NEONATOLOGY

# The fix is *in utero*

*Some genetic diseases cause damage even before a child is born. The answer to these devastating conditions could lie in gene therapy delivered while the baby is still in the womb.*

JOHN FEDELE/GETTY

Hereditary disorders that are discovered during prenatal scans could one day be cured before birth.

BY SARAH DEWEERDT

In July, an international team of researchers reported that they had used gene therapy to correct a fatal brain disorder in mice — before the mice were even born[1].

The mice had a defect in a gene known as *GBA*, which encodes an enzyme responsible for breaking down a fatty molecule called glucocerebroside. Without the enzyme, glucocerebroside builds up in the brain, causing irreversible brain damage. The mice typically die within about 14 days of birth.

The mice model a condition in humans called acute neuronopathic Gaucher's disease. Children born with this genetic mutation rarely live past the age of two.

In the study, researchers injected a virus bearing an intact copy of the *GBA* gene into the brains of fetal mice about mid-way through gestation. The treated mice were born normally, and lived for at least 18 weeks with little evidence of brain pathology. "You're talking about prolonging life significantly," says Jerry Chan, a fetal-medicine specialist at Duke-NUS Medical School in Singapore and an author of the study.

The researchers also administered the gene therapy to healthy macaque fetuses, and showed that it could transform brain tissue without serious side effects in an animal model that more closely approximates the body size and pregnancy physiology of humans.

"What we were trying to do is show the best possible experiments that would justify, if there ever was, a path to human clinical translation," says study leader Simon Waddington, a gene-therapy researcher at University College London.

Other researchers in the small field of prenatal gene therapy see the research as a leap forward, and say it provides the strongest evidence yet that the approach could be feasible in humans. "The combination of those two aspects of the study made it very, very exciting," says Bill Peranteau, a fetal surgeon at the Children's Hospital of Philadelphia in Pennsylvania.

The technical challenges, safety concerns and ethical issues of prenatal gene therapy are substantial. But this approach is more than just hotshot medicine. It could be the best way to treat a select group of devastating genetic diseases — and perhaps the only way to achieve a lasting cure.

## EARLY ADVANTAGES

Acute neuronopathic Gaucher's disease is one of the best candidates for treatment with prenatal gene therapy. That's because the build-up of glucocerebroside begins in the fetus. In the absence of any intervention, irreversible brain damage can occur even before birth. "The

main advantage is preventing the damage from occurring in the first place," Waddington says.

With other genetic diseases, the effects might not begin until sometime in infancy or early childhood. But even then, prenatal gene therapy might be more effective or efficient than waiting until after birth. "You are trying to take advantage of the normal developmental properties of the fetus to increase the efficiency and the likelihood of success of the treatment," says Peranteau, who is working on animal studies of prenatal gene therapy for metabolic diseases affecting the liver.

Before birth, the blood–brain barrier that prevents many molecules from crossing from the bloodstream into brain tissue is immature, a situation that eases delivery of genes to the central nervous system. In a 2011 paper[2], Waddington and his colleagues showed that a gene-therapy vector called AAV2/9 reaches nerve cells in the brain much more reliably in fetal mice than in those already born.

Another advantage of prenatal intervention is that the immune system is still immature. Therefore, the packaging used to deliver gene therapy — whether a virus or another type of vector — might be less likely to cause an adverse reaction. Also, the body develops immune tolerance to the vector, so if a gene therapy 'booster shot' needs to be administered later in life, it is more likely to succeed. The immune system will also accept the normal protein encoded by the gene therapy, rather than destroying it — as has sometimes been seen with postnatal gene therapy and protein-replacement therapies.

In addition, rapid fetal growth and development means more bang for the gene-therapy buck. At any given time, a large proportion of cells in the fetus is actively dividing. That yields a greater likelihood of the vector integrating into the genome. The population of corrected cells will continue to expand throughout gestation. Furthermore, to effect a cure, it is important to get replacement genes into stem cells or progenitor cells — and these long-lived cells are more abundant and more accessible before birth.

Finally, a 20-week fetus weighs roughly 300 grams, whereas a newborn tips the scales at around 3.5 kilograms. That small size translates directly into a higher therapeutic effect from a given dose of treatment. That's a big advantage because gene-therapy products can be expensive and laborious to produce.

## A RISKY BUSINESS

But the fetal time period also poses unique challenges. Any prenatal intervention is complex because it affects two people — the mother and the fetus. "You've always got to take both into consideration, and you've also got to think about the future children of the mother herself," says Anna David, a fetal-medicine specialist and gene-therapy researcher at University College London.

Generally, the delivery of prenatal gene therapy is fairly straightforward. It involves injecting the treatment into an umbilical blood vessel, the amniotic fluid or occasionally directly into fetal tissue — often with the guidance of an ultrasound probe. The techniques are similar to well-established methods used in amniocentesis, chorionic-villus sampling or umbilical-vein blood transfusion.

"The procedures themselves are relatively safe," says David. Still, they do come with a small risk of infection, preterm labour and loss of the pregnancy. All in all, she says, "it's going to be a lot safer, probably, to treat it after the baby is born when you've got the baby and you're not risking the mother".

Then there are the usual risks involved in gene therapy, such as the potential for the vector to provoke an immune reaction in the patient, or incorporate into the genome in a location where it could trigger cancer. Some of these risks are magnified in the prenatal setting. For example, if the gene therapy gets into the mother's bloodstream, it could cause a dangerous immune reaction in her body or even be incorporated into her cells.

In the fetus, especially if given early in development, the gene therapy could alter germ cells that will eventually develop into eggs and sperm, causing changes that could be passed down to eventual offspring — a possibility that many scientists consider ethically problematic. The therapy might also disrupt normal body-system development by triggering the expression of genes in an inappropriate place or at an inappropriate time. That could potentially cause lasting effects, such as organ malformation.

Parents facing an *in utero* diagnosis of a serious genetic condition must often decide whether to raise a child with a lifelong disability or terminate the pregnancy. The appeal of prenatal gene therapy is that it offers a potential third path. But these treatments also raise the stakes: what if the gene therapy doesn't work, leaving parents with a seriously ill child they weren't prepared for and would not have chosen to raise? Similarly, a gene therapy that is only partially effective could turn a disease that previously would have been fatal in infancy into one that results in long-term disability — so it could actually increase suffering for the patient and family.

As a result of such concerns, researchers are cautious about the prospect of attempting prenatal gene therapy in humans. "If there is an adequate treatment for something after birth, that is the way to go," Peranteau says.

## ORIGIN STORY

Even so, scientists have been thinking about prenatal gene therapy for nearly as long as they have been working on postnatal gene therapy. The first proof-of-concept studies[3] in animal models, showing that a gene could be introduced into an organism before birth, were published in 1995 — just a couple of years after the first human gene-therapy trial.

Often, scientists have looked to the prenatal window not just for the opportunity to treat diseases that begin before birth, but as a way around some of the difficulties of postnatal gene therapy. Charles Coutelle at Imperial College London, says that what prompted him to enter the field in the mid-1990s was, "to be quite frank, frustration with the efficiency of gene therapy at the time".

Coutelle had been involved in one of the first human trials of gene therapy for cystic fibrosis, a genetic disorder that affects the lung and other organ systems. It was difficult to deliver gene therapy to the lungs of people with cystic fibrosis because even in young children, the airways were full of viscous mucus and scar tissue; immune-system dysfunction also presented a hurdle. Coutelle thought it might be easier to correct cystic fibrosis *in utero*, when amniotic fluid moves freely in and out of the lungs.

***"You are trying to take advantage of the normal developmental properties of the fetus."***

Coutelle and his team spent several years perfecting fetal transfer techniques in mouse models, as well as working out which vectors would be best to use prenatally against cystic fibrosis or other serious diseases. The first big success — and an achievement that remains significant today — came in 2004. That year, a group including Coutelle and Waddington corrected the bleeding disorder haemophilia B in prenatal mice by injecting them with a virus bearing an intact copy of factor IX, a protein involved in blood clotting[4].

But the team soon had to switch gears. One vector used in the haemophilia work yielded only a temporary cure; another produced more lasting results but led to an increased risk of liver tumours. More importantly, the development of postnatal gene therapy for haemophilia had taken a sudden leap forward. "Once you have an established postnatal gene therapy there's no point in doing it prenatally. Or you have to have good reasons for doing it," Coutelle says.

## A SURFEIT OF TARGETS

Waddington decided to look for a more challenging target disease that causes more severe effects earlier on, which led him to Gaucher's disease. But that is just one of a fairly broad array of metabolic disorders, including Tay–Sachs disease, Niemann–Pick disease and mucopolysaccharidosis, that cause *in utero* damage and could therefore be good targets for prenatal gene therapy.

Other researchers argue that haemophilia remains a good prenatal target. Researchers led by Graça Almeida-Porada and Christopher Porada at Wake Forest University in Winston-Salem, North Carolina, are working with a sheep model of haemophilia A. This form of haemophilia accounts for about 80% of haemophilia cases in humans, but has proven

much more difficult to address with postnatal gene therapy than has haemophilia B.

One major issue is that the protein involved in haemophilia A — factor VIII — is highly immunogenic. Many people with a severe form of haemophilia A develop antibodies against factor VIII, which makes replacement therapy more costly and complicated, says Almeida-Porada. "The goal of going prior to birth is that you would induce tolerance to the protein — these patients would never develop an immune response," she explains. The team aims to cure haemophilia A in fetal sheep by collecting stem cells from the amniotic fluid, correcting the factor VIII gene and infusing the cells back into the fetus.

Studies of prenatal gene therapy in animal models are a dance between practicality and possibility. They depend on the availability of animal models for a given disease, and are shaped by the pace of advances in postnatal therapy or other experimental treatments, such as *in utero* stem-cell therapy or bone-marrow transplantation.

In June, researchers at Yale University in New Haven, Connecticut, reported that they had corrected the inherited blood disorder β-thalassaemia in fetal mice[5]. The disease is caused by mutations in the β-globin gene, which encodes a subunit of haemoglobin, the oxygen-carrying protein found in red blood cells. In β-thalassaemia, haemoglobin is less able to carry oxygen, leading to fatigue, growth stunting and damage to organs.

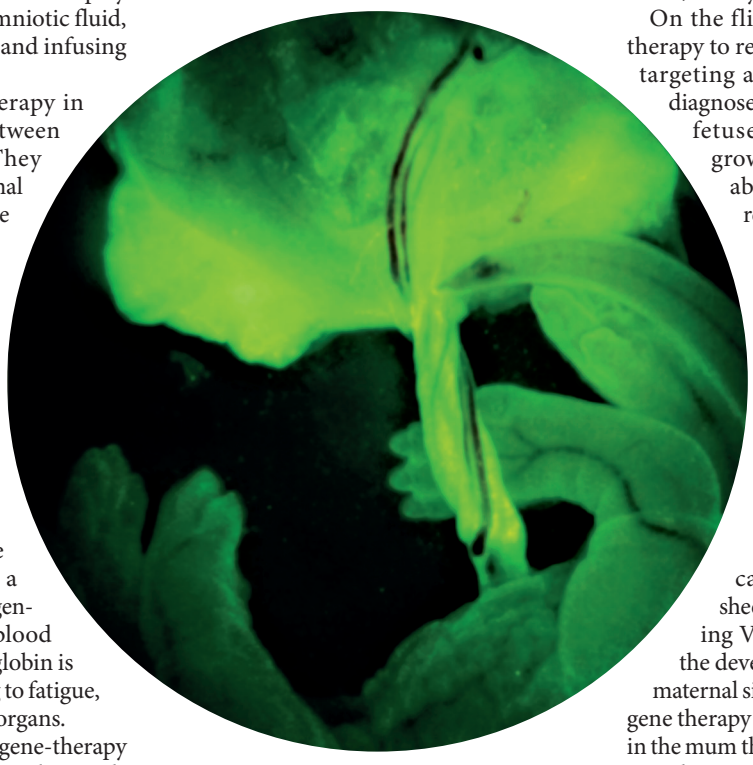
In the study, researchers used gene-therapy delivery vehicles called peptide nucleic acids (PNAs). PNAs are particles consisting of a biocompatible polymer surrounding an intact copy of the β-globin gene. "*In utero* injection of these molecules with a single injection was effective to achieve a phenotypic correction in the mice after birth," says study author Peter Glazer, a radiation oncologist and geneticist at Yale.

The PNAs make use of a cell's own DNA-repair mechanisms to incorporate the correct copy of the β-globin gene into the genome, potentially sidestepping some of the safety issues associated with gene-therapy delivery by viruses. And, crucially, the approach might be more effective prenatally than it is after birth. "In the developing fetus, the cells are more amenable to gene editing," Glazer says. "The DNA-repair capacity of the cells is revved up" because cells are dividing so rapidly, his team's data suggest.

Glazer envisions PNA-based gene therapy for thalassaemia or sickle-cell disease (another inherited blood disorder) being tried first in children, then infants and finally *in utero*. But how quickly this might happen is not clear. "For thalassaemia, a stem-cell approach is probably going to reach clinical practice much faster," says Chan. The safety of stem-cell or bone-marrow transplantation is better established than that of gene therapy, he says.

## A BOON FOR RESEARCH

But even if prenatal gene therapy doesn't reach the clinic, it could still be useful as a research tool. That's already the case with cystic fibrosis, says Marianne Carlon, a gene-therapy researcher at the Catholic University of Leuven in Belgium.



ADELE RICCIARDI/DAVID STITELMAN LAB/YALE

**Fluorescent nanoparticles reveal a mouse fetus, umbilical cord and placenta.**

Carlon and her colleagues have found that gene-therapy vectors can distribute more evenly through the lungs of fetal pigs than through the lungs of newborn pigs. The question is whether such even distribution is necessary or whether just reaching the large- and medium-sized airways is sufficient to prevent the lung damage in cystic fibrosis. *In utero* studies in animal models could also help to resolve questions about which cell types in the airways need to be targeted for gene therapy to be effective in cystic fibrosis.

"We would rather start in a neonatal setting" for attempting gene therapy on cystic fibrosis, Carlon says. Then, she adds, it would make sense to "move towards a fetal setting if you really see that you have difficulties targeting the right cell".

One reason that prenatal gene therapy for cystic fibrosis is not likely to be practical is that *in utero* screening for the disease is not widespread. As a result, the diagnosis is rarely made until after birth. "Without a prenatal diagnosis there is no prenatal gene therapy," Coutelle says.

Clinicians would need to be able not only to detect a disease before birth, but also to confidently predict that its severity would be sufficient to warrant gene therapy. These are complex questions that aren't fully resolved for all the prenatal target disorders. However, if there is no prenatal treatment for a disease, there might be little point in identifying it *in utero*.

Waddington's attitude is simply to bypass this catch-22 situation. "We'll develop the cures, and then that justifies doing the diagnoses," he says.

On the flip side, the first prenatal gene therapy to reach human trials might be one targeting a condition that is exclusively diagnosed *in utero* because it only affects fetuses before birth. Intrauterine growth restriction (IUGR) affects about 3% of all pregnancies and results in babies with dangerously low birth weight.

Unlike other prenatal gene therapy targets, IUGR is not a single-gene disorder. It occurs when, for unknown reasons, the normal remodelling of uterine arteries during pregnancy does not occur. That leaves the placenta and developing fetus starved of blood and nutrients.

David has shown that IUGR can be alleviated — at least in sheep — by delivering a gene encoding VEGF, a protein that stimulates the development of blood vessels, to the maternal side of the placenta[6]. "We're giving gene therapy to the mum, to treat a condition in the mum that causes a problem in the fetus," David says.

VEGF is expressed for only about a week, but that's long enough to trigger expansion of the placental vasculature. A similar approach has been used to stimulate the growth of blood vessels in the heart and neck, so the therapy, known as therapeutic angiogenesis, is well established postnatally. David has applied for regulatory and ethical approval to conduct a trial of the therapy in pregnant women.

"It's a major cause of cardiovascular disease and diabetes later in life," David says, referring to IUGR. "There's no treatment. And women want it, when you ask them. They're desperate to have a treatment." ■

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