OBSTETRICS

Cell-free DNA screening for trisomy 21 in twin pregnancy: a large multicenter cohort study

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BACKGROUND: Analysis of cell-free DNA from maternal blood provides effective screening for trisomy 21 in singleton pregnancies. Data on cell-free DNA screening in twin gestations are promising although limited. In previous twin studies, cell-free DNA screening was primarily performed in the second trimester and many studies did not report chorionicity.

OBJECTIVE: This study aimed to evaluate the screening performance of cell-free DNA for trisomy 21 in twin pregnancies in a large, diverse cohort. A secondary aim was to evaluate screening performance for trisomy 18 and trisomy 13.

STUDY DESIGN: This was a retrospective cohort study of twin pregnancies from 17 centers for which cell-free DNA screening was performed from December 2011 to February 2020 by one laboratory using massively parallel sequencing technology. Medical record review was conducted for all newborns and data on the birth outcome, the presence of any congenital abnormalities, phenotypic appearance at birth, and any chromosomal testing that was undertaken in the antenatal or postnatal period were extracted. Cases with a possible fetal chromosomal abnormality with no genetic test results were reviewed by a committee of maternal-fetal medicine geneticists. Cases with a vanishing twin and inadequate follow-up information were excluded. A minimum of 35 confirmed cases of trisomy 21 was required to capture a sensitivity of at least 90% with a prevalence of at least 1.9% with 80% power. Test characteristics were calculated for each outcome.

RESULTS: A total of 1764 samples were sent for twin cell-free DNA screening. Of those, 78 cases with a vanishing twin and 239 cases with inadequate follow-up were excluded, leaving a total of 1447 cases for inclusion in the analysis. The median maternal age was 35 years and the median gestational age at cell-free DNA testing was 12.3 weeks. In total, 81% of the twins were dichorionic. The median fetal fraction was 12.4%. Trisomy 21 was detected in 41 of 42 pregnancies, yielding a detection rate of 97.6% (95% confidence interval, 83.8—99.7). There was 1 false negative and no false positive cases. Trisomy 21 was detected in 38 out of 39 dichorionic twin pregnancies, yielding a detection rate of 97.4% (95% confidence interval, 82.6—99.7). Trisomy 18 was detected in 10 of the 10 affected pregnancies. There was 1 false positive case. Trisomy 13 was detected in 4 of the 5 cases, yielding a detection rate of 80% (95% confidence interval, 11.1—99.2). There was one false negative and no false positive cases. The nonreportable rate was low at 3.9 %.

CONCLUSION: Cell-free DNA testing is effective in screening for trisomy 21 in twin gestations from the first trimester of pregnancy. Detection of trisomy 21 was high in dichorionic and monochorionic twins, and the nonreportable result rates were low. This study included high numbers of cases of trisomy 18 and 13 when compared with the current literature. Although screening for these conditions in twins seems to be promising, the numbers were too small to make definitive conclusions regarding the screening efficacy for these conditions. It is possible that cell-free DNA testing performance may differ among laboratories and vary with screening methodologies.

Key words: aneuploidy, cell-free DNA, Down syndrome, noninvasive prenatal screening, noninvasive prenatal testing, screening, trisomy, trisomy 21, twin

Introduction

Analysis of cell-free DNA (cfDNA) from maternal blood provides an effective method of screening for trisomy 21 in singleton pregnancies. A meta-analysis that included 1963 cases of trisomy 21 and 223,932 nontrisomy 21 singleton pregnancies from 35 studies reported a weighted pooled detection rate and false positive rate of 99.7% (95% confidence interval [CI], 99.1—99.9) and 0.04% (95% CI, 0.02—0.07), respectively.[1]

cfDNA screening in twin gestations presents unique challenges. Aneuploidy rates in twins are relatively low,[2,3] which makes it challenging to perform an adequately powered prospective trial. In addition, there are unique technical challenges associated with cfDNA screening in twin gestations. Although the fetal fraction of cfDNA in the maternal plasma is higher in twin pregnancies, the individual contribution from each fetus is lower than for singleton pregnancies.[4—8] The overall increased cfDNA fetal fraction should lead to equivalent or improved detection rates in monozygotic twins who almost always have the same genotype. However, a lower fetal fraction will potentially make aneuploidy detection more challenging in dizygotic twins among whom aneuploidy is likely to affect only 1 fetus. Single nucleotide polymorphism (SNP) analyses in twin pregnancies have demonstrated that the individual cfDNA concentrations contributed by each fetus are only moderately correlated with each other and there is a possibility that one fetal fraction can be high and the other below the cutoff for reliable testing.4 Thus, a high contribution from a normal co-twin could mask a low fetal fraction

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AJOG at a Glance

Why was this study conducted?

Data on cell-free DNA screening for trisomy 21 in twin gestations are limited because of the small number of affected cases. Professional societies have acknowledged that there is a need for additional studies to validate cell-free DNA screening performance in twin gestations.

Key findings

Cell-free DNA screening for trisomy 21 in dichorionic and monochorionic twin gestations is effective from the first trimester and the nonreportable result rates are low.

What does this add to what is known?

This study included a large number of twin cases of trisomy 21, 18, and 13, confirming the effectiveness of screening for trisomy 21 and adding to the published cases of trisomy 18 and 13. This study demonstrates that cell-free DNA screening in twin gestations is effective in the first trimester and in dichorionic twin gestations.

of an affected fetus, leading to a false negative result.9 In addition, factors known to be associated with a lower fetal fraction and thus cfDNA test failure in singleton pregnancies, including higher maternal weight and in vitro fertilization (IVF) conception, are more common in twin pregnancies.[9−11]

Data on cfDNA screening in twins are promising although limited. The majority of published studies include small numbers of fetuses affected with trisomy 21. Furthermore, cfDNA screening was performed primarily in the second trimester in the majority of previous studies.[12−24] A prospective multicenter study that included data from 961 twin pregnancies in England reported a 100% detection rate for trisomy 21 (95% CI, 75−100).9 There were no false negative or false positive cases; however, 1 of 3 cases with no reported result was noted to have trisomy 21, and the mean gestational age at the time of cfDNA screening was 15 weeks with a range of 10 to 36 weeks. Although the study aimed to target 15 pregnancies with 1 or both fetuses affected by trisomy 21, the total number of trisomy 21 pregnancies was 13 despite increasing the initial intended study sample size. Updated data from the Fetal Medicine Research Institute on first-trimester cfDNA screening on a cohort including 1272 twin pregnancies noted a 95.0% detection rate for trisomy 21, a 90% detection rate for trisomy 18, and a 50% detection rate for trisomy 13 in the first trimester.[25]

A recent practice bulletin from the American College of Obstetricians and Gynecologists stated that cfDNA screening can be performed in twin gestations although it acknowledged that the total number of affected cases is small.[26] The International Society of Ultrasound in Obstetrics and Gynecology has acknowledged the potential role of cfDNA as a screening tool for trisomy 21 in twin pregnancies and highlighted the need for additional studies to validate its performance.[27]

The primary goal of this study was to evaluate the screening performance of cfDNA for trisomy 21 in a large, diverse cohort. A secondary aim was to evaluate the screening performance for trisomy 18 and trisomy 13.

Materials and Methods

This is an investigator-initiated (L.D.), retrospective cohort study of twin pregnancies from 17 centers for which cfDNA screening was performed from December 2011 to February 2020 by one laboratory using massively parallel sequencing technology. The test detects the relative amount of chromosomal material, an over- or underabundance of which is associated with an aneuploidy or other chromosomal abnormalities. To generate a reportable result, the sample must pass various quality control assessments including, but not limited to, coverage of the whole genome and a minimum fetal fraction requirement that was dictated by the version of the assay in use at the time when the sample had been reported. Imposed fetal fraction requirements are roughly proportional to the fetal number (ie, approximately double the requirement for twins when compared with singletons), and this minimum fetal fraction decreased with newer versions of the assay becoming available.[28] This study received institutional review board approval or exemption from all 17 centers. The study was registered with ClinicalTrials.gov under registration number NCT04488393.

All cases sent to the laboratory for twin cfDNA analysis from each of the 17 centers over the study period were included. Cases with a known vanishing twin, inadequate follow-up information, and screening for a second twin pregnancy in the same patient were excluded. Maternal demographic characteristics, pregnancy outcome data, fetal ultrasound reports, genetic test results, and newborn follow-up data were obtained through medical record review. Deidentified genetic study results, ultrasound reports, placental pathology results, and autopsy reports (when performed) were uploaded to the database. Cases with a possible fetal chromosomal abnormality with no genetic test results were reviewed by a committee of maternal-fetal medicine geneticists. Every case was reviewed by the primary study investigator (L.D.). Chorionicity was determined by ultrasonography. This was confirmed using placental pathology reports when available.

A minimum of 35 confirmed twin pregnancies with 1 or both fetuses affected by trisomy 21 was required to capture a sensitivity of at least 90% with a prevalence of at least 1.9% with 80% power. Descriptive statistics were reported using frequencies for categorical variables and medians with interquartile ranges for continuous variables. We calculated the test characteristics in

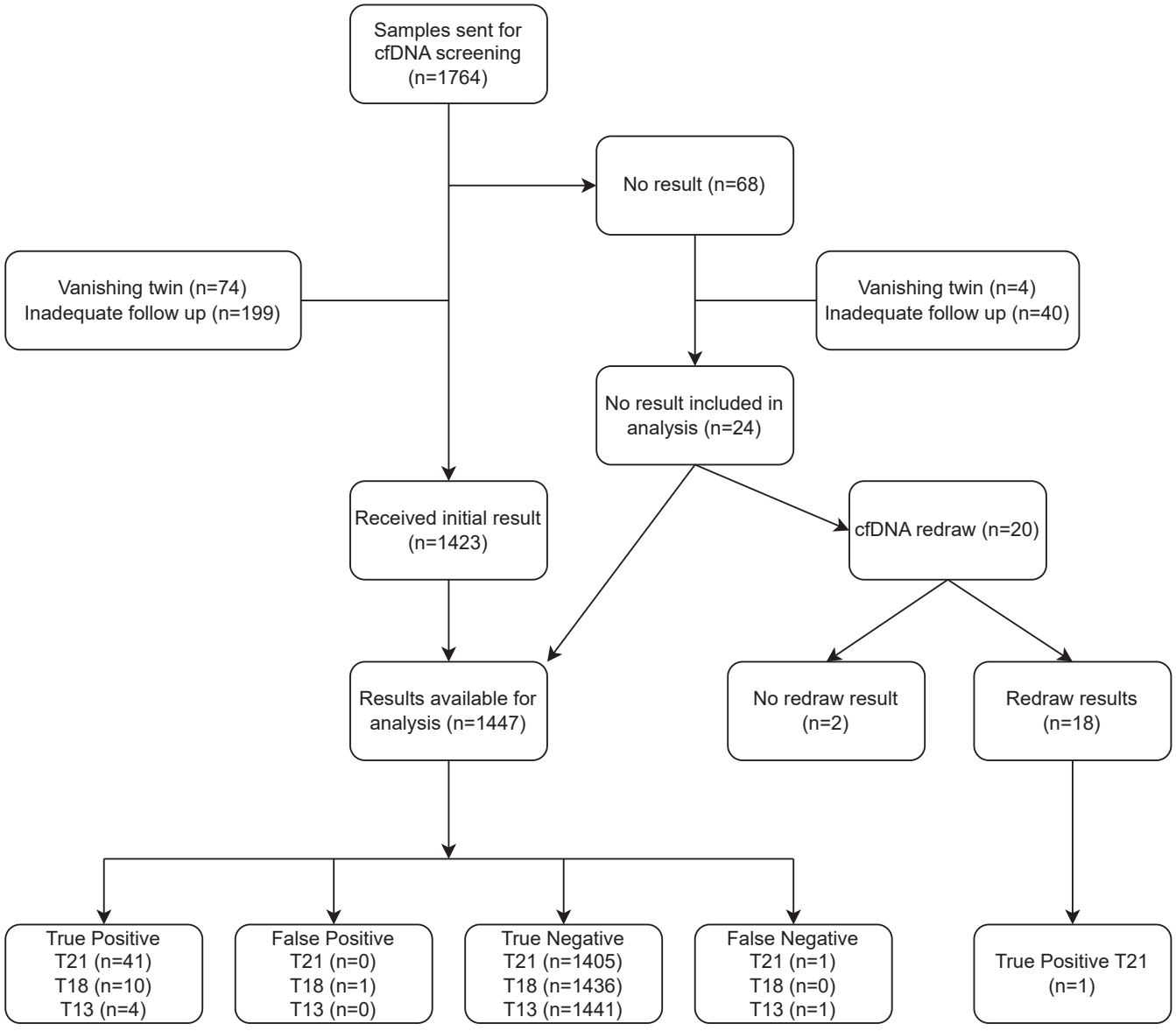
terms of sensitivity, specificity, false positive rate, and the incidence of trisomy 21, trisomy 18, and trisomy 13. For the test characteristics, 95% Wilson score CIs were calculated. Statistical analyses were performed using Stata 17.0 (StataCorp, College Station, TX).

Results

A total of 1764 samples were sent for twin cfDNA screening from the 17 centers (Figure). Of those, 78 cases with a vanishing twin and 239 cases with inadequate follow-up data were excluded, leaving a total of 1447 cases for inclusion in the analysis.

The characteristics of the study population are summarized in Table 1. The median maternal age was 35 years, and the median maternal body mass index (BMI) was 25.1 kg/m^2. The majority of pregnant individuals who underwent cfDNA testing were White with only 11% of the study population consisting of women who identified as Black and another 11% of women who identified as Asian. Of the cohort, 81% of the twins were dichorionic and 42% of the twin pregnancies were conceived via IVF. The median gestational age at the time of blood sample collection for cfDNA testing was 12.3 weeks with 79% of the cfDNA screening tests performed in the first trimester and 19% performed in the second trimester. The median fetal fraction was 12.4%. In the study

FIGURE
Flow diagram of twin cell-free DNA samples



cfDNA, cell-free DNA.

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TABLE 1
Baseline characteristics of the study population

Characteristic	Median (IQR) or n (%)
Maternal age (y)[a]	35 (33–38)
Race	
White	987 (68)
Black	155 (11)
Asian	160 (11)
Other	145 (10)
Gravidity	
1	480 (33)
2	380 (26)
≥3	587 (41)
GA at blood sample collection (wk)	12.3 (11.4–13.6)
Maternal BMI (kg/m^2)	25.1 (22.3–29.7)
Conception by IVF	611 (42)
Twin type[b]	
Monoamniotic monochorionic	18 (1)
Diamniotic monochorionic	260 (18)
Diamniotic dichorionic	1166 (81)
Fetal fraction (%)	12.4 (9.8–15.4)
Trimester of blood draw	
First trimester	1140 (79)
Second trimester	277 (19)
Third trimester	30 (2)
Abnormalities before cell-free DNA screening (n=406)	
Positive first trimester combined test or quad screen	32 (8)
Increased NT (≥3.0 mm) or cystic hygroma in 1 or both twins	21 (5)
Other structural abnormality in 1 or both twins	7 (2)

BMI, body mass index; *GA*, gestational age; *IQR*, interquartile range; *IVF*, in vitro fertilization; *NT*, nuchal translucency; *SD*, standard deviation.

[a] The mean (SD) is given; [b] Placental pathology reports were available to confirm the chorionicity for 1257 of 1447 (86.9%) cases. Of the 190 twin pregnancies with no placental pathology available, 63 cases had discordant sex consistent with dichorionic placentation. We were unable to establish chorionicity for 3 of the 1447 cases in which no ultrasound or pathology report was available and the sex of the twins was concordant.

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population, there were 57 pregnancies with the 3 common autosomal trisomies, including 42 (2.9%) with trisomy 21, 10 (0.7%) with trisomy 18, and 5 (0.3%) with trisomy 13 (Figure). There were no statistically significant differences between the study population and the 239 cases with inadequate follow-up with respect to the median maternal age, race, BMI, and gestational age at the time of blood sample collection for cfDNA testing.

The screening characteristics for trisomies 21, 18, and 13 are presented in Table 2. Of the 42 twin pregnancies with 1 or both fetuses affected by trisomy 21, 41 had true positive results and there was 1 false negative in the case of a dichorionic twin pregnancy, giving a sensitivity of 97.6% (95% CI, 83.8–99.7). Of the 41 true positive cases, 38 were dichorionic; 37 of these cases had 1 twin with trisomy 21, and in 1 case, both twins had trisomy 21. There were no false positive cases, giving a specificity of 100% (95% CI, 99.7–100). There was no difference (P=.11) in the gestational age at the time of the blood sample collection for cfDNA testing between the 42 positive trisomy 21 cases (mean, 13.3±3.4 weeks) and the 1390 cases with normal results (mean, 14.2±3.8 weeks). We were unable to obtain accurate data on which pregnancies that were conceived via IVF underwent preimplantation genetic testing for aneuploidy. A total of 18 (3.0%) cases of trisomy 21 occurred among twins conceived by IVF compared with 24 (2.9%) cases among twins conceived naturally. All 10 of the trisomy 18 cases were detected and there was 1 false positive result. All of the trisomy 18 cases were dichorionic and had 1 affected twin. Four of the 5 trisomy 13 cases were detected, and all 5 trisomy 13 cases were among dichorionic twins with 1 affected twin.

Of the initial 1764 samples, 68 (3.9%) had a nonreportable result for the first blood sample. Of these cases, 41 had a second blood sample collected and 24 (58.5%) received a result for the second sample. Of the initial 68 cases with a nonreportable result, 4 were excluded because of a vanishing twin and 40 cases were excluded because of inadequate follow-up. Of the samples included in the analysis, 24 (1.7%) had an initial nonreportable result. The median fetal fraction was 6.8% for these 24 nonreportable cases. Twenty of these patients opted to have a second blood sample taken, and 18 (90%) received a result (Figure). One of the cases with an initial nonreportable result had a true positive result for trisomy 21 for the second blood sample test (Figure). There were no fetal aneuploidies diagnosed for the 4 cases that did not have a repeat blood sample taken for cfDNA analysis after an initial nonreportable result.

Comment

Principal findings

cfDNA screening for trisomies 21, 18, and 13 in twin pregnancy was evaluated

TABLE 2
Screening characteristics for trisomies 21, 18, and 13

Trisomy	Chorionicity	True positives	True negatives	False positives	False negatives	Sensitivity (95% CI)	Specificity (95% CI)
21	—	41	1405	0	1	97.6 (83.8—99.7)	100 (99.7—100)
	Di-Di	38	1127	0	1	97.4 (82.6—99.7)	100 (99.7—100)
	Di-Mo	3	257	0	0	100 (43.9—100)	100 (98.6—100)
	Mo-Mo	0	18	0	0	—	100 (82.4—100)
18	—	10	1436	1	0	100 (72.3—100)	99.9 (99.5—100)
	Di-Di	9	1157	0	0	100 (61.0—100)	100 (99.7—100)
	Di-Mo	1[a]	258	1	0	100 (20.7—100)	99.6 (97.3—100)
	Mo-Mo	0	18	0	0	—	100 (82.4—100)
13	—	4	1441	0	1	80.0 (11.1—99.2)	100 (99.7—100)
	Di-Di	4	1160	0	1	80.0 (11.1—99.2)	100 (99.7—100)
	Di-Mo	0	260	0	0	—	100 (98.6—100)
	Mo-Mo	0	18	0	0	—	100 (82.4—100)

Di-Di, diamniotic dichorionic; *Di-Mo*, diamniotic monochorionic; *Mo-Mo*, monoamniotic monochorionic.

[a] Mosaic twin case.

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in a large, geographically diverse, retrospective cohort study of 1764 twin pregnancies evaluated at 17 centers across the United States. cfDNA screening was primarily performed in the first trimester at a median (IQR) gestational age of 12.3 weeks (11.4—13.6). Of the evaluated pregnancies, 81% were dichorionic twins, which may be more technically challenging to screen because the majority are dizygotic and will typically only have 1 twin affected by aneuploidy. Trisomy 21 was detected in 41 of 42 pregnancies, yielding a 97.6% (95% CI, 83.8—99.7) detection rate. There was 1 false negative and no false positive cases. Trisomy 18 was detected in 10 of the 10 affected pregnancies. There was 1 false positive case. Trisomy 13 was detected in 4 of the 5 cases, yielding an 80% (95% CI, 11.1—99.2) detection rate. There was 1 false negative and no false positive cases. The nonreportable rate was low at 3.9 %.

Results in the context of what is known

Our detection rate for trisomy 21 in twin gestations is consistent with previous published studies. A meta-analysis of data from 12 studies, including 137 trisomy 21 and 7507 nontrisomy 21 twin pregnancies, reported a pooled weighted detection rate of 99.0% (95% CI, 92.0—99.9) and a false positive rate of 0.02% (95% CI, 0.001—0.43).[25] In our study, cfDNA screening was primarily performed in the first trimester, whereas in previous studies, cfDNA screening was primarily performed in the second trimester. The detection rate for trisomy 21 among twin gestations in this study is close to the sensitivity reported for cfDNA screening for trisomy 21 in singletons, although our CIs are wider because of the smaller numbers of affected cases.[1] Our detection rate for trisomy 18 is comparable with the detection rate of 92.8% (95% CI, 77.6—98.0) reported in a previous pooled analysis of 50 twin cases.[9] Although all 10 cases were detected in our study, the numbers were too small, yielding wide CIs and making it difficult to draw definitive conclusions regarding the test performance. There is a paucity of reported cases of twin trisomy 13 in literature with only 1 case in a recent study by Khalil and colleagues.9 The pooled weighted detection rate in a total of 11 cases of trisomy 13 and 6290 nontrisomy 13 pregnancies was 94.7% (95% CI, 9.14—99.97).[25] Thus, although the detection for trisomy 13 seems to be promising, albeit inferior to the detection for trisomy 21, the numbers are too small to draw accurate conclusions.

This study, as well as previous studies, confirms that cfDNA screening for trisomy 21 in twin gestations yields higher detection rates and lower false positive rates than conventional screening approaches that use a combination of nuchal translucency (NT) and maternal serum analytes. A meta-analysis of 12,794 twin fetuses with 69 cases of trisomy 21 reported a pooled sensitivity of 89.3% (95% CI, 79.7—94.7) and a pooled specificity of 94.6% (95% CI, 93.3—95.7) for the combined test (NT, pregnancy associated plasma protein-A, and free beta-human chorionic gonadotrophin [hCG]) when conducted in the first trimester. The sensitivity for dichorionic twins was 86.2% (95% CI, 72.8—93.6) with a specificity of 95.2% (95% CI, 94.2—96.0).[29] Although the data are limited, the detection rates for trisomy 21 in twin gestations using second trimester screening of maternal serum alpha-fetoprotein, hCG, unconjugated estriol, and inhibin A are reported to range from 41% to 100%.30

The combination of NT and first and second trimester maternal serum analyte measurements in twins was reported to have a detection rate of 80% in all twins, 93% in monochorionic twins, and 78% in dichorionic twins for a fixed false positive rate of 5% in an analysis using statistical modeling.[31]

Clinical implications

The American College of Obstetricians and Gynecologists, the Society for Maternal-Fetal Medicine, and the International Society of Ultrasound in Obstetrics and Gynecology have acknowledged the need for additional studies to validate the cfDNA screening performance in twins.[26,27] Our study contributes a large number of twin cases of trisomy 21, 18, and 13, confirming the effectiveness of screening for trisomy 21 and adding to the published cases of trisomy 18 and 13.

This study demonstrated that cfDNA screening is effective in the first trimester. This enables patients to have the option to undergo chorionic villus sampling in the setting of a positive result. The results can also potentially provide early reassurance to couples who are reticent to undergo diagnostic testing.

Unlike many of the previous studies, we reported the performance characteristics in terms of chorionicity. Of the 41 true positive trisomy 21 results, 38 were from dichorionic twin gestations and 37 of those had 1 affected twin and were thus likely dizygotic. Although screening in cases of dizygotic twins is potentially more challenging than in monozygotic twin cases, our study indicates that effective screening can be achieved in dizygotic twins.

The no call rate in our study was relatively low. In addition, 58% of patients with an initial no call result received a result for the second blood sample, including 1 true positive result for trisomy 21. Thus, based on these data, we recommend encouraging patients to consider having a second blood sample taken in the setting of an initial no call result. This recommendation should be made in the context of the prenatal ultrasound findings and the patient's desire to proceed with diagnostic testing.

Research implications

Future studies should focus on factors that influence the fetal fraction and the incidence of nonreportable results, as well as the potential screening for additional chromosomal abnormalities and single gene disorders as has been demonstrated fo singleton gestations. In addition, future research should address the disparity in access to screening and the previously reported low uptake rate of prenatal screening in minority ethnic groups and socially deprived groups.[32]

Strengths and weaknesses

Strengths of our study include the large, geographically diverse population with a large number of confirmed trisomies. Another strength is that cfDNA screening was performed in the first trimester in 79% of the cases when the results have the greatest potential clinical value. Another strength of our study is the stratification of test screening performance based on chorionicity. The clinical implications are discussed in the previous section.

Although our study population is geographically diverse, the majority of the patients were White, and the median maternal BMI was 25.1 kg/m^2. It is possible that the test sensitivity and specificity in a twin population with a larger proportion of non-White patients with a higher incidence of obesity may be lower. Because our study was retrospective in nature, we had to rely on medical records for follow-up information although the medical records, including the genetic test results, ultrasound, and autopsy reports, were reviewed by the study principal investigator. The retrospective study design allowed us to obtain the target number of cases of trisomy 21. The increased prevalence of trisomy 21 in this cohort could lead to an increased positive predictive value and a decreased negative predictive value. All of the cfDNA tests were performed by the same laboratory. Although this allowed for consistency, it is possible that cfDNA performance may differ for other laboratories and screening metho dologies.

Conclusion

cfDNA screening is effective for identifying trisomy 21 in twin gestations from the first trimester of pregnancy. The detection rate for trisomy 21 was high in dichorionic and monochorionic twins, and the nonreportable result rates were low. This study included a high number of cases of trisomy 18 and 13 when compared with the current literature. Although screening for these conditions in twin gestations seems to be promising, the numbers were too small to make definitive conclusions regarding the screening efficacy for these conditions. ■

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