OBSTETRICS

Cell-free DNA screening for trisomies 21, 18, and 13 in pregnancies at low and high risk for aneuploidy with genetic confirmation

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BACKGROUND: Cell-free DNA noninvasive prenatal screening for trisomies 21, 18, and 13 has been rapidly adopted into clinical practice. However, previous studies are limited by a lack of follow-up genetic testing to confirm the outcomes and accurately assess test performance, particularly in women at a low risk for aneuploidy.

OBJECTIVE: To measure and compare the performance of cell-free DNA screening for trisomies 21, 18, and 13 between women at a low and high risk for aneuploidy in a large, prospective cohort with genetic confirmation of results

STUDY DESIGN: This was a multicenter prospective observational study at 21 centers in 6 countries. Women who had single-nucleotide- polymorphism-based cell-free DNA screening for trisomies 21, 18, and 13 were enrolled. Genetic confirmation was obtained from prenatal or newborn DNA samples. The test performance and test failure (no-call) rates were assessed for the cohort, and women with low and high previous risks for aneuploidy were compared. An updated cell-free DNA algorithm blinded to the pregnancy outcome was also assessed.

RESULTS: A total of 20,194 women were enrolled at a median gestational age of 12.6 weeks (interquartile range, 11.6e13.9). The genetic outcomes were confirmed in 17,851 cases (88.4%): 13,043

(73.1%) low-risk and 4808 (26.9%) high-risk cases for aneuploidy. Overall, 133 trisomies were diagnosed (100 trisomy 21; 18 trisomy 18; 15 trisomy 13). The cell-free DNA screen positive rate was lower in the low- risk vs the high-risk group (0.27% vs 2.2%; *P*<.0001). The sensitivity and specificity were similar between the groups. The positive predictive value

for the low- and high-risk groups was 85.7% vs 97.5%; *P* .058 for tri- somy 21; 50.0% vs 81.3%; *P* .283 for trisomy 18; and 62.5% vs 83.3; *P* .58 for trisomy 13, respectively. Overall, 602 (3.4%) patients had no- call result after the first draw and 287 (1.61%) after including cases with a second draw. The trisomy rate was higher in the 287 cases with no-call results than patients with a result on a first draw (2.8% vs 0.7%; *P* .001). The updated algorithm showed similar sensitivity and specificity to the study algorithm with a lower no-call rate.

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CONCLUSION: In women at a low risk for aneuploidy, single- nucleotide-polymorphism-based cell-free DNA has high sensitivity and specificity, positive predictive value of 85.7% for trisomy 21 and 74.3% for the 3 common trisomies. Patients who receive a no-call result are at an increased risk of aneuploidy and require additional investigation.

Key words: aneuploidy, cell-free DNA, prenatal screening, trisomy

# Introduction

Original Research

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Noninvasive prenatal testing using cell- free DNA (cfDNA) to screen for fetal chromosomal aneuploidy has seen rapid uptake since 2011.[1](#_bookmark7),[2](#_bookmark8) It was demon- strated to have high sensitivity and speciﬁcity[3](#_bookmark9),[4](#_bookmark10) and be superior to standard

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maternal serum analyte-based screening. Currently, most professional societies recommend cfDNA as an option for primary aneuploidy screening.[4e8](#_bookmark10)

Despite this, the routine offer of cfDNA screening to all patients has not been uniformly adopted. Cost, loss of beneﬁts associated with ultrasound- based screening, and limitations of existing studies in particular are a concern. In addition, some providers may feel that a beneﬁt of primary cfDNA screening over contingency screening in low-risk patients has not been clearly demonstrated. Initial validation studies using genetic conﬁrmation were con- ducted on small cohorts of pregnancies at a high previous risk for aneuploidy.[9](#_bookmark13),[10](#_bookmark14) Conversely, studies on large cohorts that included all-risk populations have been

limited by a lack of genetic conﬁrmation.[11e13](#_bookmark15) This left some doubt as to whether there was underreporting of trisomies and whether the measure- ment of sensitivity and positive predic- tive value, particularly in women at a low risk of aneuploidy, was accurate enough.[2e4](#_bookmark8),[12](#_bookmark16),[13](#_bookmark17) In addition, previous studies have generally excluded cases with a noninterpretable (“no-call”)

result, leaving questions about how this

impacts the overall test performance.[4](#_bookmark10),[14](#_bookmark18) The Single-nucleotide-polymorphism- based Microdeletion and Aneuploidy RegistTry (SMART) was a large pro- spective study designed to evaluate cfDNA performance for the 22q11.2 deletion syndrome and the common trisomies (trisomy 21 [T21], trisomy 18 [T18], and trisomy 13 [T13]) in a

general referral population. A unique aspect of the SMART study was the conﬁrmatory genetic testing requested in all cases through cytogenetic or cytogenomic analysis of fetal samples or chromosome microarray analysis (CMA) of newborn DNA samples, including analysis of cases with no-call cfDNA results. Here we report the re- sults of the SMART study for the pre- natal detection of T21, T18, and T13 in women at low vs high previous risk for aneuploidy.

# Materials and Methods

## Study design and participants

We enrolled pregnant women undergo- ing cfDNA screening for aneuploidy and 22q11.2DS at 21 centers in 6 countries (Supplement #1). The study was approved by each site’s institutional re- view board or ethics committee, and all the participants provided written con- sent. Eligible women who requested and

underwent screening for aneuploidy and 22q11.2 deletion syndrome were 18 years old, 9 weeks’ gestation, had a singleton pregnancy, and planned to deliver at a study site-afﬁliated hospital. Women were excluded if they received a cfDNA result before enrollment, had a history of organ transplantation, conceived using ovum donation, had a

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vanishing twin, or were unwilling or unable to provide a newborn sample. Women who had a serum screening

result for aneuploidy or sonographic detection of fetal anomalies were eligible for inclusion. Women were considered to be at a high risk for aneuploidy if they had a previous positive serum-based (ﬁrst trimester combined or second trimester triple or quadruple) screen for aneuploidy, fetal nuchal translucency (NT) 3.0 mm, an ultrasound-detected anomaly before enrollment, or if the maternal age was 35 years at delivery and no other screening results (eg, serum) were available. The participants did not receive remuneration for enrolling. The results of cfDNA screening were utilized by the providers and patients as part of clinical care.

## Outcomes

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The primary outcome was the test per- formance of single nucleotide poly- morphism (SNP)-based cfDNA for detecting T21, T18, and T13 in partici- pants with a low previous risk for aneuploidy than those at a high risk. The secondary outcomes included the rates of trisomies in cfDNA no-call cases and the test performance of an updated al- gorithm that was made available after enrollment completion.

## Procedures

The sample preparation and analysis of cfDNA were performed as previously described (Natera Inc, Austin, TX).[15](#_bookmark19) Noninvasive prenatal testing results

indicating a risk of 1/100 for a trisomy were categorized as high-risk and those

<1/100 were categorized as low-risk. In cases that did not yield a result, the pa-

Why was this study conducted?

There are limited data on the performance of cell-free DNA (cfDNA) screening for aneuploidy in low-risk populations.

Key ﬁndings

In women at low previous risk for aneuploidy, cfDNA has high sensitivity and

speci*ﬁ*city and a positive predictive value of 85.7% for trisomy 21 and of 74% for trisomies 21, 18, and 13 combined. Patients who receive a failed (no-call) result are at an increased risk of aneuploidy. An updated algorithm has a lower no-call rate while maintaining performance.

What does this add to what is known?

This is the *ﬁ*rst study to assess cfDNA screening performance using genetic con*ﬁ*rmation in a prospective obstetrical population. It adds valuable information on test performance in women at a low risk for aneuploidy and in cases with failed cfDNA tests.

AJOG at a Glance

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tients were offered repeat testing and results after a second draw were included for analysis. During enrollment, the cfDNA laboratory protocol was modi- ﬁed once[10](#_bookmark14); the results from both the periods were combined for analysis (original algorithm).

Independent of the study, the labora- tory developed an updated algorithm optimized to improve the no-call rate at a low fetal fraction using a deep neural network, which utilizes an artiﬁcial in- telligence approach. A deep learning (Tensorﬂow v1.15 [Google Inc., Moun- tain View, CA])[16](#_bookmark20) approach was used to optimally model noise using a deep mixture-of-experts neural network with multiple independent networks, combining the results into a probability score. This self-supervised algorithm leveraged 1.6 million sequenced mix- tures of mother and fetus cfDNA sam- ples, learning to harness linkage among the SNPs to make high-conﬁdence calls for a larger proportion of samples. Deeper sequencing of high-risk calls was applied to lower false positive rates. This updated protocol was assessed after enrollment completion and was blinded to the outcomes.

The genetic outcomes were assessed by CMA through the analysis of DNA from fetal (chorionic villus sampling, amniocentesis, or products of concep- tion) or infant (cord blood, buccal swab, or newborn blood spot obtained for state newborn screening) samples. Postnatal conﬁrmatory samples were obtained at the end of pregnancy in all the cases regardless of the availability of previous prenatal diagnostic genetic testing.

CMA was performed by an indepen- dent laboratory (Center for Applied Genomics, Children’s Hospital of Phila- delphia, Philadelphia, PA) and was blind to the clinical ﬁndings and cfDNA re- sults. For CMA analysis, the DNA was prepared from cord blood, buccal smear, or a dried blood spot. Copy number

variants were identiﬁed using the Illu- mina (San Diego, CA) SNP-based Inﬁnium Global Screening Array (GSA)

platform. The samples were genotyped on standard versions GSA-V1.0, GSA- V2.0, GSAMD-V1.0, or GSAMD-V2.0,

which contain >700,000 SNPs from chromosome 1 to 22 or a custom-

designed SMARTArray in which addi- tional SNPs were added to the GSA backbone. In addition, positive samples underwent conﬁrmation on the Omni 2.5-8V1-3 array and were reviewed by a clinical molecular cytogeneticist before results were generated.

If a postnatal sample for CMA conﬁrmation was not available, results from pre- or postnatal clinical testing with karyotype, quantitative ﬂuorescent polymerase chain reaction (QF-PCR), ﬂuorescence in situ hybridization (FISH), or CMA were used for genetic conﬁrmation, if available.

The cases with mosaicism were considered affected if >80% of cells were trisomic on conﬁrmatory testing. Mosaicism identiﬁed only by chorionic villus sampling (CVS) was not consid- ered as conﬁrmation of genetic outcome. The study steering committee reviewed any discordance between the conﬁrmatory tests blinded to the clin-

ical outcome to adjudicate how the re- sults should be interpreted and included in the analysis.

The neonatal DNA samples were mostly obtained in the form of dry blood spots from the States’ health de- partments; they were collected as a part of neonatal screening programs. For quality assurance, a concordance test was developed and designed to conﬁrm that cfDNA results and newborn samples were correctly paired using alignment

between SNPs in the 2 samples; any samples that could not be paired were excluded.

## Data collection

Research coordinators at each site recorded clinical data using a secured computerized tracking system developed and managed by the Data Coordinating Center at The Biostatistics Center at the George Washington University, Wash- ington, DC. We collected patient and obstetrical data, imaging reports, and aneuploidy serum screening and prena- tal diagnosis results. In addition,

information on pregnancy complica- tions; genetic testing or ultrasound ﬁndings; and newborn features sugges- tive of genetic abnormality, major mal- formations, and other adverse outcomes was collected after delivery.

## Study oversight

The study was a collaboration between the clinical investigators and the sponsor (Natera, Inc, Austin, TX). The ﬁrst and last authors designed the protocol with the sponsor and had a majority vote in study design and data interpretation. All the laboratory analyses were blinded to the outcome data. The clinical and lab- oratory results were managed by the Data Coordinating Center, which inde- pendently matched the deidentiﬁed in- formation and analyzed the results only after the pregnancy outcomes were available and testing was complete.

## Statistical analysis

The trisomy analysis was a secondary analysis, and the sample size was calcu- lated on the basis of conﬁdence intervals for the 22q11.2 deletion syndrome, with a prevalence range of 1 per 1000 to 1 per 5000. This was more than adequate to assess the detection of T21, with an ex- pected prevalence of 1 per 425, and it would provide a reasonable assessment of the detection rates of T18 (prevalence of 1/1000) and T13 (prevalence of 1/ 3000). The sensitivity, speciﬁcity, posi- tive likelihood ratio, and positive and negative predictive values of cfDNA re- sults were assessed in the entire cohort and within the risk groups. When appropriate, exact (ClopperePearson) 95% conﬁdence intervals (CIs) were re- ported. Low- and high-risk groups were compared for test performance using the

Fisher’s exact test. Participants without genetic conﬁrmation were excluded

from the analysis. The SAS Studio 9.04 software (SAS Institute, Cary, NC) was used for analysis. The MedCalc software was used to calculate the CIs for the positive likelihood ratios.[17](#_bookmark21) Continuous variables were compared using the Wil- coxon test and categorical variables were compared using the chi-square or Fisher exact test. The McNemar test was used for paired analyses.

# Results

## Study participants

A total of 25,199 pregnant individuals were assessed for eligibility, and 20,194 (80.1%) were enrolled ([Figure](#_bookmark0)); 56.6% were enrolled in the US and 43.4% in Europe or Australia. Of the enrolled participants, 285 (1.4%) had pregnancy loss without genetic conﬁrmation, 93 (0.5%) withdrew consent, 1085 (5.4%) were lost to follow-up; in 603 (3.0%), a sample for genetic conﬁrmation of aneuploidy was not obtained, and in 277 (1.4%) the conﬁrmation test failed lab- oratory quality control. The latter group included 48 cases in which the neonatal sample could not be genetically paired with a cfDNA sample. After all exclu- sions, the study cohort included 17,851 (88.4%) women for whom both cfDNA results and DNA analysis of the fetus or newborn were available.

The baseline characteristics of the entire study cohort stratiﬁed by risk groups are outlined in [Table 1](#_bookmark1). The me- dian maternal age was 34.3 years (inter- quartile range [IQR], 30.2e37.4), and the median gestational age was 12.6 weeks (IQR, 11.4e13.9). A total of 13,043 cases (73.1%) were considered low-risk for aneuploidy, including 3,873 that were 35 years old but had a low-risk result on

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a screening test before enrollment. The remaining 4808 (26.9%) were catego- rized as high-risk ([Table 1](#_bookmark1)). Most high- risk women (4010, 83.4%) were 35 years old with no previous serum screening; 616 (12.8%) were high-risk on the basis of the results of traditional serum analyte-based screening, 112 (2.3%) had cfDNA screening following the detection of a fetal abnormality on ultrasound, and 101 (2.1%) had a cystic hygroma or a NT 3 mm. Participants at a high risk for aneuploidy were enrolled at an earlier gestational age, were more likely to be enrolled in Europe, and were more likely to have conceived using in vitro fertilization. Compared with non-US participants, the US participants were younger (median 32.6 vs 35.9;

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*P*<.0001), had a higher median body mass index (BMI) (26.1 vs 24.1; *P*<.0001), and enrolled at a later mean gestational age (13.7 week vs 12.8 week; *P*<.0001).

FIGURE

Patient enrollment flowchart



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The groups were similar for sensitivity and speciﬁcity. The PPV for all 3 tri- somies was 74.3% (26/35) in the low- risk cohort and 94.2% (97/103) in the high-risk cohort (*P* .003). The PPVs for the individual trisomies among low vs high-risk cases were 85.7% vs 97.5%

(*P* .06) for T21, 50.0% vs 81.2%

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(*P* .28) for T18, and 62.5% vs 83.3%,

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(*P* .58) for T13, respectively. Within the low-risk group, the PPV for T21 was 81.8% (95% CI, 59.0e100) in women

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35 years old with a low previous risk screen and 90.0% (95% CI, 71.4e100) in women <35 years old ([Table 3](#_bookmark4)).

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In 602 (3.4%) women, cfDNA did not

yield a result after a ﬁrst draw and 10 (1.66%) of these had a trisomy. These included 5/15 (33.3%) T13s, 2/18 (11.1%) T18s, and 3/100 (3%) T21s. In

this group, the mean BMI was higher (31.3 vs 26.2; *P*<.001) and the median fetal fraction was lower (4.5% vs 9.4%; *P*<.001) than those who received a result after the ﬁrst draw. Of the 427 women who attempted a second draw, 112 (26.2%) participants remained without a result and 2 (1.8%) of these had a trisomy, T13 in both cases, comprising a total of 1.5% of all trisomy cases. The rate of trisomy in the 287 patients with failed results after a ﬁrst or second draw was higher than those with a result (2.8% vs 0.7%; *P*¼.001). The no-call rates were similar

## Primary and secondary outcomes

Among the 17,851 pregnancies in the primary analysis population, 133 (0.8%) targeted chromosomal abnormalities were identiﬁed in the cohort as follows: 100 T21 (1 in 179), 18 T18 (1 in 992),

and 15 T13 (1 in 1195). In most cases

(17,533, 98.2%), the genetic outcome was conﬁrmed after birth: 17,548 (98.3%) by postnatal CMA, 28 (0.16%) by neonatal karyotype, and 2 (0.01%) by placental karyotype. Of the remaining 288 cases, in 232 (1.3%), conﬁrmation was done by prenatal diagnostic testing and in 56 (0.3%) it was conﬁrmed from miscarriages or termination specimens.

Four cases with mosaicism, all < 80% trisomic, were identiﬁed in the cohort,

including 3 T21 cases and 1 T18 case. ([Supplemental Table 1](#_bookmark33)). These 4 cases were considered as unaffected for the purpose of the analysis.

The cfDNA results were reported as

high risk for aneuploidy in 138 patients (0.77%); 123 (89.1%) of these were conﬁrmed whereas 15 (10.8%) were false positive results (n 5 T21, n 6 T18 and n 4 T13). There were 2 false negative results (0.01%): 1 T21 and 1 T18. Test performance for the entire cohort, including sensitivity, speciﬁcity, positive predictive value (PPV), negative predic- tive value (NPV) and positive likelihood ratios, are represented in [Table 2](#_bookmark2).

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Overall, 29/133 (21.8%) trisomies were in the group with a low previous risk for aneuploidy, whereas 104/133 (78.1%) were in high-risk cases (*P*<.001). The cfDNA screen positive rate was lower in the low-risk group than the high-risk group (0.27% vs 2.2%; *P*<.0001). The performance of cfDNA screening for the different trisomies in both risk groups is presented in [Table 3](#_bookmark4).

between the high- and low-risk patients

after the ﬁrst draw (3.8% vs 3.2%, respectively; *P* .051) and after 2 draws (1.7% vs 1.6%, respectively; *P* .717).

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Of the enrolled participants (n 20,237), 352 (1.7%) had pregnancy loss either before 20 weeks (201, 1.0%) or a later fetal or neonatal demise (151, 0.7%). Of those, 27 (7.7%) had a high- risk cfDNA result for a trisomy (13 T21, 10 T18, and 4 T13). Genetic conﬁrmation was available for 108/352 (30.7%), and these cases were included in the analysis cohort. Of the 108 with genetic conﬁr- mation, 18 (16.7%) had a trisomy (7 cases with T21, 9 T18, and 2 T13).

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The overall performance of the updated algorithm was similar to that of the original algorithm in the entire cohort ([Supplemental Table 2](#_bookmark34)) and in the different risk groups ([Supplemental](#_bookmark37) [Table 3](#_bookmark37)). In the 4808 patients with a

TABLE 1

### Patient and gestational characteristics in the entire cohort and low- and high-risk groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Full cohort | Low risk | High risk | *P* valueLow vs high |
| VariableMaternal and gestational characteristics | (n¼17,851) | (n¼13,043) | (n¼4808) | risk |
| Median maternal age (IQR) - y | 34.3 (30.2e37.4) | 32.5 (28.8e35.7) | 37.6 (35.8e39.7) | <.001 |
| Nulliparity | 7876 (44.2) | 6283 (48.2) | 1593 (33.4) | <.001 |
| Median BMI (kg/m2) (IQR) | 25.0 (22.3e29.1) | 25.0 (22.3e29.3) | 25.0 (22.4e28.8) | .699 |
| Race/ethnicity |  |  |  | <.001 |
| Asian | 1532 (8.6) | 1260 (9.7) | 272 (5.7) |  |
| Black | 1569 (8.8) | 1300 (10.0) | 269 (5.6) |  |
| White | 10,811 (60.6) | 7283 (55.8) | 3528 (73.4) |  |
| Hispanic | 3331 (18.7) | 2704 (20.7) | 627 (13.0) |  |
| Other/unknown | 608 (3.4) | 496 (3.8) | 112 (2.3) |  |
| Median gestational age at enrollment (IQR)—wk | 12.6 (11.4e13.9) | 12.7 (11.9e14.0) | 11.7 (10.4e13.6) | <.001 |
| Pregnancy through assisted reproductive technology | 904 (5.1) | 582 (4.5) | 323 (6.7) | <.001 |
| Current smoker | 314 (1.8) | 257 (2.0) | 57 (1.2) | <.001 |
| Enrollment site |  |  |  | <.001 |
| United States | 10,105 (56.6) | 8345 (64.0) | 1760 (36.6) |  |
| Europe | 7331 (41.1) | 4401 (33.7) | 2930 (60.9) |  |
| Australia | 415 (2.3) | 297 (2.3) | 118 (2.5) |  |
| Prenatal screening and testing |  |  |  |  |
| Positive first trimester screen before cfDNA testing | 509 (2.9) |  | 509 (10.6) |  |  |
| NT>3 mm before cfDNA testing | 101 (0.9) |  | 101 (2.1) |  |  |
| Positive second trimester before cfDNA testing | 107 (0.6) |  | 107 (2.2) |  |  |
| Major anomaly before cfDNA testing | 112 (0.6%) |  | 112 (2.3%) |  |  |
| No call - % | 287 (1.6) | 207 (1.6) | 80 (1.7) | .717 |  |
| Mean cfDNA fetal fraction (SD) | 9.9 (4.1) | 9.9 (4.1) | 9.7 (4.2) | <.001 |  |
| Diagnostic testing (CVS and amniocentesis)—% | 544 (3.1%) | 283 (2.2) | 261 (5.4) | <.001 |  |
| Any trisomy (T13, 18, 21) | 133 (0.8%) | 29 (0.2) | 104 (2.2) | <.001 |  |
| Pregnancy and delivery outcome |  |  |  |  |  |
| Delivery outcome |  |  |  | <.001 |  |
| Miscarriage | 49 (0.3%) | 15 (0.1) | 34 (0.7) |  |  |
| Elective abortion | 159 (0.9%) | 64 (0.5) | 95 (2.0) |  |  |
| Live birth | 17,600 (98.7%) | 12,935 (99.3) | 4665 (97.1) |  |  |
| Stillbirth | 30 (0.2%) | 19 (0.2) | 11 (0.2) |  |  |
| Neonatal death | 29 (0.2%) | 16 (0.1) | 13 (0.3) | .036 |  |
| Median gestational age at delivery (IQR) - wk | 39.4 (38.4e40.3) | 39.4 (38.6e40.3) | 39.3 (38.3e40.1) | <.001 |  |
| PTB<34 wk | 459 (2.6%) | 262 (2.0) | 197 (4.1) | <.001 |  |
| Preeclampsia | 711 (4.1%) | 519 (4.1) | 192 (4.1) | .846 |  |
| Small for gestational age | 1546 (8.9%) | 1158 (9.1) | 388 (8.3) | .135 |  |
| Mean birthweight (SD) g | 3353 (555) | 3347 (544) | 3371 (586) | <.001 |  |
| Apgar 1 < 7 | 797 (5.1%) | 587 (4.9) | 210 (6.2) | .002 |  |

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| TABLE 1Patient and gestational characteristics in the entire cohort and low- and high-risk groups *(continued)* |
|  |  |  |  | *P* value |
|  | Full cohort | Low risk | High risk | Low vs high |
| Variable | (n¼17,851) | (n¼13,043) | (n¼4808) | risk |
| Apgar 5 < 7 | 154 (1.0%) | 106 (0.9) | 48 (1.4) | .006 |
| Median days to newborn discharge (IQR)—d | 2.0 (2.0e3.0) | 2.0 (2.0e3.0) | 3.0 (2.0e4.0) | <.001 |
| *BMI*, body mass index; *cfDNA*, cell-free DNA; *CVS*, chorionic villus sampling; *IQR*, interquartile range; *NT*, nuchal translucency; *PTB*, preterm birth; *SD*, standard deviation.*Dar et al. Performance of cell-free DNA screening for aneuploidy in low-risk pregnancies. Am J Obstet Gynecol 2022.* |

priori risk for aneuploidy, there were 104 trisomies (2.16%), and 100/104 (96.1%) were detected by cfDNA. In the 13,043 low-risk patients, 29 (0.22%) had a tri- somy and 27/29 (93.1%) were detected. The no-call rate was lower with the updated algorithm than the original protocol (1.4% vs 3.4% after the ﬁrst draw and 0.5% vs 1.6% after an optional second draw; *P*<.001). In the group with

a no-call result on the ﬁrst blood draw,

there were 5 trisomies (2.0%) including 1 of 100 (1.0%) T21, 3 of 15 (20.0%) T13s,

and 1 of 18 (5.6%) T18s. In the group

with 2 sequential no-call results (N 28), there were 2 (7.1%), both cases of T13.

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# Discussion

Summary of the key findings In a large prospective cohort with genetic conﬁrmation, we found that SNP-based cfDNA has high sensitivity, speciﬁcity, and PPV for the common trisomies in women who are at a high or low risk for

aneuploidy. The ﬁndings in this study are in broad agreement with previous reports that have shown high sensitivity and speciﬁcity of cfDNA screening for T21, T18, and T13 in women of all risk categories.[4](#_bookmark10),[12](#_bookmark16),[13](#_bookmark17) Because the PPV de- pends on disease prevalence, it is ex- pected to be lower in the group of women with a lower previous risk. In this study, the PPVs for T21, T18, and T13 for the low previous risk cohort were 85.7%, 50.0%, and 62.5%, respec- tively. Although the PPV for the low-risk group was somewhat lower than the PPV reported in those with a previous high risk (97.5%, 81.2%, and 83.3%, respec- tively), these differences were not statis- tically signiﬁcant, possibly because of a small sample size. Norton et al reported a PPVof 76% for T21 in women under 35 years and 50% for those with low-risk ﬁrst trimester screening, with the ascer- tainment of aneuploidy done mainly through clinical assessment.[4](#_bookmark10) Zhang

et al[13](#_bookmark17) reported a PPV of 81.3% in women at a low risk for T21 in a large prospective cohort, although conﬁrma- tion was only available for 76.7% of cases in their report. Although the PPV of > 85% for T21 and at least 50% for T18 and T13 are lower in both low- and high- risk patients, they are substantially higher than the PPV associated with

other accepted conventional aneuploidy screening tests,[18](#_bookmark22) further supporting the recommendation that patients with positive cfDNA results should be fol- lowed with conﬁrmatory testing, regardless of their previous risk status.[6](#_bookmark11),[7](#_bookmark12) Although all of the positive likelihood ratios were high, it is important to note that cfDNA should not be considered diagnostic because of conﬁned placental mosaicism and other potentially unex- pected chromosomal anomalies.

Approximately 3.4% of the cfDNA tests in the cohort did not yield a result after a ﬁrst draw, and 1.6% after an

|  |
| --- |
| TABLE 2Cell-free DNA test performance to screen for trisomies 21, 18, and 13 |
|  | T21 | T18 | T13 | T21/18/13 |
| VariableSensitivity | Full cohort (n¼17,564)97/98 | 16/17 | 10/10 | 123/125 |
|  | 98.98% (96.99e100)[a](#_bookmark3) | 94.12% (82.93e100) | 100% (69.15e100) | 98.40% (96.20e100) |
| Specificity | 17,461/17,466 | 17,541/17,547 | 17,550/17,554 | 17,424/17,439 |
|  | 99.97% (99.95e100) | 99.97% (99.94e99.99) | 99.98% (99.96e100) | 99.91% (99.87e99.96) |
| PPV | 97/102 | 16/22 | 10/14 | 123/138 |
|  | 95.10% (90.91e99.29) | 72.73% (54.12e91.34) | 71.43% (47.76e95.09) | 89.13% (83.94e94.32) |
| NPV | 17,461/17,462 | 17,541/17,542 | 17,550/17,550 | 17,424/17,426 |
|  | 99.99% (99.98e100) | 99.99% (99.98e100) | 100% (99.98e100) | 99.99% (99.97e100) |
| [b](#_bookmark3)Likelihood ratio (þ) | 3458 (1439e8308) | 2752 (1226e6180) | 4388 (1647e11,692) | 1144 (689e1898) |
| *NPV*, negative predictive value; *PPV*, positive predictive value; *T*, trisomy.a 95% Confidence interval; b Likelihood ratio (þ) e Se/(100-Sp).*Dar et al. Performance of cell-free DNA screening for aneuploidy in low-risk pregnancies. Am J Obstet Gynecol 2022.* |

TABLE 3

### Comparison of cell-free DNA test performance for trisomies 21, 18, and 13 between women at a low and high risk for aneuploidy[a](#_bookmark5)

Variable High risk[b](#_bookmark5) n¼4728

Trisomy 21 (n¼98)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sensitivity | 98.8[c](#_bookmark6)79/80 (96.3e100) | 10018/18 (81.5e100) | 1.00 | 1009/9 (66.37e100) | 1009/9 (66.37e100) |
| Specificity | 99.964646/4648 (99.90e100) | 99.9812,815/12,818 (99.95e100) | .61 | 99.953792/3794 (99.89e100) | 99.999023/9024 (99.97e100) |
| PPV | 97.5379/81 (94.15e100) | 85.7118/21 (70.75e100) | .06 | 81.829/11 (59.03e100) | 90.009/10 (71.41e100) |
| NPV | 99.984646/4647 (99.94e100) | 10012,815/12,815 (99.97e100) | .27 | 1003792/3792 (99.90e100) | 1009023/9023 (99.96e100) |
| Prevalence | 1.69[c](#_bookmark6) | 0.14 |  | 0.24 | 0.10 |
| [d](#_bookmark6)Likelihood ratio (þ) | 2295 (574e9176) | 4273 (1378e13246) |  | 1897 (475e7582) | 9024 (1271e64,057) |

Low risk n¼12,836

*P* value (High

vs low risk) Low risk ≥35 n¼3803 Low risk <35 n¼9033

Trisomy 18 (n¼17)

Sensitivity 100

13/13 (75.3e100)

Specificity 99.94

4712/4715 (99.86e100)

PPV 81.25

13/16 (62.13e100)

NPV 100

4712/4712 (99.92e100)

75.0

3/4 (32.6e100)

99.98

12,829/12,832 (99.95e100)

50.00

3/6 (10.00e90.01)

99.99

12,829/12,830 (99.98e100)

.24 100

2/2 (15.81e100)

.20 99.95

3799/3801 (99.89e100)

.28 50.00

2/4 (6.76e93.24)

1.00 100

3799/3799 (99.90e100)

50.00

1/ 2 (1.26e98.74)

99.99

9030/9031 (99.97e100)

50.00

1/ 2 (1.26e98.74)

99.99

9030/9031 (99.97e100)

Prevalence 0.27 0.03 0.05 0.02

Likelihood ratio (þ) 1572 (507e4971) 3208 (905e11,367) 1900 (475e7596) 4516 (409e49,796)

Trisomy 13 (n¼10)

Sensitivity 100

5/5 (47.8e100)

Specificity 99.98

4722/4723 (99.94e100)

PPV 83.3

5/6 (53.51e100)

NPV 100

4722/4722 (99.92e100)

100

5/5 (47.8e100)

99.98

12,828/12,831 (99.95e100)

62.50

5/8 (28.95e96.05)

100

12,828/12,828 (99.97e100)

1.00 100

1/1 (2.50e100)

1.00 99.97

3801/3802 (99.93e100)

.58 50.00

1/ 2 (1.26e98.74)

1.00 100

3801/3801 (99.92e100)

100

4/4 (39.76e100)

99.98

9027/9029 (99.95e100)

66.67

4/6 (28.95e100)

100

9027/9027 (99.96e100)

Prevalence 0.11 0.04 0.03 0.04

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optional second draw was included, a rate that is comparable to previous studies.[3](#_bookmark9) The inability of cfDNA to make a high-conﬁdence call relates most commonly to a low proportion of cfDNA of fetal origin, or “fetal frac-

Low risk <35 n¼9033

4515 (1129e18,048)

Trisomy 21,18, & 13 (n¼125)

93.33

14/15 (80.71e100)

99.96

9014/9018 (99.91e100)

77.78

14/18 (58.57e100)

99.99

9014/9015 (99.97e100)

0.17

2104 (783e5658)

Excluding no-call results; Women were considered as high-risk for aneuploidy if they had a previous positive serum-based (first trimester combined or second trimester triple or quadruple) screen for aneuploidy, fetal nuchal translucency ≥ 3.0 mm, an ultrasound-

tion,”[19](#_bookmark23) which is associated with early

TABLE 3

Comparison of cell-free DNA test performance for trisomies 21, 18, and 13 between women at a low and high risk for aneuploidya *(continued)*

gestational age and high maternal

weight.[20](#_bookmark24),[21](#_bookmark25) Previous studies have also demonstrated an association between a low fetal fraction and trisomies 13 and 18, which may be explained by a small placenta leading to reduced fetal DNA in the maternal plasma.[22](#_bookmark26),[23](#_bookmark27) Data suggest- ing an increased risk for fetal trisomy 21 among low fetal fraction cases have been conﬂicting.[4](#_bookmark10),[24](#_bookmark28) In this study, over 7.5% of the trisomies were in the no-call group and there was a 3- to 4-fold increase in the aneuploidy risk. The increased risk, particularly after 2 failed draws, was mainly attributable to T13 and to a lesser extent to T18, whereas for T21, the as- sociation did not seem to be clinically signiﬁcant. It is still important to note that although the no-call rate was not higher in the T21 group, 2 cases of T21 did have no-call results after the ﬁrst draw.

*P* value (High vs low risk)

Low risk ≥35 n¼3803

3802 (536e26,985)

96.3

26/27 (89.2e100)

99.93

12,800/12,809 (99.88e99.98)

74.29

26/35 (59.81e88.77)

99.99

12,800/12,801 (99.98e100)

0.21

1371 (710e2644)

.39

100

12/12 (73.54e100)

99.87

3786/3791 (99.79e99.99)

70.59

12/17 (48.93e99.25)

100

3786/3786 (99.92e100)

0.32

758 (316e1821)

.25

<.01

.46

c d

detected anomaly before enrollment, or if the maternal age was ≥35 years at delivery and no other screening results (eg, serum) were available; percent; Likelihood ratio (þ) - Se/(100-Sp).

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Clinical and research implications Two trisomy cases, 1 T21 and 1 T18, were reported as negative by cfDNA. Both were subsequently identiﬁed at 19 weeks after the sonographic detection of growth restriction and ventricular septal defect in the T18 fetus and ven- triculomegaly and unbalanced atrio- ventricular septal defect in the T21 fetus. Although our ﬁndings conﬁrm that false positive and negative results, particularly for T21, are uncommon, their presence clearly indicates that cfDNA is a screening and not a diag- nostic test. Pre- and post-test patient education are therefore important, and patients with a negative cfDNA result should be aware of the possibility of false negative results. The focus of this study is the performance of cfDNA screening for trisomies. However, pa- tients should be aware that though other aneuploidies and microdeletions or duplications are individually rare, they are more common than the com- mon trisomies in aggregate, and

Low risk n¼12,836

4227 (1380e13,260)

High riskb n¼4728

Variable

Likelihood ratio (þ)

4723 (665e33,523)

Sensitivity

99.0

97/98 (96.9e100)

99.87

4624/ 4630 (99.77e99.97)

94.17

97/103 (89.65e98.70)

99.98

4624/4625 (99.94e100)

2.07

763 (343e1700)

Specificity

PPV

NPV

Prevalence

Likelihood ratio (þ)

*NPV*, negative predictive value; *PPV*, positive predictive value.

a

b

options for prenatal detection, primar- ily through diagnostic testing, are available. Therefore, those who have a sonographic ﬁnding of a structural anomaly should be offered diagnostic testing despite a previous negative cfDNA result.

Technology continued to evolve dur- ing the 38 months of patient enrollment, and the laboratory developed an updated algorithm near the end of the project; we assessed this updated algorithm after enrollment was completed. Although sensitivity and speciﬁcity were compa- rable to the original algorithm, the no- call rate was signiﬁcantly lower. Never- theless, though the total number of tri- somies in the no-call group was also lower, the proportion of these trisomies among the no-call cases increased, rein- forcing the recommendation to follow up no-call reports with an additional evaluation.[7](#_bookmark12)

Strengths and limitations

The main strength of this study is the application of genetic analysis to conﬁrm outcome in a large prospective cohort undergoing prenatal screening. The requirement for conﬁrmatory genetic testing in all cases assured a complete and accurate assessment of screening performance in all risk groups and outcome of cases with no-call results. Nevertheless, this study is not without limitations. The study was designed to include an unselected population, but with a median maternal age of 34.3 years, the cohort was somewhat older than expected[25](#_bookmark29) and likely indicates the use of cfDNA screening in a higher risk popu- lation. Consequently, the prevalence of aneuploidy was also higher than ex- pected.[4](#_bookmark10),[26](#_bookmark30) Again, this difference likely represents a referral bias associated with recommendations of professional soci- eties at the time of enrollment to only offer cfDNA to women at a high risk. It is also possible that there was a higher de- mand for cfDNA, which may be viewed as an alternative to diagnostic testing by older women. Despite the overall older maternal age, subgroup analyses enabled us to assess the test performance for each of the prior risk subgroups. The inability to obtain conﬁrmatory samples after the

spontaneous loss of fetuses, likely disproportionately aneuploid, represents an unavoidable biologic limitation to any assessment of prenatal screening. The lack of genetic conﬁrmation in the 1.4% of cases because of fetal loss or neonatal demise may result in an underestimation of the actual prevalence of chromosomal abnormalities in the cohort. The rate of trisomy was 16.7% in those cases of fetal or neonatal demise that did have genetic conﬁrmation, which is consistent with evidence that common trisomies continue to play a signiﬁcant role in

pregnancy loss after 10 weeks’ gesta- tion.[27](#_bookmark31),[28](#_bookmark32) Finally, the results of this study

may not apply to all patients or be generalizable to all cfDNA laboratories, as the exclusion criteria and cfDNA analysis techniques differ.

## Conclusions

The ﬁndings in this study demonstrate that SNP-based cfDNA screening for the common trisomies performs similarly well in both high- and low-risk groups. Although technological advancements have decreased the rate of no-call results, such cases are at an increased risk of speciﬁc aneuploidies and require addi- tional investigation. The data from this study will be helpful to patients and providers when considering their pre- natal screening and diagnostic testing

options. ■

References

1. [Chitty LS, Bianchi DW. Noninvasive prenatal](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref1) [testing: the paradigm is shifting rapidly. Prenat](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref1) [Diagn 2013;33:511–3](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref1).
2. [Bianchi DW, Chiu RWK. Sequencing of](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref2)

[circulating cell-free DNA during pregnancy.](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref2) [N Engl J Med 2018;379:464–73](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref2).

1. [Gil MM, Accurti V, Santacruz B, Plana MN,](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref3)

[Nicolaides KH. Analysis of cell-free DNA in](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref3) [maternal blood in screening for aneuploidies:](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref3) [updated meta-analysis. Ultrasound Obstet](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref3) [Gynecol 2017;50:302–14](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref3).

1. [Norton ME, Jacobsson B, Swamy GK, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref4)

[Cell-free DNA analysis for noninvasive exami-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref4) [nation of trisomy. N Engl J Med 2015;372:](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref4) [1589–97](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref4).

1. [Benn P, Borrell A, Chiu RW, et al. Position](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref5)

[statement from the Chromosome Abnormality](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref5) [Screening Committee on behalf of the Board of](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref5) [the International Society for Prenatal Diagnosis.](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref5) [Prenat Diagn 2015;35:725–34](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref5).

1. [Bianchi DW, Parker RL, Wentworth J, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref6)

[DNA sequencing versus standard prenatal](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref6)

[aneuploidy screening. N Engl J Med 2014;370:](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref6) [799–808](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref6).

1. [Gregg AR, Skotko BG, Benkendorf JL, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref7)

[Noninvasive prenatal screening for fetal aneu-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref7) [ploidy, 2016 update: a position statement of the](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref7) [American College of Medical Genetics and Ge-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref7) [nomics. Genet Med 2016;18:1056–65](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref7).

1. [Screening for fetal chromosomal abnormal-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref8)

[ities: ACOG practice bulletin summary, Number](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref8) [226. Obstet Gynecol 2020;136:859–67](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref8).

1. [Nicolaides KH, Syngelaki A, Gil M,](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref9)

[Atanasova V, Markova D. Validation of targeted](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref9) [sequencing of single-nucleotide polymorphisms](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref9) [for non-invasive prenatal detection of aneuploidy](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref9) [of chromosomes 13, 18, 21, X, and Y. Prenat](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref9)

[Diagn 2013;33:575–9](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref9).

1. [Ryan A, Hunkapiller N, Banjevic M, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref10)

[Validation of an enhanced version of a single-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref10) [nucleotide polymorphism-based noninvasive](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref10) [prenatal test for detection of fetal aneuploidies.](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref10) [Fetal Diagn Ther 2016;40:219–23](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref10).

1. [Benn P, Cuckle H, Pergament E. Non-inva-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref11)

[sive prenatal diagnosis for Down syndrome: the](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref11) [paradigm will shift, but slowly. Ultrasound](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref11) [Obstet Gynecol 2012;39:127–30](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref11).

1. [Dar P, Curnow KJ, Gross SJ, et al. Clinical](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref12)

[experience and follow-up with large scale single-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref12) [nucleotide polymorphism-based noninvasive](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref12) [prenatal aneuploidy testing. Am J Obstet](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref12) [Gynecol 2014;211:527.e1–17](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref12).

1. [Zhang H, Gao Y, Jiang F, et al. Non-invasive](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref13)

[prenatal testing for trisomies 21, 18 and 13:](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref13) [clinical experience from 146,958 pregnancies.](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref13) [Ultrasound Obstet Gynecol 2015;45:530–8](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref13).

1. [Chitty LS. Use of cell-free DNA to screen for](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref14)

[Down’s syndrome. N Engl J Med 2015;372:](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref14) [1666–7](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref14).

1. [Pergament E, Cuckle H, Zimmermann B,](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref15)

[et al. Single-nucleotide polymorphism-based](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref15) [noninvasive prenatal screening in a high-risk](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref15) [and low-risk cohort. Obstet Gynecol](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref15) [2014;124:210–8](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref15).

1. Google Inc., Mountain View, CA. Available

at: [https://www.usenix.org/system/ﬁles/](https://www.usenix.org/system/files/conference/osdi16/osdi16-abadi.pdf) [conference/osdi16/osdi16-abadi.pdf](https://www.usenix.org/system/files/conference/osdi16/osdi16-abadi.pdf).

1. MedCalc. Software Ltd. Diagnostic test evaluation calculator. 2022. Available at: [https://](https://www.medcalc.org/calc/diagnostic_test.php) [www.medcalc.org/calc/diagnostic\_test.php](https://www.medcalc.org/calc/diagnostic_test.php). Accessed January 5, 2022.
2. [Malone FD, Canick JA, Ball RH, et al. First-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref17) [trimester or second-trimester screening, or](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref17) [both, for Down’s syndrome. N Engl J Med](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref17) [2005;353:2001–11](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref17).
3. [Lo YMD, Tein MSC, Lau TK, et al. Quanti-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref18) [tative analysis of fetal DNA in maternal plasma](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref18) [and serum: implications for noninvasive prenatal](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref18) [diagnosis. Am J Hum Genet 1998;62:768–75](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref18).
4. [Wang E, Batey A, Struble C, Musci T,](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref19)

[Song K, Oliphant A. Gestational age and](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref19) [maternal weight effects on fetal cell-free DNA in](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref19) [maternal plasma. Prenat Diagn 2013;33:662–6](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref19).

1. [Canick JA, Palomaki GE, Kloza EM,](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref20) [Lambert-Messerlian GM, Haddow JE. The](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref20) [impact of maternal plasma DNA fetal fraction on](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref20) [next generation sequencing tests for common](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref20) [fetal aneuploidies. Prenat Diagn 2013;33:](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref20) [667–74](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref20).
2. [McKanna T, Ryan A, Krinshpun S, et al. Fetal](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref21) [fraction-based risk algorithm for non-invasive](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref21) [prenatal testing: screening for trisomies 13 and](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref21) [18 and triploidy in women with low cell-free fetal](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref21) [DNA. Ultrasound Obstet Gynecol 2019;53:](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref21) [73–9](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref21).
3. [Wegrzyn P, Faro C, Falcon O, Peralta CF,](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref22)

[Nicolaides KH. Placental volume measured by](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref22) [three-dimensional ultrasound at 11 to 13](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref22) [6](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref22) [weeks of gestation: relation to chromosomal](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref22) [defects. Ultrasound Obstet Gynecol 2005;26:](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref22) [28–32](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref22).

þ

1. [Palomaki GE, Kloza EM, Lambert-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref23)

[Messerlian GM, et al. Circulating cell free DNA](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref23) [testing: are some test failures informative? Pre-](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref23) [nat Diagn 2015;35:289–93](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref23).

1. Organization for Economic Co-operation

and Development. OECD family database. 2021. Available at: [http://www.oecd.org/els/](http://www.oecd.org/els/family/database.htm) [family/database.htm](http://www.oecd.org/els/family/database.htm). Accessed January 20, 2021.

1. [Snijders RJ, Sundberg K, Holzgreve W,](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref25) [Henry G, Nicolaides KH. Maternal age- and](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref25) [gestation-speci](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref25)ﬁ[c risk for trisomy 21. Ultrasound](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref25)

[Obstet Gynecol 1999;13:167–70](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref25).

1. [Morris JK, Wald NJ, Watt HC. Fetal loss in](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref26)

[Down syndrome pregnancies. Prenat Diagn](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref26) [1999;19:142–5](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref26).

1. [Morris JK, Savva GM. The risk of fetal loss](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref27)

[following a prenatal diagnosis of trisomy 13 or](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref27) [trisomy 18. Am J Med Genet A 2008;146A:](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref27) [827–32](http://refhub.elsevier.com/S0002-9378%2822%2900041-2/sref27).

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B.J. reports research clinical diagnostic trials with Ariosa (completed), Vanadis (completed), Natera (ongoing), and Hologic (completed), with institutional expenditures reimbursed per patient and no personal

reimbursements. He also reports clinical probiotic studies with products provided by FukoPharma (ongoing, no funding) and BioGaia (ongoing; also pro- vided a research grant for the specific study), coordi- nation of scientific conferences and meetings with commercial partners as such as ESPBC 2016 and a Nordic educational meeting about noninvasive prenatal testing and preeclampsia screening. B.J. and Y.C. collaborated in the IMPACT study where Roche, Perkin Elmer, and Thermo Fisher provide reagents to placental growth factor analyses. R.J.W. receives research funding from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development and receives support from Illumina for research reagents.

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The trial was registered on [ClinicalTrials.gov](http://ClinicalTrials.gov/) with identifier NCT02381457, Single-nucleotide- polymorphism-based Microdeletion and Aneuploidy RegisTry (SMART).

Data sharing: Data sharing requests should be sub- mitted to the corresponding author (P.D.) for consider- ation. The requests will be considered by the study publication committee. The study protocol and statistical analysis plan will be available on request. Individual pa- tient data will not be available. Access to deidentified data may be granted following submission of a written pro- posal and a signed data sharing agreement. Files will be shared using a secure file transfer protocol.

Ethical approval: This study was designed in compli- ance with an investigational-review-board-approved protocol (Ethical and Independent Review Services Study ID, 17113; date of certification, August 28, 2017, date of renewal August 20, 2020). Written informed consent was obtained from all the study participants.

This study was presented as an oral presentation at the 41st annual meeting of the Society of Maternal and Fetal Medicine, held virtually, January 25e30, 2021.

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Supplemental information Methods Procedures

Sample preparation and analysis of cell- free DNA (cfDNA) were performed as previously described (Natera Inc, Austin, TX).[14](#_bookmark18) Noninvasive prenatal testing (NIPT) results indicating a risk of 1/ 100 for a trisomy were categorized as high risk and those at <1/100 as low risk. In the cases that did not yield a result, the patients were offered repeat testing, and

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results after a second draw were included for analysis. During enrollment, the cfDNA laboratory protocol was modi- ﬁed once[15](#_bookmark19); the results from both periods are combined for analysis (original algorithm).

Independent of the study, the labora- tory developed an updated algorithm optimized to improve the no-call rate at a low fetal fraction using a deep neural network component, which utilizes an artiﬁcial intelligence approach. A deep learning (Tensorﬂow v1.15) approach was used to optimally model noise using a deep mixture-of-experts neural network with multiple independent networks, combining the results into a probability score. This self-supervised algorithm leveraged 1.6 million sequenced mixtures of mother and fetus cfDNA samples, learning to harness linkage among the single-nucleotide-polymorphisms (SNPs) to make high-conﬁdence calls for a larger proportion of samples. Deeper sequencing of high-risk calls was applied to lower false positive rates. This updated protocol was assessed after enrollment completion and was blinded to the outcomes.

The genetic outcomes were assessed by chromosome microarray analysis (CMA) through analysis of DNA from fetal (chorionic villus sampling, amnio- centesis, or products of conception) or infant (cord blood, buccal swab, or newborn blood spot obtained for state newborn screening) samples. Postnatal conﬁrmatory samples were obtained at the end of the pregnancy in all cases, regardless of the availability of previous prenatal diagnostic genetic testing.

CMA was performed by an indepen- dent laboratory (Center for Applied Genomics, Children’s Hospital of

Philadelphia, Philadelphia, PA) and was blind to clinical ﬁndings and cfDNA re- sults. For CMA analysis, DNA was pre- pared from cord blood, buccal smear, or a dried blood spot. The copy number variants were identiﬁed using the Illu- mina (San Diego, CA) SNP-based Inﬁnium GSA platform. Samples were genotyped on standard versions GSA- V1.0, GSA-V2.0, GSAMD-V1.0, or

GSAMD-V2.0 that contain >700,000 SNP from chromosome 1e22 or a

custom-designed SMARTArray in which additional SNPs were added to the GSA backbone. In addition, positive samples underwent conﬁrmation on the Omni 2.5-8V1-3 array and were reviewed by a clinical molecular cytogeneticist before generated results.

If a postnatal sample for CMA conﬁrmation was not available, results from pre or postnatal clinical testing with karyotype, quantitative ﬂuorescent polymerase chain reaction (QF-PCR), FISH or CMA were used for genetic conﬁrmation, if available.

Cases with mosaicism were considered affected if >80% of cells were trisomic on conﬁrmatory testing. Mosaicism identi- ﬁed only by CVS was not considered as conﬁrmation of genetic outcome. The study steering committee reviewed any discordance between conﬁrmatory tests, blinded to the clinical outcome, to adju- dicate how results should be interpreted

and included in the analysis.

For quality assurance purposes, a concordance test was developed to conﬁrm that cfDNA results and newborn samples were correctly paired using alignment be- tween SNPs in the 2 samples; any samples that could not be paired were excluded.

Study design and participants Full information on study dates, including enrollment and completion are provided on [ClinicalTrials.gov](http://ClinicalTrials.gov/), Identiﬁer NCT02381457. The relevant dates are as follows: periods of recruitment: April 8, 2015 to December 12, 2019; follow-up: April 8, 2015 to July 18, 2019; data collec- tion: April 8, 2015 to September 18, 2019.

This study involved 21 locations including the following: University of California San Francisco, San Francisco, California, United States; Cooper

University Hospital, Camden, New Jersey, United States; Virtua, Mount Laurel, New Jersey, United States; St. Peter’s Univer- sity, New Brunswick, New Jersey, United States; Complete Women’s Healthcare, Garden City, New York, United States; North Shore University Hospital, Man- hasset, New York, United States; Ma- donna Perinatal, Mineola, New York, United States; Long Island Jewish Medical Center New Hyde Park, New York, United States; New York University, New York, New York, United States; Icahn School of Medicine Mt Sinai, New York, New York, United States; Columbia University, New York, New York, United States; Monteﬁore Medical Center, New

York, New York, United States; Suffolk OB, Port Jefferson, New York, United States; North Austin Maternal Fetal Medicine, Austin, Texas, United States; Zeid Women’s Health Center, Longview, Texas, United States; University of Utah, Salt Lake City, Utah, United States; Royal Prince Alfred, Camperdown, New South Wales, Australia; Royal College Surgeons

in Ireland, Dublin, Ireland, 1; Dexeus, Barcelona, Spain; Sahlgrenska University Hospital, Gothenburg, Sweden; St. George University Hospital, London, United Kingdom.

This multicenter prospective obser- vational study enrolled pregnant women who presented clinically at or after 9 weeks gestation and elected Panorama microdeletion and aneuploidy screening as part of their routine care. The primary objective was to evaluate the perfor- mance of SNP-based NIPT for 22q11.2 microdeletion in a large cohort of preg- nant women. Data collection began at enrollment and continued until patients delivered and their child was discharged from the hospital. Biospecimens were obtained from infants after birth to perform genetic diagnostic testing for 22q11.2 deletion. The results from the follow-up specimens were compared with those obtained by the Panorama screening test to determine test perfor- mance. In the event a newborn sample could not be obtained before discharge from the hospital, the participants were mailed a saliva buccal swab kit for testing at home. The samples were then shipped to Natera for testing.

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| SUPPLEMENTAL TABLE 1Outcome of cases confirmed as mosaicism |
| Case Cytogenetics (pre- or postnatal) Array (pre- or postnatal) Clinical outcome1. Prenatal amnio: Prenatal amnio: Mosaic DORV diagnosed M21/Ti21 50%/50% T21 (30%) prenatally.

Postnatal: Postnatal: Neonatal demise.* + Direct buccal FISH: T21/M21 Mosaicism with majority 50%/50% T21.
	+ Karyotype blood: T21
	+ Karyotype skin T21/M21 50%/50%
1. Prenatal amnio: none Pregnancy M18p/T18q 50%/50% Termination
2. Prenatal CVS: Prenatal: 70% T21 Pregnancy

T21 Mosaicism TerminationPrenatal amnio FISH:37% T211. Prenatal amnio: none Pregnancy 30% T21 Termination

*Alg*, algorithm; *Amnio*, Amniocentesis; *CVS*, Chorionic Villus Sampling; *DORV*, double outlet right ventricle; *FISH*, fluorescence in situ hybridization.*Dar et al. Performance of cell-free DNA screening for aneuploidy in low-risk pregnancies. Am J Obstet Gynecol 2022.* | Original | Original | Original | Updated | Updated | Updated |
| Alg T13 | Alg T18 | Alg T21 | Alg T13 | Alg T18 | Alg T21 |
| No call | No call | No call | Low risk | Low risk | Low risk |
| Low risk | High risk | Low risk | Low risk | High risk | Low risk |
| No call | No call | No call | Low risk | Low risk | Low risk |
| Low risk | Low risk | High risk | Low risk | Low risk | High risk |

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| SUPPLEMENTAL TABLE 2Cell-free DNA test performance to screen for trisomies 21, 18, and 13 with the updated algorithm |
| T21 T18 T13 | T21/18/13 |
| Updated algorithm Full cohort (n¼17,737) |  |
| Sensitivity 99/100 16/17 12/12 | 127/129 |
| 99.00% (97.05e100)[a](#_bookmark35) 94.12% (82.93e100) 100% (73.54e100) | 98.45% (96.32e100) |
| Specificity 17,630/17,637 17,716/17,720 17,722/17,725 | 17,594/17,608 |
| 99.96% (99.93e99.99) 99.98% (99.96e100) 99.98% (99.96e100) | 99.92% (99.88e99.96) |
| PPV 99/106 16/20 12/15 | 127/141 |
| 93.40% (88.67e98.12) 80.00% (62.47e97.53) 80.00% (59.76e100) | 90.07% (85.13e95.01) |
| NPV 17,630/17,631 17,716/17,717 17,722/17,722 | 17,594/17,596 |
| 99.99% (99.98e100) 99.99% (99.98e100) 100% (99.98e100) | 99.99% (99.97e100) |
| *NPV*, negative predictive value; *PPV*, positive predictive value. |  |
| a 95% confidence interval. |  |
| *Dar et al. Performance of cell-free DNA screening for aneuploidy in low-risk pregnancies. Am J Obstet Gynecol 2022.* |  |

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| SUPPLEMENTAL TABLE 3Cell-free DNA test performance to screen for trisomies 21, 18, and 13 using the updated algorithm for the entire cohort and by risk groups |
| Variable T21 T18 T13 | T21 | T18 | T13 |
| Updated |  |  |  |
| algorithm Low risk (n¼12,967) | High risk (n¼4770) |  |  |
| Sensitivity 100[a](#_bookmark36) 75.00 100 | 98.78 | 100 | 100 |
| 18/18 (81.47e100)[b](#_bookmark36) 3/4 (32.57e100) 6/6 (54.07e100) | 81/82 (96.40e100) | 13/13 (75.29e100) | 6/6 (54.07e100) |
| Specificity 99.97 12,945/12,949 (99.94e100) 99.98 99.98 | 99.94 | 99.96 | 100 |
| 12,961/12,963 (99.96e100) 12,958/12,961 (99.95e100) | 4685/4688 (99.87e100) | 4755/4757 (99.90e100) | 4764/4764 (99.92e100) |
| PPV 81.82 60.00 66.67 | 96.43 | 86.67 | 100 |
| 18/22 (65.70e97.94) 3/5 (17.06e100) 6/9 (35.87e97.46) | 81/84 (92.46e100) | 13/15 (69.46e100) | 6/6 (54.07e100) |
| NPV 100 99.99 100 | 99.98 | 100 | 100 |
| 12,945/12,945 (99.97e100) 12,961/12,962 (99.98e100) 12,958/12,958 (99.97e100) | 4685/4686 (99.94e100) | 4755/4755 (99.92e100) | 4764/4764 (99.92e100) |
| *NPV*, negative predictive value; *PPV*, positive predictive value. |  |  |  |
| a Percent; b 95% confidence interval. |  |  |  |
| *Dar et al. Performance of cell-free DNA screening for aneuploidy in low-risk pregnancies. Am J Obstet Gynecol 2022.* |  |  |  |

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