OBSTETRICS

Cell-free DNA screening for prenatal detection of 22q11.2 deletion syndrome

Pe’er Dar, MD; Bo Jacobsson, MD, PhD; Rebecca Clifton, PhD; Melissa Egbert, MS; Fergal Malone, MD; Ronald J. Wapner, MD; Ashley S. Roman, MD; Asma Khalil, MD; Revital Faro, MD; Rajeevi Madankumar, MD; Lance Edwards, MD; Noel Strong, MD; Sina Haeri, MD; Robert Silver, MD; Nidhi Vohra, MD; Jon Hyett, MD;

Zachary Demko, PhD; Kimberly Martin, MD; Matthew Rabinowitz, PhD; Karen Flood, MD; Ylva Carlsson, MD, PhD; Georgios Doulaveris, MD; Sean Daly, MD; Maria Hallingström, PhD; Cora MacPherson, PhD; Charlly Kao, PhD; Hakon Hakonarson, MD, PhD; Mary E. Norton, MD

BACKGROUND: Historically, prenatal screening has focused primarily on the detection of fetal aneuploidies. Cell-free DNA now enables noninvasive screening for subchromosomal copy number variants, including 22q11.2 deletion syndrome (or DiGeorge syndrome), which is the most common microdeletion and a leading cause of congenital heart defects and neurodevelopmental delay. Although smaller studies have demonstrated the feasibility of screening for 22q11.2 deletion syndrome, large cohort studies with confirmatory postnatal testing to assess test performance have not been reported.

OBJECTIVE: This study aimed to assess the performance of single- nucleotide polymorphismebased, prenatal cell-free DNA screening for detection of 22q11.2 deletion syndrome.

STUDY DESIGN: Patients who underwent single-nucleotide polymorphismebased prenatal cell-free DNA screening for 22q11.2 dele- tion syndrome were prospectively enrolled at 21 centers in 6 countries. Prenatal or newborn DNA samples were requested in all cases for genetic confirmation using chromosomal microarrays. The primary outcome was sensitivity, specificity, positive predictive value, and negative predictive value of cell-free DNA screening for the detection of all deletions, including the classical deletion and nested deletions that are 500 kb, in the 22q11.2 low-copy repeat A-D region. Secondary outcomes included the prevalence of 22q11.2 deletion syndrome and performance of an updated cell-free DNA algorithm that was evaluated with blinding to the pregnancy outcome.

≥

RESULTS: Of the 20,887 women enrolled, a genetic outcome was available for 18,289 (87.6%). A total of 12 22q11.2 deletion syn- drome cases were confirmed in the cohort, including 5 (41.7%) nested deletions, yielding a prevalence of 1 in 1524. In the total cohort, cell-free DNA screening identified 17,976 (98.3%) cases as low risk for 22q11.2 deletion syndrome and 38 (0.2%) cases as high risk; 275 (1.5%) cases were nonreportable. Overall, 9 of 12 cases of 22q11.2 were detected, yielding a sensitivity of 75.0% (95% confi- dence interval, 42.8e94.5); specificity of 99.84% (95% confidence interval, 99.77e99.89); positive predictive value of 23.7% (95% confidence interval, 11.44e40.24), and negative predictive value of 99.98% (95% confidence interval, 99.95e100). None of the cases with a nonreportable result was diagnosed with 22q11.2 deletion syndrome. The updated algorithm detected 10 of 12 cases (83.3%; 95% confidence interval, 51.6e97.9) with a lower false positive rate

(0.05% vs 0.16%; *P*<.001) and a positive predictive value of 52.6% (10/19; 95% confidence interval, 28.9e75.6).

CONCLUSION: Noninvasive cell-free DNA prenatal screening for 22q11.2 deletion syndrome can detect most affected cases, including smaller nested deletions, with a low false positive rate.

Key words: 22q11.2 deletion syndrome, cell-free DNA (cfDNA), DiGeorge syndrome, prenatal screening

# Introduction

Prenatal screening for genetic disorders has traditionally focused on screening for Down syndrome (T21) and other aneu- ploidies (T13 and T18) in the fetus. However, such chromosomal aneu- ploidies constitute a relatively small

Cite this article as: Dar P, Jacobsson B, Clifton R, et al. Cell-free DNA screening for prenatal detection of 22q11.2 deletion syndrome. Am J Obstet Gynecol 2022;XX:x.exex.ex.

0002-9378

ª 2022 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license ([http://](http://creativecommons.org/licenses/by-nc-nd/4.0/)

[creativecommons.org/licenses/by-nc-nd/4.0/](http://creativecommons.org/licenses/by-nc-nd/4.0/)). <https://doi.org/10.1016/j.ajog.2022.01.002>

proportion of the total number of genetic conditions that contribute to adverse in- fant and childhood outcomes. In recent years, noninvasive prenatal screening based on sequencing of circulating cell- free DNA (cfDNA) in maternal blood has introduced the potential to target any region of the genome, including an option to screen for subchromosomal copy number variants such as chromosomal microdeletions.[1e4](#_bookmark10)

Although individually rare, in aggre- gate, chromosomal microdeletions and duplications are more prevalent than the common trisomies, and because their birth incidence is not associated with

the 22q11.2 deletion syndrome (22q11.2DS), also known as DiGeorge or velocardiofacial syndrome. This condi- tion is characterized by variable features including congenital heart defects and developmental delay in most patients, a cleft palate or velopharyngeal insufﬁ- ciency, hypocalcemia, immunodeﬁ- ciency, autism, and psychiatric disorders.[7](#_bookmark14) The 22q11.2DS has been estimated to affect approximately 1 in 3000 to 6000 live births and is therefore one of the most common causes of developmental delay and congenital heart anomalies.[8e10](#_bookmark15) These mostly de novo deletions are caused by meiotic

 increasing maternal age, they are more

Original Research

[ajog.org](http://www.AJOG.org/)

common than T21 in women <30 years of age.[5](#_bookmark12),[6](#_bookmark13) The most common of these is



recombination events in 4 hot spot re-

gions known as A-D low-copy repeats (LCR) on the long arm of chromosome

22 ([Figure 1](#_bookmark0)).[11](#_bookmark17) In approximately 85% of affected individuals, the entire 2.5 to 3 Mb LCR A-D region is deleted, whereas others have smaller nested deletions within this region.[12](#_bookmark18),[13](#_bookmark19)

In addition to providing parents with important information about their pregnancy, antenatal diagnosis of 22q11.2DS has the potential to improve short- and long-term outcomes for these children.[14](#_bookmark20) Prenatal detection of congenital heart defects enables delivery at a center capable of caring for these neonates and providing timely treatment for neonatal hypocalcemia and immu- nodeﬁciency, which has been shown to improve outcomes.[15](#_bookmark21),[16](#_bookmark22) Despite these beneﬁts, the limited data on test perfor- mance have precluded prenatal screening for the syndrome from being routinely offered. Screening for 22q11.2DS has been evaluated in a few studies involving either artiﬁcially derived plasma mixtures or plasma samples from women with a high prob- ability of having a fetus with a genetic abnormality.[17e20](#_bookmark23) Retrospective analyses of clinical cohorts reported positive predictive values (PPVs) but have not performed full-cohort conﬁrmatory ge- netic testing to determine test sensitivity and speciﬁcity.[21e23](#_bookmark25)

We therefore, sought to assess the performance of single-nucleotide poly- morphism (SNP)ebased cfDNA screening for 22q11.2DS in a large pro- spective study with genetic conﬁrmation in all pregnancies.

# Materials and Methods

## Study design and participants

This was a multicenter, prospective observational study. Women with singleton gestations who underwent SNP-based cfDNA screening for aneu- ploidy and 22q11.2DS were enrolled at 21 centers in the United States, Europe, and Australia. (Supplemental Materials and Methods). The study was registered with [ClinicalTrials.gov](http://ClinicalTrials.gov/) (identiﬁer: NCT02381457; SNP-based Micro- deletion and Aneuploidy RegisTry or

SMART) and approved by each site’s institutional review board. All partici-

pants provided written consent. Eligible women were 18 years old, at 9 weeks’ gestation, had a singleton pregnancy, and planned to deliver at a study siteeafﬁliated hospital. Women were excluded if they received a cfDNA result before enrollment, underwent organ transplantation, conceived using ovum

≥ ≥

donation, or were unable to provide a newborn sample. Women who previously

underwent traditional serum screening for aneuploidy or sonographic detection of fetal anomalies were eligible for in- clusion. Participants did not receive remuneration for enrolling and were not charged for the 22q11.2DS analysis. Screening results were utilized as part of clinical care.

Genetic outcomes were assessed by analysis of prenatal (chorionic villus sampling, amniocentesis, products of conception) or infant (cord blood, buccal swab or newborn blood spot) samples. In all cases, a sample was requested at the end of pregnancy for chromosomal microarray analysis (CMA), regardless of previous prenatal testing. The postnatal CMA was per- formed by an independent laboratory (Center for Applied Genomics, Chil-

positive rate seen with cfDNA aneuploidy screening.

What does this add to what is known?

This study presents new and comprehensive information on the performance of cfDNA screening for 22q11.2DS, with results based on genetic con*ﬁ*rmation in all cases. The *ﬁ*ndings in this study demonstrate that cfDNA screening for 22q11.2 can be added to aneuploidy screening without a signi*ﬁ*cant increase in the screen positive rate.

are ≥500 kb. The test false positive rate was 0.15%, which is similar to the false

Why was this study conducted?

22q11.2 deletion syndrome (22q11.2DS or DiGeorge syndrome) is the most common microdeletion and a leading cause of congenital heart defects and neurodevelopmental delay. Although cell-free DNA (cfDNA) prenatal screening for 22q11.2DS is feasible, data on test performance are limited.

Key ﬁndings

Based on genetic con*ﬁ*rmation in all cases, the cohort prevalence of 22q11.2DS

was 1 in 1524. Single-nucleotide polymorphismebased cfDNA screening iden- ti*ﬁ*ed most cases of 22q11.2DS including both classical and nested deletions that

AJOG at a Glance

dren’s Hospital of Philadelphia, PA) that was blinded to the clinical or laboratory

results. If postnatal CMA conﬁrmation was not available, results from clinical testing with prenatal CMA, ﬂuorescence in situ hybridization (FISH), bacterial artiﬁcial chromosomes (BACs)-on- beads, or multiplex ligation-dependent probe ampliﬁcation (MLPA), if avail- able, were used for genetic conﬁrmation.

## Outcomes

The primary outcome was test perfor- mance of cfDNA screening for detection of 22q11.2 deletions 500 kb in the LCR A-D region. Secondary outcomes included the prevalence of 22q11.2DS and performance of an updated screening algorithm that was assessed after enrollment completion.

≥

## Procedures

Sample preparation and analysis of cfDNA were performed as previously described (Natera Inc, San Carlos, CA).[16](#_bookmark22) Results indicating a risk of 1 in 100 for 22q11.2DS were categorized as high risk and those indicating a risk of

≥

<1 in 100 were categorized as low risk. In cases with nonreportable results, pa-

tients were offered repeat testing and results obtained after a second blood sample collection were included; a third sample was not requested. During enrollment, the cfDNA laboratory

FIGURE 1

Depiction of the deleted 22q11.2 region in chromosome 22



The region includes 4 sets of LCR referred to as LCR-A, LCR-B, LCR-C, and LCR-D (*green boxes*). The position of the N25 and TUPLE probes used for fluorescence in situ hybridization are marked in *purple*. Deletions or variants involving T-Box Transcription Factor 1 (TBX1), 1 of 46 protein coding genes in this A-D region, are thought to be responsible for many of the clinical features of 22q11.2DS. In addition, there are 7 micro RNA (miRNA) genes and 10 noncoding genes in this region. The size and position of the typical A-D deletion and smaller, nested deletions are indicated at the bottom.

*LCR*, low-copy repeat.

*Dar et al. Performance of cell-free DNA prenatal screening for 22q11.2 deletion syndrome. Am J Obstet Gynecol 2022*.

protocol was modiﬁed once.[24](#_bookmark26),[25](#_bookmark27) Results from both periods were combined for analysis. After enrollment completion, a third updated algorithm was developed by the laboratory, optimized to identify both the full and nested deletions using a deep neural network (DNN) component and reﬂex testing of high-risk calls with deeper sequencing. A deep learning (TensorFlow v1.15, Google Brain, Mountain View, CA) 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. The self-supervised algorithm leveraged 1.6 million sequenced mix- tures of mother and fetus cfDNA sam- ples, learning to harness the linkage among the SNPs to improve call conﬁ- dence. This updated protocol was assessed with blinding to the outcomes. For conﬁrmatory CMA analysis, DNA

was prepared from the neonates’ cord blood, buccal smear, or, predominantly,

dried blood spot. Copy number variants, including aneuploidies and 22q11.2DS, were identiﬁed using the Illumina (San Diego, CA) SNP-based Inﬁnium Global Screening Array (GSA) platform. Sam- ples were genotyped in standard versions (GSA-V1.0, GSA-V2.0, GSAMD-V1.0,

or GSAMD-V2.0) or in a custom- designed SMARTArray in which addi- tional SNPs were added to the GSA backbone. Within the 22q11 region of interest (chr22:18,950,000-21,500,000;

hg19), the GSA backbone contains 600 SNPs, whereas the custom SMARTArray has 1963 SNPs including those in the backbone. A positive 22q11.2DS was conﬁrmed if a deletion 500 kb was identiﬁed within the LCR AeD interval. Positive samples underwent conﬁrma- tion with the Omni 2.5-8V1-3 array and were reviewed by a clinical cytogeneticist before the results were generated.

≥

Because neonatal DNA samples were obtained from different sources, mostly from dry blood spots that were collected

by state health departments for routine neonatal screening, we developed a concordance test for quality assurance purposes. The concordance test was designed to conﬁrm that the cfDNA re- sults and newborn samples were correctly paired by using alignment be- tween SNPs in the 2 samples; any sam- ples that could not be paired were excluded.

## Data collection

Onsite research coordinators recorded information using a secured computer- ized tracking system developed and managed by The Biostatistics Center at George Washington University, Wash- ington DC. Data that were collected included patient and obstetrical data, imaging reports, aneuploidy serum screening, and prenatal diagnosis results. After delivery, information on preg- nancy complications, genetic testing or ultrasound ﬁndings, newborn features suggestive of a genetic abnormality,

major malformations, and other adverse outcomes was collected.

## Study oversight

This study was a collaboration between the clinical investigators and the sponsor (Natera, Inc, San Carlos, CA). The ﬁrst and last authors designed the protocol in collaboration with the sponsor and had a majority vote in study design and data interpretation. There were no conﬁden- tiality agreements among the authors, sites, or sponsor. All laboratory analyses were conducted with blinding to the outcome data. Clinical and laboratory results were managed by the data coor- dinating center, which independently matched the information and de- identiﬁed and analyzed the results.

Patient and public involvement Patients and the public were not involved in the design of the study protocol, in establishing the research question, or in the outcome measures. No patients or members of the public were involved in the recruitment process or the conduct of the study. Finally, no patients or members of the public were or will be

involved in the interpretation or dissemination of the study’s results.

## Statistical analysis

Originally, a sample size of 10,000 par- ticipants was planned based on 22q11.2DS prevalence estimates that ranged from 1 in 300 to 1 in 2000.[5](#_bookmark12),[6](#_bookmark13),[17](#_bookmark23) During the trial, concerns arose that the prevalence of the 22q11.2DS may be lower and prior to unblinding, the sample size was increased to 20,000, which allowed for a higher level of pre- cision to assess performance.[9](#_bookmark16) The sensitivity, speciﬁcity, PPV, and negative predictive value (NPV) of the cfDNA results were assessed and exact (Clopper- Pearson) 95% conﬁdence intervals (CIs) were reported. Participants without cfDNA results or genetic conﬁrmation were excluded from the test performance analysis. SAS Studio 9.04 software (SAS Institute, Cary, NC) was used for anal- ysis. Continuous variables were compared using the Wilcoxon test and categorical variables were compared

using chi-square or Fisher exact tests as appropriate. McNemar test was used for paired analyses.

# Results

## Study participants

From April 2015 through January 2019, we screened 25,892 women and enrolled 20,887 ([Figure 2](#_bookmark1)). Overall, 54.8% were enrolled in the United States and 45.2% in Europe or Australia. Of the enrolled participants, 296 (1.4%) had a preg- nancy loss without genetic conﬁrmation, 1110 (5.3%) were lost to follow-up and therefore the pregnancy outcome is un- known, for 811 (3.9%), a conﬁrmatory

sample was not obtained, 94 (0.5%)

withdrew consent, and for 287 (1.4%), the conﬁrmation test failed laboratory quality control. The latter group included 49 cases that failed the concordance quality assurance test and for which the neonatal sample could not be genetically paired with a cfDNA sample. After exclusions, the study cohort included 18,289 (87.6%) partic- ipants who had both cfDNA and DNA conﬁrmation results for 22q11.2DS.

The median maternal and gestational ages at enrollment were 34.5 years and

12.6 weeks, respectively ([Table 1](#_bookmark2)). Overall, 108 (0.6%) underwent cfDNA screening after detection of a fetal anomaly on ultrasound, 95 (0.5%) after diagnosis of a cystic hygroma or nuchal translucency 3 mm, and 623 (3.4%) following a high-risk result on serum analyte screening for aneuploidy.

≥

Primary and secondary outcomes Twelve 22q11.2DS cases were diagnosed in the cohort by conﬁrmatory genetic testing, yielding a cohort prevalence of 1 in 1524. Of these, 4 (33%) cases contained the typical 3 Mb A-D deletions, 5 (41.6%) contained nested deletions, ranging from

0.73 Mb to 2 Mb, and 3 (25%) were identiﬁed by FISH or BACs-on-beads, both of which used probes speciﬁc to the A-B region, which precluded ascer- taining their precise size ([Table 2](#_bookmark7)). Most outcomes (18,195; 99.5%) were conﬁrmed by postnatal CMA and 94 (0.5%) by other pre- or postnatal genetic

testing. Three 22q11.2DS cases were conﬁrmed prenatally.

Of the 18,289 cases, based on the cfDNA screening results, 17,976 (98.3%) were categorized as low risk for 22q11.2DS, 38 (0.2%) were categorized as high risk, and 275 (1.5%) remained nonreportable despite collecting a sec- ond sample. Prenatal diagnostic testing was performed for 21 of 38 (55.3%) high-risk cfDNA cases, after which 3 22q11.2DS cases were identiﬁed.

Nine deletions, including all 4 typical deletions, the 3 deletions of uncertain size, and 2 of the 5 nested deletions were detected by cfDNA screening, yielding a sensitivity of 75.0% (95% CI,

42.8e94.5), speciﬁcity of 99.84% (95%

CI, 99.77e99.89), PPV of 23.7% (95% CI, 11.44e40.24), and NPV of 99.98%

(95% CI, 99.95e100) ([Table 3](#_bookmark8)). None of the fetuses or infants of patients with nonreportable results were conﬁrmed to have 22q11.2DS.

Fetal anomalies were detected in 7 (58.3%) patients with 22q11.2DS. Four heart anomalies were diagnosed before cfDNA screening, and 3 fetal anomalies, 2 cardiac anomalies, and 1 renal anomaly were identiﬁed after a high-risk cfDNA result was reported. In addition, a gastrointestinal anomaly was diagnosed in a fetus previously diagnosed with a cardiac anomaly. Eleven of the patients with 22q11.2DS pregnancies, including 6 patients with anomalies, underwent a ﬁrst trimester ultrasound, none of which identiﬁed any fetal anomalies or nuchal translucency 3 mm. None were at high risk for aneuploidy based on ﬁrst trimester screening and 1 patient un- derwent cfDNA screening following a high-risk result on serum screening in the second trimester.

≥

Three cases of 22q11.2DS had false negative cfDNA results with the original algorithm; 1 had a 1.5 Mb A-B deletion and 2 had 730 kb B-D deletions. Of the latter, 1 was diagnosed prenatally with unilateral renal agenesis; the deletion in this patient was detected with the updated cfDNA algorithm. Another newborn with a 730 kb B-D deletion was growth restricted and was found to have a bran- chial cleft cyst and a digital anomaly after

FIGURE 2

Patient enrollment flowchart



*Dar et al. Performance of cell-free DNA prenatal screening for 22q11.2 deletion syndrome. Am J Obstet Gynecol 2022*.

birth. All 3 had normal ﬁrst trimester ultrasound and serum screening results.

The updated algorithm identiﬁed 1 additional 22q11.2DS case, increasing the sensitivity to 83.3% (10/12; 95% CI, 51.6e98.9), and had a signiﬁcantly lower positive screening rate (19; 0.10% vs 38; 0.21%; *P*<.001) and a lower false positive

rate (9; 0.05% vs 29; 0.16%; *P*<.001),

increasing the PPV to 52.6% (95% CI,

28.9e75.6) ([Table 3](#_bookmark8)).

Overall, 2597 women did not have genetic conﬁrmation and were excluded. Compared with the included study cohort, they were younger (34.2 vs 34.5 years; *P*<.001), more likely to be Black, and less likely to be Hispanic (12.1% vs 8.5%; 15.6% vs 18.1%, respectively; *P*<.001) but had a similar body mass

index, gestational age at enrollment, and region of enrollment. In this group, 3 (0.12%) women received a high-risk cfDNA result for 22q11.2DS. One terminated because of a prenatal diag- nosis of an omphalocele and 2 had un- complicated pregnancies and no reported neonatal anomalies.

# Comment

## Principal findings and results in the context of what is known

In this multicenter prospective study, we found that prenatal screening for 22q11.22DS with SNP-based cfDNA has high sensitivity and speciﬁcity in a diverse, real-world population. These ﬁndings demonstrate that routine noninvasive prenatal screening using

cfDNA for genetic disorders beyond aneuploidy is possible with high accuracy.

Previous validation studies have also demonstrated high detection and low false positive rates when using cfDNA screening for 22q11.2DS, but most have evaluated only detection of the common 3 Mb A-D deletion.[13](#_bookmark19),[17](#_bookmark23),[18](#_bookmark24) In our cohort, at least 5 of the 12 cases involved smaller, nested deletions, a proportion that is higher than expected based on previous reports. Deletion of the LCR A-B region, which contains many 22q11.2DS critical genes, is associated with severe features and has a similar clinical presentation as that of the classical deletion. Ten of the 12 conﬁrmed deletions in our cohort included this region, and 9 of them were

TABLE 1

### Demographics and clinical characteristics of study participants[a](#_bookmark3)

Variable Study cohort (n¼18,289)

Maternal and gestational characteristics

Maternal age (y), median (IQR) 34.5 (30.4e37.5) Nulliparity, n/total, n (%) 8022/18,248 (44.0)

BMI (kg/m2), median (IQR)[b](#_bookmark3),[c](#_bookmark4) 24.9 (22.3e29.0)

|  |  |
| --- | --- |
| Race and ethnicity, n (%)[d](#_bookmark5) |  |
| Asian | 1542 (8.4) |
| Black | 1554 (8.5) |
| White | 11,272 (61.6) |
| Hispanic | 3309 (18.1) |
| Other or unknown | 612 (3.3) |

Gestational age at enrollment (wk), median (IQR) 12.6 (11.6e13.9)

detected during the screen. Although the LCR B-D region has been less well studied, clinical features associated with these deletions, including heart defects and neurodevelopmental delays, overlap with those associated with the classical deletion, and these nested deletions should be considered when calculating the overall detection rate of 22q11.2DS.[13](#_bookmark19)

The prevalence of 22q11.2DS in our diverse cohort (1 in 1524) was higher than the reported prevalence in postnatal populations, but similar to rates re- ported in prenatal studies.[5](#_bookmark12),[6](#_bookmark13),[8e10](#_bookmark15) It is possible that including the 4 cases with fetal anomalies detected before enroll- ment enriched the 22q11.2DS popula-

Pregnancy through assisted reproductive technology, n (%)

959 (5.2)

tion. Excluding these cases would lead to a prevalence of 1 in 2312, which is

Current smoker, n/total, n (%) 321/18,211 (1.8)

Enrolled at a US site, n (%) 10,005 (54.7)

Prenatal screening and testing

Positive first trimester screen before enrollment, n (%) 518 (2.8)

similar to a recently reported genetic analysis of newborn screening samples.[26](#_bookmark28) Although the rate of pregnancy loss associated with 22q11.2DS is not reportedly increased, postnatal studies

Nuchal translucency 3 mm or cystic hygroma before enrollment, n (%)

≥

Positive second trimester or integrated screen before enrollment, n (%)

95 (0.5)

105 (0.6)

may underestimate the frequency by excluding cases of 22q11.2DS that were terminated following detection of fetal anomalies.[27](#_bookmark29),[28](#_bookmark30) In addition, most post-

Major anomaly before testing, n (%) 107 (0.6)

Fetal fraction (%), mean SD[c](#_bookmark4) 9.9 4.1

Diagnostic testing, n (%) 420 (2.3)

Pregnancy and delivery outcome

Miscarriage, n/total, n (%) 5/18,281 (0.03)

Pregnancy termination, n/total, n (%) 41/18,281 (0.2) Live birth, n/total, n (%) 18,224/18,281 (99.7)

Stillbirth, n/total, n (%) 11/18,281 (0.06)

Neonatal death, n/total, n (%) 24/18,281 (0.1)

Aneuploidy (T13, 18, 21), n (%) 36 (0.2)

Gestational age at delivery (wk), median (IQR)[c](#_bookmark4) 39.4 (38.6e40.3)

|  |  |
| --- | --- |
| PTB <37 weeks’ gestation, n/total, n (%) | 1311/18,230 (7.2) |
| Preeclampsia, n/total, n (%) | 735/18,230 (4.1) |
| Birthweight (g), mean (SD)[c](#_bookmark4) | 3361 555 |
| Birthweight <10% percentile, n/total, n (%) | 1578/18,042 (8.8) |

Days to newborn discharge, median (IQR)[c](#_bookmark4) 2 (2e3)

*BMI*, body mass index; *IQR*, interquartile range; *PTB*, preterm birth; *SD*, standard deviation.

a Plus-minus values are mean standard deviation; b The body mass index is the weight in kilograms divided by the square of the height in meters; c BMI data were missing for 314 participants; fetal fraction data were missing for 76 participants because of low-level contamination, low-level fetal mosaicism, or low-level sample noise of undetermined origin; gestational age at delivery was missing for 59 participants, and birthweight data were missing for 245 infants. Days to newborn discharge were missing for 308 liveborn infants; d Race and ethnic groups were reported by the participants. If the participant did not report the information, the information from the chart was used.

*Dar et al. Performance of cell-free DNA prenatal screening for 22q11.2 deletion syndrome. Am J Obstet Gynecol 2022*.

natal reports have largely relied on earlier technologies to detect 22q11.2DS, such as FISH and BACs-on-beads, which use probes localized to the LCR A-B in- terval that do not detect some nested deletions.

Clinical and research implications Given the increasing use of cfDNA as a primary screening tool for common aneuploidies, clinical signiﬁcance and test performance are important when considering expansion of targeted con- ditions.[29](#_bookmark31) The importance of 22q11.2 is apparent given the signiﬁcant clinical sequelae and prevalence, which is higher than some of the currently screened for aneuploidies.[30](#_bookmark32) Moreover, the long-term sequalae associated with 22q11.2DS, such as autism spectrum disorder and schizophrenia, and the potential beneﬁts of early neonatal therapy for hypocalce- mia and immune deﬁciency, justify the consideration for prenatal screening.[13e15](#_bookmark19) In this study, we found that modalities such as ﬁrst trimester ultrasonography and traditional

TABLE 2

OBSTETRICS Original Research

ajog.org

MONTH 2022 American Journal of Obstetrics & Gynecology 1.e7

### Pre- and postnatal characteristics of confirmed 22q11.2 deletions >500 kb in the LCR22 A-D region

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Case | Deletion size and location | Stage of confirmation | Test | GA at cfDNA (wk) | Fetal fraction | Identified by cfDNA | First trimester ultrasound | Fetal anomaly detected before cfDNA | Fetal anomaly detected after cfDNA | Outcome | GA at delivery | Birthweight |
| 1. | A-D 2.6 Mb | Postnatal | CMA | 20 | 13.7% | Yes | Normal | Interrupted aortic arch, VSD (20 wk) | None | Live birth | Term | AGA |
| 2. | A-D 2.6 Mb | Postnatal | CMA | 31 | 9.7% | Yes | Normal | Truncus arteriosus at (31 wk) | None | Live birth | Late preterm[a](#_bookmark6) | AGA |
| 3. | A-D 2.6 Mb | Postnatal | CMA | 10 | 7.5% | Yes | Normal | None | None | Live birth | Term | SGA |
| 4. | A-D 2.6 Mb | Postnatal | CMA | 17 | 7.0% | Yes | Not done | Truncus arteriosus, VSD (17 wk) | Bowel obstruction (31 wk) | Live birth | Late preterm[a](#_bookmark6) | AGA |
| 5. | Unknown[b](#_bookmark6) | Prenatal CVS | BoB | 10 | 6.9% | Yes | Normal | None | Atrioventricular canal (20 wk) | TOP |  |  |
| 6. | Unknown[b](#_bookmark6) | Prenatal amniocentesis | BoB | 11 | 6.9% | Yes | Normal | None | No additional ultrasound | TOP |  |  |
| 7. | Unknown[b](#_bookmark6) | Postnatal | FISH | 21 | 14.4% | Yes | Normal | Tetralogy of Fallot (21 wk) | No additional ultrasound | NND | Term | SGA |
| 8. | A-C 2.06 Mb | Prenatal amniocentesis | MLPA | 10 | 7.6% | Yes | Normal | None | VSD (18 wk) | TOP |  |  |
| 9. | A-B 1.47 Mb | Postnatal | CMA | 20 | 13.3% | Yes | Normal | None | No additional ultrasound | Live birth | Term | AGA |
| 10. | A-B 1.47 Mb | Postnatal | CMA | 11 | 17.5% | No | Normal | None | None | Live birth | Term | AGA |
| 11. | B-D 0.73 Mb | Postnatal | CMA | 15 | 4.9% | No[c](#_bookmark6) | Normal | None | Unilateral renal agenesis (22 wk) | Live birth | Term | AGA |
| 12. | B-D 0.73 Mb | Postnatal | CMA | 12 | 8.5% | No | Normal | None | None | Live birth | Term | SGA |

*AGA*, appropriate for gestational age; *BoB*, bacterial artificial chromosomes (BACs)-on-Beads; *CMA*, chromosomal microarray; *CVS*, chorionic villous sampling; *FF*, fetal fraction; *FISH*, fluorescence in situ hybridization; *GA*, gestational age; *MLPA*, multiplex ligation- dependent probe amplification; *NND*, neonatal death; *SGA*, small for gestational age (birthweight <10th percentile for gestational age); *TOP*, termination of pregnancy; *VSD*, ventricular septal defect.

a Late preterm birth was defined as birth at 34 to 37 weeks’ gestation; b Probes localized to the A-B region; c This case was identified by the updated algorithm.

*Dar et al. Performance of cell-free DNA prenatal screening for 22q11.2 deletion syndrome. Am J Obstet Gynecol 2022*.

*LCR*, low-copy repeats; *NPV*, negative predictive value; *PPV*, positive predictive value.

a Positive likelihood ratio is calculated as sensitivity/100especificity and the negative likelihood ratio is calculated as 100esensitivity/specificity.

*Dar et al. Performance of cell-free DNA prenatal screening for 22q11.2 deletion syndrome. Am J Obstet Gynecol 2022*.

Updated algorithm implemented after study

completion (n¼18,043)

83.3% (10/12; 95% CI, 51.6e97.9)

99.95% (18,022/18,031; 95% CI, 99.91e99.98)

52.6% (10/19; 95% CI, 28.9e75.6)

99.99% (18,022/18,024; 95% CI, 99.96e100)

1666.00

0.17

Original algorithm used at enrollment (n¼18,014)

75.0% (9/12; 95% CI, 42.8e94.5)

99.84% (17,973/18,002; 95% CI, 99.77e99.89)

23.7% (9/38; 95% CI, 11.4e40.2)

99.98% (17,973/17,976; 95% CI, 99.95e100)

468.75

0.25

Test parameter

Sensitivity Specificity PPV

NPV

Positive likelihood ratio[a](#_bookmark9) Negative likelihood ratio[a](#_bookmark9)

TABLE 3

cfDNA test performance for detection of ‡ 500 kb 22q11.2 deletions in the LCR22 AeD region with the algorithm applied at enrollment and with the updated algorithm

aneuploidy screening are not useful for the detection of 22q11.2DS. The low prevalence of individual microdeletion syndromes and the resultant low PPVs of testing have called into question the value of screening.[30](#_bookmark32),[31](#_bookmark33) However, the PPV of cfDNA screening for 22q11.2DS is higher and the false positive rate is lower than that associated with other accepted screening tests, such as the traditional ﬁrst trimester combined screening,[3](#_bookmark11),[32](#_bookmark34),[33](#_bookmark35) and comparable with cfDNA screening for some of the aneu- ploidies.[3](#_bookmark11),[33](#_bookmark35) Finally, in the updated al- gorithm, we utilized a massively multiplexed polymerase chain reactionebased SNP analysis enhanced by postsequencing DNN analysis to further improve performance. This

detected on a second trimester anatomic survey before cfDNA screening. Although an ultrasound diagnosis of a fetal anomaly in the second trimester can be followed by a diagnostic test, leading to detection of 22q11.2DS on a micro- array, for some patients, this may be too late to consider invasive testing or preg- nancy termination. In fact, in our diverse cohort, none of the patients who were diagnosed with a fetal anomaly before cfDNA screening elected to have a diag- nostic procedure or to discontinue the pregnancy. The 3 (25%) patients who underwent a diagnostic procedure had undergone their cfDNA screening in the ﬁrst trimester. Similarly, only 2 of 7 (28.5%) patients with fetal anomalies elected to terminate the pregnancy and

In addition, the estimates of detection rates for uncommon conditions are necessarily associated with wide CIs. Finally, as a real-world study, the in- dications for testing were varied and the prevalence rates may not necessarily reﬂect the average risk population.

## Conclusions

This study identiﬁed that SNP-based cfDNA screening for 22q11.2DS can detect most affected cases, including the smaller but relatively common nested deletions, with a low false positive rate. The ﬁndings of this study provide important information when consid- ering expansion of routine prenatal ge- netic screening to include screening for 22q11.2DS for all pregnant women. ■

innovative use of machine both had their cfDNA screening and

learningebased artiﬁcial intelligence led to lower false positive rates and higher PPVs, in this case >50%, for this microdeletion. Although recognizing that prenatal screening continues to evolve with improved detection rates and lower false positive rates, pre- and posttest counseling should emphasize that, at this time, the performance of

screening tests is not equivalent to diagnostic tests and that positive screening tests should be followed by a diagnostic test.

Fetal anomalies were identiﬁed by ultrasound in 7 22q11.2DS cases, all in the second or third trimester. In 3 (25%) of the 22q11.2DS cases, the anomaly was

diagnostic test results before the anom- aly was detected.

## Strengths and limitations

The primary strength of this study is the comprehensive genetic conﬁrmation obtained on fetal or newborn DNA samples. Given that features of 22q11.2DS may not be apparent prena- tally or on clinical examination at birth, genetic testing assured complete case ascertainment. Nevertheless, this study is not without limitations. Despite the large sample size, the overall number of conﬁrmed cases was relatively low, which limits our ability to accurately assess the PPV stratiﬁed by risk factors.

References

1. [Chitty LS, Bianchi DW. Noninvasive prenatal](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref1) [testing: the paradigm is shifting rapidly. Prenat](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref1) [Diagn 2013;33:511–3](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref1).
2. [Gil MM, Accurti V, Santacruz B, Plana MN,](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref2)

[Nicolaides KH. Analysis of cell-free DNA in](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref2) [maternal blood in screening for aneuploidies:](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref2) [updated meta-analysis. Ultrasound Obstet](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref2) [Gynecol 2017;50:302–14](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref2).

1. [Norton ME, Jacobsson B, Swamy GK, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref3)

[Cell-free DNA analysis for noninvasive exami-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref3) [nation of trisomy. N Engl J Med 2015;372:](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref3) [1589–97](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref3).

1. [Dar P, Curnow KJ, Gross SJ, et al. Clinical](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref4)

[experience and follow-up with large scale single-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref4) [nucleotide polymorphism-based noninvasive](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref4) [prenatal aneuploidy testing. Am J Obstet](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref4) [Gynecol 2014;211:527.e1–17](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref4).

1. [Wapner RJ, Martin CL, Levy B, et al. Chro-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref5)

[mosomal microarray versus karyotyping for](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref5)

[prenatal diagnosis. N Engl J Med 2012;367:](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref5) [2175–84](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref5).

1. [Grati FR, Molina Gomes D, Ferreira JC, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref6)

[Prevalence of recurrent pathogenic micro-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref6) [deletions and microduplications in over 9500](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref6) [pregnancies. Prenat Diagn 2015;35:801–9](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref6).

1. [Emanuel BS, McDonald-McGinn D,](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref7)

[Saitta SC, Zackai EH. The 22q11.2 deletion](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref7) [syndrome. Adv Pediatr 2001;48:39–73](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref7).

1. [Botto LD, May K, Fernhoff PM, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref8)

[A population-based study of the 22q11.2 dele-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref8) [tion: phenotype, incidence, and contribution to](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref8) [major birth defects in the population. Pediatrics](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref8) [2003;112:101–7](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref8).

1. [Olsen L, Sparsø T, Weinsheimer SM, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref9)

[Prevalence of rearrangements in the 22q11.2](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref9) [region and population-based risk of neuropsy-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref9) [chiatric and developmental disorders in a Danish](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref9) [population: a case-cohort study. Lancet Psy-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref9) [chiatry 2018;5:573–80](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref9).

1. [McDonald-McGinn DM, Sullivan KE,](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref10)

[Marino B, et al. 22q11.2 deletion syndrome. Nat](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref10) [Rev Dis Primers 2015;1:15071](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref10).

1. [McDonald-McGinn DM, Tonnesen MK,](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref11) [Laufer-Cahana A, et al. Phenotype of the 22q11.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref11) [2 deletion in individuals identiﬁed through an](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref11) [affected relative: cast a wide FISHing net! Genet](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref11) [Med 2001;3:23–9](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref11).
2. [Edelmann L, Pandita RK, Morrow BE. Low-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref12)

[copy repeats mediate the common 3-Mb dele-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref12) [tion in patients with velo-cardio-facial syndrome.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref12) [Am J Hum Genet 1999;64:1076–86](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref12).

1. [Morrow BE, McDonald-McGinn DM,](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref13)

[Emanuel BS, Vermeesch JR, Scambler PJ.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref13) [Molecular genetics of 22q11.2 deletion syn-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref13) [drome. Am J Med Genet A 2018;176:](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref13) [2070–81](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref13).

1. [Dugoff L, Mennuti MT, McDonald-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref14)

[McGinn DM. The bene](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref14)ﬁ[ts and limitations of cell-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref14) [free DNA screening for 22q11.2 deletion](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref14) [syndrome. Prenat Diagn 2017;37:53–60](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref14).

1. [Quartermain MD, Hill KD, Goldberg DJ, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref15)

[Prenatal diagnosis in](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref15)ﬂ[uences preoperative sta-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref15) [tus in neonates with congenital heart disease: an](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref15)

[analysis of the Society of Thoracic Surgeons](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref15) [congenital heart surgery database. Pediatr](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref15) [Cardiol 2019;40:489–96](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref15).

1. [Cheung ENM, George SR, Andrade DM,](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref16)

[Chow EWC, Silversides CK, Bassett AS.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref16) [Neonatal hypocalcemia, neonatal seizures, and](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref16) [intellectual disability in 22q11.2 deletion syn-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref16) [drome. Genet Med 2014;16:40–4](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref16).

1. [Wapner RJ, Babiarz JE, Levy B, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref17)

[Expanding the scope of noninvasive prenatal](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref17) [testing: detection of fetal microdeletion syn-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref17) [dromes. Am J Obstet Gynecol 2015;212:332.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref17) [e1–9](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref17).

1. [Ravi H, McNeill G, Goel S, et al. Validation of](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref18)

[a SNP-based non-invasive prenatal test to](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref18) [detect the fetal 22q11.2 deletion in maternal](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref18) [plasma samples. PLoS One 2018;13:](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref18) [e0193476](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref18).

1. [Schmid M, Wang E, Bogard PE, et al. Pre-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref19) [natal screening for 22q11.2 deletion using a](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref19) [targeted microarray-based cell-free DNA test.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref19) [Fetal Diagn Ther 2018;44:299–304](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref19).
2. [Bevilacqua E, Jani JC, Chaoui R, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref20) [Performance of a targeted cell-free DNA pre-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref20) [natal test for 22q11.2 deletion in a large clin-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref20) [ical cohort. Ultrasound Obstet Gynecol](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref20) [2021;58:597–602](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref20).
3. [Martin K, Iyengar S, Kalyan A, et al. Clinical](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref21)

[experience with a single-nucleotide poly-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref21) [morphism-based non-invasive prenatal test for](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref21)

ﬁ[ve clinically signi](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref21)ﬁ[cant microdeletions. Clin](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref21) [Genet 2018;93:293–300](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref21).

1. [Gross SJ, Stosic M, McDonald-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref22)

[McGinn DM, et al. Clinical experience with](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref22) [single-nucleotide polymorphism-based non-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref22) [invasive prenatal screening for 22q11.2 dele-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref22) [tion syndrome. Ultrasound Obstet Gynecol](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref22) [2016;47:177–83](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref22).

1. [Helgeson J, Wardrop J, Boomer T, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref23)

[Clinical outcome of subchromosomal events](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref23) [detected by whole-genome noninvasive prena-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref23) [tal testing. Prenat Diagn 2015;35:999–1004](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref23).

1. [Zimmermann B, Hill M, Gemelos G, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24)

[Noninvasive prenatal aneuploidy testing of](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24) [chromosomes 13, 18, 21, X, and Y, using tar-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24) [geted sequencing of polymorphic loci. Prenat](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24) [Diagn 2012;32:1233–41](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24).

1. [Ryan A, Hunkapiller N, Banjevic M, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref25)

[Validation of an enhanced version of a single-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref25) [nucleotide polymorphism-based noninvasive](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref25) [prenatal test for detection of fetal aneuploidies.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref25) [Fetal Diagn Ther 2016;40:219–23](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref25).

1. [Blagojevic C, Heung T, Theriault M, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24)

[Estimate of the contemporary live-birth preva-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24) [lence of recurrent 22q11.2 deletions: a cross-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24) [sectional analysis from population-based](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24) [newborn screening. CMAJ Open 2021;9:](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24) [E802–9](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref24).

1. [Maisenbacher MK, Merrion K, Pettersen B,](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref26)

[et al. Incidence of the 22q11.2 deletion in a large](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref26) [cohort of miscarriage samples. Mol Cytogenet](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref26) [2017;10:6](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref26).

1. [Levy B, Sigurjonsson S, Pettersen B, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref27) [Genomic imbalance in products of conception:](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref27) [single-nucleotide polymorphism chromosomal](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref27) [microarray analysis. Obstet Gynecol 2014;124:](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref27) [202–9](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref27).
2. [Chitty LS, Hudgins L, Norton ME. Current](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref28)

[controversies in prenatal diagnosis 2: cell-free](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref28) [DNA prenatal screening should be used to](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref28) [identify all chromosome abnormalities. Prenat](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref28) [Diagn 2018;38:160–5](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref28).

1. [Gregg AR, Skotko BG, Benkendorf JL,](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref29)

[et al. Noninvasive prenatal screening for fetal](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref29) [aneuploidy, 2016 update: a position state-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref29) [ment of the American College of Medical](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref29) [Genetics and Genomics. Genet Med](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref29) [2016;18:1056–65](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref29).

1. [Schwartz S, Kohan M, Pasion R,](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref30)

[Papenhausen PR, Platt LD. Clinical experience](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref30) [of laboratory follow-up with noninvasive prenatal](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref30) [testing using cell-free DNA and positive micro-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref30) [deletion results in 349 cases. Prenat Diagn](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref30) [2018;38:210–8](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref30).

1. [Malone FD, Canick JA, Ball RH, et al. First-](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref31)

[trimester or second-trimester screening, or](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref31) [both, for Down’s syndrome. N Engl J Med](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref31) [2005;353:2001–11](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref31).

1. [Bianchi DW, Parker RL, Wentworth J, et al.](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref32) [DNA sequencing versus standard prenatal](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref32) [aneuploidy screening. N Engl J Med 2014;370:](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref32) [799–808](http://refhub.elsevier.com/S0002-9378%2822%2900006-0/sref32).

Author and article information

From the Department of Obstetrics and Gynecology and Women’s Health, Montefiore Medical Center, Albert Einstein College of Medicine, New York, NY (Drs Dar and Doulaveris); Department of Obstetrics and Gynecology, Sahlgrenska University Hospital, Gothenburg, Sweden (Drs Jacobsson, Carlsson, and Hallingstro¨ m); Department of Obstetrics and Gynecology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden (Dr Jacobsson); The Biostatistics Center, George Washington University, Rockville, MD (Drs Clifton and MacPherson); Natera Inc, Austin, TX (Ms Egbert and Drs Demko, Martin, and Rabinowitz); Department of Obstetrics and Gyne- cology, Rotunda Hospital, Royal College of Surgeons in Ireland, Dublin, Ireland (Drs Malone, Flood, and Daly); Department of Obstetrics and Gynecology, Columbia University Irving Medical Center, New York, NY (Dr Wapner); Department of Obstetrics and Gynecology, New York University Grossman School of Medicine, New York, NY (Dr Roman); Department of Obstetrics and Gynae- cology, St George’s Hospital, University of London, Lon- don, United Kingdom (Dr Khalil); Department of Obstetrics and Gynecology, Saint Peter’s University Hospital, New Brunswick, NJ (Dr Faro); Department of Obstetrics and Gynecology, Long Island Jewish Medical Center, Donald and Barbara Zucker School of Medicine at Hofstra/ Northwell, New Hyde Park, NY (Dr Madankumar); Suffolk Obstetrics & Gynecology, Port Jefferson, NY (Dr Edwards); Department of Obstetrics and Gynecology, Icahn School of Medicine at Mount Sinai, New York, NY (Dr Strong); Austin Maternal-Fetal Medicine, Austin, TX (Dr Haeri); Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, UT (Dr Silver); Depart- ment of Obstetrics and Gynecology, North Shore Uni- versity Hospital, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Manhasset, NY (Dr Vohra); Department of Obstetrics and Gynecology, Royal Prince Alfred Hospital, Western Sydney University, Camper- down, New South Wales, Australia (Dr Hyett); Center for Applied Genomics, Children’s Hospital of Philadelphia, Philadelphia, PA (Drs Kao and Hakonarson); and Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA (Dr Norton).

Received Oct. 20, 2021; revised Jan. 3, 2022;

accepted Jan. 5, 2022. All site principal investigators (P.D., B.J., F.M., R.J.W.,

A.R., A.K., R.F., R.M., L.E., S.H., R.S., N.V., J.H., C.M.,

R.C., and M.E.N.) received institutional research support from the funding sponsor (Natera). M.E., Z.D., and M.R. report being employed by the study’s funding sponsor (Natera) and holding stock or having options to hold stock.

K.M. is a consultant for the funding sponsor (Natera) and holds stock and options to hold stock. J.H. reports ongoing research collaboration that includes financial support for biochemical analytes from Perkin Elmer, earning honoraria and/or giving talks that were not compensated for by Natera, Roche, or Canon, and pre- viously participating in Asian and Australasian expert consultancies for Natera and Roche. B.J. reports

participating in clinical research diagnostic trials with Ariosa (completed), Vanadis (completed), Natera (ongoing), and Hologic (completed) with expenditures for each patient being reimbursed by the institution and with no personal reimbursements; participating in clinical probiotic studies with products provided by FukoPharma (ongoing, no funding) and BioGaia (ongoing; also provided a research grant for the specific study); coordinating scientific conferences and meetings with commercial partners such as the European Spontaneous Preterm Birth Congress 2016 and a Nordic educational meeting about noninvasive prenatal testing and preeclampsia screening. B.J. and Y.C. report collaborating with the IMPACT study, which received reagents for placental growth factor analyses from Roche, Perkin Elmer, and ThermoFisher Scientific. R.J.W. reports receiving research funding from the *Eunice Kennedy Shriver*

National Institute of Child Health and Human Develop- ment and receiving support from Illumina for research reagents. M.E.N. reports serving as a consultant for Invitae. All other authors report no conflict of interest.

This study was funded by Natera, Inc, San Carlos, CA. This study was a collaboration between the clinical in- vestigators and the funding sponsor. P.D., M.E.N., and

R.C. designed the protocol with the sponsor (M.E., Z.D., K.M., and M.R.). There were no confidentiality agree- ments between the authors, sites, or sponsor.

This trial was registered with [ClinicalTrials.gov](http://ClinicalTrials.gov/) under identifier NCT02381457 and with title “SNP-based Microdeletion and Aneuploidy RegisTry (SMART).”

Data sharing requests should be submitted to the corresponding author (P.D.) for consideration. Requests will be considered by the study publication committee. Study protocol and statistical analysis plan will be

available on request. Individual patient data will not be available. Access to de-identified data may be granted following submission of a written proposal and a signed data sharing agreement. Files will be shared using a secure File Transfer Protocol.

This study was designed in compliance 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 study participants.

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

Corresponding author: Pe’er Dar, MD. peerdar@ gmail.com

# Appendix

## Supplemental materials and methods

Study design and participants For full information on the study dates, including enrollment and completion, see [clinicaltrials.gov](http://clinicaltrials.gov/) identiﬁer NCT02381457. Relevant dates are as follows: period of recruitment, April 8, 2015 to December 12, 2019; follow-up,

April 8, 2015 to July 18, 2019; data

collection, April 8, 2015 to September

18, 2019.

This study involved 21 locations, including the University of California, San Francisco, San Francisco, California; Cooper University Hospital, Camden, New Jersey; Virtua, Mount Laurel, New Jersey; St. Peter’s University, New Bruns-

wick, New Jersey; Complete Women’s

Healthcare, Garden City, New York;

North Shore University Hospital, Man- hasset, New York; Madonna Perinatal,

Mineola, New York; Long Island Jewish Medical Center, New Hyde Park, New York; New York University, New York, New York; Icahn School of Medicine Mount Sinai, New York, New York; Columbia University, New York, New York; Monteﬁore Medical Center, New York, New York; Suffolk OB/GYN, Port Jefferson, New York; North Austin Maternal-Fetal Medicine, Austin, Texas;

Zeid Women’s Health Center, Longview, Texas; University of Utah, Salt Lake City,

Utah; Royal Prince Alfred, Camperdown, New South Wales, Australia; Royal Col- lege of Surgeons in Ireland, Dublin, Ireland; Dexeus, Barcelona, Spain; Sahl- grenska University Hospital, Gothen- burg, Sweden; St. George University Hospital, London, United Kingdom.

This multicenter prospective observa- tional study enrolled pregnant women who presented clinically at or after 9 weeks’ gestation and elected to undergo

Panorama microdeletion and aneuploidy screening as part of their routine care. The primary objective was to evaluate the performance of single-nucleotide poly- morphism (SNP)ebased noninvasive prenatal testing (NIPT) for the 22q11.2 microdeletion in a large cohort of preg- nant women. Data collection began at enrollment and continued until after patients delivered and their child was discharged from the hospital. Bio- specimens were obtained from infants after birth to perform genetic diagnostic testing for 22q11.2 deletion. Results from the follow-up specimens were compared with those obtained by the Panorama screening test to determine test perfor- mance. In the event that a newborn sample could not be obtained before discharge from the hospital, participants were mailed a saliva buccal swab kit for testing at home. Samples were then shipped to Natera for testing.