Understanding neurological disease

Understanding the brain is the last frontier of medical research and one of the most challenging areas of medicine. *In order to understand and potentially treat neurological diseases, scientists need to understand the structure and functioning of the healthy brain, and then explore what is happening in the brain of patients with neurological diseases.* Professor Richard Faull is Director of Auckland University’s Centre for Brain Research where interdisciplinary teams from science, medicine and the community are working together to understand and find improved ways of treating neurological disease.

While the Centre has teams looking into many different diseases, in this paper we will explore the work led by Professor Faull relating to *Huntington’s disease, a neurological disorder caused by a gene mutation on chromosome 4 which has devastating consequences for the patient and their family.*
Huntington’s disease

Huntington’s disease (HD) is an example of a neurological disease caused by an **autosomal dominant gene mutation** on chromosome 4. Because the allele associated with the mutation is dominant, a person only needs to inherit one copy of the mutated allele to inherit the disease (Fig. 1).

We now know a lot about the mutation, its pattern of inheritance and the nature of the disease it causes. Much is also known about how it damages the brain’s neurones. **However, it is currently incurable.** By finding out more about how the human brain works and how this disease affects the brain, Professor Faull’s team is contributing to an international effort to find a cure for Huntington’s disease.

![Fig. 1. Autosomal dominant inheritance pattern of HD. Every time a child is conceived from these parents, there is a 50% chance that the child will carry the allele that causes HD](image)

The phenotype

People with HD are born carrying the mutation, and initially live with no ill effects. The mutation’s damaging effects accumulate in the brain over time. Symptoms usually begin around the age of 40. These are initially quite mild, but gradually become worse as the disease progresses.

The disease progressively degenerates brain cells, reducing the person’s ability to walk, talk, reason, think and remember. Psychiatric and behavioural disturbances develop, including mood swings, irritability and depression. These eventually decline into dementia. Involuntary movements are also part of the disease, including chorea (twitching, jerking and writhing) and dystonia (muscle contractions that lead to twisting movements and abnormal postures). The symptoms eventually become so debilitating that sufferers rely totally on others for their care. The disease causes death usually about 20 years after onset.

The disease puts an enormous strain on the affected person and their family, emotionally and financially, and on health services. At-risk individuals have a high risk of suicide and suicidal thoughts. They will often have watched a parent decline and die from the disease. Because symptoms usually appear after child-bearing age, people have often potentially passed the disease to their children by the time their own symptoms appear.

**Living with Huntington’s disease**

A number of people with HD and their families have shared their stories using YouTube. In sharing their experience they hope to encourage people to understand more about the disease, and why finding a cure is so important.

You may wish to watch one:

- [NHS—Choices, Huntington’s Disease; Lee aged 39](NHS—Choices, Huntington’s Disease; Lee aged 39)
- [CBS Special—Huntington’s Disease](CBS Special—Huntington’s Disease)
- [Daniel My Brother](Daniel My Brother)
The Genotype

The affected gene that causes HD is called IT15. It is found on the short arm of chromosome 4 so is **autosomal** rather than being on a sex chromosome. Children of a carrier parent have a 50% chance of inheriting a mutated gene, and because it is **dominant** those who do inherit it will develop the disease if they live long enough (see Fig. 1 on page 2). Most people with HD are heterozygotes; homozygotes are rare and have received a mutated copy of the gene from each of their parents.

The HD gene IT15 is expressed as the protein **huntingtin** in all mammalian cells, however there are higher levels of gene expression in the brain and testicles.

All IT15 alleles have a section of trinucleotide repeats consisting of CAG (cytosine-adenine-guanine). It is usual for the number of repeats to vary but there are always less than 36 CAG repeats in the normal allele. The mutant allele has more CAG repeats than normal (Fig. 2).

**Normal allele**
- Contains between 10 to 35 CAG repeats

**Huntingtin mutation allele**
- Contains more than 36 CAG repeats

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**How does a gene mutation occur?**

**Gene mutations** are changes in the nucleotide sequence of a DNA strand. Some are point mutations, involving a change to just one nucleotide. Others, like the one responsible for HD, involve changes to longer sections of the gene. Gene mutations can involve deletions, insertion or substitutions. The **degenerate nature of the genetic code** means that in some cases several triplets code for the same amino acid. As a result, some mutations make no change to the amino acid sequence that results from translation of the gene. We say these mutations are **neutral**. However if the mutation causes a change in the amino acid sequence that is formed during translation, this will affect the protein that is formed and its function. Mutations may occur **spontaneously** or may be **induced** by external agents or **mutagens** such as radiation, some viruses and some chemicals. A low rate of mutation is a normal part of cell division.
The mutation changes the protein

CAG codes for the amino acid glutamine. During protein synthesis the HD IT15 gene is transcribed into mRNA and translated into amino acids, forming a polypeptide chain. The CAG repeats create a polyglutamate section within the polypeptide (Fig. 3). The final stage of protein synthesis is folding. The polypeptide chain folds up on itself to form the normal huntingtin protein which interacts with other proteins in the brain to support healthy brain cells and brain function.

The mutated IT15 allele that causes HD has more trinucleotide repeats than the normal allele. Carriers of the affected allele with 36 to 40 repeats may develop the disease; above 40, they definitely will develop the disease if they live long enough. The protein transcribed from the mutated allele has extra long polyglutamate chains. When these chains fold, they alter the shape of the protein, which in turns alters its function. It is these long chains that appear to do so much damage to the brain.

Fig. 3. Synthesis of the huntingtin protein
The increase in the number of CAG repeats creates an increase in the number of glutamines, affecting the folding of the protein. This changes the tertiary structure of the protein which alters the way the protein functions in the brain.
Inside the brain

The brain is a part of the central nervous system, connected to the rest of the body via the spinal cord which in turn connected to all parts of the body via the peripheral nervous system (Fig. 4). Understanding the structure of the brain and how it functions is essential if scientists are to understand the effect of HD on the brain and work out how to reverse that effect.

Brain tissue is made up of two types of cells; neurones or nerve cells, and glial cells. The glial cells have structural and support functions while the neurones are specialist cells that transmit information. With ten thousand million \(10^{10}\) neurones or nerve cells inside the brain, and the potential for each of these nerve cells to be in contact with up to 1000 other cells, the communication potential is immense.

Neurones consist of dendrites, a cell body and an axon (Fig. 5) which transmit signals carried as electrical impulses throughout the body. The dendrites receive information and the axon is used to transmit information. Glial cells provide an insulating cover around each axon called a myelin sheath (a bit like the plastic insulation that you see on electrical wiring) which helps send the electrical impulses along the axons. Glial cells also provide nutritional and physical support for the neurones.

The brain is organised into different regions (Fig. 6). The cerebral cortex makes up the outer layer of the brain. It has special regions for processing information relating to the senses (vision, smell, hearing, touch and taste) movement, language, emotion, etc. It is divided into two hemispheres, each with different functions. The left hemisphere controls the right side of the body and speech. The right hemisphere controls the left side of the body and spatial perceptions.

Deep inside the brain is a structure called the basal ganglia. Understanding this structure is important to understanding HD as it has a role in organising motor (muscle movement) and mood functions of the brain.

The cerebellum sits at the back of the brain. The cerebellum controls balance and coordination and is where learned movements are stored.
The effect of Huntington's disease inside the brain

The mutated huntingtin protein causes neurones in the brain to malfunction and then die. The first target is always the striatum, a part of the basal ganglia. When the brains of patients with HD are examined after death, the striatum and its projections are severely atrophied (Fig. 7). As the disease progresses, other areas of the brain are also ravaged and in the end no brain structure is completely spared. As an area is damaged, symptoms associated with the function of that area appear.

![Diagram of brain regions](image)

Fig. 7. Section through the forebrain of a normal brain (A) and a brain of a HD patient (B) showing major shrinkage of the basal ganglia and cortex in the HD brain.

The striatum is part of the basal ganglia. It is responsible for planning, habit-learning and modulating movement (often inhibiting it – hence the loss of its neurones leaving uninhibited, inappropriate movement). It has links to mood neurones, with mood disturbance being characteristic of the disease.

The normal huntingtin protein is important for maintaining brain cells in good health, and does this in a number of ways. It is essential for proper cell division and programmed cell death (a normal part of the life cycle of cells). Although it comes from a single gene, it interacts with many other proteins inside the brain, including those involved in transcription, cell signalling and intra-cellular transportation. The myriad interactions of huntingtin make a single cure more difficult to find.

The damage and death of brain cells is caused by the extra-long polyglutamate chains of the mutant huntingtin protein, which break off easily. Glutamine is a ‘charged’ molecule, and an excess of it causes proteins to link and clump instead of taking their normal folded form. The tangled masses that result are called protein aggregates. Both huntingtin and the proteins it interacts with – all of which are essential to brain cell health – become caught up in the aggregates. As a result, these valuable proteins not only lose the ability to carry out their proper functions, but also become toxic to brain cells, resulting in loss of brain tissue (see Fig. 7). The extent of brain cell death increases with the number of CAG repeats, which explains the earlier onset of the disease with longer CAG repeats.
How do we know all this?

HD is named after George Huntington who published a paper in 1872 comprehensively describing the disease, including its pattern of inheritance. When Mendel’s work was rediscovered in 1900, people recognised that the disease was following a Mendelian dominant pattern of inheritance.

Beginning in the 1960s, relatives of people with HD launched various organisations aimed at combating the disease. They fundraised and provided avenues for the co-operative exchange of materials and ideas between different groups of scientists.

An intense effort to discover the at-fault gene began in the early 1980s. The researchers found large families with HD, each emanating from a single individual who had the disease, and established detailed pedigrees. Using their blood samples, gene mappers found its location – on chromosome 4 – surprisingly quickly, and it was the first genetic disease to be mapped to a chromosome without any prior knowledge of its location.

In 1993 the mutation was identified and the gene was cloned. This has opened up new options for people from HD families. Previously they had to wait until after death for a diagnosis: now they can be tested to find out whether they carried the mutation before they become ill.

Genetic testing for Huntington’s disease

Strict conditions surround genetic testing. In New Zealand only people over 18 years of age are eligible, and they must be counselled before and after the test. Less than five percent of potential carriers elect to be tested, mostly because there is no cure.

Pre-implantation genetic diagnosis – where embryos created by IVF can be tested before a genetic disease before being implanted, is also available, but in New Zealand it is publically funded only in a limited way, and usually if the at-risk parent has been tested first. Fetuses can also be tested, giving parents the option to terminate the pregnancy if the baby is found to be a carrier.

Genetic fitness

HD is found in about 5–7 individuals per 100,000, although there are rare areas with a much higher prevalence. Amongst Asian and African peoples the prevalence is much lower; in Japan, for example, it is only 0.5 per 100,000.

There is no evidence for the HD mutation conferring any fitness on carriers: in other words, on average they have the same number of children as non-carriers. A mutated IT15 gene does nothing helpful. However, because its deleterious effects generally appear after carriers have had their children, it survives. Mutations whose effects take hold after child-bearing age have quite different patterns of allele survival to mutations whose effects appear earlier in life.

Some gene mutations have no effect at all on which amino acid is transcribed, so are silent, having no observable effect on the phenotype. Some mutations, however, do offer a selective advantage to their carriers. Sickle cell anaemia is one example of this. People who are heterozygous for the gene that makes their red blood cells sickle-shaped are more resistant to the effects of malaria. The sickle cell mutation is therefore relatively common in tropical countries. This selective advantage increases the likelihood of the allele surviving in the population. No such selective advantage can be identified for the Huntingtin allele.

Apart from racial heritage, the only factor that is identifiable in the patterns of Huntingtin alleles in different populations is that of founder effect. Where a population has established from a small gene pool in which the mutated gene was present, it is found in higher numbers in that population. One such population is found in Tasmania.
One gene mutation — but a variable disease

Once, it was hoped that the clear single-gene nature of HD would mean that a cure would be reasonably achievable, and that the knowledge gained would help combat other diseases. However, it is not as simple as it once seemed: although it is a single gene mutation, variation is present in both the genotype and the phenotype of HD. Understanding this variation is an important part of the process of finding answers in the HD puzzle.

In some cases, variation in the phenotype can be linked to variation in the genotype. This forms predictable patterns. In other cases, variation in the phenotype bears no relationship to variation in the genotype. When this happens scientists need to look for alternative reasons for this variation.

**Phenotype variation caused by genotype variation**

People with HD do not all have the same number of CAG repeats in their gene. The differing number of repeats is linked to two types of variation that are seen in the disease symptoms (the phenotype): the onset of disease and how quickly it progresses.

One aspect of phenotype variation that can be partially explained by genotype variation is the age of onset of the disease. Figure 8 shows that the **longer** the length of the chain of CAG repeats in the gene, the **earlier** the age of disease onset. When the chain is long enough, the disease manifests in children as young as one year of age. (In children the disease has different features: lack of movement, a rigid body and seizures). Sixty percent of the variability in age of onset is explained by length of CAG repeat, with other unknown factors contributing to the remaining 40%.

![Effect of number of CAG repeats on age of onset of Huntington's disease](image)

**Fig. 8.** Effect of increasing number of CAG repeats on age of onset of HD

Redrawn with permission from Brinkman RR, Mezei MM, Theilmann J, Almqvist E, Hayden MR. The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. Am J Hum Genet 1997; 60: 1202–1210

The age of onset of the disease can decrease with each successive generation. This can be accounted for by known instability of the IT15 gene in the testicles (where sperm are formed). In cases of 28 CAG repeats or more, the number of repeats is prone to increase during sperm formation. In this way, when genes are passed through the generations by males, chain length tends to increase. Therefore, in successive generations, the age of onset becomes increasingly younger. This is known as **genetic anticipation**.

This instability of the gene in the testicles also accounts for the rare spontaneous mutations. After some testicular hot housing, a previously normal but borderline number of 35 repeats can increase sufficiently to cause HD. People from white European races have higher frequency of huntingtin alleles with 28–35 repeats, and are therefore more vulnerable to instability during spermatogenesis. If the mutation occurs during spermatogenesis, it is a new or **de novo mutation**.
**Phenotype variation that cannot be explained by genotype variation**

Although brain cell death in HD follows a reasonably typical path, it shows significant **phenotypic variability**. Some people experience major motor (movement) symptoms, and yet their mental faculties survive remarkably well. Others have the opposite experience.

In 2010, a research team primarily from The University of Auckland, published a study using brains from the human brain bank. They took sections from 12 HD brains, about a third of whom had mainly motor symptoms and a third of whom had mainly mood symptoms, as defined by a psychologist after interviewing the families of the subjects. They also took sections from 15 normal, or **control** brains.

The team looked at the brains’ cerebral cortex, which has strong anatomical connections with the previously-mentioned striatum of the basal ganglia. They found that people with mainly motor symptoms had more damaged and lost cells in the primary motor cortex, which is the part of cerebral cortex responsible for movement. Those with mainly mood and cognitive symptoms had more cell loss in their anterior cingulate cortex, which plays an important role in mood and cognitive impairment (Fig. 9).

The researchers looked for a genetic cause for this difference by checking whether there was an association with CAG repeat, but none was found. They **concluded that the variability in symptoms is caused by the extent of cell loss in the corresponding functional regions of the cerebral cortex.**

**Human Brain Bank**

Since 1993, a very special facility called the Neurological Foundation Human Brain Bank has existed in Auckland. It holds tissue from over 400 brains, each of which is matched with a detailed history of the donor. The brains come from people who have died of brain diseases, including HD, and brains from healthy donors are also held for the purposes of comparison.

Each of the donated brains is carefully examined for signs of disease, and donors’ families are informed of the findings.

These brains provide the raw material for much of the work done by research teams in the Centre for Brain Research. Using sections from the brains, they can carry out a vast number of investigations into the disease’s effects on the brain. This is work that without the support of the patients and their families could never happen.

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**Graph showing profiles of brain tissue loss in three different groups of Huntington's disease patients.**

![Graph showing profiles of brain tissue loss in three different groups of Huntington's disease patients.](image)

Fig. 9. The symptom profile of HD patients is associated with the area in the brain where they have experienced the most tissue damage.

Epigenetics – a field of scientific exploration

The genome is the sum of all the genetic information for an individual. A copy of the entire genome is contained in the nucleus of every cell, yet only those genes required by a particular cell are turned on, or expressed in that cell. A system of controls ensures that genes are only expressed when they’re required.

Gene expression is the term used to describe the process of taking the information that is contained in the genes (the genotype), and using it to build proteins, which create the phenotype of the individual. However, the phenotype is not simply determined by the genotype. Interactions between genes and the environment impact on gene expression, and therefore on phenotype.

The epigenome sits “above the genome” and has a role in determining how messages from the environment can impact on which genes are turned on or off in a cell. The epigenome consists of chemical markers or ‘tags’ that control which genes are active, and therefore which proteins are produced in a particular cell at a particular time. While the genome does not change during a lifetime (other than through mutation), the chemical markers of the epigenome can change as the environment of the individual changes.

To understand epigenetics we need to look at how the DNA is packaged within the chromosome. There are two main ways in which the epigenome can influence which genes are turned on and off; DNA methylation and histone modification (Fig. 10). In both cases the epigenetic change alters the packaging of the DNA. If the effect makes the packaging become very tight, the gene cannot be read and will be turned off or ‘silenced’. If the epigenetic effect loosens up the packaging of the DNA, the gene will be turned on and the protein synthesised (which is potentially as bad as turning a gene off).

There is evidence that HD may involve alterations in epigenetic processes. This mainly comes from cell culture and in vivo studies showing that mutant HD allele can inactivate some essential proteins which have histone modification properties, leading to changes in the histone tails and changes in gene expression. However, whether there is a reduction in histone modification in human HD is not clear, so this remains an hypothesis.
Animal models

By studying donated brains from the Brain Bank, Professor Faull’s team have shown that neurons containing γ-aminobutyric acid (GABA), a neurotransmitter that acts as a signalling chemical between neurones, are selectively lost in the striatum of the basal ganglia of humans with HD. Unfortunately, it is not possible to treat patients with GABA because of the effect this treatment would have on the rest of the brain.

While investigating the disease in humans is crucial, the use of animal models has also become an important tool for discovering information about many aspects of HD. These animals (e.g. transgenic mice, fruit flies and nematodes) can be manipulated far more easily than humans, progress more quickly to show the symptoms of HD and have a shorter life-spans; therefore these models have a huge advantage over the study of humans as it can speed up the rate of research and information gathering. In addition, the brains of these animals can be accessed early, rather than having to wait until death occurs naturally. Animal models are being used to search for potential drug treatments, with a hope that the outcomes can be translatable to the human disease.

Professor Faull’s team use rats and induce HD by injecting the toxin quinolinic acid into the basal ganglia region of the brain. This toxin is present at higher than normal concentrations in the brains of humans with HD and induces similar symptoms in rats. In addition, the team have transplanted basal ganglia brain cells from a fetal rat into diseased adult rats and found that these cells survive and make new non-diseased GABA-like brain cells. There is hope that the transplantation of fetal brain cells may one day help humans with HD. A similar treatment for Parkinson’s disease has already been trialled in which cells producing the required neurotransmitter dopamine were transferred. Unfortunately the treatment was only partially successful as it resulted in tumour formation in some cases.

While some discoveries in animal models can be directly applied to humans, scientists are always very aware that in many cases this is not possible. Tissue culture studies are another tool to extend our understanding. The cells in tissue culture are isolated – rather than in a whole organisms – and investigating these cells in isolation can reduce the ‘noise’ associated with studying the full organism. However, these cells may behave differently when they are isolated from the whole organism, therefore information gathered from isolated cells need to be further investigated with a view to the whole organism.

While no one tool offers all the answers, it must be kept in mind that all these tools play an important part in extending our understanding of the disease and will eventually enable scientists to finding a cure for HD.
The brain’s regenerative potential

Scientists have now disproved the long-held belief that humans are born with all the brain cells they will ever have. They now know that the diseased adult human brain tries to repair itself by making new replacement brain cells, in a process is called neurogenesis.

In 2003, University of Auckland scientists published a paper showing that the brains of HD patients had been trying to regenerate. Using the Brain Bank, they took sections of normal and HD brains, and showed that there was more cell proliferation and growth in the diseased brains than in the normal brains. Their current hypothesis is that the markers of cell proliferation and growth that they discovered are due to the brain trying to grow new cells to compensate for the destroyed ones. They are hopeful that it might one day be possible to treat brain diseases like HD by augmenting this normal response.

Stem cells: a hope

The cells responsible for the cell proliferation and growth found in adult brains (and more so in HD brains) are neural stem cells, a form of an adult stem cell.

Stem cells differ from other cells in that when they go through cell division they can produce undifferentiated cells or, under the right conditions, they have the potential to differentiate to form specialised cells, with a specific function, e.g. muscle cells, skin cells, nerve cells. It is important to distinguish between embryonic stem cells and adult stem cells (Fig. 11).

Embryonic stem cells
Every cell in a zygote – the group of cells that forms soon after fertilisation – is totipotent, having the ability to form all types of cells.

Embryonic germ cells
The inner cells in a blastocyst – the next step on from a zygote – are pluripotent, having the ability to form all cell types in the body except the placenta (the outer cells form the placenta).

Adult stem cells
These are undifferentiated cells that are yet to specialise and are found in infants, children and adults. They can only produce a limited number of cell types specific to the tissue they’re found in, and are called multipotent. Tissues that have been found to contain adult stem cells include the brain, bone marrow, skeletal muscle and skin.
Embryonic stem cells, therefore, have the most flexibility. However, they are associated with major ethical challenges, because of the sources of these cells — either an embryo that is excess from IVF (which is very controversial) or more simply they can be extracted from cord blood when a child is born.

In theory adult stem cells can be extracted from an adult, grown and replaced into the same person, which avoids the ethical problems of embryonic cells. Replacing stem cells into the person they came from also avoids problems with rejection by the immune system when it recognises foreign cells, an issue that would be present in embryonic stem cell therapy other than from cord blood.

All stem cells have a ‘homing’ ability, enabling them to migrate to the area where new cells are needed. In 2007, a paper published in one of the world’s most prestigious journals, Science, first showed the route that progenitor cells stream through in the human brain. This research was led by University of Auckland scientists.

Most stem cell lines grown in labs around the world for the purposes of research are embryonic stem cells. Scientists from the Centre of Brain Research are growing stem cells cultivated from brains donated to the human brain bank, both normal and diseased. They are using them to examine things such as whether adult stem cells from HD brains are different from those in the non-diseased brains and whether they have the potential to form significant numbers of “good” new brain cells to slow (or even halt) the process of brain cell degeneration.

The potential to replace destroyed brain cells using stem cells is very exciting, but there are still many challenges to overcome. Researchers need to discover the precise environment (chemicals, growth factors and more) which will successfully direct the adult stem cells to make just the right type of new brain cells in the right numbers and in the right region of the brain. In particular, one of the dangers of stem cell therapy is the potential for stem cells to multiply uncontrollably, leading to tumours that cause more damage than the original disease.

If you or your family/whanau are affected by Huntington’s disease and you would like to know more or receive support, please contact the Huntington’s Disease Associations of New Zealand (http://www.huntingtons.org.nz)

Further Reading
Your Genes, Your Health. Huntington Disease http://www.ygyh.org/hd/whatisit.htm

For further information contact:
Centre for Brain Research | http://www.cbr.auckland.ac.nz | cbr@auckland.ac.nz
LENScience | http://lens.auckland.ac.nz | LENSscience@auckland.ac.nz
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