GENETICALLY MODIFIED HUMANS: THE X-MEN OF SCIENTIFIC RESEARCH

How far is too far when it comes to altering the human genome?

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The CRISPR/Cas9 system has been revolutionary in the world of genetic research. However, as genetic engineering moves into human applications, it's now time to ask: what benefits can this bring? And, how far is too far when it comes to altering the human genome?

Genome editing techniques have been around for decades; in 1973, the first transgenic organism was created by the insertion of antibiotic resistance genes into *Escherichia coli*, which was quickly followed by the first transgenic animal – a mouse – a year later. Since then, it has been applied across all areas of biology, from creating bacteria that could break down crude oil to increasing the shelf life of tomatoes [1].

However, it was the introduction of the CRISPR/Cas9 system in 2012 [2] that kick-started the rapid development of gene editing technology into the widely practiced technique that it is today. Thousands of papers on CRISPR are published each year, with the rate increasing annually. It seems that the applications of CRISPR know no bounds, with geneticists everywhere keen to apply the technique to anything and everything.

With use on the bacterial genome becoming old hat, researchers are turning to human use; asking how they can use this technology for a therapeutic advantage. Shifting the focus of research to the treatment of genetic diseases, laboratory advances are being made for multiple disorders and some are already being put to clinical use.

Though becoming a reality, the alteration of human DNA remains something seemingly fictional. Be it Professor X, Deadpool or Scarlett Witch, those with modified DNA or 'mutants' are still associated with the superheroes of well-known comics and films. Currently, the use of CRISPR in humans is purely therapeutic, fixing genetic mutations rather than creating them; however, such therapies are giving individuals abilities above those that the DNA they were born with gave them. They are becoming the first genetically modified humans; individuals whose DNA is being altered in order to improve their quality of life.

With trials at a variety of stages, from *in vitro* to animal models to early clinical use, the therapeutic use of CRISPR in humans is likely to increase as a field as more and more successful and beneficial effects are demonstrated. Edits in the genome are giving these individuals 'powers' as they do to the superheroes of fiction, if on a significantly smaller scale. Though unlikely to save the world, to an individual with an illness, such abilities may seem super if they can save their life.

THE POWER TO BE INVISIBLE

In a healthy individual, the immune system makes for a formidable opponent to infection. As the body's first line of defense against bacteria, parasites and viruses, it enables us to survive while in a world surrounded by pathogens. However, when it comes to patients who have undergone a transplant, it quickly turns to the dark side, acting as the villain for patients and doctors alike. The transplanted cells are perceived as foreign, initiating an immune response that leads to transplant rejection.

Currently there is no 100% effective way to prevent rejection. When matching donors to the recipient, doctors aim to reduce the likelihood of this complication by ensuring that the pair are as ►

Schrepfer and Deuse in the lab [3].

► histologically compatible as possible and by giving immunosuppressive drugs. Despite best efforts, acute rejection is something that occurs to some degree in almost all transplants, the exception being those between identical twins, unless true immunosuppression has been achieved.

However, the immunosuppression itself can then cause further problems. “We can administer drugs that suppress immune activity and make rejection less likely. Unfortunately, these immunosuppressants leave patients more susceptible to infection and cancer,” explained Sonja Schrepfer from the University of California, San Francisco (UCSF; CA, USA) [3].

Stem cell transplantation is one area where rejection is a key issue. At one time, with the introduction of the induced pluripotent stem cell (iPSC), it appeared that the problem of rejection was solved. You would think that, as these stem cells were created from the intended recipient’s own cells, they would not be perceived as foreign; the body would recognize the cells as being their own and no immune response would be triggered. Unfortunately, the results did not pan out that way.

“There are many issues with iPSC technology, but the biggest hurdles are quality control and reproducibility. We don’t know what makes some cells amenable to reprogramming, but most scientists agree it can’t yet be reliably done,” commented Tobias Deuse (UCSF). “Most approaches to individualized iPSC therapies have been abandoned because of this” [3].

In an attempt to overcome these issues, Deuse, Schrepfer and their team at UCSF created a universal iPSC, using CRISPR/Cas9

Dystrophin (red) restoration shown in a CRISPR edited DMD-affected heart muscle cell (right) relative to unedited cell (left) [11].

genome editing to alter three genes and make the cells ‘invisible’ to the immune system.

The CRISPR-controlled knockdown of two genes that code for the major histocompatibility complex class I and II family of proteins and a vector-mediated increase of the gene coding for the CD47 surface protein meant that the donor cells were not rejected by the recipient. First demonstrated by transplanting mouse iPSCs into mismatched, healthy mice and then again by transplanting human iPSCs into humanized mice, the researchers found that the cells did not elicit any form of immune response and were able to evade the radar of the immune system [4].

“This is the first time anyone has engineered cells that can be universally transplanted and can survive in immunocompetent recipients without eliciting an immune response,” commented Deuse. “Our technique solves the problem of rejection of stem cells and stem cell-derived tissues and represents a major advance for the stem cell therapy field” [3]

By granting the power of invisibility, CRISPR technology holds the potential to improve response rates to transplants and to reduce rejection. With early applications in iPSCs, expanding to other transplant areas may not be too far behind. Coupled with advances in 3D printing of organs, this application of CRISPR may result in an ‘invisible’ kidney, lung or heart.

THE POWER OF STRENGTH

An almost cliché trope of the superhero is the extreme strength they are capable of, whether it’s Superman lifting cars or the Hulk smashing his way through buildings, every good hero has the power to bench-press more than your average Joe. Though currently-used CRISPR techniques aren’t being utilized to enhance a healthy individual’s strength, they do have potential to restore it to those who are lacking.

Duchenne muscular dystrophy (DMD) is an X-linked, monogenic disorder where mutations in the DMD gene on the X chromosome prevent the production of the dystrophin protein in muscles. Displaying mendelian inheritance, it is a disorder that predominantly affects males, with females more likely to be carriers. The mutation causes premature termination of translation, resulting in a loss of the production of the protein dystrophin, without which muscles are weak, fragile and can be easily damaged [5].

Being caused by a single gene makes DMD a prime target for gene therapies. However, the DMD gene is the second largest gene known, with 2.6 million base pairs, making it unsuitable for vector-mediated insertion of a non-mutated version. In using the CRISPR/Cas9 technology to correct the mutation rather than replace it, a team from the University of Texas (UT) Southwestern (TX, USA) demon-

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strated that a correction of the mutation in a mouse zygote led to improved muscle function when the mouse was 1 month old [6].

Since their first CRISPR study, the team have improved their strategy and demonstrated the success of gene editing to restore dystrophin synthesis both *in vitro* and *in vivo*. Using 3D engineered heart muscle, they found that a single cut was enough to skip the defective exon, resulting in the restoration of dystrophin protein expression and muscle function [7]. *"We found that correcting less than half of the cardiomyocytes (heart muscle cells) was enough to rescue cardiac function to near-normal levels in human-engineered heart tissue,"* explained Chengzu Long (UT Southwestern), the study's lead author [8].

The UT Southwestern team then moved onto *in vivo* canine studies, using their single-cut CRISPR editing technique to target a mutation on exon 51, resulting in a restoration of dystrophin to 92% of its normal levels in the heart and 58% in the diaphragm [9].

"Our strategy is different from other therapeutic approaches for DMD because it edits the mutation that causes the disease and restores normal expression of the repaired dystrophin," commented Leonela Amoasii, lead author of the canine study. *"But we have more to do before we can use this clinically"* [10]

In their most recent work, the research group has demonstrated the ability of the CRISPR/Cas9 system to correct the deletion mutation of exon 44 of the DMD gene in mice and human iPSC-derived cardiomyocytes [11]. They found that when targeting this section of the gene, a different region to that targeted in their previous studies, the standard 1-to-1 ratio of Cas9 and guide RNA was not as effective.

The newly developed method involving an altered ratio boosted the efficiency of the gene editing, results that could have implications across the field of gene therapy and not just DMD. *"As we test CRISPR on other defective parts of the dystrophin gene, it may be important to tweak our formulas for optimal results,"* commented Olson, study leader and director of the research group. *"This new insight further facilitates the use of CRISPR as a therapy for Duchenne and perhaps a number of other diseases"* [12].

THE POWER OF RESISTANCE

When it comes to CRISPR, perhaps the most dramatic and controversial advance was the birth of the so-called 'CRISPR twins'. Previously, genome editing had been limited to altering somatic cells, meaning that any edits made would not be heritable. The twin girls, born November 2018, marked the start of germline edits where the effects of the CRISPR edits will not only affect their lives but also those of their future generations.

He Jiankui from the Southern University of Science and Technology (Shenzhen, China) and his research team used CRISPR technology to delete the CCR5 gene, with an overall aim of rendering the resultant offspring resistant to HIV, smallpox, and cholera.

Announcing his results in a YouTube video, Jiankui stated that *"gene surgery is another IVF advancement"* and emphasized that the

Jiankui first announced the study in a YouTube video [13].

research was for the benefit of families who could not have children otherwise and therefore *"need this technology"* [13]. The positive and wholesome message given by Jiankui did not have the reception intended, with fellow scientists being shocked by the experiment and skeptical of his family-centric approach, many calling the work selfish and fame-driven; the Center for Genetics and Society (CA, USA) labeled the work as *"a grave abuse of human rights"* [14]

Resistance to some of the world's deadliest diseases is a significant medical advantage, though in today's world, seemingly unnecessary. The benefits of treatment options currently available significantly outweigh the risks of the CRISPR procedure. However, further research has shown that immunity to disease is unlikely to be the only power granted by this edit. Aside from the as-yet-

unknown off-target effects, the editing of the CCR5 gene may also have enhanced their learning and memory. In murine models, deleting the gene has been shown to significantly improve memory and can make the animals smarter.

The link between CCR5 and cognition has been known since 2016, when a study revealed that it could act as a suppressor for cortical plasticity as well as hippocampal learning and memory [15]. Upon hearing about the twins, author of the 2016 study Alcino J Silva (University of California, Los Angeles; CA, USA) commented, *"the simplest interpretation is*

that those mutations will probably have an impact on cognitive function in the twins." The question remains as to the extent of the effect which, at this stage, is impossible to predict and, according to Silva, *"that is why it should not be done"* [16].

In his announcement video, Jiankui agreed that the technique should be used for healing and not for enhancing IQ. Despite this suggestion that there was no initial intent to improve the twin's cognitive abilities, Jiankui made no attempt to collaborate with experts to discuss the potential effect of CCR5 on cognition and, while admitting he knew of the study, said that it *"needs more independent verification"* [16].

Since the birth of the twins, new research has once again shown the effect that suppressing CCR5 can have on the brain, this time ►

"Though currently-used CRISPR techniques aren't being utilized to enhance a healthy individual's strength, they do have potential to restore it to those who are lacking."

► demonstrating it to be a therapeutic target to improve recovery from stroke or traumatic brain injury [17]. The study also highlighted a link between the gene and everyday intelligence, with those who are missing at least one copy of the gene appearing to go further in school. Not all side effects are likely to be as positive, it has also been shown that the mutated CCR5 gene can significantly increase mortality, particularly influencing susceptibility to death from the flu [18].

The backlash that occurred when Jiankui announced his study has halted the editing of germline cells for the foreseeable future. The view of Jiankui's work as reckless has led to the formation of a group of experts by the World Health Organization, tasked with setting out guidelines for future CRISPR studies and evaluating the ethics of its use. Scientists from across the world, including CRISPR co-inventor Feng Zhang, have called for a global moratorium on germline editing. This would include a freeze on any ongoing germline editing projects until an international framework for practice can be outlined [18]. However, at the summit where Jiankui first presented his results, there was a consensus that such use for gene editing was inevitable. Dean of Harvard Medical School (MA, USA), George Daley, told the conference, *"the fact that the first instance of human germline editing came forward as a misstep should not let us stick our neck in the sand. It's time to move forward from [debates on] ethical permissibility to outline the path to clinical translation"* [19].

It is unknown what the full effect of the gene surgery will be, only time will tell as the twins grow and develop; *"because one gene could affect multiple traits, and because, depending on the environment, the effects of a mutation could be quite different, I think there can be many uncertainties and unknown effects in any germline editing,"* commented Xinzhu Wei, first author of the CCR5 mortality study [21]. If predictions hold true, this case will have shown what is possible and will likely be the inspiration for future work; Pandora's box of potential has been well and truly opened and it may be too late to close it.

THE POWER TO HAVE IT ALL?

It is undeniable that the therapeutic use of CRISPR will become mainstream in the not-so-distant future. As more applications are made available, somatic gene surgery will likely become first line for the treatment of genetic disorders.

However, once the technology is available, what is there to stop geneticists following the tropes of comics and going full 'mad-scientist'? The CRISPR-twins story could be the first of many seemingly unnecessary genetic modifications that begin to occur as the technology becomes more controllable and new potential targets are identified. As advances occur, perhaps the question scientists ask themselves will change from, could we do that? to, should we do that?

It may be a stretch and seemingly ridiculous now, but as the CRISPR technology advances and the range of potential applications widens, it could lead to us asking one question; what would your superpower be?

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