

Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population

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OBJECTIVE: We sought to assess performance of noninvasive prenatal testing for fetal trisomy in a routinely screened first-trimester pregnancy population.

STUDY DESIGN: This was a cohort study of 2049 pregnant women undergoing routine screening for aneuploidies at 11-13 weeks' gestation. Plasma cell-free DNA analysis using chromosome-selective sequencing was used. Laboratory testing on a single plasma sample of 2 mL was carried out blindly and results were provided as risk score (%) for trisomies 21 and 18.

RESULTS: Trisomy risk scores were given for 95.1% (1949 of 2049) of cases including all 8 with trisomy 21 and 2 of the 3 with trisomy 18. The trisomy risk score was >99% in the 8 cases of trisomy 21 and 2 of trisomy 18 and <1% in 99.9% (1937 of 1939) of euploid cases.

CONCLUSION: Noninvasive prenatal testing using chromosome-selective sequencing in a routinely screened population identified trisomies 21 and 18 with a false-positive rate of 0.1%.

Key words: first trimester, noninvasive prenatal diagnostics, prenatal screening, trisomy 18, trisomy 21

Cite this article as: Nicolaidis KH, Syngelaki A, Ashoor G, et al. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol* 2012;207:374.e1-6.

In the last 40 years, screening and diagnosis of fetal aneuploidies has shifted from second-trimester amniocentesis for advanced maternal age, with a detection rate (DR) of trisomy 21 of 30% at false-positive rate (FPR) of 5%, to first-trimester chorionic villous sampling (CVS) in the high-risk group identified by screening with fetal nuchal translucency (NT) and serum biochemistry, with DR of about 90% at FPR of 3-5%.^{1,2} Invasive testing with CVS or

★ EDITORS' CHOICE ★

amniocentesis is diagnostic, but associated with the risk of miscarriage.

Recently, noninvasive prenatal testing (NIPT) by analysis of cell-free DNA (cfDNA) in maternal blood has shown promise for highly accurate detection of common fetal autosomal trisomies.³ Analysis of cfDNA has been validated in several clinical studies utilizing next-generation DNA sequencing technology.⁴⁻¹³ Clinical studies have primarily included women identified by prior screening, with maternal age and biochemical and/or sonographic testing in the first or second trimester of pregnancy, to be at high risk for aneuploidies. It has therefore been uncertain if the results of NIPT in such high-risk pregnancies are applicable to the general pregnancy population.

The objective of this study is to assess the performance of screening by NIPT for trisomies 21 and 18 using a chromosome-selective sequencing method of cfDNA in maternal plasma obtained from a population undergoing routine screening at 11-13 weeks' gestation.

MATERIALS AND METHODS

Study population

The data for this study were derived from analysis of stored maternal plasma obtained during prospective first-trimester

combined screening for aneuploidies in women with singleton pregnancies attending for their routine first hospital visit in pregnancy. In this visit, which was held at 11⁺⁰-13⁺⁶ weeks of gestation, we recorded maternal characteristics and medical history and performed an ultrasound scan to: firstly, determine gestational age from the measurement of the fetal crown-rump length; secondly, diagnose any major fetal abnormalities; thirdly, measure fetal NT thickness; and fourthly, assess the nasal bone (NB) as present or absent, the flow across the tricuspid valve as normal or regurgitant (tricuspid regurgitation [TR]), and the a-wave in the ductus venosus (DV) as normal or reversed.^{1,2} In addition, the maternal serum concentrations of pregnancy-associated plasma protein (PAPP)-A and free β -human chorionic gonadotrophin (hCG) were determined within 10 minutes of blood collection using automated machines (DELFIAXpress system, PerkinElmer Life and Analytical Sciences, Waltham, MA). Biophysical and biochemical markers were combined to estimate the patient-specific risk for trisomies 21, 18, and 13.^{1,2,14} Women were given their estimated individual risk for these trisomies and those considering their risk to be high were offered CVS for fetal karyotyping. Karyotype results, obtained from genetic laboratories, and details on pregnancy outcomes, ob-

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Received July 27, 2012; revised Aug. 9, 2012; accepted Aug. 22, 2012.

The study was supported by a grant from the Fetal Medicine Foundation (United Kingdom charity number 1037116). The cost of collection and analysis of the samples was covered by Ariosa Diagnostics Inc, San Jose, CA.

The authors report no conflict of interest.

Reprints not available from the authors.

0002-9378/free

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<http://dx.doi.org/10.1016/j.ajog.2012.08.033>



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tained from the maternity computerized records or the general medical practitioners of the women, were added into the database as soon as they became available.

Venous blood was obtained from women who gave written informed consent to provide samples for research into early prediction of pregnancy complications, which was approved by the National Research Ethics Service of the National Health Service. Blood was collected in EDTA BD Vacutainer tubes (Becton Dickinson UK Ltd, Oxfordshire, United Kingdom) and processed within 15 minutes of collection. The tubes were centrifuged at 2000g for 10 minutes to separate plasma from packed cells and buffy coat and subsequently at 16,000g for 10 minutes to further separate cell debris. Plasma samples were divided into 0.5-mL aliquots in separate Eppendorf tubes (Jencons Scientific Ltd, VWR International, Willard Way, Bedfordshire, UK) labeled with a unique patient identifier and stored at -80°C until subsequent analysis.

We searched our database and selected all cases with at least 2 mL of available stored plasma, collected from October 2010 through January 2011. The population consisted of 2230 singleton pregnancies. We excluded 74 pregnancies where there was no fetal karyotype and the outcome was miscarriage, stillbirth, termination ($n = 28$), or no follow-up ($n = 46$). We also excluded 7 pregnancies where CVS demonstrated that the fetal karyotype was abnormal but other than trisomy 21 or trisomy 18; 2 cases of triploidy; and 1 case each of Turner syndrome, 47,XX,+i(8)(p10), 46,XY,del(13)(q33), 46,XX,dup(10)(q26.1q24.1), and mosaic 45,X/47,XXX. There were 2149 cases after excluding for the above reasons.

Laboratory analysis

Plasma samples (4 tubes of 0.5 mL per patient) from the selected cases were sent overnight on dry ice from London, United Kingdom, to the laboratory of Ariosa Diagnostics Inc in San Jose, CA. The information provided to Ariosa Diagnostics Inc for each case was: patient unique identifier, maternal age, gestational age, date of blood collection, and fetal sex, but not fetal karyotype or birth outcome.

Prior to evaluation for fetal trisomy, Ariosa Diagnostics Inc determined 29 cases had inadequate sample volume, 1 case had tube labels that did not match the sample manifest, and 70 cases had issues of sample mixing during the manual pooling process of individual Eppendorf tubes by laboratory personnel. Therefore, the eligible study population consisted of 2049 cases.

Plasma samples from the study population were analyzed using a chromosome-selective assay (Harmony Prenatal Test, Ariosa Diagnostics Inc).^{11,15} Results were provided on the risk of trisomy 21 and trisomy 18 on each case to K.H.N. who then determined the correlation between the assay results with the fetal karyotype or birth outcome. The risk scores were represented as a percentage with ranges capped at $>99\%$ and $<0.01\%$.

Statistical analyses

Descriptive data were presented in median and interquartile range for continuous variables and in numbers and percentages for categorical variables. Comparison between the outcome groups was by χ^2 or Fisher exact test for categorical variables and Mann-Whitney U test for continuous variables. In all cases we used Bonferroni correction with adjusted P value of $< .025$.

The statistical software package SPSS 20.0 (IBM Corp, Armonk, NY) was used for data analyses.

RESULTS

Study population

The total study population included 2049 pregnancies. In 86 (4.3%) cases, the fetal karyotype was determined by CVS or amniocentesis and was normal in 75 cases, trisomy 21 in 8, and trisomy 18 in 3. The remaining 1963 pregnancies resulted in the live birth of phenotypically normal neonates assumed to be euploid and therefore, the total number of proven or assumed euploid pregnancies was 2038.

Maternal characteristics and results from conventional first-trimester screening with serum markers and ultrasound of the study population are presented in the Table. In trisomy 21, compared to the euploid pregnancies, the median maternal age, delta NT, and serum free β -hCG

were significantly higher, and there was a higher prevalence of absent NB, TR, and reversed a-wave in DV. In trisomy 18, delta NT and serum free β -hCG were higher and PAPP-A and fetal crown-rump length were lower and similar to trisomy 21, there was a higher prevalence of absent NB, TR, and reversed a-wave in DV.

The expected number of cases of trisomy 21 and trisomy 18 in our study population, on the basis of the maternal age distribution and the age-related risk for these trisomies at 11-13 weeks^{16,17} were 7.89 and 3.21, respectively, which were similar to the observed numbers of 8 and 3, respectively.

Risk score results from NIPT

Results from chromosome-selective sequencing were available for 1949 of the 2049 cases (95.1%). In 46 cases (2.2%), the fetal fraction was below the minimal requirement of 4% and in 54 cases (2.6%) there was assay failure. One of the trisomy 18 cases failed to generate an assay result. Risk scores for trisomies 21 and 18 were provided for 1949 samples, including 8 cases of trisomy 21, 2 of trisomy 18, and 1939 euploid.

In all 8 cases of trisomy 21, the risk score for trisomy 21 was $>99\%$ and the risk score for trisomy 18 was $<0.01\%$ (Figure). For the 2 cases of trisomy 18, the risk score for trisomy 18 was $>99\%$ and the risk score for trisomy 21 was $<0.01\%$.

In the 1939 proven or assumed euploid pregnancies, the risk scores for trisomies 21 and 18 were $<0.01\%$ in 1936 (99.85%) and $<1\%$ in 1937 (99.9%). In 2 euploid pregnancies the risk score for trisomy 18 was 9.8% and 11.7%, respectively. Applying a risk score cutoff of 1% to delineate high-risk from low-risk patients (based on Ariosa Diagnostics Inc cutoff for the Harmony Prenatal Test), the overall trisomy DR was 100% (10 of 10 cases) with a combined FPR of 0.1%.

In this population of 1939 euploid and 10 aneuploid pregnancies, the performance of screening by the combined test (NT, free β -hCG, and PAPP-A), at the risk cutoff of 1:150 recommended by the United Kingdom National Screening Committee, was detection of all cases of trisomies 21 and 18 at FPR of 4.5% (87/

TABLE
Characteristics of study population

Characteristic	Total n = 2049	Euploid n = 2038	Trisomy 21 n = 8	Trisomy 18 n = 3
Median maternal age, y (IQR)	31.8 (27.7–35.4)	31.8 (27.7–35.4)	39.6 (33.3–41.7) ^a	39.4 (23.4–42.3)
Median maternal weight, kg (IQR)	65.2 (58.5–76.0)	65.2 (58.5–76.0)	64.5 (60.5–88.5)	64.0 (56.0–126.0)
Median maternal height, cm (IQR)	164 (160–169)	164 (160–169)	168 (164–170)	165 (158–165)
Racial origin, n (%)				
Caucasian	1431 (69.8)	1423 (69.8)	6 (75.0)	2 (66.7)
African	422 (20.6)	419 (20.6)	2 (25.0)	1 (33.3)
South Asian	82 (4.0)	82 (4.0)	0	0
East Asian	57 (2.8)	57 (2.8)	0	0
Mixed	57 (2.8)	57 (2.8)	0	0
Cigarette smoker, n (%)	131 (6.4)	131 (6.4)	0	0
Method of conception, n (%)				
Spontaneous	2007 (98.0)	1996 (97.9)	8 (100.0)	3 (100.0)
Ovulation drugs	19 (0.9)	19 (0.9)	0	0
In vitro fertilization	23 (1.1)	23 (1.1)	0	0
Preexisting diabetes mellitus, n (%)				
Type 1	10 (0.5)	10 (0.5)	0	0
Type 2	9 (0.4)	9 (0.4)	0	0
Median fetal crown-rump length, mm (IQR)	62.4 (57.3–67.3)	62.4 (57.3–67.3)	64.1 (59.5–70.4)	47.7 (45.5–59.2) ^a
Fetal gender, n (%)				
Male	1069 (52.2)	1063 (52.2)	4 (50.0)	2 (67.7)
Female	980 (47.8)	975 (47.8)	4 (50.0)	1 (33.3)
Median PAPP-A MoM (IQR)	1.048 (0.722–1.491)	1.052 (0.724–1.494)	0.700 (0.274–1.325)	0.195 (0.152–0.300) ^a
Median free β-hCG MoM (IQR)	1.089 (0.729–1.641)	1.089 (0.730–1.631)	3.809 (1.852–8.602) ^a	0.339 (0.253–0.573) ^a
Median delta nuchal translucency (IQR)	0.112 (–0.083 to 0.349)	0.110 (–0.084 to 0.347)	1.366 (0.797–3.590) ^a	6.341 (5.872–8.721) ^a
Absent nasal bone, n (%)	122 (6.0)	115 (5.6)	4 (50.0) ^a	3 (100.0) ^a
Tricuspid regurgitation, n (%)	41 (2.0)	34 (1.7)	4 (50.0) ^a	3 (100.0) ^a
Reversed a-wave in ductus venosus, n (%)	59 (2.9)	51 (2.5)	5 (62.5) ^a	3 (100.0) ^a
Median fetal fraction, % (IQR)	10.0 (7.8–13.0)	10.0 (7.8–13.0)	12.5 (9.2–21.3)	9.3 (5.6–13.0)
Median estimated risk for trisomy 21 (range)	1:8 469 (1:2–1:23 527)	1:8 547 (1:2–1:23 527)	1:2 (1:2–1:3) ^a	1:6 (1:4–1:13) ^a
Median estimated risk for trisomy 18 (range)	1:14 894 (1:2–1:47 472)	1:14 980 (1:3–1:47 472)	1:177 (1:2–1:1 562) ^a	1:2 ^a

hCG, human chorionic gonadotrophin; IQR, interquartile range; MoM, multiple of the median; PAPP, pregnancy-associated plasma protein.

^a Comparisons between aneuploid and euploid pregnancies are by Mann-Whitney *U* test for continuous variables and by χ^2 or Fisher exact test for categorical variables, with Bonferroni correction with adjusted *P* value of < .025.

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1939). In screening by the combined test and the additional ultrasound markers (NB, TR, and DV), at the risk cutoff of 1:150, all cases of trisomies 21 and 18 were detected at FPR of 3.0% (59/1939).

COMMENT

Principal findings of this study

The study population was derived from women undergoing first-trimester screen-

ing for aneuploidies as part of their routine antenatal care in an inner-city maternity hospital. The observed number of trisomies was as expected on the basis of the maternal age distribution of the study population, which was similar to the national average in England, United Kingdom.¹⁸

This cohort study of pregnant women undergoing routine screening has shown that NIPT with a chromosome-selective

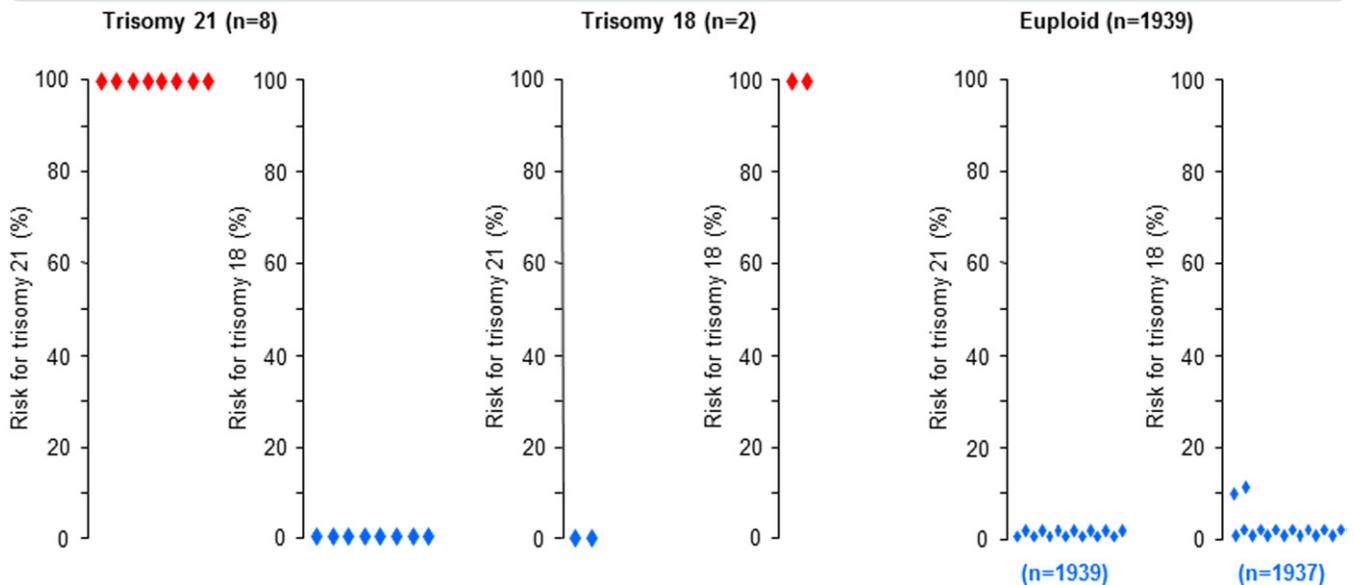
sequencing approach is highly accurate for fetal aneuploidy detection with very low FPR. The estimated trisomy risk score was >99% in all cases of trisomy 21 and trisomy 18 and <1% in 99.9% of the euploid cases.

Limitations of the study

In this study, which was based on only 1 sample of 2 mL of stored plasma per case,

FIGURE

Risk scores for trisomy 21 and 18 in pregnancies with trisomy 21, trisomy 18, and euploid fetuses



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the assay failure rate was 2.6% and there were an additional 2.2% cases for which no risk score was provided because the fetal fraction was $<4\%$. When the fraction is below this minimal requirement the small differences in circulating cfDNA between trisomic and disomic pregnancies may not be detectable.^{6,7,11} These results of assay failure and low fetal fraction are similar to the 2.8% and 1.8%, respectively, reported in a previous cohort study.¹³ The problem of low fetal fraction may be impossible to overcome by currently available NIPT techniques. The fetal fraction increases with placental mass is inversely related to maternal weight¹⁹ and the main cause of low fraction is obesity. Further study is therefore warranted to investigate the optimal method of aneuploidy screening and the role of NIPT in obese women.

A limitation of the study was that we did not perform karyotyping in all cases and the assumption of euploidy was based on the lack of phenotypic features of aneuploidy in the neonates. This was an inevitable consequence of the nature of the study that was based on a population undergoing routine screening for aneuploidies, rather than a high-risk population undergoing invasive testing.

The primary source of cfDNA in the maternal circulation is thought to be the placenta.²⁰ One possible explanation for the 2 false-positive cases in which the risk score for trisomy 18 was about 10%, rather than $<0.01\%$ as in all other assumed euploid cases, is placenta-confined mosaicism.²¹

Comparison of the findings with previous studies in the literature

Numerous studies in high-risk pregnancies reported that NIPT by cfDNA analysis of maternal plasma can detect $>99\%$ of pregnancies with fetal trisomy 21 at FPR of $<1\%$, but the performance of screening for trisomies 18 and 13 has been less robust.⁴⁻¹³ In a previous nested case-control study we used chromosome-selective sequencing to examine cfDNA in plasma obtained before CVS from 300 euploid, 50 trisomy-21, and 50 trisomy-18 pregnancies at 11-13 weeks and detected all cases of trisomy 21 and 98% of trisomy 18 with FPR of 0% for both.¹²

The findings of this study, in a population of pregnancies undergoing routine aneuploidy screening, demonstrate that the accuracy of NIPT obtained from the investigation of pregnancies at high risk for aneuploidies is applicable to the

general population where the prevalence of fetal trisomy 21 is much lower. This supports the suggestion that the ability to detect aneuploidy with cfDNA is dependent upon assay precision and fetal DNA percentage in the sample rather than the prevalence of the disease in the study population.¹²

Implications for practice

The sensitivity and specificity of NIPT is not 100% and therefore it should not be considered a diagnostic test to replace invasive testing in high-risk pregnancies. It is a new screening test that identifies a high-risk group requiring further investigation by invasive testing. The performance of this new screening method for both trisomy 21 and trisomy 18 is far superior to that of currently available screening methods with a substantial increase in DR and decrease in FPR.²

On the basis of existing data, NIPT can potentially be used in universal screening for trisomies 21 and 18 in all singleton pregnancies and the main limiting factor for such widespread application of the test at present is the associated cost. Another limiting factor in the application of NIPT in universal screening relates to the delay of 1-2 weeks between sampling and obtaining results. This

problem can be overcome by taking the blood sample 1-2 weeks before the scheduled first-trimester ultrasound examination, which is ideally performed at 12 weeks.^{1,2} At this 12-week visit, based on the results of NIPT and the ultrasound findings, the parents can be counseled concerning the option of invasive testing. In those cases where NIPT has failed to provide a result the parents would still have the option of first-trimester screening by combined testing with fetal NT and serum free β -hCG and PAPP-A and the advantage of first- rather than second-trimester termination of pregnancy should the fetus be found to be affected by a major abnormality.

In the last 40 years prenatal screening for aneuploidies has focused on trisomy 21. A beneficial consequence of such screening has been the detection of many additional clinically significant aneuploidies in the screen-positive group undergoing invasive testing and full karyotyping. Additionally the increasing application of molecular techniques in the analysis of samples obtained by invasive testing identifies many clinically significant microdeletion/duplication syndromes. Consequently, a potential criticism of replacing the traditional methods of screening for trisomy 21 by NIPT is that many clinically significant cytogenetic abnormalities that are currently detectable would be missed. However, the biomarker profile for many of the rare aneuploidies and microdeletion/duplication syndromes is not clearly defined and it is uncertain whether their incidence in the screen-positive group for trisomy 21 is higher than in the screen-negative group.

In the case of some aneuploidies, such as trisomies 18 and 13, triploidy, and Turner syndrome, their incidence in the screen-positive group for trisomy 21 is much higher than in the screen-negative group because they have a similar pattern in the expression of biophysical and biochemical markers.² The potential loss of missing some of these defects by replacing traditional methods of screening by NIPT can be overcome by firstly, expanding the conditions that can be detected by NIPT and secondly, retaining at least some of the elements of current

methods of screening as an additional method of selecting patients for invasive testing. Specifically, invasive testing should be offered to fetuses with increased NT thickness and structural defects, including exomphalos, holoprosencephaly megacystitis, and diaphragmatic hernia, even in cases with a negative NIPT result for trisomies 21 and 18.^{1,2,22-26} Consequently, the introduction of NIPT as a new high-performance test for universal screening should not be considered as a method of replacing the 11- to 13-week scan. The latter is not only useful in screening for aneuploidies but it is a diagnostic test for many major fetal defects and in combination with biochemical and other biophysical markers can provide effective early screening for pregnancy complications, including preeclampsia and preterm birth.²⁷

Conclusion

The performance of screening for trisomy 21 and trisomy 18 by NIPT using chromosome-selective sequencing in a routine population is as effective as previously reported in high-risk groups with DR >99% and FPR <1%. ■

REFERENCES

- Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* 2004;191:45-67.
- Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn* 2011;31:7-15.
- Chitty LS, Hill M, White H, Wright D, Morris S. Noninvasive prenatal testing for aneuploidy—ready for prime time? *Am J Obstet Gynecol* 2012;206:269-75.
- Chiu RW, Akolekar R, Zheng YWL, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large-scale validity study. *BMJ* 2011;342:c7401.
- Chen EZ, Chiu RWK, Sun H, et al. Noninvasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. *PLoS One* 2011;6:e21791.
- Ehrich M, Deciu C, Zwiefelhofer T, et al. Non-invasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol* 2011;204:205.e1-11.
- Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med* 2011;13:913-20.
- Sehnert AJ, Rhees B, Comstock D, et al. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. *Clin Chem* 2011;57:1042-9.
- Bianchi DW, Platt LK, Goldberg JD, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 2012;119:1-12.
- Palomaki GE, Deciu C, Kloza EM, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med* 2012;14:296-305.
- Sparks AB, Struble CA, Wange ET, et al. Non-invasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012;206:319.e1-9.
- Ashoor G, Syngelaki A, Wagner M, Birdir C, Nicolaides KH. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012;206:322.e1-5.
- Norton ME, Brar H, Weiss J, et al. Non-invasive chromosomal evaluation (NICE) study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012;207:137.e1-8.
- Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free beta-hCG and pregnancy-associated plasma protein-A. *Hum Reprod* 2008;23:1968-75.
- Sparks A, Wang E, Struble C, et al. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. *Prenat Diagn* 2012;32:1-7.
- Snijders RJM, Sebire NJ, Cuckle H, Nicolaides KH. Maternal age and gestational age-specific risks for chromosomal defects. *Fetal Diagn Ther* 1995;10:356-67.
- Snijders RJM, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age and gestation-specific risk for trisomy 21. *Ultrasound Obstet Gynecol* 1999;13:167-70.
- Office for National Statistics. Births and deaths in England and Wales, 2010. Available at: <http://www.ons.gov.uk/ons/rel/vsob1/birth-summary-tables--england-and-wales/2010/index.html>. Accessed July 23, 2012.
- Ashoor G, Poon L, Syngelaki A, Mosimann B, Nicolaides KH. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: effect of maternal and fetal factors. *Fetal Diagn Ther* 2012;31:237-43.
- Alberry M, Maddocks D, Jones M, et al. Free fetal DNA in maternal plasma in anembryonic pregnancies: confirmation that the origin is the trophoblast. *Prenat Diagn* 2007;27:415-8.
- Wirtz A, Gloning KP, Murken J. Trisomy 18 in chorionic villus sampling: problems and consequences. *Prenat Diagn* 1991;11:563-7.
- Snijders RJ, Noble P, Sebire N, Souka A,

Nicolaides KH. UK multicenter project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation: Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998;352:343-6.

23. Kagan KO, Avgidou K, Molina FS, Gajewska K, Nicolaides KH. Relation between increased fetal nuchal translucency thickness

and chromosomal defects. *Obstet Gynecol* 2006;107:6-10.

24. Chitty LS, Kagan KO, Molina FS, Waters JJ, Nicolaides KH. Fetal nuchal translucency scan and early prenatal diagnosis of chromosomal abnormalities by rapid aneuploidy screening: observational study. *BMJ* 2006;332:452-5.

25. Pergament E, Alamillo C, Sak K, Fiddler M. Genetic assessment following increased nuchal

translucency and normal karyotype. *Prenat Diagn* 2011;31:307-10.

26. Leung TY, Vogel I, Lau TK, et al. Identification of submicroscopic chromosomal aberrations in fetuses with increased nuchal translucency and apparently normal karyotype. *Ultrasound Obstet Gynecol* 2011;38:314-9.

27. Nicolaides KH. Turning the pyramid of prenatal care. *Fetal Diagn Ther* 2011;29:183-96.