Imagine you're a gene therapist

Teachers' Notes

The worksheet provides a context through which students can learn about genes, genetic disease, inheritance and DNA technology. It will introduce students to the process and method of science and the challenges that scientists face in bringing an exciting scientific idea to a clinical reality. It will also encourage students to think about the ethical issues surrounding new scientific developments.

This activity has been designed to address components of the NSW Stage 4/5 Science and HSC Biology syllabi. Completing the following activity relates well to:

Stage 4 and 5 Science
Prescribed Focus Areas 4/5.1-.5
Values and Attitudes 4/5.23, 4/5.25, 4/5.26
Skills 4/5.16-.20
Knowledge and Understanding 4/5.8, 5.12

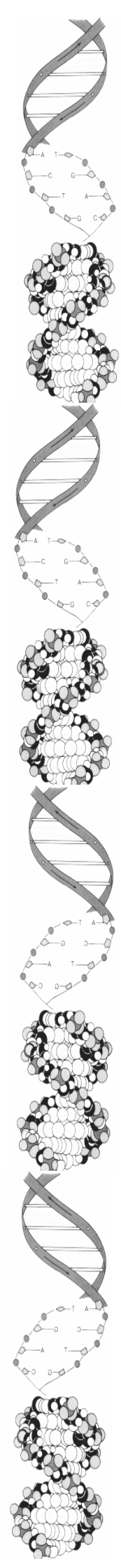
HSC Biology
Prescribed Focus Areas 1, 2, 3, 4, 5, 6
Skills 12, 13, 14, 15
Values and Attitudes 16
Blueprint of Life 9.3.2-.4
Search for better health 9.4.1
Biotechnology 9.6.4-.7
Genetics: The code broken 9.7.1,.3-.7

The activity

The students will be asked to form a team of doctors (clinical geneticists) and scientists (molecular biologists) who have decided to work together to use gene therapy to try to treat children with muscular dystrophy. They will be assigned to either the scientific or medical parts of the team and must work through the questions on the accompanying worksheets to discover what information, techniques and expertise they need before they can develop a successful gene therapy strategy to treat muscular dystrophy. Once they have done this the scientific and medical parts of the team will come back together to discuss whether they are ready to start a clinical trial of this treatment in patients.

The activity can be expanded or reduced depending on the time available.
Option 1 - The students tackle the questions using their current background knowledge. It does not matter if solutions are 'wrong' according to current research – the process of problem solving, teamwork and discovering their needs for more information is a valuable exercise that addresses many aspects of the curriculum.
Option 2 - Individual students or small teams go away to research the answers to the questions then gather back in the classroom to continue in a role-play situation to devise a gene therapy strategy as a team.

The 'scientists' and 'doctors' can present their answers/research findings to each other via oral presentation, handouts, posters or a PowerPoint slide show. This way all members of the class will learn the scientific concepts covered in both the molecular biology and clinical genetics parts of the activity.



Gene therapy – the background

The ability to manipulate DNA has been developing and improving since restriction enzymes (enzymes that cut DNA) were discovered in 1970. The concept of gene therapy – the delivery of genes to patients' cells to treat disease - has been the subject of intense research efforts for the last two decades. Despite this, there are to date only two examples of successful gene therapy in which a small number of patients have been cured. These are of the X-linked disorder Severe Combined Immunodeficiency (Boy-in-the-bubble disease or X-SCID) and a similar disorder called ADA-SCID. In both these situations gene therapy was conducted by removing bone marrow cells from the patients, exposing the cells to a modified virus which carried the healthy gene into the cells, and then returning the cells to the patient. The gene allows the bone marrow cells to respond to growth factors and mature into healthy immune cells. Sadly the X-SCID clinical trial has suffered a major setback due to a significant safety issue. Three of the 18 patients treated worldwide have developed Leukaemia. This appears to be due to the healthy gene being inserted very close to, and activating, a known leukaemia-causing gene. Research is continuing to reduce the risk of this happening again in the future.

Other gene therapy research programs are also showing promise, for example for haemophilia. But in many cases the challenge is to deliver the healthy gene to *enough* of the right kind of cells to have a clinical impact on the disease.

Despite these difficulties, gene therapists world-wide are confident that they can eventually develop strategies to overcome the technical and safety issues and that gene therapy will become a standard medical treatment for a variety of diseases.

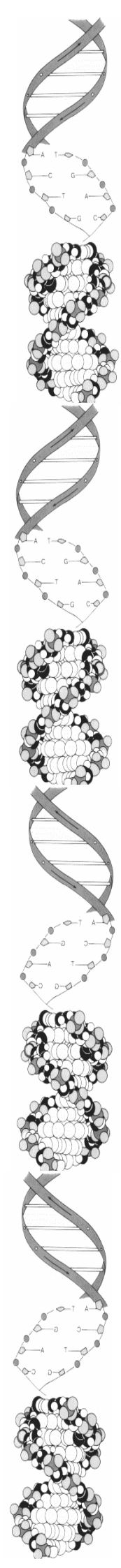
NB - Muscular dystrophy (MD) presents particular challenges to gene therapists due to the large number of cells and muscle groups that need to be treated. The questions asked in the worksheet can be easily adapted to suit other diseases as most of the difficulties faced by gene therapists are common to many diseases.

Information resources

The Centre for Genetics Education has an excellent factsheet on muscular dystrophy (http://www.genetics.com.au/pdf/factSheets/FS39.pdf) and other genetic disorders and also one on gene therapy (http://www.genetics.com.au/factsheet/25.htm).

The Muscular Dystrophy Association of Australia has very accessible information at http://www.mda.org.au/.

An excellent interactive overview of the challenges of gene therapy can also be found at http://gslc.genetics.utah.edu/units/genetherapy/.



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Guide to the questions

Molecular biologists

1. Gather information on the type of DNA mutation that causes Duchenne/Becker MD and explain to the doctors how this might cause the symptoms of MD.

Mutations in the dystrophin gene are the cause of Duchenne and Becker MD. It is a very large gene and there are many different mutations in the gene that can cause MD. Two thirds of the cases of Duchenne and Becker MD are caused by deletions of small parts of the gene (called exons) resulting in what is known as a 'frameshift mutation' – the first part of the gene 'makes sense' but the rest of the gene after the deletion is 'out of step' and cannot be read as meaningful information. The result is a non-functional protein.

The dystrophin protein is believed to be part of the scaffolding that stabilises the structure of muscle cells during contraction. An absence of, or non-functional, dystrophin leads to very fragile muscle cells that are easily damaged. Over time the muscle cells disintegrate leading to progressive loss of muscle strength.

2. Could a healthy version of the dystrophin gene restore function to the patients' muscles? Would your answer be the same if it was a dominant genetic disorder?

MD is an X-linked recessive genetic disorder. A recessive mutation means that having just one healthy version (allele) of the gene, instead of the usual two, is sufficient to produce enough protein to make the cells functional.

Females who carry a faulty dystrophin gene on one X chromosome, have a healthy version of the gene on the other copy of the X chromosome. This allows them to manufacture enough healthy dystrophin for their muscles cells to function and they do not suffer from the disease. These women are called carriers. They can pass the disorder on to their sons who have only one X chromosome. Their daughters can become carriers.

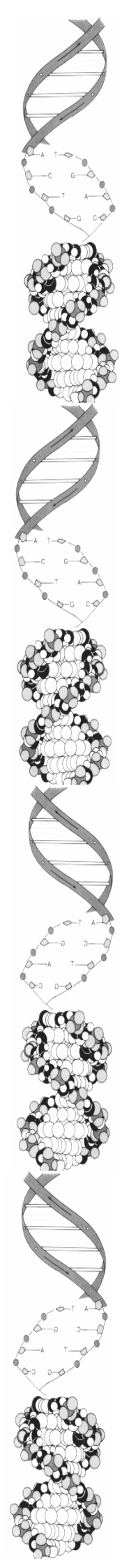
Males who inherit the faulty gene on the X chromosome they receive from their mother, have no 'back up' allele on the Y chromosome. They therefore produce no healthy protein. If it were possible to permanently deliver a healthy version of the dystrophin gene to a large proportion of the patient's muscle cells enough dystrophin protein could me made to repair the defect.

In a dominant genetic disorder, the mutation makes the protein manufactured from the faulty allele not only non-functional, but the protein also becomes toxic to the cell in some way. Putting a healthy version of the gene into a cell in this case would not overcome the genetic disorder. Other types of gene therapy are being researched for dominant disorders that will block the production of the defective protein (e.g. RNA interference).

3. How could you use your tool kit to provide a healthy dystrophin gene that could be given to the patients with MD? Draw a diagram to explain your idea to the doctors

An example of such a diagram can be found in the HSC Biology text *Biology in Context: The Spectrum of Life* (Page 425, 2000 Edition).

The key points that the students need to incorporate into their diagram:
use restriction enzymes to cut the healthy dystrophin gene from human DNA



use restriction enzymes to cut open a circular plasmid
use ligase to paste the dystrophin gene into the plasmid
put plasmid into bacteria and culture bacteria to obtain large quantities of the gene
use restriction enzymes to cut gene from plasmid
use ligase to paste gene into modified virus

Their diagram should also show consideration of how the virus will be delivered to the patient's muscle cells (intramuscular injection or intravenous injection, for example).

4. Discuss with the doctors which muscles in the body need treating. Do you think every single muscle cell will need to receive the gene for the treatment to be effective?

Gene delivery is the major challenge facing gene therapists, particularly in muscular dystrophy. In order for the treatment to have a clinical impact the gene must be delivered not only to the right cells but a large number of those cells. A single organ or muscle may be made up of several million cells!

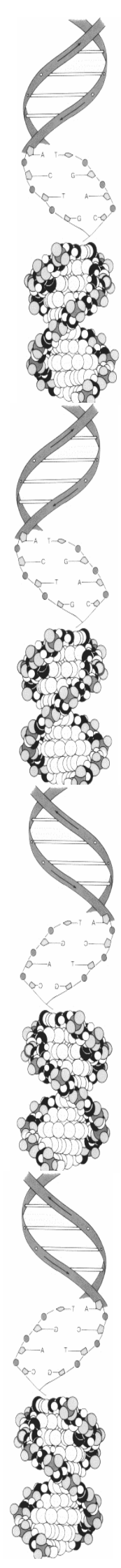
In MD many muscle groups in the body are affected and all the muscle cells within these muscle groups are affected by the disease. Any successful gene therapy strategy would need to deliver the gene to a sufficient number of muscle cells in each muscle group to restore or retain strength in that muscle. The more muscle cells that receive the gene the stronger the muscle is likely to be. *See also answer to question three in the clinical section.*

A note on gene delivery techniques
Gene delivery methods include: direct microinjection into the nucleus; delivery by liposomes (DNA enclosed in fatty droplets that fuse with the cell membrane); delivery by gene gun (DNA is attached to microparticles of gold and fired at high pressure into the cell); and viral delivery. Microinjection and gene guns directly target the correct cells, but are very labour intensive where a large number of cells are to receive the gene. Liposomes have been used in clinical trials to deliver genes via the bloodstream and via a nebuliser into the lungs, for example for cystic fibrosis, however the efficiency of gene transfer is low.

Viruses currently appear to be the most efficient at delivering genes to a large number of cells. They can be injected into the bloodstream or into tissues. Different viruses target different cells, i.e. cold and flu viruses target cells lining the respiratory passages, the hepatitis virus targets liver cells, etc. Some viruses prefer to infect dividing cells while others only infect non-dividing cells. Different viruses also have the capacity to carry different amounts of DNA. The dystrophin gene is very large so this is a significant consideration in the choice of virus. See http://gslc.genetics.utah.edu/units/genetherapy/gttools/ for a detailed description of the types of viruses that can be used. A problem with viral delivery is the immune system which may rapidly inactivate the virus. The immune system is likely to become more efficient at recognising and inactivating the virus if any subsequent rounds of treatment are required.

5. The doctors will need to persuade patients to take part in the clinical trial. Patients and their families will be concerned about the safety of the treatment and whether it will work. What do you think some of the safety problems might be with your proposed treatment? What experiments could you do to address these concerns?

The major concerns for the regulators of gene therapy (In Australia this includes the Office of the Gene Technology Regulator - http://www.ogtr.gov.au) are that:
insertion of the gene does not damage other genes in the patient's genome.



the gene is NOT delivered to the patients germ-line cells (egg and sperm producing cells) where it could be passed on to the next generation (this is called germ-line genetic modification),
and that the gene delivery method does not cause any serious side effects (e.g. a severe immune response to the virus).

DNA damage - Scientists can conduct experiments on cells growing in the laboratory (cell culture) and use DNA sequencing techniques to check where a gene has been inserted in the chromosomes. Many scientists are studying ways to design gene delivery vectors that minimise the risk of disrupting other genes. Cell culture and animal experiments will also confirm that the gene is being delivered to the correct cells and is 'switched on' (producing protein) – gene therapy will not work if the gene is delivered but does not get 'switched on'.

Germ-line genetic modification - At the current time it is considered that long term effects of germ-line genetic modification on human health are unknown and the potential risks out-weigh the potential benefits. Steps can be taken to avoid germ-line genetic modification such as designing the vector for targeted gene delivery to specific cells and the method of delivering the vector to the body, i.e. intravenous injection or direct tissue injection. Animal experiments can be used to monitor this and monitoring is continued into clinical trials in humans.

Side effects - As with safety testing for any pharmaceutical product, animal experiments and continued monitoring in clinical trials are required to check for any serious side effects prior to the gene therapy protocol being approved as a standard clinical treatment.

Clinical geneticists

1. Draw a diagram to explain to the molecular biologists the inheritance pattern of Duchenne and Becker muscular dystrophy.

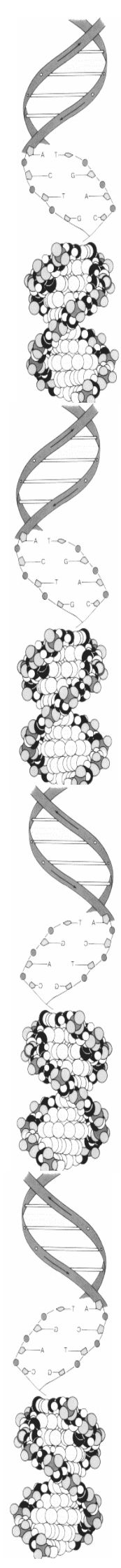
See the NSW Genetics Education Program fact sheet on muscular dystrophy at http://www.genetics.com.au/pdf/factSheets/FS39.pdf. They also have a separate worksheet on X-linked inheritance http://www.genetics.com.au/factsheet/default.htm#patterns

2. Provide the scientists with as much information as you have on the disease, its symptoms, and the progression of the disease. When do you think it will be most effective to treat the patients using gene therapy?

See the Centre for Genetics Education fact sheet on muscular dystrophy at http://www.genetics.com.au/pdf/factSheets/FS39.pdf and visit http://www.mda.org.au/.

As with any degenerative disorder it is better to prevent cells from dying rather than try to get new cells to grow. MD is a progressive disorder with more muscle groups and muscle cells becoming affected over time. The most appropriate time to treat these patients would be before they are showing any significant muscle degeneration.

Some gene therapists are researching the possibility of carrying out gene therapy on the foetus while it is still growing in the uterus (*in utero).* This would have the advantage of correcting the genetic fault in the cells while they are still developing into muscle cells. However, there



are risks and ethical issues associated with this approach and research is proceeding in animals with caution.

3. Which muscles and how many muscle cells do you think will need to receive the gene for the gene therapy treatment to have the most benefit for patients?

The muscles most affected in Duchenne and Becker muscular dystrophy are in the first instance those that control posture (arms, legs and back). In later stages of the disease the muscles involved in breathing (diaphragm and intercostal muscles between the ribs) and swallowing (in the oesophagus) are also affected. It is the involvement of these breathing and swallowing muscles that ultimately leads to death in these patients. Targetting these muscles with gene therapy would therefore be the priority for the patients' doctors. Correcting the gene defect in the other muscles would improve quality of life, but would not save the patients life. *See also answer to question 4, molecular biology section.*

4. The patients and their families will want to know if the treatment will work and it it is safe. What do you think some of the safety issues of this proposed treatment might be? Are there any reasons why it might not work? Will the scientists need to do more experiments before the clinical trial can start?

See answer to question 5 in molecular biology section.

5. Discuss the moral and ethical issues of asking patients to take part in testing a treatment that has not been tried in humans before? Who could benefit? Who could be harmed and how? Does the patient have the right to choose whether to take part?

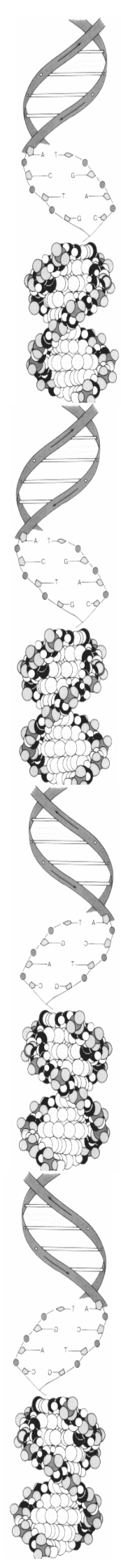
Medical ethicists (or bioethicists) approach any new problem such as this using four principles:
Respect for autonomy – recognising and enabling the right of individuals to make their own choices;
Beneficence – acting to benefit patients (primarily) and society, balancing the benefits of a treatment against the risks and costs;
Non-maleficence – avoiding harm to patients and society, balancing the risk of harm against the potential benefits to individuals and society;
Justice - distributing benefits, risks and costs fairly. Patients in similar positions should be treated in a similar manner.

There are no right or wrong answers to this question however some areas that the students may consider are the risks and benefits of genetic manipulation, germ-line genetic modification, patient safety, testing new treatments on humans/animals, the costs of this 'high-tech' treatment versus the cost (emotional and financial, to families and society) of caring for a disabled child? The students should be encouraged to think beyond the individual patient to the impact of this treatment on other family members and society.

Extension activities:

Investigate and describe the methods that might have been used to discover that mutations in the dystrophin gene were the cause of muscular dystrophy. Consider traditional methods for studying genetic inheritance (linkage maps), and modern technologies, such as genomics (the Human Genome Project) and molecular biology.

Investigate the current research that is underway to develop gene therapy for muscular dystrophy or another genetic (or non-genetic disorder). Are there any clinical trials underway? What problems have the doctors and/or scientists encountered? How do these approaches compare to the treatment you decided upon in your discussion in class today? Keep in mind



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the source of your information: did it come from the media, a scientific website or a patient support group? Do you think these sources accurately represent current progress in gene therapy?

Gather information on other methods that are being investigated for the treatment of muscular dystrophy. What are the practical and ethical difficulties associated with these potential treatments? How do the potential risks and benefits of gene therapy compare with these other forms of treatment? (A good starting point for this and the above question is the American Muscular Dystrophy Association website http://www.mdausa.org/publications/resdev.html)