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*Original Research*

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

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**Condensation**

Cell-free DNA (cfDNA) non-invasive prenatal screening detected most cases of 22q11.2DS with a low false positive rate in a large, prospective cohort

**Short Title**

Performance of cell-free DNA prenatal screening for 22q11.2DS

**AJOG at a Glance**

**A. 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 cfDNA prenatal screening for 22q11.2DS is feasible, data on test performance are limited.

**B. What are the key findings?**

- Based on genetic confirmation in all cases, the cohort prevalence of 22q11.2DS was 1 in 1524.
- SNP-based cfDNA screening identified most cases of 22q11.2DS including both classical and nested deletions that are ≥500kb
- The test false positive rate was 0.15%, which is similar to the false positive rate seen with cfDNA aneuploidy screening

**C. What does this study add to what is already known?**

The study presents new and comprehensive information on 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 significant increase in the screen positive rate.

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

## Abstract

**Background:** Prenatal screening has historically focused primarily on detection of fetal aneuploidies. Cell-free DNA (cfDNA) now enables noninvasive screening for subchromosomal copy number variants, including 22q11.2 deletion syndrome (22q11.2DS 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 22q11.2DS, large cohort studies with postnatal confirmatory testing to assess test performance have not been reported.

**Objective:** To assess the performance of SNP-based cfDNA prenatal screening for detection of 22q11.2DS.

**Study Design:** Patients who had SNP-based cfDNA prenatal screening for 22q11.2DS were prospectively enrolled at 21 centers in 6 countries. Prenatal or newborn DNA samples were requested in all cases for genetic confirmation with chromosomal microarray. The primary outcome was sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of cfDNA for detection of all deletions, including the classical deletion and nested deletions that are ≥500kb, in the 22q11.2 low copy repeat A-D region. Secondary outcomes included the prevalence of 22q11.2DS and performance of an updated cfDNA algorithm that was evaluated blinded to pregnancy outcome.

**Results:** Of 20,887 women enrolled, genetic outcome was available in 18,289 (87.6%). Twelve 22q11.2DS cases were confirmed in the cohort, including five (41.7%) nested deletions, yielding a prevalence of 1:1524. In the total cohort, cfDNA reported 17,976 (98.3%) as low risk for 22q11.2DS and 38 (0.2%) as high-risk; 275 (1.5%) were non-reportable. Overall, 9 of 12 cases of 22q11.2 were detected, 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). None of the cases with a non-reportable result was diagnosed with 22q11.2DS. The updated algorithm detected 10/12 cases (83.3%; 95% CI: 51.6-97.9) with a lower false positive rate (0.05% vs. 0.16%, p<0.001) and a PPV of 52.6% (10/19; 95% CI 28.9-75.6).

**Conclusions:** Noninvasive cfDNA prenatal screening for 22q11.2DS can detect most affected cases, including smaller nested deletions, with a low false positive rate.

**Trial Registration:** ClinicalTrials.gov: NCT02381457.

## 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 since their birth incidence is not associated with increasing maternal age, they are more common than T21 in women under 30.[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, cleft palate or velopharyngeal insufficiency, hypocalcemia, immunodeficiency, autism and psychiatric disorders.[7] The 22q11.2DS has been estimated to affect approximately 1:3,000-6000 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 four hot spot regions, known as A-D low-copy repeats (LCR), on the long arm of chromosome 22 (Figure 1).[11] In approximately 85% of affected individuals the entire 2.5-3Mb LCR A-D region is deleted, while others have smaller nested deletions within this region.[12,13]

In addition to providing parents with important information on 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 as well as 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 has 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 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 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 had SNP-based cfDNA for aneuploidy and 22q11.2DS were enrolled at 21 centers in the US, Europe, and Australia. (Supplement #1). The study was registered on ClinicalTrials.gov (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, ≥9 weeks' gestation, had a singleton pregnancy, and planned to deliver at a study site-affiliated hospital. Women were excluded if they received a cfDNA result before enrollment, had organ transplantation, conceived using ovum donation, or were unable to provide a newborn sample. Women who had had 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 prior prenatal testing. The postnatal CMA was performed by an independent laboratory (Center for Applied Genomics, Children's Hospital of Philadelphia, PA) that was blind to 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 for detection of 22q11.2 deletions ≥500kb 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 ≥1/100 for 22q11.2DS were categorized as high risk and <1/100 as low risk. In cases with non-reportable results, patients were offered repeat testing and results after a second draw were included; a third sample was not requested. During enrollment, the cfDNA laboratory 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) 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 blinded to outcomes.

For confirmatory CMA analysis, DNA was prepared from 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, USA) SNP-based Infinium Global Screening

Array (GSA) platform. Samples were genotyped on standard versions GSA-V1.0, GSA-V2.0, GSAMD-V1.0, or GSAMD-V2.0 or a custom-designed SMARTArray where 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, while the custom SMARTArray has 1963 SNPs including those in the backbone. A positive 22q11.2DS was confirmed if a deletion ≥500kb 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 results were generated.

Since neonatal DNA samples were obtained from different sources, mostly through dry blood spots that were collected by states health departments for routine neonatal screening, we developed a concordance test for quality assurance purposes. The concordance test was designed to confirm that cfDNA results and newborn samples were correctly paired, using alignment between SNPs in the two 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. Collected data included patient and obstetric 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 genetic abnormality, major malformations, and other adverse outcomes was collected.

255

256 **Study oversight**

257 The study was a collaboration between the clinical investigators and the sponsor (Natera, Inc.,
258 San Carlos, CA). The first and last authors designed the protocol with the sponsor and had a
259 majority vote in study design and data interpretation. There were no confidentiality
260 agreements between the authors, sites, or sponsor. All laboratory analyses were blinded to
261 outcome data. Clinical and laboratory results were managed by the Data Coordinating Center,
262 which independently matched the information and deidentified and analyzed the results.

263

264 **Patient and Public Involvement**

265 Patients and the public were not involved in the design of the study protocol, establishing the
266 research question or the outcome measures. No patients or members of the public were
267 involved in the recruitment process or the conduct of the study. Finally, no patients or
268 members of the public were or will be involved in the interpretation or dissemination of the
269 study's results.

270

271 **Statistical Analysis**

272 Originally, a sample size of 10,000 participants was planned based on 22q11.2DS prevalence
273 estimates that ranged from 1/300 to 1/2000.[5,6,17] During the trial, concerns arose that the
274 prevalence of the 22q11.2DS may be lower, and the sample size was increased to 20,000, which
275 allowed for a higher level of precision to assess performance.[9] The sensitivity, specificity, and
276 positive and negative predictive values of cfDNA results were assessed and exact (Clopper-

Pearson) 95% confidence intervals were reported. Participants without cfDNA results or genetic confirmation were excluded from the test performance analysis. SAS Studio 9.04 software (SAS Institute) was used for analysis. Continuous variables were compared using the Wilcoxon test and categorical variables using the chi-square test or Fisher's exact test as appropriate. McNemar's 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 US and 45.2% in Europe or Australia. Of enrolled participants, 296 (1.4%) had a pregnancy loss without genetic confirmation, 1110 (5.3%) were lost to follow-up and pregnancy outcome is unknown, in 811 (3.9%) a confirmatory sample was not obtained, 94 (0.5%) withdrew consent, and in 287 (1.4%) the confirmation test failed laboratory quality control. The latter group included 49 cases that failed the concordance quality assurance test and in 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.

Median maternal age and gestational age at enrollment were 34.5 years and 12.6 weeks, respectively (Table 1). Overall, 108 (0.6%) had cfDNA after detection of a fetal anomaly on ultrasound, 95 (0.5%) after diagnosis of a cystic hygroma or NT ≥3mm, 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 1:1524. Of these, 4 (33%) were the typical 3Mb A-D deletions, 5 (41.6%) were nested deletions, ranging 0.73Mb-2Mb, and three (25%) were identified by FISH or BACs on Beads both of which used probes in the A-B region; that 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, cfDNA reported 17,976 (98.3%) as low risk for 22q11.2DS, 38 (0.2%) as high-risk and 275 (1.5%) remained non-reportable despite a second draw. Prenatal diagnostic testing was performed in 21/38 (55.3%) high-risk cfDNA cases and identified three 22q11.2DS cases.

Nine deletions, including all four typical deletions, the 3 deletions of uncertain size and two of the five 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 non-reportable results were confirmed to have 22q11.2DS.

Fetal anomalies were detected in seven (58.3%) patients with 22q11.2DS. Four heart anomalies were diagnosed prior to cfDNA screening, and three fetal anomalies, two cardiac and one renal,

were identified after a high-risk cfDNA result was reported. In addition, a gastro-intestinal anomaly was diagnosed in a fetus previously diagnosed with a cardiac anomaly. Eleven of the patients with 22q11.2DS pregnancies, including six patients with anomalies, had first trimester ultrasound none of which identified any fetal anomalies or NT≥3mm. None were high-risk for aneuploidy on first trimester screening and one patient had cfDNA 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; one had a 1.5Mb A-B deletion and two had 730kb B-D deletions. Of the latter, one was diagnosed prenatally with unilateral renal agenesis; the deletion in this patient was detected with the updated cfDNA algorithm. Another newborn with a 730kb B-D deletion was growth restricted and found to have a branchial cleft cyst and a digital anomaly after birth. All three had normal first trimester ultrasound and serum screening.

The updated algorithm identified one additional 22q11.2DS case, increasing sensitivity to 83.3% (10/12, 95% CI 51.6, 98.9) and had a significantly lower screen positive rate (19, 0.10% vs 38, 0.21%; p<0.001) and a lower false positive rate (9, 0.05% vs. 29, 0.16%, p<0.001), increasing the PPV to 52.6% (95% CI 28.9, 75.6). (Table 3).

In all, 2597 women did not have genetic confirmation and were excluded. Compared to the study cohort, they were younger (34.2 vs 34.5, p<0.001), more likely to be Black and less likely to be Hispanic (12.1% vs 8.5%, 15.6% vs 18.1%, respectively, p<0.001) but had similar BMI,

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 due to prenatal diagnosis of an omphalocele and two 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 with cfDNA for genetic disorders beyond aneuploidy is possible with high accuracy.

Prior validation studies have also demonstrated high detection and low false positive rates of cfDNA for 22q11.2DS, but most have only evaluated detection of the common 3Mb A-D deletion.[13,17,18] In our cohort, at least five 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 to that of the classical deletion. Ten of the twelve confirmed deletions in our cohort included this region, and nine of them were detected by the screen. While the LCR B-D region has been less well studied, clinical features associated with these deletions, including heart defects and neurodevelopmental delays, overlap those seen 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 (1:1524) 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 four cases with fetal anomalies detected prior to enrollment enriched the 22q11.2DS population. Excluding these cases would result in a prevalence of 1:2312, which is still higher than prior reports. While the rate of pregnancy loss associated with 22q11.2DS is not reported to be increased, postnatal studies may underestimate the frequency by excluding cases of 22q11.2DS that were terminated following detection of fetal anomalies.[26,27] Additionally, 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 screen for common aneuploidies, clinical significance and test performance are important when considering expansion of targeted conditions.[28] The importance of 22q11.2 is evident given the significant clinical sequelae and prevalence that is higher than some of the currently screened aneuploidies.[29] 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 consideration of prenatal screening.[13-15] In this study, we found that modalities such as first trimester ultrasonography and traditional aneuploidy screening are not useful for detection of 22q11.2DS. The low prevalence of individual microdeletion syndromes,

and resultant low PPVs of testing have called into question the value of screening. [29,30] However, the PPV of cfDNA for 22q11.2DS is higher and the false positive rate is lower than that associated with other accepted screening tests such as traditional first trimester combined screening,[3,31,32] and comparable to cfDNA screening for some of the aneuploidies.[3, 32]. Finally, in the updated algorithm, we utilized a massively multiplexed PCR-based SNP analysis enhanced with post-sequencing deep neural network analysis to further improve performance. This innovative use of machine learning based artificial intelligence resulted in lower false positive rates and higher PPVs, in this case above 50%, for this microdeletion. While 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 seven 22q11.2DS cases, all in the 2nd or 3rd trimester. In three (25%) of the 22q11.2DS cases, the anomaly was detected on a second trimester anatomical survey prior to 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 for considering invasive testing or pregnancy termination. In fact, in our diverse cohort, none of the patients who was diagnosed with a fetal anomaly prior to cfDNA screening elected to have a diagnostic procedure or discontinue the pregnancy. The three (25%) patients who had a diagnostic procedure, had their cfDNA screening in the first trimester. Similarly, only 2/7 (28.5%) patients

with fetal anomalies elected to terminate the pregnancy and both had their cfDNA screening and diagnostic tests 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, the 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. Additionally, estimates of detection rates for uncommon conditions are necessarily associated with wide confidence intervals. Finally, as a real-world study, the indications for testing were varied and prevalence rates may not necessarily reflect the average risk population.

*Conclusions*

In conclusion, this study found 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 to all pregnant women.

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## Statements

### Conflicts of interest

All site principal investigators (PD, BJ, FM, RJW, AR, AK, RF, RM, LE, SH, RS, NV, JH, CM, RC and MEN) received institutional research support from the funding sponsor (Natera). ME, ZD and MR are employed by the study's funding sponsor (Natera) and hold stock or options to hold stock. KM is a consultant to the funding sponsor (Natera) and holds stock and options to hold stock. JH has an ongoing research collaboration that includes financial support for biochemical analytes from Perkin Elmer, has earned honoraria and/or given talks that were not compensated from Natera, Roche and Canon, and has participated in Asian/Australasian expert consultancies for Natera and Roche. BJ reports research clinical diagnostic trials with Ariosa (completed), Vanadis (completed), Natera (ongoing) and Hologic (completed) with expenditures institutional reimbursed per patient and no personal reimbursements; clinical probiotic studies with product provided by FukoPharma (ongoing, no funding) and BioGaia (ongoing; also provided a research grant for the specific study); coordination of scientific conferences and meetings with commercial partners as such as ESPBC 2016 and a Nordic educational meeting about NIPT and preeclampsia screening. BJ and YC collaborated in the IMPACT study where Roche, Perkin Elmer and Thermo Fisher provides reagents to PLGF analyses. RJW receive research funding from NICHD and receive support from Illumina for research reagents. MEN is a consultant to Invitae. All other authors report no conflicts of interest.

**Funding**

The study was funded by Natera, Inc, San Carlos, CA, USA. The study was a collaboration between the clinical investigators and the funding sponsor. PD, MEN and RC designed the protocol with the sponsor (ME, ZD, KM, and MR). There were no confidentiality agreements between the authors, sites, or sponsor.

**Trial Registration**

This study is registered at ClinicalTrials.gov under number NCT02381457, entitled "SNP-based Microdeletion and Aneuploidy RegisTry (SMART)".

The study was presented as an oral presentation at the Society of Maternal and Fetal Medicine (SMFM) 41st Annual Pregnancy Meeting held virtually between January 25 - 30, 2021

**Data Sharing**

Data sharing requests should be submitted to the corresponding author (PD) for consideration. Requests will be considered by the study publication committee. Study protocol and statistical analysis plan will be available upon request. Individual patient data will not be available. Access to deidentified data may be granted following submission of a written proposal and a signed data sharing agreement. Files will be shared using a secure FTP.

**Ethical approval**

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.

**Authors Contributions**

PD and MEN were the study lead investigators, designed the protocol with RC and the sponsor (representatives included ME, ZD, KM and MR), led the study steering and publication committees and had a majority vote in the committee's decisions. BJ, SH, CM, ZD and KM were also members of the publication committee. BJ, FM, RJW, ASR, AK, RF, RM, LE, NS, SH, RS, NV, JH, KF, MH, GD, SD, and YC contributed to study conduct and patient enrollment. CM and RC managed the clinical and laboratory data, deidentified and verified the data, analyzed the results and performed the statistical analysis. CK and HH were responsible for the confirmatory genetic analysis. PD, CM and MEN wrote the manuscript. All authors contributed to the interpretation of data, critically reviewed and decided to publish the manuscript.

**Figure Legend**

Figure 1 – Depiction of the approximately 3Mb deleted 22q11.2 region on the long arm of chromosome 22. The region includes four sets of low copy repeats (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 (FISH) are marked in purple. Deletions or variants involving T-Box Transcription Factor 1 (TBX1), one 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 seven miRNA genes and 10 non-coding genes in this region. The size and position of the typical A-D deletion as well as smaller, nested deletions are indicated at the bottom.

Figure 2 – Patient enrollment flow chart.

Table 1 – Demographics and clinical characteristics of study participants. *

| Variable | Study cohort (n= 18,289) |
|---|---|
| **Maternal and gestational characteristics** | |
| Median maternal age (IQR) - yr | 34.5 (30.4-37.5) |
| Nulliparity – no./total no. (%) | 8022/18248 (44.0) |
| Median BMI kg/$m^2$ (IQR)†** | 24.9 (22.3-29.0) |
| Race/Ethnicity – no. (%)‡ | |
| Asian | 1542 (8.4) |
| Black | 1554 (8.5) |
| White | 11272 (61.6) |
| Hispanic | 3309 (18.1) |
| Other/unknown | 612 (3.3) |
| Median gestational age at enrollment (IQR) - wk | 12.6 (11.6-13.9) |
| Pregnancy through assisted reproductive technology - no. (%) | 959 (5.2) |
| Current smoker - no./total no. (%) | 321/18211 (1.8) |
| Enrolled in a US site – no. (%) | 10005 (54.7) |
| **Prenatal screening and testing** | |
| Positive First trimester screen before enrollment – no. (%) | 518 (2.8) |
| NT≥3mm or cystic hygroma before enrollment – no. (%) | 95 (0.5) |
| Positive second trimester or integrated screen before enrollment – no. (%) | 105 (0.6) |

| | |
|---|---|
| Major anomaly before testing – no. (%) | 107 (0.6) |
| Fetal fraction (%) – mean (SD)** | 9.9±4.1 |
| Diagnostic testing – no. (%) | 420 (2.3) |
| **Pregnancy and delivery outcome** | |
| Miscarriage - no./total no. (%)<br>Pregnancy termination - no./total no. (%)<br>Live birth - no./total no. (%)<br>Stillbirth - no./total no. (%) | 5/18281 (0.03)<br>41/18281 (0.2)<br>18224 /18281 (99.7)<br>11/18281 (0.06) |
| Neonatal death - no./total no. (%) | 24/18281 (0.1) |
| Aneuploidy (T13, 18, 21) – no. (%) | 36 (0.2) |
| Median gestational age at delivery (IQR) – wk** | 39.4 (38.6-40.3) |
| PTB <37 weeks - no./total no. (%) | 1311/18230 (7.2) |
| Preeclampsia - no./total no. (%) | 735/18230 (4.1) |
| Birth weight (grams) – mean (SD)** | 3361±555 |
| Birth weight <10% percentile - no./total no. (%) | 1578/18042 (8.8) |
| Median days to newborn discharge (IQR) – d** | 2 (2-3) |

*Plus-minus values are means ±SD. IQR denotes interquartile range.

†The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡Race and ethnic groups were reported by the participants. If the participant did not report the information, the information from the chart was used.

**BMI data were missing for 314 participants, fetal fraction data were missing for 76 participants due to: 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 was missing for 245 infants. Days to newborn discharge were missing for 308 liveborn infants

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

| Case | Deletion size and location | Stage of confirmation | Test | GA at cfDNA | Fetal fraction | Identified by cfDNA | First trimester ultrasound | Fetal anomaly detected before cfDNA | Fetal anomaly detected after cfDNA | Outcome | GA at delivery | Birth weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | A-D 2.6 Mb | Postnatal | CMA | 20 | 13.7% | Yes | Normal | Interrupted aortic arch, VSD (20w) | None | Live birth | Term | AGA |
| 2. | A-D 2.6 Mb | Postnatal | CMA | 31 | 9.7% | Yes | Normal | Truncus arteriosus at (31w) | None | Live birth | Late preterm^ | 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 (17w) | Bowel obstruction (31w) | Live birth | Late preterm^ | AGA |
| 5. | Unknown§ | Prenatal CVS | BoB | 10 | 6.9% | Yes | Normal | None | Atrioventricular canal (20w) | TOP | | . |
| 6. | Unknown§ | Prenatal Amniocentesis | BoB | 11 | 6.9% | Yes | Normal | None | No additional ultrasound | TOP | | |
| 7. | Unknown§ | Postnatal | FISH | 21 | 14.4% | Yes | Normal | Tetralogy of Fallot (21w) | No additional ultrasound | NND | Term | SGA |
| 8. | A-C 2.06 Mb | Prenatal Amniocentesis | MLPA | 10 | 7.6% | Yes | Normal | None | VSD (18w) | 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* | Normal | None | Unilateral renal agenesis (22w) | Live birth | Term | AGA |
| 12. | B-D 0.73 Mb | Postnatal | CMA | 12 | 8.5% | No | Normal | None | None | Live birth | Term | SGA |

GA= Gestational age at enrollment; FF=Fetal fraction; CVS=Chorionic villous sampling; VSD= Ventricular septal defect; TOP= Termination of pregnancy; BoB=BACs-on-Beads; FISH=Fluorescence in Situ Hybridization; MLPA= Multiplex ligation-dependent probe amplification; CMA=Chromosomal Microarray; NND= neonatal death; AGA=Appropriate for Gestational Age; SGA-Small for Gestational Age (birth weight <10% percentile for gestational age);

§ probes located in the A-B region

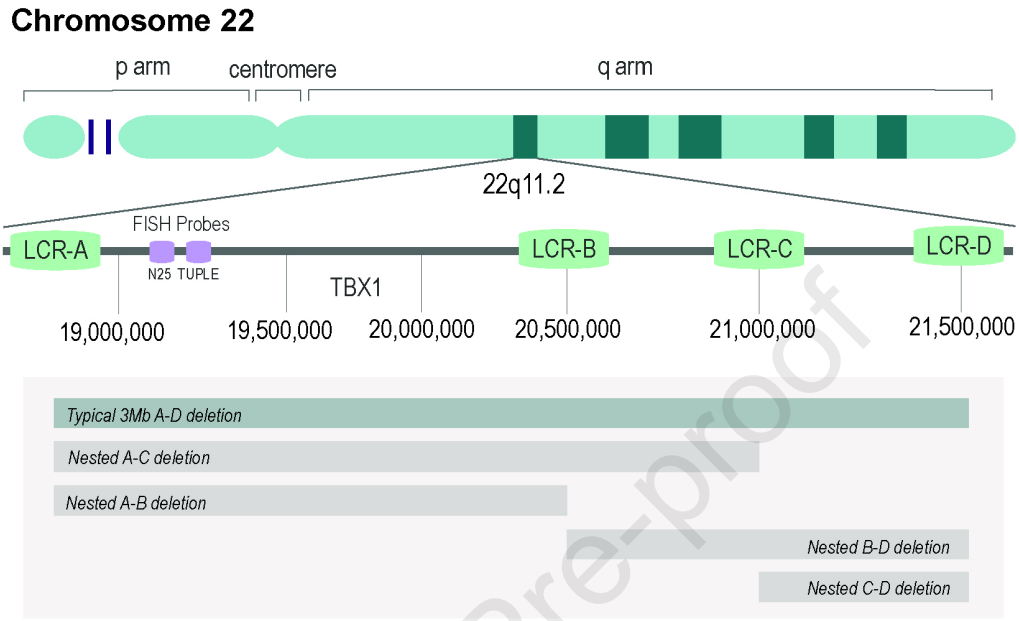
^Late preterm birth= 34-37 weeks' gestation.

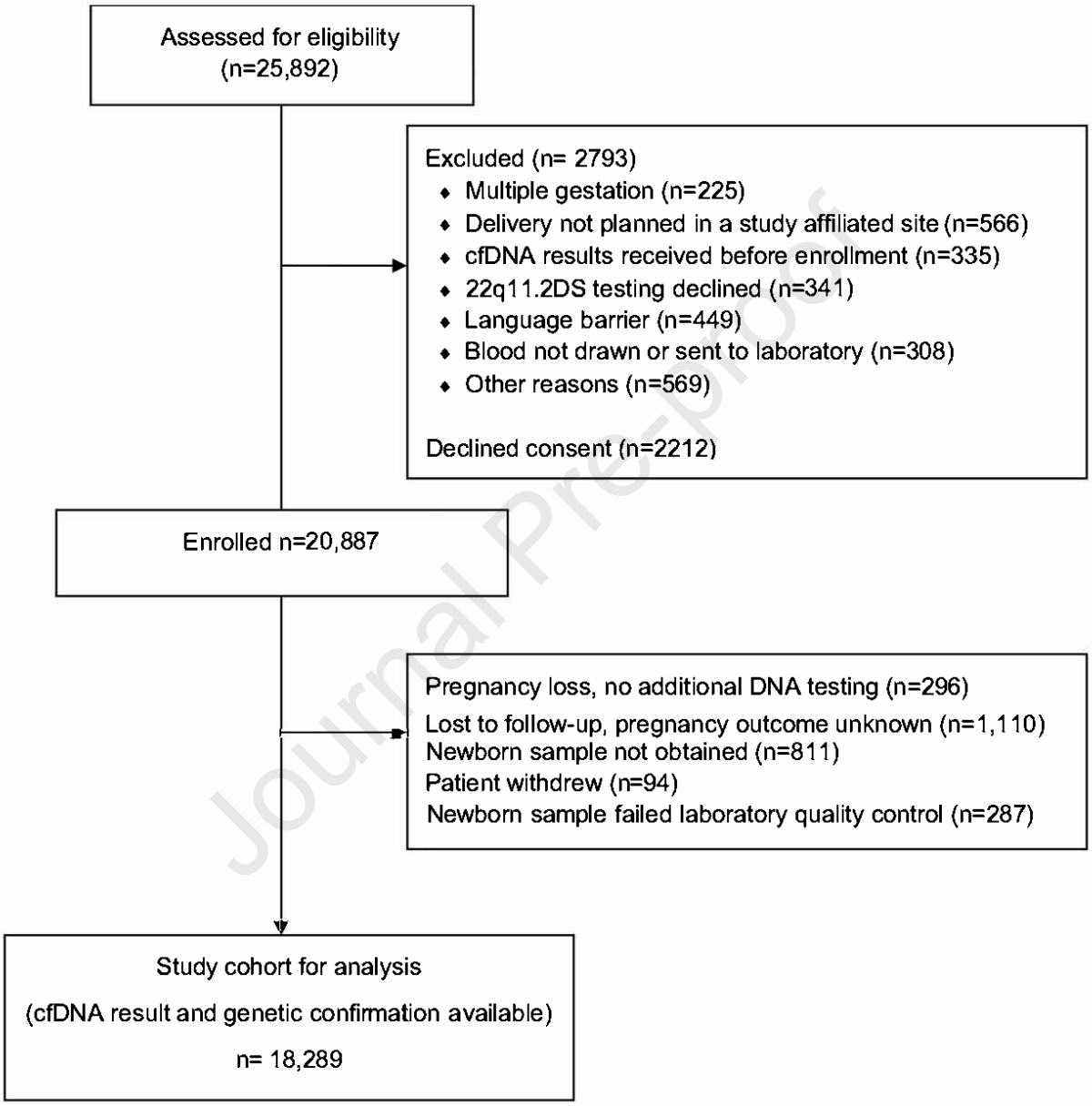
* This case was identified by the updated algorithm

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

| | 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[£] | 468.75 | 1666.00 |
| Negative likelihood ratio[§] | 0.25 | 0.17 |

*PPV=Positive predictive value; NPV=Negative predictive value; [£] Positive likelihood ratio- (sensitivity/100-specificity); [§] Negative likelihood ratio- (100–sensitivity/specificity)





*Supplementary Information*

## Methods

### Study design and participants

For full information on study dates, including enrollment and completion, see clinicaltrials.gov identifier NCT02381457. Relevant dates are as follows: Periods of recruitment: 4/8/2015 – 12/12/2019; Follow-up: 4/8/2015 – 7/18/2019; Data collection: 4/8/2015 – 9/18/2019.

This study involved 21 locations, including: University of California San Francisco, San Francisco, California, United States, 94158; Cooper University Hospital, Camden, New Jersey, United States, 08103; Virtua, Mount Laurel, New Jersey, United States, 08054; St. Peter's University, New Brunswick, New Jersey, United States, 08901; Complete Women's Healthcare, Garden City, New York, United States, 11530; North Shore University Hospital, Manhasset, New York, United States, 11030; Madonna Perinatal, Mineola, New York, United States, 11501; Long Island Jewish Medical Center New Hyde Park, New York, United States, 11040; New York University, New York, New York, United States, 10016; Icahn School of Medicine Mt Sinai, New York, New York, United States, 10029; Columbia University, New York, New York, United States, 10032; Montefiore Medical Center, New York, New York, United States, 10461; Suffolk OB, Port Jefferson, New York, United States, 11777; North Austin Maternal Fetal Medicine, Austin, Texas, United States, 78758; Zeid Women's Health Center, Longview, Texas, United States, 75601; University of Utah, Salt Lake City, Utah, United States, 84132; Royal Prince Alfred, Camperdown, New South Wales, Australia, 2050; Royal College Surgeons in Ireland, Dublin, Ireland, 1; Dexeus, Barcelona, Spain, 08028; Sahlgrenska University Hospital, Gothenburg, Sweden, SE-416 85; St. George University Hospital, London, United Kingdom, SW17 0QT.

This multi-center prospective observational study enrolled pregnant women who presented clinically at or after 9 weeks gestation and elected Panorama microdeletion and aneuploidy

screening as part of their routine care. The primary objective was to evaluate the performance of Single Nucleotide Polymorphism (SNP)-based Non Invasive Prenatal Testing (NIPT) for 22q11.2 microdeletion in a large cohort of pregnant women. Data collection began at enrollment and continued until patients delivered and their child was discharged from the hospital. Biospecimens were obtained from infants after birth to perform genetic diagnostic testing for 22q11.2 deletion. Results from the follow-up specimens were compared to those obtained by the Panorama screening test to determine test performance. In the event a newborn sample could not be obtained before discharge from the hospital, participants were mailed a salvia buccal swab kit for testing at home. Samples were then shipped to Natera for testing.