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REVIEW ARTICLE

Current Concepts in Noninvasive Prenatal Screening (NIPS)

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Abstract Noninvasive prenatal screening (NIPS) represents a significant advance in prenatal screening for trisomy 21 and other conditions. Like any new and rapidly developing technology, it is important for healthcare providers to keep up to date with current and developing issues to help ensure that users of tests such as NIPS are well informed. This review intends to outline and explain some of the main current issues with regards to NIPS and to look ahead to the future, in order to increase understanding and inform debate.

Keywords Noninvasive prenatal screening · SNP · Fetal fraction · Expanded screening

Introduction

Noninvasive prenatal screening (NIPS), originally designed as a more accurate screening test for trisomy 21, 13, and 18, has now been available for several years. One of the major advantages of NIPS its strong positive predictive value (PPV) as regards trisomy 21, the most common chromosomal aneuploidy with a live birth prevalence of 14.2 per 10,000 [1]. In the absence of screening, prevalence at 20 weeks has been estimated at between 13 per 10,000 and 35 per 10,000 [2]. Traditional screening using a combined first-trimester approach gives a PPV of less than 4% [3], meaning that more than 96% of women given high-risk results for trisomy 21 will have an unaffected baby. Reducing the number of false positives reduces the anxieties associated with high-risk results and the need for invasive procedures such as amniocentesis and the attendant risks and anxieties. NIPS, besides having a specificity of 99.9%, also has a superior detection rate to combined first-trimester screening, so that over 99% of pregnancies affected by trisomy 21 can be identified as compared to the 78.9% detected by combined first-trimester screening [3, 4].

Since the first large-scale clinical evaluations of NIPS in 2011 [5], there have been innovations and additions aimed at increasing the scope of the test and improving the methods by which it is performed. Although new tests join the market regularly, they utilize a similar method of assessing the risk for chromosome abnormalities, commonly referred to as "counting". Only one completely new method has been made clinically available to date, which analyzes single nucleotide polymorphisms (SNPs) to assess the risk for aneuploidy. In addition to assessing the likelihood of the presence of whole chromosome aneuploidies, a number of tests claim to be able to detect a broad range of microscopic and submicroscopic deletions and duplications [6–8]. It is likely that this effort to obtain further information through prenatal screening will continue. Future goals are likely to include screening for specific single-gene disorders and even whole genome sequencing. Such expanded forms of testing bring not only the promise of detailed information but also provide technical and ethical challenges which need to be addressed.

In these times of rapid advances in the field of prenatal genetics, it is important for healthcare providers to keep up to date with current and developing issues to help ensure that users of tests such as NIPS are well informed. This review aims to outline and explain some of the main issues with regards to NIPS and to look ahead to the future, in order to increase understanding and inform debate.

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Will NIPS Ever Be Considered a Diagnostic Test?

The high sensitivity and specificity of NIPS has incorrectly led some providers to believe that NIPS tests are diagnostic or 'virtually' diagnostic. This is *not* a correct assumption. One important reason for this is that the cell-free DNA analyzed by NIPS originates from the placenta, predominantly the cytotrophoblast [9]. In most pregnancies the chromosomal make-up of the placenta is identical to that of the fetus. However, in a small proportion of cases a mutation will have occurred after the point at which the cells destined to become the fetus have separated from the cells destined to become the placenta [10]. When this form of discordance occurs, it is termed 'confined placental mosaicism' or 'confined fetal mosaicism' depending on the location of the mosaic cells. Chorionic villus sampling (CVS) also analyzes the placenta as a proxy for the fetus, and yet is considered diagnostic, which can cause confusion. However, CVS analysis usually assesses two different cell layers from the placenta (cytotrophoblast and mesenchyme), increasing the opportunity to detect mosaicism. It has been established that concordance rates between the fetus and placental cells analyzed after cell culture (mesenchymal cells) are much higher than those obtained after direct prep (cytotrophoblast) analysis alone, and that irreversible decisions should not be made based upon evaluation of cytotrophoblast alone [11]. Abnormal NIPS results, similarly, require confirmation before interruption of pregnancy is considered.

Other reasons for discordant results from NIPS include the presence of cell-free DNA from a vanished twin, or karyotype anomalies in the mother which are assumed to represent abnormalities in the fetus [12]. These sources of false positive results are more likely when a counting methodology is used, rather than a SNP-based approach (detailed below).

Are All NIPS Tests the Same?

Broadly speaking, two major approaches to NIPS have been developed. The first method which became clinically available in 2011, can be termed "counting". This method assesses the total amount of cell free DNA (cfDNA) that is found in a maternal plasma sample, and compares the amounts originating from the chromosomes of interest with those originating from a reference chromosome. The observed ratio of material between these chromosomes is compared to the expected ratio, and if a greater amount of material than expected is found originating from a chromosome of interest, this is assumed to be fetal in origin and a "high-risk" or "positive" call would be made. This method is generally effective for the detection of whole chromosome trisomies but the methodology has some limitations. Because the counting methodology does not distinguish between placental and maternal DNA, it cannot recognize a number of situations which may confound the results. The first of these potential confounders is vanishing twins. Vanishing twins occur in 27–41% of pregnancies where two sacs have been identified on ultrasound [13]. The DNA from vanishing twins can persist for at least 8 weeks after the demise of the twin [14], and can lead to incorrect results, as persisting cfDNA from the vanished twin is counted along with that from the ongoing pregnancy. Secondly, maternal chromosomal abnormalities can confound the results. A study of discordant high risk sex chromosome abnormality calls made using a counting NIPS test demonstrated that 8.6% of them were in fact due to an abnormal maternal karyotype [15]. It is recognized that a number of women undergo loss of an X-chromosome in a certain proportion of cells with age [16]. The resulting maternal mosaicism cannot be detected by a test that counts all cfDNA and does not distinguish between the sources. Finally, counting methods are unable to detect triploidy, as they rely on seeking a difference in ratios between the chromosome of interest and a reference chromosome. If three copies of all chromosomes are present, as in triploidy, there is no ratio change and a false-negative call is likely.

The more recently developed method of performing NIPS, commercially available since 2013, uses an evaluation of single nucleotide polymorphisms (SNPs) to distinguish between the cfDNA of maternal and of fetal (placental) origin [17]. SNPs are benign variations of single bases in the DNA sequence which occur frequently in the general population. By sequencing over 13,000 SNPs covering the 5 chromosomes of interest (21, 13, 18, X, and Y), an evaluation is made of the allele ratios at each SNP site, and advanced bioinformatics are utilized to determine the likelihood that a copy number variation involving one of these chromosomes is present in the pregnancy. This determination permits a calculation of the probability that the fetus has trisomy 21, 13, 18, or monosomy X. The maternal allele ratios are evaluated to rule out a maternal copy number variation. cfDNA from an additional source, such as a vanishing twin may also be identified [14] and will not be analyzed for aneuploidy, avoiding this potential cause of discordant results. The SNP method can also determine the presence of triploidy and the parent of origin [14].

Fetal Fraction

Fetal fraction has been identified as a vital quality metric for accurate NIPS analysis [18]. Fetal fraction is the proportion of the total cfDNA in a plasma sample which

originates from the placenta rather than the mother, expressed as a percentage. During the period that NIPS is typically performed, the average fetal fraction is 10–12% [19]. If the fetal fraction is too low, it becomes difficult to accurately distinguish disomy from trisomy in the fetus, and low fetal fraction has been identified as a major factor in the few false negatives associated with NIPS [20]. Until recently, many NIPS laboratories did not measure fetal fraction. The ACMG, in its 2016 statement, asserted that fetal fraction should be measured and reported when NIPS is performed [21].

Fetal fraction can be measured in a number of different ways. One method involves assessing the presence of material from the Y chromosome. Whilst this is effective for determining fetal fraction for male fetuses, it cannot do so for females. This method should therefore not be used as the only way of assessing fetal fraction. Some methods work for both male and female fetuses, but are indirect approaches which exploit characteristics that vary depending on the origin of the DNA. For example, the average length of the cfDNA fragments in a sample has been used to estimate the fetal fraction (fetal fragments are, on average, shorter than those of maternal origin [22]) as has the presence of methylation (fetal fragments are more likely to be methylated than maternal ones [23]). Using SNPs to distinguish fetal from maternal DNA allows a more direct assessment of the proportion of the DNA that is of placental origin. This SNP method works equally well for male and female fetuses, and furthermore it evaluates an absolute distinguishing factor between maternal and fetal DNA—the presence of alleles of paternal origin.

Factors Affecting Fetal Fraction

A number of factors are known to affect fetal fraction. Fetal fraction changes with gestational age (the fetal fraction increases throughout the pregnancy but is relatively stable between 12 and 17 weeks [19, 24]). Maternal obesity is associated with lower fetal fraction. This finding is believed to be due to a dilutional effect related to increased contribution of cfDNA from maternal adipocytes [5]. Other maternal factors such as hypertension may also lower the fetal fraction [25]. Importantly, it has been noted that low fetal fraction is associated with an increased risk of aneuploidy, in particular trisomies 13 and 18 [17, 25]. For this reason, the ACMG has recommended that diagnostic testing be offered to patients who are unable to obtain results from NIPS due to low fetal fraction [21].

Using the Technologies: Where Does NIPS Fit In?

Can NIPS Replace First Trimester Screening?

First trimester screening is typically performed by a combination of ultrasound to evaluate the nuchal translucency and a maternal serum test to evaluate levels of PAPP-A and β-hCG. A first trimester scan can confirm the presence of a live fetus, exclude twins or higher multiples (or if present can determine chorionicity), and may identify major structural defects such as anencephaly as well as measuring nuchal translucency. The serum component seeks not only to screen for trisomy 21, 13, and 18, but also spina bifida.

NIPS is focused on screening for chromosomal abnormalities. It thus does not replace the other aspects of first trimester screening. In order to evaluate nonchromosomal aspects, NIPS should be integrated into a program which includes ultrasonography and spina bifida detection.

Screening for Trisomy 21

As the most common chromosomal disorder and the commonest single cause of learning disability [26], trisomy 21 is a major focus of prenatal screening. The combined first trimester test brings together maternal serum screening and a measurement of the nuchal translucency to give a risk score for trisomy 21 and can detect approximately 80% of cases of trisomy 21 [3]. However, the *specificity* of this screening is very low, and only around 4% of 'high-risk' pregnancies identified through this process will actually be affected with trisomy 21 [3]. Prior to the introduction of NIPS, when women received a high-risk first-trimester combined screening result, the only option was to offer invasive testing. However, this option carries a miscarriage risk, which, even if low, is enough to deter some women from going ahead with testing, particularly as the vast majority will not in fact have an affected fetus. As NIPS has far greater specificity, offering NIPS to this group of women with high-risk first-trimester screening results will greatly reduce the number of false positives for trisomy 21 [3]. Part of the advantage of NIPS however is that it is also more sensitive for detecting trisomy 21 than combined serum screening tests, with detection rates of over 99% [4]. If NIPS is used as a second-line screening test for trisomy 21, this additional sensitivity is lost.

Expanding the Paradigm to Other Disorders

One criticism of NIPS is that because it is targeted at certain specific abnormalities it would not detect other abnormalities that may have been detected via the original

screening pathway with invasive testing for high-risk first-trimester screening results [27]. The argument is sometimes made that some women who were detected as 'high-risk' through combined first-trimester screening will occasionally have fetuses with chromosomal abnormalities other than those screened for by NIPS, and that these will be fortuitously detected on karyotyping or chromosomal microarray (CMA) at amniocentesis or CVS.

When no ultrasound anomalies are present, karyotype detects abnormalities in an additional 0.1% of pregnancies [28]. Most of the chromosomal abnormalities that are not associated with structural anomalies in the fetus are under 5–7 Mb, the lower limits of detection of the karyotype [28].

Chromosomal microarray (CMA), which detects smaller deletions and duplications of chromosomal material but no single gene mutations, would yield clinically significant results in an additional 1.7% of cases where no ultrasound anomaly is detected [8]. However CMA is not at present offered to all women who have a high-risk serum screening result with an ultrasonographically normal fetus. Only those women who are offered CMA when they have an invasive test will benefit from the discovery of submicroscopic deletions and duplications following a high-risk first trimester combined screening result.

Criticisms regarding the specific focus of NIPS have been in part answered by broadening the scope of this screening, retaining the increased sensitivity and specificity for the common trisomies but offering the possibility of picking up a wider range of other conditions. At present, options include tests which target specific microdeletions as well as those that offer a genome-wide scan for larger deletions and duplications [7, 29].

Targeting certain microdeletions allows the possibility of specifically detecting syndromes which are of known clinical significance such as the 22q11.2 deletion; the most common microdeletion in humans [30]. Recent studies have indicated that the prevalence of 22q11.2 syndrome is as high as 1 in 1000 [10]. Unlike the trisomies, the risk of microdeletions does not vary with maternal age. Therefore in younger women the risk of having a child with a microdeletion is greater than the risk of having a child with Down syndrome [8].

Another option is the performance of a genome-wide scan for large deletions and duplications. The currently-available test of this type offers the possibility of detecting deletions and duplications of greater than or equal to 7 Mb, a similar size to those which can be detected using a standard karyotype [7].

How Far Should Testing Go?

As the number of anomalies that can be detected prenatally increases, so do concerns about the possible negative consequences of this [31, 32]. Whilst trisomies 21, 13, and 18 are well described and there is a wealth of information available to use for counseling parents about the range of possible outcomes, the same is not true for all of the anomalies which may be detected prenatally. Chromosomal microarrays will detect a number of copy number variants (deletions or duplications of chromosomal material) for which the significance is unknown [33]. Such variants are termed "variants of unknown significance", or VOUS. These VOUS are particularly problematic when detected prenatally as they present parents and healthcare professionals with a dilemma—an anomaly has been detected but the likely outcome for the baby is difficult to predict. Some chromosomal abnormalities that can be detected prenatally are associated with known disorders but the condition may be so rare or so variable that it is difficult to provide clear information to parents [34].

Some chromosomal conditions which may be detected prenatally are associated with pregnancy loss, and are generally incompatible with life, such as rare autosomal trisomies, or frequently inherited and of variable significance, such as marker chromosomes [28]. There is thus an argument that offering widespread screening for such conditions is of limited use. However, some parents may wish to have this information.

There are a number of challenges related to expanding NIPT beyond large chromosomal anomalies. The first challenge is that small deletions and duplications are harder to detect, and individually rarer, and so false positive rates and false negative rates are higher than for the common trisomies. A second challenge is that although more deletions and duplications of clinical significance can be detected, so too can deletions and duplications of uncertain significance. Finding chromosomal abnormalities of uncertain significance during pregnancy poses serious counseling challenges. The use of a targeted test can help to limit the discovery of such VOUS as only known abnormalities are sought.

The ACMG guidelines recommend informing all pregnant women of the availability of screening for clinically significant copy number variations provided that a number of conditions can be met, such as having discussed with the patient whether they want prenatal screening or diagnostic testing. They do not support genome-wide copy number variant screening by NIPS, recommending instead diagnostic testing with CVS or amniocentesis and chromosomal microarray for women requiring this depth of information [21].

The Future

As technology advances, it is likely that increasingly detailed prenatal screening tests will be offered. These more detailed tests will most likely include single gene testing for a broad variety of conditions, and increasingly detailed copy number variant detection. Ultimately, it is possible that whole genome sequencing will be offered on a noninvasive basis. Such testing brings a number of ethical challenges. Whole genome sequencing can identify not only conditions which can have a significant impact in the prenatal period, but also conditions that will only manifest in adulthood if at all, carrier status for a number of conditions and a large number of variants of unknown significance [35]. It has been argued that too much information given prenatally, rather than being of benefit, can actually hamper autonomous choice [36]. There is thus, a need for reflection on the types of information which are of value and ethically justifiable as a prenatal screen, and consideration of how parents may be counseled so that they can reach an informed decision as to the extent of the information that they wish to receive during pregnancy.

Conclusion

NIPS represents a major advance in the field of prenatal screening, not only in allowing greater sensitivity and specificity for trisomy 21 in comparison to combined first-trimester trisomy screening, but also in the capacity to screen for a broader range of conditions. However, it is important that the limitations as well as the advantages of the technology are understood so that test users can make informed decisions about their prenatal care. No matter which test is chosen, it is important that healthcare providers understand the capabilities of that specific test, and are aware of the data supporting it. A broader range of screening options are now available, and it is likely that these will continue to expand. Therefore, there is a need to continue developing methods of counseling which facilitate the process of providing this information to pregnant women and their partners.

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Compliance with Ethical Standards

Conflict of interest SL is an employee of Natera Inc.

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