Jeff Carroll was concerned about passing Huntington's disease on to his children.

TAEHOON KIM FOR *NATURE*

CRISPR BABIES

When will the world be ready?

Scientists say efforts to make heritable changes to the human genome are premature and fraught with uncertainty. Here's what it could take to make the technique safe and acceptable.

BY HEIDI LEDFORD

Jeff Carroll had been married for six months when he and his wife decided not to have children. Carroll, 25 years old and a former corporal in the US Army, had just found out that he had the mutation that causes Huntington's disease, a genetic disorder that ravages the brain and nervous system and invariably ends in an early death. He had learnt that his mother had the disease about four years earlier, and now he knew that he was all but certain to develop it, too.

Faced with a 50% chance of passing on the same grim fate to their children, the couple decided that kids were out of the question. "We just kind of shut that down," says Carroll.

But he had begun studying biology in the army in the hope of learning more about the disease. He found out about a process called preimplantation genetic diagnosis or PGD. By conceiving through *in vitro* fertilization (IVF) and screening the embryos, Carroll and his wife could all but eliminate the chance of passing on the mutation. They decided to give it a shot, and had twins free of the Huntington's mutation in 2006.

Now Carroll is a researcher at Western Washington University in Bellingham, where he uses another technique that might help couples in his position: CRISPR gene editing. He has been using the powerful tool to tweak expression of the gene responsible for Huntington's disease in mouse cells. Because it is caused by a single gene and is so devastating, Huntington's is sometimes held up as an example of a condition in which gene editing a human embryo — controversial because it would cause changes that would be inherited by future generations — could be really powerful. But the prospect of using CRISPR to alter the gene in human embryos still worries Carroll. "That's a big red line," he says. "I get that people want to go over it — I do, too. But we have to be super humble about this stuff." There could be many unintended consequences, both for the health of individuals and for society. It would take decades of research, he says, before the technology could be used safely.

Public opinion on gene editing to prevent disease is largely positive. But Carroll's reticence is common among scientists. When news broke last year that a Chinese biophysicist had used genome editing in an attempt to make children more resistant to HIV, many scientists were quick to condemn the move as premature and irresponsible.

Several researchers and scientific societies have since called for a moratorium on heritable genome editing in humans. But such a moratorium raises an important question, says embryologist Tony Perry of the University of Bath, UK. "When would it stop?" he asks. "What conditions would you need to meet?"

Nature asked researchers and other stakeholders what hurdles remain before heritable gene editing could become acceptable as a clinical tool. Although some scientific challenges are probably surmountable, approval on a grand scale is likely to require changes to how clinical trials are run, as well as a broader consensus about the technology .

Off-target edits: how many 'mistakes' are too many?

Genome editing presents many difficult technical challenges, but the spectre of creating unwanted genetic changes has probably received the most attention, says Martin Pera, a stem-cell researcher at the Jackson Laboratory in Bar Harbor, Maine. And yet, he adds, this challenge might also be the easiest to surmount.

The most popular way to edit genes relies on a system called CRISPR–Cas9. Co-opted from a mechanism that some microbes use to defend themselves against viruses, it uses an enzyme called Cas9 to make cuts to DNA. A scientist can supply a snippet of RNA to guide Cas9 to a specific site in the genome. But Cas9 and enzymes like it have been known to cut DNA at other sites, too, particularly when there are DNA sequences in the genome similar to the target (see 'Off-target effects'). Such 'off target' cuts could result in health problems: a change to a gene that suppresses tumour growth, for example, might lead to cancer.

Researchers have looked to develop alternatives to the Cas9 enzyme, some of which might be less error-prone. They have also engineered versions of Cas9 that have lower error rates[1].

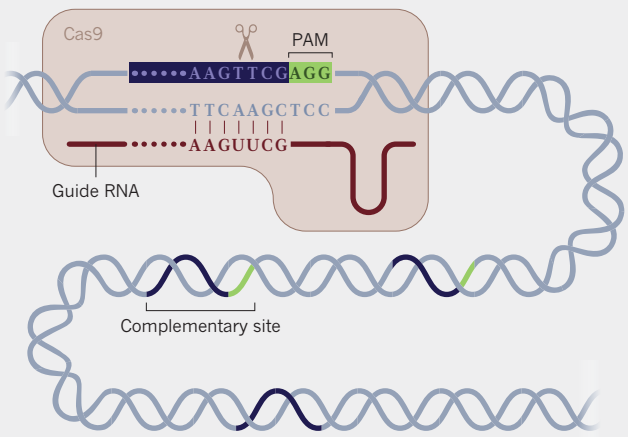
Error rates vary depending on what site in the genome is targeted. And many of the gene-editing enzymes have been studied only in mice or in human cells grown in culture — not in human embryos. The rate of mistakes could differ between mice and human cells, and between mature cells and embryos.

The number of errors might not need to be zero. A small number of DNA changes occur naturally every time a cell divides. Some say that a few background changes could be acceptable, especially if the technique is being used to prevent or treat a serious disease.

Some researchers already consider the error rate for CRISPR to be sufficiently low, says Perry. "But, and I think it's quite a big 'but', we don't really have a handle on the editing specificity in human oocytes and embryos," he says.

Off-target effects

The Cas9 protein works like a pair of molecular scissors. A guide RNA sequence binds to a complementary DNA sequence that is adjacent to a string of letters known as the proto-spacer adjacent motif (PAM). But there can be many sites in the genome that contain the same or similar sequences, so Cas9 might cut in the wrong places.



On-target, but wrong: how precise does gene editing need to be?

A bigger problem than off-target effects might be DNA changes that are on-target but unwanted. After Cas9 or a similar enzyme cuts DNA, it is up to the cell to heal the wound. But the cell's repair processes are unpredictable.

One form of repair, called non-homologous end joining, often deletes some DNA letters at the cut site — a process that could be useful if the goal of the edit is to shut down expression of a mutant gene.

Another form of repair, called homology-directed repair, allows researchers to rewrite a DNA sequence, by supplying a template that gets copied in at the site of the cut. This could be used to correct a disease such as cystic fibrosis, which is generally caused by short deletions in the *CFTR* gene (see 'On-target effects').

Both processes are difficult to control. The deletions caused by non-homologous end joining can vary in size, producing different DNA sequences. Homology-directed repair gives more control over the editing process, but it occurs at a much lower frequency than deletions in many cell types. Research in mice can make CRISPR gene editing seem more precise and efficient than it is now, says Andy Greenfield, a geneticist at the UK Medical Research Council's Harwell Institute near Oxford. Mouse litters are large, and so researchers have a lot of shots at goal to get the right edit — discarding all errors. The same would not be true for human embryos.

It is not yet clear how efficient homology-directed repair would be in humans, or even how it would work. In 2017, one team used

CRISPR–Cas9 in human embryos to correct gene variants associated with heart failure[2]. The embryos were never implanted, but the results suggested that the modified cells had used the mother's genome as their template for DNA repair, rather than the DNA template that the researchers had provided. That could be a more reliable way to edit DNA in human embryos. But other researchers have since reported that they have been unable to repeat the results[3]. "At this point, we don't really understand how embryos deal with DNA repair," says Jennifer Doudna, a molecular biologist at the University of California, Berkeley. "A lot of work needs to be done in other kinds of embryos, just to understand the fundamentals."

Researchers are developing ways around the problems associated with DNA repair. Two reports published in June discuss a CRISPR system that can insert DNA into the genome without breaking both strands, thereby bypassing the reliance on DNA repair mechanisms. If the systems hold up to further testing, they could offer researchers greater control over what they edit[4,5].

Another approach is to use a technique called base editing. Base editors fuse a disabled Cas9 to an enzyme that can convert one DNA letter to another[6]. The disabled Cas9 directs the base editor to a site in the genome where it chemically changes the DNA directly, rather than by making a break. Studies published in April have shown that some of these base editors are prone to making off-target changes, too[7,8], but work is ongoing to try to improve their fidelity.

"Base editing doesn't currently meet our criteria," says Matthew Porteus, a paediatric haematologist at Stanford University in California. "But one can imagine it getting better and better."

Wanted, but dangerous: which edits are safe?

Even if the targeting and precision of changes in genome editing were perfect, there would still be a question about what kinds of changes to the human germ line are likely to be safe. In 2017, an international effort spearheaded by the US National Academies of Sciences, Engineering, and Medicine outlined the conditions that should be met before editing a human embryo that is destined for implantation[9]. One of the criteria was that the DNA sequence created by the edit already be common in the population, and carry no known risk of disease.

That requirement alone would put heritable gene editing in people out of reach for the near future, says Porteus. It is not only difficult to predict the precise sequence of an edit, but also hard to know with certainty that a variant will not increase the risk of disease.

Some mutations in a gene called *PCSK9*, for example, are associated with lower cholesterol levels and therefore a reduced risk of heart disease. The gene is sometimes suggested as a candidate for editing. But only a small number of people have those protective mutations, notes Porteus. The people known to have it are healthy, but researchers don't know how many others might have had the mutation and died.

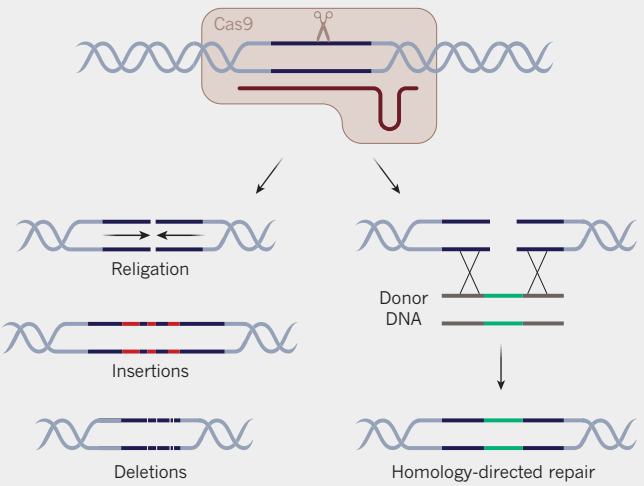
The first known attempt at heritable gene editing in humans was an effort to disable a gene called *CCR5*, which produces an immune-cell receptor that allows HIV to infect humans. Break the gene, and the children should be resistant to the virus, reasoned He Jiankui, then at the Southern University of Science and Technology in Shenzhen, China. He attempted to create a *CCR5* mutation that is found naturally in some people of European descent and is associated with HIV resistance. But a study published this month using data from the UK Biobank found that the deletion might also shorten lifespan[10].

The effects of some genetic variants can also depend on the environment and on other variants present in the genome. The *CCR5* mutation, for example, is very rare in Chinese populations, raising concerns that the gene could be important for protection against viruses that people would be more likely to encounter in Asia.

That kind of confusion can cause trouble for heritable gene editing, notes Cletus Tandoh Andoh, a bioethicist at the University of Yaoundé in Cameroon. "The majority of studies of genetic association with disease have been performed in Europeans," he says. To deploy heritable genome editing in Africa, for example, extensive gene and environment studies would first need to be done in African populations, he says.

On-target effects

After Cas9 cuts DNA, the cell tries to repair the damage, but the processes it uses are unpredictable. The fissure can be repaired perfectly (religation) or some letters could be inserted or deleted. Researchers can also introduce donor DNA for the cell to use as a template in homology-directed repair. This process is more precise, but less efficient.



Patchwork babies: how can researchers prevent mosaics?

Sometimes genes differ not only between individuals in a population, but also among the cells of an individual. The advent of cheap and rapid genome sequencing has revealed that this condition, known as mosaicism, is more common than once thought.

Mosaicism might pose problems for gene editing. An embryo tweaked to correct a gene that causes Huntington's disease could contain a mix of corrected and uncorrected cells. How that affects the health of the resulting child would depend on which cells were edited and which were not — something that could be difficult to predict in advance.

Rudolf Jaenisch, a stem-cell scientist at the Whitehead Institute in Cambridge, Massachusetts, doubts that researchers will ever be able to rule out the possibility of mosaicism in an embryo. And methods to assay the DNA sequence in an embryo rely on removing a small number of cells for testing, and then destroying them. Researchers can't test the cells that remain. "Even if you do preimplantation diagnosis," he says, "it is impossible to decide whether it was a success."

Some researchers have reported injecting the CRISPR–Cas9 machinery into embryos at very early stages of development[2], when they are still only a single cell. This technique eliminated mosaicism, the authors said. But it will need to be tested many more times to be sure, says Perry.

And genome editing so early in development creates a new problem: there is no way to distinguish embryos that carry the genetic disease from those that do not at the single-cell stage, cautions Jaenisch. "You will, by definition, manipulate healthy embryos," says Jaenisch, and so expose them to unnecessary risk (see 'Mosaicism').

Would any degree of mosaicism be tolerable? It might depend on the condition being treated, says Krishanu Saha, a bioengineer at the University of Wisconsin–Madison. "If we have 30% of the liver edited and we're trying to treat, let's say, a retinal disease, is that ok?" he says. "In some cases it could be."

Testing times: how should clinical trials be designed?

With all these technological barriers still to cross, there has been comparatively little discussion of how heritable genome editing would be tested in clinical trials, and what data would be needed before the technique can make that step. The requirements should be high, because the changes could be passed on to future generations, says Guoping Feng, a neuroscientist at the Massachusetts Institute of Technology in Cambridge. "This is not like an 'I'm going to have a cramp in my

stomach' side effect," he says. "This is permanent."

Some are looking to the example set by the UK Human Fertilisation and Embryology Authority (HFEA), which spent 14 years analysing data from animals and people before it decided to conditionally allow a technique called mitochondrial donation. The technique allows women with disease-causing mutations in the DNA of the cell's power plants — its mitochondria — to use mitochondria from the egg of a healthy donor during IVF. As with gene editing, it could allow parents to avoid passing along dangerous mutations. And there are still questions about the safety of this procedure — some countries, including the United States, do not allow it. Even so, many more data were available about that technique than there are now for CRISPR–Cas9 editing in embryos, says Greenfield, who served on the HFEA panel. (IVF took more than 30 years to move from laboratory testing to a healthy pregnancy.)

Human clinical trials would present a host of fresh challenges. For example, for how long will genome-edited children need to be followed up before the technique can be considered safe? How will researchers track the children of those children to look for transgenerational effects? "It's going to be a mess," says Bryan Cwik, a bioethicist at Portland State University in Oregon.

On 22 May, the US National Academy of Sciences, the US National Academy of Medicine and the UK Royal Society announced a committee to study these aspects of heritable gene editing. The panel aims to publish a report next year. "There is really a need to have a much more in-depth set of criteria in place," says Doudna. "I think we all wish that would have happened faster than it had."

The biggest question: is the world ready?

Despite the sizeable scientific barriers to heritable gene editing, the more difficult issues are likely to be ethical and social. Consultations have been ongoing, and reports and position statements have been pouring in from scientific societies around the world. In March, a panel convened by World Health Organization (WHO) concluded that it would currently be irresponsible to make heritable edits to the genome in humans. Authors writing in *Nature* have called for a global moratorium[11], and members of the US National Academy of Sciences, the US National Academy of Medicine and the Royal Society have said that "we must achieve broad societal consensus before making any decisions".

Achieving global consensus is a daunting task and, at present, most of the consultations have been conducted in wealthy, Western countries. Kewal Krishan, an anthropologist at Panjab University in Chandigarh, India, says that there has been little discussion of heritable gene editing in India, for example. And Andoh notes that in some African cultures, the pressure to have children is intense, and women can be ostracized from the community for failing to do so. This could foster demand.

Demand is another question entirely. For now, there is not a huge clamour among people affected by disease, says Sharon Terry, president and chief executive of Genetic Alliance, an advocacy group in Washington DC. Initial enthusiasm has been tempered over time, both as debates advanced and as patient advocates realized that treatments were not imminent, she says. Many families at risk of passing on genetic diseases tell her that, for now, they just want a way to screen their embryos for mutations. But screening is hardly a panacea. It won't work for all couples.

Such decisions are intensely personal, says Andrew Imparato, executive director of the Association of University Centers on Disabilities in Silver Spring, Maryland. Some members of the deaf community, for example, welcome the thought of having children who are deaf, and might be concerned that ways to edit deafness mutations from the genome would increase pressure on families to do so.

Public surveys often find support for heritable genome editing — if it is shown to be safe and used to treat genetic diseases. A UK survey conducted by the Royal Society found that 83% of participants were in favour of editing the germ line to treat incurable disease. But many drew the line at editing for 'enhancement': 60%, for example, were opposed to the idea of using heritable gene editing to improve intelligence.

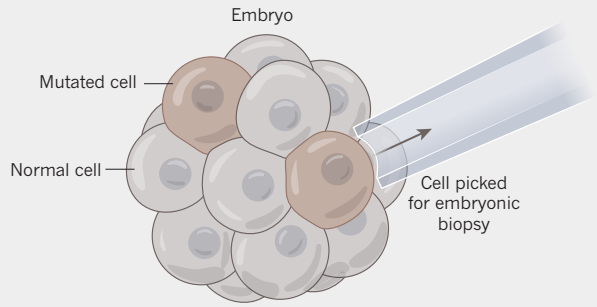
Many scientists and ethicists make a similar distinction, between modifying the genome to enhance athletic ability, for example, or to change eye colour, versus treating or preventing disease. And even then, there is debate about which diseases might warrant such an approach. Fatal conditions with a strong, clear-cut genetic contribution — such as Huntington's disease, which is almost inevitable when the mutation is present — are the most common examples given. But when it comes to editing a gene such as *PCSK9* to prevent high cholesterol and potentially stave off heart disease, things are decidedly more grey, says Feng. Ultimately, Porteus hopes to see a registry of conditions that have been evaluated by specialists and deemed worthy of intervention with heritable gene editing, much as the United Kingdom now maintains for PGD.

Still, some people might be quietly moving towards the idea of more gene-edited children. This month, a Russian scientist announced his interest in pursuing a project to edit the genes of human embryos. And the US media company STAT reported late last month that a fertility clinic in Dubai had reached out to He for advice on gene editing shortly after he made his announcement.

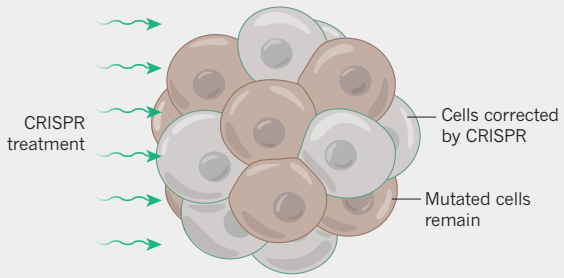
Abha Saxena, a bioethicist at the University of Geneva, Switzerland, and former adviser to the WHO, hopes that consultations will continue, even if the ultimate goal of reaching a global consensus might not be possible. "Are we ever going to be ready? It's difficult to say," Saxena says. "But humanity has always been adventurous." ■

Mosaicism

Mosaicism can cause two sorts of problems. If a developing embryo contains just a few cells with risky mutations, then a biopsy that picks up a mutated cell might lead to unnecessary manipulations.



The CRISPR–Cas9 process is inefficient, and might leave too many cells uncorrected to treat the disease.



Heidi Ledford *is a senior reporter for* Nature *in London, UK.*

1. Kleinstiver, B. P. *et al. Nature* **529**, 490–495 (2016).
2. Ma, H. *et al. Nature* **548**, 413–419 (2017).
3. Egli, D. *et al. Nature* **560**, E5–E7 (2018).
4. Strecker, J. *et al. Science* https://doi.org/10.1126/science.aax9181 (2019).
5. Klompe, S. E., Vo, P. L., Halpin-Healy, T. S. & Sternberg, S. H. *Nature* https://doi.org/10.1038/s41586-019-1323-z (2019).
6. Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. & Liu, D. R. *Nature* **533**, 420–424 (2016).
7. Zuo, E. *et al. Science* **364**, 289–292 (2019).
8. Jin, S. *et al. Science* **364**, 292–295 (2019).
9. National Academies of Sciences, Engineering, and Medicine. *Human Genome Editing: Science, Ethics, and Governance* (National Academies Press, 2017).
10. Wei, X. & Nielsen, R. *Nature Med.* **25**, 909–910 (2019).
11. Lander, E. *et al. Nature* **567**, 165–168 (2019).

CORRECTION

The News Feature 'CRISPR babies: when will the world be ready?' (*Nature* **570**, 293–296; 2019) gave the wrong name for the gene associated with lower cholesterol levels and cited an inappropriate reference for the finding. It also gave the wrong reference for the study based on UK Biobank data: the correct reference is X. Wei and R. Nielsen *Nature Med.* **25**, 909–910 (2019).