Womb with a view

The intricate development of the fetus is yielding its long-held secrets to state-of-the-art molecular technologies.

BY CLAIRE AINSWORTH

ILLUSTRATION BY STEPHAN SCHMITZ

Life starts with a puzzle. Out of sight in a mother's womb, 3 billion letters of DNA code somehow turn into 3D bodies, all in the space of a mere 40 weeks. Fetuses form eyes, brains, hearts, fingers and toes — in processes that are meticulously coordinated in both time and space. Biologists have pieced together parts of this puzzle, but many gaps remain.

Now, a crop of molecular technologies is giving scientists tantalizing hints about how to fill in those gaps. Improved ways of reading and interpreting the information in fetal genetic material are uncovering a raft of genes involved in human development, and letting researchers eavesdrop on the hum of gene activity before birth. They can see which genes turn on or off at pivotal moments, and sense how the environment nurtures or intrudes on this.

Even the vital life-support system that we jettison at birth — the

placenta — is laying bare its secrets. "It really is this great mystery in reproduction," says Zev Williams, a reproductive endocrinologist and infertility specialist at the Albert Einstein College of Medicine in New York City. "It's obviously such a critical part of human development, but it's been so understudied."

Until now, much of the work has relied on amniotic or placental samples obtained during routine invasive tests such as amniocentesis. But scientists are eyeing the next step: studies that are non-invasive for the fetus and are done on a teaspoonful of blood drawn from a pregnant woman's arm. In this way, researchers could monitor fetuses as they develop and, down the line, develop non-invasive tests for a broad range of conditions, in both fetus and mother.

Physicians are already moving towards treating fetuses in the womb on the basis of such diagnoses. "It's an exciting time," says Mark Kilby, a fetal-medicine specialist at the University of Birmingham, UK.

But it won't be plain sailing. The technologies are developing so quickly that scientists are struggling to interpret the information they yield and are facing knotty ethical quandaries. What, for example, should doctors do if non-invasive prenatal testing (NIPT) reveals a DNA sequence that sometimes causes disease — but not always? "That is what we have to discuss as a whole community," says clinician and geneticist Dennis Lo of the Chinese University of Hong Kong, who was the first to find fetal DNA in a mother's blood[1].

DEVELOPMENT WORK

Probing fetal development starts, naturally enough, with DNA, the recipe for life. Developmental biologists have already gathered a trove of information here, through studies of laboratory animals from worms to mice, identifying many genes and processes that have human equivalents. Painstaking detective work on families with inherited genetic diseases has yielded even more insight.

But the advent of next-generation DNA sequencing is transforming the field. It is now relatively easy to sequence genomes, in whole or in part, to look for the causes of rare genetic disorders. And discoveries are piling up: how key signalling proteins help cells to adopt their myriad identities, and how the packaging of DNA influences brain development, to name just two. "We are currently in a very rich vein of accelerated understanding," says Matthew Hurles, a geneticist at the Wellcome Trust Sanger Institute near Cambridge, UK.

Most studies so far have parsed genomes after birth. But researchers are pushing to use the same methods on fetuses in the womb, in the hope of improving the diagnoses and prognoses they can offer to expectant parents. Hurles and his colleagues, for example, are studying 1,000 fetuses with structural abnormalities spotted through ultrasounds. Using cells from fetus, mother and father, the team is sequencing the 1–2% of the genome that carries instructions for making proteins (the exome), as well as the entire genomes of a smaller subset, to try to identify the genetics behind the disorders.

Researchers want to go still further, and sequence entire fetal genomes using blood from the mother. This would give them ready access to DNA at nearly all stages of fetal development, in healthy fetuses as well as ones that may have problems.

The approach is realistic, they say. The field is racing ahead: a flurry of papers, from Lo[2,3], Stephen Quake at Stanford University in California[4,5] and genome scientist Jay Shendure at the University of Washington in Seattle[6] have honed the resolution with which scientists can analyse a fetal genome from tiny bits of DNA floating in the mother's blood. They can now count the number of chromosomes in a fetus[2,4], and are developing ever-more-accurate ways to sequence genomes. In principle, they can now detect single-letter variants in the DNA sequence that might cause inherited diseases, and are building up their ability to find mutations that underlie some developmental disorders but are not present in either parent. Several companies have been formed to develop the technologies.

There are barriers to overcome before the newest technologies will see widespread use in lab or clinic. One is the cost. Whole-genome sequencing is getting cheaper, but researchers often need to repeat it many times to boost the resolution of their results[7]. But researchers are confident that these roadblocks won't remain. "There's work to be done here, but it's not an unsolvable problem from a technical perspective," says Shendure.

Interpreting the results will be another sticking point. Not all DNA changes cause disorders. And even if an individual carries a specific mutation, scientists cannot yet be sure that it will always result in disease.

But as costs drop, scientists say, they will be able to sequence enough genomes to learn which mutations predict disorders with high probability. They then hope to see non-invasive whole-genome sequencing applied as a screening tool during pregnancy. "This is the kind of thing you could imagine would be incredibly useful in diagnosing metabolic and immune disorders where you want to treat the baby right when they're born," says Quake.

> *"We are currently in a very rich vein of accelerated understanding."*

And even before birth might be an option. A team of scientists is already using next-generation sequencing of specific genes to diagnose brittle-bone disease in fetuses as part of a clinical trial that uses stem cells to treat the condition in the womb. The researchers are currently obtaining the fetal cells through invasive sampling techniques, but aim to switch to non-invasive testing.

FULL TRANSCRIPT

DNA is but the start of the story of human development. Researchers are keen to understand how instructions in the genome are deployed in time and space as a fetus grows, and how this goes wrong during disease. Many are therefore focusing on the molecule RNA, which the cell uses to copy — and then act on — a given set of DNA instructions. And that presents fresh challenges. RNA breaks down very quickly, so it is harder to work with than DNA, especially when trying to untangle a fetus's output of RNA — its transcriptome — from the mother's.

To simplify things, clinician and geneticist Diana Bianchi, now director of the National Institute of Child Health and Human Development in Bethesda, Maryland, began by studying the transcriptome of amniotic fluid, which contains freely floating RNA from fetus and placenta. Over the past decade, her team has built up intriguing snapshots of gene activity through the second and third trimesters (from discarded samples taken during amniocentesis tests), and at term (from samples gleaned during Caesarean sections), as well as some work with maternal blood, which bears free-floating RNA fragments from fetus, mother and placenta.

She has shown how a full-term fetus switches on just the sorts of genes that might be expected for a baby gearing up to be born — including ones involved in lung and gut physiology, energy metabolism, the immune system and the eye[8]. Genes involved in smell ramp up, too, "which we think has some evolutionary advantage", says Bianchi, "because the baby needs to know the smell of its own mother, for survival reasons".

Much of Bianchi's work has focused on amniotic-fluid samples from fetuses affected by chromosomal abnormalities, such as Down's syndrome (an extra chromosome 21) and Edward's syndrome (an extra chromosome 18). She finds that gene activity is abnormal across the whole genome, not just on the extra chromosome, and even in genes needed for brain development[9]. She's also found that cells of fetuses with Down's incur damage from the by-products of metabolism, a condition known as oxidative stress[10].

This raises the provocative possibility of treating fetuses in the womb to ameliorate the cognitive impairment associated with Down's. To explore this, Bianchi's team compared transcriptome data from fetuses

with and without Down's, and mouse models of the syndrome, to pinpoint patterns associated with the condition[11]. Then they scoured a database for molecules that might reverse some of the abnormal patterns, including some drugs that are already approved for human use.

They fed one of these molecules, called apigenin, to pregnant 'Down's syndrome' mice and in unpublished data found that the offspring had improved memory and met developmental milestones sooner than those whose mothers did not get the compound. "It's not that everything gets better, but certain areas do improve," says Bianchi. "We are very encouraged."

Bianchi and others in the field are now seeking ways to get more detailed information, non-invasively, about fetal RNA. Until recently, the work has been done using devices called microarrays, which allow scientists to detect known RNA sequences. Although valuable, they offer limited insight because much about the transcriptome remains

"It's not that everything gets better, but certain areas do improve."

mysterious. A version of next-generation DNA sequencing called RNA-seq reveals the transcriptome in all its complex glory, and quantifies each RNA type much more accurately.

Researchers have shown that such an approach is possible[12,13]. In 2014, for example, Quake's team examined blood samples from pregnant women using RNA-seq, in combination with other methods, to detect RNAs that probably originated in the fetus and placenta[12]. They could track the ebbs and flows of transcripts through all three trimesters, including the activity of genes that are crucial for normal brain development. Now they are hunting for transcripts that could yield insight into conditions associated with pregnancy such as pre-eclampsia, in which problems with the placenta cause dangerously high blood pressure in the mother.

The placenta is also the focus of an RNA-seq project led by Williams and RNA biologist Thomas Tuschl at the Rockefeller University in New York City. They are focusing on microRNA (miRNA), a kind of RNA that's known to control the activity of genes, in the hope of uncovering insight into placental biology and devising early-warning tests for pre-eclampsia and other pregnancy conditions. Existing tests, such as looking for protein in the mother's urine, don't reveal the disease until the mother has already started to develop organ damage, says Williams. His team hopes to use miRNA to monitor the placenta non-invasively, and detect pre-eclampsia before damage takes hold.

But such methods still need more work to ensure accuracy and reproducibility before their full potential can be realized, he says.

ENVIRONMENTAL IMPACT

The third piece of the puzzle is how conditions in the womb affect fetal development. Researchers have long known that environmental exposures during this delicate time can influence an individual's lifelong health. Babies whose mothers smoke during pregnancy, for example, grow more slowly in the womb and have an increased risk of developing respiratory diseases and obesity, and studies[14] suggest that smoking alters the transcriptome of the placenta.

One way in which the environment exerts such effects is by altering chemical marks on DNA and the proteins that package up the genome — thereby altering the activities of genes without changing the DNA sequence. The best-studied of these 'epigenetic' marks are methyl groups that, when added to or removed from DNA, boost or silence gene activity. Researchers are using microarrays as well as a form of DNA sequencing known as bisulfite sequencing to lay bare these methylation patterns across the whole genome in samples from maternal blood and fetuses.

That includes the all-important placenta. One surprise from studies of placental tissue is just how dynamic placental methylation is, says Wendy Robinson, a developmental biologist at the University of British Columbia in Vancouver, Canada. The most striking trimester-to-trimester changes are in genes related to immune-system functions, possibly reflecting the placenta's role as a peacemaker between the mother's immune cells and the fetus.

Researchers are itching to understand changes in DNA methylation in pregnancy conditions and after environmental assaults such as smoking. Indeed, studies already suggest that smoking during pregnancy may lead to altered methylation patterns in placental DNA[15]. Lo's group has shown that it can do bisulfite sequencing on fetal DNA in blood samples. But the complexity of unravelling links between environment and epigenetics makes it hard to draw definitive conclusions for these samples yet, says Robinson. Researchers are therefore focusing on studies of placental tissue for now.

THORNY ETHICS

The promises of all these technologies raise issues that should be debated sooner rather than later, say scientists and bioethicists — not least because there are concerns about the NIPT tests already on the market. These tests have spread fast: since becoming commercially available in 2011, NIPT for missing or extra chromosomes (aneuploidies) is now being used in at least 90 countries. And millions of women have had the tests.

NIPT for aneuploidy is a dramatic improvement, says Bianchi. Globally, it has led to a 70% reduction in invasive procedures such as amniocentesis, which carry a small risk of triggering miscarriage. But NIPT can't diagnose aneuploidies reliably, she says: it is a screen, and other, more-invasive diagnostic tests must be used to follow up on the findings. Nonetheless, some women have opted to terminate their pregnancies on the basis of NIPT results alone. Concerns such as these have led several societies to publish position statements that give recommendations for how to counsel patients.

The situation stands to get even murkier. Careless talk about the epigenetics of pregnancy risks scapegoating women for their babies' ill health, when problems such as obesity and gestational diabetes can stem from many factors, including poverty and poor access to health care, say social scientists.

Women must also prepare for unexpected findings, researchers say — and not just about their fetuses. In several cases, non-invasive fetal screening has picked up undiagnosed cancers and diseases such as lupus in pregnant women. And sequencing will sometimes reveal fetal DNA variants that increase the risk of conditions later in life, such as breast cancer or neurodegenerative diseases. Medical researchers say that clinicians must prepare what to share with patients, even as the light-speed pace of invention and discovery outstrips their ability to interpret the findings.

It is always hard to balance the right to know against the potential harm of revealing the presence of a DNA variant — especially if scientists can't be sure what the effect of that variant will be, says Shendure. "It's just going to get really tricky." ■

Claire Ainsworth *is a freelance science journalist in Hampshire, UK.*

1. Lo, Y. M. D. *et al. Lancet* **350,** 485–487 (1997).
2. Chiu, R. W. K. *et al. Proc. Natl Acad. Sci. USA* **105,** 20458–20463 (2008).
3. Lo, Y. M. D. *et al. Sci. Transl. Med.* **2,** 61ra91 (2010).
4. Fan, H. C., Blumenfeld, Y. J., Chitkara, U., Hudgins, L. & Quake, S. R. *Proc. Natl Acad. Sci. USA* **105,** 16266–16271 (2008).
5. Fan, H. C. A. *et al. Nature* **487,** 320–324 (2012).
6. Kitzman, J. O. *et al. Sci. Transl. Med.* **4,** 137ra76 (2012).
7. Chan, K. C. *et al. Proc. Natl Acad. Sci. USA* **113,** E8159–E8168 (2016).
8. Zwemer, L. M. & Bianchi, D. W. *Cold Spring Harb. Perspect. Med.* **5,** a023101 (2015).
9. Hui, L. *et al. Hum. Genet.* **131,** 1751–1759 (2012).
10. Slonim, D. K. *et al. Proc. Natl Acad. Sci. USA* **106,** 9425–9429 (2009).
11. Guedj, F. *et al. Sci. Rep.* **6,** 32353 (2016).
12. Koh, W. *et al. Proc. Natl Acad. Sci. USA* **111,** 7361–7366 (2014).
13. Tsui, N. B. Y. *et al. Clin. Chem.* **60,** 954–962 (2014).
14. Bruchova, H. *et al. Placenta* **31,** 186–191 (2010).
15. Maccani, J. Z. J. & Maccani, M. A. *Adv. Genomics Genet.* **2015,** 205–214 (2015).