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REVIEW ARTICLE

Noninvasive Prenatal Testing for Fetal Aneuploidy

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ABSTRACT

After decades of research with a wide range of putative methodologies, at last a commercially viable technique has emerged for the noninvasive prenatal testing (NIPT) for the most common fetal aneuploidies, the massively parallel shotgun sequencing (MPSS). Recently, a number of groups have validated this technology to accurately detect most common trisomies as early as the 10th week of pregnancy with results available 1 to 2 weeks after maternal sampling. Several molecular techniques have been proposed for the detection of trisomies 21, 18 and 13, mainly by two different approaches in analyzing the cell-free fetal (cff) DNA: quantitative and single-nucleotide polymorphism (SNP)-based methods. Among them and to address some of the limitations of counting techniques, a new method called NATUS algorithm (Next-generation Aneuploidy Testing Using SNPs) has been recently introduced. This approach, as a targeted and noncounting technique, offers numerous advantages, although more evidence is needed from large prospective studies. Published studies have demonstrated that diagnostic parameters of NIPT are better than those of the current first trimester prenatal screening risk assessment for fetal trisomy 21. NIPT of trisomy 21 by MPS with or without preselection of chromosomes is promising and likely to replace the prenatal serum screening test that is currently combined with nuchal translucency measurement in the first trimester of pregnancy. However, before NIPT can be introduced as a screening test, more evidence is needed from large prospective diagnostic accuracy studies in first trimester pregnancies.

Keywords: Noninvasive prenatal testing, Cell-free fetal DNA, Down syndrome, First-trimester screening.

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INTRODUCTION

In developed countries, the two-step prenatal care system includes a noninvasive risk assessment for the most common aneuploidies, before invasive prenatal procedures are offered. At the moment, the most used noninvasive screening test for individual trisomy 21 (T21) risk calculation worldwide is the combined test, which combines maternal age, serum screening (pregnancy-associated plasma protein-A and free beta-human chorionic gonadotropin) and nuchal translucency measurement. However, the combined test is considered safe but has a poor accuracy, with a false-negative rates between 12 and 23% and false-positive rates between 1.9 and 5.2%.[1,2] Only about 5% of the 'high-risk' pregnant women indeed carry a fetus with T21 while the remaining are normal, giving a positive predictive value of about 1 in 20. In case of a priori high risk or a positive individual assessment of T21, invasive prenatal diagnosis by fetal karyotyping or rapid aneuploidy detection is performed. These invasive procedures carry a risk of miscarriage ranging from 0.6% (within 14 days of the procedure) to 2% (for total pregnancy loss).[3] To overcome the low accuracy of the current strategies and to avert this risk of miscarriage of invasive procedures, there is a continuous drive to search for a diagnostic test without risk of miscarriage or a screening test with a better performance. That is the reason why there has been an increasing demand for a reliable and safe noninvasive prenatal test that is applicable as early in pregnancy as possible.

Since, the discovery of the presence of cell-free fetal (cff) DNA and cff placental-specific mRNA in maternal plasma, the possibility of using this as the target for noninvasive prenatal testing (NIPT) of fetal genetic conditions is being widely explored.[4-6] Decades of research on noninvasive DNA-based prenatal testing are finally reaching fruition. A variety of strategies have been explored, basically through two different approaches in analyzing the cffDNA: quantitative and single-nucleotide polymorphism (SNP)-based methods. At the moment, commercial NIPT of T21 by quantitative techniques has become available for high-risk pregnant women and includes the MaterniT21PLUS™ test from Sequenom (http://www.sequenomcmm.com), the PrenaTest® from their European partner LifeCodexx (http://www.lifecodexx.com), the Verifi® prenatal test from Verinata (http://www.verinata.com) and the Harmony™ prenatal test from Ariosa (http://www.ariosadx.com). In another line, commercial NIPT by noncounting targeted techniques is offered by Natera through the Panorama test™ (http://www.panoramatest.com/natera_news). In the short term, these tests offer a more accurate alternative or adjunct to serum or combined screens. Currently, NIPT that uses cffDNA from the plasma of pregnant women offers tremendous potential as a screening tool for fetal aneuploidy.

TECHNOLOGY

Initial research efforts targeted the isolation and subsequent analysis of circulating fetal cells from maternal blood. Given

the low ratio between circulating fetal and maternal cells, these approaches struggled to reliable detect and isolate fetal cells and have largely been unsuccessful.[7-9] More recent efforts focused on analyzing cffDNA in maternal plasma. Circulating cffDNA, which comprises approximately 3 to 13% of the total cell free maternal DNA, is thought to be derived primarily from the placenta, and is cleared from the maternal blood within hours after childbirth.[4]

After decades of research with a wide range of putative methodologies, at last a commercially viable technique has emerged for the NIPT for the most common fetal aneuploidies. The technique in question is massively parallel shotgun sequencing (MPSS). Recently, a number of groups have validated this technology to accurately detect most common trisomies as early as the 10th week of pregnancy with results available 1 to 2 weeks after maternal sampling.[10-18] MPSS detects higher relative amounts of DNA in maternal plasma from the trisomic chromosome compared with reference chromosomes: a slightly higher than expected percentage of chromosome 21 fragments indicates that the fetus has a third chromosome 21. The MPSS method shows good accuracy for detecting T21 and trisomy 18 (T18) given sufficient cffDNA levels; however, detection of trisomy 13 (T13) and sex chromosome abnormalities is more limited because some chromosomes are represented in sequencing data with high variability.[10,14-20] Detecting T18 and T13 by sequencing was proved to be more difficult than detecting T21 because the measurement coefficient of variances for chromosome 18 and 13 were much larger than that for chromosome 21. This limits the scope of chromosome abnormalities that can be accurately detected with these purely quantitative methods.[10,19-21] This limitation is exacerbated in samples drawn in the first trimester, as they tend to have lower cffDNA fraction in maternal plasma. However, when adjusted with GC content, it was documented that T18 and T13 can be detected effectively and accurately.[17]

Recently, two massively parallel sequencing (MPS) techniques, in which only the chromosomes of clinical interest are sequenced, have been described. In these targeted MPS techniques, preselection of chromosomes leads to less unutilized sequencing data and a significant increase in sequencing efficiency. Moreover, rapid next-generation sequencing devices can be used, altering the costs, turnaround time and the number of patients who can be tested per week. A summary of the main methodologies used in NIPT of main trisomies is shown in Figure 1.

One of these techniques is digital analysis of selected regions (DANSR), in which selected nonpolymorphic loci on chromosomes of clinical interest are simultaneously quantified.[22-26] However, as with all purely quantitative methods, the approach depends on low chromosomal amplification variation between target and reference chromosomes, thus limiting its diagnostic accuracy for some chromosomes. Liao et al recently described a method that selectively sequences SNPs and determines copy number by comparing fetal to maternal SNP ratios between target and reference chromosomes.[27] The use of SNPs may mitigate chromosome-to-chromosome amplification variability; however, the need for a reference chromosome partly negates this advantage.

The other technique is Parental Support™, based on NATUS (Next-generation Aneuploidy Testing Using SNPs) algorithm, in which the observed allele distribution after sequencing of polymorphic loci on the chromosomes of clinical interest is compared with the expected allele distribution based on parental genotypes.[28] This method determines fetal copy number from maternal blood samples at chromosomes 13, 18, 21, X and Y with high accuracy at all chromosomes. This technique uses parental genotypes, data from the HapMap database and the observed number of sequence reads associated with each of the relevant alleles at SNP loci.[29,30] A key novel feature of NATUS algorithm

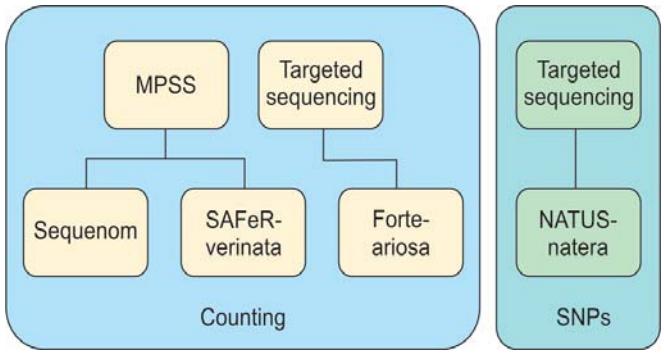

Fig. 1: Summary of NIPT methodologies

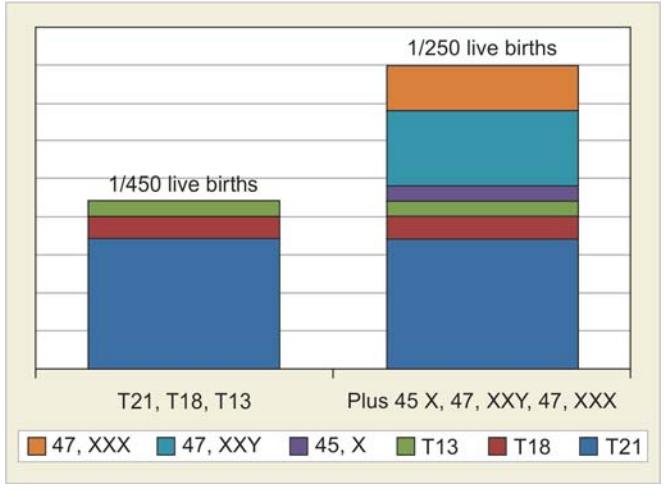

Fig. 2: At-birth prevalence of aneuploidy

is that it calculates a pertest, perchromosome accuracy for each sample, offering clinicians an individualized risk score for each patient. Preliminary results of this methodology have been recently published as a proof-of-principle of this technology.[28] This approach, as a targeted and noncounting method, offers numerous advantages, including greater clinical coverage and sample-specific calculated accuracies.

NATUS Algorithm Technology

NATUS algorithm represents a novel, promising method for prenatal aneuploidy testing. Here, chromosome copy number was determined at chromosomes 13, 18, 21, X and Y with 100% sensitivity and 100% specificity for all samples passing the quality test. The NATUS algorithm method, as a noncounting and targeted method, obviates issues with amplification variation and generates a more powerful sample-specific calculated accuracy for samples with low fetal fractions of cffDNA. Together, this holds promise for the development of a noninvasive screening test with accuracy and scope comparable to current invasive testing.

Advantages of NATUS Algorithm

The NATUS algorithm increases clinical coverage of viable chromosomal abnormalities by approximately 2-fold, with comparable accuracies at each chromosome compared with previously reported methods (Fig. 2).[22,25-27] Whereas most published methods focus only on detecting autosomal trisomies, the combined at-birth prevalence of sex chromosome abnormalities is slightly higher than that of autosomal trisomies, emphasizing the need for methods that detect sex chromosome abnormalities during pregnancy.[31,32]

Differently from traditional models, NATUS calculates a sample-specific accuracy for each chromosome copy number call, a feature that informs which individual calls are highly reliable and which ones may require follow-up. This approach models data distribution associated with both euploid and aneuploid hypotheses to optimize decision thresholds and produce sample-specific accuracy calculations. A benefit of using sample-specific *vs* cohort-based accuracy calculations is illustrated by comparing sensitivity rates for MPSS and NATUS algorithm for aneuploid samples with low fetal fraction. At the similar lower detection limits of MPSS-based and NATUS-based methods,[14] MPSS tends to make incorrect calls on low quality samples, whereas the NATUS method tends to make no-calls. The presumption is that a no call is preferred to a false, negative result, as a no call simply requires a redraw and retest, whereas a miscall can result in lifelong consequences.

Calculating accuracies is particularly beneficial in early gestational age pregnancies. Prenatal testing in early pregnancy is typically preferred as it facilitates earlier decision making; the drawback is typically lower fetal fractions, which correlate with an increased error rate. This is especially acute in single hypothesis rejection-based tests (in quantitative methods, e.g. MPSS and DANSR) that were validated using a cohort with a significantly higher average gestational age.[10,17,18,24,25] NATUS algorithm identifies samples for which incorrect results are likely, for example, due to low amounts or quality of fetal DNA, thus decreasing the chance of false negatives.

NATUS algorithm also offers various other benefits over previous methods. Because it relies on comparing the relative amounts of alleles at a set of loci, it obviates problems with chromosome-to-chromosome amplification variation that generate poor accuracies for chromosomes 13, X and Y in previous methods.[11,19-21] Using allelic data obviates the requirement for a reference chromosome that is presumed to be euploid, and noncounting algorithm is therefore uniquely expected to detect triploidy. Moreover, incorporating parental data allows NATUS algorithm to detect abnormalities that preserve chromosome copy number, such as uniparental disomy.

Lastly, because NATUS informatics maximally utilizes available information in the data set, combining it with high fidelity parental allelic information and HapMap data, it generates more powerful test statistics with narrower distributions, similar to a diagnostic. Indeed, 90.1% of these results return a calculated aneuploidy probability of either $\leq 0.1\%$ or $\geq 99.9\%$.[28]

Taken together, NATUS algorithm is an encouraging, novel method for detecting fetal chromosomal abnormalities noninvasively.

Limitations of NATUS Algorithm

Although this study demonstrates the promise of targeted cffDNA sequence analysis, there are several caveats.

The prevalence of confined placental mosaicism and its impact on prenatal screening is unclear. Assuming that a significant portion of the fetal DNA present in maternal blood is derived from the placenta, the presence of placental mosaicism could undermine the significance of any algorithm-generated accuracies in each sample. Importantly, no method relying on cffDNA found in maternal plasma could overcome this limitation.

Specifically related to the Zimmerman study,[28] the promising results must take into consideration some limitations, as some samples are collected >20 weeks, which do not represent the early stages of pregnancy for which this method is intended; some aneuploid samples were confirmed prior to blood draw using invasive procedures, which increases cffDNA in maternal blood; and importantly,

a relatively high 12.6% no-call rate, typically due to low fetal fraction and poor DNA quality. Note that previous methods report accuracies for calls only on a subset of chromosomes, not on all five (13, 18, 21, X and Y), and usually exclude the sex chromosomes.[14,17,18,22] Interestingly, the NATUS algorithm method enables a fast turnaround time (<1 week), thus it allows for redraws and reanalyses with sufficient time to avoid invasive procedures after a no call.

Future Directions of NATUS Algorithm

In order to validate the preliminary results regarding this promising method for prenatal aneuploidy testing, currently a large-scale clinical trial is underway (NCT01545674).

Moreover, Nicolaides has recently published an externally blinded validation study on this technique in a series of 242 singleton pregnancies, including 32 chromosomal abnormalities.[33] This study shows preliminary data from an improved method involving an increase in the number of PCR assays to 19,500, an increase in reaction concentration, and an updated version of the NATUS algorithm, showing a no-call rate of significantly below 10%, in line with other commercially available tests, without a change in the accuracy. This externally blinded validation study has demonstrated that SNP-based analysis of cfDNA in maternal blood obtained at 11 to 13 weeks' gestation from high-risk singleton pregnancies correctly identified all cases of trisomies 21, 18 and 13, Turner syndrome and triploidy, with no false-positives or negatives and correctly determined the fetal sex in all cases. The test did not provide results in 5.4% of cases. These recent results overcome some of the limitations of the Zimmermann' study previously described,[28] demonstrating that targeted sequencing of SNPs at chromosomes 13, 18, 21, X and Y holds promise for accurate detection of fetal autosomal aneuploidies, sex chromosome aneuploidies and triploidy.

Because NATUS algorithm uses a targeted amplification approach, future efforts could target panels for detection of submicroscopic imbalances (microdeletions/microduplications).[34,35] Additionally, NATUS focuses on polymorphic loci, which allows for parental haplotype reconstruction, and thus detection of fetal inheritance of individual disease-linked loci. This is not possible for quantitative methods that utilize sequence counts of nonpolymorphic loci.

OVERVIEW OF NIPT OF TRISOMY 21

To provide an up-to-date overview of NIPT of T21, an evaluation of the methodological quality and outcomes of diagnostic accuracy studies has been recently published.[36] A total of 16 studies were included and possible bias and applicability was evaluated utilizing the revised tool for Quality Assessment of Diagnostic Accuracy (QUADAS-2). Meta-analysis could not be performed due to the use of six different molecular techniques and different cutoff points. In the majority of the studies, high-risk pregnancies were included, although the inclusion criteria were often not clearly described, and different definitions of 'high risk of T21' were applied. Blood sampling took place throughout pregnancy, including sampling in the third trimester. The diagnostic accuracy of NIPT was compared with karyotyping, although in some studies in combination with another reference standard test. The 16 included studies, dating from 2007 to 2012, applied six different molecular genetic techniques for NIPT of T21 in a high-risk population. Seven of the included studies were recently published in large cohort studies that examined MPSS, with or without preselection of chromosomes, and reported sensitivities between 98.58% [95% confidence interval (CI): 95.9-99.5%] and 100% (95% CI: 96-100%) and specificities between 97.95% (95% CI: 94.1-99.3%) and 100% (95% CI: 99.1-100%).[6,12,14,18,22,24,37] None of these seven large studies had an overall low risk of bias and low concerns regarding applicability. The other nine cohort studies were too small to give precise estimates and were not included in the discussion. MPS with or without preselection of chromosomes exhibits an excellent negative predictive value (100%) in conditions with disease odds from 1:1,500 to 1:200. However, positive predictive values were lower, even in high-risk pregnancies (19.7-100%). The authors conclude that in the future, NIPT by MPS with and without preselection of chromosomes should be further explored, focusing on the inclusion of a consecutive sample early in the first trimester of pregnancy and the incorporation of all samples in the analysis. Large prospective studies will give more certainty about the predictive values in the high-risk group.

PROFESSIONAL SOCIETY STATEMENTS

Professional societies are beginning to make statements about the use of NIPT.

The American College of Obstetricians and Gynecologists Committee on Genetics and the Society for Maternal-Fetal Medicine Publications Committee[38] (Table 1)

NIPT that uses cffDNA from the plasma of pregnant women offers tremendous potential as a screening tool for fetal aneuploidy. The cffDNA testing should be an informed patient choice after pretest counseling and should not be

part of routine prenatal laboratory assessment. The cffDNA testing should not be offered to low-risk women or women with multiple gestations because it has not been sufficiently evaluated in these groups. A negative cffDNA test result does not ensure an unaffected pregnancy. A patient with a positive test result should be referred for genetic counseling and should be offered invasive prenatal diagnosis for confirmation of test results.

The American College of Obstetricians and Gynecologists has recommended that women, regardless of maternal age, be offered prenatal assessment for aneuploidy either by screening or invasive prenatal diagnosis regardless of maternal age; cffDNA is one option that can be used as a primary screening test in women at increased risk of aneuploidy. This includes women aged 35 years or older, fetuses with ultrasonographic findings that indicate an increased risk of aneuploidy, women with a history of a child affected with a trisomy, or a parent carrying a balanced Robertsonian translocation with increased risk of T13 or T21. It also can be used as a follow-up test for women with a positive first-trimester or second-trimester screening test result.

International Society of Prenatal Diagnosis[39,40]

The International Society of Prenatal Diagnosis (ISPD) formulated, on 24 October 2011 in a rapid response statement, its considerations and recommendations for the clinical use of the commercial MPS tests in women at high risk and at lower risk.[39] A final position statement on screening for fetal aneuploidy of ISPD has been recently released.[40] According to this statement, cfDNA screening can be offered to women classified as high risk by any of the other optimal first or second-trimester protocol options. cfDNA screening can also be considered for additional groups of women who did not receive any other screening and who are considered to be high risk on the basis of maternal age; presence of an ultrasound abnormality suggestive of T21, T18 or T13; family history of a chromosome abnormality that could result in full T21, 18 or 13; and history of a previous pregnancy/live birth with T21, T18 or T13. Local economic considerations and access to sonography, invasive testing and counseling resources should be considered when deciding on the use of NIPT-MPS in additional groups of women.

National Society of Genetic Counsellors[41]

National Society of Genetic Counsellors (NSGC) supports NIPT as an option for patients whose pregnancies are considered to be increased risk for certain chromosome abnormalities. NSGC urges that NIPT only be offered in the context of informed consent, education, and counseling by a qualified provider, such as a certified genetic counsellor. Patients whose NIPT results are abnormal, or who have other factors suggestive of a chromosome abnormality, should receive genetic counseling and be given the option of standard confirmatory diagnostic testing.

American College of Medical Genetics and Genomics[42]

According to American College of Medical Genetics and Genomics (ACMG), 'NIPS for fetal aneuploidy has arrived; however, as with most new technologies, there is room for refinement. The ACMG encourages providers of NIPT technology to make serious efforts to provide the more clinically relevant metrics. This can be accomplished through a funded registry at which efforts are made to confirm and archive not only true positives, but also false

Table 1: Recommendations of the American College of Obstetricians and Gynecologists Committee on Genetics and the Society for Maternal-Fetal Medicine Publications Committee

- Patients at increased risk of aneuploidy can be offered testing with cffDNA. This technology can be expected to identify approximately 98% of cases of Down syndrome with a false-positive rate of less than 0.5%.
- cffDNA testing should not be part of routine prenatal laboratory assessment, but should be an informed patient choice after pretest counseling.
- cffDNA testing should not be offered to low-risk women or women with multiple gestations because it has not been sufficiently evaluated in these groups.
- Pretest counseling should include a review that although the cffDNA test is not a diagnostic test, it has high sensitivity and specificity. The test will only screen for the common trisomies and, at the present time, gives no other genetic information about the pregnancy.
- A family history should be obtained before the use of this test to determine if the patient should be offered other forms of screening or prenatal diagnosis for familial genetic disease.
- If a fetal structural anomaly is identified on ultrasound examination, invasive prenatal diagnosis should be offered.
- A negative cffDNA test result does not ensure an unaffected pregnancy.
- A patient with a positive test result should be referred for genetic counseling and offered invasive prenatal diagnosis for confirmation of test results.
- cffDNA does not replace the accuracy and diagnostic precision of prenatal diagnosis with chorionic villus sampling or amniocentesis, which remain an option for women.

positives and true negatives. The ethical principle of distributive justice causes us to reflect on who will pay for NIPS and who should be insured for the procedure. No doubt NIPT costs will come down; however, for NIPT to establish roots in the perinatal aneuploidy screening paradigm, cost as a barrier to population-based screening must be minimized. NIPT technology is perhaps only a few steps removed from an eventual whole-genome array, whole-genome sequencing, or whole-exome sequencing of noninvasively isolated cffDNA. Whether this best comes about by simultaneously amplifying maternal sequence and subtracting this from fetal sequence, or after isolation and amplification of fetal sequences unique from maternal, is yet to be resolved.'

Interestingly, on April 4, 2013, both the International Society for Prenatal Diagnosis (ISPD) and the American College of Genetics and Genomics (ACMG) publicly made available their respective organization's policy statements on the newest form of prenatal testing for Down syndrome.[40,42]

A New Name

What had been referred to as NIPT for 'noninvasive prenatal testing' has been formally renamed. ISPD refers to the new testing offered by US companies like Sequenom, Ariosa, Verinata, and Natera as 'Maternal cfDNA', meaning cfDNA found in the mother's blood stream. Note the removal of 'fetal' from the traditional acronym of cffDNA. This is because most of the DNA tested is not, in fact, from the fetus. ACMG makes this point, stating that the tested material 'is derived from the placenta'. ACMG provides the clearer acronym, referring to the new testing as NIPS for 'noninvasive prenatal screening', emphasizing that the new testing remains a screening test, not a diagnostic test.

Emphasis on Counseling

Both ACMG and ISPD emphasize the need for pretest and post-test counseling. In both of the new policy statements is an emphasis on the need to provide pretest counseling on the limitations of NIPS and post-test counseling on the need for confirmation of NIPS results through diagnostic testing.

A Call for Quality Control

Both organizations call for quality control and adherence to a set of laboratory standards. The ISPD is particularly notable in both its message and its messengers on this issue. From the ISPD position statement: 'Although rapid progress is being made in the development and validation of this technology, demonstration that in actual clinical practice the testing is sufficiently accurate, has low failure rates and can be provided in a timely fashion has not yet been provided.'

Recognized Resources for Patients

The ACMG statement is further noteworthy in providing recognized resources of accurate information about Down syndrome that should be provided to patients.

FUTURE DIRECTIONS

Low-risk Population

The sensitivity and specificity of NIPT were recently examined in a population of low-risk pregnant women and in a mixed population.[23,37] Mersy' study demonstrates that the diagnostic parameters of NIPT are better than those of the current first trimester prenatal screening risk assessment for fetal T21.[36] Therefore, NIPT is likely to replace the prenatal serum screening test that is currently combined with nuchal translucency measurement in the first trimester of pregnancy. In that case, less invasive procedures would be needed, only to verify a positive NIPT result and to confirm noninheritable or inheritable forms of Down syndrome, using the gold standard that is still karyotyping. However, there is still more evidence needed before NIPT of T21 can be introduced in routine prenatal care. Preferably, large prospective diagnostic accuracy studies, including low-risk pregnant women recruited in a clinical setting early in pregnancy will have to be performed before this will become a reality in the public, social insurance health care systems. One study included examined diagnostic accuracy in low-risk pregnant women, demonstrating that the performance of NIPT in a routine population is as effective as previously reported in high-risk groups, with a detection rate higher than 99% and a false-positive rate lower than 1%.[23] However, a 5 to 10 times larger sample size than the 1,949 samples analyzed in this study is needed for a reliable estimation of the sensitivity in *a priori* low-risk first trimester pregnancies. Additional accuracy studies are currently designed and ongoing. Moreover, NIPT should be provided in a cost-effective, timely and equitable manner. Finally, further ethical exploration and evaluation of the current opinion of pregnant women and the formulation of proper informed consent information are needed.

Spectrum of Chromosomal Condition Detected

The testing for T21 and T18 is the most clinically relevant because other numerical autosomal chromosome aberrations are rare after 12 weeks. However, other than main autosomal

trisomies, current data demonstrate the potential of expanding the scope of screening to include sex chromosome aneuploidies and triploidy, particularly in the noncounting targeted method. With increasing sequencing depth, MPS test has the potential to detect more fetal chromosomal abnormalities. In principle, MPS-based methods could detect all kinds of fetal aneuploidy and microdeletions/microduplications. Additionally, noncounting targeted method focused on polymorphic loci allows for parental haplotype reconstruction, and thus detection of fetal inheritance of individual disease-linked loci. On the other hand, the detection of other chromosomal abnormalities such as mosaic, structural variations or even single-gene disorders still remains challenging for this new MPS-based test.

Ethical Considerations

With the revolutionary progress of noninvasive testing technology, new ethical questions are emerging.[43] One of the most difficult issues relates to the fact that such new approaches can count sex chromosomes and therefore contain information of fetal gender. Would the availability of fetal sex information result in sex selection behavior? Because part of sex chromosomal abnormalities are not associated with major disabilities, should a woman be informed if there is a suspicion of sex chromosomal abnormalities? In this sense, the views are quite contradictory. The ability to detect fetal sex prenatally may include some risks, especially in certain geographic areas.[37] For those pregnancies at risk for X-linked disorders, knowing sex can remove risks and worry if fetus is female and, moreover, allow decisions about prenatal diagnosis if fetus is male. It can also inform maternal medication use when known to affect certain genders *in utero*. Additionally, it can assist in identifying intersex conditions. Moreover, the combined at-birth prevalence of sex chromosome abnormalities is slightly higher than of autosomal trisomies,[31,32] emphasizing the need for methods that detect sex chromosome abnormalities during pregnancy (Fig. 2). Undoubtedly; the prenatal detection of certain sexual abnormalities, especially Turner syndrome, is clearly beneficial. Thus, the key concern is what information could or should be provided to pregnant women. As for sex chromosomal abnormalities, it may be time to consider a more comprehensive informed consent process to allow pregnant women to make well-informed decisions on requesting this additional information or not.

Costs

MPS-based test as a screening method is undoubtedly more efficient than any other existing noninvasive screening tests for fetal T21 and T18. Although widespread use of this technology is currently limited by the cost and reporting time at the moment, the situation probably will change quickly. The cost of MPS-based noninvasive test varies between countries, even states. In the United States, this MPS-based test is charged $795 to $2762, while it takes around $500 to $1000 in China. The cost will decrease with increasing numbers, advancing technology and more efficient methods, such as targeting testing. With the rapid development of high throughput sequencing technology, the cost of this test will drop to levels probably lower than that of conventional invasive diagnosis procedures in the near future.

New Technology

The technology applied to this field progresses rapidly. New platforms are being tested. For example, platforms based on proteomics using mass spectrometry techniques is gaining considerable acceptance for the identification of different markers associated with fetal chromosome abnormalities. The current practice of using single protein biomarkers will most likely give way to the use of multiplexed biomarkers, as they promise better sensibility and specificity.[44] On the other side, Liang recently has demonstrated that, with appropriate GC correction algorithm, detecting aneuploidies of all the 24 chromosomes in one single sequencing event is achievable.[45] These are only two examples of new technologies, but there is worldwide emerging continuously a large number of new methodologies in this field. More trials using these new approaches need to be performed to ascertain the efficacy of these new platforms.

New Indications

Preliminary studies suggest that NIPT seems to work well in twin pregnancies.[46,47] Although the results are very preliminary, possibly technology allows twin implementation in the near future.

New Prenatal Clinical Strategies

It is clear that this new technology will radically change the current clinical model of prenatal screening and diagnosis in the near future. However, the proper application of the MPS-based test in clinical settings needs careful consideration to integrate with established obstetric practice and workflow, and the right of informed consent and choice of pregnant women should be fully respected in this process.

The extent to which cfDNA testing could be applied as a universal screening tool for aneuploidies in all pregnant

women would depend on whether access to this technique becomes comparable with that of current methods of sonographic and biochemical testing. In the meantime, cfDNA testing would be useful as a secondary test contingent on the results of a more universally applicable primary method of screening. In such cases, the use of cfDNA testing would considerably reduce the number of unnecessary invasive tests and eliminate their associated risk of causing miscarriage. Another population that may benefit from screening by maternal plasma cfDNA is the one identified by the combined test as being at intermediate risk for autosomal trisomies, because in these cases, their risk will be revised to either very high or very low, thereby making their decision in favour or against invasive testing easier.[33]

CONCLUSION

- Finally, after decades of research, noninvasive prenatal trisomy testing is now a clinical reality. The technique which makes it possible is MPSS. Currently, research on NIPT of fetal T21 is developing tremendously fast and several commercial tests have become worldwide available.
- Currently, NIPT that uses cffDNA from the plasma of pregnant women offers tremendous potential as a screening tool for fetal aneuploidy. The diagnostic parameters of NIPT of T21 are definitively better than those of the current first trimester assessment. Nevertheless, considering the limited size and quality of the currents published studies, additional large prospective studies will allow more precise estimates about sensitivity, specificity and predictive values in high-risk and low-risk pregnancies.
- Several molecular techniques have been proposed for the detection of trisomies 21, 18 and 13. Regarding the counting techniques, although the sensitivity and specificity of these assays are considerably improved over serum screens and ultrasound, they currently do not achieve the same scope and accuracy as amniocentesis or chorionic villus sampling. Regarding to the object of interest, targeted techniques, in which only the chromosomes of clinical interest are sequenced, significantly increase sequencing efficiency. To address some of the limitations of counting techniques, a new method called NATUS algorithm has been recently introduced. This approach, as a targeted and noncounting technique, offers numerous advantages, although more evidence is needed from large prospective studies.
- NIPT of T21 by MPS with or without preselection of chromosomes is promising and likely to replace the prenatal serum screening test that is currently combined with nuchal translucency measurement in the first trimester of pregnancy. Before NIPT can be introduced as a screening test in a social insurance health care system, more evidence is needed from large prospective diagnostic accuracy studies in first trimester pregnancies. Moreover, NIPT should be provided in a cost-effective, timely and equitable manner. Finally, further ethical exploration and evaluation of the current opinion of pregnant women and the formulation of proper informed consent information are needed.

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