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Q&A

Emerging Considerations for Noninvasive Prenatal Testing

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Approaches to prenatal screening for common fetal chromosomal aneuploidies are undergoing a dynamic transformation in response to a greater understanding surrounding advances in the clinical utilities and limitations of noninvasive prenatal testing (NIPT).[10] NIPT has been clinically adopted as a screening tool for aneuploidies, such as Down, Edwards, and Patau syndromes, and methodologies are primarily based on next generation sequencing of cell-free DNA (cfDNA) from maternal plasma. The cfDNA comprises maternal DNA fragments as well as placental DNA fragments that serve as a fetal surrogate marker. While NIPT was initially recommended as a screening option for high-risk women from about 10 weeks of gestation, recent clinical studies demonstrate that NIPT outperforms conventional screening approaches (e.g., first trimester combined test) regardless of the maternal age spectrum. The American College of Medical Genetics and Genomics updated recommendations in 2016 to include informing all pregnant women that NIPT is a screening option for conventionally screened aneuploidies. However, the potential expansion of NIPT utilization in prenatal care practices is faced with evolving challenges. Depending upon the laboratory, test methodology, and bioinformatics processes used, NIPT result reporting is not standardized. The decision of whether to use NIPT screening is ultimately that of the informed patient. However, it will also be driven by the ability to clearly communicate the risks and benefits of screening approaches by the clinical care team. This highlights the need for multidisciplinary collaboration in the clinical implementation of NIPT. To address these exciting advancements and emerging considerations, we invited a group of experts and early adopters of NIPT screening from multiple disciplines (genetic counseling, obstetrics, genomics, ethics, and clinical chemistry) to share their views on this topic.

Does NIPT have a role beyond screening for fetal chromosomal aneuploidies in high-risk pregnancies?

Judith Jackson: NIPT plays an important role in screening for aneuploidy in both high-risk and low-risk pregnancies. When it became available clinically in the US in 2011 (high-risk) and 2012 (low-risk), my department was one of the early adopters. We have ordered NIPT for over 2600 low-risk pregnancies. Although some studies indicate the positive predictive value (PPV) is lower in low-risk populations compared to high-risk populations, clinical validation studies clearly demonstrate that NIPT significantly outperforms traditional analyte screening. The decision to utilize NIPT was not a difficult one for us.

Furthermore, low-risk women have the greatest number of pregnancies affected with Down syndrome (DS), trisomy 13 (T13), and T18. In our experience, using NIPT in this population specifically resulted in an earlier diagnosis of aneuploidy and may have prevented missed diagnoses. Parental anxiety from false-positive screening results and soft aneuploidy markers is an important consideration. Aside from the effects of stress on

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[10] Nonstandard abbreviations: NIPT, noninvasive prenatal testing; cfDNA, cell-free DNA; PPV, positive predictive value; DS, Down syndrome; T13, Trisomy 13; CNV, copy number variation; CVS, chorionic villus sampling; aCGH, array comparative genomic hybridization; NT, nuchal translucency; 45, XO, Turner syndrome; EQA, external quality assurance; NIPD, noninvasive prenatal diagnosis.

the pregnancy, these findings require substantial counseling support time and contribute to patient dissatisfaction. NIPT used in the first trimester minimizes the likelihood of parental anxiety related to these factors. Although cost efficacy is difficult to measure, using NIPT for all patients has essentially eliminated the time required to support couples who have received false-positive results.

Mark Evans: NIPT is a very good screening test for DS and other well-characterized aneuploidies such as T18. The clinical performance and PPV of NIPT is much lower for T13, sex chromosomes, and subchromosomal deletions and duplications. It is important to note that at age 30, only about one-third of common aneuploidies diagnosed prenatally are DS. At this age group, incidence of DS is about 1/1000 while the yield of abnormal microarrays is 1/100. Thus, the incidence of the other aneuploidies, including copy number variations (CNV), is 10× greater than that of DS. Thus, universal NIPT is simply not a reasonable frontline approach at this point for all pregnancies. When NIPT can reliably do everything that can be accomplished with chorionic villus sampling (CVS) and array comparative genomic hybridization (aCGH), then my opinion will change. From a public health perspective, currently NIPT performed in younger women is essentially ignoring the major issue and focusing on the minor one.

While NIPT has higher clinical sensitivity when used as a screening test for DS in younger women, the estimated cost to find the first case of DS detected via NIPT, which would be missed through multimarker screening [free β hCG (human chorionic gonadotropin), PAPP-A (pregnancy-associated plasma protein A), and nuchal translucency (NT)], would be about US $3 million. Given that the cost of caring for and raising a DS child is about $1 million, from the perspective of a minister of health for many countries or managed care director in the US, this approach is simply far too expensive.

My opinion is that that all women regardless of age should be offered diagnostic testing and aCGH because (*a*) the minimum 1.0% risk of an abnormal CNV by aCGH is far above the gold standard comparison of 0.5% risk at age 35 years, (*b*) the yield of abnormalities found is substantially higher by aCGH than by NIPT, and (*c*) the risks of an adverse event from diagnostic procedures in experienced hands is much less than often quoted by NIPT companies (1/500 for both CVS and amniocentesis).

Subhashini Chandrasekharan: cfDNA screening for common chromosomal aneuploidies has a role beyond high-risk pregnancies, especially when women and families are able to make an informed decision to opt for such screening. Clinical validation studies indicate the clinical specificity of NIPT for detecting T21, T18, and T13 in average-risk pregnancies is comparable to that in high-risk pregnancies. Compared to first trimester serum screening, NIPT would reduce the number of unnecessary invasive procedures performed in an average-risk pregnancy population and decrease procedural risks/adverse outcomes, due to its lower false-positive rate. While coverage for NIPT in low-risk pregnancies is increasing by most private health insurance providers in the US, there still remain questions about whether all state-based payers will cover NIPT. This raises concerns about inequity in access to and quality of prenatal care, especially for women from low socioeconomic groups who cannot afford out-of-pocket costs for NIPT. Equitable use of NIPT for common aneuploidy screening in all women will require further reductions in test cost. Public payers will need to include NIPT as part of medically necessary prenatal services covered for all women independent of their risk. Provision of pretest and posttest counseling as standard of care for all women will also be necessary to ensure appropriate use of NIPT. However, this raises important and difficult questions about various resources needed by the health systems.

Lyn Chitty: Yes, NIPT does have a role, particularly in remote geographical areas where access to good ultrasound scanning for NT measurement is poor. In these circumstances, assuming a dating scan can be performed, NIPT alone may be the best option for trisomy screening. NIPT implementation for all pregnancies may also be preferable where there is no existing prenatal screening program upon which to base the offer of NIPT as a contingent test. Much will depend on cost and local healthcare pol-

icies. At present, it is cost that precludes offering it to all women in a fully publically-funded health sector with a well-established screening program. These costs include not only the direct cost of the test but also pre- and posttest counseling, which is essential, time consuming, and often not considered adequately in economic analyses or funding allocations.

Glenn Palomaki: NIPT via next generation sequencing of cfDNA in maternal plasma will likely become a first line prenatal screening test for common aneuploidies in the US in the near future. In 2016, both the American College of Obstetricians and Gynecologists and the Society of Maternal Fetal Medicine, as well as the American College of Medical Genetics and Genomics, published updated recommendations to indicate that NIPT may be offered to women regardless of their risk category. The transition time from routine serum screening to routine NIPT screening will likely be driven by these clinical recommendations, costs of testing, and whether broad insurance coverage is available. Recently, several US and global clinical laboratories have licensed the needed intellectual property to allow for NIPT to be implemented in as many as 15 US laboratories by the end of 2017. Should the insurance reimbursement (not cost) drop below $200 or even $300, the transition might occur within 2 or 3 years. Alternatively, if the charges remain high and variable (from $400 to over $2000), then it will be more problematic for insurance companies to cover these costs for the 2–2.5 million women in the US who are likely to be offered such testing in the future.

How often do you encounter incidental findings associated with NIPT? What conditions are associated with incidental findings? Would you consider this as an advantage or a limitation of NIPT? Should laboratories report incidental findings found with NIPT?

Mark Evans: As director of a clinical center for prenatal diagnosis and screening and not an NIPT laboratory, we only receive information the laboratory chooses to report. As a clinician, I proceed according to the report. However, for many years I was the medical director of a cytogenetics laboratory and I understand there are often nebulous issues that require internal interpretation and judgements that cannot be easily understood by noncytogenetically trained physicians or patients.

The same principles must be applied to NIPT. As with all new technologies, we get the numerators of problems far before we understand the denominators. Uncertainty is common and can only be resolved with years of experience and case interpretations to determine the likelihood of each laboratory discovering its own variation as to whether it is clinically pathological or not. We have seen this for CVS vs amnio cytogenetics, diethylstilbesterol cancer risks, choroid plexus cysts, intracardiac echogenic focus, and numerous others ultrasound markers. Abnormal clinical cases get published first, but we only finally get realistic risk estimates after years of finding markers in cases that don't have clinical pathology. The same issue also exists for NIPT, it is just part of the maturing process of this technology.

Judith Jackson: We order approximately 1500 NIPT tests annually in high and low-risk patients. We have had several unexpected results that have changed the course of our care.

In 3 maternal mosaic Turner syndrome (45, XO) cases, NIPT results indicating increased risk for 45, XO were not confirmed in the fetuses but were confirmed in their mothers. The information allowed these patients to have genetics and cardiology consultations for managing the increased chance for heart defects and aortic dissection. These women also expressed relief to learn about a possible underlying reason for their learning disabilities.

NIPT positive findings not confirmed in the fetus or mother are presumed to be confined placental mosaicism. Placental dysfunction has a well-known relationship with poor outcomes. One of our confined placental mosaicism cases was for Trisomy 16, and the awareness led to heightened surveillance and lifesaving preterm intervention. The mother developed intrauterine growth restriction, severe sudden-onset preeclampsia, and HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome, ultimately becoming comatose after renal and hepatic failure and was considered for liver transplant. Subsequently, she and her preterm daughter fully recovered. Intensive management of this patient's pregnancy, because of the early awareness, was critical to a favorable outcome.

In another case, NIPT in the first trimester indicated a male fetus. At the second trimester ultrasound examination, the fetus appeared female. After genetic counseling, the patient underwent amniocentesis, and the fetus was confirmed 46, XY male and ultimately diagnosed with androgen insensitivity syndrome. The mother sought additional genetic consultation and will raise the child as a female with specialized postnatal care.

Though we have not had a finding from NIPT that revealed potential risk for maternal cancer, we would certainly want to know this information.

Subhashini Chandrasekharan: I understand that inci-

dental findings can be associated with testing for any of the conditions included in current cfDNA screening tests. Whether this is an advantage or a limitation really depends on whether women and families are fully prepared for the possibility of such incidental findings through adequate pretest counseling when they opt for cfDNA screening, as well as the quality of posttest counseling and diagnostic follow-up. It also depends on the nature of the information itself since detection of a maternal tumor may be lifesaving even if this incidental finding creates anxiety. However, identifying a previously undetected sex chromosomal anomaly in the mother may have less clinical utility and may even be confusing to the family. I believe laboratories should report incidental findings but only within the context of robust pre- and posttest counseling and in accordance with the wishes of the patient as expressed through informed consent or when deemed medically appropriate.

What key factors should be included in the informed consent process for NIPT?

Lyn Chitty: I could write an essay on this! I think the first thing to say is that what is included will depend on the platform being used and what is being offered. The following points should be included:

1. NIPT is a screening test, and a positive result requires confirmation by invasive testing.

2. Specifically what the test is screening for (including "extras" if tested for—subchromosomal anomalies, sex chromosome aneuploidies).

3. Why results may be discordant and not accurately reflect the fetal karyotype.

4. A description of the major trisomies, using information regarding DS that is balanced and including the fact that Edwards and Pataus are usually but not always lethal.

5. Clinical sensitivity and specificity for the trisomies tested.

6. Pretest counseling needs to be based on the platform being used, so if there is a risk of incidental findings these should be discussed.

7. Whether testing for fetal sex is an option and if so do they want this. If yes, then the possibilities of detecting sex chromosome anomalies and what these are need to be discussed, together with the possibility of detecting maternal sex chromosome anomalies. What the clinical sensitivity and specificity is for fetal sex and sex chromosome anomalies.

8. How long the results will take to come back and how the result will be fed back.

9. The test may fail or give an inconclusive result—and what the options are then.

10. That it is the parents' choice whether or not to have NIPT, and if positive it will be their choice what to do next—invasive test or not.

11. If offering this as an alternative to invasive testing or in the presence of an ultrasound anomaly, the fact that a substantial number of potentially pathogenic chromosomal rearrangements will not be detected by NIPT.

Mark Evans: A major difference exists in the ordering process for NIPT compared to virtually all other laboratory tests in that the order form signed by the physician states that the physician has completely counselled the patient about limitations of NIPT. There is no doubt that 99% of ordering physicians do not actually know these limitations nor have they spent considerable time with the patient counseling them on these issues. Thus, the laboratories are establishing a defense to blame the physician for the inevitable situations when NIPT does not report accurate results.

The primary issue that must be addressed is the difference between screening and diagnostic tests, i.e., the former only provides an odds adjustment vs a definitive answer. NIPT counseling should include all the standard options available to patients, including taking appropriate personal and family histories, demographics, ethnicity, and explanation of the types of genetic problems (Mendelian, multifactorial, chromosomal). Patients should be offered the option of having no screening, Mendelian panels of varying sizes, combined biochemical and ultrasound, NIPT, or diagnostic procedures and laboratory tests for definitive answers.

We routinely see patients in our program who have an abnormal NIPT for a sex chromosome abnormality or subchromosomal CNV such as DiGeorge syndrome, who are convinced that NIPT has definitively diagnosed the abnormality. They are astounded to discover that the PPV is not the same as clinical sensitivity, and their risk may actually be as little as 5%. On the other end, many patients believe that NIPT can find "everything," and there is no additional information that can be obtained by diagnostic procedures. A major educational effort for both physicians and patients is required. For the frontline clinician who has neither the time nor expertise for such extensive counseling, it would be far better to have genetic consultations/counseling performed by an independent genetics center.

Judith Jackson: In our department all patients considering aneuploidy screening meet with a counselor for pretest counseling. Our objective is to have well informed patients who understand all options for aneuploidy testing, including diagnostic testing, and can then make the best choice for themselves in their current pregnancy. We collectively determined that our standard NIPT profile would include microdeletions and sex chromosome ab-

normalities and do not customarily offer NIPT testing without these additional assessments.

Our mission of patient advocacy means that we devote substantial counseling resources to this education, and patients spend an average of thirty minutes with the counselor. We function as a referral facility for obstetrical practices using our hospital to ensure thorough discussion and consent for testing and that current and accurate information is provided to patients. The process of informed consent for NIPT is dynamic. Information, recommendations, and availability of NIPT change at an astounding pace, and achieving informed consent, including documentation, requires vigilance.

When NIPT is made available to all pregnant patients, many will have their blood drawn at their obstetrician's office where counseling may be limited. Continued development and use of counseling aids such as those provided by the Genetic Support Foundation, which offer nondirective videos related to prenatal testing and specifically NIPT, will be essential. Laboratories providing NIPT tests also have a responsibility to support development of these counseling aids.

Glenn Palomaki: The information to be included in the consent process has been well described in recommendations from several professional organizations. I would like to provide the insights gained since my academic group offered NIPT as a routine first line screening test offered through primary obstetrical care offices to the general pregnancy population in Rhode Island. We enrolled providers, made informal presentations, and supplied patient educational materials we developed. These materials included information suggested by professional organizations, were at the 8th grade reading level, and subject to modifications after input from focus groups of pregnant women. We evaluated the educational and consent process using structured interviews with 100 women having NIPT and their provider's responses on a questionnaire. Both groups agreed that the interaction between provider and patients averaged about 5 to 6 minutes, but women reported this amount of time was sufficient (97%), they had their questions answered (97%), and felt the provider conveyed that the test was optional (99%). The providers reported that they and their staff felt prepared to offer NIPT as part of their routine practice. Over 10 months, nearly 3000 women were screened while testing was offered at no charge to the patient or her insurance.

Subhashini Chandrasekharan: There is a lot of information that needs to be conveyed to women and families about the strengths, limitations, and utility of cfDNA screening especially for the different conditions included. This information is also likely to keep changing as test options expand. I think it can be particularly challenging to convey all that information during the consent process given how little time most healthcare providers have to spend with patients during prenatal appointments. I propose that more attention be focused on the process of informed decision-making, with formal informed consent perhaps as the last step. Robust informed decision-making means providing information about NIPT to families in ways that are easy to understand, giving them sufficient time to digest and contemplate all their choices and options (including not getting tested at all), and enabling them to provide true "informed" consent. This will require more research on effective methods for educating patients and families and rethinking how we educate healthcare providers, especially obstetricians and gynecologists, nurse midwives, family practitioners, and others at the frontline of prenatal care, so they are empowered to participate in shared informed decision-making.

A recent 2016 position statement by the American College of Medical Genetics and Genomics recommends reporting of clinical performance characteristics by laboratories performing NIPT; moreover, laboratories should not offer NIPT screening for Patau, Edwards, and Down syndromes if they cannot report detection rate, clinical specificity, and PPV. What challenges, if any, may this impose for laboratories performing NIPT?

Lyn Chitty: The main challenge is that laboratories will need to perform very large validation sets to deliver accurate clinical specificity and personalized PPVs. While validating and determining clinical sensitivity and specificity (with confidence limits) for T21 may not be so challenging as this is a common trisomy, getting sufficient numbers for the other trisomies, T13 in particular, will be more challenging.

Glenn Palomaki: Laboratories in the US are required to validate their laboratory-developed tests before offering a test clinically. Such validation includes analytical and clinical validation (clinical sensitivity and specificity), with clinical validation often performed using samples with known karyotypes. This is a relatively straightforward process for validation of common trisomies since these have been the basis of serum and ultrasound screening for decades. However, these clinical performance estimates have not always been available for the less common disorders (e.g., select microdeletions, sex chromosome aneuploidies) before introduction into clinical practice.

For each disorder of interest (e.g., T21) there is only 1 detection/false positive for a given methodology. However, the PPV associated with that test depends on the population being tested. NIPT in a general pregnancy

population would be expected to have a lower PPV than that same test in a high-risk population. More important than the PPV is the ability to provide appropriate patient-specific risks (an individualized PPV), which is the process used for decades in serum screening. Modeling can help generate such risks, but they must be validated. Some clinical laboratories do assign an apparent "risk" for each pregnancy, but it is not clear whether it is an average risk for all those with a positive result (e.g., a PPV) or an individualized risk for that specific pregnancy. Some reported risks are as high as "99 in 100," indicating that among 100 screen positive pregnancies, 99 will be true positives with only 1 false positive (99:1 if expressed as an odds). However, follow-up of pregnancy outcome from those same laboratories show that the group of women with such risks are only correct 50% to 80% of the time (odds 1:1–4:1). This indicates that the reported risks of 99:1 are far too high.

Mark Evans: In the 1980's, when in vitro fertilization moved from a purely experimental technique done at only a few centers to multiple centers throughout the world, there were very few regulatory controls. In the US, by law the government could have no involvement including regulatory oversight because no federal funds could be spent on the procedure, and it became a "wild west" scenario of unfettered claims with little data to back them up. Centers that had never achieved even a single pregnancy would quote national statistics implying that those applied to them. Many patients were, in fact, deliberately mislead.

For NIPT, clinical services see the claims of some laboratories that report having fewer "no calls" and "incorrect calls" nationally that are fewer than we have seen in our own centers. Reported statistics are only as good as their reliability. To date these have been very problematic. As NIPT expands its scope to rarer and more difficult conditions, the validity of performance will be even more difficult to obtain under the best circumstances, and the ethical integrity of the laboratories will be even more paramount in believability of reported data.

Judith Jackson: Laboratories performing NIPT should report detection rates, clinical specificity, and PPV, which should be automatically included on all positive results. In a clinical setting it is unrealistic to expect a provider to understand how to calculate PPV.

What challenges exist in monitoring the quality performance of a NIPT service?

Glenn Palomaki: A challenging aspect of cfDNA testing is the need to develop a reliable external quality assessment/external proficiency testing scheme due to the wide variety of methods used by NIPT laboratories to extract information from the cfDNA in maternal plasma. Unlike most molecular genetic testing, the goal of NIPT is not to identify a specific variant(s) of interest, but to extract information in such a way that allows a highly confident estimate of the fetal genome at the chromosome level. Methods have relied on regions with differential methylation, counting fragment reads aligned with each chromosome, targeted counting of chromosomes of interest, targeting highly polymorphic single nucleotide polymorphisms, and examining fragment lengths by chromosome region. The task of creating an artificial proficiency testing sample that can mimic the cfDNA patterns relied upon for the current (and future) NIPT modalities is daunting. One alternative solution is to use plasma from women with a known pregnancy outcome, but this strategy is also complicated by a different set of barriers. The College of American Pathologists has already implemented quality assessment guidelines (laboratory checklist questions) as well as explored options for implementing external proficiency testing.

Lyn Chitty: There are no external quality assurance (EQA) schemes currently in place for NIPT. Any scheme will need to include the laboratory-specific performance characteristics and flexible result reporting schemes to allow EQA results to be reported identical to patient results. The production of sufficient material for an EQA scheme for NIPT will be challenging. In Europe, preliminary studies that used "spiked" maternal plasma for a NIPT EQA scheme for fetal sex determination did not deliver results as well as using pooled maternal plasma, and the single nucleotide polymorphism-based NIPT platforms cannot perform testing using pooled plasma. The challenge here is either going to be development of an artificial product for testing or use multiple small volumes distributed to groups of laboratories. Such a scheme is currently being piloted in a small group of laboratories in Europe. A survey of laboratory NIPT reports has been conducted and discussed at a recent International Society for Prenatal Diagnosis meeting and a consensus report discussing what information to include on reports will be published and ultimately provide guidance for a formal EQA.

Subhashini Chandrasekharan: There is currently no way to compare the performance of different services that use different methods or proprietary algorithms for analysis of cfDNA. Unlike most other genetic tests, to the best of my knowledge there is no independent proficiency testing available for NIPT. Such quality assurance schemes are particularly relevant for assessing the performance of NIPT for rare aneuploidies, sex chromosome aneuploidies, and CNVs like microdeletions. These have much lower PPV compared to that for the 3 common aneuploidies, but are increasingly offered by many labo-

ratories. Statements from professional societies also do not offer guidelines on how results should be reported, particularly for creating a more uniform reporting format that may foster better quality control assessment.

Mark Evans: Many clinical and laboratory services in prenatal diagnosis and screening (e.g., CVS, NT assessment, α fetoprotein, ultrasound, aCGH, and now whole-exome sequencing) in the US were introduced through NIH-funded trials by vetted investigators and produced refereed publications before extended commercialization. NIPT, on the other hand, has been largely promoted by companies often headed by engineers, rather than physicians, who do not come with a traditional background in medical ethics and are unable to provide support to clinicians in complex clinical situations. The strategy used by some companies is to attack the tertiary centers with sales staff, telling frontline generalists that they no longer need such centers to perform counseling and NT assessments, and that diagnostic procedures are so dangerous and can be avoided. Quality performance evaluation must ultimately include assessment of the entire spectrum of interactions and data which can only be as reliable as the integrity of those providing it and regulatory mechanisms to ensure its accuracy.

In your opinion, what newer NIPT application(s) would be the next most likely to become widely available clinically?

Lyn Chitty: I think we need to be clear regarding terminology here. NIPT for aneuploidy is NIPT and is a screening test. There are already other applications based on analysis of cfDNA in maternal plasma in use, but these are diagnostic and should therefore be referred to as noninvasive prenatal diagnosis (NIPD). These include PCR-based tests for fetal sex determination where Y-chromosome sequences are targeted and fetal RHD typing in RhD negative mothers. Other NIPD tests are increasingly becoming available and now frequently use sequencing based technology, include NIPD for monogenic disorders. In our laboratory, when offered in pregnancies at increased risk because of a family history or ultrasound findings, these tests are considered diagnostic and do not require confirmation by invasive testing. These are the tests that should become more widely available for families at high genetic risk, but development is largely focused on academic or public sector laboratories because of lack of commercial interest to date since they are relatively small volume and potentially expensive tests. Furthermore, at present, for rare diseases many of these tests must be developed on a bespoke, family-specific basis. Which tests are likely to be developed next—possibly NIPD for the more common monogenic conditions such as sickle cell anemia? In some countries, screening low-risk pregnancies for more common pathogenic new dominant mutations may become available. This is likely to be costly and will require sequencing of the mother as well as the cfDNA extracted from plasma. Validation may be challenging since large numbers of cases will be required, and confirmation by invasive testing is likely to be needed for some time. Advances in NIPD will also raise important ethical issues with the barriers to testing presented by invasive procedures removed if the scope of testing becomes broader.

Subhashini Chandrasekharan: NIPT for detecting genome wide CNV is already possible and may become widely available clinically. In addition, NIPD for several single gene disorders is already being offered as a clinical service in the UK. I believe that NIPD for sets of common Mendelian disorders like β-thalassemia, cystic fibrosis, and other conditions currently included in preconception carrier screening will likely become clinically available as well. Although technically possible with cfDNA at this time, the cost of such testing is prohibitive for actual clinical use. These applications may alternately become fetal cell–based as demonstrated by noninvasive prenatal genomic profiling of fetal trophoblasts. Noninvasive fetal whole-exome and whole-genome sequencing may become more widely available and even cheaper going forward, but current practice guidelines from all professional societies do not recommend clinical use of these applications, partly due to ethical concerns.

Glenn Palomaki: Proof of concept studies have shown that it is possible to reliably perform diagnostic testing for serious single gene disorders using cfDNA. One could envision selecting 50–80 such disorders where the woman would initially undergo screening to identify carrier status (perhaps using the buffy coat from the plasma collection tube). For most women, the screen would be negative, but others would be carriers for 1, 2, or, rarely, 3 or more disorders. At that point, focused testing of the cfDNA for only those disorders for which the mother is a carrier could be undertaken. Hopefully, such testing could be reliably performed without the need for identifying and sampling the biological father.

Judith Jackson: From our perspective, the next widely available clinical application associated with NIPT is likely genome-wide CNVs. We currently offer this technology for specific situations.

Mark Evans: Most people don't anticipate the changes that create a new paradigm they hadn't previously seen or considered. Five chromosome NIPT has caught up to florescence in situ hybridization and qfPCR (quantitative fluorescence–PCR) of the 90's. Now 24 chromosome NIPT has been introduced and has caught up to the karyotype. This is the next test that will become widely available over the next

couple of years. Diagnostic laboratory tests have moved on to increased clarity and discrimination with microarrays, selective sequencing, and now whole exome sequencing. Until NIPT catches up to diagnostic tests, there will always be a diagnostic advantage to them.

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