Original Research

Cell-Free DNA Analysis in Maternal Plasma in Cases of Fetal Abnormalities Detected on Ultrasound Examination

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OBJECTIVE: To evaluate the utility of noninvasive prenatal testing using cell-free circulating fetal DNA for detection of the three main autosomal fetal trisomies in the setting of ultrasonographically identified fetal anomalies.

METHODS: Nine hundred patients at risk for fetal aneuploidy with or without ultrasonography anomalies and who underwent invasive procedures were included in the study. Cell-free DNA analysis was performed by massive parallel sequencing during a multicenter, noninterventional, prospective study and the results were compared with a fetal karyotype.

RESULTS: Among all 900 pregnancies, cell-free DNA identified 76 of 76 (100%) fetal Down syndrome, 22 of 25 (88%) trisomy 18, and 12 of 12 (100%) trisomy 13. In those with a normal ultrasonogram and normal cell-free DNA analysis, karyotype identified 2 of 483 (0.4%) additional aneuploidies other than trisomies 13, 18, and 21. In those with an abnormal ultrasonogram and a normal cell-free DNA analysis, there were 23 of 290 (7.9%) additional pathogenic karyotypes. These additional aneuploidies included sex chromosome abnormalities and triploidy. The rates of additional aneuploidies not identifiable by standard cell-free DNA screening in the two groups is significantly different at $P<.01$.

CONCLUSION: In women with fetal abnormalities by ultrasonography, the rate of pathogenic chromosome abnormalities missed by cell-free DNA was 8%. Noninvasive prenatal testing should not be offered to women with fetal abnormalities because a negative result is falsely reassuring.

(Obstet Gynecol 2015;125:1330–7)

DOI: 10.1097/AOG.0000000000000874

LEVEL OF EVIDENCE: III

Cell-free fetal DNA analysis for aneuploidy screening is now widely used in a clinical setting, especially in North America and China since 2011, but also very recently in Europe where the use of the test is slowly spreading. First evaluated in a so-called high-risk population, noninvasive prenatal testing is now used in some clinics as a primary screening option. Testing has spread, although recommendations may vary from one country to another.[1–5]

The American College of Obstetricians and Gynecologists (the College), the Society for Maternal-Fetal Medicine, and the American College of Medical Genetics and Genomics issued an opinion statement in 2012 and 2013 regarding the use of these tests.[1,4] The indications presently accepted for these tests are: 1) maternal age 35 years or older at delivery; 2) fetal ultrasound findings indicating an increased

**For a list of members in the Collaborative SEHDA Study Group, see the Appendix online at http://links.lww.com/AOG/A642.*

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The authors thank Martine Olivi, Sandrine Moukoury, and Ramdane Mallek for their technical assistance and David Marsh for language editing.

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Financial Disclosure

Jean-Marc Costa is an employee of CERBA, in which he is also a shareholder. The other authors did not report any potential conflicts of interest.

ISSN: 0029-7844/15

risk of aneuploidy; 3) history of a prior pregnancy with trisomy; 4) positive test result for aneuploidy, including first-trimester screening; and 5) parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21. Therefore, cell-free DNA testing could be offered to women with these risk factors for fetal aneuploidy as a screening test after counseling. In this population the prevalence of trisomy 13, trisomy 18, and trisomy 21 is higher than in the general population. However, the question of whether cell-free DNA should be offered to any patient with a high risk for fetal aneuploidy must be clearly answered. Clinical evaluation is needed to define the target population and optimize clinical implementation. We designed a study to evaluate the performance of the noninvasive prenatal test (using cell-free circulating fetal DNA for detection of the three main autosomal fetal trisomies) in a very high-risk population of patients whose fetuses display ultrasonographically identified anomalies by comparing the results with those obtained by conventional fetal karyotyping.

MATERIALS AND METHODS

A multicenter, noninterventional, prospective study was performed in 29 French fetal medicine centers to avoid bias resulting from patient selection. The local institutional review boards approved the study and written informed consent was obtained for all patients.

From December 2012 to October 2013, pregnant women, with or without fetal ultrasound findings (structural or "soft markers" whenever fetal karyotyping was thought necessary), who were considered at high risk for fetal aneuploidies based on maternal age alone (older than 38 years, standard French maternal age cutoff), maternal serum screening (first-trimester combined test or second trimester), or a history of pregnancy with trisomy and who were willing to undergo invasive procedures were included in the study. Patients had to be at least 18 years old. All patients were more than 10 weeks of gestation and had a singleton or twin pregnancy. Maternal blood samples were collected in all patients just before the invasive procedure. Noninvasive prenatal testing was performed by massive parallel sequencing by using a whole-genome approach as described elsewhere.[6] Maternal blood was collected in two cell-free DNA BCT Streck tubes (10 mL for each) and sent at room temperature to the clinical laboratory where plasma was isolated within 72 hours after collection by a double centrifugation procedure and stored frozen at −70°C or less until further processing. Total DNA was extracted from 4 mL of plasma with the QIAamp DSP Circulating Nucleic Acid Kit after thawing and centrifugation of the samples and eluted with 55 microliters of elution buffer according to the manufacturer's instructions. The DNA libraries were then prepared in semiautomated 96-microplate format without any size selection starting from 50 microliters of extracted DNA solution using the TruSeq DNA Sample Prep reagents. After quantification on the LabChip GX microfluidic platform, the libraries from 12 different samples were pooled and sequenced on each lane of an Illumina V3 flow-cell on a HiSeq1500 instrument with the Truseq SBS kit V3-HS reagent for 27 cycles followed by seven cycles to read each sample index. During each run of the experiment, no template controls, plasma pooled from euploid pregnancies, or low positive controls prepared by mixing plasma from nonpregnant women and trisomy 13, 18, or 21 libraries were run simultaneously with the patient samples. Finally, sequence reads were mapped to the UCSC hg19 version of the human genome using Bowtie version 2; Z-scores were calculated for the targeted chromosomes 13, 18, and 21 as described[7]; and the fetal fraction was evaluated as described by Sung Kim et al (personal communication, AGTB Meeting, Marco Island, Florida, February 25–28, 2015). The results are expressed as "positive" or "negative" when the experiments fulfilled the following metric criteria: library concentration 7.5 nM or greater, total number of aligned sequence reads nine million or greater, no amplification bias, and estimated fetal DNA fraction 4% or greater. The latter metric is of particular importance because it might influence test performance for noninvasive prenatal testing assays based on counting methods. A 4% value is often used as a cutoff based on the work by Fan et al.[8] The classification was based on a standard normal transformed cutoff value of $z=3$ for chromosome 21 and $z=3.95$ for chromosomes 18 and 13.[7]

The noninvasive prenatal test was performed blindly without any knowledge of the fetal karyotype. Because the noninvasive prenatal testing samples were stored and the analysis was performed retrospectively, their results were not used for the management of the patient.

Only those cases with a karyotype and cell-free DNA analysis were used to evaluate the performance of the noninvasive prenatal test. Descriptive results were reported as percentages for categorical variables and as median and interquartile range values for quantitative variables. The performance of the test was characterized by sensitivity and specificity. Exact 95% confidence intervals (CIs) were computed with the binomial distribution. Percentages were compared

using the χ^2 test or Fisher's test and means using Student's *t* test.

RESULTS

Nine hundred patients were enrolled in the study from the 29 centers. All were at high risk for fetal aneuploidy based on the criteria given. The number of samples per center varied from two to 67 and none was withdrawn from the laboratory analysis because of insufficient quality criteria. The demographic characteristics of the patients are shown in Table 1. A body mass index (BMI, calculated as weight (kg)/[height (m)]^2) of 30 or greater was recorded in 16.3% of patients.

Definitive karyotype results were not available for eight patients who were excluded from analysis. One patient declined invasive sampling after the blood test; the noninvasive prenatal testing result was negative and the newborn was clinically unaffected. For two patients, fetal death occurred before sampling; one was positive for trisomy 21 with cell-free DNA testing and the other was positive for trisomy 18 by the same test. Fetal tissue culture failed in four additional cases and one was lost to follow-up; for all of them the noninvasive prenatal testing result was negative. Finally, 892 patients were available for a comparative analysis between noninvasive prenatal testing and conventional karyotyping (Fig. 1).

In these 892 patients, there were 113 (12.6%) cases of trisomy 21, 18, or 13 (76 cases of trisomy 21, 25 cases of trisomy 18, and 12 cases of trisomy 13). For trisomy 21 cell-free DNA, sensitivity was 100% (95% CI 95.3–100%) and specificity 99.9% (95% CI 99.3–100%). For trisomy 18, sensitivity 88.0% (95% CI 68.8–97.5%) and specificity 99.9% (95% CI 99.4–100%). For trisomy 13, sensitivity was 100% (95% CI 73.5–100%) and specificity 99.9% (95% CI 99.4–100%). Twenty-five of 773 (3.2%) had karyotype showing pathogenic results involving aneuploidies not screened for by cell-free DNA testing, including sex chromosome abnormalities, deletions, and triploidy.

Table 1. Demographics of the Patients

Parameter	Value
Maternal age (y)	35 (30–39)
BMI (kg/m^2)	23 (21–27)
Ethnic group	
Caucasian	758 (84.2)
Black or Caribbean	41 (4.6)
Asian	18 (2.0)
Mixed	51 (5.7)
Unknown	32 (3.5)
Gestational age at sampling (wk)	15.1 (10.2–34.6)
1st trimester	316 (35.1)
2nd trimester	546 (60.6)
3rd trimester	38 (4.3)
Singleton	893
Twin	7

BMI, body mass index.
Data are median (interquartile range), n (%), or n.

Cell-free DNA test results were not available for 6 of 892 patients (0.7%) with a singleton pregnancy, either because the estimated fetal fraction was below the minimum 4% required for interpretation or because the result appeared atypical (ie, positive Z-score for more than one chromosome). Three of these six patients had abnormal karyotypes (two triploidy and one monosomy X), four of six had low fetal fractions, and three of six had BMIs greater than 34. Only one of six had a normal karyotype, a fetal fraction greater than 4%, and a BMI of 22, but the z-scores were atypical. The characteristics of these patients are shown in Table 2.

For the other 886 patients, a definitive positive or negative result was reported; among those 42 (4.7%) for whom the test had to be run twice because of technical issues during the first assay (ie, failure in library preparation). Seven patients had a twin pregnancy with a reportable result for all of them; two were positive for trisomy 21.

For further data analysis, the patients were separated into two groups depending on their risk of having an aneuploid fetal karyotype. Group 1 included 513 patients without abnormal fetal ultrasound findings and who were high risk as a result of maternal age alone (older than 38 years) (n=33), abnormal results at maternal serum screening (first-trimester combined test or second trimester) (n=444), a history of a pregnancy with trisomy (n=28), one parent carrying a balanced Robertsonian translocation (n=4), a cytomegalovirus infection (n=1), or prenatal diagnosis for monogenic disorder (n=3). Group 2 included 387 patients at very high risk of having an aneuploid fetal karyotype with the fetus displaying increased nuchal translucency (n=193) or malformations other than nuchal translucency (n=194) identified at any gestational age. For group 1, the prevalence of trisomy 21 and trisomy 18 was 4.1% (21/510) and 0.8% (4/510), respectively (Table 3).

In one patient a positive noninvasive prenatal test result for chromosome 18 was observed, although on karyotype the abnormality was present as low-level mosaicism (12.5%) and consisted of partial tetrasomy. For another patient, noninvasive prenatal testing gave an unambiguous positive result for chromosome 21 (Z-score 12.02), whereas the fetus did not have trisomy 21. Sample exchange was excluded for this

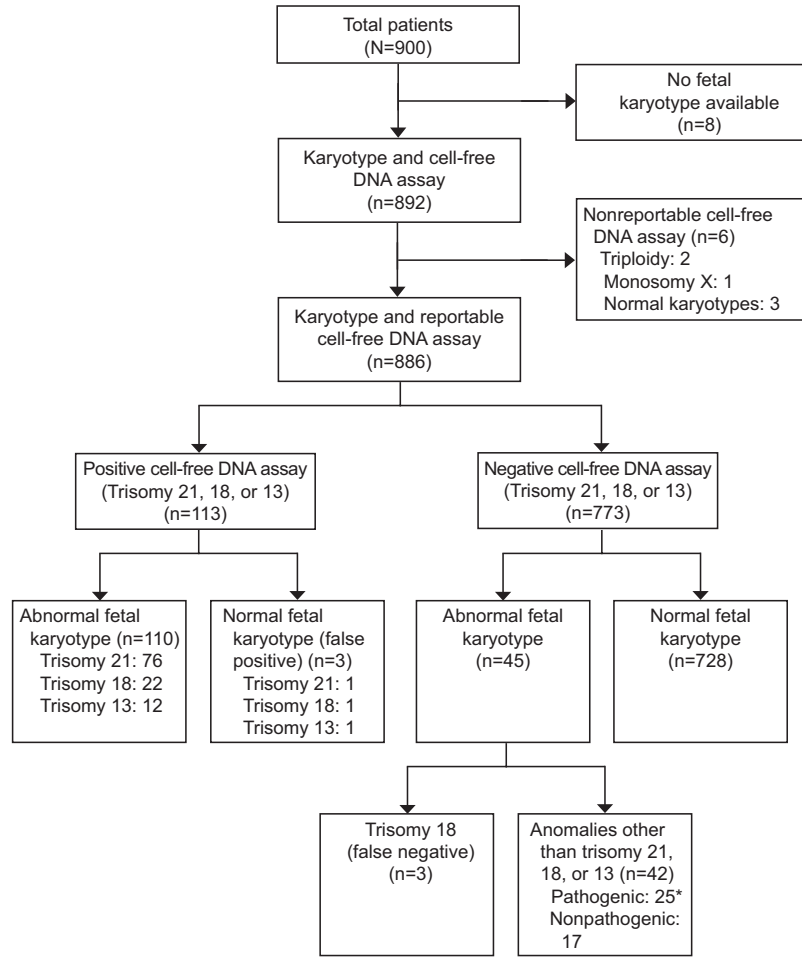

Fig. 1. Flow chart. *The pathogenic group included 14 sex chromosomal anomalies.

Benachi. Noninvasive Prenatal Testing and Ultrasound Findings. Obstet Gynecol 2015.

case, which remained unexplained. The possibility of a vanishing twin was ruled out because a scan had been performed at 7 weeks of gestation and showed a single embryo. The cell-free DNA study was performed at 12 weeks of gestation. The plasma of the patient after delivery could not be examined to exclude a possible maternal constitutional mosaicism.

Because all fetal karyotypes were available and reviewed by a cytogeneticist, we noted that in the 510 patients in group 1, there were 25 cases of trisomy 13, 18, or 21 and there were 15 cases (2.7%) of chromosomal anomalies other than trisomy 21, trisomy 18, and trisomy 13. Nine were balanced rearrangements (inherited 8, de novo 1), whereas six were aneuploidy. Two of these six were considered nonpathogenic

Table 2. Characteristics of the Six Nonreportable Noninvasive Prenatal Test Results

Reason for Referral	Week of Gestation	Fetal Fraction (%)	BMI (kg/m^2)	Z-Score			Fetal Karyotype	Tissue
				21	18	13		
Nuchal translucency	11 1/7	2.7	23	−2.39	−3.69	−2.47	45,X	CVS
Nuchal translucency	12 2/7	2.7	34	0.48	−0.14	−1.26	69,XXX	CVS
Nuchal translucency	13 0/7	2.9	43	−0.44	0.67	1.29	46,XX	CVS
Ultrasound findings	12 5/7	3.7	37	−0.30	0.97	1.38	46,XY	CVS
Nuchal translucency	13 0/7	4.5	21	6.32	10.29	5.30	46,XX	CVS
Nuchal translucency	14 4/7	7.7	23	3.56	3.85	3.38	69,XXX	CVS

BMI, body mass index; CVS, chorionic villus sampling.

Table 3. Comparison of Noninvasive Prenatal Testing Results With Conventional Fetal Karyotype According to the Absence (Group 1) or Presence (Group 2) of Fetal Findings at Ultrasound Examination

Groups	Fetal Karyotype				
Group 1 (n=510)		47,XX+21 or 47,XY+21	47,XX+18 or 47,XY+18	47,XX+13 or 47,XY+13	Other*
NIPT result	Normal				
Positive trisomy 21	0	21	0	0	1
Positive trisomy 18	0	0	4	0	1
Positive trisomy 13	0	0	0	0	0
Negative	470	0	0	0	13
Group 2 (n=376)					
NIPT result	Normal				
Positive trisomy 21	0	55	0	0	0
Positive trisomy 18	0	0	18	0	0
Positive trisomy 13	1	0	0	12	0
Negative	258	0	3	0	29

NIPT, noninvasive prenatal testing.
* Chromosomal abnormalities other than trisomy 13, 18, or 21.

(Table 4). Two of the pathogenic cases were false-positives, one for trisomy 21 and one for trisomy 18. Thus, 2 of 483 (0.4%; 95% CI 0.05–1.5%) of the group 1 patients had a pathogenic unbalanced chromosomal abnormality with a negative cell-free DNA test.

In the 376 group 2 patients (those with ultrasound abnormalities) (Table 3), the prevalences of trisomy 21, trisomy 18, and trisomy 13 were 14.6% (55/376), 5.6% (21/376), and 3.2% (12/376), respectively, for a total aneuploidy rate of 31.1%. There were three false-negative cell-free DNA results for trisomy 18 with respective Z-score values of 2.43, 1.55, and 3.27 and respective fetal fractions of 4.2%, 6.1%, and 4.5%. One of these can be explained by mosaicism, the cytotrophoblasts (direct preparation) being normal, whereas the mesenchyme (long-term culture) was abnormal. The other two remained unexplained because cytotrophoblastic cells could not be examined. One false-positive result was observed (Z-score 13.52; fetal fraction 6.4%) for trisomy 13. Again this result could not be elucidated because direct examination of cytotrophoblasts failed.

In group 2, 29 fetuses (7.7%) had a chromosomal abnormality other than trisomy 21, trisomy 18, and trisomy 13, of which 13 were related to sex chromosome aneuploidies. All of those 29 anomalies were aneuploidy with 23 considered as pathogenic and negative for noninvasive prenatal testing (7.9%; 95% CI 5.1–11.6%). This rate was significantly higher than in group 1 ($P<.001$). It is interesting to note that the overall rate of sex chromosome aneuploidy is low, but that sex chromosome aneuploidies represent a high proportion (13/29 [44.8%]) of aneuploid chromosomal

Table 4. Characteristics of the Six Patients From Group 1 With an Unbalanced Fetal Karyotype

NIPT Result	Fetal Fraction (%)	Z-Score 21	Z-Score 18	Z-Score 13	Fetal Karyotype	Tissue	Clinical Significance
Positive trisomy 18	10.4	1.04	5.06	−0.54	47,XY,+mar.ish i(18)(p10)[3]/46,XY [24]	AF	Pathogenic
Positive trisomy 21	17.1	12.02	−2.75	−0.47	46,XY,del(4)(q35)	CVS	Pathogenic
Negative	11.9	1.50	−0.08	−0.01	46,XX,der(8)(p12->p23:p23->qter)	CVS	Pathogenic
Negative	6.5	0.68	0.22	1.92	47,XXY	AF	Pathogenic
Negative	10.3	−0.70	−0.83	−1.88	46,XX.ish rec(X)del(X)(p22?)dup(X)(q28?)mat	CVS	Nonpathogenic
Negative	6.7	1.18	−1.39	−0.40	47,XX,+22[2]/46,XX[13]	CVS	Nonpathogenic

NIPT, noninvasive prenatal testing; AF, amniotic fluid; CVS, chorionic villus sampling.
Overall in this group, 15 cases showed other karyotypes then trisomy 21, 18, and 13, but 9 of 15 were balanced rearrangements and have not been added in this table.

anomalies, especially in group 2 with ultrasound findings. See Table 5 for details.

Mean fetal fraction was 10.9% and 11.2% in groups 1 and 2, respectively, and mean BMI was 24.8 and 24.4, respectively. These parameters did not differ significantly between the two groups, as already reported by others.[9]

DISCUSSION

All initial studies that led to the introduction of noninvasive prenatal testing in clinical practice have been conducted in patients at high risk for fetal aneuploidy. This selection of patients was necessary to obtain a sufficient number of affected fetuses to assess the sensitivity and specificity of the tests accurately because of the low prevalence in the general population even of the most common chromosomal abnormalities, trisomies 13, 18, and 21. In this respect, the inclusion of patients whose fetuses had an ultrasound abnormality was particularly interesting because of the high prevalence of chromosomal abnormalities in this group. As a consequence, the very good published results suggest, intentionally or not, that the criteria for inclusion in these studies, mentioned by numerous professional societies, were becoming the eligibility criteria for noninvasive prenatal testing to be offered to these different groups of high-risk patients.

To clarify this, we report a clinical study to define the best eligibility criteria before its implementation in a clinical setting. The study design was very similar to that of the first published studies. Patients who chose invasive testing were asked to participate in a noninterventional manner according to the recommendations of the College.[1] Mean maternal age at inclusion was high (35 years), as expected for this selected population. Mean gestational age at sampling was 16.2 weeks, which is low by comparison with most studies and is explained by the paucity of third-trimester cases. The overall results of the technique were consistent with performance expectations of previous published screening in a high-risk population,[10–15] especially for trisomy 21 where sensitivity and specificity were greater than 99.9%. Because fetal fraction is lower during the first trimester, the lower gestational age at sampling in our study highlights the performance of the assay, a rerun control being performed in less than 5% of cases. The low rate of samplings that needed to be run twice is particularly important in clinical practice to avoid delays in patient management. Interestingly, the number of nonreportable cases was remarkably low (6/892 [0.7%]) when compared with another study.[16] One explanation might be the lower BMI in the French population; only 12.5% of the patients had BMIs greater than 30, unlike other populations, where average BMI is higher such as in the United States or French Polynesia, where a higher rate of nonreportable results is therefore expected. Moreover, the use of a nonsingle nucleotide polymorphism-based technology may also contribute to this low rate of nonreportable results, contrary to what is often observed for single nucleotide polymorphism-based assays.[16,17] This should be kept in mind for the management of the patient, as discussed recently by Palomaki et al.[18] The rate of aneuploidy in the nonreportable group is high (three of six) and a follow-up test (targeted scanning or preferably invasive procedure) should be performed in this group as already shown by Pergament et al.[17]

The strength of our study includes the multisite collection of paired noninvasive prenatal testing and karyotype results from a large number of women. We have shown that noninvasive prenatal testing in a French population has similar efficacy for detection of trisomies 13, 18, and 21 as reported in studies done elsewhere. There is a lower rate of "no report" results but a similarly increased rate of aneuploidy among those patients. In addition, we had a large enough sample of women with fetal abnormalities that we could compare the test results in those high-risk patients with and without fetal abnormalities. Further strengths included that the results of the noninvasive prenatal test were not available to the clinician or patient.

Physicians, but more importantly patients, should be aware that approximately 8% of fetuses will have chromosomal anomalies other than trisomy 21, trisomy 18, and trisomy 13 when increased nuchal translucency or another fetal anomaly is observed on ultrasound examination. Moreover, an additional argument against using noninvasive prenatal testing as opposed to invasive testing when there are anomalies is that one cannot obtain fetal DNA testing results such as with microarrays or infectious evaluations such as with polymerase chain reaction for infectious diseases. These data highlight the need for clear and comprehensive information, which should be delivered to the patients before sampling for noninvasive prenatal testing.

The relatively small size of our study population compared with others may appear to be a weakness,[19] but our aim was to evaluate noninvasive prenatal testing screening in the very high-risk group, which has not yet been studied separately. Only one other study has evaluated the genetic abnormalities that would not have been detected by noninvasive prenatal testing in the setting of positive aneuploidy, but this study was

Table 5. Characteristics of the 29 Patients From Group 2 With an Unbalanced Fetal Karyotype

NIPT Result	Fetal Fraction (%)	Z-Score			Fetal Karyotype	Tissue	Clinical Significance	Reason for Referral
		21	18	13				
Negative	5.3	−0.75	0.41	−0.12	45,X	CVS	Pathogenic	Ultrasound findings
Negative	9.7	−2.19	0.93	−0.03	45,X	CVS	Pathogenic	Nuchal translucency
Negative	5.7	0.52	−0.57	−0.28	45,X	CVS	Pathogenic	Ultrasound findings
Negative	6.5	0.25	0.55	−0.56	45,X	CVS	Pathogenic	Ultrasound findings
Negative	8.1	1.13	1.20	0.56	45,X	CVS	Pathogenic	Nuchal translucency
Negative	9.4	−0.78	−0.74	0.09	45,X	CVS	Pathogenic	Nuchal translucency
Negative	6.8	0.12	−0.52	1.66	45,X	Amniotic fluid	Pathogenic	Nuchal translucency
Negative	7.9	0.71	−0.34	−0.42	45,X	CVS	Pathogenic	Ultrasound findings
Negative	4.6	−1.89	−1.04	−1.22	45,X	CVS	Pathogenic	Ultrasound findings
Negative	10.4	1.71	0.46	0.36	47,XXY	CVS	Pathogenic	Ultrasound findings
Negative	6.1	1.28	−1.22	0.87	45,XX,der(18)t(18;21)(q10;q10),-21	Amniotic fluid	Pathogenic	Nuchal translucency
Negative	5.4	−1.37	−0.65	−0.77	46,XX [4]/46,XX,add(17)(p13)[12]	CVS	Pathogenic	Ultrasound findings
Negative	9.0	0.88	−1.03	1.14	46,XX,der(5)t(2;5)	CVS	Pathogenic	Ultrasound findings
Negative	5.2	−0.06	−1.01	−0.48	46,XX,add(7)(q3?2)	CVS	Pathogenic	Ultrasound findings
Negative	9.2	0.20	2.36	3.01	46,XX,del(4)(p15.2)	Amniotic fluid	Pathogenic	Ultrasound findings
Negative	13.9	−2.55	0.89	−0.31	46,XX.ish dup(22)(q11.22q11.22)	CVS	Pathogenic	Nuchal translucency
Negative	7.0	0.34	−1.50	−0.32	47,XX,+15	CVS	Pathogenic	Nuchal translucency
Negative	7.7	−0.65	1.60	0.98	47,XY,+22	CVS	Pathogenic	Nuchal translucency
Negative	7.5	−2.22	−3.58	−0.43	47,XY,+22	CVS	Pathogenic	Nuchal translucency
Negative	6.5	−0.54	0.42	0.21	69,XXX	Amniotic fluid	Pathogenic	Ultrasound findings
Negative	6.3	−1.49	1.94	0.89	69,XXY	Amniotic fluid	Pathogenic	Nuchal translucency
Negative	7.5	−0.72	0.52	0.42	69,XXY	Amniotic fluid	Pathogenic	Ultrasound findings
Negative	13.3	−1.64	0.15	0.92	69,XXY	CVS	Pathogenic	Ultrasound findings
Negative	48.0	0.49	−1.49	0.73	46,X,der(X)t(X;Y)(p22;q12.2)mat	Amniotic fluid	Nonpathogenic	Ultrasound findings
Negative	7.5	−1.58	−0.33	−0.24	47,XX,+16[16]/46,XX[26]	CVS	Nonpathogenic	Nuchal translucency
Negative	26.5	−0.09	0.67	0.33	47,XY,+20[6]/46,XY[44]	Amniotic fluid	Nonpathogenic	Ultrasound findings
Negative	8.9	1.17	−0.04	0.22	47,XYY	Amniotic fluid	Nonpathogenic	Ultrasound findings
Negative	15.6	−1.97	−0.91	−0.71	47,XYY	CVS	Nonpathogenic	Nuchal translucency
Negative	10.8	−0.12	−1.80	−2.05	47,XYY	CVS	Nonpathogenic	Nuchal translucency

NIPT, noninvasive prenatal testing; AF, amniotic fluid; CVS, chorionic villus sampling.

a simulation. Only 1.6% of abnormal karyotypes would have been missed, but no detail is given on the ultrasound results.[20] Our laboratory chooses not to study sex chromosome aneuploidies. Even if they were detected with 100% sensitivity, our data show that the residual risk of a fetus being affected would remain high (10/277 [3.6%]) after negative noninvasive prenatal testing results in the case of abnormal ultrasound findings. It should also be emphasized that noninvasive prenatal testing for sexual chromosomal aneuploidies is of poor positive predictive value[10,13] and could lead to a significant increase in the number of invasive procedures.[21] As long as cell-free DNA testing remains a screening test, abnormal results should be verified with an invasive procedure to define the "real" fetal status by conventional karyotyping or microarray.

In the case of abnormal nuchal translucency or other ultrasound findings, an invasive procedure should first be offered to the patient, either to confirm trisomy 21, trisomy 18, or trisomy 13 or, in the case of a negative noninvasive prenatal test result, to look for other chromosomal anomalies. Although the College and the Society for Maternal-Fetal Medicine recommend abnormal fetal ultrasound findings as an indication for considering the use of cell-free DNA, patients in this group should be informed that this testing will miss approximately 8% of karyotypic abnormalities that would be detected if invasive testing were performed. Moreover, because microarray-based tests are tending to replace conventional karyotyping, the rate of pathogenic chromosome abnormalities missed by noninvasive prenatal testing would certainly be even

higher. The French College of Obstetrics and Gynecology recommended in January 2013 that cell-free DNA testing should only be offered to the population considered at risk after first- or second-trimester screening and with nuchal translucency less than 3.5 mm. Cell-free DNA testing should not be offered to patients with nuchal translucency greater than 3.5 mm and fetal anomalies.[22,23] These recommendations are also in concordance with a cost-effectiveness goal sought by all countries. Local economic considerations and access to ultrasonography, invasive testing, and counseling resources should be considered when deciding on the use of maternal plasma massive parallel sequencing screening.[2]

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