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Building Global Partnerships in Genetics and Fetal Care

Position Statement from the Chromosome Abnormality Screening Committee on Behalf of the Board of the International Society for Prenatal Diagnosis

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BACKGROUND

Prenatal diagnosis of chromosome abnormalities through the analysis of amniocytes or chorionic villus samples (CVS) is an accepted part of prenatal care. Chromosome numerical changes (aneuploidy, polyploidy), large deletions and duplications, and rearrangements can be detected through conventional chromosome analysis (karyotyping) and smaller copy number variations can be detected using microarrays.[1] Use of chromosome microarrays can be particularly informative when there is ultrasonographic evidence of fetal anatomic abnormalies.[2]

Amniocentesis and CVS procedures carry some degree of risk for miscarriage or other pregnancy complications.[3,4] Therefore, in most developed countries it is routine practice to provide a woman's personal risk for specific fetal aneuploidies (screening) and to offer definitive diagnosis through amniocentesis or CVS if the risk is considered to be elevated. Risk for chromosome abnormalities can be evaluated on the basis of various combinations of maternal age, prior affected pregnancy or family history, maternal serum biochemical tests, fetal ultrasound markers and analysis of cell-free DNA (cfDNA) in maternal plasma. Very high sensitivities and specificities have been reported with cfDNA screening for some aneuploidies, notably for fetal trisomy 21.[5] However, it is important to recognize that all screening tests, including cfDNA testing, are not fully diagnostic and follow-up confirmatory studies are necessary for positive screening results. In addition, screening only targets specific chromosomal imbalances and does not identify all of the abnormalities identifiable through invasive testing.[6] When women receive screening for specific chromosome abnormalities, they should be informed about their risk for all detectable abnormalities; not just those included in the screening panel.

All approaches to risk assessment provide an opportunity to re-assure most women that their fetus is unlikely to be affected by a chromosomal disorder (and thereby reduce the number of invasive procedures performed) while identifying those women at highest risk for an affected pregnancy. Potential follow up options for women who are identified as being at elevated risk based on any of these screening options can include further counseling, additional testing and appropriate follow-up obstetric care.

Because Down syndrome (trisomy 21) is the most common significant aneuploidy, prenatal screening has emphasized the detection of this disorder. However, it is recognized that many of the screening tests have a variable potential to detect other aneuploidies, some other genetic disorders, specific fetal anatomic abnormalities, and pregnancy complications such as preeclampsia. This Position Statement only considers the utility of screening tests for detecting fetal chromosome abnormalities. Our purpose is to review key issues and recommend best practices from a global perspective.

GOAL OF FETAL ANEUPLOIDY RISK EVALUATION

Every pregnant woman should have the opportunity to receive the best possible estimate of her personal risk for fetal chromosome abnormalities. Programs involved in risk evaluation aim to provide timely and accurate individual patient-specific estimates of risk for the most common and clinically significant fetal chromosome abnormalities.

THE PROVISION OF PRENATAL SCREENING FOR CHROMOSOME ABNORMALITIES

Chromosome abnormality risk assessment is a component of a broad set of prenatal clinical services that should be offered from 9-13 weeks gestational age whenever possible. Services can include genetic counseling, screening for pregnancy complications and other fetal conditions, diagnostic testing (chromosome analysis, microarray analysis, other genetic testing), midwifery and obstetrical interventions. For women who only come into care after the first trimester, risk assessment testing should be made available as soon as possible.

Prenatal screening for chromosome abnormalities should be provided by a medical healthcare professional. It should not be independently offered as a direct-to-consumer test by laboratories but instead be integrated and coordinated with the overall prenatal care that is provided by the clinician. Prior to undergoing prenatal screening, women should be given information on the screening process and be provided with an opportunity to discuss this with the health professional before making a personal decision to accept or decline screening or diagnostic testing.

ISPD recognizes the challenge associated with explaining the expanding range of disorders that can be included in screening panels as well as the complexity of the various testing alternatives. To help meet this growing need, we support additional professional education for obstetricians and other healthcare personnel involved in screening, development of patient educational materials, and increased availability of genetic counseling.

When there are results from more than one screening approach in the same pregnancy for a specific disorder, where possible the information should be combined into a unified risk assessment. If a patient-specific risk is not available, it may be appropriate to explain screening results in the context of a population based positive predictive value (PPV) or odds of being affected given a positive result (OAPR), recognizing that this will not take into consideration an individual woman's specific results and any additional risk factors. Following the screening, results should be explained in the context of the hazards and benefits of definitive diagnosis through amniocentesis and CVS, including the possibility of detecting clinically significant copy number variants by microarray. Information must be provided through non-directive counseling. Each woman should make her own determination as to whether she wishes to receive screening and diagnostic services. Respect for ethical and cultural values, sensitivities and the decisions made by each patient are of key importance in the provision of prenatal testing services.

Prenatal chromosome abnormality risk assessment services can vary according to the healthcare systems present in different countries. Furthermore, the services chosen may differ based on an individual women's clinical conditions such as reduced fertility, past obstetrical history, co-existing risk for other genetic disorders, or their moral and ethical values. Other programmatic differences include the use of different risk cut-offs in recommendations for diagnostic testing

and sequential versus concomitant offers of screening and diagnostic testing. Providers may have differing opinions on these standards of care and differing access to the economic resources needed to provide risk assessment services. It is recognized that there are diverse approaches to these patient services that are compatible with beneficence to both individual women and to the populations served.

ISPD supports the wide availability of affordable, high quality prenatal screening for clinically significant chromosome abnormalities together with appropriate patient counseling and follow-up diagnostic testing.

MEASURING EFFICACY OF PROTOCOLS

The efficacies of screening protocols are assessed by consideration of the detection rate (DR, or sensitivity), false-positive rate (FPR), and positive predictive value (PPV), or odds of being affected given a positive result (OAPR). These population-based screening performance indices are of considerable value in comparing different protocols. PPV and OAPR are strongly dependent on the prevalence of the disorder.

GENERAL CONSIDERATIONS FOR cfDNA SCREENING

Most validation studies (Table 1) have evaluated cfDNA screening performance on the basis of maternal plasma samples derived from pregnancies where the clinical diagnoses were established through amniocentesis, CVS, livebirth studies or phenotype. In many studies, cases with mosaicism, complex karyotypes and maternal samples with low fetal fraction were excluded. For reasons discussed below (section (c)), the DRs and FPRs from these studies could overstate actual clinical performance. Large, comprehensive, cohort studies on unselected populations have not been carried out to fully assess cfDNA screening from the perspective of all of the chromosome abnormalities that are now detectable through invasive testing.

(a) Validated methodologies for trisomies 21, 18 and 13

Three approaches to maternal plasma cfDNA screening for fetal aneuploidy have been clinically validated; "shotgun" massively parallel sequencing (s-MPS) followed by counting of the DNA sequences;[7-17] a "targeted" sequencing (t-MPS) with counting of specific DNA sequences;[18-24] and a method based on the analysis of single nucleotide polymorphisms (SNPs)[25,26] Table 1 summarizes the DR and FPR for the common trisomies derived from clinical validation studies using these three approaches. For t-MPS, replacement of sequencing by a chromosome microarray is under consideration.[27] Other approaches to aneuploidy screening that take advantage of cfDNA and cfRNA have also been proposed but these are not considered sufficiently validated at this time.

Performance of cfDNA screening is considerably greater than conventional screening, with a very high OAPR, but it falls far short of a diagnostic test. For example, for a total population, the birth OAPR for trisomy 21 based on Table 1 data for all cfDNA screening methods combined is approximately 1:1.2 (PPV of 45%). This can be compared to OAPRs of 1:25 to 1:41 (PPV 2-4%) using conventional serum and ultrasound markers (Table 2). cfDNA screening for trisomy 18 and trisomy 13 is also associated with relatively high OAPRs.

The performance of the three approaches to use cfDNA to screen for trisomies 21, 18 and 13 were initially established in studies on women who were at high-risk on the basis of maternal age and/or maternal serum and ultrasound markers. There is now increasing evidence to show that the testing can also be applied to women with average risk.[13,17,20,24,26,28-30]

(b) Sex chromosome abnormalities

The fetal sex chromosome complement can be evaluated by cfDNA analysis. Table 1 summarizes results for monosomy X. Robust estimates are not available for the detection rates and false-positive rates for 47,XXX, 47,XXY, 47,XYY and the mosaic or variant Turner syndrome karyotypes seen in livebirths. When women are offered cfDNA screening for fetal sex chromosome abnormality they should be informed that testing for fetal sex chromosomes could involve potential discovery of both fetal and maternal sex chromosome abnormalities including those that may be of minor, or no, clinical significance.[34] Evaluating the significance of a positive result may involve both invasive testing and additional studies on the mother. When women are offered cfDNA screening and X- and Y-chromosome analysis is available, women should have the option to separately accept or reject the sex chromosome analysis. In some countries, this component of the testing may be restricted.

(c) Reasons for discordancy between cfDNA testing and the true fetal karyotype.

cfDNA is primarily derived from trophoblasts and the chromosome complement present in these cells does not always correspond to that present in the fetus.[35] This is also known as confined placental mosaicism. This fetal/placental discordancy contributes to both false-positive and false-negative screening results.[36,37] True fetal mosaicism may also cause discordancy.[38,39]

All methods of cfDNA screening require sufficient fetal (placental) cfDNA in the maternal plasma and many laboratories have a minimum requirement for test interpretation.[5] Moreover, low fetal fraction appears to be associated an increased risk for trisomy 18, 13, monosomy X and triploidy.[26,29,40-43] A robust estimate for the incidence of chromosome abnormalities in cases with very low fetal fraction is not yet available. Failure to measure the fetal fraction may be falsely reassuring if the cfDNA screening test is not sufficiently sensitive to recognize abnormality in a sample with a very low fetal fraction. The extent to which low fetal fractions contribute to false-positive and false-negative rates is currently unknown.

s-MPS and t-MPS based cfDNA screening do not distinguish between maternal and fetal chromosome imbalances and therefore maternal chromosome abnormalities (constitutional or somatically acquired), including small copy number variants may contribute to the false-positive rate.[13,30,34,44-47] These methods may also be susceptible to false-positive results or an incorrect gender call that can be attributed to abnormal cfDNA from an undetected vanishing twin.[16,48,49]

(d) No results

In reports from several large laboratories, cfDNA screening was unsuccessful in approximately 1.9-6.4% of cases.[29,48,50] Test failure rates can depend on a variety of factors including the fetal fraction, which in turn is dependent on gestational age, maternal weight, and the policies used by the laboratory. In cases where there is no result due to a low fetal fraction, repeating the sampling can be successful although up to one-third may fail again.[51] An implication of a low fetal fraction is the added risk for trisomy 18, 13, monosomy X and triploidy but the magnitude

of this risk is currently poorly defined (see (c) above).[43] Hence, in such cases prior to redrawing, a re-appraisal of the use of cfDNA versus alternative testing is indicated taking into consideration the gestational age, abnormal ultrasound findings, or maternal serum screening markers consistent with trisomy 18, 13, monosomy X or triploidy, as well as patient preferences for follow up testing.

(e) Optimal gestational age for testing

cfDNA testing can be provided from as early as 9 or 10 weeks gestational age. The gestational age of cfDNA testing will depend on whether cfDNA testing is offered subsequent to conventional screening. In some practices an early ultrasound examination for fetal abnormalities is carried out and postponing cfDNA until this is completed is a consideration.[52]

It is also important to consider the availability or choice of CVS versus amniocentesis for positive test cases. There are cases where positive cfDNA screening results have been confirmed in studies on CVS or placenta but analyses on amniotic fluid cells and/or newborns have failed to confirm the abnormality.[28,35,40,50,51] Both cfDNA and CVS are based on placental cells and these discordances can be explained by the presence of confined placental mosaicism. The use of CVS following positive cfDNA results therefore should be undertaken with consideration of the potential for confined placental mosaicism. Analysis of amniotic fluid cells is considered to be a reliable indicator of the true fetal karyotype. Early provision of cfDNA screening offers the benefits of earlier identification and potential intervention but this has to be weighed against the difficulty of achieving early definitive diagnosis.

(f) Multiple gestational pregnancies

Testing has also been extended to twin pregnancies. Provided the cfDNA test is interpretable, performance in twins concordant for aneuploidy is expected to be equivalent to that for singletons. A meta-analysis of published studies found sensitivity in discordant twins was similar to that found for singletons.[31] The specificity for all twins was also similar to singletons. However, when fetal fraction is measured for each fetus and the lowest value used to decide on interpretability, the failure rate will be higher than in singletons.[31]

(g) Other autosomal trisomies

Some laboratories have extended the testing to other autosomal trisomies.[32] In the first trimester, this may identify pregnancies at high risk for spontaneous fetal loss but in the second trimester the clinical significance of a positive result is unclear.[33] Some positive cases will be associated with confined placental mosaicism but in rare instances true fetal mosaicism could be present. In general, the phenotypes for each of these rare true mosaic conditions range from normal to a highly variable set of abnormalities that do not constitute well-defined syndromes. Moreover, phenotypes cannot be accurately predicted from follow-up cytogenetic analysis of amniotic fluid cells.[33] A clinically significant uniparental disomy could also be present for chromosomes 6, 7, 11, 14, 15, and 20. The prevalences, detection rates, false-positive rates and positive predictive values for each of the additional autosomal trisomies are currently unknown. Testing for these rare trisomies should only be offered when there is sufficient information available to allow test-positive women to be provided with a clear indication of their risk for fetal death or serious fetal abnormality or other adverse pregnancy outcome.

(h) Microdeletions and microduplications

cfDNA screening is also being extended by some laboratories to include microdeletion and microduplication syndromes.[55,56] Where this is offered, the testing should be limited to clinically significant disorders with a well-defined severe phenotype. For many of these disorders, there are alternative molecular mechanisms reported other than copy number changes and not all cases are therefore detectable; this information should be included in estimated detection rates for each disorder. There remains some uncertainty about the prenatal prevalence of the disorders currently included in microdeletion syndrome panels. Testing for microdeletions and microduplications may be associated with false-positives and the cumulative false-positive rate for all testing in the panel needs to be low. For each individual disorder the positive predictive value also needs to be compatible with other disorders where prenatal screening is offered. Physicians and their patients are likely to be unfamiliar with these syndromes and therefore patient information materials and counseling need to be available (see (j) below).

(i) General issues associated with expanded cfDNA screening

It is recognized that validating test detection rates and false-positive rates for additional rare disorders is problematic because alternative comparative screening methods do not exist and test samples from affected pregnancies may be unavailable. Where screening performance is based on limited numbers of samples, experimental data, or extrapolation of screening performance for other conditions, explanatory information and data should be available to healthcare professionals and prospective parents.

(j) Patient counseling

Pre-test information should describe the hazards and benefits. This includes the scope and nature of disorders being tested; the detection, false-positive, and no call rates; an explanation that false-positive results can be common (particularly when testing for rare disorders); the need to confirm results through additional testing; the potential to detect maternal chromosome abnormalities, and the uncertainties associated with mosaicism, sex chromosome aneuploidy and unexpected findings. Following a positive result, counseling should include additional information about the disorder, issues associated with confirmation through either amniocentesis, CVS, or neonatal studies and the possible need for additional ultrasound studies for those cases established as false-positives following invasive testing.

In rare instances, cfDNA screening may result in the incidental identification of additional clinically significant maternal or fetal constitutional chromosome abnormalities or acquired cytogenetic abnormalities (including some associated with malignancy). The testing may also identify a copy number variant of uncertain significance that the laboratory medical director judges necessary to report.[30,45] Genetic counseling and/or patient referral recommendations should be available for these situations.

(k) Quality control and quality assurance

Laboratories providing cfDNA screening must adhere to specific standards for test requisitions, laboratory procedures, reporting, sample and data storage and the protection of patient information confidentiality. They should be prepared to provide ongoing details on performance based on epidemiologic monitoring, test failure rates, and turn-around time. In addition, they should participate in proficiency testing.

ISPD recognizes specific guidelines for quality control and quality assurance for cfDNA screening have not yet been developed. In the mean time, we strongly recommend that providers utilize laboratory services that meet national guidelines for quality control and proficiency testing consistent with that available for other molecular tests. We recommend the development of specific requirements for cfDNA screening.

GENERAL CONSIDERATIONS FOR CONVENTIONAL BIOCHEMICAL AND ULTRASOUND SCREENING

(a) Comparing protocols

The relative efficacy of different maternal serum biochemical and ultrasound screening protocols can be assessed by either fixing the FPR (between 1% and 5%) and comparing the DR, or fixing the DR (between 75% and 90%) and comparing the FPR. For a fixed risk cut-off both the DR and FPR will vary between protocols. Statistical modeling using observational data is a reliable way of estimating the DR, FPR and OAPR of different screening protocols.

A range of maternal serum biochemical and fetal ultrasound markers have well-documented efficacy in distinguishing between affected and unaffected pregnancies. Each has validity within a specified time interval in pregnancy and should not be offered at earlier or later gestational ages. Combination of markers is valid, provided the correlation between them has been taken into consideration in the risk calculation. Table 2 presents the modeled performance of conventional Down syndrome screening for various serum and ultrasound protocols for a fixed 3% FPR. Results are based on nuchal translucency (NT) at 12 weeks gestational age. This is generally preferred over 11 weeks in order to facilitate optimal patient scheduling, because fetal anatomy is more clearly visualized and is better than 13 weeks because the screening performance is superior.

Intervention studies can overestimate the screening performance but may provide important information on the practicality of a specific protocol. All protocols lead to 'incidental' diagnosis of trisomies 18 and 13, and monosomy X and there are also specific algorithms for calculating risk for trisomy 18 and 13. For protocols other than 2a and b, models predict that the DR exceeds that of Down syndrome even without using additional aneuploidy-specific cut-offs.

(b) The first trimester Combined test

First trimester aneuploidy screening (the 'Combined' test) generally involves the measurement of NT, pregnancy associated plasma protein-A (PAPPA) and human chorionic gonadotropin (hCG). NT is considered to be a particularly important marker because of the additional associations of large NT with cardiac defects and other serious fetal defects.[64]

The Combined test is more advantageous than second trimester screening (the 'Quadruple' test) not only because information is available earlier in pregnancy but also because the screening has greater efficacy (compare protocols 1a, b, c, d with 2a, b in Table 1). The performance of Combined test can be further improved by adding addition serum markers such as placental growth factor (PlGF) and alpha fetoprotein (AFP) (1e, 1f).

NT is measured at 11-13 weeks while the first trimester serum tests are usually carried out at 9-13 weeks (depending on the marker combination used).

(c) The second trimester Quadruple test
The Quadruple test can be provided from 14 to 21 weeks' gestation but 15-19 weeks is preferred because 15-19 weeks is optimal for open neural tube screening using (AFP).

(d) Sequential first and second trimester tests
Many women who receive a first trimester risk estimate that is intermediate between very high or moderately low risk may benefit from the provision of additional serum and ultrasound screening tests in the second trimester ('Contingent' screening) and this can be associated with highly effective screening (protocols 3a,b). Additional testing for those with low first trimester risks ('step-wise' screening) can also be considered (protocols 3c,d) although this should not be needed for the majority of cases with very low first trimester risks (e.g. <1 in 1,500 at term). For both contingent and step-wise screening, it is essential that the second trimester risk estimation incorporate both the first and second trimester tests that have been performed. The provision of separate risk assessments based on first trimester markers alone and second trimester markers alone ('independent' screening) should not be carried out as it is associated with a significantly higher overall false-positive rate and difficulties with second trimester counseling using two separate risk estimates. Protocols that include first and second trimester tests but only provide a risk figure after all screening tests are complete ('integrated' screening) are also associated with a high detection rate and low false-positive rate but will delay reassurance and/or restrict women's options in the first trimester (protocols 4a,b). When the same marker is tested in both trimesters ('repeat measures') there can be an additional benefit (protocols 4c, d).

(e) Additional first trimester sonographic markers
The provision of additional first trimester sonographic markers can obviate the need for second trimester aneuploidy screening.[59] The most widely used markers are absence of a fetal nasal bone (NB), tricuspid regurgitation (TR) determined by pulse wave Doppler ultrasound and abnormal blood flow in the ductus venosus (DV). The routine use of these markers can substantially increase detection (protocol 5a), but comparable results can be obtained when this is done contingently at specialist centers (protocols 5b, c, d).

(f) Additional second trimester sonographic markers
Additional second trimester ultrasound markers can also improve aneuploidy screening. One approach is to measure three facial profile markers concurrently with the quadruple test.[60] These facial profile markers are nuchal fold thickness (NF), nasal bone length (NBL) and prenasal thickness (PT). The model predicted results are comparable with a first trimester combined test (protocol 6a, b). NF is the most widely used marker.

In centers that routinely perform a 'genetic sonogram' or 'anomaly scan' at 18-23 weeks, presence or absence of a number of specific characteristics can be combined to assess risk.[61,65] Findings reported to be useful in modifying aneuploidy risk (abnormalities, anomalies and "markers") include major malformations (MM), increased nuchal fold thickness (NF), short femur or humerus length (FL or HL), echogenic intracardiac focus (EIF), pylectasis (P), echogenic bowel (EB), ventriculomegaly (V), and absent or hypoplastic nasal bone (NB), NF,

FL, and HL should be expressed as continuous variables (e.g. with results expressed as MoMs) rather than categorical (i.e. on the basis of a value above or below a specific cut-off) because use of continuous variables maximizes the discriminatory power of the test and results in more specific information for each woman. Presence of EIF, P, and EB need to be based on objective criteria. Regional policies vary considerably with respect to the perceived value of the genetic sonogram and the individual markers that may be included (see for example, policies adopted by the UK and Canada[66,67]).

The genetic sonogram can be used for women who have received first trimester screening (protocols 7a, b), second trimester screening (protocols 8b, c) or both (protocols 9a, b). Although the second trimester anomaly scan can be used simply to modify the maternal age-specific aneuploidy risk alone, it is not a very effective screening test (protocol 8a). Using it to modify the risk following other aneuploidy screening can improve detection but when, as often happens, this is restricted to women with screen-positive results, it can actually reduce detection. The genetic sonogram in combination with maternal age can be useful for women first receiving prenatal care at 21-23 weeks' gestation, where rapid information about risk may be required.

(g) Multiple gestational pregnancies

Aneuploidy risks based on both NT and serum markers can be provided for twin pregnancies, despite poorer performance of the serum markers than in singletons. First trimester screening should take into consideration chorionicity; monochorionic twins are assumed to be monozygotic with an identical risk for each fetus while the majority of dichorionic twins are dizygotic and will be provided with separate risks for each fetus. First trimester serum markers require the use of gestation-specific and chorionicity-specific correction factors.[68] Second trimester screening with serum markers alone is considerably less accurate than that in singleton pregnancies. For triplets and higher multiplies, risks should be based on ultrasound markers alone. In the situation where there has been an early fetal loss ("vanishing twin"), the serum markers need to be interpreted cautiously.[69,70]

(h) Quality control and quality assurance.

Laboratories providing maternal serum screening tests must participate in proficiency testing and monitor their performance through epidemiologic monitoring. Computer programs used in calculating risk should be checked for design accuracy.

NT measurement should be performed in centers with experience and demonstrated proficiency. Ultrasonographers performing NT ultrasound must participate in an on-going audit of performance. Use of ultrasound needs to be consistent with fetal safety recommendations; i.e. with an ultrasound exposure that is as low as reasonably achievable.[71]

SCREENING PROTOCOL RECOMMENDATIONS

Recommendations are based on our assessment of the current state-of-the-art of the various technologies, best practices for overall prenatal healthcare, and optimal use of resources. It is recognized that areas of testing are rapidly changing with respect to the range of chromosome abnormalities detectable, the demonstrated applicability to additional groups of women, and the costs of testing. As these developments evolve, new protocols or the inclusion of more women in contingent steps of certain protocols may be appropriate.

There may also be limitations in the availability of reproductive genetic services, including but not limited to proficient sonographers, certified genetic counselors and physicians or requisite computer programs used to calculate risks. Early pregnancy referral patterns and economic considerations are also likely to result in geographic differences in the protocols used. Currently, cfDNA screening is relatively expensive and may not be easily accessed in some countries. The choice of protocol also must take into consideration the need to screen for open neural tube defects either through second trimester AFP or second trimester ultrasound.

Individual women perceive risk differently, may prefer particular approaches, or may choose to personally finance their testing. Patient requests for testing that fall outside recommendations should not be the sole basis for the denial of testing.

The following protocol options are currently considered appropriate:

1. cfDNA screening as a primary test offered to all pregnant women.
2. cfDNA secondary to a high risk assessment based on serum and ultrasound screening protocols (options 4-9 below).
3. cf-DNA contingently offered to a broader group of women ascertained as having high or intermediate risks by conventional screening. Contingent provision of cfDNA, could also include a protocol in which women with very high risks are offered invasive prenatal diagnosis while those with intermediate risk are offered cfDNA.
4. Ultrasound nuchal translucency at 11-13 completed weeks[1] combined with serum markers at 9-13 weeks' gestation.
5. Extending option (4) to include other first trimester serum or sonographic markers. Ultrasound performance needs to be prospectively validated by the center where the screening is performed.
6. A contingent test whereby women with borderline risks from option (4) have option (5) at a specialist center and risk is subsequently modified.
7. Four maternal serum markers (quadruple test) at 15-19 weeks, for women who first attend after 13 weeks 6 days gestation.
8. Combining options (4) and (7) in either a stepwise or contingent protocol - provided that all screening test data are included in the final risk assessment. Integrated screening can be offered when CVS is not available. A serum integrated test when NT measurement is unavailable.
9. Contingent second trimester ultrasound to modify risks for aneuploidy for women having options (4), (7) or (8). Ultrasound performance must be prospectively validated by the center where the screening is performed.

Except in exceptional circumstances, the following are not recommended:

1. The use of maternal age as a sole criterion for aneuploidy risk assessment.
2. First trimester measurement of NT with no additional tests.

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[1] Completed weeks (e.g. 10=10 weeks 0 days to 10 weeks 6 days).

3. Conventional screening tests for chromosome abnormalities following successful and unambiguous cfDNA screening.

Exceptional circumstances could include situations where tests are not applicable (e.g. triplets and higher pregnancy multiples, co-existing additional fetal or maternal conditions), test failures, and the need for urgent risk assessment.

SUMMARY

I. High sensitivities and specificities are potentially achievable with cfDNA screening for some fetal aneuploidies, notably trisomy 21.
II. Definitive diagnosis of Down syndrome and other fetal chromosome abnormalities can only be achieved through testing on cells obtained by amniocentesis or CVS.
III. The use of maternal age alone to assess fetal Down syndrome risk in pregnant women is not recommended.
IV. A combination of ultrasound NT measurement and maternal serum markers in the first trimester should be available to women who want an early risk assessment and for whom cfDNA screening cannot be provided.
V. A four-marker serum test should be available to women who first attend for their prenatal care after 13 weeks 6 days of pregnancy and where cfDNA screening cannot be provided.
VI. Protocols that combine first trimester and second trimester conventional markers are valid.
VII. Second trimester ultrasound can be a useful adjunct to conventional aneuploidy screening protocols.
VIII. When cfDNA screening is extended to microdeletion and microduplication syndromes or rare trisomies the testing should be limited to clinically significant disorders or well-defined severe conditions. There should be defined estimates for the detection rates, false-positive rates, and information about the clinical significance of a positive test for each disorder being screened.

CONFLICTS OF INTEREST S

P. Benn is a consultant to Natera Inc. A. Borrell's hospital received payments from Sequenom for samples sent for a collaborative study in the years 2009-11. R Chiu has received research funding from, consults for, and has equity in Sequenom. She has equity in and is on the board of Xcelom. Patent portfolios of The Chinese University of Hong Kong on noninvasive prenatal testing have been licensed to Illumina, Sequenom and Xcelom. She has received travel support and research funding from Roche. H. Cuckle is a consultant to PerkinElmer Inc., Ariosa Diagnostics Inc., and Natera Inc., a director of Genome Ltd and holds International Patent number PCT/GB88/00557 on "The use of oestriol, progesterone, 16alpha-hydroxy-DHEAS and DHEAS in screening for Down's syndrome." L Dugoff has no current conflicts of interest. In the past she has received institutional research support from PerkinElmer Inc. B. Faas has no conflicts of interest. S. Gross is an employee of Natera, Inc. In the past she has received institutional research support from PerkinElmer Inc. M. Norton is a principal investigator in clinical trial NCT0145167, sponsored by Ariosa Diagnostics Inc, and has received research support from Natera. R Maymon has no conflicts of interest. A Odebo has been a consultant for Ariosa Diagnostics. P Schielen has no conflicts of interest. K. Spencer is a consultant for Brahms GmbH and his institution receives research funding from PerkinElmer Inc. D Wright

has received research support from Public Health England, the RAPID cfDNA study, and the Fetal Medicine Foundation. Y. Yaron is a consultant for Teva Pharmaceuticals, distributor of Illumina's Verifi test in Israel and a consultant for FugeneGenetics, distributor of Ariosa's Harmony in Israel.

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Table 1. Clinical trials of cfDNA screening for fetal trisomy 21, 18, 13 and monosomy X.

Study	**Method**	**Trisomy 21**		**Trisomy 18**		**Trisomy 13**		**Monosomy X**	
		DR (%)	**FPR (%)**	**DR (%)**	**FPR (%)**	**DR (%)**	**FPR (%)**	**DR (%)**	**FPR (%)**
Chiu *et al* (2011)[7]	s-MPS	86/86 (100)	3/146 (2.1)						
Ehrich *et al* (2011)[8]	s-MPS	39/39 (100)	1/410 (0.2)						
Palomaki *et al* (2011, 2012)[9,10]	s-MPS	209/212 (98.6)	3/1471 (0.2)	59/59 (100)	5/1688 (0.3)	11/12 (91.7)	16/1688 (0.9)		
Bianchi *et al* (2012)(a)[11]	s-MPS	89/90 (98.9)	0/410 (0)	35/38 (92.1)	0/463 (0)	11/16 (68.8)	0/485 (0)	15/20 (75)	1/462 (0.2)
Liang *et al* (2013)[12]	s-MPS	40/40 (100)	0/372 (0)	14/14 (100)	0/398 (0)	4/4 (100)	1/408 (0.2)	5/5 (100)	1/407 (0.2)
Song *et al* (2013)[13]	s-MPS	8/8 (100)	0/1733 (0)	2/2 (100)	1/1739 (0.1)	1/1 (100)	0/1740 (0)	2/3 (66.7)	0/1737 (0)
Mazloom *et al* (2013)[14]	s-MPS							17/18 (94.4)	11/393 (2.8)
Stumm *et al* (2014)[15]	s-MPS	40/41 (97.6)	0/430 (0)	8/8 (100)	1/463 (0.2)	5/5 (100)	0/466 (0)		
Porreco *et al* (2014)[16]	s-MPS	137/137(100)	3/3185 (0.1)	36/39 (92.3)	0/3283 (0)	14/16 (87.5)	0/3306 (0)	9/9 (100)	11/3269 (0.3)
Bianchi *et al* (2014)[17]	s-MPS	5/5 (100)	6/1904 (0.3)	2/2 (100)	3/1903 (0.2)	1/1 (100)	3/1913 (0.2)		
TOTAL	**s-MPS**	**653/658 (99.2)**	**16/10061 (0.2)**	**156/162 (96.3)**	**10/9937 (0.1)**	**47/56 (83.9)**	**20/10006 (0.2)**	**48/55 (87.3)**	**14/6268 (0.2)**
Ashoor *et al* (2012; 2013)[18,21]	t-MPS	50/50 (100)	0/297 (0)	49/50 (98.0)	0/297 (0)	8/10 (80.0)	1/1939 (0.1)		
Norton *et al* (2012)[19]	t-MPS	81/81 (100)	1/2887 (0.1)	37/38 (97.4)	2/2888 (0.1)				
Nicolaides *et al* (2012, 2014)[20,23]	t-MPS	8/8 (100)	0/1941 (0)	2/2 (100)	2/1947 (0.1)			43/47 (91.5)	0/125 (0)
Verweij *et al* (2013)[22]	t-MPS	17/18 (94.4)	0/486 (0)						
Norton *et al* (2015)[24]	t-MPS	38/38 (100)	9/15803 (0.1)	9/10 (90.0)	1/15831 (0.0)	2/2 (100)	2/11183 (0.0)		

TOTAL	**t-MPS**	**194/195 (99.5)**	**10/21415 (0.0)**	**97/100 (97.0)**	**5/20963 (0.0)**	**10/12 (83.3)**	**3/13122 (0.0)**	**43/47 (91.5)**	**0/125 (0)**
Nicolaides *et al* (2013)[25]	SNP	25/25 (100)	0/204 (0)	3/3 (100)	0/226 (0)	1/1 (100)	0/228 (0)	2/2 (100)	0/227 (0)
Pergament *et al* (2014)[26]	SNP	58/58 (100)	0/905 (0)	24/25 (96.0)	1/939 (0.1)	12/12 (100)	0/953 (0)	9/10 (90.0)	1/955 (0.1)
TOTAL	**SNP**	**83/83 (100)**	**0/1109 (0)**	**27/28 (96.4)**	**1/1165 (0.1)**	**13/13 (100)**	**0/1181 (0)**	**11/12 (91.7%)**	**1/1182 (0.1)**
TOTAL	**ALL**	**930/936 (99.4)**	**26/32585 (0.16)**	**280/290 (96.6)**	**16/32065 (0.05)**	**70/81 (86.4)**	**23/24309 (0.09)**	**102/114 (89.5)**	**15/7575 (0.20)**

Retrospective trials only; prospective trials were excluded due to incomplete ascertainment and viability bias.

(a) Unclassified cases counted as negative

Table 2. Model predicted Down syndrome detection rate for a 3% false-positive rate and positive predictive value for various screening protocols

	Protocol (completed weeks*)	DR	OAPR 1:n
1a	PAPPA+freeβ (10), NT (12)	82%	29
1b	PAPPA+hCG (10), NT (12)	80%	29
1c	PAPPA+freeβ (12), NT (12)	80%	29
1d	PAPPA+hCG (12), NT (12)	79%	30
1d	PAPPA+freeβ+PlGF+AFP(12), NT (12)	85%	27
1e	PAPPA+hCG+PlGF+AFP(12), NT (12)	83%	27
2a	AFP+freeβ+uE_3+InhA (15-19)	64%	36
2b	AFP+hCG+uE_3+ InhA (15-19)	60%	39
3a	PAPPA+freeβ (10), NT (12), contingent AFP+freeβ+uE_3+InhA (15-19)	90%	26
3b	PAPPA+hCG (10), NT (12), contingent AFP+hCG+uE_3+InhA (15-21)	88%	27
3c	PAPPA+freeβ (10), NT (12), stepwise AFP+freeβ+uE_3+InhA (15-21)	92%	25
3d	PAPPA+hCG (10), NT (12), stepwise AFP+hCG+uE_3+InhA (15-21)	91%	26
4a	PAPPA (10), NT (12), AFP+freeβ+uE_3+InhA (15-19)	91%	26
4b	PAPPA (10), NT (12), AFP+hCG+uE_3+InhA (15-19)	89%	26
4c	PAPPA+freeβ (10), NT (12), AFP+freeβ+uE_3+InhA (15-19)	93%	25
4d	PAPPA+hCG (10), NT (12), AFP+hCG+uE_3+InhA (15-19)	91%	26
4e	PAPPA+freeβ (10), AFP+freeβ+uE_3+InhA (15-19)	80%	29
4f	PAPPA+hCG (10), AFP+hCG+uE_3+InhA (15-19)	75%	33
5a	PAPPA+freeβ (10), NT+NB (12)	91%	26
5b	PAPPA+freeβ (10), NT (12), contingent NB	89%	26
5c	PAPPA+freeβ (10), NT (12), contingent TR	88%	27
5d	PAPPA+freeβ (10), NT (12), contingent DV	88%	27
6a	AFP+freeβ+uE_3+InhA+NF+NBL+PT (15-19)	90%	26
6b	AFP+hCG+uE_3+InhA+NF+NBL+PT (15-19)	89%	27
7a	PAPPA+freeβ (10), NT (12), ANOMALY (18+)	88%	27
7b	PAPPA+hCG (10), NT (12), ANOMALY (18+)	86%	27
8a	ANOMALY (18+)	56%	41
8b	AFP+freeβ+uE_3+InhA (15-19), ANOMALY (18+)	80%	29
8c	AFP+freeβ+uE_3+InhA (15-19), contingent ANOMALY (18+)	77%	30

9a	PAPPA+freeβ (10), NT (12), AFP+freeβ+uE_3+InhA (15-19), ANOMALY (18+)	96%	25
9b	PAPPA+hCG (10), NT (12), AFP+hCG+uE_3+InhA (15-19), ANOMALY (18+)	95%	25

The rates specified are for the purposes of comparison of protocols and do not necessarily indicate optimal cut-offs. NT=nuchal translucency, NB=nasal bone absence, TR=tricuspid regurgitation, DV=ductus venosus, NF=nuchal skinfold, NBL=nasal bone length, PT=prenasal thickness, contingent=1 in 50-1500 borderline risks (at term, equivalent to 1 in 38-1200 at mid-trimester), stepwise=borderline or lower risks, ANOMALY=major malformation, large NF, short femur, echogenic intracardiac focus, pyelectasis, echogenic bowel and ventriculomegaly, completed weeks, e.g. 10=10 weeks 0 days to 10 weeks 6 days (see recommendations for optimal times to provide tests). Predicted performance is based on published statistical parameters for NT PAPP-A, freeβ and hCG[57] NB,[58] TCR and DV,[59] NF, NBL and PT,[60] ANOMALY,[61] meta-analysis for PlGF and AFP based on publications cited in 62 and a standardized maternal age distribution.[63] Rates are based on prevalence at birth.