Disclosure Slide

Financial Disclosure for:
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Clinical utilization of a cfDNA expanded content 'reflex' pathway: A case series

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Introduction

Increased clinical adoption of expanded genome-wide content on cfDNA screening has allowed patients and providers to receive additional information regarding risk for fetal chromosome conditions. In most cases, initial cfDNA screening for trisomies 21, 18, 13 and sex chromosome aneuploidies will return with negative, or low-risk results. However, later in a pregnancy, ultrasound findings, contributory updates to personal or family history, or other new data may lead to a desire for additional information. Ideally, diagnostic testing via amniocentesis or chorionic villus sampling should be performed in these cases, but when patients decline diagnostic testing, they are left without non-invasive options (other than ultrasound) to provide additional information. The introduction of an expanded genome-wide "reflex" pathway, allows patients who decline diagnostic testing to obtain additional information about their pregnancies. These case examples illustrate the clinical utilization of genome-wide cfDNA screening when additional information is desired subsequent to negative initial cfDNA results.

Methods

Maternal blood samples were submitted for cfDNA screening of the core trisomies (trisomies 21, 18, and 13), subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing as previously described.[1] Sequencing data were analyzed using novel algorithms as previously described.[2] Residual specimen is retained until approximately 42 weeks gestation based on the information provided on the test requisition form. At the request of the provider, a deeper genome-wide re-sequencing was performed and data analyzed using a novel algorithm as previously described.[3] In two cases, residual sample was available and tested; in one case (case 2), a new sample was requested as no residual specimen was available. Clinical outcomes were requested from ordering providers as part of routine follow-up of positive cases.

In addition to screening for core trisomies, the 'standard' cfDNA assay allows providers to order sex chromosome aneuploidies and/or the 'enhanced sequencing series' (ESS) encompassing microdeletion syndromes located at 1p36, 4p16.3, 5p15.2, 8q23.2q24.1, 11q24.1, 15q11q13, and 22q11.2 as well as trisomies 16 and 22 as an 'opt in' to testing. Case 1 did not order sex chromosome aneuploidies (SCAs) but opted to include ESS. Case 2 opted to include SCAs but did not order ESS. Case 3 did not opt in to either ESS or SCAs.

Results

Table 1 summarizes the clinical details for the cases described in the series. **Figures 1, 2, 3** show the cfDNA traces for each of the cases. For the genome-wide (black and blue) traces, the arrows show the deviations prompting the positive results.

Table 1: Summary of clinical details for each case

	Maternal age	Gestational age at cfDNA orders	cfDNA indications	cfDNA turnaround time	cfDNA results	Diagnostic testing and pregnancy outcome
Case 1	33	**Initial cfDNA sample:** 20+0	**Initial sample:** None listed	**Initial sample:** 2.00 days	**Initial result:** Normal	Amniocentesis with array confirmed 2.83 Mb terminal deletion of 4pter->4p16.3 (Wolf-Hirschhorn syndrome) and 9.73 Mb terminal duplication of 22q13.2->qter, suggestive of an unbalanced translocation in the fetus; parental studies via FISH recommended and were normal.
		Genome-wide cfDNA: 23+3	**Reason for genome-wide cfDNA:** IUGR and cardiac defect	**Genome-wide cfDNA:** 3.21 days	**Genome-wide cfDNA result:** Abnormal, suggesting ~9.65 Mb dup on 22q13.2-q13.33 and ~2.2 Mb deletion of 4p16.3	**True positive**
Case 2	18	**Initial cfDNA sample:** 11+4	**Initial sample:** None listed	**Initial sample:** 2.09 days	**Initial result:** Normal	Pregnancy termination due to cardiac anomaly; testing on products of conception (POC) confirmed 3.15 Mb deletion of 22q11.2 (originally ordered karyotype on POC but canceled and changed to array due to findings on cfDNA).
		Genome-wide cfDNA: 21+1	**Reason for genome-wide cfDNA:** Tetralogy of Fallot	**Genome-wide cfDNA:** 3.09 days	**Genome-wide cfDNA result:** Abnormal, suggesting ~2.75 Mb deletion of 22q11.2	**True positive**
Case 3	34	**Initial cfDNA sample:** 18+0	**Initial sample:** Abnormal cfDNA at another lab (details not given, but desired more information), increased NT	**Initial sample:** 2.80 days	**Initial result:** Normal	Previous miscarriage with different partner but no testing completed; Amniocentesis with array confirmed 70.8 Mb terminal deletion of Xpter->q13.1 and 27.1 Mb terminal duplication of 17pter->q11.2
		Genome-wide cfDNA: 20+0	**Reason for genome-wide cfDNA:** Same as initial cfDNA	**Genome-wide cfDNA:** 5.00 days	**Genome-wide cfDNA result:** Abnormal, suggesting ~27.05 Mb dup of 17p13-q11.2 and ~68.35 Mb del of Xp22.33-q13	Maternal chromosomes confirmed she carries the translocation 46,X,t(X;17)(q12;q11.2); Fetal results updated to reflect this: 46,X,-X,+der(17)t(X;17)(q12;q11.2)mat **True positive**

Figure 1: Case 1 genome-wide cfDNA traces

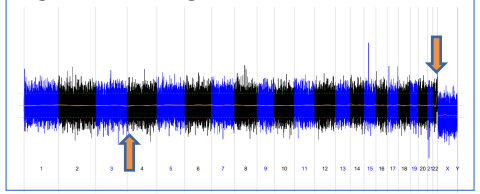


Figure 2: Case 2 genome-wide cfDNA traces

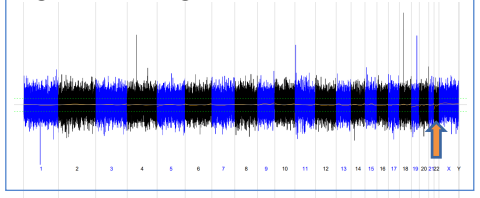
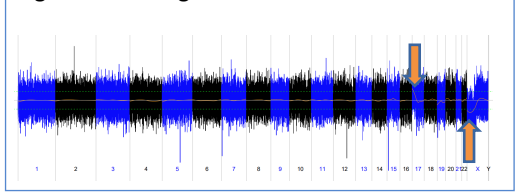


Figure 3: Case 3 genome-wide cfDNA traces



Conclusions

This case series demonstrates clinical examples in which expanded analysis via genome-wide cfDNA provided additional information to the patient and provider. While prenatal microarray or other diagnostic testing provides the most complete information about fetal status for chromosome abnormalities, patients may decline this option and/or wish to exhaust all screening options before considering diagnostic testing. A pathway for obtaining expanded information after a traditional cfDNA test allows patients to have flexibility in decision making and offers providers another tool for patient care.

References:

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3. 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. Gynecol*. doi:http://dx.doi.org/10.1016/j. ajog.2016.02.03.