The increasing ability to analyze fetal DNA from maternal blood should lead to better prenatal diagnoses of genetic disease—and confront future parents with tough information and choices

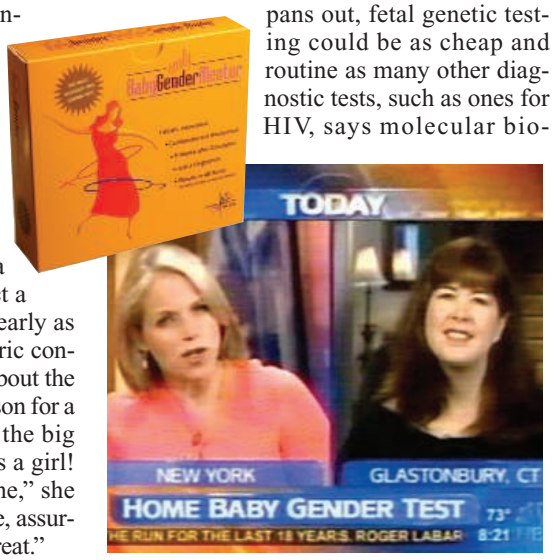
An Earlier Look At Baby's Genes

The smiling, dark-haired woman chatting with Katie Couric on NBC's popular *Today* show explains why she wants to know the sex of her third baby just 7 weeks into her pregnancy. Holly Osburn of Glastonbury, Connecticut, the mother of two daughters, says her house is full of pink, purple, and green, and "we're anxious to find out if we're going to … maybe have to paint the nursery blue."

So Osburn has sent dried spots of her blood to a Massachusetts company offering Baby Gender Mentor, a new $275 test that promises to detect a fetus's sex from maternal blood as early as 5 weeks after conception. After Couric conducts a discussion with a physician about the pros and cons of the test, a spokesperson for a company selling it online delivers the big news live to millions of viewers: It's a girl! Osburn's smile wavers. "Another one," she says. Then she regains her composure, assuring the TV audience that "a third is great."

While watching this in June, "my jaw dropped," says Diana Bianchi, a prenatal geneticist at Tufts University School of Medicine in Boston and one of a small number of researchers who have spent more than a decade trying to detect sex and genetic disorders from fetal cells and DNA in a mother's blood. She notes that "at home" fetal DNA tests such as Baby Gender Mentor aren't yet considered scientifically and ethically vetted. "I'm concerned about whether this is ready for prime time," says Bianchi.

Ready or not, noninvasive fetal diagnosis is here. Tests based on fetal DNA circulating in a woman's blood are expected to replace invasive prenatal tests, such as amniocentesis, that are typically done later in pregnancy and pose a small risk of miscarriage. Researchers have already used fetal DNA from maternal blood to successfully test for genes inherited from a father that cause diseases such as cystic fibrosis and the blood disorder thalassemia. They are now refining their techniques and moving on to bigger challenges, such as identifying Down syndrome. If this work pans out, fetal genetic testing could be as cheap and routine as many other diagnostic tests, such as ones for HIV, says molecular biologist Sinuhe Hahn of the University Women's Hospital in Basel, Switzerland.



Broadcast news. Through a new test (*inset*), expectant mother Holly Osburn, along with Katie Couric and *Today* viewers, learned the apparent gender of her 7-week-old fetus.

Earlier and easier fetal DNA testing will certainly raise ethical questions. For example, some researchers worry that gender tests will lead to abortions by parents who desire a baby of a specific gender. The ethically explosive applications extend beyond sex selection. If fetal DNA testing can one day routinely reveal whether an early fetus has genes that predispose it to cancer or other diseases, parents-to-be could be facing much more difficult decisions than what color to paint the nursery.

For now, researchers are grappling with how to get a clear, consistent signal from a relatively few molecules of fetal DNA sequence floating in a sea of maternal DNA. When a diagnosis could lead parents to end a pregnancy, they note, accuracy is crucial. "It's very important that we get it right," says medical geneticist Maj Hulten of the University of Warwick, U.K.

One in a million

Researchers have known for more than 3 decades that a few fetal cells of various types are present in a pregnant woman's blood. While there may only be about two to six fetal cells per milliliter of blood during pregnancy, some of these cells can linger for several decades after birth and may even contribute to postnatal tissue repair or disease in the mother (*Science*, 21 June 2002, p. 2169). The first proof that such cells could be used to diagnose a fetal condition came in 1991 from Joe Leigh Simpson's lab at Baylor College of Medicine in Houston, Texas. Using an antibody called CD71 that tends to bind to red blood cells of fetal origin, his team separated these cells from most maternal blood cells. They then used fluorescence in situ hybridization (FISH), in which colored probes bind to chromosomes, to detect Down syndrome, which is caused by an extra chromosome 21, and another chromosomal disorder.

Other labs soon reported similar results, exciting researchers who saw the technique as a promising alternative to amniocentesis and chorionic villus sampling (CVS). These diagnostic tests, which collect fetal cells by inserting a needle into the womb either late in the first trimester or during the second trimester, carry up to a 1% risk of miscarriage. In 1994, the National Institute for Child Health and Development (NICHD) launched a validation study in which five labs used fetal cells from maternal blood to look for Down syndrome in 2744 pregnancies. The results, published in 2002, were just modestly encouraging: The researchers found only enough fetal cells to detect 74% of Down syndrome cases. In contrast,

CREDITS (TOP TO BOTTOM): G. MOSCOSO/PHOTO RESEARCHERS INC.; (INSET) ACU-GEN BIOLAB INC.

Published by AAAS

CVS and amniocentesis are 99% accurate.

The authors of the NICHD study concluded that the current techniques—which involve physically separating the fetal and maternal cells—would have to improve before blood-borne fetal cells could provide reliable diagnoses. The key will be an antibody or other compound that can more efficiently separate out the fetal cells, which make up only about one out of every million cells in a mother's blood, says Simpson. "Once that occurs, the field will turn around overnight," he says.

A few teams, including Simpson's at Baylor, and at least two companies are also pursuing an alternative approach, attempting to isolate fetal cells, called trophoblasts, from cervical swabs of pregnant women. The trophoblasts make up about 1 in 100,000 cells in a swab, and so should be easier to distinguish from maternal cells than fetal blood cells, says Farideh Bischoff of Simpson's group. Yet to be proved is whether researchers can extract enough cells without sampling so high in a woman's cervix that the technique becomes invasive, Bianchi notes.

Free and easy

Noninvasive fetal testing took off in a new direction several years ago after Dennis Lo, now at the Chinese University of Hong Kong, and co-workers discovered that maternal blood contains more than fetal cells. There's also fetal DNA floating freely, outside of cells, he found. Lo was inspired to look by two 1996 *Nature Medicine* articles on detecting tumor DNA in the blood of cancer patients. He reasoned that like a tumor, the fetus-derived placenta is a fast-growing tissue that might shed DNA.

The hunch paid off: Using a form of polymerase chain reaction (PCR) to detect a gene called *SRY* on the Y chromosome of male fetuses, Lo's group reported in 1998 that fetal DNA is much more plentiful in a future mom's bloodstream than are fetal cells. Levels rise during pregnancy to as much as 3% to 6% of the cell-free DNA in a mother's plasma, then plummet in 2 hours after a baby is born. The fetal DNA seems to come mainly from the placenta, Bianchi and others have shown.

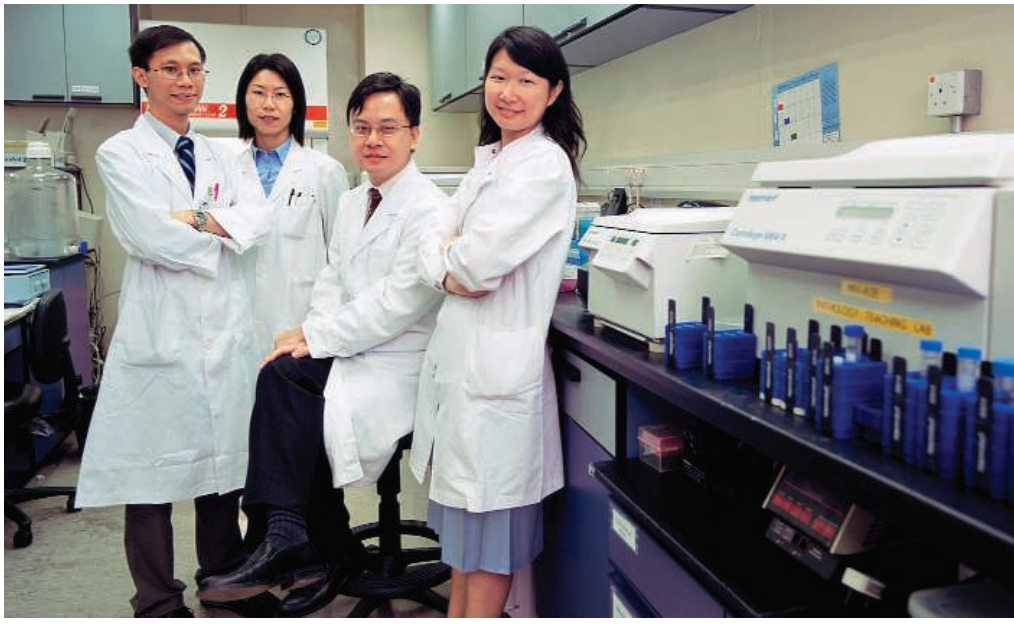
Lo's group soon showed that this fetal DNA could be used to diagnose potentially lethal conflicts in Rh factor, a protein on the surface of red blood cells. If an Rh-negative woman carries an Rh-positive fetus, her immune system can create antibodies against the baby's blood cells, causing anemia for the fetus. This sensitization can be prevented by injecting the pregnant mother at certain points in pregnancy with Rh immunoglobulin, a step often taken as a precaution without knowing the fetus's Rh status. But many research groups have now shown they can reliably test the blood of Rh-negative pregnant women for fetal DNA that reveals the functional form of the *Rh* gene. Such a test has been offered since 2001 by a few research labs in Europe.

Several groups have since reported they can detect other disease mutations passed on from the father, such as ones causing cystic fibrosis, beta-thalassemia, a type of dwarfism, and Huntington's disease. The results haven't always been reproducible, partly because smaller mutations are difficult to pick up from a mixture of fetal and maternal DNA. Other promising findings are still being debated. Lo's group reported in 2000 that intact fetal DNA in fragments of dying cells could be analyzed for Down syndrome, and last year a biotech company claimed that treating maternal blood with formaldehyde could boost the amount of fetal DNA recovered. Only some labs have been able to replicate these experiments.

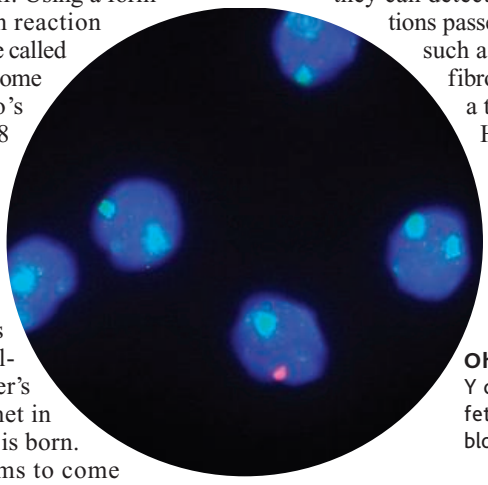
Two advances in the past year have clearly boosted the potential reliability of fetal DNA tests, however. Both involved studies looking for mutations that trigger beta-thalassemia, which leads to severe anemia and is most common in people of Asian and Mediterranean descent. Last summer, a report in the *Proceedings of the National Academy of Sciences* by Lo's team and the San Diego–based firm Sequenom Inc. said that inherited beta-thalassemia point mutations could be diagnosed in 12 fetuses much more reliably if mass spectrometry and PCR, rather than PCR alone, were used to analyze the fetal DNA.

Earlier this year in the *Journal of the American Medical Association*, Hahn's team in Basel reported another approach for detecting beta-thalassemia mutations comprising a single nucleotide change. The group took advantage of a finding by Lo's group that the fragments of fetal DNA found in the mother's blood are typically less than 300 basepairs in size, compared with more than 500 basepairs for cell-free maternal DNA. By using electrophoresis to increase the ratio of the shorter segments in blood samples, the Swiss team successfully detected the presence or absence of four common beta-thalassemia point mutations in 28 of 31 fetuses. While the mass spectrometer needed for the Sequenom-Lo method costs $300,000, the Swiss team notes that its approach could cost as little as $8 per sample, within the economic reach of developing countries.

Several teams are now racing to try these techniques—or combine them—to reliably detect cystic fibrosis and other genetic dis-



Detective squad. Dennis Lo (*center*) and his group at Chinese University of Hong Kong have pioneered noninvasive prenatal testing using cell-free fetal DNA.

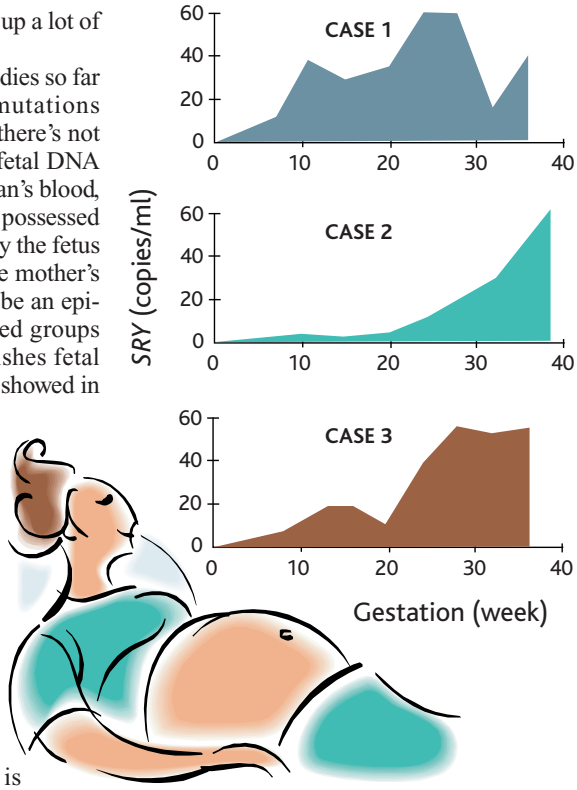


Oh, boy. Red marks the Y chromosome in a male fetal cell amid maternal blood cells.

CREDITS (TOP TO BOTTOM): THE CHINESE UNIVERSITY OF HONG KONG; T. BABOCHKINA/UNIVERSITY WOMEN'S HOSPITAL, SWITZERLAND

eases, says Hahn. "They will open up a lot of new applications," Lo agrees.

One major caveat is that the studies so far have only been able to detect mutations passed on by the father. Because there's not yet a way to completely separate fetal DNA from the maternal DNA in a woman's blood, it's not possible to tell if a mutation possessed by the mother has been inherited by the fetus or if researchers are just seeing the mother's DNA. One possible solution may be an epigenetic marker, such as methylated groups attached to a gene, that distinguishes fetal DNA from a mother's. Lo's group showed in 2002 that they could make such a distinction. Another potential strategy is to use messenger RNA molecules produced only by the fetus and not the mother. Several groups have recently shown, for example, that RNA produced by placental genes can be detected in maternal blood.



Baby signs. Cell-free fetal DNA levels rise during pregnancy, as shown in three future moms.

Seeing double

Diagnosing Down syndrome noninvasively through fetal DNA is the big prize luring researchers. The potential demand for such a test is huge, says Boston University's Charles Cantor, chief scientific officer of Sequenom Inc., because the rate of Down syndrome is at least 1 in 270 for mothers over 35. Doctors can screen for the disorder in the first trimester by using ultrasound to measure the dimensions of the fetus's neck and checking the levels of several protein markers in maternal blood; this combination picks up 85% of cases, albeit with a false positive rate of 2% to 6%. The International Down Syndrome Screening group last year called for this noninvasive strategy to be offered to all women, but a firm diagnosis still requires subsequent amniocentesis or CVS. The $1000 or more cost of these two tests limits routine use to women over 35, which means most Down syndrome births now occur in younger women.

Yet while Down syndrome is easy to detect if fetal cells are in hand, it's harder using cell-free DNA. The reason is that this condition is caused by an extra chromosome, rather than a mutation that can be detected with PCR. So far, for Down syndrome, fetal DNA can be used to only slightly improve screening: Overall fetal DNA levels are higher in women carrying fetuses with Down syndrome and some other aneuploidies. Adding a fetal DNA quantity test to other serum markers for Down syndrome would boost the detection rate from 81% to 85%, Bianchi's group has shown.

Still, the real prize is a straightforward, noninvasive fetal DNA diagnostic for Down syndrome that's as accurate as amniocentesis and CVS. One possible solution is to discover an epigenetic marker for Down syndrome that would allow Down-specific DNA sequences to be amplified with PCR. Another is to look for fetal mRNA from a gene expressed by chromosome 21 but not by the mother's cells. Cantor estimates that two dozen groups are working on the problem and predicts it will be solved in 3 years.

Ethical minefield

Indeed, while research on noninvasive fetal testing is very competitive—Lo and other investigators have certainly applied for many patents—cooperation is common. Cross-lab studies like the one sponsored by NICHD have nurtured the field, and they are continuing thanks to a new 5-year, €12 million European Union project called Special Advances in Fetal Evaluation (SAFE) that involves 52 institutional partners. "I think this is a positive example of a new technology being rigorously investigated before it filters into practice," says gynecologist Wolfgang Holzgreve of the Basel group.

The need for caution makes some scientists uncomfortable with Baby Gender Mentor. The company offering the test, Acu-Gen Biolabs in Lowell, Massachusetts, claims it works at 5 weeks of gestation at 99.9% accuracy. But Bianchi questions that figure, noting that a cross-lab study of gender detection published last year found that sensitivity varied widely among labs. A company spokesperson says the 99.9% figure is based on 20,000 births but notes that the company won't publish results until it has patented its technology.

There's little chance for outside experts to scrutinize that accuracy claim. Food and Drug Administration approval is not needed as long as the blood sample goes to a lab and the test is sold as a service rather than as a kit. Like other genetic tests, "[this] is opening up gaps in the oversight system," says Kathy Hudson, director of the Genetics and Public Policy Center at Johns Hopkins University in Baltimore, Maryland. It's not just the U.S. that does not regulate such testing. A Canadian company called Paragon Genetics has been offering a fetal DNA gender test for more than 2 years. The firm's quiet marketing of it hasn't drawn as much criticism as Baby Gender Mentor, in part because it follows the practice of many fetal DNA researchers by using fresh maternal blood, instead of dried blood spots. It also suggests that samples be taken 10 weeks into pregnancy.

As for concerns that some couples could use fetal DNA gender tests to end a pregnancy, Paragon Genetics lab director Yuri Melekhovets argues that parents can already do that based on ultrasound tests early in the second trimester. Still, Lo's group has gone so far as to stipulate in licensing agreements with companies that its technology can't be used for sex selection. The SAFE project, meanwhile, is funding a study of the implications of using early fetal DNA testing, especially if costs fall enough to make it feasible for couples in countries such as India and China where female children may be viewed as less desirable. "Especially in 'one child' countries, there is a risk that this [test] can be abused," says Hulten, the SAFE project's coordinator.

Another troubling ethical issue for some is how abortion rates could be affected by the advent of widespread, accurate fetal DNA testing for many genetic diseases. Although abortions may increase, Bianchi points out that mothers who keep a child with a disease could also benefit from the prenatal diagnosis. A survey by her group found that mothers who went to term after learning that they were carrying a fetus with Down syndrome were better able to cope psychologically once the child was born than mothers who learned of their baby's disorder at birth. Based on that finding, if fetal DNA testing fully comes of age, it may provide many potential parents with news that's difficult to hear, but it could also give them time to decide what's right for them and accept their decision.

—JOCELYN KAISER

SOURCE: D. LO, *AM. HUM. GENET.* **62**, 768–775 (1998); ILLUSTRATION: JUPITER IMAGES