

# When ultrasound anomalies are present: An estimation of the frequency of chromosome abnormalities not detected by cell-free DNA aneuploidy screens

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## Abstract

**Objectives:** This study characterizes cytogenetic abnormalities with ultrasound findings to refine counseling following negative cell-free DNA (cfDNA).

**Methods:** A retrospective cohort of pregnancies with chromosome abnormalities and ultrasound findings was examined to determine the residual risk following negative cfDNA. Cytogenetic data was categorized as cfDNA detectable for aneuploidies of chromosomes 13, 18, 21, X, or Y or non-cfDNA detectable for other chromosome abnormalities. Ultrasound reports were categorized as structural anomaly, nuchal translucency (NT)  $\geq 3.0$  mm, or other "soft markers". Results were compared using chi squared and Fishers exact tests.

**Results:** Of the 498 fetuses with cytogenetic abnormalities and ultrasound findings, 16.3% (81/498) had non-cfDNA detectable results. In the first, second, and third trimesters, 12.4% (32/259), 19.5% (42/215), and 29.2% (7/24) had non-cfDNA detectable results respectively. The first trimester non-cfDNA detectable results reduced to 7.7% (19/246) if triploidy was detectable by cfDNA testing. For isolated first trimester NT of 3.0–3.49 mm, 15.8% (6/38) had non-cfDNA detectable results, while for NT  $\geq 3.5$  mm, it was 12.3% (20/162). For cystic hygroma, 4.3% (4/94) had non-cfDNA detectable results.

**Conclusions:** Counseling for residual risk following cfDNA in the presence of an ultrasound finding is impacted by gestational age, ultrasound finding, and cfDNA detection of triploidy.

## 1 | INTRODUCTION

Since its introduction in October of 2011, prenatal genetic screening for aneuploidy through analysis of cell-free DNA (cfDNA) from maternal serum has been established as a sensitive and specific screening method for autosomal trisomy of chromosomes 13, 18, and 21.<sup>1,2</sup> CfDNA was initially indicated for "high risk" women including those 35 years of age and older, a history of a fetus affected by trisomy of covered chromosomes, a parental balanced Robertsonian translocation involving chromosomes 13 or 21, a fetal ultrasound indicating increased risk of aneuploidy, and positive first or second trimester maternal serum screening results.<sup>3,4</sup> These indications were based on eligibility criteria in the initial validation studies for cfDNA, not upon epidemiologic studies of population-wide screening.<sup>5</sup>

In 2015, the Society for Maternal-Fetal Medicine and the American College of Obstetricians and Gynecologists published a joint

Committee Opinion with updated recommendations, stating that if an anomaly is identified on ultrasound a diagnostic test should be offered rather than cfDNA.<sup>6</sup> This change followed a paper by Benachi et al noting that in patients with an abnormal ultrasound and low risk cfDNA, 7.9% of the fetuses had an undetected abnormal cytogenetic finding on karyotype (8.5% if the 3 cases of false negative trisomy 18 are accounted for). The characteristics of these remaining chromosomal aberrancies have received less attention.<sup>5</sup> Additionally, microarray findings also contribute to the overall rate of chromosomal disorders among fetuses with ultrasound anomalies.<sup>7-10</sup>

Since the introduction of cfDNA, reports of decreasing numbers of diagnostic procedures have emerged from several facilities.<sup>11-13</sup> Many women will accept cfDNA but not diagnostic prenatal testing due to the small risks of fetal loss associated with diagnostic procedures.<sup>14,15</sup>

If women with an ultrasound finding forgo diagnostic studies for cfDNA screening, these less common but clinically significant

chromosome abnormalities will remain undetected until newborn or pediatric evaluation.

The objective of this study was to examine, in a retrospective fashion, cytogenetic results following diagnostic prenatal testing initiated for an ultrasound finding. The intent was to examine variables influencing the distribution of cytogenetic anomalies detectable by cfDNA and to provide estimates of residual risk for incorporation into patient counseling.

## 2 | METHODS

We performed a retrospective cohort study of all fetuses with cytogenetic testing, isolating those with an abnormal result (fluorescent in situ hybridization [FISH], karyotype, microarray) between January 2005 and December 2014 at the Center for Advanced Molecular Diagnostic Cytogenetics laboratory in Boston, Massachusetts, which receives samples primarily from Brigham and Women's Hospital and Massachusetts General Hospital. Data were abstracted from internal databases containing records of all samples tested in the time frame. Study samples were retained for initial statistical analysis if they met the following criteria: a prenatal sample such as amniotic fluid or chorionic villi and an abnormal cytogenetic or array finding and a fetal ultrasound finding. The experience of cfDNA in this population and our aneuploidy screening protocol has previously been described by Dobson et al, and in the time frame there were 114 women with positive cfDNA of whom 71 (62%) underwent diagnostic testing.<sup>16</sup>

Abnormal cytogenetic findings included mosaicism, deletions, duplications, aneuploidy, inversions, and translocations identified through karyotype, FISH, or microarray analysis. Microarray findings included known pathogenic, likely pathogenic, and variants of uncertain significance copy number variants (CNVs) as well as large regions of homozygosity greater than 10 Mb in size. Fetal chromosome studies were performed on samples obtained by amniocentesis or chorionic villus sampling by institutional protocol. Giemsa banded karyotypes and FISH studies were obtained by standard protocols. Microarray investigations were initiated in 2014 utilizing an Affymetrix CytoScan HD platform. We performed a retrospective review of microarray results to review variants of unknown significance (HMS). Inherited balanced translocations and common inversions (eg, common pericentric inversions of chromosomes 1, 9, 16) were excluded given their limited clinical significance to the underlying ultrasound finding after cytogeneticist review (HMS). Apparently balanced de novo rearrangements were retained in the abnormal population given their higher risk for birth defects and cognitive impairment.<sup>17</sup> Aneuploidy of whole chromosome changes of 13, 18, 21, X, and Y was considered detectable by cfDNA without correction for sensitivity. Mosaics, deletions, duplications, triploidy, markers, and de novo balanced and unbalanced inversions and translocations were assumed to be non-cfDNA detectable. Case reports suggest that detection of mosaicism by cfDNA is not reliable.<sup>18-20</sup> Additionally, there is no data available to suggest that cfDNA can detect unbalanced or de novo balanced rearrangements. Multiple pregnancies were included in the analysis and categorized as cfDNA detectable or not based on their genetic findings.

### What's already known about this topic?

- Maternal serum screening for aneuploidies has changed since the introduction of cell-free DNA (cfDNA) in 2011.
- Diagnostic testing with chorionic villus sampling or amniocentesis is recommended in the presence of a structural ultrasound finding.

### What does this study add?

- When ultrasound findings are present, the rate of cytogenetic anomalies not detectable by cfDNA increases with increasing fetal gestational age.
- The size of first trimester nuchal translucency is inversely related to the likelihood of cfDNA detected aneuploidy.
- 16.3% of cytogenetic abnormalities would likely be missed by cfDNA in the presence of an ultrasound finding.

Ultrasounds were performed in accordance to national guidelines, by registered diagnostic medical sonographers with final interpretation and report by Maternal Fetal Medicine or Radiology attending physicians.<sup>21</sup> Ultrasound reports were reviewed, and patients were categorized into 1 of 3 groups: (1) no recognized anomaly or soft marker, (2) presence of a structural ultrasound anomaly, or (3) soft marker for aneuploidy. Those with no ultrasound findings were excluded from the study. All ultrasounds in the pregnancy were reviewed. The second trimester soft markers included those associated with aneuploidy according to the Eunice Kennedy Shriver National Institute of Child Health and Human Development Joint Report and were categorized as isolated or multiple.<sup>22</sup> Fetuses with both a structural anomaly and a soft marker were categorized as having a structural anomaly. Nuchal translucency (NT) greater than 3.0 mm for fetuses with a crown-rump length between 34 and 84 mm and fetuses with cystic hygroma were included in the category of structural anomalies. Two clinicians (RMR, LWH) independently reviewed categorizations to verify accuracy. Ultrasounds were categorized by first trimester (11 weeks and 0 days through 13 weeks and 6 days), second trimester (14 weeks and 0 days through 27 weeks and 6 days), or third trimester (28 weeks and above).

Statistical analyses included parametric and nonparametric statistics with Student's T tests for continuous variables and chi-square analysis with Fisher exact correction for categorical variables with *P*-values of <0.05 considered significant. All analyses were conducted using the statistical package SPSS version 24.0 (SPSS Inc, Chicago, IL). The Partners Human Research Committee Institutional Review Board authorized and approved this study (protocol 2015O000148 approved 2/10/2015). Patient consent was waived in this retrospective study, and data were housed in a de-identified secure database.

### 3 | RESULTS

There were 4725 women who underwent prenatal diagnostic testing from 2005 to 2014 (Figure 1). Upon clinical review, the ultrasounds of the karyotypically abnormal studies ( $N = 651$ ) included those with a structural anomaly ( $N = 446$ ), soft markers ( $N = 52$ ), and no ultrasound anomaly or soft marker ( $N = 153$ ) (Figure 1). The resultant confirmed abnormal ultrasound cohort contained 498 patients with chromosomal abnormalities detected by FISH or karyotype (see Table 1). The mean maternal age was 33.9 (range 16–49). There were 238 (47.8%) women 35 years of age or older. The mean gestational age at diagnostic testing was 15.7 (range 10.1–37.9) weeks gestational age. There were 254 diagnostic tests performed after ultrasound from 11 + 0/7 through 13 + 6/7 weeks gestational age. There were 273 (54.8%) samples from chorionic villi and 225 (45.2%) from amniotic fluid (Table 1).

Due to the limited sample size of patients with sonographic findings and microarray analysis, these were removed from the overall cohort and presented separately. Additionally, a broader time period with inclusion until February of 2016 was reviewed. From 2014 to 2016, 12.6% (12/95) of fetuses with ultrasound findings had a microdeletion, microduplication, or large region of homozygosity (>10 Mbs) not detectable by routine karyotype analysis. Six of the CNVs were classified as variants of uncertain significance, which brings the known pathogenic CNV percentage to 6.3% (6/95). Only 1 of the 12 aberrations identified is currently available from cfDNA testing facilities (Table 2, Supplementary Table 2). The balanced translocations and isochromosomes detected by karyotype were identified prior to the introduction of microarray in our population.

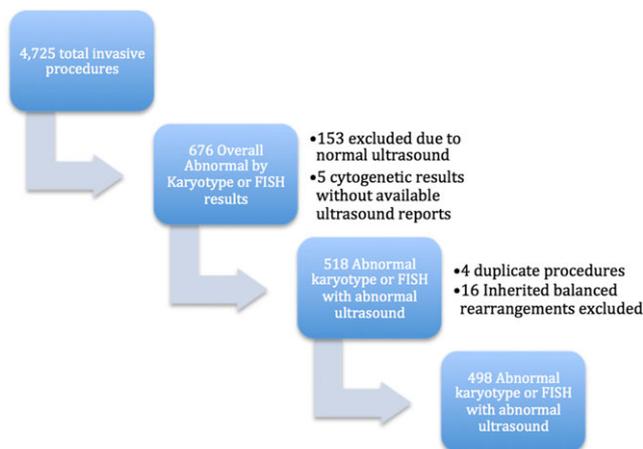
In the 498 patients with a sonographic finding and abnormal karyotype, 216 (43.4%) had trisomy 21 (either as a nondisjunction event or an unbalanced Robertsonian translocation), 102 (20.5%) had trisomy 18, 43 (8.6%) had trisomy 13, and 56 (11.2%) had sex chromosome aneuploidy. There were 81 patients (16.3%) with non-cfDNA detectable fetal karyotype abnormalities by the previously stated assumptions (see methods). These 81 non-cfDNA detectable abnormalities

**TABLE 1** Demographic characteristics of the 498 pregnancies diagnosed with genetic abnormalities by amniocentesis or chorionic villus sampling from 1/1/2005 to 12/31/2014 at 2 academic medical centers

Characteristics	<i>n</i> (%) or $\mu \pm SD$
Overall cohort <i>n</i> (%)	
Maternal age at diagnostic testing	
Mean (y)	33.9 $\pm$ 6.1
Minimum-maximum (y)	16–49
< 35 years old	260 (52.2)
$\geq$ 35 years old	238 (47.8)
Gestational age at diagnostic testing	
Mean (weeks)	15.7 $\pm$ 4.8
Minimum-maximum (weeks)	10.1–37.9
First trimester test	259 (52.1)
Second trimester test	215 (43.2)
Third trimester test	24 (4.8)
Specimen type	
Amniotic fluid	225 (45.2)
Chorionic villi	273 (54.8)
Genetic studies	
Karyotype only	135 (27.1)
Karyotype and FISH	363 (72.9)
Chorionicity	
Singleton	473 (95.0)
Twins	23 (4.6)
Triples	2 (0.4)

included the following (some samples had more than 1 genetic finding identified): 21 (25.9%) triploidy, 20 (24.7%) mosaic for any chromosome, 15 (18.5%) rare autosomal trisomy, and 36 (44.4%) duplications, deletions, markers, rings, isochromosomes, unbalanced rearrangements, or de novo balanced translocations. All de novo balanced translocations and markers were identified prior to the introduction of microarray studies. Of the mosaics, 13/20 (65.0%) involved mosaicism of chromosomes 13, 18, 21, X, or Y (Table 3). Overall, 25 of the non-cfDNA detectable abnormalities included mosaicism, deletions, duplications, rings, or marker chromosomes with genetic material from chromosomes 13, 18, 21, X, or Y and an additional 3 fetuses had deletions at chromosome 22q11. If these were detected by cfDNA, then the non-cfDNA detectable percentage would become 11.2%.

The abnormal ultrasound cohort was further analyzed by subcategories reflecting the type of ultrasound indication (structural anomaly or soft marker) and gestational age at recognition of the ultrasound anomaly. Analysis of structural anomalies compared with second trimester soft markers revealed no significant difference in the distribution between cfDNA-detectable and non-cfDNA detectable findings ( $P = 0.21$ ). However, trisomy 21 and trisomy 18 considered separately were significantly more associated with structural anomalies. The trimester at diagnosis of an ultrasound finding had a significant difference in the likelihood of a non-cfDNA detectable aneuploidy. When an ultrasound finding (inclusive of nuchal translucency >3.0 mm) was detected in the first trimester, a non-cfDNA detectable chromosome abnormality was present in 12.4% (32/259), whereas in the second and third trimesters, this non-cfDNA detectable



**FIGURE 1** Flowchart of inclusion and exclusion from the cohort of diagnostic procedures with ultrasound anomalies in the multicenter cohort from the years 2005 to 2014 [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 2** Microarray abnormalities in fetus with sonographic findings and a normal karyotype from 2014 to 2016

Microarray Abnormality	Indication	Size and Clinical Implications
arr[hg19] 22q11.21(18626108-21800797)×3	Hydrocephalus, suspect absent cerebellum at 14 + 0/7 weeks	3.17-Mb gain, pathogenic a (microduplication syndrome)
rr[hg19]2q31.1(176,917,342-177,073,747)×3-4	Cystic placenta, tetralogy of Fallot, short forearms and abnormal hands, hyperextended legs, possible hemivertebrae, dislocated elbows at 17 + 4/7 weeks	156-kb gain duplication, VUS
arr[hg19] 7q21.12q21.13(88,158,041-89,913,566)×3-4	Micrognathia, hydrops at 18 + 3/7 weeks	1.75-Mb gain, VUS
arr[hg19] Xq11.1q13.3(61,932,503-75,285,868)hmz	Suspect molar or cystic placenta at 18 + 4/7 weeks	13.35-Mb region AOH, VUS
arr[hg19]1q41q42.11(223,318,279-224,491,618)×1	Congenital diaphragmatic hernia at 14 + 0/7 weeks	1.17-Mb deletion, likely pathogenic (microdeletion syndrome)
arr[hg19] 10p13p11.21(14,413,424-34,728,086)hmz,Xq21.33q24(97,183,583-118,843,904)hmz	Complex congenital heart defect and umbilical cord mass at 20 + 6/7 weeks	2 regions of AOH > 20 Mb, VUS
arr[hg19] 6p21.1(45,291,520-45,307,250)×1	Clubbed foot and trigonocephalic skull shape, short long bones at 13 + 3/7 weeks	15.7-kb deletion, likely pathogenic (contains RUNX2 associated with Cleidocranial dysplasia)
arr[hg19] mos 2p25.3p21(15,702-42,505,301)hmz	Double outlet right ventricle with ventricular septal defect and transposition of the great vessels at 19 + 1/7 weeks	42.5-Mb region of AOH, VUS
arr[hg19] Yq11.223q11.23(25,640,805-27,811,878)×1,16p11.2(29,591,078-30,177,807)×3	High grade aortic stenosis and small ascending aortic arch at 22 + 1/7 weeks	1.3-Mb deletion, 587-kb gain, pathogenic (azoospermia, microduplication syndrome)
arr[hg19] Xp22.33p22.33(401660-12999178)×1	Absent corpus callosum, pes excavatum, congenital diaphragmatic hernia, displaced kidney, and intrauterine growth restriction in the 0 percentile at 30 + 3/7 weeks	12.6-Mb deletion (56 genes, including SHOX, STS, KAL1), pathogenic
arr[hg19]14q23.1q24.2(62,001,355-72,747,523)×2 hmz	Complex congenital heart defect including hypoplastic left ventricle, possible ventricular septal defect with hypoplastic overriding aorta at 29 + 0/7 weeks	10.5-Mb AOH, VUS
arr[hg19] 11q24.1q25(123,422,107-134,938,470)×1	Duodenal atresia, polyhydramnios, and single ventricle heart at 34 + 0/7 weeks	11.5-Mb deletion, pathogenic (Jacobson syndrome)

Abbreviations: AOH, absence of heterozygosity; VUS, variant of uncertain significance.

<sup>a</sup>Deletions at this locus are available on some cfDNA platforms. While a gain may be detected, it is unlikely to be reported. Please see Supplementary Table 2 for copy number variants available at the time of publication.

percentage was 19.5% (42/215) and 29.2% (7/24), respectively ( $P = 0.02$ ). Triploidy comprised a significantly greater percentage of non-cfDNA detectable findings in the first as opposed to the second trimester: 13/32 (40.6%) vs 8/42 (19.0%) ( $P = 0.03$ ), respectively.

For the pregnancies with isolated second trimester soft markers, there were 7 fetuses with an isolated soft marker and non-cfDNA detectable cytogenetic findings (Table 4). In 4 of these 7, there was an abnormal serum screen prior to the detailed ultrasound showing an isolated soft marker. Four of these patients chose a selective reduction or termination based upon their cytogenetic testing and ultrasound findings, 1 patient with a de novo balanced translocation between chromosomes 15 and 18 had a vaginal delivery of a phenotypically normal infant after normal microarray, 1 patient with mosaic sex chromosome aneuploidy 45,X<sup>3</sup>/46,XY<sup>23</sup> delivered a term phenotypically normal male, and 1 patient did not have available outcome data.

As seen in Table 4C, there were 21 pregnancies with cfDNA-detectable findings and an isolated second trimester soft marker. Most these cases were trisomy 21; however, there was 1 trisomy 18 case with an isolated choroid plexus cyst in a patient with a high-risk serum screen. In this patient, the ultrasound was done at 17 + 6/7 weeks gestational age, and both hands were reported to be open (Table 4C).

As seen in Table 4B, when looking at first trimester nuchal translucency, there were 294 patients with an increased nuchal translucency

of  $\geq 3.0$  mm or cystic hygroma with crown-rump length between 34 and 84 mm. Of these 294 fetuses, 38 had a nuchal translucency between 3.0 and 3.4 mm, 162 had a nuchal translucency  $\geq 3.5$  mm without a cystic hygroma, and 94 ultrasounds reported a cystic hygroma. For the ultrasounds with increased nuchal translucency (3.0 mm–3.4 mm vs  $\geq 3.5$  mm without a cystic hygroma), there was no difference in proportions of cfDNA detectable and non-cfDNA detectable results ( $P = 0.59$ ). However, both of these categories were significantly different from the cystic hygroma group, which was more likely to have trisomy 18 or sex chromosome aneuploidy and less likely to have trisomy 21, trisomy 13, or non-cfDNA detectable changes ( $P < 0.001$ , Table 4B). For an isolated first trimester nuchal translucency of 3.0 to 3.49 mm, the chromosome abnormality was detectable by cfDNA in 84.2% (32/38), whereas for an isolated first trimester nuchal translucency of  $\geq 3.5$  mm without a cystic hygroma, it was 87.7% (142/162) detectable by cfDNA, and for first trimester cystic hygroma, it was 95.7% (90/94) detectable by cfDNA (Table 4B).

## 4 | DISCUSSION

This study describes the ultrasound findings and prenatal cytogenetic abnormalities in a cohort of 498 patients from January 2005 to

**TABLE 3** Cytogenetic abnormalities detected through amniocentesis and chorionic villus sampling after ultrasound finding from 1/1/2005 to 12/31/2014

Cytogenetic Abnormality	n	%
Number of fetuses with cytogenetic abnormalities	498	100.0%
Common aneuploidies	417	83.7%
Trisomy 21 and Robertsonian translocations	216	43.4%
Trisomy 18 <sup>(a)</sup>	102	20.5%
Trisomy 13	43	8.6%
Sex chromosome aneuploidy	56	11.2%
Number of fetuses with non-cfDNA detectable cytogenetic abnormalities	81	16.3%
Triploidy	21	4.2%
Deletions	18	3.6%
Trisomy <sup>(b)</sup>	15	3.0%
Duplications	8	1.6%
Ring or marker chromosomes	6	1.2%
Monosomy <sup>(b)</sup>	5	1.0%
Unbalanced rearrangements	4	0.8%
Balanced de novo non-Robertsonian translocations	2	0.4%
Isochromosomes	2	0.4%
Mosaic <sup>(c)</sup>	20	4.0%

<sup>a</sup>The following 2 samples were categorized under trisomy 18 but had an additional cfDNA detectable abnormality: 48,XXY,+18, 48,XXX,+18.

<sup>b</sup>The following samples had more than 1 category of cytogenetic finding, the assigned category is shown in parentheses: mos 45,X [13]/46,X,+mar [7] der(Y) (monosomy); 48,XY,+10,+18 (trisomy); 47,XX,+21 [16]/48,XX,+12,+21 [4] (trisomy); 47,XXX [6]/45,X [4]/46,XX [13] (monosomy).

<sup>c</sup>Mosaic samples are represented in their category of primary abnormality.

December 2014 at multiple academic medical centers in Boston, Massachusetts. The information presented can be useful for counseling patients about the residual risk of a karyotype abnormality after cfDNA taking ultrasound information into account. If assuming 100% sensitivity for detection of the major aneuploidies by cfDNA (13,18,21,X, Y), in 16.3% of these fetuses with ultrasound findings, there was a non-cfDNA detectable karyotype abnormality. The sensitivity of cfDNA for the major chromosomes varies by chromosome and by testing methodology.<sup>24</sup> If incorporating false negatives and no call results into the estimate, we anticipate the rate of undiagnosed karyotype anomalies will be higher than the 16.3% noted.

Our analysis provides a larger sample size and expands the clinical findings presented by Benachi et al in a multicenter trial performed to evaluate the eligibility criteria for cfDNA for prenatal screening<sup>5</sup> (Supplementary Table 3). Among their subset with abnormal ultrasounds ( $n = 376$ ), they found 7.9% (29/290) fetuses with negative cfDNA had a cytogenetic finding other than trisomy 13, 18, or 21. Their cfDNA assay did not include sex chromosome aneuploidy. Additionally, they reported 3 false negative trisomy 18 and 1 false positive trisomy 13 cfDNA results. Among these 29 cases, there were 13 cases with sex chromosome aneuploidy and 6 cases with nonpathogenic CNVs. When considering a comparable population to ours (all abnormal karyotypes in fetuses with ultrasound findings), 13.6% (16/117) of patients with ultrasound abnormalities would have clinically

relevant cytogenetic changes not detectable by cfDNA. This rate is lower than our estimate of 16.3%. However, the distribution of karyotype findings among the ultrasound abnormal population mirrored our findings with trisomy 21 being the most common followed by trisomy 18, trisomy 13, and sex chromosome aneuploidy.

Further literature contains a single center study by Shani et al that identified 220 cases of cytogenetic findings following a referral indication of an ultrasound finding (Supplementary Table 3). Forty-five percent, (99/220) were predicted non-cfDNA detectable with correction for assay sensitivity. Additionally, of these 99 cases, 47 were submicroscopic changes identified by microarray.<sup>23</sup> For comparability to our estimates, if the microarray findings, balanced translocations, and confined placental mosaicism are removed from this study, 27.7% of their cytogenetic findings would not have been detected by cfDNA without correction for sensitivity. The distribution of cytogenetic findings among non-cfDNA detectable also differed. Microarray-detected submicroscopic chromosomal changes were the most prevalent, followed by mosaicism, unbalanced structural rearrangements, then triploidy. These differences likely reflect different indications for testing and referral patterns.

Overall, our findings add to the literature on the performance of cfDNA as a screening test for the common aneuploidies but with limitations in settings when other chromosome abnormalities may play a role. Three findings in particular have implications for clinical care. Among fetuses with ultrasound findings, 16.3% of clinically relevant karyotypic abnormalities were non-cfDNA detectable. This included 21 fetuses with triploidy. While considered not detectable in our study, some SNP-based platforms provide coverage for triploidy,<sup>25,26</sup> If triploidy is ascertained by the cfDNA platform, our overall rate of not detectable cytogenetic anomalies drops further to 12.0% overall and 7.3% (19/259) following first trimester ultrasound findings. Secondly, gestational age at recognition of the ultrasound abnormality is related to the likelihood of a cfDNA detectable chromosome abnormality. With first trimester ultrasound findings, the majority of which represent increased nuchal translucency ( $\geq 3.0$  mm), the occurrence of the common aneuploidies detectable by cfDNA is higher than in the second trimester (87.6% and 80.5% respectively). Lastly, among the studies initiated for second trimester ultrasound findings, the proportion of cytogenetic results considered non-cfDNA detectable did not differ between those with a structural anomaly compared with those with single or multiple soft markers. As our overall sample size with soft markers and a non-cfDNA detectable abnormality is small and our study did not include normal karyotypes, this last finding may have further clinical relevance if replicated in a larger study. Whether to pursue amniocentesis for definitive diagnosis in the setting of a soft marker and a negative cfDNA will require further investigation with larger populations.

Our analysis of microarray findings is limited by small sample size. However, the rate of additional abnormality detected in the presence of an ultrasound anomaly (12.6%) is similar to that reported in a recent meta-analysis by Hillman et al (11.2%).<sup>8</sup> While the coverage for microduplications and deletions continues to expand for cfDNA, only 1 of the 12 identified submicroscopic findings in this ultrasound indicated cohort is present on the currently available cfDNA platforms and the sensitivity and specificity is lower than that for whole

**TABLE 4** Distribution of genetic abnormalities by gestational age and sonographic findings among 498 fetuses with ultrasound findings and chromosomal abnormalities from 2005 through 2014

A) Trimester of Ultrasound Finding by type of genetic abnormality	First Trimester (n = 259) n(%)	Second Trimester (n = 215) n(%)	Third Trimester (n = 24) n(%)	P <sup>a</sup>	
Detectable by cfDNA (n = 417)	227 (87.6)	173 (80.5)	17 (70.8)	P = 0.02	
Trisomy 21 (n = 216)	115 (44.4)	95 (44.2)	6 (25.0)	P = 0.17	
Trisomy 18 (n = 102)	60 (23.2)	38 (17.7)	4 (16.7)	P = 0.30	
Trisomy 13 (n = 43)	18 (6.9)	19 (8.8)	6 (25.0)	P = 0.01	
Sex chromosome aneuploidy (n = 56)	34 (13.1)	21 (9.8)	1 (4.2)	P = 0.27	
Not detectable by cfDNA (n = 81)	32 (12.4)	42 (19.5)	7 (29.2)	P = 0.02	
B) Nuchal translucency by type of genetic finding <sup>b</sup>	First trimester NT 3.0–3.49 mm (n = 38) n(%)	First trimester NT ≥ 3.5 mm without cystic hygroma (n = 162) n(%)	First trimester cystic hygroma (n = 94) n(%)	P <sup>a</sup>	
Overall detectable by cfDNA (n = 264)	32 (84.2)	142 (87.7)	90 (95.7)	P < 0.0001	
Trisomy 21 (n = 140)	25 (65.8)	84 (51.9)	31 (35.1)	P < 0.0001	
Trisomy 18 (n = 63)	2 (5.3)	29 (17.9)	32 (34.0)	P = 0.001	
Trisomy 13 (n = 16)	4 (10.5)	9 (5.6)	3 (3.2)	P = 0.01	
Sex chromosome aneuploidy (n = 43)	1 (2.6)	20 (12.3)	22 (23.4)	P < 0.0001	
Not detectable by cfDNA (n = 30)	6 (15.8)	20 (12.3)	4 (4.3)	P < 0.0001	
C) Ultrasound finding by type of genetic abnormality	Structural anomalies including NT ≥ 3.0 mm (n = 446) n(%)	Isolated second trimester soft markers (n = 28) n(%)	Multiple second trimester soft markers (n = 12) n(%)	First trimester absent nasal bone or EIF (n = 12) n(%)	P <sup>a</sup>
Detectable by cfDNA (n = 417)	373 (83.6)	21 (75.0)	12 (100)	11 (91.7)	P = 0.22
Trisomy 21 (n = 216)	178 (40.0)	17 (60.7)	12 (100)	9 (69.2)	P < 0.0001
Trisomy 18 (n = 102)	99 (22.2)	1 (3.6)	0	2 (16.7)	P = 0.03
Trisomy 13 (n = 43)	43 (9.6)	0	0	0	P = 0.14
Sex chromosome aneuploidy (n = 56)	53 (11.9)	3 (10.7)	0	0	P = 0.36
Not detectable by cfDNA (n = 81)	73 (16.4)	7 (25.0)	0	1 (8.3)	P = 0.22

<sup>a</sup>P values for differences in trimesters, nuchal translucency, and ultrasound findings across categories were obtained by Chi squared test for categorical variables. NT, nuchal translucency; EIF, echogenic intracardiac focus; first trimester ultrasounds were performed between 11 + 6/7 and 13 + 6/7 weeks gestational age, second trimester ultrasounds were performed between 14 + 0/7 and 27 + 6/7 weeks gestational age.

<sup>b</sup>Only fetuses with a crown-rump length between 34 and 84 mm and NT ≥ 3.0 mm included in analysis.

chromosome changes. The other findings identified in this study by microarray are unlikely to be added to current cfDNA platforms because of their small size or low incidence (Table 2). This emphasizes the point that while collectively microarray abnormalities occur at a rate of 1 in 1000, for the majority their location is scattered throughout the genome.

This study was not designed to be an assessment of the performance of cfDNA versus standard screening; many other authors have addressed this question.<sup>2,27,28</sup> Assumptions of detection of aneuploidy by cfDNA were made without correction for sensitivity. As testing cut-offs and methodologies can change, the results presented here represent the gold standard of cytogenetic assessment and can be applied to future screening models with varying performance. However, modeling of cfDNA performance is less useful than large studies with

cfDNA and diagnostic testing. While the detection rate of cfDNA for trisomy 21 is high (99.7%), sensitivities for the remainder of the common aneuploidies and the sex chromosomes are lower (80%–90%),<sup>6,24</sup> As such, the rates established in this study should be used with caution when applied to aneuploidy other than trisomy 21.

This study benefits from clinical review of ultrasounds and delineation of specific ultrasound findings rather than reliance upon lab requisitions or diagnosis codes. Detailed review of the cytogenetic database enabled distinctions such as the relevance of inherited versus de novo balanced translocations which enriched the detail and relevance of the cytogenetic anomalies included. For limitations, while the sample size for the karyotype assessment is robust, the sample size of microarray studies is limited and thus was presented separately. This highlights an area of future focus and but limits the utility of microarray

data in this study. Additionally, as the initial cohort was defined as those with an abnormal prenatal cytogenetic result, women who terminated based on an abnormal ultrasound alone or those who had postnatal diagnosis were not captured in this study. This could introduce bias and limit the generalizability of our results. This paper represents the experience of a single large cytogenetics lab that serves several hospitals in the greater Boston area, which further limits the generalizability to other populations.

## 5 | CONCLUSIONS

In a cytogenetically abnormal cohort ascertained due to ultrasound findings, we found a 16.3% rate of karyotype abnormalities of clinical significance that were non-cfDNA detectable. This rate lowers to 12.0% with triploidy assessment by SNP-based platforms. This rate differs by gestational age with the lowest rate of non-cfDNA detectable aneuploidy in the first trimester (12.4% if triploidy is not detected and 7.3% in triploidy is detected). Abnormal chromosome results in pregnancies with a first trimester cystic hygroma or NT  $\geq 3.5$  mm were more likely to be detected by cfDNA than NT 3.0 to 3.5 mm. However, the true rate is likely higher than our estimate as cfDNA has less than 100% sensitivity especially for trisomies 13 and 18 and the sex chromosomes. Microarray analysis further contributes important clinical information with a wider distribution of deletion and duplication aberrancies than available currently through cfDNA.

## DECLARATIONS

- The authors report no conflicts of interest.
- There was no funding received for this project.
- Some portions of the study were presented at the 2015 International Society for Prenatal Diagnosis meeting in Washington, D. C, July 14<sup>th</sup>, 2015 under the title "Characterization of ultrasound abnormalities associated with the common aneuploidies detectable by Noninvasive Prenatal testing (NIPT)."

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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