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Screening for fetal chromosomal and subchromosomal disorders

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SUMMARY

Screening for fetal chromosomal disorders has evolved greatly over the last four decades. Initially, only maternal age-related risks of aneuploidy were provided to patients. This was followed by screening with maternal serum analytes and ultrasound markers, followed by the introduction and rapid uptake of maternal plasma cell-free DNA-based screening. Studies continue to demonstrate that cfDNA screening for common aneuploidies has impressive detection rates with low false-positive rates. The technology continues to push the boundaries of prenatal screening as it is now possible to screen for less common aneuploidies and subchromosomal disorders. The optimal method for incorporating cfDNA screening into existing programs continues to be debated. It is important that obstetricians understand the biological foundations and limitations of this technology and provide patients with up-to-date information regarding cfDNA screening.

Keywords

Screening; Non-invasive prenatal testing; Cell-free DNA; Aneuploidy; Genetic counseling

1. Introduction

Prenatal screening for fetal chromosomal disorders has been offered clinically for more than 40 years. The goal of aneuploidy screening is to identify pregnancies at increased risk for chromosomal disorders using screening tests that have high detection rates while minimizing the number of false-positive results. Individuals identified to be at increased risk are subsequently offered diagnostic testing. Diagnostic confirmation of fetal chromosomal

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Conflict of interest statement

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disorders requires obtaining fetal cells through invasive procedures, such as amniocentesis or chorionic villus sampling. Whereas these procedures are relatively safe, they carry a small risk for miscarriage [1]. Prenatal screening and diagnostic testing give women the opportunity to make informed reproductive decisions and optimize their pregnancy outcomes. In this review, we discuss the evolution of screening for chromosomal disorders from its modest beginnings with maternal serum analytes through the development of cell-free DNA (cfDNA) screening. We focus our discussion on the clinical applications of cfDNA, its limitations, and how it continues to revolutionize the field of prenatal screening.

2. Historical perspective

Traditionally, prenatal screening has focused on detecting cases of trisomy 21 (Down syndrome), the most common chromosome condition affecting live births. It is well established that the risk for trisomy 21 increases with maternal age. The risk of a live term infant with trisomy 21 increases from ~1 in 1500 at age 20 years to 1 in 85 at age 40 years [2]. In the USA and other high-income countries, pregnant women aged >35 years have been offered amniocentesis based on their age-related risk of trisomy 21 since the 1970s. At that time, screening based solely on maternal age allowed the detection of 30% of fetuses with trisomy 21 by offering diagnostic testing to ~6% of pregnant women [3]. Now, with the availability of maternal blood and ultrasound-based markers, screening and follow-up diagnostic testing is offered to all pregnant women regardless of age, based on the patient's desire for information about genetic conditions.

Screening for chromosomal abnormalities in women age <35 years was introduced in the 1980s when reports were published linking low alpha-fetoprotein levels in maternal serum to pregnancies with trisomy 21 [3]. Over time, additional maternal serum markers, including human chorionic gonadotropin (hCG), dimeric inhibin A (DIA), and unconjugated estriol (uE3), were added to the screening algorithm to improve detection rates. Combined first-trimester screening further improved detection rates by incorporating analysis of serum free β -hCG or total hCG along with pregnancy-associated plasma protein A (PAPP-A) in addition to an ultrasound measurement of the nuchal translucency [4,5]. Table 1 summarizes the evolution of screening for trisomy 21.

While conventional screening methods were developed to identify fetuses with trisomy 21, other aneuploidies, including trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome) and monosomy X (Turner syndrome), were also being detected with the widespread use of these screening tests. Screening algorithms were developed for trisomy 18 using the analytes and ultrasound components developed for trisomy 21 detection [6]. However, screening for trisomy 13 and monosomy X remained more difficult.

In 2011, the paradigm of prenatal screening shifted with the introduction of cell-free DNA (cfDNA) screening. This new technology allowed for significant improvements in the detection of pregnancies with trisomy 21 as well as the less common chromosomal disorders.

3. Development of cell-free DNA screening

Driven by the desire to produce an accurate test without risk, researchers have worked towards developing a non-invasive method of analyzing fetal DNA. Early studies examining fetal DNA from intact fetal cells circulating in maternal blood were disappointing due to the difficulty in isolating these rare fetal cells [7]. A landmark study by Lo et al. demonstrated that a portion of the cell-free DNA found within the maternal plasma was of fetal origin [8]. This finding paved the way for the development of cell-free DNA-based screening. Plasma cfDNA fragments originate from apoptosis of various cells, a large proportion of which come from hematopoietic cells [9]. During pregnancy, a woman has a combination of both maternally derived and feto-placental-derived cfDNA circulating in her plasma. Fetal cfDNA is thought to originate from the placental trophoblasts [10] and makes up ~10% of the cell-free DNA found in maternal circulation [11]. Maternal and fetal cell-free DNA can be differentiated based on fragment length, with fetal cell-free DNA having shorter fragments [12].

In 2008, two research groups demonstrated their ability to correctly identify fetuses with chromosomal aneuploidy using massively parallel sequencing (MPS) of cell-free DNA in maternal plasma [13,14]. MPS followed by bioinformatics analysis allowed for the detection of genetic material from the chromosomes of interest. In the case of a fetus with trisomy 21, one would expect to identify extra genetic material from chromosome 21. Different methods of sequencing have been reported, including whole genome MPS-, chromosome-selective MPS-, and single nucleotide polymorphism (SNP)-based approaches [15–19]. Various statistical methods are then used to determine whether any extra or missing chromosome material is present (see Fig. 1 for a comparison of common platforms). There are pros and cons to each of these methods. The whole genome sequencing method allows for detection of differences on any chromosome and, depending on the depth of sequencing, allows for detection of less common aneuploidies and subchromosomal abnormalities [20]. Targeted sequencing approaches reduce the risk of incidental findings by only reporting on chromosomes associated with the common aneuploidies. The SNP-based approach is the only method that can detect cases of triploidy; however, due to the need to compare maternal and fetal genotypes, it cannot be used in twin pregnancies, pregnancies conceived with a donor oocyte, nor in women who have previously undergone organ transplantation.

Between 2011 and 2012, several large clinical validation studies reported on their success in detecting common chromosomal disorders with impressive sensitivity and specificity [15–19]. With these publications came the rapid clinical implementation of this new screening test.

4. Clinical performance

4.1. Clinical validity

cfDNA screening has the highest detection rate with the lowest false-positive rate, when compared to other conventional screening tests (Table 2). An updated meta-analysis, which included studies between January 2011 to January 2015, was published. The pooled weighted detection rates for common aneuploidies are as follows: trisomy 21 (99.7%),

trisomy 18 (98.2%), and trisomy 13 (99%), with a combined false-positive rate (FPR) of 0.13% (Table 3). They also report pooled weighted detection rate of 95.8% for monosomy X with a false-positive rate of 0.14%. Sex chromosome aneuploidies other than monosomy X had a pooled weighted detection rate of 100% and FPR of 0.003%. However, the authors conclude that the number of reported cases was too small for accurate assessment of performance of screening for sex chromosome conditions (Table 4). There was no significant difference found between testing platforms in this meta-analysis [22].

4.2. Clinical utility

Whereas high detection rate and low FPR confirm the validity of a clinical test, the usefulness of a screening test also depends on prevalence of the condition in the population and its impact on clinical management. The positive predictive value (PPV) is a measure of clinical utility and relies both on the sensitivity/specificity of a test as well as on the prevalence, or pre-test probability, of the condition. PPV helps patients and clinicians understand the chance that a pregnancy is affected with the condition following a positive result. Conversely, the negative predictive value (NPV) can be useful for understanding the likelihood that the pregnancy is truly unaffected after a negative test.

When cfDNA screening was introduced, there was concern that clinicians and patients were misinterpreting the reported sensitivities and specificities to mean that cfDNA screening functioned at the same level as a diagnostic test. In one study, 6.2% of patients with an abnormal cfDNA screening result elected to terminate the pregnancy without confirmatory testing [23]. Many experts in the field expressed concern and there was a push for laboratories to begin reporting PPV to improve interpretation of test results [24].

Table 5 demonstrates the range of post-test PPVs possible for cfDNA screening with various pre-test probabilities. Sensitivities and false-positive rates from the most recent meta-analysis for trisomy 21 (99.7% and 0.04%), trisomy 18 (98.2% and 0.05%), and trisomy 13 (99% and 0.04%) were used [22]. A positive cfDNA screening result is clearly most significant in women with the highest a-priori risk. The NPV is >99% across all trisomies and pre-test risk groups. One study compared the PPV of cfDNA screening and conventional screening in a group of low-risk pregnant patients. For trisomy 21 the PPV was significantly higher for cfDNA screening at 45.5%, as compared to conventional screening with a PPV of 4.2% [16].

Two online calculators have been developed to assist with patient counseling (http://www.med.unc.edu/obgyn/Patient_Care/specialty-services/maternal-fetal-medicine/nips_calc.html; <https://www.perinatalquality.org/vendors/nsgc/nipt/>). However, PPV calculators have limitations, and providing individual post-test risks may be less useful for some patients. This is particularly true when post-test risks are calculated for less common conditions in which the true prevalence is not well established and the false-positive rate is undefined. Providers need to consider several factors, including how the prior risk estimates were made, the specificity value used in the calculation, and whether providing the estimate will improve the patient's understanding and aid in her decision-making process [25]. It is crucial that patients are counseled appropriately following a positive test result so that they can understand the value and limitations of their results.

4.3. Fetal fraction

Fetal fraction (FF) is another component of cfDNA screening that can affect clinical performance. Whereas ~10% of cfDNA in maternal circulation is of placental/fetal origin [11], a range of FF values are seen in pregnancy. It is more challenging to detect aneuploidy in a pregnancy with low FF as the statistical separation between fetal disomic and trisomic status is smaller. Low FF may result in an increased rate of false-negative results, or “no call” result, in which the laboratory is unable to report any result. The rate of “no call” results due to low FF varies by assay platform and FF estimation method, and ranges from 0.1% to 6.1% of samples [22]. Failure rates have been shown to vary by testing approach, with SNP-based approaches having the highest failure rate at 6.29%, followed by chromosome-selective MPS at 3.56% and whole genome MPS at 1.58% [26].

Fetal fraction can be influenced by gestational age, maternal weight, and fetal aneuploidy [27]. FF increases throughout gestation, with a rate of ~0.1% per week between 10 and 21 weeks and ~1% per week after 21 weeks gestation [28]. FF has an inverse relationship with maternal weight. Within the body mass index (BMI) range of 20–40 kg/m², there is a decrease of 1.17% in fetal fraction for every increase in BMI of 5 kg/m². At BMI >40 kg/m², the median fetal fraction is estimated between 5.99 to 5.27 [27]. There is also evidence that FF tends to be slightly elevated in pregnancies with trisomy 21 and decreased in pregnancies with trisomy 18 and 13, when compared to euploid pregnancies [27,29]. It is estimated that a “no call” result secondary to low FF has a PPV for aneuploidy of 7% in the general pregnant population and up to 30% in high-risk pregnancies [30]. Clinical management after a “no call” result should include a detailed ultrasound to confirm fetal viability and to investigate for structural abnormality, growth restriction, or other high-risk feature that would prompt a recommendation for diagnostic testing. Maternal factors that increase the risk of a failed result, such as low molecular weight heparin therapy [31] or obesity [32] should be reviewed. In the presence of structurally normal fetus, an individualized approach with either repeat cfDNA screening [33], an alternative form of screening (such as the combined first trimester, or second trimester serum screening tests) or diagnostic testing may be offered.

5. Clinical practice

The clinical implementation of cell-free DNA screening occurred rapidly and was driven by extensive marketing from the commercial laboratories that helped develop and offer the test [34]. Since its inception, there has been much debate about the appropriate use of this new technology. Initially, practice guidelines recommended that cfDNA screening be limited to high-risk populations [21,35,36]. However, there has been a shift in practice after studies showed that cfDNA performed with equivalent sensitivity and specificity in low-risk pregnant women, though the PPV was correspondingly lower [16]. The value of the 11–13-week ultrasound, independent of nuchal translucency measurement, has continued to be emphasized in the cfDNA era [35,37,38]. cfDNA screening continues to push boundaries as it is now technically feasible to screen for conditions other than trisomies 21, 18, and 13, including the sex chromosomes, microdeletions, and rare autosomal trisomies.

5.1. Traditionally screened chromosome disorders

Multiple options are available for screening for the common aneuploidies in pregnancy. Trisomies 21, 18, and 13 and the most common autosomal trisomies are associated with predictable patterns of intellectual disability and birth defects. Pregnant women should be informed of their options for screening and have the opportunity to select a screening or diagnostic test based on their own personal goals and preferences [21,35,39]. Table 2 compares common aneuploidy screening tests.

Various models for incorporating cfDNA screening into existing screening protocols have been proposed (Fig. 2). Recent practice guidelines have recommended that cfDNA screening be offered to patients based on its superior detection rates [21,35,39]. However, cfDNA screening remains expensive, limiting the universal acceptance of it as a first-line screening test. For this reason, many practices use cfDNA screening as a second-line screening test and continue to offer combined first-trimester screening (cFTS) as the initial test to women at average risk for chromosome conditions. cFTS also has the advantage of early structural assessment of the fetus. In the Netherlands, the TRIDENT (Trial by Dutch laboratories for evaluation of non-invasive prenatal testing) study showed support for using cfDNA screening as a second-tier screening test for women at high risk for aneuploidy. This study confirmed the high accuracy of cfDNA screening and demonstrated a high uptake (86%) in their high-risk cohort with a significant reduction in number of diagnostic tests [40]. The TRIDENT-2 study is currently underway, in which all pregnant patients will be offered the option between conventional screening or cfDNA screening. In the UK contingency model, patients are stratified into three different risk categories based on results of cFTS. Women with a risk >1 in 100 for trisomy 21, 18, or 13 are considered high risk and are offered diagnostic testing, cfDNA testing, or no further testing. Women with an intermediate risk, which is defined as a risk of 1 in 101 to 1 in 2500, are offered cfDNA testing or no further testing. Women with a low risk, <1 in 2500, are offered reassurance and no further testing. This method allowed for the detection of 91.5% (43/47) of cases of trisomy 21 and 100% (28/28) of trisomies 18 or 13. The authors concluded that the contingency model would allow for the detection of the majority of trisomic fetuses while substantially reducing the need for diagnostic procedures [41]. Both of these studies were completed in countries with universal health coverage and established national prenatal screening programs. It is less clear how these models could be applied in the USA and other countries where patients choose their own health insurance, and where insurance coverage and access to care is variable.

5.2. Rare autosomal trisomies

It is technically possible to detect aneuploidy for chromosomes other than 21, 18, and 13 using cfDNA screening. Some laboratories have added rare autosomal trisomies, including trisomies 16 and 22, to their testing panels [42]. The vast majority of these rare aneuploidies result in early pregnancy loss; thus, the clinical value of screening in a viable pregnancy is less clear. Other cases have been associated with confined placental mosaicism and severe fetal growth restriction requiring preterm delivery [43]; cfDNA screening thus facilitated identification of those fetuses requiring additional growth surveillance. At this time, most

professional organizations do not recommend routine screening for these conditions given their rarity and lack of data on detection with cfDNA [21,35,39].

5.3. Sex chromosome disorders

Sex chromosome disorders describe conditions affecting the number or structure of the X and Y chromosome. The most common sex chromosome aneuploidies (SCAs) include 47,XXX, 47,XXY, 47,XYY, and 45,X. The range of phenotypes associated with sex chromosome conditions is highly variable, from early miscarriage to fully functioning, non-dysmorphic adults [44].

With the exception of 45,X (Turner syndrome), most SCAs are not associated with a prenatal phenotype and are not detectable by conventional screening methods. Although technically feasible to screen for these conditions with cfDNA, the data on the detection rates and false-positive rates are less robust, making counseling more difficult. Testing for fetal SCA with cfDNA screening also has the potential to uncover cases of maternal SCA. In one study, 8.6% of positive cfDNA results for SCA were due to an abnormal maternal karyotype involving the X chromosome [45]. Recent guidelines recommend that patients be informed of the availability of screening for sex chromosome conditions with cfDNA screening, but patients should have the option of declining this portion of testing [35,39].

5.4. Subchromosomal disorders

Subchromosomal disorders result from submicroscopic copy number variants that are too small to be detected by standard karyotyping. The phenotypes of conditions caused by copy number changes are highly variable and depend on the specific chromosome region and the amount of genetic material that is duplicated or deleted. 22q11.2 deletion syndrome is the most common subchromosomal disorder. It is associated with a widely variable phenotype, but may include congenital heart defects, palatal abnormalities, characteristic facial features, and learning difficulties [46]. Unlike the common aneuploidies, the incidence of subchromosomal disorders is not affected by maternal age. Chromosomal microarray analysis (CMA) is now the first-line test for the postnatal genetic evaluation of individuals with developmental delay, intellectual disability, or congenital anomalies due to its ability to detect more clinically significant changes [47]. Approximately 6% of fetuses with anomalies and 1.7% of structurally normal fetuses with a normal karyotype carry a clinically significant copy number variant on CMA [48]. Experts recommend that patients undergoing prenatal diagnosis testing be offered the option of CMA [1].

Proof-of-concept studies have reported the possibility of expanding cfDNA screening to include well-defined microdeletion syndromes [20,49–51]. However, some of these studies were limited by the use of a small number of synthetically designed samples. Researchers generated artificial pregnancy plasma mixtures using DNA from individuals with microdeletion syndromes diluted into matched maternal DNA to create a simulated fetal fraction [49]. However, following these early reports, several commercial laboratories began offering expanded panels including several microdeletion syndromes. Although this testing is offered clinically, fewer data have been published on screening for 22q11.2 deletion syndrome compared with the common aneuploidies. Two retrospective cohort studies have

been published [52,53]; however, due to the small number of affected fetuses, heterogeneous populations, and incomplete outcome data, the true sensitivity and NPVs in clinical practice are unknown. No prospective clinical trials have been completed for the use of this technology for the detection of microdeletion syndromes. At this time, estimates of PPV vary considerably between studies with reports for 22q11.2 deletion syndrome ranging from 5.3% [49] to 18% [52] to 96% [53].

As would be expected with this rapidly progressing field, researchers continue to work to expand the use of this technology. Studies have demonstrated the use of genome-wide cfDNA screening to detect rare autosomal trisomies or structural chromosome anomalies that would not be detectable with conventional cfDNA screening [54,55]. In a recent clinical utility study, genome-wide cfDNA screening was completed in 12,078 samples. Clinically relevant chromosomal abnormalities were identified in 196 (1.6%) pregnancies, including 151 common aneuploidies, 10 rare autosomal trisomies, and eight segmental imbalances [56]. Notably, of the 10 rare autosomal trisomies that were identified, seven resulted in early miscarriage, which raises the question of the clinical utility of screening for rare aneuploidies. Genome-wide cfDNA screening at similar resolution as a G-banded karyotype is now clinically available; however, additional clinical validation studies are needed to better characterize this technology. Both national and international societies do not recommend genome-wide screening [21,35,39]. Patients who desire this level of information should pursue diagnostic testing [39].

5.5. Screening in multifetal gestations

Fewer data are available regarding the performance of aneuploidy screening in multifetal pregnancies. Screening with any maternal blood-based marker can be challenging in twin pregnancies, especially those that are discordant for trisomy. In both biochemical and DNA-based screening, the abnormal levels of analytes or DNA fragments from an aneuploid fetus may be masked by the euploid co-twin. Combined first trimester screening in twin pregnancies has lower detection rates when compared to singletons [57]. Only five studies have reported on the performance of cfDNA screening in twin pregnancies. Whereas pooled detection rates in the most recent meta-analysis are reported to be 100%, the number of studies included limit the generalizability of these results [22]. Because there is no data regarding the performance of cfDNA aneuploidy screening in higher-order multifetal gestations (triplets or higher), it is not offered clinically. Aneuploidy screening in these pregnancies is limited to ultrasound markers alone.

6. Counseling

6.1. Pre-test counseling

All patients should be informed that screening and testing in pregnancy are optional. Pre-test counseling should be individualized to each patient to assure informed decision-making. The discussion should include information about commonly detected chromosome conditions and personal risk factors. A review of available testing options with emphasis on the difference between screening and diagnostic testing is also crucial. Information about

detection rates, false-positive rates, and positive and negative predictive values should also be shared [58].

6.2. Post-test counseling

When results from prenatal screening tests are discussed with patients, providers should supply a brief review of what the test does and does not screen for. For patients with negative results, reassurance should be provided along with a discussion that a negative result does not eliminate the possibility that the pregnancy has a genetic condition or birth defect. Patients with positive results on any screening test should be referred for additional genetic counseling to discuss available diagnostic testing options. A discussion of the test's positive predictive value should be included. The decision to undergo diagnostic testing is very personal and dependent on an individual patient's values and goals.

6.3. Counseling dilemmas

Incidental findings, detected when abnormal cfDNA results are followed by discordant diagnostic test results, may lead to counseling challenges. There are various explanations for these discordant results ("biological false-positives"), including confined placental mosaicism, demise of a previously undetected co-twin [59], maternal copy number variants [60], or maternal aneuploidy (e.g. sex chromosome abnormalities). The incidental identification of maternal cancers has been reported when multiple aneuploidies are found on cfDNA screening [61,62].

Due to the wide range of potential causes of discordant results, it is important that providers offering cfDNA screening have a basic understanding of these possible etiologies. All patients with discordant results should be referred to an appropriately trained professional for further counseling and review of additional testing options.

7. Future directions

cfDNA screening has transformed the landscape of prenatal screening for chromosomal disorders. The technology continues to expand rapidly with researchers working to achieve comprehensive screening for genome-wide alterations. It is important that providers and patients understand the benefits and limitations of this screening test. The menu of testing options for pregnant women continues to expand, so it is of the utmost importance that obstetric providers receive appropriate genetics training and that clear and concise patient educational materials are available. New counseling tools, such as decision aids, should be piloted and studied to determine whether such aids can improve patient understanding of the various screening and diagnostic testing options.

cfDNA screening has already had a significant population-wide impact on the uptake of conventional screening and diagnostic testing, and is rapidly becoming a first-line screening test in some high-income populations [63]. These trends have been associated with improvements in the diagnostic yield of invasive testing, now reported at 20%. Government-funding screening programs are rapidly incorporating the use of this new technology into their screening algorithms [40,64]. In countries without universal health coverage, there is concern that the high cost of cfDNA screening may limit its use only to women of higher

socio-economic status [65]. Currently in the USA, insurance coverage varies, but many private insurance companies are covering the test for high-risk pregnancies.

An important component of any screening program is the availability of an accurate diagnostic test, which in the case of prenatal screening is an invasive diagnostic procedure. The very low false-positive rates associated with cfDNA screening have led to a widespread decline in the number of diagnostic procedures performed. One study in the USA reported that the number of amniocenteses performed fell by 23.6–50% and CVS by 14.2–65.7% after the introduction of cfDNA screening [66]. Similarly, in Australia the total number of amniocenteses fell by 51% and CVS by 37%, representing the largest fall in number of invasive procedures in 20 years [67]. It is concerning that this decline may result in an increase in complications from the procedures, due to the lack of availability of experienced providers. It is essential that obstetricians providing prenatal diagnosis services remain well trained in diagnostic procedures [68].

8. Conclusion

Screening for chromosomal disorders has evolved from its limited beginnings of age-based population screening to the new frontier of DNA-based non-invasive testing. Although cfDNA screening has shown great success in detecting common aneuploidies, clinicians should proceed with caution with the ever-expanding scope of prenatal screening. Many questions remain to be answered across technical, clinical, economic, and ethical dimensions. Continued research on best practice and clinical implementation of cfDNA screening is essential for the responsible application of genomic advances to prenatal care.

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Practice points

- cfDNA screening for common aneuploidies has high detection rates with low false-positive rates.
- Screening for subchromosomal disorders is technically feasible, but additional research is needed before it is routinely offered to all women.
- False-positive and false-negative results are possible with all screening tests, including cfDNA screening.
- High-risk cfDNA results should be confirmed by diagnostic testing prior to any pregnancy management decisions.
- Providers should be aware of possible etiologies of discordant results in order to guide appropriate follow-up care.

Fig. 1.

Comparison of cell-free DNA screening platforms. MPS, massively parallel sequencing; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction.

Fig. 2.

Various models for the incorporation of cell-free DNA (cfDNA) screening into aneuploidy screening programs. US, ultrasound; cFDS, combined first-trimester screening.

Table 1

Evolution of screening tests for trisomy 21.

Year	Event
1970s	Maternal age >35 years Detection rate: 30%
1980s	Introduction of maternal serum screening with AFP AFP and maternal age Detection rate 25–30%
1990s	Addition of multiple maternal serum markers Triple screen (hCG, AFP, uE3) Detection rate: 69% GA: 15–22 weeks Quadruple screen (hCG, AFP, uE3, DIA) Detection rate 81% GA: 15–22 weeks
2000s	First trimester screening First trimester combined screening (hCG, PAPP-A + NT measurement) Detection rate: 82–87% GA: 10–13 ^{6/7} weeks
2011	cfDNA screening introduced to clinical practice cfDNA measured in maternal plasma Detection rate: >99%

AFP, alpha-fetoprotein; hCG, human chorionic gonadotropin; uE3, unconjugated estriol; GA, gestational age; DIA, dimeric inhibin-A; PAPP-A, pregnancy-associated plasma protein A; NT, nuchal translucency; cfDNA, cell-free DNA.

Table 2

Comparison of widely used screening tests for chromosomal disorders.

Screening test	Components	Gestational age	Detection rate	False-positive rate	Advantages	Disadvantages
First trimester						
NT ultrasound	NT	10–14 weeks	Tri 21: 64–70%	–	Useful in multifetal pregnancies	NT certified provider
Combined first-trimester screen	NT PAPP-A, hCG	10 – 14 weeks	Tri 21: 82 – 87% Tri 18/13: 82%	5%	Results in 1 st trimester	NT certified provider
cfDNA screening	Sequencing of DNA	>10 weeks	Tri 21: >99% Tri 18: 98% Tri 13: 99%	0.13%	Highest DR Low FPR in high risk women	Expensive PPV/NPV not reported
Second trimester						
Quad screen	hCG, AFP, uE3, DIA	15 – 22 weeks	Tri 21: 81% Tri 18: 69%	5%	Relatively inexpensive No US required	Results delayed to 2 nd trimester; Lowest DR
Anatomy ultrasound	Fetal anatomy	18 – 20 weeks	Tri 21: 50%	–	Identify structural anomalies	Lack of standardization Variation between providers

NT, nuchal translucency; tri 21, trisomy 21; tri 18, trisomy 18; PAPP-A, pregnancy-associated plasma protein A; hCG, human chorionic gonadotropin; cfDNA, cell-free DNA; DR, detection rate; FPR, false-positive rate; PPV, positive predictive value; NPV, negative predictive value; AFP, alpha-fetoprotein; uE3, unconjugated estriol; DIA dimeric inhibin A; US, ultrasound.

Source: American College of Obstetricians and Gynecologists [21]; Gil et al. [22].

Table 3

Meta-analysis and performance of screening for common aneuploidies.

Aneuploidy	Included data	Detection rate^a (95% CI)	False positive rate^a (95% CI)
Trisomy 21	30 studies 1963 cases of trisomy 21 225,032 non-trisomy 21 singleton pregnancies	99.7% (99.1 – 99.9)	0.04% (0.02 – 0.08)
Trisomy 18	25 studies 560 cases of trisomy 18 212,019 non-trisomy 18 singleton pregnancies	98.2% (95.5–99.2%)	0.05% (0.03–0.07%)
Trisomy 13	23 studies 119 cases of trisomy 13 212,883 non-trisomy 13 singleton pregnancies	99.0% (CI 65.8–100%)	0.04% (0.02–0.07%)

^aPooled weighted detection rate and false-positive rate from Gil et al. [22].

Table 4

Meta-analysis and performance of screening for sex chromosome aneuploidies.

Aneuploidy	Included data	Detection rate^a (95% CI)	False-positive rate^a (95% CI)
Monosomy X	11 studies 36 cases of monosomy X 7677 with no monosomy X Singleton pregnancies	95.8% (70.3–99.5%)	0.14% (0.05–0.38%)
Other sex chromosomal aneuploidy	8 studies 17 affected sex chromosome aneuploidies 5383 non-sex chromosome aneuploidies singleton pregnancies	100% (83.6–100%)	0.003% (0–0.07%)

^aPooled weighted detection rate and false-positive rate from Gil et al. [22].

Table 5

Range of post-test positive predictive values possible for cell-free DNA screening with various pre-test probabilities.

Pre-test probability	Post-test positive predictive value		
	Trisomy 21	Trisomy 18	Trisomy 13
1 in 10	99%	99%	99%
1 in 100	96%	95%	96%
1 in 100	71%	66%	71%
1 in 10,000	20%	16%	20%

Data from Gil et al. [22].