Experience with resequencing and reanalysis of genome-wide cell-free DNA specimens for expanded content

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1. Introduction

Genome-wide cell-free DNA (cfDNA) screening provides information about traditionally-screened chromosome abnormalities as well as subchromosomal copy number variants (CNVs) and rare autosomal aneuploidies. At least one commercial laboratory offers an option to resequence and reanalyze a sample initially tested using traditional cfDNA screening, employing genome-wide analysis when clinical concern is identified later in the patient's pregnancy. Here we describe the outcome of the first 782 reportable samples utilizing this testing pathway.

2. Methods

Maternal blood samples were submitted for traditional cfDNA screening (for trisomies 21, 18, and 13) via massively parallel sequencing with "opt-in" analysis of sex chromosome aneuploidies and microdeletions/trisomies 16 and 22. After results from traditional screening were reported, residual specimen was banked for the duration of the patient's pregnancy in case a reanalysis was requested by the clinician. For this reanalysis, samples were resequenced at a higher depth of read, and sequencing data were analyzed using a novel algorithm to detect aneuploidies and other subchromosomal events as described by Lefkowitz et al.[1] Cases were compiled from internal databases and outcome data were collected, when available, from ordering providers.

3. Results

- Analysis of the first 782 reportable cases yielded 34 positive results, for a positivity rate of 4.3%.
- The average fetal fraction in these 782 cases was 9.8%. The average gestational age of rescreened patients was 16.4 weeks (n=632), and the average maternal age was 31 years (n=633). (**Table 1**).
- The indication for resequencing was provided for 354 cases, with 27% of cases indicating ultrasound findings (either as an isolated indication for testing or in combination with other high-risk indications). (**Figure 1**)
- The positive findings from resequencing and reanalysis consisted of: (15) subchromosomal CNVs ≥7Mb, (4) rare autosomal trisomies (including one case of trisomy 22), (9) monosomy X, (4) 22q11.2 deletions, (1) trisomy 21, and (1) trisomy 18. (**Figure 2**)
- Of the 34 positive results, 18 (53%) provided information not detectable by traditional cfDNA screening.
- The laboratory had access to diagnostic outcomes for 10 of the 34 positive cases: (8) CNVs – all confirmed, (1) 22q11.2 deletion – confirmed, and (1) trisomy 7 – not confirmed. (**Figure 3**)

Positivity rate of resequenced and reanalyzed cases
4.3%

41% of the positive findings from resequencing had the opportunity to be identified by an expanded version of the traditional cfDNA assay.*

Had the originally-ordered test included expanded analysis, patients could have received positive results an average of
40 days earlier

*Expanded: cfDNA screening that includes analysis for sex chromosome abnormalities, microdeletions, and trisomy 16 and 22

4. Conclusions

A review of cfDNA specimens that underwent resequencing and reanalysis via genome-wide testing showed a range of findings, including disorders not identifiable by traditional screening, and conditions that may have been identified earlier in pregnancy had an expanded version of traditional cfDNA been ordered. This data may assist clinicians in making more informed decisions about cfDNA screening options.

Learning objective

The participant shall be able to assess the clinical utility of a genome-wide resequencing and reanalysis pathway for cell-free DNA screening following traditional cell-free DNA analysis.

Tables + Figures

Table 1. Metrics from resequencing and reanalysis cases

Metrics of reportable cases	
Average gestational age (n=632)	16.4 weeks
Average maternal age (n=633)	31 years
Average fetal fraction (n=782)	9.8%

Figure 1. Indication for testing in resequencing cases (n=354)

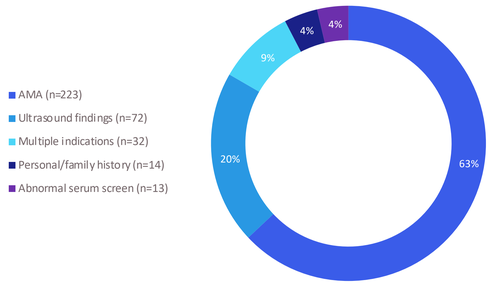
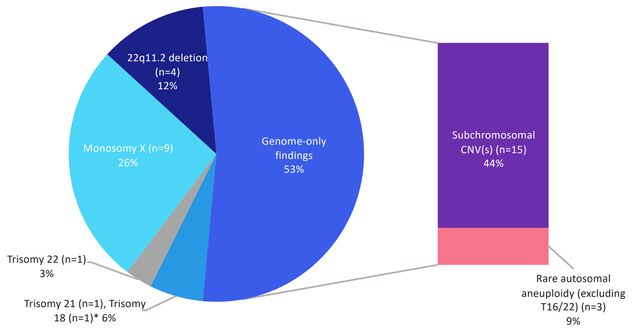
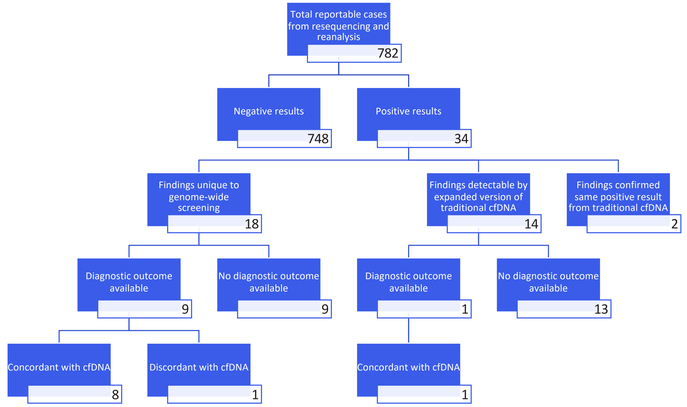


Figure 2. cfDNA findings in positive resequencing and reanalysis cases (n=34)



*Results consistent with previously-reported aneuploidy from initial analysis

Figure 3. Overview of resequencing and reanalysis cases, including diagnostic outcomes



References

1. 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

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