MND Factsheet 26
Current Research Areas

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The motor nerves of people affected by MND show a range of different changes and the changes which are seen at any one time may vary according to the stage of the disease affecting the particular nerve. The challenge for scientists investigating these has been to understand both their significance within the disease process and their underlying causes.

In an effort to understand these issues researchers use a variety of models which can be manipulated in a way that would be impossible in living patients. These models include mice, fruit flies, fish and yeasts as well as cell cultures grown from the cells of people affected by MND.

These models, and clinical observations, have shown that the main changes encountered in the disease process are varied and include changes to, or disruption of, processes such as those controlling inflammation, mitochondrial functioning, cellular transport, protein metabolism, RNA metabolism, axonal outgrowth, cellular signalling, and oxidative stress.

At the cellular level each of these processes would normally be controlled by genes being switched on or off at the appropriate times. Sometimes a gene may be switched on to make something a cell needs; sometimes groups of genes may be switched on to make a whole series of things that can be assembled into more complex structures required to maintain or grow the cell; and at other times genes that make products which can break things down are switched on if there is too much of something or it is no longer required.

In 1993 the "Copper-Zinc Superoxide-dismutase 1" (SOD1) gene was identified and confirmed to be associated with inherited or familial ALS (FALS). Estimates from various sources suggest SOD1 accounts for around one in five familial cases. It took more than a decade for any other significant genes related to this form of ALS to be identified.

Since then, with improvements in analytical techniques, knowledge of genes, RNA and DNA has grown exponentially. However, almost 25 years after the confirmation of SOD 1 as a FALS gene, in the UK the cause of inherited ALS in between a quarter and a third of familial cases is still unexplained.

Most people affected by ALS have no family history of the disease yet a substantial number, one authority sets this figure at more than a quarter, have mutations to the same genes that are found in FALS.

When motor neurones damaged by MND are investigated a number of different pathologies are seen, suggesting that all cases of MND do not result from only one common disease process but from any of several possible disease processes.

Biological processes usually involve series, or chains, of controlled chemical reactions which take place in sequence.

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The products of the genes involved in MND are probably links in different biological chains which, if any single process is interrupted, eventually causes the whole chain to fail and results in the death of the affected motor neurones.

The following list identifies some areas of motor neurone function that are thought to be disrupted in ALS and the genes thought to be associated with that disruption when mutated.

RNA metabolism
C9orf72
TARDBP (TAR DNA-binding protein)
FUS
hnRNPA1
hnRNPA2B1
SETX
TAF15
ANG

Antioxidant
SOD1

Cellular transport
ALS2
VAPB

Protein metabolism
SQSTM1
VCP
UBQLN2
OPTN

Axonal outgrowth
PFN1

Glutamatergic signalling
DAO

Genes that appear to modify ALS risk or progression

RNA metabolism
ATXN2

Cytoskeletal protein
NEFH

Protein metabolism
GRN

Angiogenesis
VEGF

Neurotransmission
UNC13A

As a result of discovering so many genes that can either independently or in conjunction with others cause ALS when mutated, many scientists are now questioning whether MND is one disease or many different diseases.

Free Radicals and Oxidative Stress
All cells generate toxic metabolites (think of these as the "exhaust" from your car and the consequences to yourself and your passengers should your car not be properly ventilated). In the cell, these "exhaust products" are carbon dioxide, water and sometimes "free radicals."

Free radicals in this context are a destructive form of oxygen that can also be used by the cell under normal circumstances to fight disease. However,

an overproduction of free oxygen radicals can result in cell damage and death. As might be expected, several defences against such a process exist. A major one is the superoxide dismutase enzyme (SOD-1) discussed elsewhere.

Mutations in the SOD-1 gene could allow excessive free radical accumulation in the cell, damaging the neuron. Researchers have documented the existence of excessive levels of proteins damaged by free oxygen radicals within neurons in ALS, suggesting that either the neuron synthesises excessive levels of free radicals or it is incapable of disposing of those that are normally produced.

Therapies geared to reducing oxidative stress are in development, including gene therapy and new drugs. The properties of certain foods and vitamins are also studied for their ability to reduce free radicals. Vitamin E's antioxidant properties made it the prescribed therapy for people with MND one hundred years ago and it is still in use today, although with little apparent effect in hindering the course of the disease.

In recent years, researchers have considered how injured motor neurons might spur an immune response that could contribute to, and even perpetuate, a cascade of cell death in the nervous system. The nervous system's immune cells, called microglial cells and astrocytes, can respond to an injured neuron in a way that can be either beneficial or harmful. This response by microglial cells has been implicated as a trigger of programmed cell death (PCD), a mechanism that is useful on the small scale to clear away damaged cells, but devastating on a large scale as it ripples through the nervous system killing motor neurons.

Neurotrophic Factors

These important chemicals help in the growth and maintenance of motor neurons and have been shown to enhance motor neuron survival in mice with a variety of motor neuron disorders. Although it is not clear how deficiencies of neurotrophic factors may affect human motor neurons, several attempts have been made to determine whether neurotrophic factors can slow the rate of progression of MND by first testing these agents in animal models of the disease.

Researchers are also investigating how the neurotrophic factors including brain derived neurotrophic factor (BDNF) and "cytokine ciliary neurotrophic factor" (CNTF) interact with metal ions in cells, and how metal ions can thereby have enhanced toxic effects in a cell depending on the neurotrophic factors present. By developing drugs to manipulate the interactions of neurotrophic factors, researchers hope to learn about and gain some control over this potentially useful aspect of the disease process.

Gene therapies are also in development to promote the levels of beneficial neurotrophic factors. The gene for insulin-like growth factor 1 (IGF-1) was successfully delivered in ALS mice using a viral vector, with the successful result of prolonging the life of the mice.

Viral Vectors

Viruses are parasites which reproduce themselves by hijacking the reproductive mechanisms of a host cell to manufacture virus particles. They do this by injecting their own DNA into the cells of the host organism where the viral DNA inserts itself into the DNA of the host cell. Once the viral DNA is in place it releases signals which cause the host cell's reproductive apparatus to copy the viral

instructions over an over again making more and more virus particles. In the case of the virus responsible for cold-sores (Herpes simplex) the host cell makes so many copies of the virus that the host cell swells up until it bursts releasing cell fluid loaded with newly made virus particles.

The ability of viruses to insert their DNA into that of a host cell makes them ideal carriers (vectors) for genes we might want to insert into a healthy cell. In theory a healthy copy of a human gene is inserted into the DNA of a harmless virus so that when the virus DNA inserts itself into the host DNA the healthy copy of the human gene is used.

There are dangers in using viral DNA as a vector in this way. A number of genes are known to make biochemicals which prevent the growth and proliferation of tumours in the healthy body. The dangers come from the possibility that the vector might insert itself into any one of these tumour suppressor genes and consequently switch it off, allowing the infected cell to become cancerous. Amongst other areas viral vector research is also looking at ways of directing where such vectors are inserted into human DNA to try to reduce these dangers.
Recent tests, in which a virus was used to carry a length of genetic material into the cells of mice carrying a mutated SOD1 gene, with the intention of blocking the product of that gene, gave impressive extensions to average life expectancy of the mice. This technology is now being evaluated for early human trials.

Altered Protein and Neurofilament Metabolism
Advances in technology in the field of protein research have opened up new avenues into understanding the protein mechanisms involved in MND. Mass spectrometry has become very advanced enabling scientists to detect which proteins are present in an extremely small sample. Mass spectrometry is also providing researchers with the tools to develop a desperately needed simple, rapid test to diagnose MND by identifying proteins generated as a result of the disease. Tests of this kind are undergoing clinical trials at the moment to test how reliable and effective the process actually is. There are hopes that this kind of test can be used as part of the diagnostic process in the future when a small sample of cerebrospinal fluid could be taken and tested for the levels of certain proteins, so indicating whether or not MND is present and possibly which subtype it may be.

Powerful electron microscopy can reveal the configuration and behaviour of proteins. Such technological advances provide insights into how proteins fold and unfold and interact with their environment.

A signature feature of ALS is the accumulation of neurofilaments in the motor neurons. These key neuronal proteins are believed to be responsible for maintaining the normal structure and shape in healthy neurons. Studies making use of transgenic models to alter neurofilament expression reveal that abnormalities in the metabolism of neurofilaments, or the way in which neurofilaments interact with each other - or with other proteins, could play a role in the development of MND.

Many types of cellular proteins and enzymes may play a role in MND. Protein kinases are enzymes that regulate many cellular functions. Studies have revealed that abnormal levels of

protein kinases exist in the nerve tissue of people who died of MND. By comparing this tissue with mouse models, researchers are investigating how abnormal levels of these important regulatory enzymes may trigger cell death in the motor neurons. They hope to find a way to counterbalance the protein kinase ratios and so prevent cell death from being triggered.

Proteosomes and protein chaperones are bodies that shuttle proteins around and can chop them up into their basic components in order to clear away damaged proteins in the cell. Researchers are investigating these enzymes in mouse models and cell lines to understand their role in the pathogenesis of ALS and how they might be used therapeutically to hinder the disease.

By studying the proteins affected by genetic mutations in familial ALS, and the behaviour and interactions of the proteins involved in neurofilament aggregation in cells, researchers are gaining a better understanding of the role of abnormal protein mechanisms in MND.

Glutamate Excitotoxicity
Abnormalities in the handling of excitatory amino acids, particularly glutamate, by the nervous system may be critical to the occurrence of MND. Damage to the normal "transporter" mechanisms, by which glutamate is removed from the nervous system, allows excessive glutamate to accumulate.

When motor neurons receive glutamate at their receptors, there is an influx of calcium ions into the cell. The motor neurons may not be able to deal with the excessive levels of calcium flooding in, resulting in damage.

Researchers are investigating ways to help the nervous system handle calcium and glutamate. Riluzole, the only drug currently available for the treatment of MND, shows very modest results. Its action is not well understood but is thought to perhaps affect glutamate uptake mechanisms in the motor neurone.

Genetic Factors
It is genetic material which ultimately controls the processes of life and when genes and DNA “go wrong,” so these vital processes can also go wrong.

Mistakes can be incorporated into DNA in many different ways, but can happen most often when the DNA is being copied to make new cells or when an existing cell is exposed to chemicals or radiation that can cause changes in genetic material. These changes to genes are described as mutations.

Mutations can take many forms such as one letter of the genetic code being changed to another; stretches of DNA being deleted, moved to a wrong area or duplicated – sometimes many times over; other stretches of DNA being reversed; bits of chromosomes being detached from one and attached to another. There are cellular processes that can silence genetic material that is meant to be active and activate genetic material that is meant to be silent. The word “mutation” encompasses many possible ways in which DNA may be changed.

When DNA is changed, particularly if the DNA is part of a gene, the change can have one of several possible consequences.

Depending on where the mutation occurs the mutation might be harmless and have no effect on the gene's function at all. A similar kind of mutation in another place on the gene might mean that its product may simply no longer do its job, whatever that may be. This is often referred to as loss of function. On the other hand, the change could result in the gene product itself becoming toxic to the cell, which would be a gain of function. Even if the gene loses function it might allow materials it should have processed to build up and poison the cell.

Although more than two dozen genes have been identified as being directly involved in some way in different people with MND; the precise details of their involvement has not been fully discovered for most of them. Some are thought to directly cause MND when they contain a mutation which affects their normal functioning while others are thought to perhaps either promote or inhibit the rate at which the disease progresses.

Current ALS genetic research is working on several fronts. Most obviously to identify which genes are involved in the disease process and what their role is. In some cases, such as ALS caused by the SOD1 gene, inheritance of a single mutated and disease causing gene can result in the person developing ALS.

It is also possible that two or more different mutated genes might need to be inherited in some forms of MND.

Situations like this are more difficult to identify in families since the chances of inheriting both faulty genes from a parent are ½ x ½ which is ¼ or a 1 in 4 chance of inheriting both copies. If three genes are involved the chances of inheriting all three faulty genes are ½ x ½ x ½, or 1 in 8. Four faulty genes would be 1 in 16 and so on.
Relatively low risks of inheriting all the faulty genes like this would make a familial form passed on this way very difficult to identify when the average family size is between two and three children and may lie at the root of many supposedly sporadic ALS cases.

Identifying the genes responsible when people are unrelated, such as in sporadic ALS, is even more difficult, and it is for this reason that a number of studies, including the MND Scotland Register, are collecting genetic material from as many people as possible.

The theory is that when the DNA of many unrelated people, who all have a condition like ALS, is compared with the DNA of people who do not have the condition then mutations to genes associated with ALS will be much more common in the group with the disease than in the group who do not have the disease. By looking for genes where the number of mutations is greater in the ALS group than in the group who don't have ALS there is a greater chance that the genes concerned have some part to play in ALS.

The more people included in the studies the greater the chances that rare and previously unknown mutations will be represented in numbers sufficiently large to stand out and be noticed. One study published in 2016 identified 4 genes that had not previously been linked to ALS, however to do so they screened the DNA of more than 15,000 people with ALS and 26,000 controls.

In the year 2000 such large scale studies involving tens of thousands of people would have been unthinkable due to the

time and costs involved, but new technologies and techniques that have evolved since then have both reduced the time taken to decode someone's DNA and made it much more affordable.

In 2016 a person's complete DNA can be read in a matter of days at a cost of around £800. Compare that to decoding the first complete human DNA which had taken 9 years and cost £2 billion in the last decade of the 20th century. This reduction in time and costs has opened new areas not only for research, but also for medicine.

Non-coding RNAs

Most people with some knowledge of biology will be aware of the standard description that when a gene is active the double helix of DNA "unzips" and the sense strand of DNA is transcribed into a single strand of messenger RNA (mRNA). The single stranded mRNA is read by a structure called a Ribosome and a type of RNA known as transfer RNA (tRNA) brings amino acids to the ribosome to be assembled into a protein.

Some may be aware that not everything coded within a gene is translated into the final protein product. A gene has a short, special, code at the beginning which indicates the start point and another which indicates the end. Between these two points there are alternating blocks of material known as "introns" and "exons" which make up the body of the gene. When the messenger RNA strand is transcribed from the genetic DNA those parts of the mRNA that are derived from the introns are spliced out of the strand and only the mRNA from the exons is read by the ribosome and translated into the protein that is the gene's product.

Since they are not translated into proteins the RNA derived from the introns, which is spliced out from the mRNA strand, the RNA which makes up about 60% of the ribosome and the tRNA are all known as non-coding RNA. Since the 1980s our knowledge of non-coding RNA (ncRNA) has increased and many sub-types of non-coding RNA are now recognised.

With the discovery of the C9orf72* molecule, which is described as a non-coding region, (i.e. it can be transcribed into an RNA molecule which is not translated into a protein) the role of ncRNA's in MND, and in ALS in particular, took on an added significance. In Finland, for example, mutations to the C9orf72 product account for over 40% of familial ALS cases. More recently a new non-coding region at C21orf2* which interacts with the NEK1 gene product has been discovered. (*These names are geneticists' code for the Chromosome number, in these examples C9 and C21, and the position on the chromosome of the "open reading frame" in these examples, position 72 and position 2 respectively.)

The number of types of ncRNAs coded for in human DNA is unknown; however, recent studies suggest the existence of thousands of ncRNAs. The expression of many thousands of genes are regulated by ncRNAs and there is increasing evidence that a special type of ncRNAs called enhancer RNAs, transcribed from the enhancer region of a gene, act to promote gene expression. As with proteins, mutations or imbalances in the ncRNA repertoire within the body can cause a variety of diseases and it is probable that this class of disorder will become increasingly important in understanding MND and ALS in particular.

Many ncRNAs show abnormal expression patterns in cancerous tissues as some types are involved in the large scale regulation of many protein coding genes while others are important for initiating DNA replication. A study of 17 micro RNAs (a particular kind of non-coding RNA) that have been predicted to regulate a number of breast cancer associated genes found variations in two of them in particular amongst patients; these patients did not have mutations to their BRCA1 or BRCA2 genes, suggesting the possibility that some familial breast cancers may be caused by variation in these miRNAs. This mechanism is a plausible explanation of the 1/3 of familial ALS cases for which a gene is unknown and the ¾ of sporadic cases where the cause is unknown.

Medicine and the Future

For more than a century it was assumed that MND was three distinct conditions that affected upper motor neurones only (Primary Lateral Sclerosis), lower motor neurones only (Progressive Muscular Atrophy), or both upper and lower motor neurones (ALS).

In the past drug trials treated each of these as three separate conditions and when drugs which worked to prolong the lives of experimental mice were tested on humans with ALS the researchers were generally unable to identify any benefits for the trial participants. It is now obvious from the genetic data that there are different forms of ALS and each one will have its own treatment or possibly treatments, for example:

- ALS caused by either the SOD1 gene or the VAPB gene is not associated with dementia, whereas C9orf72 ALS is;
- ALS caused by the FUS gene, like the SOD1 gene, does not cause deposits of the TDP-43 protein in cells of the spinal cord, whereas the disease associated with most other genes is associated with these deposits.
- Deposits of the Ubiquilin 2 protein are only found when the Ubiquilin2 gene is mutated and is the cause of the disease.

How many sub-types of ALS exist will not be known until all of the genes and non-coding RNAs involved in ALS have been identified and their disease causing mechanisms unravelled. However, it is anticipated that in the near future people with complex diseases such as ALS will be offered genetic tests to identify which of their ALS-associated genes or ncRNA are mutated. The treatment the person receives (once treatments have been developed) will be prescribed not only by what is mutated, but by the nature of the mutation. In effect, ALS will require individual or personalised medicine.

Developments of this kind, known as “Stratified Medicine,” are already an important tool in the fight against cancer where the location of the cancer is only part of the information required in order to know how best to treat it. In many cases the exact genetic mutations now dictate which chemotherapy will be given. It seems probable we will see similar developments in the treatments for MND.

Further Reading

Factsheet 16	Stem Cells
Factsheet 18	Clinical Trials

Factsheet 22 Riluzole