Genetic Transfer in Medicine: Batten Disease

Inheriting small mutations can sometimes have devastating effects on people's lives.

Otago Department of Biochemistry scientistw Dr Stephanie Hughes and her research team study a rare genetic neurodegenerative disorder called Batten disease.

They are developing a treatment using gene therapy, and the help of some sheep that also have the disease.

Read through these pages to find out more about Batten disease and Dr Hughes's research to develop a treatment.

We have provided links to web pages where you can learn more about some topics. The URLs for all links are provided at the end of this pdf.

What is Batten disease?

A handful of children from around New Zealand suffer from a cruel inherited disorder known as Batten disease.

At first these children are healthy and normal. Then between the ages of about 5 and 10 years, they start to develop symptoms including vision problems, seizures, personality and behaviour changes, slow learning, clumsiness, or stumbling.

The symptoms gradually get worse, until eventually the children become blind, bedridden and demented. They usually die before they reach their late teens or twenties. There are currently no cures or effective treatments.

Brad Timms of Timaru (pictured right) has Batten disease. His older sister JordynRose also had the disease and died when she was 19. You can read more about what his life is like in the Otago Daily Times and Stuff.co.nz.

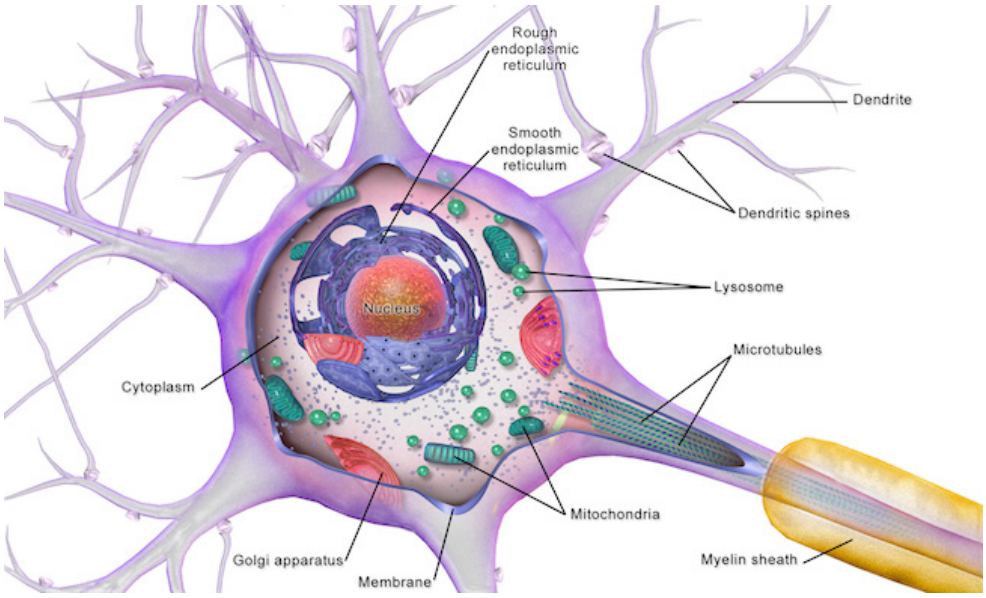
More information on Batten disease and gene therapy:

- Lysosomal Diseases New Zealand
- Batten Disease Support and Research Association (U.S.A.)
- Understanding gene therapy (U.S. N.L.M.)

What causes Batten disease?

Batten disease is a rare recessive genetic disorder caused by a mutation in one of 13 different genes.

One of these genes is called CLN5 (neuronal ceroid lipofuscinosis 5). The CLN5 gene encodes a protein (that means it is a 'recipe' for making a protein) The protein is also known as CLN5.



Neuron cell body (by BruceBlaus [CC BY-SA 4.0], from Wikimedia Commons).

Where do you find the CLN5 protein?

You will find the CLN5 protein inside your cells, in part of the cell's recycling centre, called the lysosome.

The lysosome receives waste from around the cell and breaks it up into small pieces that can be used again.

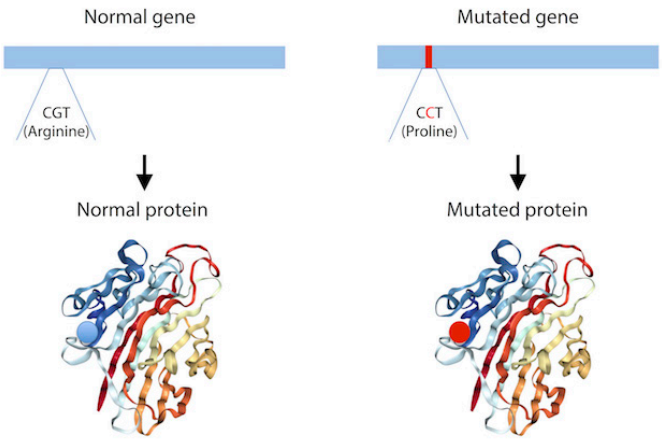
Lysosomes are the small, round, green organelles in the picture above of a nerve cell or neuron

A video from Nucleus Medical Media explains what the parts of a cell are. Lysosomes are described at time code 4:04.

What does the CLN5 protein do?

We don't know exactly what the CLN5 protein does yet, but we do know that it helps the lysosome do its recycling job correctly.

When the CLN5 gene has a mutation, one of the amino acids in the CLN5 protein is changed. This mutated CLN5 protein does not work properly.



When the CLN5 protein does not work properly, the lysosome cannot recycle waste for the cell. This leads to a build-up of waste proteins and lipids (fats and oils) that clog the cell, so that eventually it doesn't work properly either.

How does this affect someone with a CLN5 mutation?

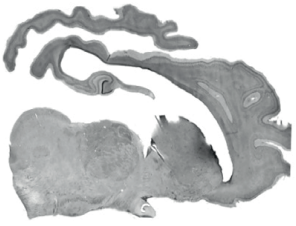
If a person has a Batten disease mutation, it causes their neurons to stop working. These cells have problems communicating with each other and die.

The person with the mutation will struggle to do anything that needs nerve cells, so will develop vision problems, seizures, personality and behaviour changes, slow learning, clumsiness, or stumbling. Eventually they will not be able to move at all or even breathe.

Here is a picture of slices through the brains of some two-year-old sheep. On the left is a brain from a normal sheep, on the right is a brain from a sheep with a mutation in its CLN5 gene. You can see how the mutation has caused the brain to shrink.

Normal brain

Brain with mutation in CLN5



Recessive genetic disorders, family trees, and genetic testing

Batten disease is a recessive genetic disorder. This means that a child needs to inherit the mutant gene from both parents before they will get the disease. The mutation in the gene means that the protein made from from the gene will be either absent or inactive.

If one parent passes on the mutant gene and the other a normal gene, then there will be enough normal protein for the child's cells to function properly.

Blue eyes are another example of a recessive genetic 'disorder', but not a serious one! They are the result of having

two mutant/inactive copies of a gene responsible for brown pigmentation in the eyes.

Finding the mutation in a family with a recessive genetic disorder

This tree of a family affected by a recessive genetic disorder shows that some members must be carrying the mutant gene. They have passed the disorder to their children, but they do not have the disorder themselves. Remember, both parents of an affected person must carry the gene.

Squares are male and circles are female. Black circles and squares are family members affected by the disorder. Double lines indicate in-family breeding. The roman numerals on the left hand side are the generations.

Since 1, 2, 5, and 7 of generation V have the disorder, they must each have two mutant genes (-/-). Their parents (IV 1, 2, and 3, and III 7) must have one good gene and one mutant gene each (+/-).

- What can we tell about the other people?
- Is there anything we can guess?

Genetic testing

We use genetic testing to find out what mutation is present in a specific gene.

Because families can have genetic disorders with similar symptoms but caused by different mutations in the same or different genes, each family needs to be assessed separately.

There are now hundreds of different genetic tests available, each designed to detect a specific mutation or analyse multiple genes to determine the risk of developing a disease or disorder.

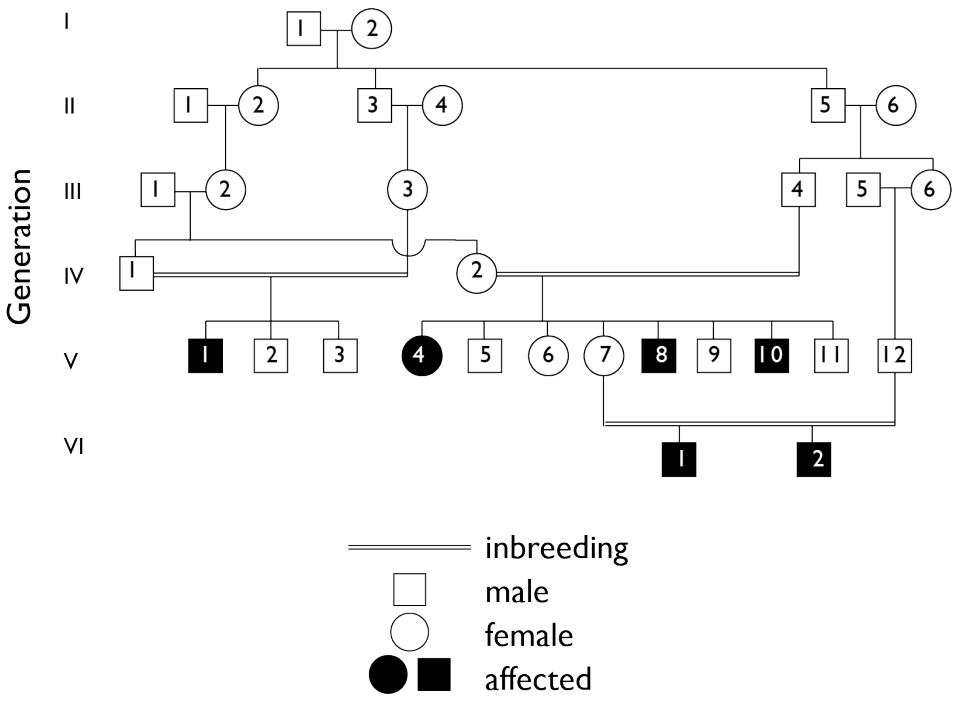
People who come from families with a specific genetic disorder may decide to be tested prior to having children. If they and their partner both carry the gene, they may decide not to have children. If they do decide to have children, they may have a test early in pregnancy to find out whether the foetus is affected and then decide whether or not to abort.

Before getting tested for a genetic disorder, people go through a counselling process to make sure they are properly prepared to make any unpleasant decisions afterwards.

- Can you think of any reasons why, after counselling, a person may decide not to have a genetic test done?

You can learn more about genetic disorders on the Learn. Genetics website and studying genetic diseases on the Science Learning Hub.

Family tree

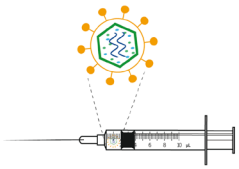


Gene therapy - a cure for Batten disease?

What is gene therapy?

In a genetic disorder, a mutated gene produces a faulty protein that can't do its job properly. Gene therapy aims to introduce a normal version of that gene into a cell, which produces a protein that works.

There are two types of gene therapy that are theoretically possible for the treatment of recessive genetic disorders: somatic gene therapy and germ line gene therapy.



Somatic gene therapy is where a mutated gene is edited or replaced in the DNA of cells in the affected body part only. In the case of Batten disease this would be the brain. The modification would not be passed down to any children.

Here is a link to short video that explains how somatic gene therapy works. It is produced by Applied Genetic Technologies Corporation (AGCT), a company that produces gene therapy for genetic disorders that affect eyesight.

Gene Therapy Explained, a video by AGCT

Briefly, the video explains gene therapy like this:

When a disease has a genetic basis, it means that DNA of the patient contains a mutated gene. The mutation provides the cell with incorrect instructions to make a specific protein, causing the cell to produce either an abnormal, nonfunctioning protein or even not make the protein at all.

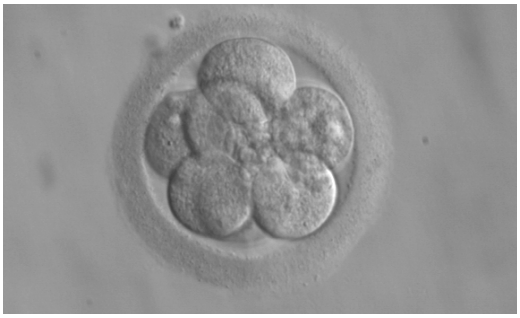
In gene therapy, a functional copy of the gene is delivered to the patient's own cells, so the cells can produce a normal, functioning protein, which will (hopefully) correct the underlying cause of the disease.

Gene therapy uses engineered viruses, called viral vectors, to deliver the functional gene to cells.

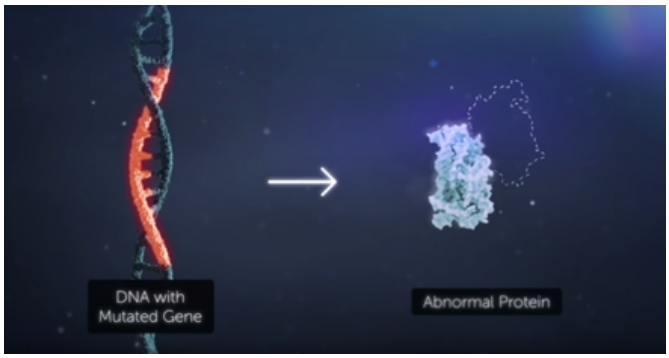
Once administered, the viral vector enters the patient's cells and delivers a functional gene directly into the nucleus.

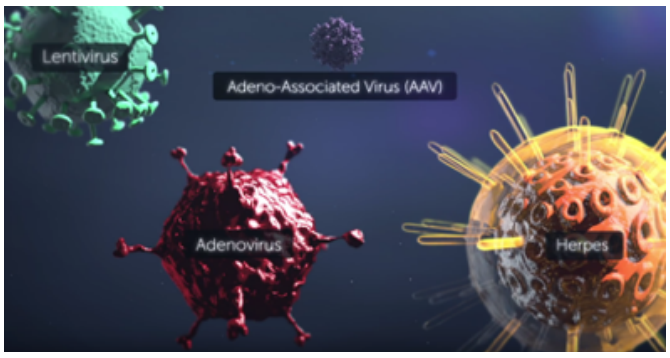
Germ line gene therapy is where a mutated gene is edited or replaced in the DNA of all of the cells of an embryo. All of the baby's cells would then be genetically modified, and it would pass the modification on to its children when it had them.

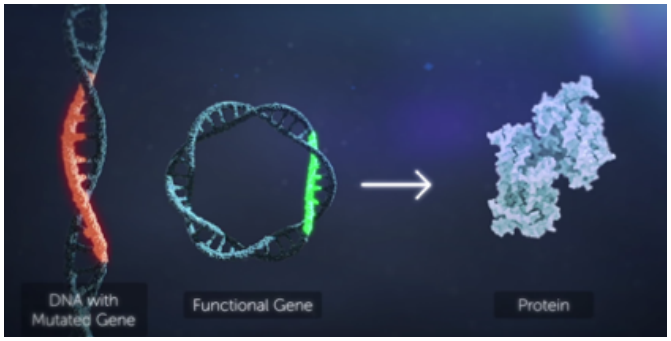
This type of modification has only recently been done in human embryos, but these embryos are not allowed to develop into babies as there are many ethical issues involved.

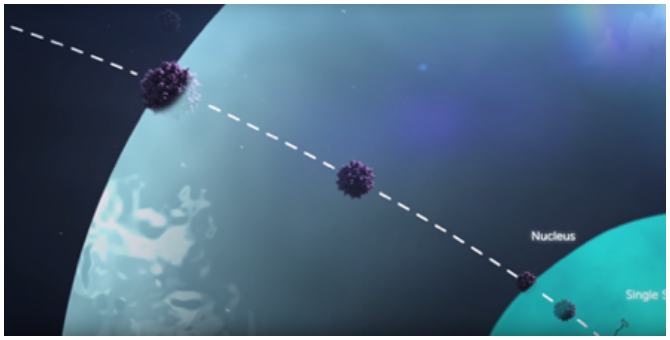


Eight cell human embryo. (By ekem, Courtesy: RWJMS IVF Program [Public domain], via Wikimedia Commons).









Screen shots from the AGCT video explaining gene therapy

Testing gene therapy for Batten disease in sheep

Dr Stephanie Hughes and her colleagues in the Otago Department of Biochemistry have been developing special viruses as a gene therapy treatment for Batten disease.

To correct for the mutation in the CLN5 gene of people with Batten disease, the normal gene needs to be injected into thousands or even millions of cells where it is needed.

If you simply insert a gene directly into a cell, it usually does not function. Viruses naturally inject their DNA into cells in a way that allows the genes in the DNA to help make proteins.

Read more about viruses on the Science Learning Hub

The viruses used in gene therapy are modified so they can't cause disease.

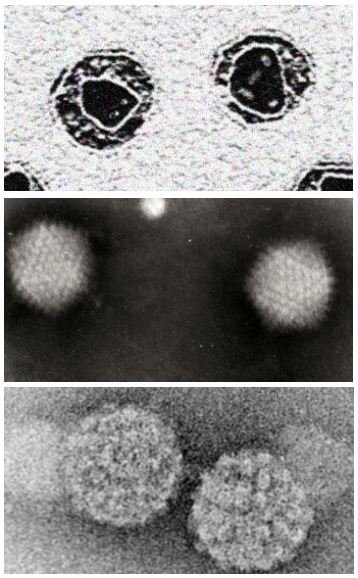
Three types of viruses can be used: retrovirus, adenovirus, or adeno-associated virus (most commonly used). All of these viruses introduce their DNA into the nucleus of a cell; retroviruses integrate their DNA into one of the cell's chromosomes, whereas adenoviruses and adeno-associated viruses do not.

Dr Hughes has been using lentivirus, a type of retrovirus, and adeno-associated virus to create and test gene therapy for treating Batten disease in sheep and mice.

Before the gene therapy can be tested on human patients, a lot of research needs to be done in the lab.

There are three main parts to this research:

- Make the gene therapy virus.
- Test the gene therapy on cells cultured in a dish.
- Test the gene therapy in an animal model, in this case on sheep who naturally already have the Batten disease mutation.



*Top: Electron micrograph of **retroviruses** (GrahamColm / English Wikipedia / CC BY 3.0). Middle: Electron micrograph of **adenoviruses** (PhD Dre / English Wikipedia / GFDL or CC BY-SA 3.0 / via Wikimedia Commons). Bottom: Electron micrograph of **adeno-associated viruses** (Kenneth Raj Research Group, formerly of the NIMR, UK; MicrobeWiki).*

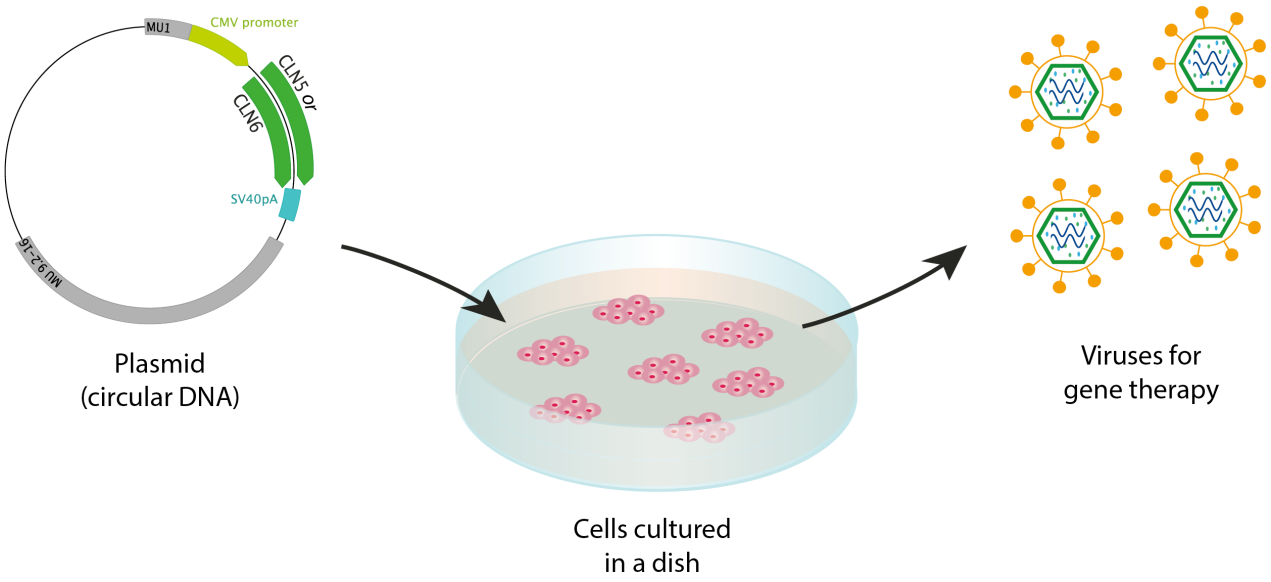
1) Make the gene therapy virus

To make the virus, the researchers need a DNA plasmid (a circle of DNA) which contains the correct CLN5 gene, and cells cultured (grown) in a dish inside an incubator. The cells sit in media, which is a liquid containing the nutrients they need to stay alive.

The researchers make the plasmid using:

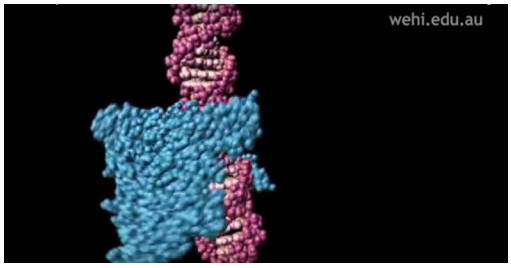
- restriction enzymes - 'scissor' proteins that cut DNA into pieces
- ligases - 'glue' proteins that stick pieces of DNA together
- polymerase chain reaction (PCR) or DNA cloning - techniques which make multiple copies of the plasmid

From left: Dr Stephanie Hughes and students Stephanie Mercer, Sophie Mathison and Oluwatobi Eboda in the Neurodegenerative and Lysosomal Disease Lab.



Making viruses using plasmids and cultured cells.

[Watch an animation of a restriction enzyme and a ligase at work on a DNA plasmid here.]



Next, the researchers add the plasmid to the dish of cultured cells, along with another plasmid that contains genes that help build the lentivirus, and a chemical that makes the cell membranes a little leaky.

The cells take up the plasmid DNA through their leaky membranes, and turn into little virus-making factories, spitting out the finished virus particles into the media.

Finally, the researchers harvest the viruses from the media, which are ready to be used in gene therapy trials.

2) Test the gene therapy on cells

To see whether their gene therapy viruses work, the researchers add them to a different culture of cells. These cells were taken from sheep with Batten disease and cultured in a dish. They have a mutated version of the CLN5 gene and are not well; you can see that their lysosomes don't work very well if you look at them using a microscope and special fluorescent dyes.

If it works, the virus transfers the correct CLN5 gene to the nucleus of the cells, integrating it into their chromosomes. The cells then start to produce the correct CLN5 protein:

The researchers are able to see whether the gene therapy works successfully by comparing treated sick cells with untreated sick cells and untreated normal cells under a microscope. In their experiments, the gene therapy-treated cells started to look more like normal cells.

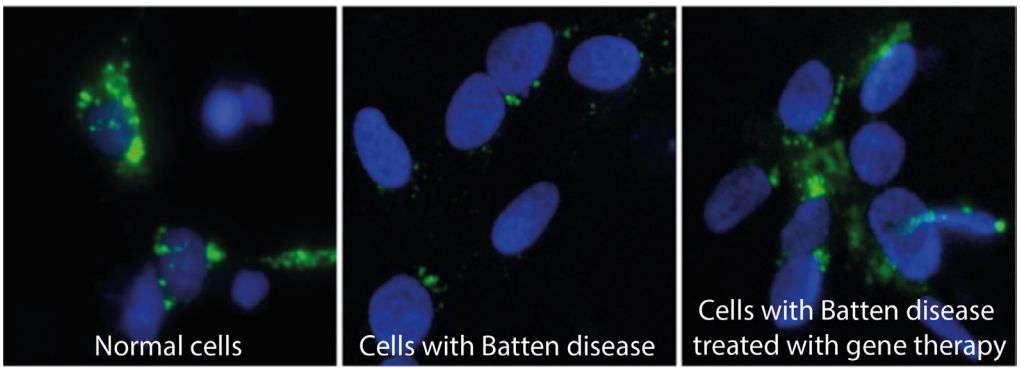
Below are photos of cells from one of the experiments in the Hughes Lab:

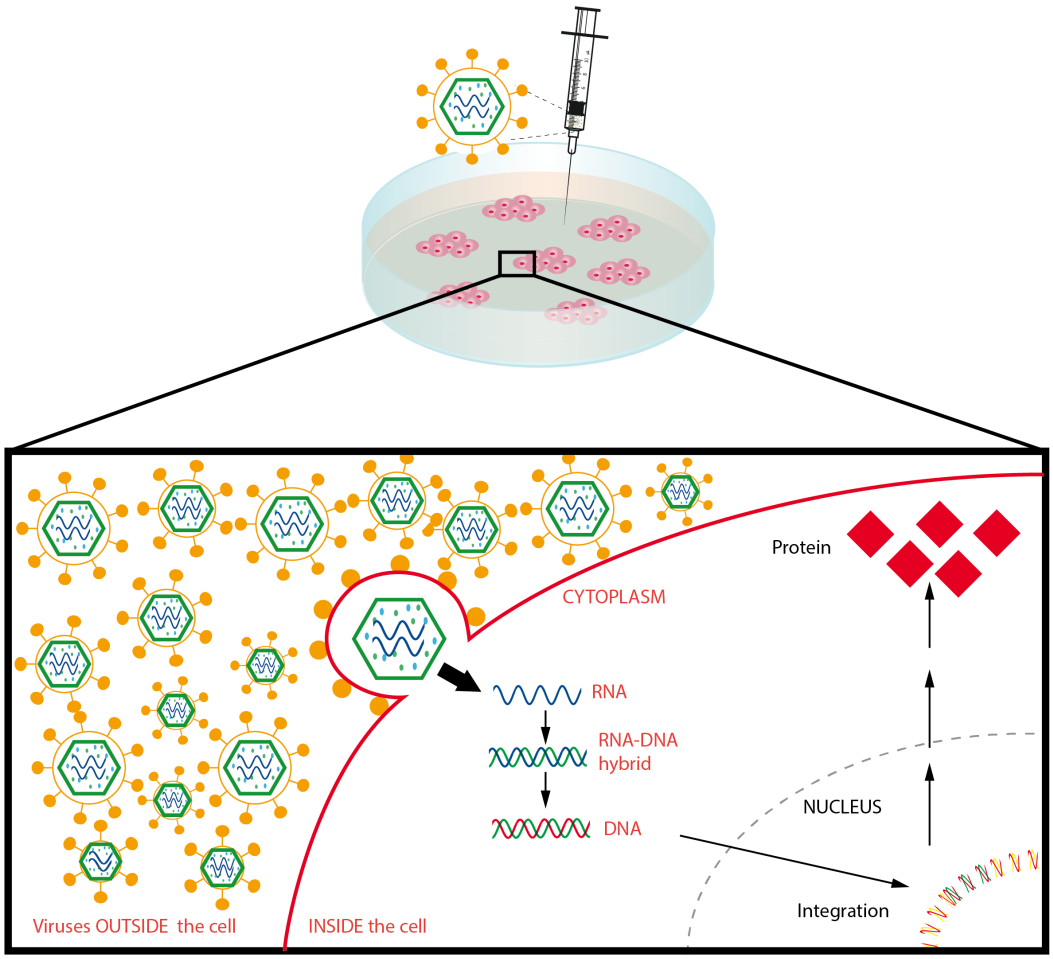
The blue round shapes are the nuclei of cells, stained blue using a fluorescent dye. The green spots show where lysosomes are working properly, carrying out a process called autophagy.

You can see that there are lots of green spots on the picture of the normal cells (left), i.e. the lysosomes in those cells are working normally.

In the cells with Batten disease (middle), the lysosomes are not working very well and have very few green spots.

The cells with Batten disease that have been treated with the virus gene therapy have lots of green spots, and look much more like the normal cells.





How the gene therapy virus works on cells to correct the mutated gene.

3) Test the gene therapy on an animal model

Once it was clear the gene therapy viruses were working on cells cultured in a dish, Dr Hughes and her collaborators next tested the gene therapy viruses on a flock of sheep at Lincoln University, New Zealand, that naturally develop Batten disease.

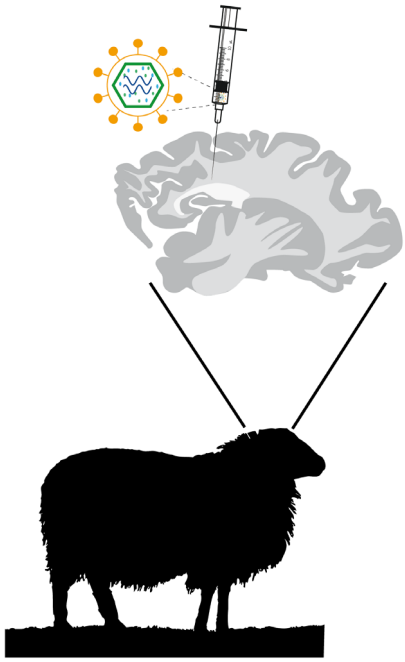
The animals have a mutation in their CLN5 gene, as do many humans with Batten disease, and share many of the features of the human disease, including neurodegeneration, blindness and premature death.

The sheep chosen for treatment had not yet developed symptoms of Batten disease.

The researchers injected the viruses into the fluid-filled spaces in the sheep brains. The viruses then "infected" the brain cells, integrating the normal CLN5 gene into the cells' genomes.

Promisingly, the treated sheep lived longer than the untreated sheep, and they did not develop most of the Batten disease problems, apart from some loss of vision.

A clinical trial has now started for the CLN6 form of the disease on human patients with collaborators in the USA (see also curebatten.org).



Implications of gene therapy

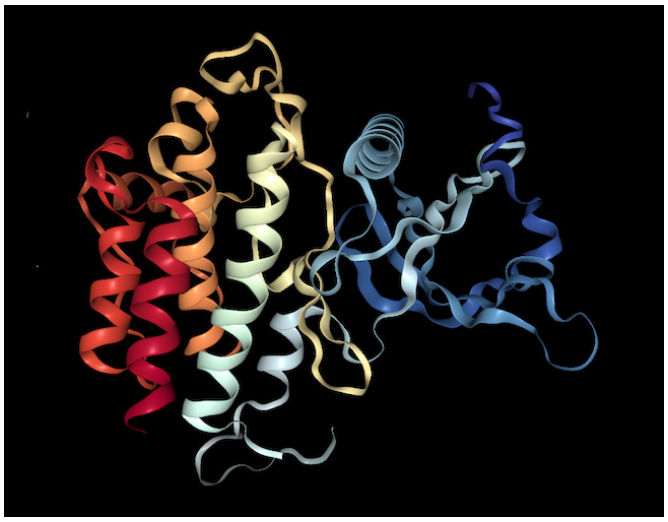
A genetic-based treatment for inherited genetic diseases initially sounds like a great idea, but can you think of any ethical issues with these treatments?

Here are just a few of many you can consider. There are no sure answers – when an issue is two-sided, only you can make up your mind.

What do you treat?

Although Batten disease is clearly an undesirable disease that causes a lot of suffering, the status of other inherited conditions is not so clear.

- What do you consider to be a disease, and what a trait (ie a genetically determined characteristic or condition)? For example, is dwarfism disorder a disease that needs to be treated, or a trait that is just part of human variation?
- What about just short height?
- Should any inherited diseases be treated at all, even clearly awful ones, especially when the cost of developing a treatment is extremely high?



Part of the fibroblast growth factor receptor 3 protein. Mutations in the gene encoding this protein can cause achondroplasia, the genetic disorder that results in dwarfism.

Genetic testing

Genetic testing identifies changes in chromosomes, genes, or proteins. The results can help determine whether a person has a suspected genetic condition or might develop a genetic condition. The results can also inform a person's chance of passing on a genetic disorder.

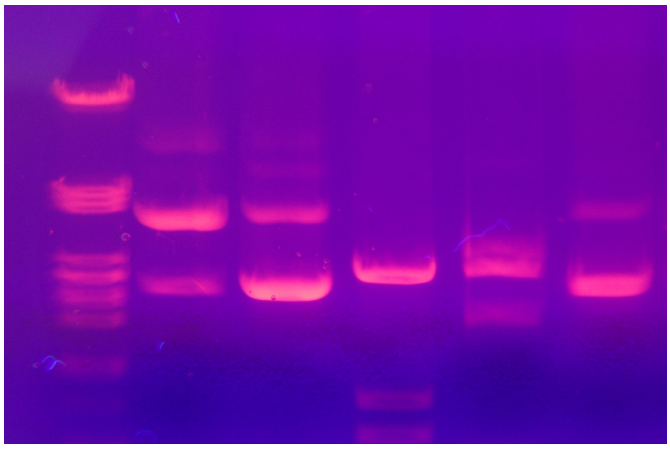
Genetic testing has limitations: some tests can't detect all of the possible mutations that can cause a condition, or the mutations that can be identified may only lead to a condition only some of the time.

- If screening for Batten disease was available, what implications would there be for potential patients and their parents?

Imagine if you find out you have a mutation, one that might give you a disease in the future, and you could pass on to your children.

- What happens when you are looking for someone to marry, or when you want to get health insurance, or when you are deciding on a career?
- Would you want to find out about that mutation if there was no treatment for the disease?

The National Institute of Health in the USA provides an overview of different genetic tests:



A DNA electrophoresis gel, which scientists use to separate pieces of DNA of different sizes. Some genetic tests use DNA electrophoresis to reveal gene mutations.

Germline vs somatic gene therapy

Somatic gene therapy is DNA treatment targeted at cells in a person's body that are not passed on to the person's children, ie somatic cells. The treatment that Dr. Hughes is developing for children with Batten disease is an example of somatic gene therapy.

Germline gene therapy is DNA treatment targeted at a person's cells that produce reproductive cells, eggs or sperm. The treatment is then passed on to that person's children and grandchildren.

- What would be the advantages of germline therapy?
- What problems can you think of that might make germline therapy unacceptable?
- Do you think germline therapy should be used in humans?

An editorial in the Nature journal talks about the ethics around germline editing used in human embryos. It refers to a recent example of genetic editing of human embryos where scientists used the technique to find out about how a gene works (the embryos were not allowed to develop into babies):

'Take stock of research ethics in human genome editing.'

Taking away the choice of offspring to decide

There is now a new tool available that can very accurately edit the DNA in a cell without using viruses. It is called CRISPR-Cas9, and has been adopted from bacteria. In theory, it could be used to fix mutations in an embryo.

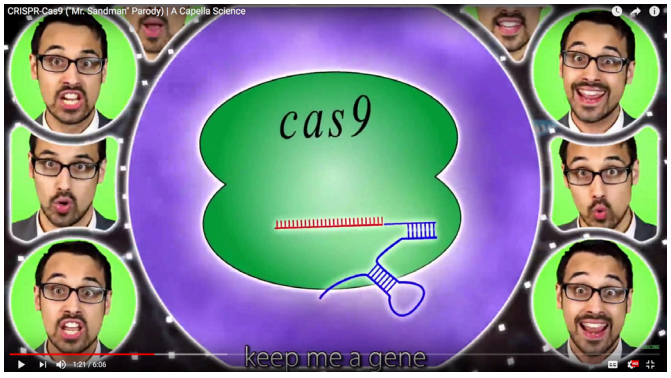
Issie Robertson, a teenager from Dunedin, wondered if modifying a human embryo to extend its life could be considered ethical. The sixteen-year-old even wrote an essay about these concerns that was published in the Journal of Medical Ethics.

Listen to Issie talk about her concerns on Radio New Zealand National here.

You can read the abstract of her essay here.

You can watch a news item about Issie's essay here:

Watch a musical tribute/explanation/extravaganza about CRISPR-Cas9 here:

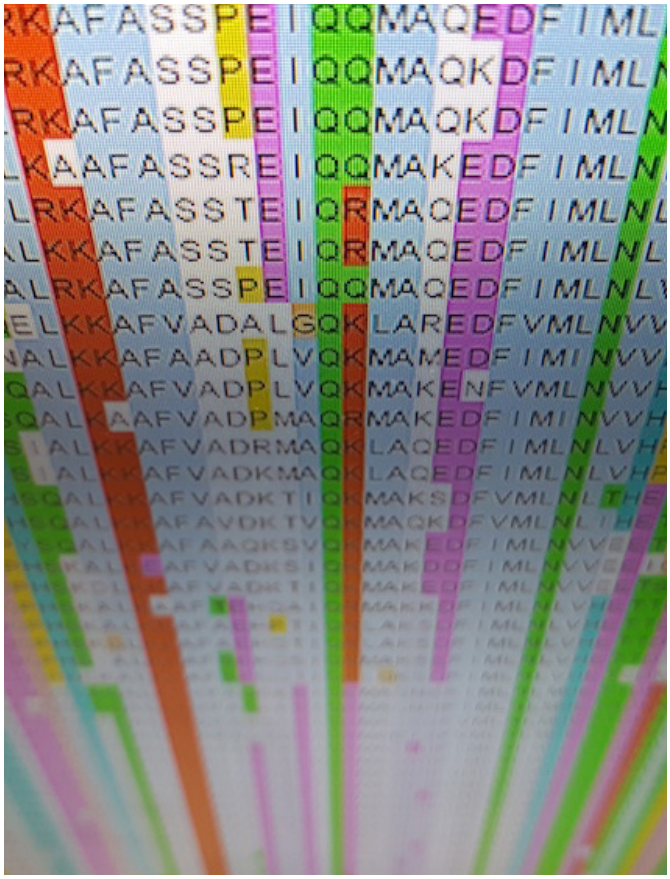


Social impact

- Will the high cost of gene therapy exclude people on middle and low wages, so that only the rich can access the technology?

More about the ethics of gene therapy:

- Gene editing could eradicate dozens of genetic diseases - video from One News, TVNZ
- What are the ethical issues surrounding gene therapy? - resource from the U.S. National Library of Medicine
- 'That's $425,000 right there' — the anxious launch of a gene therapy with a record sticker price - Luxterna is the first "true" gene therapy (in which a functional gene does the job of a defective one) to be approved for use in the USA
- Gene therapy: issues and impacts - A page exploring issues surrounding gene therapy, including ethical, social, legal, economic, and political issues (USA-based)



Batten disease: list of link urls

Links to find this story online

Otago Biochemistry: Resources for high school students
otago.ac.nz/biochemistry/research/otago663017.html

Genetic transfer in medicine: Batten disease
otago.ac.nz/biochemistry/research/otago663025.html

Dr Stephanie Hughes
otago.ac.nz/biochemistry/people/profile/index.html?id=398

More information on Batten disease and gene therapy

Lysosomal Diseases New Zealand
ldnz.org.nz/

Batten Disease Support and Research Association (U.S.A.)
bdsra.org/

Understanding gene therapy (U.S. National Library of Medicine)
ghr.nlm.nih.gov/primer/therapy/genetherapy

What is Batten disease?

Brad Timms article from the Otago Daily Times
odt.co.nz/lifestyle/magazine/glimmer-hope

Brad Timms article from Stuff.co.nz
stuff.co.nz/national/health/96749081/mum-clinging-to-hope-that-cannabis-extract-could-help-her-son-who-has-batten-disease-a-rare-brain-condition

Katie Archer video from CureKidsNZ
youtube.com/watch?v=GloL2eual2E

Katie Archer video from the NZ Herald
nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=11920609

What causes Batten disease?

Video from Nucleus Medical Media which explains what the parts of a cell are.
youtube.com/watch?v=URUJD5NEXC8

Recessive genetic disorders, family trees, and genetic testing

Learn more about genetic disorders on the Learn.Genetics website
learn.genetics.utah.edu/content/disorders/

Learn more about studying genetic diseases on the Science Learning Hub
sciencelearn.org.nz/resources/2050-studying-genetic-diseases-finding-out-about-the-genes

Gene therapy - a cure for Batten disease?

Video about how gene therapy works. from Applied Genetic Technologies Corporation.
youtube.com/watch?v=xOQFJJOBGM0

Testing gene therapy for Batten disease in sheep

More about viruses on the Science Learning Hub
sciencelearn.org.nz/resources/184-virus-strains

About restriction enzymes
sciencelearn.org.nz/resources/2035-restriction-enzymes

About ligases
sciencelearn.org.nz/resources/2034-dna-ligation

About the polymerase chain reaction (PCR)
sciencelearn.org.nz/resources/2347-what-is-pcr

About DNA cloning
sciencelearn.org.nz/resources/2031-dna-cloning

An animation of a restriction enzyme and a ligase at work on a DNA plasmid
youtube.com/watch?time_continue=1&v=aA5fyWJh5S0

About a clinical trial for the CLN6 form of Batten disease in human patients
curebatten.org/service/gene-therapy/

Implications of gene therapy

What are the types of genetics tests?
ghr.nlm.nih.gov/primer/testing/uses

Nature editorial: 'Take stock of research ethics in human genome editing.'
nature.com/news/take-stock-of-research-ethics-in-human-genome-editing-1.22632

Issie Robertson talking about her concerns about modifying a human embryo on Radio New Zealand National
radionz.co.nz/national/programmes/saturday/audio/201850380/issie-robertson-teen-takes-on-bioethics

Issie Robertson's essay abstract
jme.bmj.com/content/43/9/645

News item about Issie's essay on Channel 39
youtu.be/hvhNwqPT5y0

a musical tribute/explanation/extravaganza about CRISPR-Cas9
youtu.be/k99bMtg4zRk

Gene editing could eradicate dozens of genetic diseases - video from One News, TVNZ
tvnz.co.nz/one-news/new-zealand/gene-editing-could-eradicate-dozens-genetic-diseases

What are the ethical issues surrounding gene therapy? - resource from the U.S. National Library of Medicine
ghr.nlm.nih.gov/primer/therapy/ethics

'That's $425,000 right there' — the anxious launch of a gene therapy with a record sticker price
statnews.com/2018/03/21/gene-therapy-luxturna-launch/

Gene therapy: issues and impacts - A page exploring issues surrounding gene therapy
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