OBSTETRICS

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

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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 polymorphism–based, prenatal cell-free DNA screening for detection of 22q11.2 deletion syndrome.

STUDY DESIGN: Patients who underwent single-nucleotide polymorphism–based prenatal cell-free DNA screening for 22q11.2 deletion 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 syndrome 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% confidence interval, 42.8–94.5); specificity of 99.84% (95% confidence interval, 99.77–99.89); positive predictive value of 23.7% (95% confidence interval, 11.44–40.24), and negative predictive value of 99.98% (95% confidence interval, 99.95–100). 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, 51.6–97.9) 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, 28.9–75.6).

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 aneuploidies (T13 and T18) in the fetus. However, such chromosomal aneuploidies constitute a relatively small proportion of the total number of genetic conditions that contribute to adverse infant 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.[1–4]

Although individually rare, in aggregate, chromosomal microdeletions and duplications are more prevalent than the common trisomies, and because their birth incidence is not associated with increasing maternal age, they are more common than T21 in women <30 years of age.[5,6] The most common of these is the 22q11.2 deletion syndrome (22q11.2DS), also known as DiGeorge or velocardiofacial syndrome. This condition is characterized by variable features including congenital heart defects and developmental delay in most patients, a cleft palate or velopharyngeal insufficiency, hypocalcemia, immunodeficiency, autism, and psychiatric disorders.[7] 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.[8–10] These mostly de novo deletions are caused by meiotic recombination events in 4 hot spot regions known as A-D low-copy repeats (LCR) on the long arm of chromosome

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AJOG at a Glance

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 confirmation in all cases, the cohort prevalence of 22q11.2DS was 1 in 1524. Single-nucleotide polymorphism–based cfDNA screening identified most cases of 22q11.2DS including both classical and nested deletions that are ≥500 kb. The test false positive rate was 0.15%, which is similar to the false 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 confirmation in all cases. The findings in this study demonstrate that cfDNA screening for 22q11.2 can be added to aneuploidy screening without a significant increase in the screen positive rate.

22 (Figure 1).[11] 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.[12,13]

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] 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 immunodeficiency, which has been shown to improve outcomes.[15,16] Despite these benefits, the limited data on test performance 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 probability of having a fetus with a genetic abnormality.[17–20] Retrospective analyses of clinical cohorts reported positive predictive values (PPVs) but have not performed full-cohort confirmatory genetic testing to determine test sensitivity and specificity.[21–23]

We therefore, sought to assess the performance of single-nucleotide polymorphism (SNP)–based cfDNA screening for 22q11.2DS in a large prospective 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 aneuploidy 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 (identifier: NCT02381457; SNP-based Microdeletion and Aneuploidy RegisTry or SMART) and approved by each site's institutional review board. All participants provided written consent. Eligible women were ≥18 years old, at ≥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, 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 inclusion. 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 performed by an independent laboratory (Center for Applied Genomics, Children'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 available, were used for genetic confirmation.

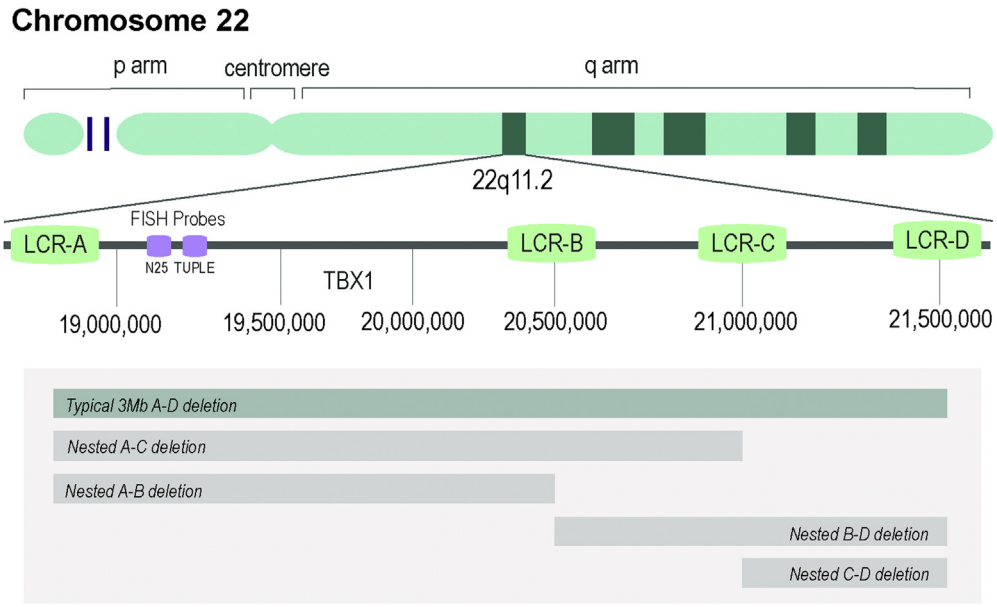
Outcomes

The primary outcome was test performance 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] 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, patients 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.

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protocol was modified once.[24,25] 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 mixtures of mother and fetus cfDNA samples, learning to harness the linkage among the SNPs to improve call confidence. 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. Samples 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 additional 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 $\geq$500 kb was identified within the LCR A−D interval. Positive samples underwent confirmation 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 results and newborn samples were correctly paired by using alignment between SNPs in the 2 samples; any samples that could not be paired were excluded.

Data collection

Onsite research coordinators recorded information using a secured computerized tracking system developed and managed by The Biostatistics Center at George Washington University, Washington DC. Data that were collected included patient and obstetrical data, imaging reports, aneuploidy serum screening, and prenatal diagnosis results. After delivery, information on pregnancy 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 confidentiality 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 coordinating 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 participants was planned based on 22q11.2DS prevalence estimates that ranged from 1 in 300 to 1 in 2000.[5,6,17] 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 precision to assess performance.[9] 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 analysis. 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). 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 pregnancy loss without genetic confirmation, 1110 (5.3%) were lost to follow-up and therefore the pregnancy outcome is unknown, 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%) participants 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). 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 $\geq$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 ascertaining their precise size (Table 2). 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.

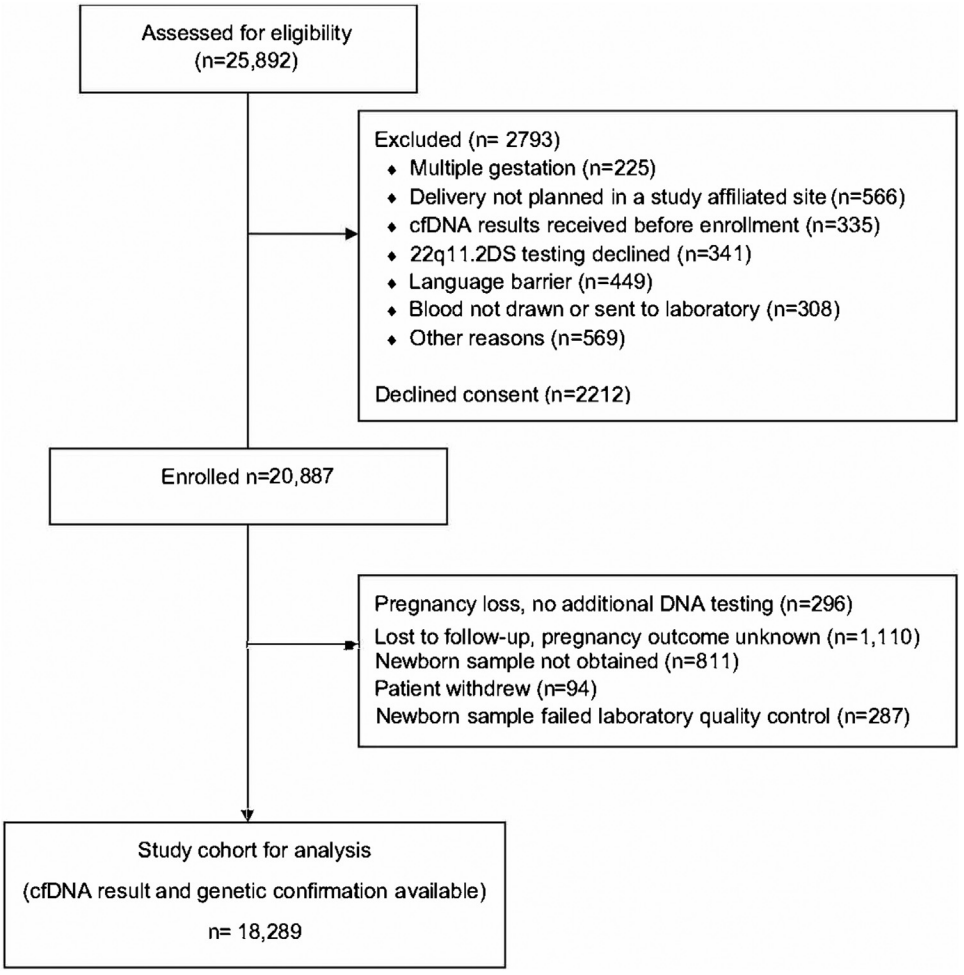
Of the 18,289 cases, based on the cfDNA screening results, 17,976 (98.3%) were categorized as low risk for 22q11.2DS, 38 (0.2%) were categorized as high risk, and 275 (1.5%) remained nonreportable despite collecting a second sample. Prenatal diagnostic testing was performed for 21 of 38 (55.3%) high-risk 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, 42.8—94.5), specificity of 99.84% (95% CI, 99.77—99.89), PPV of 23.7% (95% CI, 11.44—40.24), and NPV of 99.98% (95% CI, 99.95—100) (Table 3). 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 $\geq$3 mm. None were at high risk for aneuploidy based on first trimester screening and 1 patient underwent 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 branchial cleft cyst and a digital anomaly after

FIGURE 2
Patient enrollment flowchart



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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, 51.6—98.9), 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, 28.9—75.6) (Table 3).

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 diagnosis of an omphalocele and 2 had uncomplicated 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.[13,17,18] 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]

Variable	Study cohort (n=18,289)
Maternal and gestational characteristics	
Maternal age (y), median (IQR)	34.5 (30.4–37.5)
Nulliparity, n/total, n (%)	8022/18,248 (44.0)
BMI (kg/m^2), median (IQR)[b,c]	24.9 (22.3–29.0)
Race and ethnicity, n (%)[d]	
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 (11.6–13.9)
Pregnancy through assisted reproductive technology, n (%)	959 (5.2)
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)
Nuchal translucency ≥3 mm or cystic hygroma before enrollment, n (%)	95 (0.5)
Positive second trimester or integrated screen before enrollment, n (%)	105 (0.6)
Major anomaly before testing, n (%)	107 (0.6)
Fetal fraction (%), mean±SD[c]	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]	39.4 (38.6–40.3)
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]	3361±555
Birthweight <10% percentile, n/total, n (%)	1578/18,042 (8.8)
Days to newborn discharge, median (IQR)[c]	2 (2–3)

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.

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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]

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 reported in prenatal studies.[5,6,8–10] It is possible that including the 4 cases with fetal anomalies detected before enrollment enriched the 22q11.2DS population. Excluding these cases would lead to a prevalence of 1 in 2312, which is similar to a recently reported genetic analysis of newborn screening samples.[26] Although the rate of pregnancy loss associated with 22q11.2DS is not reportedly increased, postnatal studies may underestimate the frequency by excluding cases of 22q11.2DS that were terminated following detection of fetal anomalies.[27,28] In addition, most postnatal 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 interval 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 conditions.[29] 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] 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 hypocalcemia and immune deficiency, justify the consideration for prenatal screening.[13–15] In this study, we found that modalities such as first trimester ultrasonography and traditional

TABLE 2
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]	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]	AGA
5.	Unknown[b]	Prenatal CVS	BoB	10	6.9%	Yes	Normal	None	Atrioventricular canal (20 wk)	TOP		
6.	Unknown[b]	Prenatal amniocentesis	BoB	11	6.9%	Yes	Normal	None	No additional ultrasound	TOP		
7.	Unknown[b]	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]	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.

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TABLE 3
cfDNA test performance for detection of ≥ 500 kb 22q11.2 deletions in the LCR22 A−D region with the algorithm applied at enrollment and with the updated algorithm

Test parameter	Original algorithm used at enrollment (n=18,014)	Updated algorithm implemented after study completion (n=18,043)
Sensitivity	75.0% (9/12; 95% CI, 42.8−94.5)	83.3% (10/12; 95% CI, 51.6−97.9)
Specificity	99.84% (17,973/18,002; 95% CI, 99.77−99.89)	99.95% (18,022/18,031; 95% CI, 99.91−99.98)
PPV	23.7% (9/38; 95% CI, 11.4−40.2)	52.6% (10/19; 95% CI, 28.9−75.6)
NPV	99.98% (17,973/17,976; 95% CI, 99.95−100)	99.99% (18,022/18,024; 95% CI, 99.96−100)
Positive likelihood ratio[a]	468.75	1666.00
Negative likelihood ratio[a]	0.25	0.17

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

[a] Positive likelihood ratio is calculated as sensitivity/100−specificity and the negative likelihood ratio is calculated as 100−sensitivity/specificity.

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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.[30,31] 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,[3,32,33] and comparable with cfDNA screening for some of the aneuploidies.[3,33] Finally, in the updated algorithm, we utilized a massively multiplexed polymerase chain reaction−based SNP analysis enhanced by postsequencing DNN analysis to further improve performance. This innovative use of machine learning−based 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 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 microarray, for some patients, this may be too late to consider invasive testing or pregnancy 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 diagnostic 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 both had their cfDNA screening and diagnostic test results before the anomaly 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 prenatally 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. In addition, the estimates of detection rates for uncommon conditions are necessarily associated with wide CIs. Finally, as a real-world study, the indications 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 considering expansion of routine prenatal genetic screening to include screening for 22q11.2DS for all pregnant women. ■

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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 Development 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.

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This trial was registered with 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 25–30, 2021.

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Appendix

Supplemental materials and methods

Study design and participants

For full information on the study dates, including enrollment and completion, see 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 Brunswick, New Jersey; Complete Women's Healthcare, Garden City, New York; North Shore University Hospital, Manhasset, 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 College of Surgeons in Ireland, Dublin, Ireland; Dexeus, Barcelona, Spain; Sahlgrenska University Hospital, Gothenburg, Sweden; St. George University Hospital, London, United Kingdom.

This multicenter prospective observational 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 polymorphism (SNP)—based noninvasive prenatal testing (NIPT) for the 22q11.2 microdeletion in a large cohort of pregnant women. Data collection began at enrollment and continued until after 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. Results from the follow-up specimens were compared with those obtained by the Panorama screening test to determine test performance. 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.