Fetal Fraction Amplification in Noninvasive Prenatal Screening: Impact on Fetal Sex Chromosome Analysis

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INTRODUCTION

- Noninvasive prenatal screening (NIPS) is widely used to screen for common aneuploidies (Trisomy 21, 18, 13) as well as to analyze the sex chromosomes for detection of aneuploidy and prediction of fetal sex. The proportion of cell-free DNA originating from the placenta, also known as the fetal fraction (FF), is a quality control metric which can impact accuracy of NIPS, with low FF resulting in test failures in many laboratories.
- A custom whole genome sequencing (WGS) NIPS utilizing FF amplification (FFA) has been shown to improve accuracy of aneuploidy detection compared to standard NIPS (Welker, 2020) and to virtually eliminate low FF samples.
- The clinical impact of FFA on fetal sex chromosome analysis and fetal sex prediction is investigated in the case series presented here.

RESULTS

- Five samples met the criteria for inclusion.

Table 1. Impact of FFA on Concordance of Sex Chromosome Results

	Fetal Fraction (Original)	Sex Chromosome Result (Original)	Fetal Fraction with FFA	Sex Chromosome Result with FFA	Clinical Details	Clinical Concordance with FFA Result
Patient 1	6.2%	XX	9.6%	XY	Male by ultrasound	Concordant
Patient 2	8.9%	XY	21.6%	XY	PGT-A Female; Male by ultrasound	Concordant
Patient 3	2.1%	XX	4.6%	XY	Male by ultrasound	Concordant
Patient 4	8.2%	XX	16.4%	XO	45,X/46,XX by amnio	Concordant
Patient 5	12.3%	XX	32.3%	XO	Male by ultrasound	Discordant

METHODS

- Samples received for clinical NIPS were evaluated on a customized WGS-based platform prior to implementation of FFA.
- Healthcare providers and patients were able to report fetal sex call discordance to the laboratory.
- Samples reported as being discordant for fetal sex call were selected for reanalysis with FFA by the following criteria:
  - NIPS report was screen negative with fetal sex prediction of XX or XY
  - No ultrasound abnormalities were indicated
  - Sufficient sample was available in the laboratory for reanalysis
- Eligible samples were reanalyzed with FFA.

DISCUSSION

- NIPS is the most accurate prenatal screening method for aneuploidy detection. However, both biological and technical limitations ensure its status as a screening test rather than a replacement for diagnostic methods.
- The technical limitation of NIPS' ability to discern fetal chromosome status at lower FF can be largely overcome with FFA technology, and this is illustrated by the patient examples that became clear concordant calls with FFA.
- Biological limitations such as mosaicism (confined to placenta or present in the fetus) contribute to discrepancies that will persist with NIPS regardless of NIPS platform used or FF of the analyzed sample. Even so, FFA did improve concordance even in the setting of confirmed fetal mosaicism (Patient 4).
- One sample was reported as discordant with PGT-A analysis. Analysis of this sample with FFA did not change the fetal sex call; however, later ultrasound at 20 weeks was concordant with NIPS (Patient 2). This seemingly highlights the limitations of PGT rather than NIPS. PGT is also not diagnostic and higher confidence in fetal sex chromosome calling with FFA may alert providers in this scenario to confer early with the fertility center to investigate potential sources of discordance.
- The lone sample (Patient 5) that remained discordant after FFA reanalysis (45,X call in male pregnancy by ultrasound) may be an example of an insurmountable biological limitation of NIPS, as this case had the highest FF in the series (32.3%) and extremely high confidence.

CONCLUSION

- The clinical case series presented here provides concrete examples of improved fetal sex chromosome calling with FFA technology, a direct result of the achievement of increased FF combined with a highly customized WGS-based NIPS.
- Four of the 5 reported discrepancies were concordant with FFA reanalysis.
- The improvement in concordance in this series offers clinical evidence that FFA improves fetal sex prediction as well as detection of fetal sex chromosome abnormality.

REFERENCES: **1.** Welker, N.C., et al. *Genet Med.* 2020. https://doi.org/10.1038/s41436-020-01009-5