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Permalink

<https://escholarship.org/uc/item/2nt9b4x8>

Journal

Human Reproduction, 27(11)

ISSN

0268-1161

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Publication Date

2012-11-01

DOI

10.1093/humrep/des286

Peer reviewed

Cell-free fetal DNA testing for fetal aneuploidy and beyond: clinical integration challenges in the US context

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ABSTRACT: The recent release of new, non-invasive prenatal tests for fetal aneuploidy using cell-free fetal DNA (cffDNA) has been hailed as a revolution in prenatal testing and has triggered significant commercial interest in the field. Ongoing research portends the arrival of a wide range of cffDNA tests. However, it is not yet clear how these tests will be integrated into well-established prenatal testing strategies in the USA, as the timing of such testing and the degree to which new non-invasive tests will supplement or replace existing screening and diagnostic tools remain uncertain. We argue that there is an urgent need for policy-makers, regulators and professional societies to provide guidance on the most efficient and ethical manner for such tests to be introduced into clinical practice in the USA.

Key words: ethics / prenatal diagnosis / aneuploidy

Introduction

Since the 1997 discovery of cell-free fetal DNA (cffDNA) floating in maternal serum (Lo *et al.*, 1997), there has been a concerted push on several fronts to employ cffDNA in non-invasive prenatal tests for a variety of conditions. Several years ago, Sequenom, Inc., the licensee of initial work on analyzing cffDNA, released a non-invasive test for Rhesus D status of the fetus (Milunsky and Milunsky, 2010). Sequenom's non-invasive test for trisomy 21 (Down syndrome) entered the commercial market in late 2011 with much fanfare (Sequenom, 2011), and its PLUS, which includes testing for trisomy 13 and 18 (Palomaki *et al.*, 2012a,b), was released in 2012 (Sequenom, 2012). Other companies, including Verinata Health, Natera and Ariosa Diagnostics, are in the process of validating and releasing similar tests or have already released these tests to the public (Leuty, 2011; Heger, 2011a; Ariosa Diagnostics, 2012a). Ongoing research anticipates the availability of additional cffDNA tests for a variety of conditions, including a broader range of chromosomal abnormalities or single-gene conditions (Lun *et al.*, 2008).

The news that a cffDNA-based test had been released drew widespread media attention to prenatal testing and predictions of a 'revolution' in prenatal testing (Greely, 2011; Palomaki *et al.*, 2011; Roan,

2011; Staff, 2011). Various publicity announcements have claimed that cffDNA tests are 'the first universal, non-invasive tests for Down syndrome [that] should put an end to invasive testing procedures' (Staff, 2008), 'test[s] that can greatly simplify the standard of care for pregnant women and give providers and patients confidence as a result of... highly accurate results', a 'universal screening tool' (Aria Diagnostics, 2012a,b), and capable of 'chang[ing] human pregnancy forever' (Darnovsky, 2011). Even the National Society for Genetic Counselors (NSGC) concedes that 'NIPT's introduction into clinical practice has the potential to significantly shift the paradigm of prenatal diagnosis and screening for all women' (Devers *et al.*, 2012). This possibility has engendered considerable attention to more general concerns about the potential ethical implications of these new forms of testing, including the normalization of prenatal testing and the stigmatization of individuals and families living with genetic conditions (Newson, 2008; Benn and Chapman, 2009, 2010; Schmitz *et al.*, 2009a,b; de Jong *et al.*, 2010, 2011; Hall *et al.*, 2010; Lewis *et al.*, 2012). While many of these ethical questions have been addressed with respect to prior technologies, we argue that the unique features of this testing—non-invasiveness, early timing for use and theoretical access to the entire fetal genome—provide a sense of urgency in considering the more concrete translational context of cffDNA testing.

The details of how this testing will be integrated into existing prenatal testing strategies in the unique regulatory and health care context of the USA are far from settled. Currently, in the USA, commercially available cffDNA tests for aneuploidy have been primarily offered to high-risk populations as a secondary screening test requiring invasive testing for confirmation, although there are signs that testing may soon be extended into low-risk populations (Ariosa Diagnostics, 2012b). The International Society of Prenatal Diagnosis has introduced preliminary recommendations on the timing and clinical integration of cffDNA testing (Benn et al., 2012a,b) and the NSGC has likewise endorsed the use of NIPT as a second-tier screen in populations that are at high risk of chromosome abnormality (Devers et al., 2012). In Europe, projects such as the RAPID project (Reliable Accurate Prenatal non-Invasive Diagnosis) and SAFE NOE (Special Non-invasive Advances in Fetal and Neonatal Evaluation Network of Excellence) Framework 6 initiatives have attempted to address these issues in a European context. However, the larger professional societies in the USA, such as the American Congress of Obstetricians and Gynecologists, the American College of Medical Genetics and Genomics (ACMG) and the International Society for Human Genetics (ISHG) are still developing guidance on how such tests should be employed in prenatal practice. This is significant because, unlike various European countries, most notably the UK (Pennings, 2009), the USA has very limited direct regulation of either reproductive technologies or genetic testing and no national health care system. As a result, large professional societies, private medical insurers and for-profit companies largely govern the uptake and integration of new technologies in prenatal practice (Adamson, 2005).

Preliminary studies of health care practitioners in the USA reveal that many believe that they will offer non-invasive prenatal testing in the relatively near future. However, respondents also reported a lack of comprehensive knowledge about these tests and a desire for clinical and regulatory guidance (Sayres et al., 2011). Companies offering such tests have limited themselves to publishing the results of pilot studies and large-scale clinical trials in an effort to demonstrate the efficacy of their tests to providers and patients (Gene Security Network, 2011; Palomaki et al., 2011, 2012a,b; Bianchi et al., 2012a,b; Norton et al., 2012). However, while clinical and analytic validation is an important piece of the introduction of a novel technology, it is not the only element of successful translation. More contextual, but no less important, issues of consistent and systematic validation, timing, risk and scope of cffDNA testing still need to be resolved. Here, we analyze the expected trajectory of cffDNA testing in the US context in terms of its current uses and future applications. We suggest that while the capabilities of currently available tests may limit the immediate clinical impact of cffDNA technologies to aneuploidy screening, there is an urgent need for professional societies, regulators and other policy-makers to anticipate the arrival of a wide variety of future tests and provide guidance on the most efficient and ethical manner for such tests to be integrated into clinical practice in the USA and abroad.

Current needs: validation

The US Food and Drug Administration's (FDA) continued ambivalence towards regulating genetic tests over the last 20 years places the emergence of non-invasive prenatal testing in a complex

context. There has been considerable ongoing debate about the desirability of comprehensive state or federal oversight of genetic tests. The Department of Health and Human Services' 'U.S. System of Oversight of Genetic Testing: A Response to the Charge of the Secretary of Health and Human Services', chronicles efforts, both regulatory and legislative, to expand FDA and/or Centers for Medicare and Medicaid Services (CMS) supervision of genetic tests. Despite these efforts, until recently, the agency has only initiated validation procedures to confirm safety and efficacy for a very limited number of genetic laboratory developed tests (LDTs) (Dept. of Health and Human Services, 2008). Recently, however, representatives have indicated that the agency is reconsidering its enforcement discretion for cffDNA tests because these tests involve complex software and non-transparent automation and their clinical validity is not well understood. Furthermore, the FDA is somewhat concerned that these tests are broadly advertised at a national level and aggressively marketed with direct-to-consumer advertising despite their lack of comprehensive validation (Gutierrez, 2012).

If regulations were implemented, it would be applied first to currently available tests for aneuploidy. These tests have an advantage in as much as they can be reliably validated against existing screening or diagnostic methods with well-documented clinical validity (Wald and Huttly, 1999; Meier et al., 2002; Harris et al., 2004; Cuckle et al., 2008). However, the expansion of cffDNA testing applications to other diseases and conditions and their use in guiding critical reproductive decisions will challenge regulatory discretion and heighten the need for highly trustworthy validation of tests before they are placed into clinical practice. Clinical testing for an expanded range of genetic conditions, such as achondroplasia and thanatophoric dysplasia, is already being offered on a small scale in the UK (Lench et al., 2012). The low risk and relative ease of obtaining blood samples for cffDNA testing combined with the possibility of obtaining a large amount of genetic information may make it tempting to use these tests even in the absence of comprehensive validation of findings. From a clinical standpoint, it is difficult enough for health care practitioners to explain the complexities of prenatal risk factors and testing characteristics without also having to explain the uncertainties of unvalidated or insufficiently validated tests. As the conditions testable through cffDNA testing expand, clinicians may find themselves in the difficult (and inappropriate) position of bearing fiduciary responsibility for inaccurate or misleading results. Given that pregnancy termination is an option in the setting of a positive test for fetal abnormality, even when cffDNA tests are currently recommended only as a non-diagnostic screening tool, clinicians bear a heavy burden to ensure the analytic and clinical validity of any tests to which they refer. To address this concern, health care providers and patients must have access to transparent information regarding the validity and quality of all cffDNA tests on the market. How this social obligation is to be fulfilled is the necessary subject of social policy and professional review.

At least one of the companies providing non-invasive prenatal tests, Sequenom, has asserted that there is no requirement for premarket approval by the FDA because its products are LDTs, and as such the company's activities should be regulated by CMS as part of the Clinical Laboratory Improvement Amendments of 1988 (CLIA). However, CLIA regulations are restricted to certifying internal procedures and qualifications of laboratories rather than the safety and

efficacy of LDTs specifically (Centers for Disease Control and Prevention, 2004). As such, FDA regulation would most likely result in more restrictive regulation of LDTs than currently required through CLIA. If the FDA does begin to require approval of LDTs, Sequenom admits that its existing validation studies would be ineligible for submission to the FDA because the protocols were not approved in advance (Heger, 2011b). The Institute of Medicine (IOM) has recommended advance discussion with the FDA prior to initiation of validation studies for LDTs in a recent draft report (Micheel *et al.*, 2012), and the company does state that it is in discussion with the FDA about further validation. However, given the recent launch of its MaterniT21 and MaterniT21 PLUS tests, Sequenom clearly does not believe that such validation is a necessary precursor to the commercial offering of tests (Staff, 2010). Sequenom does state that its subsidiary laboratories are fully compliant with CLIA, but CLIA regulations of genetic tests are designed to ensure procedural compliance at laboratory level and do not extend to validation of specific tests (Centers for Disease Control and Prevention). Other companies have followed similar routes to commercialization—conducting validation studies without FDA input and launching tests without premarket approval (but with CLIA regulation of laboratory facilities).

However, this may soon change. The IOM now recommends that clinical utility of medical LDTs be assessed, ideally through clinical trials, and that the FDA be consulted regarding the use of investigational device exemption, which allows the investigational device to be used in a clinical study in order to collect data on safety and effectiveness required to support a Premarket Approval application (Caulfield and McGuire, 2012). Although the IOM can offer only recommendations, it joins a growing chorus of other stakeholders who believe that FDA approval may be beneficial to cffDNA testing. Given that they involve complex algorithms for analyzing fetal DNA, cffDNA tests are also subject to FDA regulation as *in vitro* diagnostic multivariate index assays (IVDMIA) (US Food and Drug Administration, 2007). This possibility will differ on the basis of the precise methods used. Companies that use multivariate index assays would most likely qualify them for IVDMIA status. Other companies that use chips produced by outside manufacturers are likewise potentially open to regulation since the presence of outside materials weakens the test's status as an LDT. Companies that use only in-house materials in their tests are least exposed to the possibility of regulation (Gutierrez, 2012).

Clearly, these arrangements differ from the more structured systems in place in many European countries. Although there is no coordinated regulation across the European Union, most individual countries have their own regulatory structures in place (Hogarth *et al.*, 2008). In the UK, for instance, the Medicines and Healthcare products Regulatory Agency (MHRA) accepts jurisdiction over all medicines and medical devices. This direct regulation reduces uncertainty in the translation process and potentially offers greater resources to test developers who have a fixed set of requirements to meet. Failing such direct regulation in the US, professional societies should encourage registration of new prenatal tests with the National Institute of Health's Genetic Test Registry. Although participation is voluntary, registration would promote the documentation of clinical and analytic validity of new tests before active use. Professional societies should also contribute by issuing guidelines that encourage physicians to review validity data before ordering tests and recommend minimum levels of clinical utility (Hockett and Close, 2010).

Near future: risk and timing

Risk

Another highly touted feature of cffDNA testing is its non-invasive nature. Diagnostic tests for fetal abnormalities, including amniocentesis and chorionic villus sampling (CVS), involve invasive procedures to remove fetal DNA for genetic analysis. Although risk calculations vary depending on the experience of the practitioner, most calculations put the risk of fetal miscarriage as a result of these procedures as somewhere between 0.1 and 1.0%, although CVS may carry a slightly higher risk, especially if practitioners are inexperienced (Evans and Wapner, 2005; Caughey *et al.*, 2006; Tabor and Alfirevic, 2010). Many pregnant women find the idea of any risk to the fetus, although small, to be undesirable or unacceptable. A sizeable proportion, estimated in some models at 10–25%, decline invasive tests even when demographics or preliminary screening have shown them to be at an elevated risk of carrying a fetus with a genetic condition (Markens *et al.*, 1999). CffDNA testing affords the potential of receiving results with significantly higher specificity and sensitivity than existing screens but without the risk to the fetus that occurs with invasive testing.

At present, however, cffDNA tests have not achieved sufficient specificity and sensitivity to replace existing invasive tests as a diagnostic tool. Demonstrated detection rates for trisomy 18 and 21 have all shown a >99% sensitivity with a false-positive rate ranging between 1 and 1% (Palomaki *et al.*, 2011; Bianchi *et al.*, 2012a,b; Norton *et al.*, 2012). For trisomy 13, the detection rates are lower, ranging from 78 to 91% sensitivity with a 0–9% false-positives rate (Palomaki *et al.*, 2011; Bianchi *et al.*, 2012a,b). These numbers show a significant improvement over existing integrated screening regimes, which generally show rates between 88 and 92% sensitivity and 4.5 and 5.1% false positives (Malone *et al.*, 2005; Benn *et al.*, 2012a,b), depending on how the first and the second trimester screening are combined. Nevertheless, they do not match the near-perfect diagnostic capabilities of invasive tests (Kay *et al.*, 2011).

Furthermore, existing clinical validation trials have taken place only in high-risk populations. It is unclear whether acceptable positive and negative predictive values can be attained in lower risk populations (Benn *et al.*, 2012a,b; Palomaki *et al.*, 2012a,b).

Pregnant women are in a position to make extremely sensitive decisions about termination of pregnancy on the basis of such testing, and it is unclear whether some pregnant women will act upon the less-than-perfect positive predictive value of cffDNA testing, despite recommendations to confirm positive results with invasive prenatal diagnosis. Certainly, for the approximately 10 to 25% of women who decline invasive procedures regardless of their risk status, this test represents an additional non-invasive option to improve upon existing screening. Increased uptake of non-invasive prenatal testing and trust in its abilities have the potential to result in as much as a 10-fold reduction in the number of invasive procedures performed.

Although a variety of social and subjective factors are involved in women's decisions on whether or not to undergo prenatal testing, the advice of clinicians remains a significant factor, especially since clinicians may have direct control over which tests are available (Browner and Press, 1995; Helm *et al.*, 1998; Bishop *et al.*, 2004a,b; Sheppard *et al.*, 2004; Kuppermann *et al.*, 2006; Favre *et al.*, 2007; Benn *et al.*, 2012a,b). However, until relevant professional guidance is released

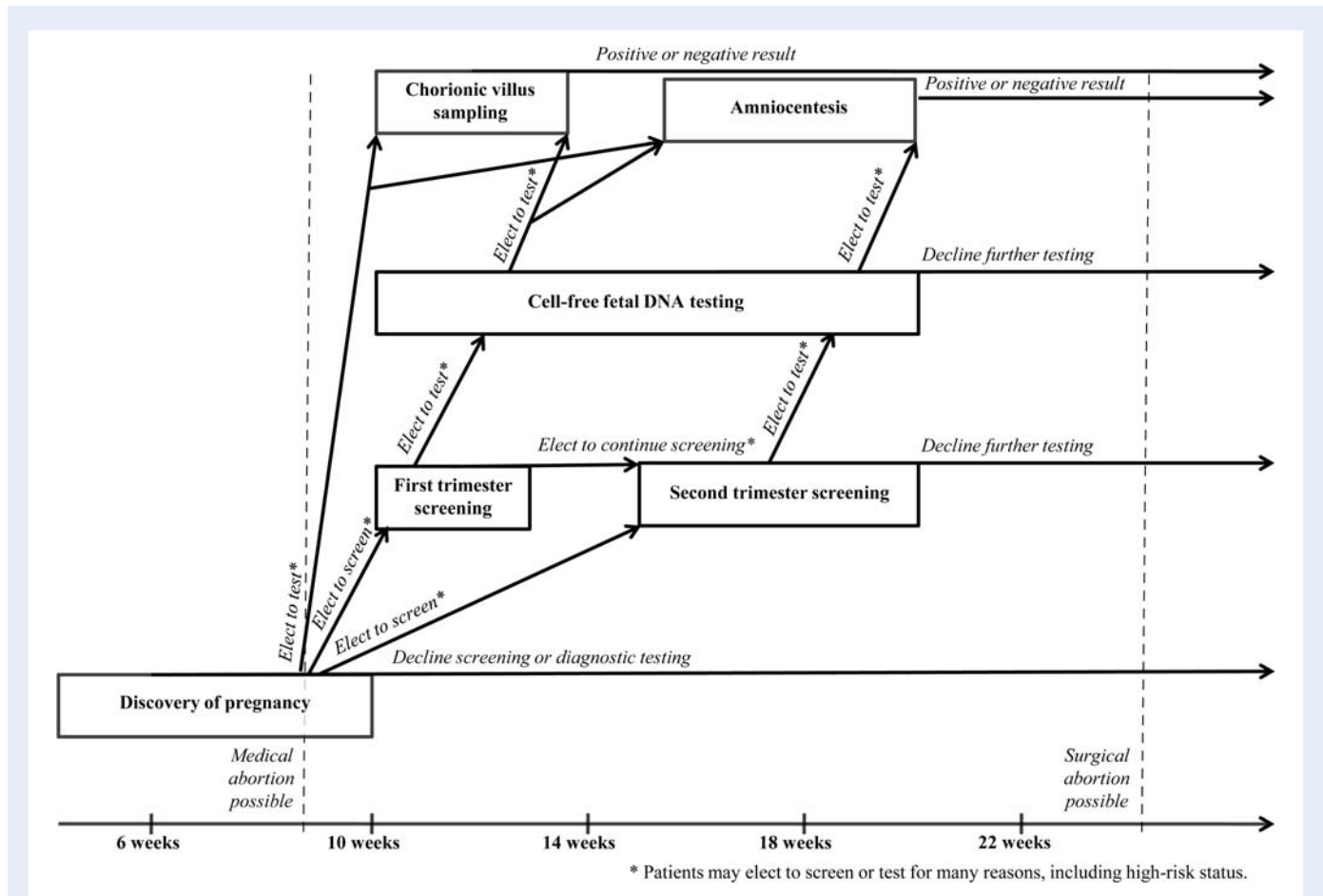


Figure 1 Prenatal screening and testing schematic incorporating cfDNA testing.

and clinicians change their practice patterns, it is unclear what the accepted standard of care for prenatal diagnosis of trisomy will be. Even when in compliance with standard of care, doctors are under tremendous pressure to deliver the most accurate results. Indeed, a recent court decision resulted in payments of \$2.9 million for a wrongful birth suit in which doctors failed to detect trisomy 21 prenatally (Ariel and Deborah v. Legacy Health; Steinbock, 2011). Prenatal tests for less well-defined conditions, including microarray comparative genomic hybridization, will create even more complications surrounding how to handle the trade-off between risk and sensitivity (Wapner et al., 2012). This increases the ethical and clinical need for guidance for clinicians as to how risk should be calculated in these increasingly complex scenarios.

Timing

Another commonly espoused feature of non-invasive tests using cfDNA is its potential to detect fetal DNA beginning at 10 weeks of gestation. Currently, the most accurate, widely available prenatal screening regimes, involving sequential or integrated screening, include an initial blood serum screen and an ultrasound between 10 and 14 weeks of gestation, followed by a second trimester serum screen between 15 and 20 weeks of gestation (see Fig. 1) (Malone et al., 2005). Women identified as high-risk or who otherwise

desire further testing are then given the option to have invasive prenatal diagnostic testing through CVS at 10–13 weeks of gestation, or amniocentesis at 15–20 weeks of gestation. While cfDNA testing is currently available only at 10 weeks or later in gestation, future non-invasive prenatal testing could potentially return results on trisomy status as early as 7–10 weeks. Supporters of cfDNA testing argue that early detection of trisomy without a risk of miscarriage could reduce the anxiety of many pregnant women, especially women with high-risk pregnancies (Ravitsky, 2009). Alternatively, if a woman received a positive result from these tests, she would have additional time to consider her options and seek out appropriate resources and guidance. Since first trimester termination involves fewer complications, this may provide positive health benefits to the patient (Niinimäki et al., 2009). Although it is still recommended that positive cfDNA tests be confirmed prior to termination of a pregnancy, if a woman did decide to terminate her pregnancy on the basis of a cfDNA test, the earlier timing may allow her to have an abortion before 9 weeks of gestation.

However, these simplistic scenarios do not take into account several complex practicalities. On the one hand, some women may want cfDNA testing to enable them to terminate a non-viable pregnancy as early as possible to avoid physical and emotional discomfort. However, a majority of pregnancies with trisomy 13, 18 and 21 spontaneously abort during the first trimester (Hassold and Chiu, 1985).

Conducting early testing to recognize a trisomic pregnancy could require women to make wrenching decisions about termination and generate considerable guilt and stress that might have been avoided had the fetus spontaneously aborted. In addition to the psychosocial effects, this process would also entail spending considerable medical resources on prenatal care for non-viable pregnancies.

As a further complexity in the timing of non-invasive testing, Fig. 1 shows that in order to gain the full benefits of early testing scenarios, a pregnant woman would have to become aware of her pregnancy early, obtain an appointment with an obstetrician or other relevant care provider soon after and receive testing immediately. One advantage to this scenario might be that women only have to receive two sets of test results, one from cffDNA testing and one from an optional invasive follow-up; current screening and diagnostic strategies elongate the return of results over several stages of screening even before invasive follow-up is generally offered, potentially enhancing parental anxiety and leading to confusion regarding conflicting test results from sequential screening in the first and second trimesters, as well as findings on the ultrasound. Early cffDNA testing also shifts the informed consent and counseling process, which would normally be extended over several weeks as various stages of testing are completed, much earlier into the pregnancy and into a highly condensed time frame (de Jong *et al.*, 2010; Farrell *et al.*, 2011).

However, if patients do not see their care provider early in the pregnancy, it is possible that the timing advantages of cffDNA tests may be eroded; medical abortions are available only until 9 weeks of gestation and current cffDNA testing methods require between 7 and 10 days to receive results. Furthermore, unlike European countries, in which laws on the availability of termination are generally enforced nation-wide, US states have a patchwork of abortion availability laws which make it difficult to generalize the potential benefits that earlier application may provide (Joyce *et al.*, 2009). Thus, although current practice of using cffDNA as an increasingly accurate screening tool succeeds in reducing the risk of false positives, the realistic advantages of early testing may currently be less than has been suggested, at least for patients with positive results. The greatest benefit of early testing will be realized by the large majority of patients who receive normal results, with the reduced waiting time to receive final, reassuring results or lessened anxiety while waiting for confirmatory diagnostic follow-up of what ultimately are found to be false-positive results.

While obstetric providers have some experience in dealing with these issues in the context of aneuploidy and neural tube defects, the importance of adequate pretest counseling and informed consent will increase as the positive predictive value of testing increases (Press and Browner, 1997; Asch and Wasserman, 2009; Deans and Newson, 2011; King, 2011). Although cffDNA tests are currently being used as part of a two-step screening process, in combination with invasive measures, it is technically possible that cffDNA testing may eventually attain diagnostic capabilities. This could potentially collapse the prenatal testing process into a one-step event. While it is true that the ability to deliver one set of test results, rather than the multiple stages of results inherent to the current prenatal testing sequence, will simplify counseling procedures, this will also increase the necessity of ensuring that patients achieve comprehensive understanding of those results and their implications before cffDNA tests are performed. Even with current testing strategies, there are questions about the adequacy of the informed consent process for

preliminary screening measures (Seror and Ville, 2009). While the invasiveness of diagnostic tests for aneuploidy require clear consent from the pregnant woman, the multitude of tests administered on multiple blood draws early in the pregnancy often lead to confusion and imperfect informed consent among pregnant women (van den Heuvel *et al.*, 2010). Whether the addition of non-invasive tests will simplify or complicate genetic counseling will depend on whether the tests are added to existing screening regimes as an additional test or used on their own as a first-tier test. Adding yet another set of risk factors and non-diagnostic results to the counseling process will require additional counseling resources. On the other hand, using cffDNA as a first-tier test would reduce the amount of risk information pregnant women are required to integrate. Either way, women should arguably receive genetic counseling before undergoing non-invasive testing, as well as after, as is traditionally done with current diagnostic mechanisms in order to preserve the integrity of the informed-consent process, as recommended by the NSGC. We believe that guidance regarding informed consent issues from larger professional organizations would help to standardize and equalize the availability of cffDNA testing from a clinical perspective and enhance the informed-consent process, which is essential from an ethical perspective.

Long-term applications: scope and cost creep

The range of conditions potentially detectable by future applications of cffDNA technology is very broad, and theoretically includes any genetic disorder for which a molecular test is available. Currently available tests are limited to fetal sex, Rh blood type and trisomy 13, 18 and 21 (Bianchi *et al.*, 2012a,b; Palomaki *et al.*, 2012a,b). In addition, proof-of-concept has been provided for the detection of microdeletions in the fetal genome (Peters *et al.*, 2011) and several single-gene disorders, including myotonic dystrophy, Huntington's disease and achondroplasia (Lo *et al.*, 2010; Sayres and Cho, 2011). Moreover, at least three groups have demonstrated the feasibility of mapping the whole fetal genome using sequencing methods (Fan and Quake, 2010; Liao *et al.*, 2011; Kitzman *et al.*, 2012). In theory, the ability to detect genetic conditions in the fetus will be limited only by our understanding of the relevance of genomic variants.

Regardless of the breadth of our knowledge of genetics, however, cffDNA testing will almost certainly never identify all relevant prenatal conditions that can be detected by ultrasound and other means, including structural disorders such as congenital heart defects and other physical malformations (Milunsky and Milunsky, 2010). Furthermore, there is the vexed question of the 3–5% failure rate of cffDNA testing due to low volume of fetal DNA and correlations with maternal obesity (Vora *et al.*, 2012; Bianchi *et al.*, 2012a,b), which raises serious questions about its potential as a first-tier screen or diagnostic test. While the inclusion of non-invasive testing may reduce the number of invasive procedures performed—estimates vary from 66 to as many as 98% avoided—it also means that until the procedure is diagnostic, or patients and doctors are prepared to treat it as such, payers may be asked to pay for an additional procedure (Chiu and Lo, 2012; Garfield and Armstrong, 2012). The current price of Sequenom's MaterniT21 test is ~\$1900, although Sequenom

estimates that the majority of this cost should be borne by third-party payers such that insured patients pay only \$235 out-of-pocket (Heger, 2011b). Other companies price cffDNA testing for aneuploidy at \$795 and \$1100, although it is not yet clear what percentage of this cost will be borne by private insurers. The cost of first and second trimester screening varies by location. For example, in California it is only \$162, but cost effectiveness estimates place the combination of screening and ultrasound at approximately \$500. Cost-effectiveness models generally place the cost of existing invasive diagnostic procedures in the ballpark of \$1277 (Little et al., 2010). Until cffDNA testing can reliably detect conditions that are not covered by the current screening regime or becomes the first-tier test for trisomy, the justification for the additional expenditure is unsettled. The California Prenatal Screening Program, for instance, has stated that it is unlikely to move to cffDNA testing as a first-tier test for several years (Goldman, 2012). This uncertainty contrasts clearly with countries such as the UK, which have a nationalized health care system in which reimbursement for new technologies is standardized by a central agency, reducing physician uncertainty surrounding the advisability of providing new tests (Rawlins and Culyer, 2004).

Furthermore, the timeline for the arrival of tests for a broader range of conditions, including single-gene disorders, on the market is unclear. As more tests become available, there is an additional danger of scope creep in prenatal testing (Schmitz et al., 2009a,b). Owing to the non-invasiveness of cffDNA testing, some clinicians or pregnant women may perceive 'no downside' to initiating as many tests as possible (Bailey et al., 2008). Indeed, there have been claims that the existence of non-invasive prenatal testing will make it desirable to test all pregnancies, not only high-risk pregnancies, for a variety of conditions. This raises many of the same issues that have been identified with expanded prenatal testing and carrier screening (Grosse et al., 2010). Among them, the concern that while additional testing may serve to alleviate anxiety in some high-risk pregnant women or allow them the reassurance of knowing that they have received every test available to them, it is equally possible that anticipating and interpreting additional tests may undesirably increase anxiety and stress on pregnant women in lower risk pregnancies. In addition, as discussed earlier, not all fetal abnormalities can be detected through non-invasive prenatal testing. However, without continual and comprehensive genetic counseling, undue emphasis on the scope and accuracy of such testing may lead women to overestimate the capabilities of testing, leading to the mistaken impression that there will be 'nothing wrong' with their baby (King, 2011). The accompanying stress and lack of trust in the medical establishment may have significant implications in the long term.

Conclusion

Clearly, there are complex and difficult calculations that must be made around the successful expansion of cffDNA testing. The existence of well-established screening mechanisms and a well-defined high-risk population may restrict the immediate clinical impact of such testing for aneuploidy to high-risk women as a second-tier screen. It is reasonably foreseeable, however, that aneuploidy testing is only the prologue to an expanding portfolio of prenatal tests, the ethical and clinical significance of which are considerable. In particular, there are vexed questions of validation, risk, timing and scope that need to be

addressed in the context of the best interest of the patient and medical practice as a whole. This situation may present a preview of potential difficulties in other countries, such as China and India, which have similarly dispersed and potentially privatized health care systems (Berman, 1998; Fan, 2008). Further research is encouraged on the impact of introducing these new, non-invasive tests in countries with high birth rates and decentralized health care.

Although we believe that in the absence of formal regulation or professional guidance, companies and clinicians offering cffDNA tests should adopt codes of best practices for the provision of these tests, this is not sufficient. There is an urgent need for regulators and policy-makers, including professional societies, to undertake a careful analysis of the anticipatable complexities we have laid out and derive standardized regulations and guidelines that can harness the potential benefits and minimize the risks of non-invasive prenatal testing.

Acknowledgements

The authors thank the two anonymous reviewers who provided valuable feedback on the manuscript.

Authors' roles

M.A. drafted and edited the manuscript and contributed substantially to the content. L.C.S., J.S.K., M.E.N. and M.K.C. reviewed and edited the manuscript and contributed substantially to the content.

Funding

This work was supported by NIH grant P50 HG003389 (Center for Integrating Ethics and Genetic Research). M.K.C. is additionally supported by NIH grant 1 U54 RR024374-01A1 (Stanford Center for Clinical and Translational Education and Research).

Conflict of interest

M.E.N. is a principal investigator on clinical trial NCT0145167, sponsored by Ariosa Diagnostics. The other authors have none to declare.

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