Genomic testing reaches into the womb

Malorye Allison

Next-generation sequencing has provided long-sought-after noninvasive tests for prenatal screening, and a small cadre of companies has stepped up to push it into the clinic at warp speed.

Competition is heating up in the noninvasive prenatal test (NIPT) market, now valued at over $1 billion. Sequenom (San Diego), which was first to the US market in October 2011, racked up its 100,000th MaterniT21 test sale in April and expects to sell more than 150,000 tests this year alone. The other three US competitors are not releasing sales figures, but all are reporting growing demand and a steady stream of new deals. US insurers are also covering the test in growing numbers and specialty medical societies are releasing guidelines about them at record speed. Meanwhile, China’s Beijing-based BGI inked a NIPT deal last fall with Singapore-based Inex Innovations Exchange, which provides a gateway to marketing in South and Southeast Asia, the Middle East and Australasia. NIPTs are spreading around the world.

So far, the tests screen for only a handful of the most common aneuploidies, including Down syndrome and defects associated with sex chromosomes, but NIPT makers and others are quickly developing ever-more comprehensive tests to gain market advantage. It is one of those classic cases where the science limped along, and then achieved breakthroughs that suddenly made conceivable what once seemed inconceivable. Developers predict that they’ll quickly move from routinely detecting aneuploidies to detecting subchromosomal aberrations and then single-gene disorders, but it’s not clear yet which specific technology is going to win this contest.

The success of NIPTs underscores the huge unmet need for better noninvasive genetic-disease screening. “The pace at which this technology has been integrated into clinical care is unprecedented,” says Diana Bianchi, executive director of the Mother Infant Research Institute at Tufts Medical Center (Boston), and an advisory board member at NIPT-maker Verinata Health (Redwood City, CA, USA), which was acquired by Illumina (San Diego) for $450 million at the start of this year. Noninvasive prenatal testing was described as “one of the most rapidly growing areas utilizing next-generation sequencing,” in a press release about that deal. The rollout has not been without hiccups (**Box 1**), but the field has tremendous momentum.

A brief history

Researchers have been trying to develop better noninvasive prenatal genetic screening tools for decades. At first they tried to use whole fetal cells from the maternal bloodstream. Success for such tests has proved elusive so far because just a tiny number of such cells exist—about 10 for every 200,000 billion of the mother’s. Most groups have abandoned that approach, although at least one company, CellScape (Newark, CA, USA) is still pursuing it and says it expects to launch its test by 2014.

In the late 1990s, researchers discovered that cell-free fetal DNA (cffDNA), which is released when placental cells break down, is a much more plentiful source of fetal DNA, comprising a generous 5–10% of the genetic material in a pregnant woman’s bloodstream. At that percentage, cffDNA can easily be detected, using standard PCR, as early as 7 weeks’ gestation. The first clinical application of cffDNA was in detecting Rh-positive fetuses in Rh-negative women—done routinely in Europe now—followed by paternity and sex determination.

Since then, next-generation sequencing (NGS) has come of age, allowing faster and cheaper sequencing. In 2008, two groups published pivotal papers, demonstrating that by sequencing cffDNA, researchers could accurately detect aneuploidies. Both groups, Stephen Quake’s at the Department of Bioengineering at Stanford University (Stanford, CA, USA)[1], and Y.M. Dennis Lo’s at the Chinese University of Hong Kong[2], used massively parallel shotgun sequencing (MPSS) to look at fragments across the entire fetal genome. They sequenced and mapped 5- to 10-million short (25–36 base pair) sequence tags to individual chromosomes and then counted how many came from each chromosome. Where aneuploidy occurs, proportionally more fragments originate from the extra chromosome. Detecting aneuploidy by MPSS, as a result, becomes essentially a counting problem (**Fig. 1**).

Noninvasive prenatal testing. Moving from aneuploidies to whole genomes. It’s just a matter of time, some say.

Entrepreneurs saw this as an opportunity to replace older prenatal screening tests, which rely on blood biomarkers and ultrasound findings. Those tests find less than 20% of possible genetic defects when used in the first trimester. Even for trisomy 21, the most common

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Box 1 NIPTs accuracy questioned

An article published in the *Wall Street Journal* in April described alarming anecdotal reports of healthy pregnancies being terminated in one case (test maker not identified) and almost terminated in another (involving Sequenom's test), as a result of false-positive NIPT results[16]. That article also described a case in which a Down syndrome diagnosis was missed by a NIPT (Verinata's), but later detected on ultrasound. It suggested the tests might not work as well in real "work-a-day medicine" as they had in studies and highlighted the fact that some women think of the tests as being diagnostic, not screening tests, and are not getting the recommended follow-up to confirm positive findings.

NIPT makers strongly dispute the first of those charges. "The results in clinical practice are exactly what we predicted," says Sequenom's CMO Allen Bombard. False positives and negatives are extremely rare, but expected, he says. "Positive results are supposed to be confirmed." Clearly, not everyone is taking that step, and some critics worry that as NIPTs become more popular, the number of false positives could become substantial. "No one has done a national analysis, but it's believed that the results of some 5% to 10% of positive screens are not being confirmed," says Michael Watson, director of the American College of Medical Genetics and Genomics.

NIPT developers concede that there is confusion about what screening means, but point out that their tests are much more accurate than the biomarker-based screens they are replacing. Some women terminate pregnancies based on the older screens, which also require confirmation. "The conventional screening tool has a 5% false positive, whereas with a NIPT you get less than 1 in a 1,000 false positives," says Ariosa's CEO Ken Song. "That means 50 times fewer women will be falsely told there is a high risk of a fetal genetic defect." Song adds that his company has tried to alleviate confusion by reporting not a "positive or negative result, but a relative risk." They also include information about the amount of fetal DNA in the sample, which affects the reliability of results.

Some false positives, possibly as much as 1% of them, could be the result of a condition called confined placental mosaicism, which is caused by genetic mishaps, such as nondisjunction of chromosomes, in trophoblasts that make up the placenta. "When you see trisomy 21 in the placenta, you usually see it in the fetus also, but there is less concordance with other trisomies or sex chromosome aneuploidies," Song explains.

A few weeks after the *Wall Street Journal* article was published, Verinata released clinical laboratory performance data collected from the first 9 months of selling their verifi prenatal test. The report covered almost 6,000 tests and found five (0.08%) false-negative results and 14 (0.2%) false positives. Meanwhile, newcomer Natera claims its Panorama test has "best-in-class" sensitivity (99%) and specificity (100%). The product is unique because it is the only one so far that works by analyzing SNPs rather than by sequencing. Verifiable 100% specificity could be an attractive selling point. *MA*

live-birth aneuploidy, such screening has only a 77–86% detection rate and a 3.2–5.6% false-positive rate. Although a 5% false-positive rate may seem small, it results in a substantial number of unnecessary tests to find relatively few cases of real defects. Trisomy 21 occurs in about 1 in 691 live births, according to the National Down Syndrome Society (New York).

With traditional screening, large numbers of women are offered invasive follow-up procedures that require sampling either placental fluid (amniocentesis) or tissue (chorionic villus sampling; CVS) for karyotyping, or, more recently, chromosomal microarray analysis (CMA), which has been established as superior to karyotyping[3]. Many women are skittish about these invasive tests, which involve inserting a long needle into the pregnant women's abdomen or snaking a catheter through her cervix. They also come with a slight risk of miscarriage. As a result, some women forgo amniocentesis or CVS despite being advised to undergo it. It's estimated that 60,000 invasive prenatal genetic diagnoses were carried out in 2009 in the United States, just over 1% of the 5.2 million women in the country who were pregnant that year.

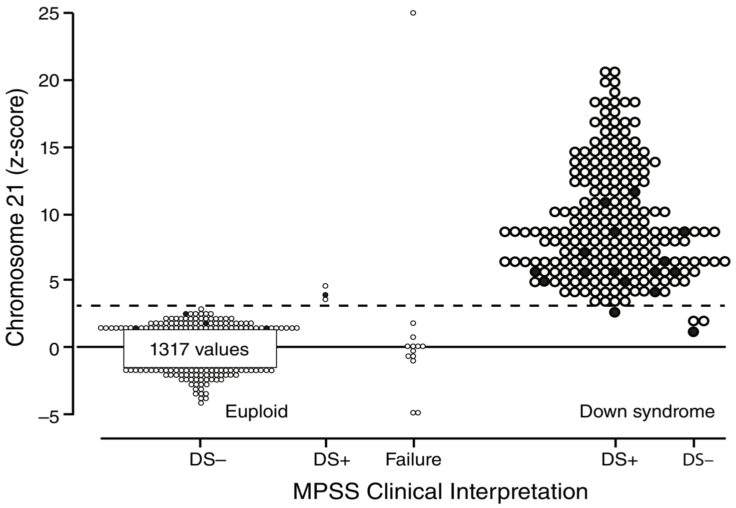

Figure 1 Clinical validation of noninvasive trisomy 21 testing. Of 1,696 samples tested, 210 out of 212 trisomy 21 cases were detected (sensitivity 99.1%). DS, Down syndrome. (Source: Sequenom)

In 2011, results from several large-scale NIPT clinical trials were released[4–6]. Not only were the new NIPTs more accurate at flagging chromosomal defects, but they also had false-positive rates close to zero. Based on those results, the tests took off. The clinical data were so compelling it prompted the American College of Obstetrics and Gynecology (Washington, DC) to issue an opinion on the tests more quickly than is typical. In November 2012, the group recommended that NIPTs should be routinely offered to women at high risk of carrying a baby affected by aneuploidy, based on maternal age and medical history. "The rapidity of that statement was unprecedented," Bianchi notes.

NIPT offers the perfect solution to a long-standing challenge. "Women are very motivated to get information about their fetus," Bianchi explains. "But they are even more motivated to not harm the fetus."

Many women not deemed at high risk are still receiving the traditional screening test, and as a result, the rate of invasive tests is still much higher than it would be if all women were getting NIPT. But NIPTs are currently still only recommended for women deemed

Table 1 Commercial landscape of noninvasive prenatal testing for fetal aneuploidy in the United States

	Sequenom	Verinata Health	Ariosa Diagnostics	Natera
Test name	MaterniT21 Plus and SensiGene Fetal RhD Genotyping Test	verifi	Harmony Prenatal Test	Panorama Prenatal Test
Platform	SEQureDx technology incorporating massively parallel shotgun sequencing	Massively parallel sequencing using SAFeR algorithm	DANSR technology incorporating targeted sequencing and FORTE algorithm	Next-generation SNP-based Targeted Aneuploidy Testing
Conditions	Trisomy 13, trisomy 18, trisomy 21 and sex chromosome aneuploidies	Trisomy 13, trisomy 18, trisomy 21, sex chromosome aneuploidies and fetal sex	Trisomy 21, trisomy 18, trisomy 13, sex chromosome aneuploidies and fetal sex	Trisomy 13, trisomy 18, trisomy 21, triploidy and sex chromosome aneuploidies
Cost	$200 or less the majority of the time	$1,500. Covered as an in-network benefit by most of the top insurers (Aetna, UnitedHealthcare), $200 Patient Access to Care program, Financial Assistance program, and multiple self pay options available	$795 list price; typical patient pays $0–200	Out-of-pocket and list price varies based on service lab
Specificity	99.9% trisomy 21, 99.6% trisomy 18, 99.7% trisomy 13, 99.7% sex aneuploidies, >99.9% multiple gestations	99.8% trisomy 21, 99.6% trisomy 18, >99.9% trisomy 13 97.2% XX, 98.9% XY, 99.0% multiple XY	<0.1% false-positive rate for each trisomy	100% trisomy 21, 100% trisomy 18, 100% trisomy 13 and 91.7% 45,X
Turnaround	7 days	Average 4 days (95% within 8 days)	8–10 calendar days	10 days
Market entry	October 2011	March 2012	May 2012	December 2012
Marketing	Through physicians	Through physicians	Through physicians	Through physicians
Regulatory status	CAP accredited, CLIA certified, plans to submit premarket approval for *in vitro* diagnostic application	CAP accredited, CLIA certified	CAP accredited, CLIA certified	CAP accredited, CLIA certified
Primary publications	Refs. 5 and 11	Refs. 6 and 12	Refs. 13 and 14	Ref. 7

Adapted from ref. 15.

at high risk of carrying a fetus with a defect. Studies suggest NIPTs could bring down the rates of such testing by as much as 70%. And in places where the rates have been examined, use of invasive tests is indeed going down. Bianchi, for example, reports that her institution saw over a 30% reduction in amniocentesis after they started offering NIPTs. That number is likely to drop even further. News about NIPTs has traveled fast on social media, and women not at high risk are starting to ask for the tests. “At our institution, we’ve decided that if women request it we’ll give it to them, regardless of their risk level,” Bianchi says. “We decided that should be their decision to make.”

The US market

Sequenom licensed Lo’s technology to develop MaterniT21, the first US commercial NIPT. Other developers were fast to follow, including a couple with new types of tests. In March 2012, Verinata, which has licensed Quake’s MPSS technology, launched the ‘verifi’ test for detecting aneuploidies 21, 18 and 13. That

Box 2 Too much of a good thing

Although companies are reporting increased demand for their NIPTs from patients and providers, social and ethical issues persist. “NIPT presents providers with numerous ethical questions regarding which tests to offer, what information patients need to give an informed consent to testing and how to counsel patients regarding the results of the tests,” says Jaime King, an ethicist and associate professor of law at the University of California Hastings College of the Law in Berkeley.

Informed consent—the process for getting a patient’s permission for a medical intervention—emerges as the dominant concern. For consent to be truly informed and voluntary, patients should understand the risks and benefits of the procedure being offered. Decision making about NIPTs depends not only on a provider’s ability to explain the technology, but also on women’s knowledge of the conditions tested, their understanding of risk, their own personal and family priorities, and the predictive accuracy of certain tests. Studies have shown that patients have difficulty understanding statistical risk information, that women are overwhelmed by information during prenatal care, and that the very ease with which testing is administered may lead women to feel pressured and anxious about their pregnancy[17,18].

“Women are relieved to have access to a highly accurate noninvasive screening test,” says Jessica Mozersky, a social scientist at the University of Pennsylvania. And, tests with greater predictive power lead to better healthcare outcomes. However, she cautions “similar to serum screening, while uptake is high, women who receive negative results may feel overly reassured about the health of their baby, and do not necessarily understand the test limitations or recall all the conditions being screened for.”

The possible combination of NIPTs with other routine blood draws creates other problems for relaying information to women in the context of their prenatal care. When a typical amniocentesis is ordered in a hospital setting, a series of procedures are triggered that include a routinized informed consent and genetic counseling. This is not usually the case with blood draws and women may be shocked when a positive NIPT result is returned. As a result, the American Congress of Obstetricians and Gynecologists’ committee opinion recommends that NIPTs should not be part of routine prenatal laboratory work[19]. That, and the increased predictive value of these tests suggest that the informed consent process be held to a higher standard than those of standard screening measures[20].

(continued)

Box 2 Too much of a good thing (continued)

Some argue that the spread of NIPTs will enhance genetic counseling and informed consent because the technology relies on a single blood draw and only one return of results. By contrast, integrated screening involves two stages of testing, both of which may involve communicating complicated risk information to patients. Yet as the range of genetic conditions screened by tests broadens, it complicates matters for parents and healthcare providers. Although commercially available NIPTs are currently limited to detecting common aneuploidies, researchers are working to extend testing into single-gene disorders and microdeletions. Indeed, the prospect for fetal whole-genome sequencing introduces increasingly complex issues surrounding the prenatal decision-making process—which conditions should be tested and under what circumstances tests should be conducted. In children, for example, testing for adult-onset conditions has been controversial owing to concerns over the impact on the child of increased medical surveillance and parental concern.

The worry is that expanding the number and range of simple tests may cause parents to select against conditions that may not be life threatening but may be considered socially disabling, such as deafness or dwarfism. Down syndrome (chromosome 21 triploidy), for example, typically results in cognitive impairment and an increased risk of heart malfunctions, but in the US, these individuals often lead healthy lives well into their sixties. According to Brian Skotko, co-director of the Down Syndrome Program at Massachusetts General Hospital in Boston, the increasing use of highly accurate, noninvasive testing for conditions like Down syndrome will mean more expectant parents will have to decide whether to continue or terminate such a pregnancy. As Skotko puts it, "As a society, we must also decide how best to represent the voices of people with Down syndrome in our prenatal counseling sessions. Approximately 99% of people with Down syndrome say that they are happy with their lives. When and how do you share this with an expectant couple?"

Although many of these concerns are not unique to NIPTs, a commercial model that encourages widespread distribution of tests such as NIPTs may emphasize clinical uptake over clinical utility. Though one result might be the elimination of crippling genetic disorders, some tests may yield genetic information that is of limited clinical value or, worse, may reveal information that can't be effectively acted upon.

Together, the ethical and social impacts of NIPTs suggest that greater regulation is needed. The US-based companies have Clinical Laboratory Improvement Amendments (CLIA) certification and have received accreditation from the College of American Pathologists (CAP). However, there is currently no US legal requirement to demonstrate safety and effectiveness for laboratory-developed tests. Finally, off-site testing raises the possibility that standards may be needed to ensure tests are legal in both the jurisdiction of the company and the one in which the patient resides.

These market forces mean the US Food and Drug Administration (FDA) is stepping up its efforts to regulate these laboratory-developed technologies as devices. Alberto Gutierrez, FDA's director of the Office of *In vitro* Diagnostics in the Center for Devices and Radiological Health is on record as saying that companies that sell collection kits or instruments that are used for clinical relevance are subject to approval by the FDA. This puts the sequencer used by the four US NIPT players—and made by Illumina—squarely in the FDA crosshairs. The Illumina machine is approved for research use only (RUO). Illumina maintains that it cannot control how people use its machines, but now that Illumina has bought Verinata, the company must reckon with its foray into NIPTs. A 2011 FDA draft guidance states[21] that RUO equipment manufacturers "should not sell them to laboratories that they know use the product for clinical diagnostic use outside of a clinical investigation." Without naming Illumina, Gutierrez said at a recent meeting that if one large company acquires another, and that firm is using the parent firm's RUO products in a clinical setting, then it's obvious that the parent company was aware of how its products were being used[22].

High expectations surrounding the profitability of NIPT have also encouraged an active and contentious legal battle over intellectual property. All four providers of NIPT in the United States are involved in litigation against their competitors. The implications of this battle are potentially problematic. "IP-related issues, such as royalty fees or the cost of inventing around patents, could affect how tests are priced," says Subhashini Chandrasekharan of the Institute for Genome Sciences & Policy at Duke University (Durham, NC, USA). "High test prices could reduce access to testing, especially for those without insurance and who cannot afford out-of-pocket costs. This raises concerns that low-income families may be disproportionately limited in using noninvasive tests, exacerbating disparities in prenatal care."

The tendency toward ever-broader testing in the context of prenatal screening raises ethical and social questions about patient access, satisfactory informed consent, regulation and reproductive choice, especially with respect to the termination of pregnancy. NIPTs have potential to significantly change prenatal genetic testing because of its noninvasiveness and use earlier in pregnancy. However, such changes would not come without considerable ethical, legal and social concerns. It remains to be seen if noninvasive prenatal testing can fulfill its considerable technical potential in an ethical and socially positive manner.

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was quickly followed by Ariosa's Harmony test, which is also based on sequencing technology but uses directed amplification of selected regions on the three chromosomes. Natera's Panorama test was launched in March 2013. It uses a proprietary single-nucleotide polymorphism (SNP)-based approach for detecting the three common aneuploidies and some sex-chromosome abnormalities as well.

All of these products offer prenatal screening based on a simple blood draw done as early as 9- or 10-weeks' gestation. The companies are now working feverishly to differentiate their products based on cost, convenience, comprehensiveness, accuracy and availability. Each company has specialized bioinformatics, which are crucial (**Table 1**).

And the technology continues to advance. All of the companies now offer screening for the most common chromosomal defects: Down (trisomy 21), Edwards (trisomy 18) and Patau (trisomy 13) syndromes, as well as for aneuploidies involving sex chromosomes, which cause conditions that include Turner and Klinefelter syndromes (and incidentally reveals the baby's sex).

In contrast to the lackluster market for adult genome sequencing, orders for NIPTs are increasing rapidly . As noted earlier, Sequenom alone is on track to sell about 150,000 tests this year. Even at 120,000, which accounts for is ~3% of all pregnancies in the United States, forecasters put the US prenatal testing market at $1.5–2 billion. The tests' developers are eagerly eyeing new markets as well. Sequenom has already made several deals in European

Table 2 US patents and applications for institutions commercializing noninvasive prenatal tests

Institution	US patents	US applications
Natera	0	8
Verinata Health	2	19
Ravgen (Columbia, MD, USA)	4	6
NIPD Genetics (Nicosia, Cyprus)	0	1
Sequenom	4	17
Stanford University	5	18
Chinese University, Hong Kong	12	18
Isis Innovation (Oxford, UK)	1	1
University of Louisville (Louisville, KY, USA)	1	5

Adapted from ref. 15.

markets. Besides the BGI Inex collaboration, China's Berry Genomics (Beijing) is partnering with Baylor College of Medicine in Houston, to offer NIPTs in China. Demand for NIPTs is expected to be particularly high in China, which has a one-child policy and where an estimated 17-million babies are born each year. The country currently has a relatively low rate of amniocentesis in part due to low availability, although that figure is steadily climbing.

Not surprisingly, lawsuits are piling up around intellectual property (**Table 2** and **Table 3**) and investors are trying to gauge the market's full potential. Because the tests cost up to about $3,000, insurance coverage is a key issue. Insurers were at first slow to approve the tests, but some of the biggest, including Aetna and UnitedHealthcare, have issued policy decisions in favor of such tests for pregnancies at high risk of aneuploidy. Although a policy decision isn't the same thing as guaranteed coverage, analysts are optimistic that the tests will start being widely covered.

Differentiating themselves

Competition among NIPT developers is already intense, and it's not yet clear how much technology matters. Ariosa (San Jose) CEO Ken Song argues that if you are looking just at chromosomes 21, 18 and 13, the company's approach of amplifying regions from those chromosomes, has advantages. Targeted sequencing is cheaper, because more different clinical samples can be run in a single sequencing run. Verinata CEO Jeff Bird agrees but counters that amplification is not necessary; it increases the rate at which tests fail and must be repeated, requiring more time and collection of additional samples.

In addition, Bird says, "Sequencing costs are already coming down and we do not think [those costs] will ultimately be the primary driver of overall test cost." Bird maintains that MPSS is more robust, pointing out that both Verinata and Sequenom have failure rates below 1% now. Song argues that Ariosa's test is the only one that includes information about how much cffDNA is present in the sample, which he claims is crucial in understanding how reliable the result is. He also says that Harmony's low cost (it's the only one under $1,000) makes it most cost effective, and it is the only test that has been widely tested in pregnancies that are not high risk.

Natera (San Carlos, CA, USA), the most recent entrant, developed its test based on its established technology to screen embryos created by *in vitro* fertilization (IVF), before implantation. The company's parental support platform uses bioinformatics and SNP detection to analyze fetal chromosomes, which their marketing material says is the most accurate method because SNPs are specific to the individual. The company "takes a heavy statistics" approach to sort out the maternal or paternal contribution to the fetal genome according to Jonathan Sheena, Natera's chief technology officer. "The power of this approach becomes especially evident when you have low amounts of fetal DNA," says Sheena. Given the low false-negative and false-positive rates other companies are reporting, Natera will have to demonstrate consistently better numbers to claim the top spot.

In April, researchers at King's College Hospital in collaboration with the Fetal Medicine Foundation (both in London) published a prospective study of 229 patients using Natera's test that yielded 100% detection of trisomies 21, 18, 13, as well as Turner's syndrome and fetal triploidy. The company says that they will explore triploidy for eventual inclusion in their test, which will set them apart from the others[7].

Distinguishing between the tests is difficult, even for experts. "They all have proprietary informatics and that makes it hard to compare between them and determine which is the best test for a patient," says Michael Watson, director of the American College of Medical Genetics and Genomics (Bethesda, MD, USA).

Companies are taking additional steps to outrun their competitors, including offering new tests. "We'd like to quickly deliver a much more comprehensive test and wrap that around with all the services physicians want, such as rapid turnaround, exquisite accuracy and a low to nonexistent failure rate," says Sequenom chief medical officer Allan Bombard. Natera, likewise, is offering products for a range of circumstances, including carrier testing, miscarriage analysis and pre-implantation testing.

It's pretty clear that NIPTs are hot properties now. Acquiring Verinata, "made sense" for Illumina because it creates "a comprehensive genetics portfolio," says Greg Heath, senior vice president and general manager of Illumina's diagnostics business. Given Illumina's internal products, which include a carrier and diagnostics test for cystic fibrosis, and its recent acquisition of BlueGnome (Cambridge, UK), the company can do everything from carrier testing and IVF screening to "following the baby genetically throughout his or her life," Heath says.

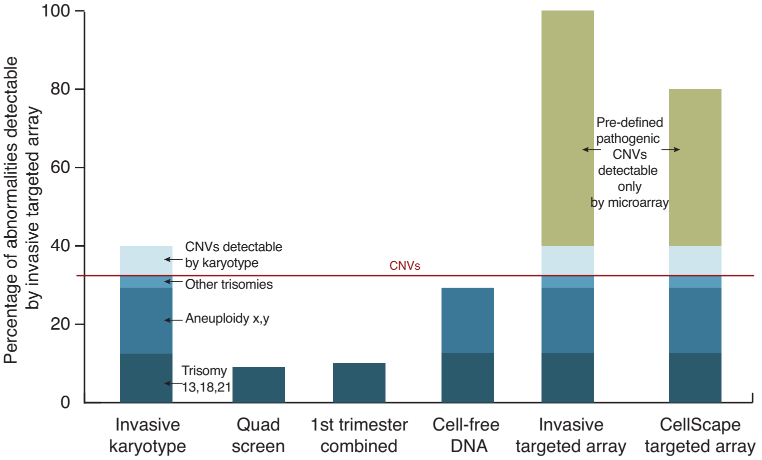

Figure 2 Genetic disorders detectable by prenatal testing methods. CMAs are the most informative test for cytogenetic defects as compared to karyotyping, cffDNA testing, quad screening (which measures four substances in blood) and first-trimester screening (which combines maternal blood screening for two pregnancy-specific molecules with ultrasound evaluation)[3]. (Source: CellScape)

Table 3 Recent patents issued in the noninvasive prenatal testing landscape

Patent No	Date of issue	Assignee	Title
US7645576	01/12/2010	The Chinese University of Hong Kong	Method for detection of chromosomal aneuploidies
US7655399	02/02/2010	Boston University	Methods for prenatal diagnosis of chromosomal abnormalities
US7709194	05/04/2010	The Chinese University of Hong Kong	Marker for prenatal diagnosis and monitoring
US7718367	05/18/2010	The Chinese University of Hong Kong	Markers for prenatal diagnosis and monitoring
US7718370	05/18/2010	Ravgen	Methods for detection of genetic disorders
US7727720	06/01/2010	Ravgen	Methods for detection of genetic disorders
US7754428	07/13/2010	The Chinese University of Hong Kong	Fetal methylation markers
US7785798	08/31/2010	Boston University	Methods for prenatal diagnosis of chromosomal abnormalities
US7799531	09/21/2010	University of Louisville Research Foundation, Louisville, KY, USA	Detecting fetal chromosome abnormalities using tandem single-nucleotide polymorphisms
US7829285	11/09/2010	The Chinese University of Hong Kong	Circulating mRNA as a diagnostic marker
US7838647	11/23/2010	Sequenom	Noninvasive testing of fetal genetic traits
US7888017	02/15/2011	Stanford University	Noninvasive fetal genetic screening by digital analysis
US7901884	03/08/2011	The Chinese University of Hong Kong	Markers for prenatal diagnosis and monitoring
US8008018	08/30/2011	Stanford University	Determination of fetal aneuploidies by massively parallel DNA sequencing
US8026067	09/27/2011	The Chinese University of Hong Kong	Marker for prenatal diagnosis and monitoring
US8133701	03/13/2012	Sequenom	Detection and quantification of biomolecules using mass spectrometry
US8137912	03/20/2012	General Hospital Corporation Boston	Methods for diagnosis of fetal abnormalities
US8168382	05/01/2012	The Chinese University of Hong Kong	Methods for detecting DNA originating from different individuals
US8168389	05/01/2012	General Hospital Corporation	Fetal cell analysis using sample splitting
US8173370	05/08/2012	Sequenom	Nucleic acid–based tests for RHD typing, gender determination and nucleic acid quantification
US8195415	06/5/2012	Stanford University	Noninvasive diagnosis of fetal aneuploidy by sequencing
US8206926	06/26/2012	Sequenom	Restriction endonuclease enhanced polymorphic sequence detection
US8288100	10/16/2012	The Chinese University of Hong Kong	Methods for detecting fetal DNA in plasma or serum sample from a pregnant woman
US8293470	10/23/2012	Stanford University	Noninvasive fetal genetic screening by digital analysis
US8296076	10/23/2012	Stanford University	Noninvasive diagnosis of fetal aneuploidy by sequencing
US8304187	11/06/2012	Strack Omaha, NE, USA	Preservation of cell-free RNA in blood samples

Adapted from ref. 15.

Next-generation prenatal tests

Given the huge success of this first wave of NIPT, the big question is who or what will move the bar further? The American College of Obstetrics and Gynecology has already recommended that all pregnant women, regardless of age and other risk factors, should be offered information and access to invasive prenatal diagnosis. Last December's publication of a large-scale comparison of chromosomal microarrays (CMAs) and karyotyping has also altered the landscape[3]. That study, led by Ronald Wapner of New York-based Columbia University Medical Center, determined that CMAs are superior and are thus considered by some to be the future technology of choice for prenatal diagnosis. What is surprising is that the study found the incidence of genetic abnormalities among women of all risk levels to be greater than previously believed. In about 1 in 60 pregnancies the fetus has a genetic defect, some of which will not be apparent until after infancy (**Fig. 2**).

Together these factors mean that more women may want prenatal diagnostic testing, and with that, the potential market for such tests is now every pregnant woman in the country.

Two groups have demonstrated that whole-genome sequencing of cffDNA is also now possible, though still too expensive and cumbersome to be adopted, although that is expected to change[8,9]. Jay Shendure at the University of Washington (Seattle) did deep sequencing of cffDNA, and combined it with haplotype sequencing of the mother and shotgun sequencing of the father[8], whereas Quake's group modified their chromosome counting technique to counting haplotype blocks to deduce the fetal genome[9], Shendure thinks it's more likely than not that whole genome prediction during pregnancy will happen and be routine. "Whether it's 5, 10 or 20 years is more difficult to say," he says.

In the meantime, the next phase of this market is likely to involve expansion of targeted sequencing (that is, the current NIPT technology) and the increased use of CMAs, even among low-risk pregnancies. The dark horse in this race is CellScape, which aims to analyze circulating fetal cells, rather than cffDNA. Their test collects fetal nucleated erythrocytes from maternal blood, enriches them by means of osmolarity and/or detergent-mediated lysis of enucleated maternal erythrocytes, subsequent cell sorting and/or optical identification of nucleated erythrocytes using fetus-specific antibody markers and assessment of genetic status by fluorescence *in situ* hybridization, PCR, ligase chain reaction or another method.

One advantage of using these specific fetal cells, they point out, is that they do not divide and so do not persist from earlier pregnancies. In a study presented as a poster at this year's American College of Medical Genetics and Genomics annual meeting in Phoenix, in March, the company reported that fetal nucleated erythrocytes were present in all tested samples at a sufficient level for one megabase resolution on CMAs. That's the level of resolution needed to detect most serious defects. The test is currently in clinical trials and expected to launch in 2014. CellScape CEO Karen Drexler says the company plans to launch their product initially as a screening test, "We will still have limited data at the time of launch, and so the prudent thing will be to get confirmation." However, they expect to eventually add the diagnostic label with sufficient data.

With MPSS, meanwhile, the challenge is simply to determine if cffDNA provides enough DNA to allow accurate and affordable detection of defects of interest. In February, Verinata published a study demonstrating detection of fetal subchromosomal abnormalities from a maternal sample[10]. They analyzed cffDNA from the plasma of 11 women carrying fetuses known to have such genetic defects by karyotype analysis. Seven cases of microdeletions, duplications, translocations and a trisomy 20 were detected, including a deletion as small as 300 kb. The researchers used 25-mer tags, which they reported "mapped with high efficiency across the genome" and allowed them to obtain one billion tags for less than $1,000 per sample. They said their study proves that MPSS can be as accurate as CMAs. "We're now collecting more samples to do more studies and decide when we should offer this," says Richard Rava, CSO at Verinata.

Sequenom is likewise aiming to make its test equal or superior to CMAs. Song says that Ariosa will take a more strategic approach. He's not sure women and their doctors are prepared yet for truly comprehensive genetic diagnosis.

Determining what to test for is also a moving target because so many diagnostic tests are being developed. In the CMA study[3], Wapner's group, for example, is doing long-term follow up on children who were identified as having small deletions or duplications of unknown significance. During that study, the researchers initially identified 94 copy-number variants of "uncertain clinical significance." Within a year, they were able to reclassify 38 of those variants as either benign or significant. "By following children with uncategorized variants, we will be able to determine whether there are problems associated with these or not," says David H. Ledbetter, executive vice president and CSO at Geisinger Health System (Danville, PA, USA). Hospitals and other groups, including the International Standards for Cytogenomic Arrays Consortium, are eagerly amassing such data.

Troubling questions

The field is rife with ethical concerns (**Box 2**), including worries about increased abortions, eugenics, distorted expectations and uncertainty about how parents will react to 'unknown' genetic variations in their babies. But now that the technology is available, it might be hard to contain demand, at least for those who can pay for it.

Ethicists have been left wondering how some of the most troubling situations, such as sex selection can be avoided. With greater use of CMAs, more and more parents will have to endure the anxiety of being told their baby has a novel genetic variation that may or may not be consequential. But there are benefits that are sometimes overlooked, too. "An important goal is to also eventually be able to prevent and treat some of these conditions, possibly even in the womb," says Wapner. Bianchi agrees, adding "Some families also just want to be prepared."

Another question is whether this will lead to the gradual eradication of certain genetic conditions. Tay Sachs, a deadly condition once common among Ashkenazi Jews, is rarely seen in that population now, because of carrier and prenatal testing. According to the Genetic Alliance (Washington, DC), prenatal diagnostics have raised concerns among advocates for people with certain genetic diseases, such as Down's syndrome and dwarfism. They worry whether wider use of prenatal tests could ultimately affect research into these conditions. Natasha Bonhomme, the alliance's vice president of strategic development, sees a lost opportunity here. "There is such a wide range of places that people can get information," she says. "Advocacy groups that represent people with these conditions would like to be part of the discussion around what test results really mean."

Bonhomme would like to see more of a societal conversation going on. "What does this mean to parents, to society? To people with genetic diseases and to research into those conditions?" she asks. "It would be nice if we had a chance to talk about that."

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