid fetuses of mixed-sex

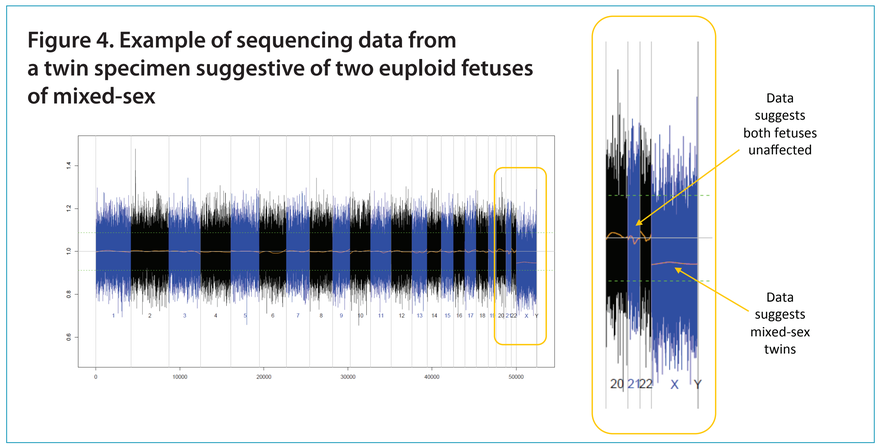


Figure 5. Example of sequencing data from a twin specimen suggestive of two female fetuses, one with trisomy 21

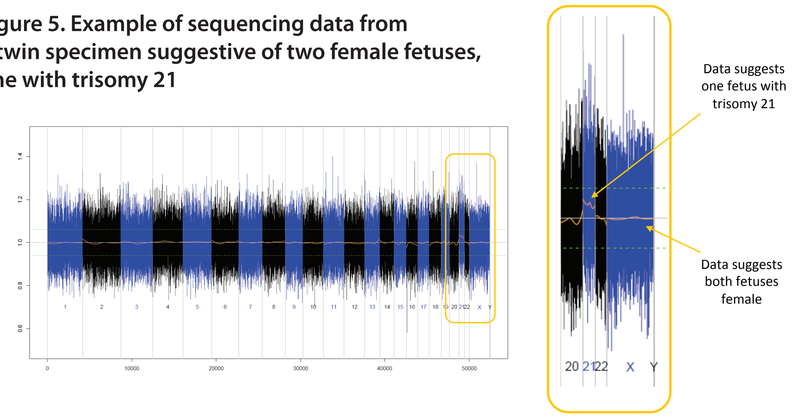
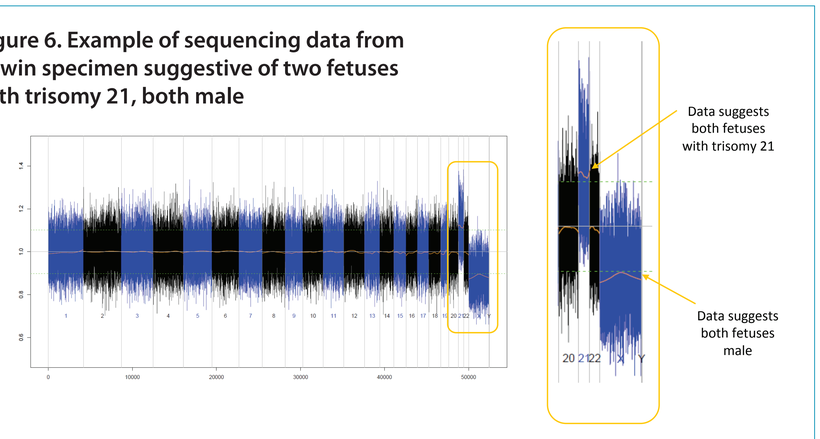


Figure 6. Example of sequencing data from a twin specimen suggestive of two fetuses with trisomy 21, both male



III. Discussion

Mosaicism ratio is a metric already used in the analysis of MaterniT® 21 PLUS data to identify abnormal results that may be impacted by mosaicism, either because of placental/fetal mosaicism or the demise of a co-twin. This same metric has potential to be applied to pregnancies involving multifetal gestations to: (1) predict when one vs. more than one fetus is affected with aneuploidy, and (2) provide information about the anticipated sex of the fetuses.

The cases presented here demonstrate that MR has potential clinical utility for individuals carrying twins or higher order multiples. These cases also support the need for additional studies comparing the mean and standard deviation of MR in multifetal gestations with one vs. multiple fetuses affected, as well as studies of how MR is impacted by other aneuploidies that are more commonly seen in mosaic form (e.g. trisomy 13 and trisomy 18).

IV. References

1. Wardrop J, et al. Mosaicism Ratio in cfDNA Testing: A Potential Tool to Identify Discordant Results. Poster presented at: *2017 ACMG Annual Clinical Genetics Meeting*; 2017 Mar 21-25; Phoenix, AZ.

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Poster presented at the *38th NSGC Annual Conference*; 2019 November 5-8; Salt Lake City, UT.