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The maternal X-factor: Maternal X chromosome deletions' influence on cfDNA analysis of sex chromosome aneuploidy status in the fetus

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1. Introduction

Cell free DNA (cfDNA) testing is a valuable screening tool to assess pregnancies at risk for sex chromosome aneuploidies (SCAs). Maternal mosaicism for SCA or maternal copy number variants (CNVs) on the X chromosome can complicate the interpretation of cfDNA results and may preclude fetal assessment for fetal sex or SCA status. We describe three cases of maternal X chromosome deletions impacting the cfDNA sequencing data and analysis of sex chromosome aneuploidy in the fetus.

2. Methods

Maternal blood samples submitted for MaterniT®21 PLUS or MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing as described by Jensen et al[1] and Lefkowitz et al[2]. Additional clinical information and case review was elicited from or supplied by the clinical provider via phone or email.

3. Results

Case 1: 40-year-old pregnant patient undergoing cfDNA screening for MaterniT®Genome at 11 weeks gestation. The ordering clinician reported a known 6.82 Mb maternal deletion at Xq24 with negative preimplantation genetic testing for the deletion in the embryo. Additionally, there was history of a prior child with Prader-Willi syndrome. Pre-test counseling included a discussion of the limitation to assess for fetal findings in the setting of a known maternal chromosome abnormality. As expected, the overall X chromosome z-score was abnormal=-6.5; identified as monosomy X on the SCA plot. Further detailed review of the sequencing data and X chromosome traces identified a deletion that was presumed to be the known maternal deletion. The cfDNA sequencing data identified a 6.85 Mb deletion likely of maternal origin near Xq23-q24; deletion z-score=-75.3. No other positive findings on the X chromosome were identified. However, the overall depressed z-score for the X chromosome was presumed to be influenced by the strong signal/z-score from the maternal deletion event. Therefore, SCA in the fetus was reported as uninformative due to maternal deletion.

Case 2: 35-year-old pregnant patient undergoing cfDNA screening at 12 weeks gestation. The clinician called the laboratory to review the patient history after the sample was received and relayed a known 25 Mb mosaic deletion at Xp22. After discussion with the laboratory and in light of the known maternal mosaic deletion on the X chromosome the order was updated to exclude SCA reporting. As expected, MaterniT®21 data was consistent with a depressed X chromosome z-score=-2.8 and SCA analysis in the fetus was uninformative. Further detailed review of the sequencing data and individual X chromosome traces did identify a likely maternal deletion approximately 13.6 Mb near Xp22.33-p22.2; deletion z-score=-12.3.

Case 3: 30-year-old pregnant patient undergoing cfDNA screening for MaterniT®Genome at 14 weeks gestation. The requisition listed indication for screening as prior children with chromosome abnormality. Additional clinical information reported a history of five spontaneous abortions, one healthy daughter, one male stillbirth with significant renal abnormalities, and one living male child delivered at 33 weeks with renal abnormalities with significant impairments. Upon review of the cfDNA data, sex chromosome aneuploidy analysis was uninformative. The overall z-score of the X chromosome was depressed=-3.8. This, however was below positive reporting criteria for monosomy X. Additional review of cfDNA sequencing data identified an approximately 6.5 Mb deletion, likely maternal in origin, near Xp22.33-p22.31; deleted region z-score=-57.1. This sample was reported as positive for a deletion on the X chromosome likely of maternal origin, precluding fetal assessment of this locus. After reporting this to the clinician, subsequent review of patient records confirmed a known 9.3 Mb deletion of Xp22.31 in the patient. The pregnancy was confirmed to have inherited the maternal X deletion on microarray, and this data is representative of both a maternal and fetal deletion.

Figures 1-3: Depicted for each case is the SCA plot, with red arrow indicating patient data, representing chromosome X and Y z-scores on the X and Y axis, respectively. Case 1 was represented as monosomy X, while SCA data for cases 2 and 3 were uninformative or non-reportable. Next, the 50 kb sequencing trace which depicts raw genome-wide sequencing data for the sample. The corresponding chromosome is listed below the raw data, the patient's normalized sequencing data is represented by the orange/yellow line, the solid gray line in the middle of the image is where normal or euploid data would plot. The top dashed green line represents trisomy (over representation) and the bottom dashed green line represents monosomy (under representation) relative to the fetal fraction measured for the sample. When signals for over or under-representation reach far beyond the dashed green lines, one possible explanation is a maternal finding. The last graphic represents the individual X chromosome traces that depict the specific deletions identified for each sample highlighted in purple.

4. Conclusions

Maternal X chromosome CNVs can impact interpretation of cfDNA results leading to discordant or uninformative screening of SCA in the fetus. These cases demonstrate that analysis of cfDNA data is multifaceted and cannot be simplified to an abnormal z-score for positive reporting criteria. For example, case 1 had a positive z-score meeting reporting criteria for monosomy X and could have been resulted this way, supported by clinical validation. However, additional review of the sequencing data identified a likely maternal deletion possibly impacting the overall z-score of the X chromosome, and was reported as such. Depending on the methodology used, cfDNA results for patients with maternal X chromosome deletions could be misinterpreted as positive fetal monosomy X, uninformative, or reported as atypical findings. Additional information regarding the potential for a maternal event may not always be supplied by the laboratory in these cases. Collaboration between the laboratory and provider are crucial for elucidating the underlying explanation and accurately assessing the risk to the fetus. Counseling of patients with positive or uninformative results for SCA, specifically involving the X chromosome, should emphasize the importance of confirmatory diagnostic testing and clinical correlation. In the event of normal fetal and/or placental diagnostic outcome, a wider range of clinical origins to include maternal chromosome abnormality should be considered.

Key Points:

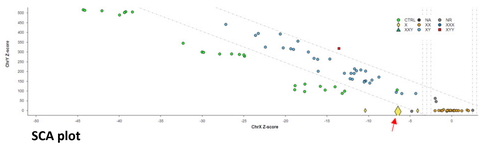
- Assessment of fetal SCA with cfDNA screening may be dependent on maternal chromosome findings.
- Laboratories should utilize multifaceted analysis of SCA data to help identify likely maternal findings impacting fetal screening.
- Laboratories should report information regarding likely maternal findings whenever possible to aid in counseling and the discussion of diagnostic testing following an abnormal or uninformative result on cfDNA screening.

References

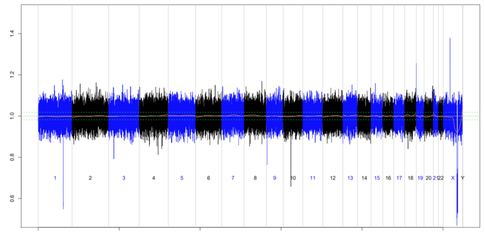
1. Jensen TJ, Zwiefelhofer T, Tim RC, et al. High-throughput massively parallel sequencing for fetal aneuploidy detection from maternal plasma. *PLoS One*. 2013;8(3):e57381. doi: 10.1371/journal.pone.0057381. Epub 2013 Mar 6.
2. Lefkowitz RB, Tynan J, Liu T, et al. Clinical validation of a non-invasive prenatal test for genome-wide detection of fetal copy number variants. *Am J Obstet Gynec*. doi: http://dx.doi.org/10.1016/j. ajog.2016.02.03.

Tables + Figures

Figure 1. Case 1



SCA plot



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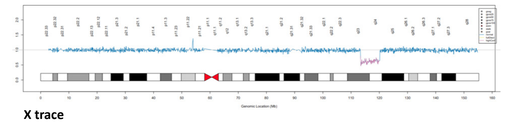
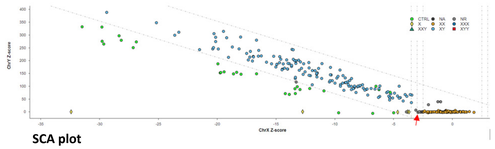


X trace

Figure 2. Case 2



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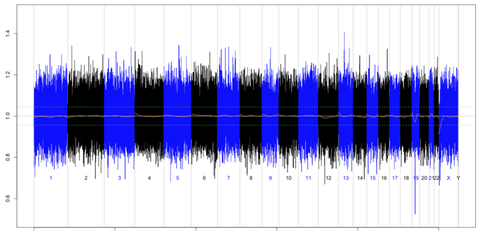

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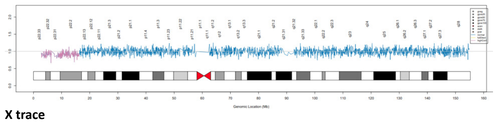
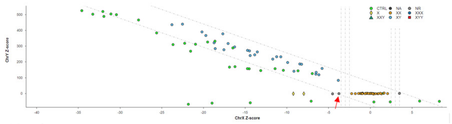


X trace

Figure 3. Case 3



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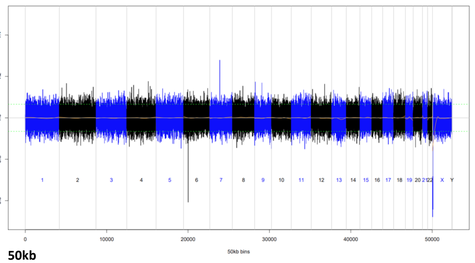

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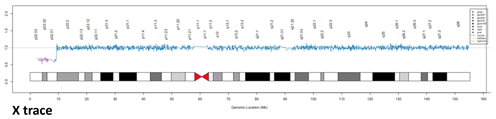

X trace

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