CHILDREN'S HOSPITAL OF PHILADELPHIA

Beverly Davidson and Alex Monteys are using gene editing to inactivate the mutated gene huntingtin.

GENE EDITING

To cut is to cure

One-off treatments that target the brain in Huntington's disease must meet strict safety and efficacy requirements.

BY MICHAEL EISENSTEIN

Beverly Davidson is well insulated from the hype of gene therapy, having spent decades working in the field. During that time, she has grappled with the harsh realities of turning flashy and potentially transformational technologies into clinical applications. But when she heard about the genome-editing technology CRISPR, she was instantly intrigued. "As soon as those first papers came out, we started playing with it," says Davidson, a specialist in neurodegenerative disease at the Children's Hospital of Philadelphia in Pennsylvania.

Like most other neurological disorders, Huntington's disease has proved to be a costly and frustrating target for drug developers. But it also has distinctive features that make it a good match for treatments that target genes. It arises from a mutation in a single gene that encodes the protein huntingtin, and a disease-causing copy of the gene can be readily distinguished from a normal copy by the presence of an overlong stretch of a repeated triplet of nucleotides, CAG (see page S36). Before turning to CRISPR, Davidson and her colleagues had some success in treating animal models of Huntington's disease with RNA interference (RNAi), which uses synthetic molecules of RNA to prevent the production of mutant huntingtin — although it took them a considerable amount of time to get there. "We've focused the last 17 years on RNAi-based approaches," says Davidson. However, both this and a promising related treatment for Huntington's disease that involves antisense oligonucleotides (S39) will probably require long-term, repeated administration to provide sustained benefits.

By contrast, CRISPR could achieve the same benefits through a single dose that permanently inactivates the defective gene with remarkable efficiency, as Davidson's team demonstrated last year[1], both in cells from people with Huntington's disease and in mouse models of the condition. "I was surprised how easy it was — I think that's the beauty of the system," she says. In the past five years, several teams of researchers have independently shown that genome editing can reliably eliminate the gene that encodes mutant huntingtin, thereby halting the production of the toxic protein and its accumulation into clumps in experimental models.

But clearing protein clumps in mice is of questionable value when researchers often struggle to translate such findings into treatments for people — in general, potential therapies for brain disorders have a long history of failure and disappointment in clinical trials. Accordingly, the early adopters of CRISPR are trying to obtain clearer evidence of its probable clinical benefits while grappling with thorny questions related to its safety, efficacy and delivery that it is crucial to answer before trials in people can take place. "I believe we can now seriously consider clinical strategies to edit huntingtin," says Nicole Déglon, a neurologist at the Lausanne University Hospital in Switzerland, "but I would say we are still at the very beginning of the story."

TO THE LETTER

The targeted DNA-snipping capabilities of CRISPR evolved in bacteria as a defence against viruses that shoehorn their genomic material into their microbial hosts. The system uses a short sequence of RNA known as a guide RNA, which can pair with a complementary DNA sequence. Researchers have learnt how to target almost any genomic sequence by engineering an appropriate guide RNA. They couple it with an enzyme called Cas9, which can then cut both strands of a DNA sequence of interest at a specific site. Because the DNA-repair mechanism of cells is sloppy, it typically produces insertions or deletions that inactivate the affected gene.

One of the first decisions that would-be editors have to make is whether to eliminate the gene that encodes huntingtin altogether, or to selectively target the repeat-laden mutated copy. Although the function of huntingtin remains poorly understood (S36), it is crucial for early development. "If you knock out huntingtin in mice, they die in the womb," says Jong-Min Lee, a neurogeneticist at Massachusetts General Hospital in Boston. However, Xiao-Jiang Li and colleagues at Emory University School of Medicine in Atlanta, Georgia, have obtained evidence from mouse studies[2] that the depletion of huntingtin in the brain might not be detrimental when it occurs in adulthood. His team subsequently demonstrated[3] that a CRISPR–Cas9 approach that eliminates huntingtin can clear clumps of the protein from the brain with no apparent adverse effects in a mouse model of Huntington's disease (see 'Cutting down on huntingtin'), although he is cautious about drawing too firm a conclusion. "We didn't find any obvious phenotype or neuropathology, but we still don't know whether there was some sort of functional impact," says Li.

Most researchers are therefore erring on the side of caution by designing guide RNAs that recognize sequences found only in the mutated gene. This was the approach that Davidson's team pursued, and Lee and colleagues also showed that they could make edits with remarkable accuracy in cells that were collected from a person with Huntington's disease, by designing guide RNAs that recognize sequence

variations found only on the chromosome that contains the mutated gene[4]. "The specificity is excellent," says Lee, noting that the chromosome that bears the normal copy of the gene is consistently unaffected in treated cells. Achieving this in a clinical setting would require a level of personalization, but Lee has collected genomic data from more than 4,000 people with Huntington's disease and identified some informative patterns of sequence variation that are strongly associated with mutated copies of the gene that encodes huntingtin. "With just a couple of CRISPR designs, you could easily target more than 50% of patients," he says.

Another concern is off-target editing, in which genes other than the target are modified inadvertently — with potentially disastrous consequences. Software can be used to predict probable off-target edits and to help researchers pick distinctive guide RNAs with reasonable confidence. But clinical researchers do need to consider the effects that CRISPR might have when used over the longer term. "We should not apply this to humans if we have permanent expression of Cas9 in the brain," says Déglon. Unfortunately, most systems for getting the CRISPR machinery into the brain rely on its delivery by viral vector, which could lead to Cas9 being produced indefinitely. Over time, the enzyme might wreak irreparable genomic damage on healthy neurons. A possible solution entails using synthetic nanoparticles to facilitate the one-time delivery of the enzyme and guide RNA, although this work is still at an early stage.

Déglon's team has devised a promising alternative to CRISPR called KamiCas9, which includes a self-destruct button for Cas9. It uses two guide RNAs — one to target the gene encoding huntingtin, and another to target the gene encoding Cas9. This means that, after a brief flurry of activity by Cas9, production of the DNA-dicing enzyme is inactivated permanently, which dramatically reduces the risk of collateral damage. She notes that several weeks after conventional CRISPR–Cas9 was applied to neural cells derived from people with Huntington's disease, low levels of off-target editing were detected — roughly 2% of modified cells received unwanted edits at a site that is particularly susceptible to off-target editing[5]. By using KamiCas9, her team was able to reduce that effect dramatically — only 0.5% of such modified cells had off-target edits. "We did not see any difference in terms of efficacy, which is really good news," says Déglon.

BURDEN OF PROOF

Such concerns are of little relevance unless editing with CRISPR can be shown to change the course of a disease — something that is difficult to demonstrate through experiments with mice. Li's team has been able to alter Huntington's disease at the molecular level[3] by sharply reducing the production of mutant huntingtin, which forms the toxic clumps that drive the progression of the condition. "We have shown that, in an injected area of the mouse brain, probably more than 90% of cells do not contain huntingtin aggregates," says Li. This effect was accompanied by modest yet measurable improvements in motor function. However, as with many animal models developed for other diseases, the mouse models that researchers use to investigate Huntington's disease are poor surrogates for what happens in people with the condition. "Our model has mild motor phenotypes that show up later in life," says Davidson. "It doesn't have any overt, robust neurodegeneration like you would see in a human patient."

> ***"I believe we can now seriously consider clinical strategies to edit huntingtin."***

To some extent, this issue reflects the lifespan of mice — one or two years is not enough time in which to accurately map a degenerative disease that normally unfolds over decades. And there are also fundamental differences in the function and organization of rodent brains compared with those of larger mammals. However, Li's team has developed a promising pig model[6] of the condition that reflects the neurodegeneration and the motor and behavioral defects observed in people more closely than any mouse model so far. "Small animals and large animals exhibit very different pathological changes and behavioural changes," says Li.

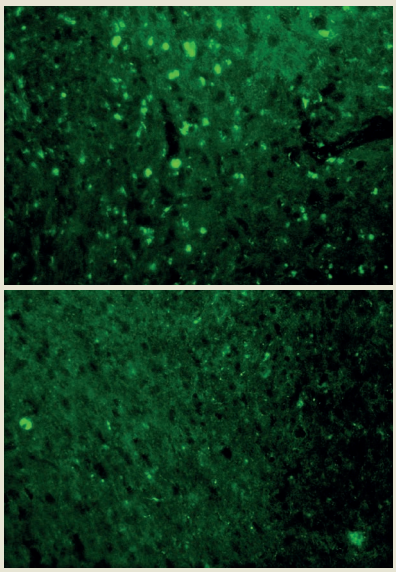
These improved models will also help researchers to get a handle on how many brain cells must undergo gene editing to obtain clinical gains — useful information given the impracticality and undesirability of bathing the brain in CRISPR-laden viral vectors. The striatum, a brain structure that governs both movement and cognition, is a prominent casualty of Huntington's disease, and work with antisense oligonucleotides and RNAi suggests that efficient targeting with CRISPR could help to prevent the death of neurons in the area. Davidson thinks that cutting the production of mutant huntingtin in the striatum by half might be sufficient to halt disease progression or even prevent its onset.

For those already in the grip of Huntington's disease, there are hints that genomic repair could provide a partial rebound. "Neurons may have a lot of capacity to get rid of mutant protein if you break the continuous formation of new aggregates," says Li. A preventive approach, however, could one day enable individuals to avert their genetic destiny, long before the onset of disease. Indeed, Huntington's disease is among the few disorders that can be confidently predicted using a genetic red flag. But even if CRISPR-based treatment amasses a strong body of preclinical data to support its use in Huntington's disease, initial clinical testing will almost certainly focus on people with symptoms, for whom improvements in motor and cognitive function can be measured in a reasonable timeframe. "Then, based on the results of the first trials showing the absence of potential side effects, they might consider early-stage or even presymptomatic patients," says Déglon.

The brain will not be the first clinical proving ground for CRISPR. Instead, initial forays will probably be aimed at conditions such as haemophilia, which can be treated with cells that have already been genetically manipulated in the laboratory. The brain remains a daunting target because of its biological complexity, relative inaccessibility and irreplaceable function. But the parallel surge in the clinical development of gene therapy and oligonucleotide-based interventions has cleared a path for testing the potential of CRISPR in treating Huntington's disease. Even at this early stage, Davidson is optimistic. She is collaborating with Intellia Therapeutics in Cambridge, Massachusetts, which was co-founded by CRISPR pioneer Jennifer Doudna, to address the technical challenges that are involved in moving her research into the clinic. "I hate to say this, because I probably gave these sorts of numbers for RNAi, but with further advances in delivery, I could envision doing clinical testing within five years," says Davidson. "I don't think it's particularly far off." ■

CUTTING DOWN ON HUNTINGTIN

The mutant protein huntingtin (green fluorescence) is abundant in brain tissue gathered from a mouse model of Huntington's disease (top), but a CRISPR-based intervention that targets the gene encoding huntingtin greatly reduces production of the toxic protein (bottom).



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