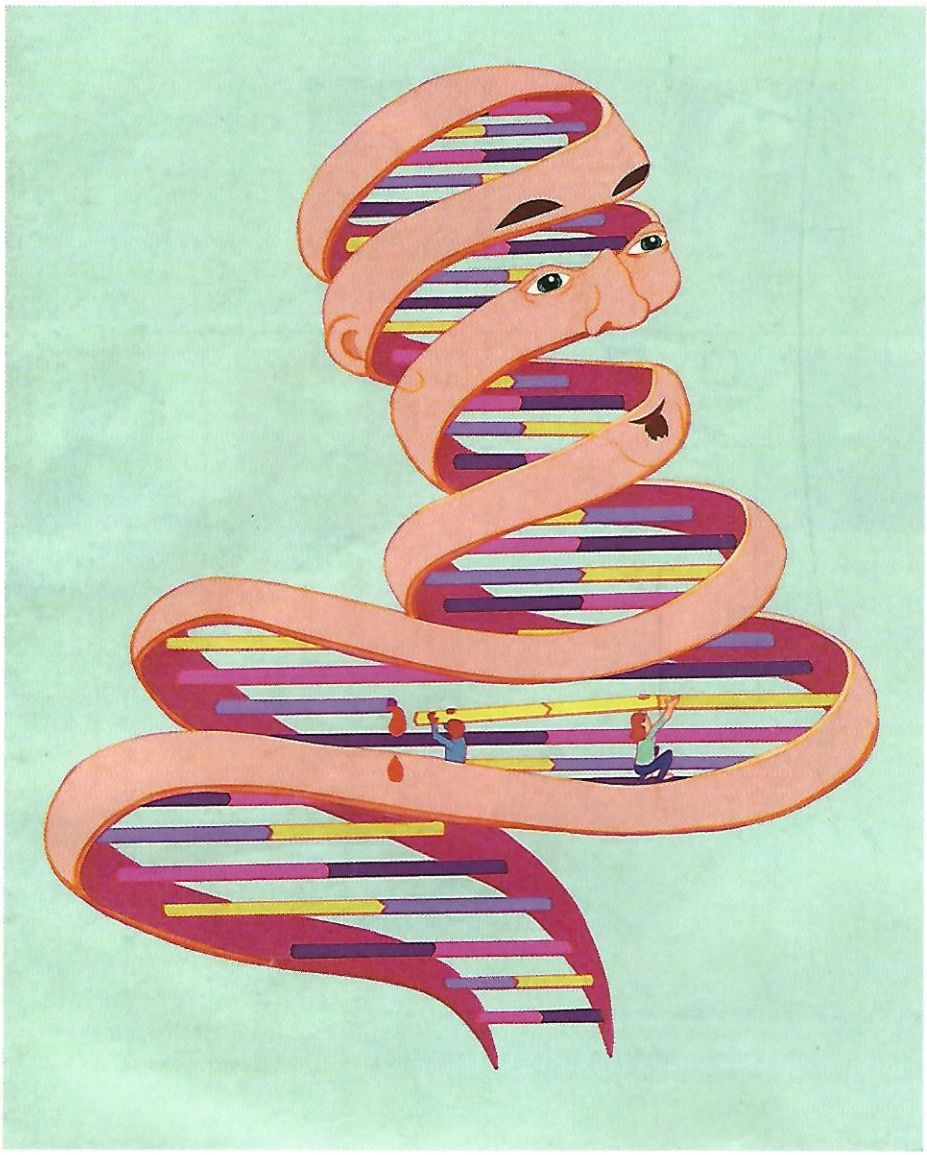

JESSICA FORTNER

GENE THERAPY

Genie in a vector

Repairing the faulty genes that cause haemophilia could ultimately cure the disease, but it will be a tough challenge.

BY JULIE GOULD

Martin never learned to ride a bike, could not play football with his friends and wore a crash helmet when playing in the garden, just in case he bumped his head. His parents had good reason to be protective: his severe haemophilia B meant that the gentlest touch could lead to a serious, debilitating bleed. "It's very frustrating, growing up with haemophilia," says Martin. "You want to be like the other kids, but you can't."

As a result of an inherited genetic mutation, people with haemophilia B lack a protein called factor IX that is crucial for forming blood clots (see page S4). Currently, patients are treated several times a week with infusions of a concentrated version of the protein. This stops the bleeding, but it does not address the underlying cause of the disease nor does it fully remove its debilitating symptoms.

A few years ago, Martin had to stop his work as a truck driver. "I was letting the company down because I couldn't make it into work," he says. "The bleeding into my joints had made it very painful for me to move." In 2011, after 37 years of pain and joint degeneration caused by internal bleeding, Martin signed up for a clinical trial of a gene-therapy treatment at the Royal Free Hospital in London, hoping that it would provide some relief.

Rather than infusing functional clotting factors, the therapy aims to get the body to create its own. DNA with a functional factor IX gene was bundled into the molecular wrapper of a virus — known as AAV8 — then shuttled into liver cells, where factor IX is normally made.

Of the six patients who enrolled, four were able to discontinue their infusion treatments after the therapy[1]. Martin was one of them: his factor IX levels increased significantly, taking him out of the severe haemophiliac range and into the moderate group. His clotting factor levels have remained stable ever since.

The success was a crucial stepping stone for Edward Tuddenham, emeritus professor of haemophilia at University College London, who led the clinical trial. He wants to find a treatment not just for haemophilia B but for the much more common haemophilia A — but that is turning out to be a challenge.

FREEDOM OF EXPRESSION

The viral vehicle AAV8 is ideal for treating haemophilia B, but it works less well for haemophilia A. This is because the DNA encoding the clotting factor that is missing in the latter — factor VIII — is about six times larger than for factor IX, so it doesn't fit into AAV8. To make it fit, researchers often cut 4,500 base pairs out of the factor VIII gene sequence. The section they delete encodes a specific region of the protein — called the B-domain — that ensures efficient secretion of factor VIII. In its place, Tuddenham and his colleagues tried inserting a DNA sequence that is one-fiftieth of the size, but has the same function. But in a 2010 study of haemophiliac mice, these B-domain-modified treatments did not increase the level of factor VIII expressed in the blood[2]. Since then, Tuddenham has not only been trying to fix the gene but also to improve its expression.

The rate at which the factor VIII gene produces its protein is affected in part by the placement of the triplets of DNA bases — codons — that dictate where translation of the genetic material into protein should start and stop. The start and stop codons in the DNA sequence of a normal mouse or human factor VIII gene, did not promote vigorous protein production. "So we replaced them with better ones," says Tuddenham. When that was done, expression levels in a mouse model of haemophilia went from about 2% of that found in healthy mice to about 2,000%. The increase produced by the codon optimization was "enormous, truly stunning", he says.

In 2015, Tuddenham and his team hope to lead trials to test safety

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and efficacy of their optimized factor VIII gene therapy for people with haemophilia A. The number of people in the trials is likely to be between 10 and 20, but even if the factor is expressed effectively in humans, there are still hurdles to overcome.

DELIVERY ON A PLATELET

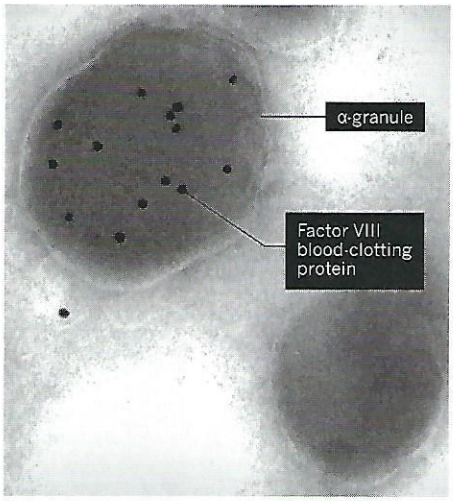
One hurdle is that AAV8 can be administered only once, because the virus triggers a strong immune response. "After one treatment with AAV8, you can't ever have a repeat dose. You are immunized against it," says Tuddenham. So although gene therapy could be a one-off cure, if the immune response is triggered before the therapy reaches the target, it is useless.

David Wilcox at the Children's Hospital of Wisconsin Research Institute in Milwaukee, hopes to get around this problem by using the body's own cells to deliver factor VIII. He is developing a way to insert functional factor VIII into structures called α-granules, which are found inside blood cells called platelets (see image, right). Platelets are the first cells to arrive at a wound site, where they rapidly begin to help form blood clots by releasing chemical messengers. Wilcox is working on modifying platelets to also release functioning factor VIII. "This removes the problem of having AAVs and factor VIII proteins floating around the rest of the body," says Wilcox, "thus avoiding any immune reactions."

"We've still got a lot to learn about gene editing in large animals before we even think about trying it in adult humans."

First, however, Wilcox has to harvest blood stem cells from the patient. He uses growth factors to coax stem cells in the bone marrow out into peripheral blood vessels, where they can easily be collected. The stem cells, which make up 2–5% of the peripheral blood sample, are then separated out in a procedure called peripheral blood stem cell apheresis and undergo gene therapy so that they contain the working factor VIII. The patient then has chemotherapy to partially suppress their existing bone-marrow stem cells before receiving a transfusion of the engineered stem cells into the blood. These cells find their way back to the bone marrow, where they will eventually produce platelets that contain functioning factor VIII.

In 2013, Wilcox tested the procedure on three dogs with severe haemophilia A, using a human factor VIII gene — and two of the dogs no longer require the usual treatment with infused factor VIII[3]. As predicted, none of the dogs showed signs of developing antibodies to the human factor VIII proteins — when the dogs received a cut, blood clots formed faster than they had without the gene therapy. "We think that the factor VIII is secreted from the platelets so quickly at the trauma site that the immune system does not have time to react before the factor VIII can start repairing the vascular injury," says Wilcox. Like Tuddenham, Wilcox's team hopes to start clinical trials inext year.



DAVID A. WILCOX & PAQUITA NURDEN

Researchers are modifying platelets to release factor VIII from α-granules at the site of injury.

But even if platelets can offer an alternative delivery vehicle, it could be an unpleasant one for patients. "I think they have a viable approach for patients with antibodies to AAVs or those affected by HIV and hepatitis," says Tuddenham, "but the doses of chemotherapy treatment before the stem-cell transplant aren't a walk in the park".

CORRECTING IN PLACE

So far, gene-therapy trials have focused on adults with the disease, but haemophilia is an inherited disease, affecting a person from birth. Unfortunately, the technique is not a viable option for children. If a child's liver were to be infused with factor VIII genes introduced through AAV, there would be an initial increase in the levels of clotting factor in the blood, as with the adults in Tuddenham's 2011 trial. But as the child grows, the expression levels would decrease when new liver cells are produced without the functioning factor VIII gene are produced, says haematologist Katherine High, at the Children's Hospital of Philadelphia in Pennsylvania.

In theory, Wilcox's method might work in children because the functional clotting-factor genes have been integrated into the stem cell's genome and will be passed on to daughter cells. In practice, however, no responsible physician would expose an infant or child with a non-lethal disease such as haemophilia to chemotherapy.

A promising way to avoid these problems is *in vivo* genome editing, in which mutant genes are corrected *in situ* rather than replaced. This could potentially work at any age — but the earlier in life such a treatment is available, the better, as the benefit would be lifelong.

Conceptually, this approach is as simple as setting up a biological tool to cut out the mutated area of the genome, then another to insert a corrected template, says Merlin Crossley of the University of New South Wales, Sydney, Australia. Crossley sees gene-editing therapies as the best potential tool for curing haemophilia.

This could be particularly beneficial for children: as the liver grows, the new daughter cells would contain the functioning clotting-factor gene. The clotting factor would then be recognized as part of the body, and could ultimately eliminate the child's haemophilia. "The replacement template is cloned from healthy patients and wouldn't be attacked by the immune system because it isn't considered as foreign," Crossley says.

A 2011 study in mice[4] by High provided strong evidence that genome editing is a viable option. Immediately after birth, one set of mice was given Tuddenham's style of gene-transfer therapy; a second set was given the genome-editing treatment. High discovered that the levels of functioning clotting proteins in mice receiving the genome-editing treatment stayed high even after a portion of their liver was surgically removed; in the mice receiving gene therapy, by contrast, factor levels decreased. "This is the advantage of this treatment, especially for children," says High.

Genome editing has to be precisely targeted to the mutation to be repaired, and the sheer number of mutations for haemophilia A — more than 2,000 — makes this a challenge.

Both High and Tuddenham believe that in the short term, genome editing is not the answer. "The gap between proof-of-principle experiments in mice to clinical trials in humans for gene-transfer therapy was 14 years," says High. "And we've still got a lot to learn about gene editing in large animals before we even think about trying it in adult humans, let alone infants."

Having his haemophilia reduced to a moderate level has improved Martin's quality of life tremendously. He has needed the standard infusion treatment fewer than ten times since the gene therapy, and says that "only one of those occasions was a serious bleed". He says that signing up for the trial was not an easy decision, because there were not any other similar trials on which to base his decision. But he believes that his successful experience should help to encourage people to participte in future studies. "You go from a position of knowing what you are, how you are and how to deal with it, to a position of complete uncertainty," he says. "So I hope that the uncertainty is reduced for other patients when they hear about our experiences." ■

Julie Gould *is the editor of Naturejobs.*

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3. Du, L. M. *et al. Nature Comm.* 4, 2773 (2013).
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