Could ingenious gene therapy prevent health issues associated with Down syndrome?

Down syndrome is a genetic developmental disorder that leads to a range of health issues. It is caused when someone inherits three copies of chromosome 21, rather than two. **Dr Volney Sheen** and his team at the **Beth Israel Deaconess Medical Center** in the US are investigating methods to silence the extra copy of this chromosome. If this gene therapy can be used in humans, it could reduce the negative health impacts faced by people with Down syndrome.

Dr Volney Sheen

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Fields of research
Neuroscience, neurology

Research project
Using gene editing techniques to silence the expression of the extra chromosome that causes Down syndrome

Funder
US National Institutes of Health (NIH) Grants 1R01HD109794-01 and 5R21NS115593-02

DOI: 10.33424/FUTURUM586

PROFILE

Talk like a ...neuroscientist

Chromosome — the structure made of proteins and DNA that carries genetic information in cells

CRISPR-Cas9 — a gene editing technology

Down syndrome — a genetic condition in which a person is born with an extra copy of chromosome 21, leading to health issues such as cognitive impairments and early-onset dementia

Expression — the process by which proteins are created from genes

Gene editing — deliberately altering the DNA of an organism

Gene therapy — the application of gene editing to address a disease

Genome — all the genetic information in an organism

Postnatal — after birth

Prenatal — before birth

Progenitor cell — a cell that will divide and differentiate into specialised cells

Tissue culture — living cells grown in a lab

XIST — an RNA molecule that inactivates one X chromosome in females

Down syndrome is the world's most common genetic developmental disorder, affecting as many as 1 in 1,000 births. "People with Down syndrome suffer from a range of neurological and physiological issues," says Dr Volney Sheen. "These include cognitive impairments, early-onset dementia, blood cancers, heart defects and vulnerability to infection." There is currently no cure for Down syndrome, but at the Beth Israel Deaconess Medical Center, Volney and his team are developing techniques that could address the syndrome at its genetic source.

Humans have 23 pairs of chromosomes in every cell, which together make up our genome and contain the genetic information that makes each of us unique. During conception, we inherit one chromosome in each pair from each of our biological parents. However, sometimes an extra chromosome can be inherited, which can lead to genetic diseases. "Down syndrome is caused by the inheritance of three, rather than two, copies of chromosome 21," says Dr Gewai Lian, who oversees the project. "This causes overexpression of the genes present on this chromosome." Gene expression involves cellular machinery using a gene's code to create the proteins that make our body function. Overexpression, however, as in the case of Down syndrome, causes

medical issues. If it was possible to prevent expression of this extra chromosome, these issues could potentially be avoided.

How to silence a chromosome

Elsewhere in our genome, chromosome 23 determines our sex: XX for females or XY for males. "As females have two X chromosomes, one copy of this chromosome is naturally silenced to allow normal gene expression," explains Volney. "A large RNA molecule, called XIST, is responsible for this task." Volney's hypothesis is that if XIST could be inserted into chromosome 21 in people with Down syndrome, it would silence the extra copy of chromosome 21 and so prevent its overexpression. This involves some clever genetic manipulation, which is only now becoming possible.

Gene editing technology has exploded in recent years, especially due to a technique known as CRISPR-Cas9. "CRISPR-Cas9 involves using tailored guide RNAs to direct a Cas9 protein to cut the DNA of a cell at a specific point," says Volney. "We are using a modified CRISPR-Cas9 approach that not only cuts the DNA in chromosome 21 at a certain point but also integrates XIST when it does so." The team is designing specific guide RNAs that can insert the XIST RNA molecule into the DNA on the third copy of chromosome 21 and activate it, which silences the extra chromosome and prevents it from being expressed.

Testing the theory

While this technique sounds great in theory, it needs to work in a substantial proportion of the body's cells at once to be effective in a whole organism. Volney and his team needed to test whether cells were successfully incorporating XIST and silencing the extra chromosome after being treated with their modified CRISPR-Cas9 approach. They began by inserting XIST into tissue cultures. "XIST can be linked to a reporter gene, which signals whether it's been incorporated into the chromosome or not," says Volney. "For instance, the reporter gene GFP makes cells fluoresce green if they have incorporated it into their DNA." Using this technique alongside others, the researchers found they could get about 30-45% of cells in a tissue culture to integrate XIST into their DNA.

> ***"Mice with Down syndrome performed better in cognitive tests after being given the XIST gene therapy."***

With these promising results in hand, the team moved on to test the technique on mice with the mouse equivalent of Down syndrome, specifically targeting their hippocampus (the region of the brain responsible for memory). In people with Down syndrome, the hippocampus does not grow fully and is more prone to degradation, leading to cognitive impairments and dementia. "We have a range of evaluation methods to test the effectiveness of this technique," says Dr Abdalla Khabazeh, a medical doctor working in the lab. "This includes genetic methods that directly test whether XIST is taken up into their DNA, and behavioural methods to see if a mouse's cognitive functions improve." On the latter, the team found that mice with Down syndrome performed better in cognitive tests, such as navigating mazes and recognising objects, after being given the XIST gene therapy.

What happens next?

The researchers suspect that their results could be better if the gene therapy was applied earlier in development, in other words, before the mice are born. "The number of cells that could take up XIST is thousands of times higher in prenatal mice than postnatal mice," says Dr Eunju Cho, a postdoctoral researcher. This is because, if XIST is inserted earlier in development, it will be incorporated into the genome of progenitor cells. These cells will then divide to form the brain, and every cell produced from them will also contain XIST. This means more cells will undergo silencing.

"Having proven that our modified CRISPR-Cas9 approach allows us to efficiently insert XIST into the genome, the next hurdle will be getting it into as many cells as possible," explains Volney. "This means we need to find reliable delivery methods (which could be viruses, nanoparticles or lipid-based delivery) and discover the optimal areas of the brain to deliver XIST into." Once these issues have been solved, it will pave the way for XIST gene therapy to become a clinical reality for people with Down syndrome.

About *neuroscience and neurology*

Neuroscience is the scientific study of the nervous system (brain, spinal cord and nerves), while neurology is the branch of medicine that that deals with the study, diagnosis and treatment of disorders of the nervous system. These two fields are closely linked, as neuroscience research is applied in the clinical practice of neurology.

Volney works both as neuroscientist and neurologist, splitting his time between laboratory research and clinical work in hospital. "As a neuroscientist, I spend most of my time talking with lab members to oversee their experiments and reading scientific literature," he says. "As a neurologist, I treat patients with neurological conditions, ranging from epilepsy and stroke to headaches and back pain." This dual role allows him to conduct translational research in the lab that he then applies in the hospital to treat his patients. In both roles, Volney teaches the next generation of neuroscientists and neurologists.

"A typical day in the lab involves a combination of experiments and data analysis," says Dr Abdalla Khabazeh, who hopes to train as a neurosurgeon. "The dynamic nature of the lab allows me to integrate clinical perspectives with translational research, which I find particularly rewarding." Dr Gewei Lian, who oversees lab operations comments that, "My role is dynamic and requires a combination of technical expertise, problem-solving and collaboration to drive meaningful scientific discoveries." For Dr Eunju Cho, a neuroscience researcher, "The most enjoyable moments are when we can confidently say that our results support our hypotheses."

Pathway from school to *neuroscience and neurology*

At school, gain a solid foundation in science subjects, such as biology, chemistry, physics and mathematics.

If you want to pursue a research career in neuroscience, then consider studying neuroscience at university, or a related degree such as biology or biochemistry.

If you want to pursue a clinical career in neurology, you will need to study a medical degree at university and to then specialise in neurology during your clinical training.

"Many disciplines, from engineering to mathematics to biological sciences, have relevance in neurology and neuroscience," says Volney. "Each provides different expertise needed to tackle neurological problems."

Explore careers in *neuroscience and neurology*

A clinical career in neurology will involve treating patients with neurological conditions, while a research career will involve investigating these conditions in a laboratory. You could also do both, like Volney.

Volney recommends seeking internships or work experience opportunities in a neuroscience laboratory or with a clinical neurologist to get a sense of what the work involves and whether it interests you. Pathways to Science has a directory of potential opportunities to get this experience, including for high school students: **pathwaystoscience.org/Discipline.aspx?sort=MED-NeuroSci_Neuroscience**

Harvard Medical School, where Volney works, runs programmes for high school students to get experience in laboratories and the medical sciences: **dicp.hms.harvard.edu/dicp-programs/k-12/high-school-programs**

Meet *the team*

Dr Volney Sheen

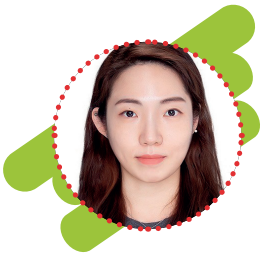
My path to neurology was not direct. At high school, I enjoyed science classes and lab work. I went to university to study biomedical engineering as I was interested in robotics and the human-engineering interface, but I realised this was still far from a practical reality. So, I shifted towards the biological sciences and did an undergraduate research project with a mentor who was a neuroscientist. Then, when I did medical training, neurology was the natural fit for me.

I have a wonderful job. As a clinical neurologist, I get to help patients with neurological disorders. As a research neuroscientist, I get to think of new and interesting questions related to neurology and how to find answers to them.

Dr Gewei Lian

The excitement of discovery has driven my dedication to neuroscience over the years. I experienced intense academic competition throughout my education in China, from high school to graduate studies, but on every step of my journey, my curiosity for the unknown has been my greatest motivator.

As a neuroscientist, I most enjoy uncovering new insights into the brain and its complexities. The process of designing experiments, analysing data and interpreting results is both challenging and rewarding. I also find great satisfaction in problem-solving and contributing to scientific discussions that advance our understanding of neurological diseases. Ultimately, the potential impact of our work, whether improving treatments or deepening our knowledge of the brain, drives my passion for neuroscience.



Dr Eunju Cho

My curiosity about the brain was sparked by reading *The Ultimate Secret*, a science-fiction book by Bernard Werber, and by learning about neurodegenerative diseases in a cell biology class. When my grandmother was later diagnosed with a neurodegenerative disease, I was inspired to pursue a career as a neuroscientist to help ensure patients and their families don't go through the same painful experiences.

I trust in the power of being hopeful and I value giving hope to others. There have been many challenges on my journey to becoming a neuroscientist, and I am sure I will face more in the future. But thinking about patients and their loving families reminds me not to give up, because as a neuroscientist, I can give them hope.

Dr Abdalla Khabazeh

I've always had a deep fascination with the human brain: its complexity, resilience and capacity for transformation. Growing up in Syria, where medical resources were often limited, I witnessed first-hand the devastating effects of neurological disorders and the urgent need for advancements in this field. This solidified my determination to contribute to neurosurgery. Despite the challenges of studying in a country severely affected by civil war, I remained committed to excelling in neurology, knowing that this could serve as a pathway to impact people's lives.

I love the opportunity to unravel the mysteries of the brain while making a tangible impact on patients' lives. Neurology is a field where scientific inquiry meets compassionate care. This duality makes neurology an inspiring and ever-evolving field for me.