

Gene therapy

Are we nearly there yet?

Gene therapy — curing disease by genetic engineering — has been on the research agenda for more than 30 years. Now, after years of false dawns and disappointments, we are seeing the first real successes. At last, patients are being cured of life-threatening diseases by genetic manipulation. This article considers the problems and prospects for this technology.

KEY WORDS

- Gain of function
- Gene augmentation
- Germline
- Loss of function
- Somatic cell
- Vector

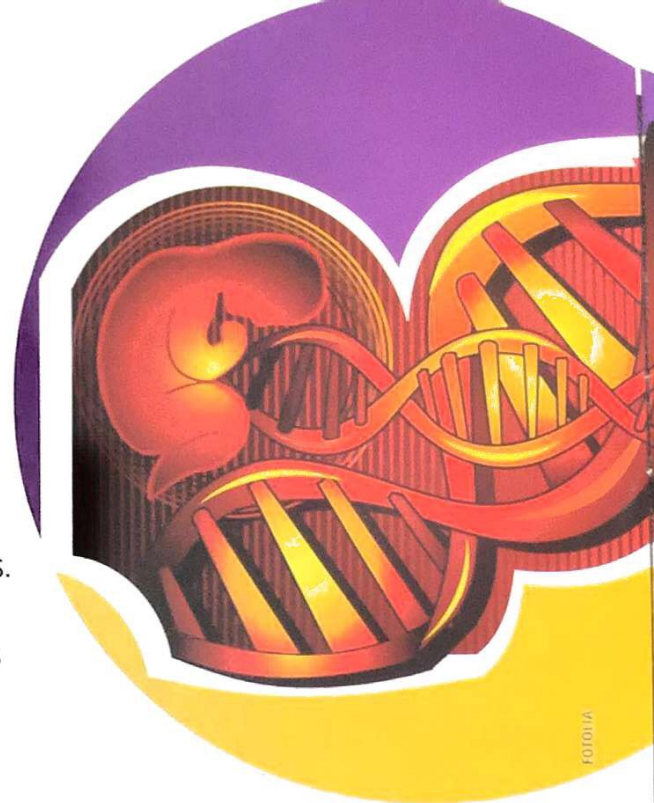
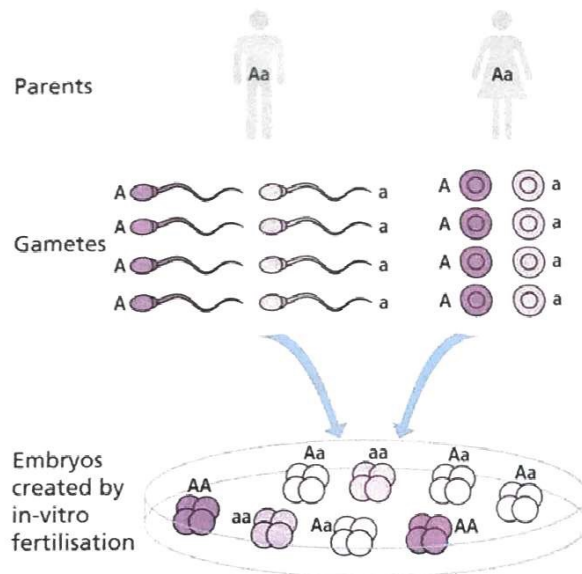
The idea behind gene therapy is simple. Non-functioning single genes cause many genetic diseases. These diseases include muscular dystrophy and cystic fibrosis (see *BIOLOGICAL SCIENCES REVIEW*, Vol. 21, No. 1, pp. 21–25). Genetic engineering allows us to isolate genes and insert them into cells. So why not isolate a working copy of the defective gene and put it back into the cells of the patient? Surely that would cure the disease? And it's not just genetic diseases (those inherited as **autosomal dominant**, **autosomal recessive** or **X-linked** conditions) that could benefit. Many non-inherited diseases are caused by cells misbehaving. Genes give cells their instructions. Even cancer is caused by genetic mutations that happen in individual cells some time during a person's life. So reprogramming cells by introducing beneficial genes looks like a good way to treat all sorts of diseases.

Germline or somatic cell therapy?

One important problem is choosing which cells to treat. It would seem logical to choose the fertilised egg as the target for gene therapy, at least for inherited diseases. That way the repaired gene will be copied faithfully into each daughter cell at every mitosis, and every cell in the baby's body will have been treated. Moreover, the mutant gene will have been eliminated not just from that person but also from all his or her offspring. But there are two objections to this strategy, an ethical one and a practical one. The ethical question is whether we have the right to alter the genes of future generations. Many people would say we do not have this right — our well-intentioned manipulations might do some unforeseen harm. In line with this view, genetic manipulation of the germline is prohibited in Great Britain.

Even without the ethical uncertainties, practical difficulty rules out gene therapy of the fertilised egg at present. You can see why in Figure 1. Suppose both partners carry the cystic fibrosis mutant gene in heterozygous form. They are completely healthy themselves but they are at risk of having affected children. Eggs would be obtained by the same procedure that is used for test-tube babies. Typically half a dozen eggs would be obtained and fertilised in the laboratory with the father's sperm. They would be grown in culture for about a day and tested to see whether or not they carry the CF mutation. On average, only one in four of the fertilised eggs would be homozygous for the CF mutation; the other three would develop into healthy normal people without any need for genetic manipulation. One or two of the fertilised eggs would be re-implanted in the mother, the rest discarded. Nobody would be so perverse as to select the affected eggs, subject them to an experimental gene therapy

Figure 1 Embryos obtained by in-vitro fertilisation from a couple, both of whom are carriers of an autosomal recessive disease. Only one in four of the embryos carries the disease in homozygous form (aa); the remaining three in four are heterozygous (Aa) or homozygous for the wild-type allele (AA) and would develop into unaffected babies.



procedure and re-implant them, while discarding the three in four eggs that would have developed normally anyway. Surely you would discard the potentially affected embryos and select one or more of the three in four genetically normal embryos for implantation? That would solve the problem but it wouldn't have involved any gene therapy.

Given the ethical and practical objections to germline gene therapy, all present proposals concern the alternative, less dramatic option of somatic cell gene therapy.

Loss of function diseases

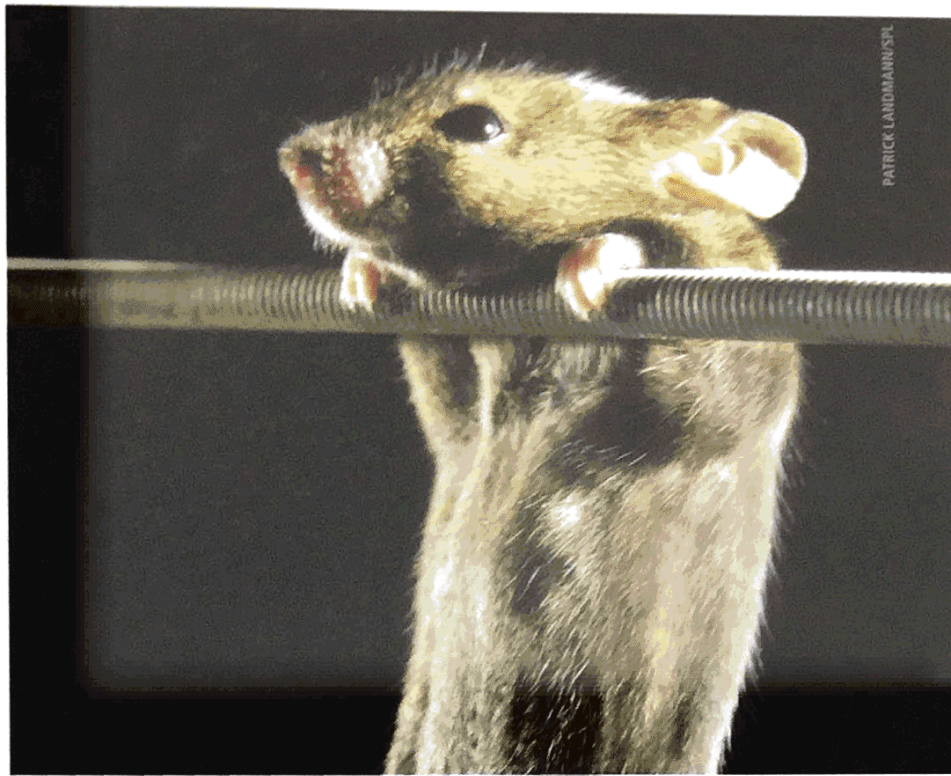
Some diseases happen because a vital gene is not working. In cystic fibrosis, for example, a gene that encodes a protein that forms an essential chloride ion channel in cell membranes has undergone some change in its DNA sequence so that it no longer encodes a working channel. Such 'loss of function' diseases are usually recessive, because in most cases cells can get by with a single working copy of a gene. It is only when neither copy works (that is, in homozygous mutants) that disease results. In principle, such diseases could be cured by equipping cells with a working version of the gene. The resident non-functional gene can be left in place; it is doing no positive harm. This is gene augmentation, which is the most straightforward form of gene therapy.

Gain of function diseases

In some diseases the altered gene does something positively wrong. Maybe it makes a product that is toxic to the cell, or causes the cell to respond in a wrong way to external signals. These are 'gain of function' diseases. For example, in Huntington's disease a normal cellular protein undergoes a genetic alteration that makes it toxic to neurones. The mutant protein accumulates and gradually kills neurones, causing a progressive neurodegenerative disease. Like most gain of function diseases, Huntington's disease is dominant and affects heterozygotes. There is one normal copy of the gene in each cell already, but that does not prevent the mutant gene exerting its deleterious effect. For gene therapy it will not be sufficient just to provide a correctly functioning copy of the gene. We would somehow have to inactivate, repair or replace the mutant gene. This is a much more difficult task than simply adding a functional gene to a cell. There are many ideas about how this might be done, but successful clinical applications still lie in the future.

Hopes and disappointments

Even gene augmentation is not simple. The whole gene therapy field has run a roller-coaster course between hope and disappointment for most of the past three decades. There are two main problems:



Gene therapy research on mice. This mouse is a mammalian model for Duchenne muscular dystrophy, a condition in humans typified by muscle wasting and loss of function. The mouse has an abnormal gene that, left untreated, caused the muscles in its legs to waste, making it unable to walk. Here, it has been treated by a method of gene therapy known as exon skipping. It is undergoing tests to determine how much its muscles have recovered after treatment.

- To cure the disease you need to get your desired gene into many or most of the cells where the malfunction is causing problems. So, for cystic fibrosis you would need to 'transfect' (see Box 1) large numbers of cells in the patient's airways, lungs and pancreatic duct — the major sites of problems for CF patients. For muscular dystrophy you would need to transfect cells in many different muscles distributed around the patient's body. It is difficult to transfect large numbers of cells in tissues or organs deep inside a patient's body.

- Even if you could get your working gene into the right cells, you need to keep it working long term. When a gene is artificially introduced into a cell, it tends to work

BOX 1

Getting genes into cells

In the laboratory there are ways to get cells to take up pieces of naked DNA, but they don't work very well. So most practical ideas for gene therapy rely on vectors. These exploit the tricks viruses use to infect cells. Viruses like HIV are packages of rogue genes that can get into cells and take them over. Gene therapy vectors are natural viruses that have been modified by replacing the pathogenic virus genes with the desired therapeutic gene. It requires a great deal of clever genetic engineering to design a vector that will deliver the therapeutic gene reliably into the right cells ('transfecting' them) without causing any ill effects. Most of the progress in gene therapy over the past 20 years has been in designing better vectors. Surprisingly, complex viruses related to HIV (lentiviruses) are proving better vectors than the smaller and simpler **retroviruses** used in many earlier trials.

FURTHER READING

The US Department of Energy website has a good explanation of gene therapy, with details of many trials:

www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml

For data on numbers, diseases and countries involved in clinical trials of gene therapy, see: <http://tinyurl.com/c3zu43q>

The pie chart in Box 2 is from this website.

For information on gene therapy for an inherited eye disease see:

www.candleinthedark.be/disease

This page includes two short videos showing an affected boy navigating a maze before and after gene therapy for his eye disease.

TERMS EXPLAINED

Autosomal dominant A disorder where one mutated copy of a gene is sufficient for a person to be affected.

Autosomal recessive A disorder where two mutated copies of a gene must be present for a person to be affected. An affected person usually has unaffected parents who are carriers (each parent carries a single copy of the mutated gene).

Germline The sperm and/or egg, and other cells that give rise to them. Genetic changes to the germline can be transmitted to future generations; changes in somatic cells cannot.

Retrovirus A virus that has RNA as its nucleic acid. When it infects a cell from its host, it is able to make a copy of this RNA using the enzyme reverse transcriptase. This DNA copy is then incorporated into the hosts's genetic material.

Somatic cells Any cells of the body that are not part of the germline.

X-linked Conditions caused by mutations in genes carried on the X chromosome.

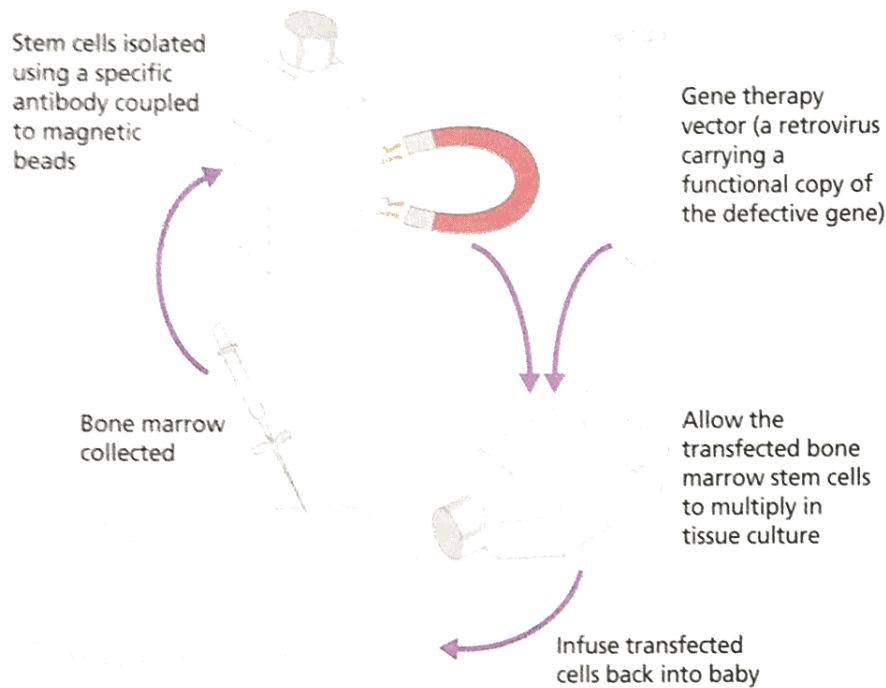


Figure 2 Gene therapy by ex-vivo gene augmentation. Because of a genetic mutation, this baby's bone marrow is unable to make functioning white blood cells to resist infection. Bone marrow cells were removed and exposed in the laboratory to a vector containing a working version of the defective gene. Cells that had taken up the gene were selected and grown in culture, then used to replace the baby's defective bone marrow.

for only a short time, typically a few days. Cells are on the alert for foreign genes, because these usually come from viruses, and the cells have defence mechanisms to shut them down.

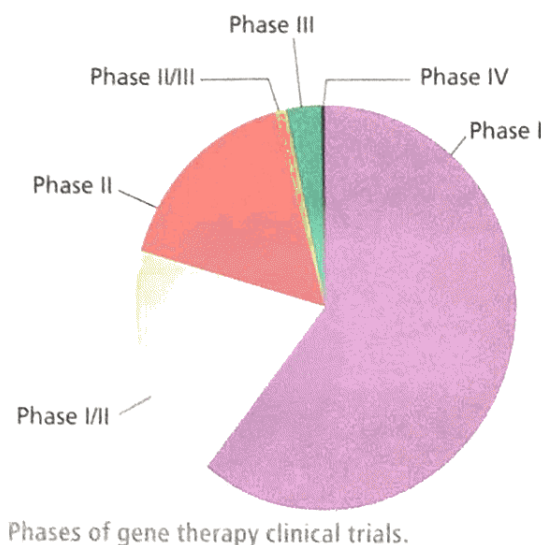
Neither of these problems has yet been solved, but over the years there has been significant progress. A big high in the roller-coaster ride came in 2002. Doctors in Paris used gene therapy to cure young boys who suffered from a lethal X-linked 'immunodeficiency syndrome'. Because of a faulty gene, these boys had no functioning immune system. They were completely unable to resist any infection, and each had to live in a sterile plastic bubble. Gene therapy was their only hope for long-term survival. Because the basic fault is in bone marrow cells, there was a possibility of getting around the transfection problem. Bone marrow cells were removed from each patient and transfected in the laboratory. Only a tiny proportion of the cells was successfully transfected but the scientists were able to pick out those cells and make them multiply in cell culture. Their descendents all carried the working gene. Once sufficient transfected cells had been grown, the patient's own bone marrow was partly destroyed by drug treatment and replaced by the transfected cells (see Figure 2). In 17 of 20 boys treated in Paris and in a similar trial at Great Ormond Street Hospital in London, the transfected cells made fully functional bone marrow. Those boys had working immune systems, and they could come out of their plastic bubbles and begin to live normal lives.

Every roller-coaster has downs as well as ups, and 2 years later disaster struck. First two, then eventually five of the boys developed leukaemia. This was successfully treated in four of them but a fifth boy died. Studies of their DNA proved that it was the gene therapy that had caused the leukaemia. The vector, a modified virus, was designed to insert its own DNA into the DNA of the boys' chromosomes. But in those five boys it had done

BOX 2

Clinical trials of gene therapy

Phase I trials test only the safety and side effects of a procedure. Phase II and III trials test both safety and efficacy — phase II in a small number of people, phase III in much larger numbers. Phase IV trials cover procedures that have been already licensed. The chart shows the phases of all the gene therapy clinical trials that have ever been run, starting in 1989 — over 1700. In 2010, 79 new trials were approved worldwide. A lot is happening!



so next door to a gene called *LMO2* that controlled the growth of the cells. The vector turned on *LMO2* as well as the therapeutic gene. This tipped the cells towards growing too fast, ultimately causing leukaemia. But despite this setback, several boys who would otherwise have died are now living healthy lives. Hopefully, clever modifications of the vector can overcome the *LMO2* problem. More recently a different form of immunodeficiency (adenosine deaminase deficiency) has been successfully treated in 25 patients at hospitals in Italy, London and Los Angeles.

Another high came with the successful gene therapy of a form of blindness. In 2009, after lengthy trials in dogs, doctors at the Moorfields Hospital in London and in the USA succeeded in restoring some degree of vision in patients with a rare form of blindness — Leber's congenital amaurosis — by injecting a vector containing a working version of the mutated gene *RPE65* into their retinas. Over 30 patients have now been treated.

The lesson from this history is that there is no one-size-fits-all method for gene therapy. Vectors must be laboriously designed for each specific gene to target each specific disease. But the potential rewards of success are great, and hundreds of laboratories worldwide are working on a variety of approaches for all sorts of diseases. This is reflected in the large number of clinical trials currently underway (see Box 2). Most of these

are testing just the safety of a procedure rather than the clinical efficacy, but gradually progress is inching towards clinical utility (phase III and IV trials). So are we nearly there yet? Well, gene therapy certainly won't provide a universal panacea for all genetic diseases, progress is slow and there will be more setbacks, but for a steadily increasing number of patients, yes, we are at least beginning to get there.

Professor Andrew Read works in human genetics at the University of Manchester. He has worked on identifying the genes that go wrong in various hereditary diseases, particularly hereditary deafness. He was involved in setting up the DNA diagnostic laboratory at St Mary's Hospital, Manchester, which provides NHS diagnostic services to 5 million people in the northwest of England.

KEY POINTS

- Gene therapy seeks to cure disease by changing the genetic instructions in the cells of a patient.
- Gene therapy is not just for inherited diseases, but could potentially be used for any disease where the behaviour of cells needs to be changed, such as cancer.
- Most gene therapy involves using a genetically engineered virus as a vector to ferry therapeutic genes into cells of the patient.
- The main problems in gene therapy are getting the vector into enough of the right cells in the patient, and keeping the therapeutic gene working once it is in a cell.
- For many years attempts at gene therapy did not really work, but with advances in genetic engineering technology we are now seeing the first true successes.