No Chromosome Abnormality Left Behind: When Karyotype Following Positive cfDNA Screening Does Not Tell the Full Story

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Introduction

Cell-free fetal DNA (cfDNA) testing is now a routine part of prenatal care for many patients. This testing can be performed by several laboratory technologies, including targeted analysis using SNP or modified array (DANSR) techniques and massively parallel sequencing (MPS). ACOG practice bulletin 226 recommends diagnostic testing with chromosomal microarray for patients who desire comprehensive prenatal testing. However, currently no guidelines exist on what type of testing, karyotype or chromosomal microarray is recommended after a positive cfDNA result. The goal of this study was to determine the incidence of microarray abnormalities in patients with a normal fetal karyotype after positive cfDNA testing.

Methods

This study included patients who were referred for prenatal genetic counseling from January to December 2020 for positive cfDNA results, elected diagnostic testing and had a normal karyotype followed by SNP chromosomal microarray testing (n=209). Positive cfDNA results came from a variety of laboratories performing testing by different methodologies (targeted and MPS). Patients referred for cfDNA results consistent with "low fetal fraction" and CNVs (including deletions of 22q11.21) were excluded from the study (n= 34), leaving 175 patients.

Results

The 175 patients who met inclusion criteria ranged from 20 to 44 years of age. The positive cfDNA results included common trisomies (21, 18 and 13) (n= 26); "atypical finding" (59); 1/17 risk for trisomy 18, trisomy 13 or triploidy (31); 45, X (33), XXY, XYY and XXX (19); rare autosomal trisomies (17) and triploidy (11). Diagnostic testing was performed by either CVS or amniocentesis. Microarray analysis was abnormal in 31/175 (17%) of these patients. The majority (70%) of patients with abnormal microarray results had cfDNA analysis performed through SNP-based technology. Details of the abnormal microarray results are presented in Table 1. The abnormal microarray results included a variety of findings illustrated by some examples (Table 2).

Conclusions

In this study of patients who had positive cfDNA results, normal karyotype, and chromosomal microarray testing, 31 out of 175 (17%) of cases were abnormal. This number may be an underestimation given not all patients with a normal karyotype had microarray analysis performed. Based on these findings, microarray analysis provides clinically significant information as a follow-up to abnormal cfDNA results. Microarray analysis should be considered as a first-tier test in patients with positive cfDNA results when undergoing diagnostic testing. In the 31 abnormal microarray results, 22 patients (70%) had cfDNA performed by SNP-based methodology. This highlights the importance of performing microarray analysis particularly when patients have had cfDNA testing through this method. As cfDNA testing continues to be offered to more patients through a variety of technologies, results should be evaluated carefully in order to choose the follow-up testing that will provide the most useful clinical information.

Table 1. Abnormal Microarray Results (n= 31)

cfDNA Result	Number of Cases	NIPT Methodology	Microarray Results
Atypical Finding	15	15 SNP	10 CNV 3 SCA 2 ROH
45, X	5	2 SNP 1 MPS 2 Targeted	2 SCA 2 CNV 1 ROH
1/17 risk for: tri18, tri13 or triploidy	3	3 SNP	3 CNV
Trisomy 21	3	1 SNP 1 MPS 1 Targeted	2 CNV 1 Mosaic T21
Trisomy 13	2	2 MPS	1 Mosaic T13 1 ROH
47, XXY	1	1 SNP	1 SCA
Trisomy 22	1	1 Targeted 1 MPS	2 UPD Chrom 22
Trisomy 4	1	1 MPS	1 UPD Chrom 11

Key: CNV (copy number variant), SCA (sex chromosome abnormality), ROH (regions of homozygosity), MPS (massively parallel sequencing), UPD (uniparental disomy).

Table 2. Selection of Abnormal Microarray Results

cfDNA Result	45, X	45, X	Atypical Finding	Trisomy 13	Trisomy 21
Microarray Result	Mosaic loss of an entire X chromosome (mosaic Turner syndrome)	73 KB deletion of 22q12.1->22q.12.1 (likely pathogenic deletion of CHEK2	2.5 MB terminal deletion of 18q23->q23 (pathogenic deletion associated with multiple anomalies)	Mosaic gain of an entire chrosome 13 (mosaic trisomy 13)	1.12 MB deletion of 8p22->8p22 (deletion of uncertain significance; overlapping deletions associated with intellectual disability)

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