Autosomal Dominant Leukodystrophy with Autonomic Symptoms and Rippling Muscle Disease

Translational Studies of Two Neurogenetic Diseases

JIMMY SUNDBLOM
Abstract


There is a large variety of diseases caused by single-gene mutations. Although most of these conditions are rare, together they impose a significant burden to the population. This thesis describes clinical and genetic studies of two single-gene diseases:

1) Adult-onset autosomal dominant leukodystrophy with autonomic symptoms (ADLD) caused by LMNB1 gene duplications, and characterized by autonomic, pyramidal and cerebellar symptoms. Spinal cords of patients with ADLD were studied by MRI and found to be thin, with high signal intensity in white matter. Histopathology showed loss of myelinated fibres with some reactive gliosis. DNA samples from four different families with ADLD were obtained, and the LMNB1 gene was screened for duplications. Single nucleotide polymorphism array revealed LMNB1 duplications in all ADLD families. LMNB1 mRNA and protein levels were assessed in white blood cells using quantitative polymerase chain reaction and Western blot, and increased levels of LMNB1 mRNA and lamin B1 protein could be demonstrated. We concluded that spinal cord atrophy in patients with ADLD is a valuable differential diagnostic sign, and that increased levels of LMNB1 can be detected in peripheral blood.

2) Rippling muscle disease (RMD) is caused by CAV3 gene mutations. Clinical features are percussion-induced muscle mounding, rapid contractions and undulating muscle contractions (rippling). The CAV3 gene was sequenced in 38 members of a family with RMD. Twenty-two individuals had clinical features of RMD. No muscle weakness was seen. All patients with signs of RMD carried the p.A46T CAV3 mutation, showing that the p.A46T mutation was benign and that the diagnosis can be made clinically. In vitro contracture test results from 10 of the subjects were collected, but no association between pathological test results and RMD was found.

Keywords: Inborn genetic diseases, Leukoencephalopathies, Lamin type B, Muscular disease, Caveolin 3

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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<tr>
<td>ADLD</td>
<td>Adult-onset autosomal dominant leukodystrophy with autonomic symptoms</td>
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<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
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<tr>
<td>BACT</td>
<td>beta-actin</td>
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<td>CADASIL</td>
<td>Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy</td>
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<td>CCD</td>
<td>Central core disease</td>
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<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
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<tr>
<td>CK</td>
<td>Creatine kinase</td>
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<tr>
<td>CNV</td>
<td>Copy number variation</td>
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<td>CT</td>
<td>Computerized tomography</td>
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<td>DHPR</td>
<td>Dihydropyridine receptor</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>ECC</td>
<td>Excitation-coupled contraction</td>
</tr>
<tr>
<td>ECCE</td>
<td>Excitation-coupled Ca(^{2+})-entry</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
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<td>Electroencephalogram</td>
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<tr>
<td>EMG</td>
<td>Electromyogram</td>
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<tr>
<td>FD</td>
<td>Familial dysautonomia</td>
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<td>HD</td>
<td>Huntington's disease</td>
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<td>HDAC</td>
<td>Histone deacetylase</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<tr>
<td>iPSC</td>
<td>Induced pluripotent stem cells</td>
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<td>IVCT</td>
<td>In vitro contracture test</td>
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<tr>
<td>kb</td>
<td>kilobase</td>
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<tr>
<td>LGMD</td>
<td>Limb-girdle muscle dystrophy</td>
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<tr>
<td>LOD</td>
<td>Logarithm of the odds</td>
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<tr>
<td>L-MAG</td>
<td>Myelin-associated glycoprotein, large isoform</td>
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<tr>
<td>MBP</td>
<td>Myelin basic protein</td>
</tr>
<tr>
<td>MERFF</td>
<td>Myoclonic epilepsy with ragged-red fibers</td>
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<tr>
<td>MG</td>
<td>Myasthenia gravis</td>
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<td>MmCD</td>
<td>Multi-minicore disease</td>
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<td>MRI</td>
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<td>MS</td>
<td>Multiple sclerosis</td>
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<td>MSA</td>
<td>Multiple system atrophy</td>
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<td>MUP</td>
<td>Motor unit potential</td>
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<td>N-CAM</td>
<td>Neural cell adhesion molecule</td>
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<td>OH</td>
<td>Orthostatic hypotension</td>
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<td>OMIM</td>
<td>Online Mendelian inheritance in Man</td>
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<td>PAF</td>
<td>Pure autonomic failure</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PMD</td>
<td>Pelizaeus-Merzebacher disease</td>
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<td>PIMM</td>
<td>Percussion-induced muscle mounding</td>
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<td>Ribonucleic acid</td>
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<td>Ryanodine receptor</td>
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<td>SCA</td>
<td>Spinocerebellar ataxia</td>
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<tr>
<td>SEP</td>
<td>Sensory evoked potential</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
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<tr>
<td>SI</td>
<td>Signal intensity</td>
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<tr>
<td>VEP</td>
<td>Visual evoked potential</td>
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Twin Stars of happy omen, named Releasers, have gone up.  
May they  
Loose, of inherited disease, the uppermost and lowest bond.  
Vanish this Night, extinct in Dawn! Let those who weave their spells depart.  
So let the plague-destroying Plant remove inherited disease.

Excerpt from a charm against hereditary disease

Hymns of the Atharva Veda, (1200-150 BC).

The Atharva Veda is the fourth Samhita in the Vedas, ancient sacred texts from the Indian subcontinent.
Introduction

“Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature by careful investigation of cases of rarer forms of disease. For it has been found, in almost all things, that what they contain of useful or applicable nature is hardly perceived unless we are deprived of them, or they become deranged in some way.”

William Harvey, 1652

Here follows a thesis concerning two neurogenetic diseases: adult-onset autosomal dominant leukodystrophy with autonomic symptoms (ADLD) and rippling muscle disease (RMD).

Neurogenetic diseases are diseases of the brain, nervous system and muscle that are inherited in a Mendelian fashion – that is, they are monogenic, caused by mutations in a single gene which can be transmitted through the germline in a dominant or a recessive pattern, autosomal or X-linked.

The obvious reason for studying these disorders is to further our understanding of the diseases in question, in order to improve clinical diagnostics and hopefully to find better treatments. The other, less obvious, rationale is to use the knowledge we gain from studies of these diseases to broaden our understanding of the normal functions of the genes involved. This may help us in finding treatment options and biomarkers for other similar disorders, not necessarily hereditary ones.

Genetic diseases are as a group a large burden on health worldwide. WHO estimates put the incidence of single gene diseases from birth to age 30 in a typical developed country at 7/1,000 births\(^1\). This figure includes neurogenetic disease, but also the more common “non-neurological” genetic diseases such as hemochromatosis, cystic fibrosis (CF) and thalassemia. Exact figures
for neurogenetic disease are difficult to find, as the spectrum is very broad, from very severe diseases apparent at birth to adult-onset disorders. It must be kept in mind that although a single disorder may be very rare, perhaps only described in a few families worldwide, this group of diseases collectively constitute a large burden on the health care system, and a larger one yet on the families concerned.

I will initially provide a background and history of genetics and medical applications before introducing some concepts of neurogenetics - not focusing on any specific diseases but highlighting some important points. Short remarks on ADLD and RMD will follow before the main part of this thesis, which intends to describe certain clinical features of ADLD and RMD as well as discuss possible implications for basic research into muscle physiology and nervous system development and degeneration.
Background and history

“This memoir, very beautiful for its time, has been misunderstood and then forgotten.”
Hugo de Vries, 1900

Hereditary disease has been acknowledged since ancient times. In the Jewish scripture Talmud (additions from around 200 AD) and in the Kitab al-Tasrif by the Arab physician Albuqasis (936-1013 AD), accounts concerning haemophilia emphasizing certain facts about the condition, such as it only affecting males, can be found. Also, more generally, there is a hymn in the sanskrit scripture Atharva Veda (1200-150 BC), read in order to protect against hereditary disease.

The nature of heredity, the inheritance of certain traits and distinguishing features, has been debated in the occidental tradition since the time of the Greek philosophers. Different views were proposed, but the most long-lived ones were those of Aristotle (384 BC – 322 BC). In his work On the Generation of Animals, he stated that male and female both contributed to the generation of offspring, with the female providing basic structure and the male, through the sperm, the specific content. His theories lived on through medieval times much thanks to the Christian church, which incorporated his views in its official doctrine.

Then, until the eighteenth century, most of the thinking about heredity was of practical concern. Improvements in plant breeding and domestication of animals were achieved, but without much thought to the underlying mechanisms.

Early studies of hereditary disease

From the seventeenth century and onwards, more detailed investigations into families afflicted by certain hereditary diseases were performed. The first described ones concerned easily identifiable phenotypes, such as the descriptions of “Double thumb” by Digby (1645) and of polydactyly by Maupertuis (1753). Pedigrees were drawn, but formal predictions were not made and inheritance patterns were not classified further.
Generally, physicians of the nineteenth century were aware of hereditary factors in disease, but considered it mostly as “diathesis”, a predisposition for a certain disease, rather than a proximate cause of disease. Nevertheless, further conditions were described sometimes with great detail or with insights into inheritance patterns that predated the theoretical framework for the practical observations.

One such account was that of George Huntington, who in 1872 described a hereditary form of chorea, afflicting certain families on Long Island. The disease, which later bore his name, was described in good detail, but he was not the first to describe neither the disease nor its hereditary nature. The insight of Huntington's paper was that he realized the disease could not skip generations: in unaffected children “the thread is broken and the grandchildren and great-grandchildren of the original shakers may rest assured that they are free from the disease.”

Darwin and Mendel

The second half of the nineteenth century is considered to be the true starting point of modern genetics. Charles Darwin was very interested in the matter of heredity; of course, it was evident that his theory of evolution was based on inheritance, a concept poorly understood. In his work “Variation of Animals and Plants under Domestication”, he concerned himself with the matter in detail. He drew on a multitude of examples, including many hereditary conditions in humans, to formulate his theory of pangenesis, introducing the concept of gemmules, small particles coming from all parts of the body and carrying information of their characteristics. He, as most thinkers, considered inheritance as essentially qualitative in nature.

Predating both the works of Darwin and Huntington was that of Gregor Mendel. Working in the gardens of a monastery in Brno, he studied the variations and frequencies of seven different traits, and treated them quantitatively. This led him to formulate specific laws of heredity, and to realize that the hereditary factors were preserved intact through generations – not “blended” into the normal phenotype. His results were published in 1866, but no serious notice was taken at the time.

Mendel rediscovered: genetics evolving

In the year 1900, Mendel's work was rediscovered independently by at least three researchers: Hugo De Vries, Erich von Tschermak and Carl Corren, all plant researchers, and the work's importance for studies of inheritance was finally appreciated. One early convert was zoologist William Bateson, who together with the physician Archibald Garrod in 1902 could classify the
first human disease obeying Mendel's laws: autosomal recessive alkaptonuria. Further diseases would soon follow, with brachydactyly being the first autosomal dominant human condition classified. Sex chromosome linked inheritance proved initially more difficult to reconcile with Mendelian inheritance.

The first years of the new century also saw the naming of the hereditary unit, the gene, by Wilhelm Johannsen. The basic nomenclature was by now defined:

- Allele: monogenic traits are delivered in pairs, two alleles (Johannsen).
- Dominant: a trait manifested when the individual carries one allele (Mendel).
- Recessive: a trait manifested when the individual carries both alleles (Mendel).
- Mutation: a change in the alleles, which alters the manifestation (de Vries).

The new framework, and the possibility to formulate problems mathematically, would allow for an enormous explosion in the field of genetics. Studies on the common fruit fly, Drosophila Melanogaster, by Thomas Hunt Morgan and his team, proved the concept of genetic linkage (when two alleles are physically close, they tend to be inherited together) and were the basis for the first genetic map, of the Drosophila X-chromosome. They also showed the first examples of gene duplication and unequal crossing over.

Population genetics was also developed during these years, and after some early hesitation it attracted the interest of skilled mathematicians.

**Eugenics: dark legacy**

The first decades of the twentieth century would also see the rise of eugenics, the study and subsequent applications of methods to improve the genetic composition of the population. The ideas were not at all new, but the advances in the field of genetics gave the eugenics movement possibility to formalize predictions and interventions to devastating effect.

Early advocates include Charles Davenport, founder of the American eugenics association, and in the Nordic countries, Alfred Mjøen and Herman Lundborg. Initially, eugenics was considered a legitimate scientific endeavor, but from the 1920’s and onward, criticism of it’s slight to nonexistent scientific underpinnings and inhumane consequences was expressed, notably by J. B. S. Haldane and Hermann Muller.

Tragically, the eugenic theories had struck home in Germany, and for political reasons but also with help from German physicians and geneticists, they were now implemented to an abominable extent, including not only sterilization but also outright killing of individuals possibly carrying “un-
desirable” traits. A passage such as this, from a German study on Huntington’s disease (HD) by Panse in 1942, sums up the situation with a, in hindsight, chilling statement: “79 cases located and diagnosed by us were reported to the health administration. They have been passed on to the Genetic Health procedure, if they were of an age to procreate.”

The extent of the atrocious policies in Nazi Germany is thankfully unmatched. It must be remembered however, that forced sterilization was still in use, especially in the Nordic countries, well into the 1970’s.

Birth of molecular genetics

Despite the fast development in genetics before the Second World War, a giant gap in knowledge remained. What was the thing passed on? What was the gene, and how did it work? The substance nuclein, composed of nucleic acid and albumin was suspected to affect inheritance as early as 1895 by E. B. Wilson.

Nucleic acid consists of deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA). Phoebus Levene identified the different DNA bases guanine, cytosine, thymine and adenine in 1919, linked by sugar and phosphate groups. That this molecule would be the basis of inheritance was still far from clear, but further research, for example in bacteriophage biology pointed in that direction. In 1944, the “Avery-Macleod-McCarty” experiment investigated transformation of bacteria from one form to another by protein-free extracts. The results strongly suggested that DNA was the main molecule involved in inheritance. How such a relatively simple molecule could achieve this function was unclear.

One of the most well-known scientific discoveries of the modern age is the DNA structure. It has proved a starting point for molecular biology and modern genetics, and the “race” to find the right structure, with different groups involved, lent a sense of drama to the whole matter. In 1953, James Watson and Francis Crick published a paper in Nature suggesting a double-helical DNA structure with complimentary base pairing (adenine always pairs with thymine and guanine with cytosine) which allowed for the copying and transmission of genetic material. Crick and Watson used model building to come up with the hypothesis, doing scant laboratory research about DNA on their own. The built upon, and checked their results against, X-ray diffraction photos taken by Rosalind Franklin and Maurice Wilkins, who published papers supporting Watson and Cricks hypothesis in the very same issue of Nature.

Crick continued by working on deciphering the DNA code, and this was achieved during the 1960’s by combined efforts from several centers. It was discovered that DNA is read in triplets, a string of three bases code for one amino acid, or for a stop codon terminating the translation. By now, the
“central dogma” of genetics was laid down: DNA makes RNA, RNA makes protein, in a one-way only process\textsuperscript{22}.

Human molecular genetics

In human genetics, cytogenetics developed with the discovery of chromosome banding and more and more clinically useful applications emanated. During the 1970’s the discovery of restriction fragment length polymorphisms started the application of molecular genetics to human genetics. Single genes could now be mapped with higher resolution, and in 1977 the human beta-globin gene was cloned. However, the protein structure of beta-globin was already known at the time – the cloning of an “unknown” disease-causing gene would come later in 1989, when the gene causing CF was cloned\textsuperscript{3}.

In between, one of the most powerful tools in molecular genetics was developed. In 1985, a paper was published concerning sickle-cell anemia, where a new enzymatic amplification technique was used: the polymerase chain reaction (PCR), designed by Kary Mullis\textsuperscript{23}. PCR enabled DNA to be copied and amplified to an incredible extent, substantially simplifying molecular genetic research and clinical practice.

Human molecular genetics had now become sufficiently advanced for a previously unthinkable prospect to appear possible: the mapping and sequencing of the complete human genome\textsuperscript{24,25}. This endeavor will certainly go down as one of the great achievements in science, remarkable not only in scope but in the great extent of international collaboration. And, with that pursuit completed, history is now.

Of course, the picture proves to be ever more complex.

RNA, in the central dogma described as a simple intermediary messaging substance, has a wide range of functions of its own. The vast majority of the genome consists of non-coding (“junk”) DNA, i.e., DNA that is not translated into protein, but still with potential to influence transcription and thus gene expression as well as to exert RNA-mediated effects. Alternative splicing opens up endless possibilities within the genome itself. Gene-gene interactions, even between chromosomes, prove to be more complex than imagined. And, probably not finally, epigenetic modification can change the expression pattern of genes in several different ways\textsuperscript{13}.

Unfulfilled hopes?

Fascinating and impressive as all these achievements are, it must be acknowledged that the great promise held up by these enormous developments in molecular genetics during the last half-century still stands very much unful-
filled from a patient's point of view\textsuperscript{26}. Advances lay mainly in the fields of genetic counseling, screening and prenatal testing.

Genetic counseling, for instance, has reduced the frequency of Familial Dysautonomia (FD) among Ashkenazi jews\textsuperscript{27}. This is a devastating autosomal recessive condition caused by a mutation in the \textit{IKBKAP} gene, resulting in poor development and progressive degeneration of unmyelinated sensory and autonomic neurons\textsuperscript{28}.

The Ashkenazim, a Hasidic Jewish population, are frequent carriers of both this and other recessive diseases; the best known may be Tay-Sachs disease\textsuperscript{29}. Today, individuals who wish to marry within the group commonly undergo a screening for the mutations, and abstain from marrying if there is a risk of serious disease, alternatively using prenatal testing to avoid bearing a child with disease. In the case of FD, this has led to a reduction in frequency from about 10-20 children born with FD/year in the mid-nineties to 2-3 children born/year\textsuperscript{27}.

The case of FD illustrates the greatest achievements in this field – knowledge and subsequent counseling reduced the frequency of a devastating condition. However, this raises profound issues to which we do not have any clear-cut answers. What does it say about people living with the condition when one chooses not to have children bearing the causative gene? When is a disease devastating enough to warrant elective childbearing?
Remarks on Medical Genetics

"Your father had it. Now you've got it too."
Ian McEwan, Saturday 2005

To some extent, all diseases (as all biological variation) have a genetic origin. Diseases may be considered along a continuum, with diseases with a slight genetic component at one end, and diseases with a prominent genetic component at the other. Most common diseases are found in the middle.

Figure 1. The environmental – genetic continuum

Among diseases not strongly affected by genetic factors are infectious and environmental diseases, such as human immunodeficiency virus (HIV)-infection and asbestosis. However strong microbial or environmental causes are for a disease, disease-modifying factors in the individual genome are still of importance.30,31

At the genetic end of the continuum, one finds diseases such as HD, CF, Downs syndrome, Duchenne muscular dystrophy (DMD) and myoclonic epilepsy with ragged red fibers (MERRF) as well as the two diseases discussed in this thesis. These diseases are caused by mutations in single autosomal or X-linked genes, chromosomal aberrations or mitochondrial gene mutations. Thus, the risk for disease is fully explained by genetic factors. Even so, environmental and other non-genetic factors may still contribute to the individual phenotype.32

Here, we also find variants of more common multi-factorial diseases caused by single gene mutations.33
Stroke, hypertension, asthma and multiple sclerosis are all examples of diseases in the middle, where both environmental factors and the genetic background are of importance. Many gene variants are associated with these diseases, but no single mutation or genetic variant completely determines the risk for disease\textsuperscript{33}.

An interesting exception to this continuum is malignant hyperthermia (MH), a pharmacogenetic disease which may be placed at either extreme end of the scale – persons carrying a MH-causing mutation are completely healthy individuals, but at risk for a life-threatening MH reaction when exposed to potent anesthetics during surgery\textsuperscript{34}. 

Neurogenetics

“(The disease)... has been transmitted to them, an heirloom from generations away back in the dim past.”

George Huntington, On Chorea 1872

In every major patient group within the broad field of neurology, there is a place for clinical neurogenetics. Single gene disorders can be found among movement disorders, epilepsies, polyneuropathies, ataxias, muscle diseases, dementias and cerebrovascular disease, while in diseases such as multiple sclerosis (MS) and cerebral palsy there might exist a considerable “reservoir” of misdiagnosed patients who may instead have a neurogenetic disease

Also, it must be remembered that several of the more common diseases such as Parkinson’s disease and amyotrophic lateral sclerosis (ALS) have familial variants, where single gene mutations have been shown to cause the disease.

Clinical pointers

The clinical work-up of a suspected neurogenetic disorder starts with a thorough investigation of the patient, including history and a detailed family history, including ethnic origin. A pedigree, if possible, is mandatory, and helpful in classifying inheritance pattern.

Several different medical specialties may be involved. Cooperation with a skilled clinical genetics department is of course important, but initial help in finding a likely diagnosis may come from neuroradiologists, pathologists, neurophysiologists or specialists in any field where the disease manifests itself. It is only when the disease has been thoroughly reviewed clinically and there is reason to pinpoint the genetic cause in the individual that it is advisable to involve the genetics department – otherwise genetic testing may quickly soak up most of a mid-sized neurology department’s budget.
Finding disease-causing mutations

Finding the gene behind a certain disease starts by broadly locating it in the genome. This can be done by linkage studies, where one takes advantage of the fact that there is recombination of the genetic material in form of crossover between chromosomes.

With the use of polymorphic markers (genetic variants with a known chromosomal position), one can establish the likelihood that the disease-causing mutation is located close to a marker, since recombination is more likely to occur the longer the physical distance between the mutation and the marker. The probability that the two loci are linked is calculated as an odds ratio, and the common logarithm is taken to acquire a LOD score. A score of 3.0 means the odds are a thousand to one that the result is not achieved by chance, and is conventionally accepted as evidence of linkage. For this, of course, one needs several samples, both from patients carrying the disease and healthy relatives.

Physical mapping can be a very cumbersome process, albeit one that has been made considerably easier by recent technological developments, such as the sequencing of the whole human genome and the development of microarray technology.

When the chromosomal position is obtained with as great resolution as possible, the next step is usually looking for candidate genes in the region. A candidate gene is a gene with a known function pertaining to the specific features of the disease in question. For instance, focusing on genes coding for a protein expressed in skeletal muscle when looking for a gene causing a certain disorder of muscle function.

Once candidate genes are identified, they can be sequenced, and one can screen for mutations by comparing the obtained sequence with the published genome.

The genome is large, and most of it is non-coding, not translated into protein. Nearly all known disease-causing mutations are located in the coding regions, which are collectively referred to as the exome. A more convenient way of finding disease genes is thus possible: by looking only in the exome. The DHODH gene causing Miller syndrome was the first found by exome sequencing.

Genetic testing for diseases where the mutation is known is of course a much simpler prospect. The need to screen for many different mutations in many different genes may still be there, but this has been made easier by the development of massively parallel sequencing. The power of new sequencing technology is of course also very useful in detecting new disease-associated mutations.
Types of mutations

When primarily looking at the genome level, a disease-causing mutation can be almost any kind of change in the DNA sequence. Broadly, mutations can be divided into:

- Base-pair substitutions. One base is replaced by another (ex A<T). These can be classified as silent (when the mutation does not change the amino acid sequence), missense (resulting in a change in the amino acid sequence) or nonsense (the substitution results in a premature stop codon, alternatively altering an existing stop codon)44.

- Expansions. In the genome, there are many repetitive sequences. Depending on length and complexity, they may be classified as micro- or minisatellites, short tandem repeats or tandem repeats45. Trinucleotide repeats are associated with many different diseases46. These may sometimes expand, indeed, the longer they are the more unstable they seem46, and when they reach a certain length they cause disease. HD is caused by expansion of a CAG repeat in exon 1 of the HTT gene (encoding huntingtin). More than 36 of these polyQ-repeats (so named because they code for glutamine (Q)) may cause the disease, more than 39 invariably do35.

- Insertions/Deletions. One or several bases are inserted into a sequence. If the insertion/deletion is divisible by 3 bp and correctly placed, it inserts extra amino acids/deletes whole amino acids. More commonly, it disrupts the sequence downstream completely (a frameshift mutation), often resulting in a premature stop codon44.

- Copy number variations (CNV). These large-scale genetic variants are today given a great deal of interest as factors in our genetic susceptibility for disease. CNV:s are large-scale duplications and deletions, a subgroup of the genome structural variants which are kilobase (kb)- to megabase-sized deletions, inversions, duplications and insertions. When these span entire genes, they lead to different gene copy numbers between individuals. CNV:s tend to affect specific functional categories, such as genes related to environmental response, while they are more uncommon in genes related to more basic cellular processes47.

- Aneuploidy. The human genome is ordered into 46 chromosomes, 44 autosomes and two sex-chromosomes. 23 of these are inherited from each parent. If an individual for some reason has more (or fewer) chromosomes, specific syndromes appear. Most are lethal, but Down's syndrome, caused by trisomy 21, is one of the most common causes of intellectual disability. Other trisomies that appear in live births are 18 (Edwards syndrome) and 13 (Patau syndrome; rare)48. Full monosomy of autosomes is lethal, while large deletions of parts of chromosomes can cause certain specific syndromes. Aneuploidy of the sex chromosomes also causes disease48.
Mechanisms affecting pathogenesis

Mutations have different effects depending on what kind of mutation it is and where it is located. Often, the clinical characteristics can give clues as to the mutation type.

Expansions, for instance, often lead to accumulation of a faulty product and subsequently often present themselves clinically in adulthood. The phenomenon of anticipation can be present, as the expansion may grow longer in subsequent generations, leading to earlier onset of symptoms\(^4\).

Considering the gene product and its use may be of help – a mutated gene expressed mainly in peripheral nerves will probably lead to disruption of function of the peripheral nerves.

Gain of function is a term used to describe instances where the mutated protein acquires a new, abnormal function. These mutations often give rise to a dominant phenotype\(^4\).

Conversely, loss of function-mutations can cause dysfunction of the protein. These mutations may be recessive, or, if one functioning allele is not enough to create a sufficient amount of protein, dominant. In the latter case, the term haploinsufficiency is used. Premature stop codons cause the protein to be truncated and lose function\(^4\).

Alternative splicing ratios denote the fact that some mutations at splice sites may affect the efficacy of splicing and change the ratio of the splice products, occurring, for instance, in CF and possibly explaining some of the phenotypic variability in the disease\(^5\). Aberrant splicing elements may also play a part in other diseases not clearly identified as genetic in nature\(^5\).

Over-expression of a gene, perhaps caused by an increased copy number or mutations in transcription factors, can lead to increased gene dosage that can cause disease\(^5\).

Mitochondrial disease should perhaps be considered separately. A plasmid containing DNA (mtDNA) is located in the mitochondrion. MtDNA is always inherited from the mother. The few genes located there almost invariably code for proteins used in the oxidative phosphorylation, and mutations often present with symptoms from multiple organ systems, especially with neurologic and myopathic features\(^5\). Diseases include Leber's hereditary optic neuropathy, MERRF and Leigh syndrome (causing psychomotor delay in infants followed by severe neurological manifestations such as ataxia, seizures and weakness)\(^5\). Interestingly, disturbed mitochondrial function has been described in several neurodegenerative disorders not necessarily genetic in origin, such as ALS\(^5\).

Finally, prion disorders, such as Creutzfeld-Jakob disease, should be mentioned. These disorders are unique, as the are both infectious and genetic. Certain isoforms of the prion protein are pathogenic and cause disease by inducing misfolding in existing, correctly folded isoforms. The pathogenic isoforms can be transmitted or inherited\(^4,5\).
Treating neurogenetic disorders

What possibilities do we have to alleviate the suffering these diseases cause? Different roads have been and are pursued, from attacking the disease at its roots, to appeasing the symptoms as well as possible.

Care and symptomatic relief

This is the basis for all treatment of these conditions. For the sufferers of some conditions such as HD or the hereditary spastic paraplegias, physiotherapy increases quality of life\textsuperscript{57,58}, while symptomatic medical treatment can make life easier for many individuals for instance with hereditary seizure diseases. We have already touched upon the symptomatic care of FD patients, which improved life expectancy astonishingly.

Still, it is not likely that we in the foreseeable future should stumble upon such tools as to completely eradicate either these diseases or their symptoms – the most important task for anyone working in this field will thus remain the comforting and caring one.

Here, I also want to acknowledge the relief that many patients experience just by virtue of receiving a distinct diagnosis. As mentioned, many of these diseases are rare and not well known. The symptoms may thus be misunderstood (or worse, wrongly interpreted as functional or “hysteroid” in nature). Knowledge of these, sometimes rare, entities can be of great help to the patient, if “only” to remove the haunting uncertainty of symptoms not understood.

Screening and monitoring

The identification of disease-causing genes has made genetic counseling more powerful, with screening and presymptomatic testing of specific diseases distinct possibilities.

A different situation is the testing of persons at risk of developing diseases themselves. The psychological and existential implications for, at the moment, healthy persons who are at risk of serious disease can be profound – whatever the outcome is. Here, mentioning HD is again warranted. When the disease-causing gene was identified, guidelines for testing were quickly developed and the views not only of scientists and clinicians, but also of representatives for patient organizations, were taken into account\textsuperscript{3,59}.

New technology has made previously unthinkable options possible, such as pre-implantation screening of fertilized eggs in families with HD to ensure that children born do not carry the disease-causing gene even without the mother(or father)-to-be knowing whether she carries the gene or not\textsuperscript{60}.
In this category I also include monitoring of known disease. Screening programs can increase the life expectancy of patients with hereditary tumor diseases such as von Hippel-Lindau disease\textsuperscript{61}.

**Replacement/elimination therapy**

Some genetic diseases result in errors of metabolism, when enzymes responsible for different tasks in the metabolic process are affected. In some cases, this is treatable by means of administration of the correct enzyme, alternatively by lifestyle restrictions to prevent the accumulation of substances the faulty enzyme cannot metabolize.

Examples of the former are the treatment of Hurler’s disease (mucopolysaccharidose type 1; deficiency of the enzyme alpha-L-iduronidase) with laronidase, or Gaucher’s disease type 1 and 3, which can be treated by administration of recombinant glucocerebrosidase\textsuperscript{62}.

Of the latter, Refsum’s disease (caused by faulty enzymes during the alpha-oxidation of phytanic acid), which results in accumulation of phytanic acid, merits a mention – eliminating dairy products, certain meats and fatty fish from the diet improves symptoms\textsuperscript{63}. Also phenylketonuria, one of the first “success stories” of genetic disease screening, is still treated by dietary restrictions, although other options are emerging\textsuperscript{64}.

**Medical treatment targeting the genetic machinery**

Certain types of mutations may carry biochemical properties, which can make them targets for medical therapy.

A commonly used class of antibiotics, aminoglycosides, has the ability to force read-through of premature stop codons. This could be used as a treatment for diseases caused by premature stop codons, such as DMD or CF. Clinical trials have been performed, but results are so far equivocal at best. However, there is hope that chemicals with similar and stronger effects could prove more efficient\textsuperscript{65}.

Kinetin, a plant cytokinin, enhances exon 20 inclusion in the *IKBKAP* gene in several types of mutations associated with FD\textsuperscript{66}.

Transcriptional dysregulation is a feature of HD (and other polyQ diseases). Histone deacetylase (HDAC) inhibitors modulate transcriptional regulation by affecting chromatin structure and may ameliorate symptoms. This approach has been proved fruitful in a mouse model of HD\textsuperscript{67}, and, recently, given a boost by the finding of a potential biomarker ameliorated by HDAC inhibition in humans\textsuperscript{68}.
Gene therapy

Perhaps the most appealing and elegant treatment option is to correct a mutation at the gene level. This, however, is charged with difficulties. Altering the genetic material in a cell is not difficult today – altering it in all (or at least in a sufficient amount of) cells affected by a certain disease is a different challenge.

Perhaps not unsurprisingly, gene therapy has been most successful when blood cells have been targeted, since blood cells are constantly produced, easily accessed and have a high turnover rate – all of which (depending on vector type) make them ideal candidates for gene therapy. Also, some success has been seen in skin disease – of course, the advantages seen in blood can also be applied to skin.

When it comes to neurogenetics, a problem appears. Brain and nervous tissue are neither constantly produced, easily accessed nor have high turnover rates. Thus, correcting a faulty gene in neurons seems difficult. The success so far has been in altering the genes of white blood cells in diseases where hematopoietic stem cell transplantation is effective – recently, two patients with X-linked adrenoleukodystrophy improved substantially after just this treatment. This is thought to be partly mediated by the crossing of cells from the blood stream into the central nervous system followed by a differentiation into microglia, cells which then produce the correct version of the faulty enzyme. A similar approach is being pursued also in metachromatic leukodystrophy and globoid cell leukodystrophy (Krabbe's disease).

A mention is also warranted of the treatment of Leber’s congenital amaurosis (where mutations in different genes expressed in the retinal pigment epithelium cause progressively impaired vision and subsequent blindness) by subretinal injection.

Table 1. Gene therapy successes (Adjusted from Fischer)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cell type</th>
<th>Effect</th>
<th>Toxicity</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leber’s congenital amaurosis</td>
<td>Retinal</td>
<td>Stable</td>
<td>?</td>
<td>Adeno-associated virus</td>
</tr>
<tr>
<td>SCID</td>
<td>CD34 cells</td>
<td>Stable</td>
<td>Insertional mutagenesis</td>
<td>γ-retrovirus</td>
</tr>
<tr>
<td>X-linked adrenoleukodystrophy</td>
<td>White blood cells</td>
<td>Stable</td>
<td>?</td>
<td>Lentivirus</td>
</tr>
<tr>
<td>Epidermolysis bullosa</td>
<td>Skin</td>
<td>Stable</td>
<td>?</td>
<td>γ-retrovirus</td>
</tr>
<tr>
<td>Chronic granulomatous disease</td>
<td>CD34 cells</td>
<td>Transient</td>
<td>Insertional mutagenesis</td>
<td>γ-retrovirus</td>
</tr>
<tr>
<td>Haemophilia B</td>
<td>Liver, muscle</td>
<td>Transient</td>
<td>?</td>
<td>Adeno-associated virus</td>
</tr>
<tr>
<td>Adenosine deaminase deficiency</td>
<td>CD34 cells</td>
<td>Stable</td>
<td>?</td>
<td>γ-retrovirus</td>
</tr>
</tbody>
</table>
Different types of vectors have been used in gene therapy trials. The majority, and so far all successful ones, have been viral vectors.

RNA viruses, such as γ-retrovirus and lentivirus, have the advantage of inserting genetic material into the genome, thus preserving it after cell division. This is a concern for safety, since there is a risk of insertional mutagenesis.

DNA viruses do not insert genetic material into the genome – they just co-opt the cells transcriptional machinery. Adenoviruses and adeno-associated viruses are the most popular candidates for gene therapy. The possibilities are limited, since only post-mitotic cells can be targeted – the inserted gene is not preserved through cell division. The same holds true for plasmid vectors. Specifically when targeting neurons, DNA viruses and plasmids may be of interest, since all cells are post-mitotic and thus no loss of material at cell division would occur.

A potentially important development in this field is genome editing. This method uses different zinc finger nucleases to induce double-stranded breaks at relevant sites, and, with co-administration of a DNA fragment preceded by a splice acceptor site, in vivo correction of the genetic defect. This was recently done successfully in a mouse model of haemophilia. The advantages of genome editing are that the defect can be corrected in vivo, and at the same time probably decreasing the risk of insertional mutagenesis significantly.

Cell replacement technology

Stem cells have captured the imagination of researchers and laymen for the last decade. The potential to replace damaged tissue with fresh, newly-produced cells sounds promising indeed, and successful trials have been performed in different types of tissue, including the central nervous system.

Potentially, cell replacement techniques would of course be of great use in almost all disease types, ranging from cardiovascular insults to degenerative conditions. The reason for mentioning it here, is that it may be necessary to use this for treating many hereditary conditions even though the genetic defect has been corrected. The combination of stem cell therapy and gene therapy would also be a very attractive proposition, where you could correct the faulty gene in vitro in extracted stem cells or induced pluripotent stem cells (iPSC) cells before implanting them in vivo. The further development of iPSC technology would also bypass one important constraint on the use of stem cell technology, since there is no need to harvest existing stem cells.

RNA silencing

Since the discovery of RNA molecules’ ability to interact with each other, it has been proposed that small antisense RNA fragments, inhibiting transcript-
tion of the corresponding mRNA, could have therapeutic potential. There has been great interest in developing therapeutic molecules, and different approaches have been tried, using different delivery systems\(^7\).

One approach is using so-called Morpholinos, small molecules with the same bases as the RNA sequence but with a synthetic backbone. This makes the molecule unrecognizable by cellular proteins – nucleases do not degrade them, neither do they elicit inflammatory responses. This adds to their potential as therapeutic molecules\(^8\). Therapeutic trials are already underway, for instance in DMD\(^9\).

**Translational applications**

The study of hereditary diseases has yielded valuable insights into gene and cellular function in general. The porphyrias, for instance, have yielded valuable insights into the metabolism of heme. Certain metabolic steps were initially uncovered by studies of patient samples\(^8\).

Specifically in neurogenetics, the most useful animal model of ALS is the \(SOD1\)-mice – made possible by the identification of the gene behind many familial ALS cases\(^8\).

As will be discussed later, the understanding of function and physiology of muscle tissue has been greatly enhanced by the clinical study of different muscular diseases, such as channelopathies.

The study of hereditary tumor diseases such as retinoblastoma and Li-Fraumeni disease have given researchers greater understanding of the genetic machinery involved in tumor development\(^8\,8^3\).

Understanding hereditary causes and associations of common diseases can also give us tools such as cell cultures and animal models in which we may study the diseases in more detail. With the advent of powerful technologies such as genome-wide association studies, we may gain clues into affected pathways in all diseases where genetic variation plays a part (i.e. almost all known diseases), exemplified by a recent study of MS\(^8\).
Adult-onset autosomal dominant leukodystrophy with autonomic symptoms (ADLD)

Introduction

ADLD is a leukodystrophy, a genetic disease of the white matter of the central nervous system caused by an defect in myelin development or maintenance. The broader term leukoencephalopathy is used to describe all diseases of white matter, whether hereditary or other. The total incidence of leukodystrophies as a group was estimated in Germany to 2/100,000.

The long name of the disease implies some features uncommon for this class of disease. Usually, they strike early in life, affecting mainly infants and children, with a dismal prognosis. Adult onset forms are more rare. Most forms are inherited in an autosomal recessive pattern.

ADLD was identified and first described by Eldridge et al in 1984. The clinical picture was similar to primary progressive MS: indeed, 20 of the 21 described patients had been diagnosed with MS before computerized tomography (CT) scans revealed findings distinct from MS and the family history was reviewed. The preferential involvement of the autonomic nervous system causing bladder and bowel dysfunction and orthostatic hypotension was noted, and seemed to precede cerebellar and pyramidal symptoms.

The inheritance pattern was autosomal dominant, and initially some researchers feared that this could be quite a common hereditary disease, rivaling HD in the genetic, social and emotional situation created.

Fortunately, this proved not to be the case, since the disease, in most patients, progresses slowly and usually doesn’t affect cognition. It has so far also proved to be quite a rare disease – it is impossible to calculate any incidence rates. At the same time, it is very possible that it is a most under-diagnosed disorder.

Since the 80’s, genetically confirmed cases also have been reported from Canada, Germany, Italy, Japan and Sweden.

The development of MRI technology during the last decades have meant a great deal in diagnostics of white matter disorders and much attention has been paid to MRI characteristics of leukodystrophies. ADLD is no exception, and several authors have published accounts concerning specific abnormalities enabling a probable diagnosis to be made without genetic analysis.
Genetics

Linkage studies were able to locate the genetic defect causing ADLD to an area on chromosome 5q<sup>90,97</sup>. In 2006, Padiath et al showed by examination of candidate genes that duplication of the LMNB1 gene, located on chromosome 5q23 and consisting of 11 exons, causes the disorder<sup>91</sup>. Different duplications have been reported, all spanning the LMNB1 gene in its entirety, suggesting that a founder effect is not likely. The size of the duplication varies from 107 to 341 kb in different reported families<sup>89,91-93</sup>. It also proves very unlikely that duplication of genes in the upstream or downstream region affect phenotype, since different duplications involve these variably. The reason for this situation, with the same duplication of different size arising independently in several families, has been suggested to be a sequence motif predisposition in the flanking region<sup>89</sup>, as described previously for instance in Pelizaeus-Merzbacher disease.

Interestingly, a similar phenotype although without the striking involvement of the autonomic nervous system has been mapped to the same region, but without LMNB1 duplication<sup>98</sup>. A possible explanation is an altered gene dosage depending on a mutation in transcription factors, since it was shown that patients from this family over-expressed LMNB1 mRNA.

Clinical findings

Onset of symptoms is usually in the fourth or fifth decade of life. Some patients may exhibit symptoms already when in their thirties. Almost invariably, there is a history of autonomic dysfunction such as impotence, orthostatic hypotension (OH) or inability to sweat evident at presentation. Symptoms are often reported to increase during stress such as infections, perhaps giving the impression of relapsing-remitting disease. Temperature dysregulation may be present, with one report of severe hypothermia (32) that might have been the cause of death in one patient<sup>89</sup>

The autonomic dysfunction has been investigated in detail by different authors. Guaraldi describes an absence of sympathetic activity by microneurography, along with morphologically abnormal and depleted noradrenergic dopamine-b-hydroxylase fibers<sup>99</sup>. Cholinergic fibers were spared. This is in accordance with studies by Brown, who assessed responses to norepinephrine and insulin-induced hypoglycemia and concluded a distal lesion of sympathetic noradrenergic neurons was likely<sup>100</sup>

Except for the autonomic disturbances, possible findings upon investigation are pyramidal signs with extensor plantar reflexes, spasticity with hyperreflexia, dysmetria/ataxia and weakness. No sensory affliction has been described with the exception of one family, where mild disturbances of vibration and proprioception was noted<sup>94</sup>. Cognitive exams are normal, although
cases with slight-to-moderate impairment have been described, and one pedigree was recently reported with more prominent cognitive disturbances and neuropsychiatric symptoms. We have noted retinal myelination in one patient, which may occur in healthy individuals but also is one prominent feature of the spastic ataxia of Charlevoix-Saguenay.

Routine blood samples are normal. Analysis of spinal fluid has been normal in the majority of patients, but significantly, two patients have been reported with elevated IgG-levels, while increased protein has been reported in several patients.

Mild hearing loss has been reported from one pedigree. Neurophysiological examination can be used to quantify the autonomic disturbances. R-R intervals during deep breathing may be abnormal and head tilt test often reveal severe OH. EMG and neurography is normal, although single cases with slight disturbances have been reported. Visual and sensory evoked potentials (VEP and SEP) are normal, although VEP in one patient is reported as prolonged. EEG is normal or shows diffuse slowing but no epileptic discharges.

MRI reveals distinct findings. In asymptomatic patients, subtle changes in the central corticospinal tract can be seen. Later, signal intensity changes are most prominent in the frontoparietal white matter and in the cerebellar peduncles. The whole length of the corticospinal tract is usually involved, as is the corpus callosum. Myelin is better preserved close to the lateral ventricles than in the surrounding white matter. Atrophy is generally slight, if present at all. The corpus callosum and the brain stem are thin in symptomatic subjects.

Studies on autopsy material from brain reveal a prominent reduction of myelin, but with no significant loss of oligodendrocytes. Myelin is better preserved around blood vessels. There is remarkably little reactive gliosis, and there is no significant axonal loss.

Differential diagnosis

As noted, many patients with ADLD have initially received a diagnosis of MS. As the risk of MS is approximately 0.3% among people of northern European background, differential diagnosis in this case is mostly a case of recognizing patients with suspected MS who may instead have ADLD. Using a normal clinical workup, excluding MS should pose little problem since MR findings and spinal fluid analysis usually differ greatly. There has been one case reported from an ADLD family where the MRI findings had similarities with MS. Cases of MS sometimes cluster in families, giving the appearance of hereditary disease. Genetic risk factors for MS, such as SNP’s associated with increased risk for disease, have been found, but so far no single gene mutation has been found that fully confers the MS
phenotype. Families with MS and possible autosomal dominant inheritance have been screened for LMNB1 duplications, but without results\textsuperscript{106}.

In patients presenting before the onset of other symptoms, ADLD must be distinguished from other neurodegenerative diseases with prominent autonomic dysfunction. Parkinson’s disease often includes symptoms of autonomic failure; however, the motor symptoms and typical clinical appearance of PD should not present a diagnostic challenge. Other synucleinopathies such as Lewy-body dementia, multiple system atrophy (MSA) and pure autonomic failure (PAF; Bradbury-Eggleston syndrome) may exhibit autonomic symptoms initially similar to ADLD\textsuperscript{107}. Cognitive decline distinguishes Lewy-body dementia from ADLD, while true PAF do not present with other neurological findings. MSA cause other neurological disturbances, such as parkinsonism and dementia\textsuperscript{107}, which are not usually seen in ADLD. Again, MRI can easily distinguish ADLD from these entities.

The diagnostic difficulty lies mainly in distinguishing ADLD from other hereditary adult-onset leukoencephalopathies. Table 2 comprises a summary of most hitherto described forms. It must be acknowledged that yet other leukodystrophies have been described, but not as extensively. Among them, one described by Autti et al merits mention, mostly because it has some features which may mimic ADLD, notably extensive white matter abnormalities in cognitively intact persons with slight neurological findings and autosomal dominant inheritance\textsuperscript{108}. 
Table 2. Genetically characterized hereditary leukoencephalopathies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>MOI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenoleukodystrophy</td>
<td>ALDP</td>
<td>X-L</td>
</tr>
<tr>
<td>Salla disease</td>
<td>SLC17A5</td>
<td>AR</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td>ARSA</td>
<td>AR</td>
</tr>
<tr>
<td>Multiple sulfatase deficiency</td>
<td>SUFM1</td>
<td>AR</td>
</tr>
<tr>
<td>Krabbe disease</td>
<td>GALC</td>
<td>AR</td>
</tr>
<tr>
<td>Nasu-Hakola disease</td>
<td>TREM2/TYRO</td>
<td>AR</td>
</tr>
<tr>
<td></td>
<td>BP/DAP12</td>
<td>AR</td>
</tr>
<tr>
<td>Megalencephalic leukodystrophy with subcortical cysts</td>
<td>MLC1</td>
<td>AR</td>
</tr>
<tr>
<td>Canavan's disease</td>
<td>ASPA</td>
<td>AR</td>
</tr>
<tr>
<td>Alexander disease</td>
<td>GFAP</td>
<td>AD</td>
</tr>
<tr>
<td>Vanishing white matter disease</td>
<td>EIF2B1-5</td>
<td>AR</td>
</tr>
<tr>
<td>Pelizaeus-Merzbacher disease</td>
<td>PLP1</td>
<td>X-L</td>
</tr>
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<td>PM-like disease</td>
<td>GJA12</td>
<td>AR</td>
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<td>Sjögren-Larsson disease</td>
<td>ALDH3A2</td>
<td>AR</td>
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<tr>
<td>Cockayne disease</td>
<td>CKN1/ERCC6</td>
<td>AR</td>
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<tr>
<td>Cerebrotendinous xanthomatosis</td>
<td>CYP27A1</td>
<td>AR</td>
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<tr>
<td>ADLD with autonomic symptoms</td>
<td>LMNB1</td>
<td>AD</td>
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<tr>
<td>Autosomal dominant retinal vasculopathy with cerebral leukodystrophy</td>
<td>TREX1</td>
<td>AD</td>
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<tr>
<td>Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)</td>
<td>NOTCH3</td>
<td>AD</td>
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<tr>
<td>Leukoencephalopathy with brainstem and spinal cord involvement and high lactate</td>
<td>DARS2</td>
<td>AR</td>
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Some of the forms, as for instance metachromatic leukodystrophy, Krabbe disease, Alexander disease and Vanishing white matter disease, have adult-onset forms\(^{109-112}\). These may be initially confused with ADLD. This is also true for CADASIL, which manifests itself in adults\(^{113}\). The clinical and radiological picture is however sufficiently distinct to enable a diagnosis. A diagnostic MRI-based logarithm for diagnosing white matter disease has been proposed which may be of aid\(^{114}\). Most forms are recessively inherited, thus ADLD is in many ways a rarity among rarities.

In discussing differential diagnosis, other non-leukoencephalopathic hereditary diseases which may cause some of the symptoms of ADLD must be mentioned. The hereditary spastic paraplegias may exhibit similar pyramidal symptoms\(^{115}\). Some of the spino-cerebellar ataxias (SCA; to this date 30 variants are known) may show some likeness. They are inherited in an autosomal dominant fashion, and similar initial symptoms, which may constitute spasticity and/or pyramidal signs (forms 1, 3, 7, 8, 12, 17) can be seen in ADLD. Age of onset varies, as anticipation is common in SCA. Ataxia is more pronounced in the SCA spectrum than in ADLD, as is eye movement disturbances\(^{116}\). Autonomic symptoms are present in several SCA types\(^{117,118}\), but the typical MRI findings in ADLD can easily differentiate it from the SCA's.
Outside factors (drug use, certain medications and environmental toxins) may also cause leukoencephalopathy. Severe leukoencephalopathy with autonomic disturbances has been reported with vitamin B12-deficiency, and it is of course mandatory to rule out this, since it is easily treated and the prognosis is good\textsuperscript{119}. One case has also been reported with normal B12, methylmalonic acid and homocystein levels, but with positive intrinsic factor antibodies, who recovered by administration of B12 injections\textsuperscript{119}.

In short, the main dilemma in diagnosing ADLD is to suspect this quite rare disease. The clinical picture may resemble primary-progressive MS, but workup including MRI and spinal fluid analysis should exclude MS. Autosomal dominant leukodystrophy could lead to suspicion of adult-onset Alexander disease, which also may exhibit significant autonomic symptoms\textsuperscript{120}, but the MRI findings are sufficiently distinct to enable a correct diagnosis.

**Molecular mechanisms**

\textit{LMNB1} encodes the lamin B1-protein. Lamins are structural components of the nuclear lamina, divided into A- and B-type lamins. They are type V intermediate filament proteins with a short N-terminal head domain, a long alpha-helical coiled-coil rod domain and a globular tail domain\textsuperscript{121}.

Lamins are the main components of nuclear architecture, existing in both the inner nuclear membrane as a network of polymers and within the cell nucleus as stable complexes (internal lamina). An increasing number of functions are supported by lamins, through interactions with proteins in many diverse pathways, including transcriptional machinery and the NF-κβ-pathway\textsuperscript{121,122}.

\textit{LMNA} mutations can cause diseases of a few varieties: diseases of striated muscle, peripheral neuropathy, lipodystrophy syndromes or accelerated aging disorders\textsuperscript{123}.

Mutations in the genes encoding B-type lamins cause at least two distinct disorders: ADLD and Barraquer-Simons syndrome, a progressive lipodystrophy, that has been linked to \textit{LMNB2}\textsuperscript{124}.

ADLD seems to be a disease mainly affecting myelin. Other organ manifestations are secondary to the nervous system degeneration. Whether the exact cause is degeneration of myelin or if there is a defect present already at birth is unclear, but the extent of abnormalities seen in MR examinations even before symptom onset suggests perhaps a combination of the two.

How does the nuclear envelope link to myelin? The process of myelination is complex and not fully understood. The oligodendrocyte, the myelinating cell of the central nervous system, must spread and differentiate by forming ramified processes. Then, cell-cell interactions between oligodendrocytes and neurons must be established and stabilized, in order for membrane sheets and primary membrane wraps around the axons to be formed. Finally,
the many layers of wraps must be compacted to create the mature myelin sheet\textsuperscript{125}. Exactly how the wrapping, perhaps the most central mechanism, takes place is unclear. However, it is self-evident that the enormous morphological changes demand rigorous control of the cytoskeleton, responsible for cell shape alterations.

Lamin B1 is developmentally regulated in the brain. Levels peak at birth, followed by a gradual decrease. \textit{LMNB1} mRNA is also down-regulated during oligodendrocyte maturation\textsuperscript{126}, raising the possibility that lamin B1 regulates oligodendrocyte maturation. The levels of lamin B1 are inversely correlated to levels of miR-23, a microRNA that may enhance oligodendrogenesis. Excessive levels of lamin B1 have \textit{in vitro} been shown to reduce the number of oligodendrocytes expressing mature markers and lower levels of mature myelin proteins. This effect can evidently be reversed by miR-23\textsuperscript{127}.

Two reported subjects with ADLD and linkage to 5q lacked a short peptide at the C-terminus region of the large isoform of myelin-associated glycoprotein (L-MAG), associated with a deficient myelin basic protein (MBP)\textsuperscript{128}. Altered levels of neural cell adhesion molecules (N-CAM) and an accumulation of polysialylated N-CAM was found in the same subjects, along with increased levels of the 180 isoform of N-CAM and a decrease of the 120 isoform. This was considered to suggest a pathogenic mechanism affecting cross-talk between axonal membrane and myelin, with adverse effects on cell-cell interactions\textsuperscript{129}. How, and if, lamin B1 duplications cause these alterations is unclear.

\section*{Treatment options}

Treatment of patients with ADLD is purely symptomatic.

There are rational reasons for exercise and physical therapy, although no studies have been performed. OH can in some cases be successfully managed with mineral corticoids such as fludrocortisone (Florinef) or vasopressors such as midodrine hydrochloride (Gutrone). Spasmolytics as solifenacine succinate (Vesicare) can be tried for treatment of micturition urgency. Urinary tract infections are common and should be treated promptly, since symptoms often worsen during infections. Also, antipyretics should be used freely during infections.

Sexual dysfunction in males can be alleviated by medical therapy such as sildenafil (Viagra) or by injection.

In the future, a possible option could be RNA-based therapeutics with siRNA/Morpholino approaches silencing the production of excess lamin B1. By gene therapy, up-regulating levels of miR-23 might ameliorate symptoms. It is possible that the damage to the myelin sheet is already too extensive when the individual has presented with symptoms, making cell-replacement technology focused on oligodendrocytes an attractive option.
Presymptomatic testing may be a possibility. We do not have any experience of this for ADLD at our centre, but it is conceivable that there is no need for \textit{LMNB1} duplication analysis, since MR abnormalities have been shown in asymptomatic individuals. Whether a subclinical autonomic dysfunction is present in patients from birth is not known at this stage, but it may well be that presymptomatic testing in ADLD is an oxymoron.

Prenatal diagnostics is theoretically possible in families with a known mutation. Since this is a sensitive and complex issue, it should be thoroughly discussed among physicians and families – after all, it is sometimes offered to known carriers of other genetic diseases with adult onset.
Rippling muscle disease

Introduction

RMD is a disorder of voluntary muscle, its name derived from peculiar undulating contractions, triggered by muscle stretching or percussion that may be observed in this disorder.

Torbergsen was the first to describe a family with RMD. The initial report described 5 individuals with muscular symptoms (also noting 7 further with idiomuscular responses to percussion) in one family. He noted as distinct symptoms and findings myotonia-like phenomena, muscular hypertrophy and increased muscular irritability. An elevation in serum creatine kinase (CK) was present, and the phenotype was largely benign\textsuperscript{130}.

In one case, strange, rolling movements of percussed muscle was seen. Later, this “rippling” phenomenon would give the disease its name, as suggested by Ricker\textsuperscript{131}.

Other hallmarks of the disease are percussion-induced muscle mounding (PIMM) (also known as myoedema) and rapid contractions (PIRC). PIMM:s are seen as localized, hard swellings of stricken muscle, persistent for about ten seconds. PIRC:s are easy to visualize in the dorsal forearm, where a brisk tap on the muscles causes wrist extension. Also, after tapping, the wrist only slowly returns to its relaxed position, a phenomenon similar to myotonia. True myotonia is absent in RMD\textsuperscript{132}.

Mutations in the CA V3-gene, encoding a caveolin protein, were shown to cause RMD. The inheritance is usually autosomal dominant, but cases with an autosomal recessive inheritance pattern and a more severe phenotype have been described\textsuperscript{133-135}.

RMD can also arise as an autoimmune condition, most commonly in connection with myasthenia gravis\textsuperscript{136,137} (MG) or with thymoma without signs of MG. It has also been described as a para-malignant phenomenon\textsuperscript{138}. Recently, cases with a mosaic caveolin-3 deficiency was described following severe viral infection and pregnancy\textsuperscript{139}. In some of these cases, autoantibodies directed against caveolin-3 or other muscle proteins have been found\textsuperscript{137,140}.

Rippling, PIMM and PIRC are electrically silent\textsuperscript{130,131}, and this feature has given rise to debate on the true nature of these phenomena. Torbergsen concluded in a review\textsuperscript{132}, that the basis is a disruption of intracellular calcium homeostasis by disturbance of sarcoplasmic reticulum (SR) calcium chan-
nels. Lamb has put forth the suggestion that mutated caveolin-3 in its interaction with the transverse (T-) tubular system can cause “silent” action potentials, traveling beneath the plasma membrane. The slow speed at which the contractions are spreading (roughly 60 cm/s) suggest this type of mechanism\(^{141}\). Studies on cultured myotubes by Ullrich et al have revealed alterations in the excitation-contraction coupling (ECC) and the excitation-coupled Ca\(^{2+}\) entry (ECCE) in cells derived form RMD patients, although how this relates to the rippling phenomenon is presently unclear\(^{142}\).

Whatever the mechanism, autosomal dominant RMD usually seem to have a benign phenotype. Note should however be taken of at least one phenotype, described in an Italian family, with cardiac conduction defects along with RMD\(^{143}\).

**Malignant hyperthermia**

A feature of hereditary muscle diseases recently given some attention is the risk of MH reactions\(^{144}\). MH is a rare but dreaded side effect of general anesthesia, characterized by disturbances in intracellular Ca-homeostasis, leading to increased metabolism and elevated body temperature, muscular rigidity and destruction of the cell membrane\(^{34}\).

The incidence of MH is estimated to 1 in 4,200-6,000 general anesthesias, with a higher incidence in younger individuals. In the event of a MH reaction, treatment consists of discontinuation of anesthetic agents, hyperventilation, administration of dantrolene sodium and cooling of the patient. Mutations in the RyRI gene, coding for the ryanodine receptor (RyR), are the most common causes of MH reactions, while mutations in the gene coding for the dihydropyridine receptor (DHPR) are responsible in some cases\(^{145}\). Clinical myopathies with MH-sensitivity as a feature are central core disease (CCD), multi-minicore disease (MmCD), Evans myopathy and King-Denborough syndrome\(^{146,147}\).

The standard for diagnosing a person with MHS is the *in vitro* contracture test (IVCT), whereas a muscle bundle, usually from the vastus lateralis, is mounted in an organ bath and the contraction force measured upon exposure to different concentrations of caffeine and halothane. Thereby, a person can be classified as MHS, MH equivocal (MHE) or MH-negative (MHN)\(^{148}\).

MHS has not been shown to be a feature of RMD, but the possibility has been discussed\(^{149}\).

**Genetics**

The *CAV3* gene is located on chromosome 3p25 and consists of 2 exons. Cloning has revealed a cDNA encoding an open reading frame of 150 amino
acids. The mRNA is found exclusively in cardiac and skeletal muscle cells.

The caveolin gene family also includes \textit{CAV1} and \textit{CAV2}, most abundantly expressed in adipose tissue. Both are also expressed in the nervous system. They share a single stretch of amino acids (FEDVIAP) which may constitute a “signature sequence” for caveolin proteins.

Mutations in the \textit{CAV3} gene give rise to a variety of different phenotypes. In the Online Mendelian inheritance in man (OMIM) database, limb-girdle muscular dystrophy (LGMD) type 1C, distal myopathy, hyperCKemia, hypertrophic cardiomyopathy, long QT syndrome and RMD are listed. There is allelic overlap between the phenotypes of LGMD 1C, RMD and hyperCKemia. A case has also been reported of unilateral calf atrophy with myopathy, caused by a p.A46T mutation which also can give rise to LGMD 1C and RMD.

More than ten different CAV3 mutations have been described in connection with the RMD/LGMD1C phenotype, most consisting of base-pair substitutions, where the p.A46V mutation should be noted as associated with cardiac conduction defects, and also with sudden death in another family. One deletion (p.103-104) has been described with a typical RMD phenotype.

Most described mutations are dominant. Cases of recessive RMD with a more severe phenotype including weakness have been described in connection with homozygous p.A93T and p.L87P mutations. Extra-ocular muscle paresis with RMD has been described in one homozygote for a premature stop codon. A case of compound heterozygote with a frameshift mutation along with a large deletion of CAV3 has been described. Also, a family with an undefined mutation, autosomal recessive inheritance pattern and the rippling phenotype with cardiac conduction defects and short stature has been reported.

It may well be that the diseases, even within single families, should be considered along a spectrum, constituting hyperCKemia, RMD, LGMD1 and distal myopathy, with other, unknown factors influencing the individual phenotype.

Note should be taken that the numbering system used for the different mutations is taken from Fulizio et al. Some authors may use different numbering (e.g. the p.A46T mutation was initially numbered as p.A45T).

Clinical findings

In our experience, the diagnosis of RMD is a purely clinical one. The distinct features of PIMM and PIRC must be present, while muscle rippling may or may not be observed. Calf hypertrophy may be observed in hereditary cases. Weakness is not significant in RMD, note here the allelic overlap between
The phenotypes of LGMD 1C, distal myopathy and RMD. The hereditary form of the disease is manifested already from childhood, and reports have described toe-walking in young children as a finding\(^{154}\). Toe-walking is commonly idiopathic and benign, but may be caused by short Achilles tendons, spasticity or other neuromuscular disorders such as Becker’s muscular dystrophy\(^{163}\).

**Differential diagnosis**

There is one report of misdiagnosis in the literature. A patient given a diagnosis of acid maltase deficiency was reevaluated after her son was diagnosed with RMD and found to have RMD herself\(^ {164}\).

The findings may be mistaken for true myotonia, and lead to excessive investigations in young children. The cardinal features can also be interpreted as fasciculations, myokymia or neuromyotonia. Neurophysiological examination can help distinguish these from PIMM, PIRC and rippling, which are considered electrically silent, although one recently reported case showed short-duration low-amplitude spikes resembling single motor unit discharges\(^ {165}\). We have also observed myokymic discharges and myotonic runs in RMD, although temporally distinct from the aforementioned phenomena. PIMM, or myoedema, is also seen in cachexia, hypothyroidism and malnutrition\(^ {132}\). In itself, it should not be considered of pathological significance.

A prudent workup in a patient presenting with signs of RMD is suggested below:

**History**
- Muscle specific questions (including for instance weakness, development, temperature sensitivity, stiffness after rest).
- Experienced any weight loss, fatigability?
- Heredity for peculiar muscle phenomena, perhaps only noted by the family itself?

**Clinical examination**
- PIMM, PIRC and rippling?
- Weakness present/absent? Distal/central?
- Muscle atrophy/hypertrophy?
- Other neurological findings?
- Cardiac arrhythmia?
- Fatigue testing.
- Lymph node evaluation.
Laboratory studies

- Routine blood samples including CK, thyroid hormones, liver enzymes, analysis of MG-associated antibodies if there are signs of fatigability.
- Electrocardiogram.
- Electromyogram (EMG) with muscle percussion, single-fiber EMG if fatigability is present.
- CT-scan of the chest if positive family history is absent.
- Muscle biopsy, specific staining for caveolin-3.
- Sequencing of the CAV3 gene.

Molecular mechanisms

Caveolae, “little caves”, are 50-100 nm invaginations of the plasma membrane present in most cells, participating in signal transduction and intracellular trafficking of cellular components. There are three members of the caveolin family of proteins (caveolin-1, -2 and -3), and they are essential for the formation of caveolae in different cell types.

Caveolin-3, encoded by the CAV3 gene, is mainly expressed in heart and skeletal muscle. It has shown interactions with dystrophin and dysferlin, proteins affected in different muscular dystrophies. It also regulates myostatin signaling, and interacts directly with the RyR sarcoplasmic reticulum calcium release channel. Mutated caveolin-3 in muscle modifies the function of the DHPR. The DHPR and RyR both affect Ca\(^{2+}\)-homeostasis, and thus it is not surprising that a number of reports have suggested that caveolin-3 also play a role in maintaining Ca\(^{2+}\)-levels. The expression pattern of other proteins known to be involved in Ca\(^{2+}\)-regulation (including DHPR and RyR) is not altered in patients lacking caveolin-3. RMD myotubes show lower voltage-induced Ca\(^{2+}\)-release from the SR compared to controls. Plasma membrane depolarization in muscle myotubes is accompanied by Ca\(^{2+}\)-influx mediated by the DHPR and also dependent on the RyR. RMD myotubes have a significantly smaller Ca\(^{2+}\)-influx than controls, possibly mediated by loss of co-localization of the RyR and the DHPR. Exactly how this gives rise to rippling, PIMM and PIRC is not clear, but it supports the view that RMD is caused by dysfunctional Ca\(^{2+}\)-regulation.

A study of autoantibodies in autoimmune RMD suggested that a disruption of the RyR-DHPR complex could lead to increased mechanosensitivity, causing rippling, PIMM and PIRC. Interestingly, serological studies on patients with autoimmune RMD show that a major auto-antigen in these cases is titin, which provide elasticity and support in the sarcomere and is suggested to interact with T-cap stretch-activated processes, possibly playing a role in the rippling phenotype of...
these patients\textsuperscript{136}. No physiological interactions between titin and caveolin-3 have been described as of yet.

**Treatment options**

Treatment for RMD is usually not considered, since the phenotype is benign and seems non-progressive. It has been reported that the Ca\textsuperscript{2+}-blocker nifedipine has relieved symptoms in one patient\textsuperscript{172}, while dantrolene sodium gave some symptomatic relief in another. Dantrolene, though, aggravated weakness in that patient and had to be discontinued\textsuperscript{131}.

A strategy pursued in the search for treatment of LGMD1C caused by CAV3 mutations is using proteasomal inhibitors to prevent degradation of mutant caveolin-3 and thus also rescuing wild-type caveolin-3. This has been found effective in 3T3 cells, derived from mice embryonic fibroblasts\textsuperscript{173}. Muscular atrophy in transgenic mice with mutated caveolin-3 can be ameliorated by myostatin inhibition, also a promising treatment for LGMD1C patients\textsuperscript{174}. 
Aims

The aim of the thesis is to describe clinical and molecular characteristics of these disorders, in order to increase diagnostic accuracy and the understanding of affected pathways.

Paper I
To investigate the radiological and pathological features of the spinal cord in patients with ADLD and correlate findings with earlier results in brain imaging of ADLD. Also, to review the literature concerning spinal cord manifestations in other adult-onset leukodystrophies.

Paper II
To find the specific disease-causing mutation in four families with ADLD and investigate whether altered expression patterns are detectable in peripheral blood cells.

Paper III
To describe the clinical and genetic features of a large Swedish family with RMD and assess genotype-phenotype correlations.

Paper IV
To cross-reference our findings from Paper III with earlier investigations on the same family concerning MH and investigate and discuss possible links between the two disorders.
Methods

All subjects were, unless otherwise stated, enrolled through the outpatients clinic at the Neurology department, Uppsala University hospital. All studies were approved by the regional ethics committee, and written informed consent was obtained in all cases.

For detailed technical data, including manufacturers, please refer to the papers in the appendix.

Paper I

Subjects

Twelve persons from two unrelated Swedish families with adult-onset ADLD with autonomic symptoms were enrolled in this study. The disease was identified by clinical characteristics as described earlier. One patient did not undergo brain MR. He had symptoms and signs of the disease, including bladder and bowel dysfunction preceding pyramidal signs, and ataxia.

Genetic studies in one of the families had confirmed linkage to chromosome 5q23, the genetic region harboring the LMNB1 gene.

Magnetic resonance imaging (MRI)

All subjects were examined with sagittal spin echo (SE) T1- and fast SE T2-weighted sequences through the entire spinal cord. Transverse T2-weighted images were obtained using a 3D SE sequence at the levels of C2, C5, T3, T6, T9 and conus in 8 subjects. In 4 subjects, the transverse images were taken with varying T2-weighted SE or gradient echo sequences in varying levels.

Signal intensity (SI) was assessed visually. When the SI was higher in white than in gray matter, the white matter SI was considered pathologic. Spinal cord anteroposterior and coronal diameters and cross-sectional areas were measured at C2-, T6- and T11-levels, and the measures were compared to those in values from healthy controls, taken from literature. Measures out of the range of ±2 SD were considered pathological.
A MRI examination of the brain was obtained with a routine technique at the same day as the spinal examination in all but one case.

**Histopathology**

The spinal cord of a female patient who died at the age of 56 was investigated. The upper part of the cervical spinal cord was available for neuropathological examination.

Paraffin sections were stained for basic histopathology with hematoxylin & eosin and for myelin with Luxol fast blue - cresyl violet. Astrocytes were demonstrated immunohistochemically with antibodies to glial fibrillar acidic protein. Neurons and their processes were identified with neurofilament antibody and phagocytic cells (microglial cells and macrophages) with CD 68 antibody. The bound primary antibodies were visualized using peroxidase-labelled secondary antibodies, diaminobenzidine as the chromogen and hematoxylin as the counterstain.

**Paper II**

**Subjects**

Individuals from the two Swedish families with ADLD were recruited for this study. Linkage analysis had confirmed linkage to the region on chromosome 5 harboring the LMNB1-gene in one of these.

In addition, ADLD patients from a German and an Israeli family were included, diagnosed by clinical findings, including a pattern of autosomal dominant inheritance, and MR imaging.

**Duplication analysis**

Genomic DNA samples from selected individuals were analyzed for copy-number variations using a genome-wide human single nucleotide polymorphism array.

**Quantitative reverse transcriptase PCR (qRT-PCR) and western blot analyses of lamin B1 in nucleated blood cells**

Total RNA was extracted from EDTA-treated peripheral blood samples obtained from probands and healthy controls. RNA quality was assessed by gel electrophoresis and RNA concentration was measured by spectrometry before cDNA synthesis using a cDNA synthesis kit.

Primers for qRT-PCR were designed for two different amplicons of the LMNB1 cDNA. In addition, primers were designed for qRT-PCR analysis of
the *MARCH3*-gene, since previous reports have described duplications extending at least partly over it. The amplicons was normalized to β-actin (*BACT*) and glyceraldehyde 3-phosphate dehydrogenase. The PCR products were analyzed in triplicate using a fluorescent RT-PCR kit and a qPCR thermocycler. The experiment was repeated three times using independent samples from each individual.

For western blot analysis\textsuperscript{178} we pelleted white blood cells from one affected individual of each of the two Swedish families, as well as from three healthy controls. The cells were dissolved in radioimmunoprecipitation assay buffer containing protease inhibitor cocktail and stored on ice for 30 minutes and centrifuged at 13,000 g. The supernatant, containing protein, were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis and transferred to polyvinyl difluoride membranes. Lamin B1 was detected using primary α-lamin B1 and α-BACT antibodies, respectively. Proteins were visualized using infrared-labeled secondary antibodies, respectively and analyzed using an infrared imaging system, determining integrated intensities for lamin B1\textsuperscript{179}. BACT was measured in the same samples for internal normalization. Each sample was analyzed on four independent blots and the average of the independent measurements was used for further analysis to rule out experimental error.

**Statistical analysis**

Expression of *LMNB1* determined by qRT-PCR and Western blot analysis was compared to levels in controls by student’s two-tailed t-test.

**Paper III**

**Subjects**

The proband, a 26 year-old woman, was referred to the neurology outpatient clinic, Uppsala University Hospital. She had difficulty maintaining her handwriting for longer periods and frequent attacks of migraine. She had hypertrophy of the calves, slight muscle weakness as judged by difficulty performing a sit-up, and serum CK level was elevated. On follow-up, her mother accompanied and reported rolling and wave-like phenomena in muscles experienced by several family members including herself. This information lead us to suspect RMD and to examine further family members.

Altogether, 39 individuals (aged 1 to 67 years, median age 31 years, 20 females and 19 males) from three generations of the family underwent clinical physical and neurological examination. Skeletal muscle was percussed in order to elicit PIMM, PIRC, and rippling.
Serum CK levels were measured in 29 individuals. ECGs were obtained in 10 family members.

Neurophysiological studies
Twelve family members underwent studies at the neurophysiology laboratory at the Uppsala University Hospital. Eleven of the 12 members showed findings consistent with RMD on clinical investigation.

Nerve conduction studies of motor and sensory arm and leg nerves were performed with routine techniques using surface electrodes for stimulation and recording.

Conduction velocity, F- latency, amplitude of compound muscle action potential (CMAP) and sensory signals, signs of dispersion, extra discharges and signs of conduction block were assessed.

For EMG, concentric needle electrode was used. Commercial EMG equipment with software for quantitative EMG was used. Reference values for motor unit potential (MUP) parameters and for interference pattern analysis were taken from the database in the laboratory.

Different muscles were studied, partly dependent on the clinical picture. Extensor digitorum communis was always included, and often tibialis anterior, rectus femoris but also vastus medialis, lateralis and biceps brachii.

First the electrode was inserted in the muscle at rest, and the duration of insertional activity was assessed. The insertional activity was studied in the resting muscle. It was also studied in relation to the percussion mounds, either with the needle in the muscle before the percussion at the same place, or just after percussion while the PIMM was present. In cases where rippling was seen, the EMG needle was inserted here.

Finally, the EMG during strong force was studied, so called interference pattern analysis by means of turn amplitude analysis.

EMG investigation was performed twice in 2 patients, 6 months apart.

Muscle pathology
Muscle biopsy specimens were obtained from three patients at 25, 45, and 53 years of age, from the vastus lateralis muscle in two and the deltoid muscle in one.

Routine stainings for muscle pathology were performed, as well as specific immunostainings for caveolin-3 as well as for fetal, neonatal, fast and slow myosin heavy chains and selected muscular dystrophy associated molecules. Electron microscopy was performed in one case. Quantitative analysis of myofibers, including calculation of the atrophy and hypertrophy factors, was performed using a computerized muscle biopsy analyzer. A pathology report of a patient investigated at the age of 1 was obtained, and
further immunostainings including western blotting was performed on frozen tissue samples.

Genetic analysis.
Peripheral blood samples for DNA extraction were obtained from 38 individuals. Primers for the two exons of the $CAV3$-gene were designed. The $CAV3$-gene was amplified by PCR, and samples were sequenced on a genetic analyzer.

Paper IV
Ten individuals from the family described in paper III with 23 cases of RMD and the p.A46T mutation in the $CAV3$ gene had been investigated concerning MHS because of elevated CK-values and a fatal MH reaction that occurred in a patient from the village where this family originates.

The investigation for MHS was initiated before the clinical diagnosis of RMD was made in this family. The MHS-proband had a history of suspected myocarditis, muscular fatigue, muscle cramps, hyperCKemia, and, during childhood, a 5-10 minute episode of generalized muscle stiffness elicited by local anesthesia. Nine further individuals in this family were subjected to IVCT$^{148}$. No analysis of known MH-causing mutations was performed.

We cross-referenced the results of the IVCT tests in the family with the diagnosis of RMD. Clinical data including laboratory, pathology and EMG reports were compared in order to establish whether the RMD phenotype was influenced in persons with an IVCT result suggestive of MHS. Exposures to general anesthesia were noted in the patient histories, including also patients with RMD who had not been subjected to IVCT (n=17).
Results

Paper I
Measurements of the spinal cord
In all patients and the clinically unaffected family member, a pronounced thinning of the spinal cord was manifest. All the anteroposterior and coronal diameters, as well as the cross-sectional areas, were significantly smaller than in the control material.

Signal intensity of the spinal cord
A subtle increase in SI was observed along the posterior part of the spinal cord in sagittal T2-weighted images of the cervical and/or thoracic spine in four of the twelve patients. In the clinically most severely affected patient, distinct but diffuse SI changes were seen.

T2-weighted transverse images suggested a homogeneous increase of the SI of white matter along the whole spinal cord in all patients, as well as in the asymptomatic family member. Images of the conus were difficult to interpret and motion (pulsation) artifacts disturbed evaluation of SI homogeneity in some stray slices.

MRI of the brain showed the characteristic features as described by Melberg et al in the same families. The subjects included in this study presented various severity grades of the radiological brain involvement. In all subjects, increased SI was seen in the cerebral white matter beneath the motor cortex, and along the corticospinal tract down to the medulla oblongata. Images of the clinically affected patients also showed large frontoparietal white matter changes. Seven patients had white matter changes also in the upper and middle cerebellar peduncles. Some atrophy was found in ten subjects, most often in the corpus callosum (seven subjects) and in the medulla oblongata (eight subjects).

Histopathological findings
In the cervical spinal cord of the autopsied patient no obvious loss of myelinated fibers was visible at a low magnification. However, at a higher magnification, such a loss was observed subpially at the periphery of the spinal
cord with both myelin and neurofilament stainings. The loss of nerve fibers had induced reactive astrogliosis, whereas the number of microglial cells was not increased. The MRI of this patient showed atrophy and white matter changes consistent with findings in the other patients. We were not able to see any SI difference between the most peripheral and the other parts of white matter.

Parietal sections of the brain from the same patient were also examined. Myelin was reduced, rarefied, and vacuolated with better preservation around blood vessels. Despite a prominent reduction of myelin, there was only minimal reactive gliosis.

Paper II
Clinical and neuroradiological features
Clinical characteristics of the two Swedish families are described in Paper I.

The family histories of the German and Israeli families indicate that four patients were affected in the German over two generations and that four patients were affected in the Israeli family over three generations. Patients from these families had an onset in their sixth decade with autonomic symptoms, pyramidal signs and had brain MRI signs characteristic of this type of leukodystrophy. Peripheral nerve conduction studies in two affected members of the German family were normal, whereas the proband of the Israeli family had mild electrophysiological signs of peripheral neuropathy and levo-dopa responsive parkinsonism. These features are not previously described in ADLD with autonomic symptoms but the patient’s autonomic symptoms and MRI signs are compatible with this type of ADLD.

Analysis of gene copy number variation (CNV)
The analysis of CNVs showed a pattern consistent with duplications spanning the entire LMNB1 gene on chromosome 5q in all four probands. Each proband has a distinct duplication.

The patient from the large Swedish pedigree has a duplication with an estimated size of 187 kb; while the other Swedish patient has a duplication with an estimated size of 107 kb; the German patient has a duplication with an estimated size of 218 kb and the Israeli patient has a duplication with an estimated size of 194 kb. The duplicated segments extend over part of the MARCH3-gene in all four cases.
**LMNB1 expression**

The levels of \textit{LMNB1} mRNA in white blood cells from the Swedish ADLD patients were significantly increased when compared to the levels from healthy controls. This pattern was observed for both amplicons detecting the \textit{LMNB1} transcript. Similarly, the lamin B1 protein levels were significantly increased in white blood cells from patients compared to healthy controls. The increase was uniformly higher in patient samples by a two- to four-fold factor. The levels of \textit{MARCH3} mRNA were similar when comparing patients and controls.

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**Paper III**

**Clinical investigation**

Of the 39 patients examined, 23 were clinically affected as judged by the presence of PIMM and PIRC. The age range was 1 to 67 years, median age 28 years, 12 females and 11 males.

PIMM was consistently elicited in the thenar eminence in all, but could also be observed in the biceps brachii, triceps, pectoral, quadriceps, and trapezius muscles in many individuals. In the one-year-old boy, PIMM was evaluated in the brachioradial muscle. PIRC was observed in all evaluated musculature as an easily induced rapid contraction. Rippling was elicited in eleven patients, most readily in the quadriceps femoris, less frequently in the gastrocnemius, deltoid or latissimus dorsi muscles. Fifteen patients had noticed PIMM and/or rippling prior to examination by us. Several patients were able to elicit rippling on request. Calf muscle hypertrophy was found in nine patients. Two patients had generalized muscular hypertrophy, one to an extreme extent appearing as a muscular athlete, without any significant history of physical exercise. Significant muscle atrophy or muscle weakness was not observed in any patient.

Three patients with RMD were asymptomatic. The remaining 20 patients had symptoms of muscle stiffness, myalgia or cramps. Nineteen patients reported stiffness, typically worsening after periods of rest following exercise. Two patients had noted tiptoe walking in the morning. Four had a history of tiptoe walking in early childhood. None had tiptoe walking on clinical examination. Two patients experienced difficulties with fine motor skills during youth. Difficulty writing by hand for longer periods was reported by two. In all, only two adults sought medical attention because of muscular symptoms. Two infants had been investigated because of concerns about muscle stiffness raised by parents or daycare workers. At least one patient had sought advice from a physical therapist.

Serum CK levels in the affected patients ranged from 2,8 to 60,2 µkat/l (mean value 14,85; reference intervals: male 0,7-4,7, female 0,6-3,5). Three
patients with RMD had normal serum CK levels. The ECGs showed no signs of cardiac hypertrophy or long QT-intervals.

Several individuals lacking signs of RMD reported myalgia and muscle stiffness. They lacked a history of worsening of stiffness on exertion after rest or morning stiffness. One clinically normal man had elevated serum CK, 5.4 µkat/l.

Neurophysiological studies

Nerve conduction studies were performed in 3 patients, repeating the examination in one of these who had normal results on both studies. In one subject, temporal dispersion and low CMAP amplitude was observed from the peroneal nerve. No other signs of neuropathy were observed. In the peroneal nerve, there were also irregular late components, directly after the CMAP of the type that may indicate a very distal generation of the activity. In the third subject, stimulation of the median nerve gave rise to activation of lower limb muscles after 90 msec, recorded in rectus femoris with intramuscular needle electrode. No response could be recorded from the frontalis muscle. This was interpreted as an inter-limb reflex.

EMG was performed in 12 subjects. Insertional activity was increased in 5 (in one rather pronounced with activity duration of more than 100 msec), borderline in 2 and normal in 5 persons. Insertional activity was also studied inside the PIMM, either with the electrode already in place, or inserted just after percussion. The activity was increased in 2 cases (and these had also increased activity at rest). In 1 case, there was a definite impression of less insertional activity than normal. In the remaining 8 investigated family members the insertional activity was unremarkable.

There was no ongoing motor unit activity in the PIMM. The muscle was judged electrically silent. In 3 studies of 2 patients fibrillation and positive waves were seen separated from the insertion of the electrode. In these patients myokymic discharges were seen in 2 and myotonic runs in 3.

The EMG during slight or strong voluntary activation was interpreted as myopathic in 3 and normal in 9. In the overall interpretation, 6 EMGs were considered to indicate myopathy (in 3 cases based on spontaneous activity as fibrillations, positive waves and myokymia). The changes were of moderate degree only in one case, in the others they were graded as slight.

EMG in the patient investigated at another centre at age 1 showed slight signs of myopathy with normal nerve conduction velocities.

Muscle pathology

Routine examination of the muscle biopsy specimens revealed only mild myopathic findings of unspecific character in all three patients, but there were some differences between the three biopsies.
All biopsy specimens showed markedly increased fiber size variation. In two patients’ biopsy specimens definite fiber hypertrophy was noted. It was somewhat greater in the type 2 fast fibers than in the type 1 slow fibers. Thus, the hypertrophy factors for type 2 fibers were clearly increased in both patients, but for type 1 fibers only in the youngest individual. The third patient’s fibers were either of normal size (type 1 fibers) or slightly atrophic (type 2 fibers. In addition, there were scattered minifibers and some hypertrophic fibers containing central nuclei. In the third subject’s biopsy the mean fiber sizes were approximately normal. In none of the biopsy specimens fibers stained positively for fetal myosin, excluding recent necrotizing lesions, in concordance with the lack of any signs of necrosis, phagocytosis, inflammation or fibrosis. In the proband’s biopsy specimen, several fibers showed positive staining for neonatal myosin. Most of these fibers were approximately of mean size, but some were minifibers. In the second one hypertrophic fiber and several minifibers were positive for neonatal myosin, whereas in the third no fibers were positive for neonatal myosin. All these findings indicate altered expression pattern of myosins.

Specific immunostainings for caveolin 3 revealed markedly weaker immunopositivity of caveolin 3 as compared to controls in all three patients’ biopsy specimens. Further immunostainings performed using antibodies to dystrophin 2, β-sarcoglycan, and β-dystroglycan yielded normal results.

The pathology report of the muscle biopsy specimen from the patient investigated during infancy described only a marked variability in muscle fiber size in routine stainings, but further immunostaining revealed markedly weaker caveolin-3 immunopositivity than in the controls.

This patient was clinically affected by RMD, and genetic analysis was not performed.

A western blot analysis revealed significant loss of caveolin 3 compared to controls.

Most fibers in the electron microscopic analysis of the youngest individual’s muscle biopsy specimen appeared by and large normal, but closer scrutiny of the myofiber plasma membrane showed decreased numbers of sarcolemma associated caveolae.

Genetic analysis
Of the 38 genetically investigated family members, 22 were found to have an A>G transition resulting in an amino acid exchange from alanine (A) to threonine (T) at amino acid position 46 in the CAV3 sequence. The mutation is localized in the caveolin signature sequence (FEDVIAP)\textsuperscript{162}. No other mutations shown to cause RMD or other caveolinopathies were found in any of the tested individuals. The 22 affected patients who were genetically tested harbored the mutation. The mutation was absent in individuals who did not have clinical signs of RMD.
Paper IV

Ten patients in the family had been investigated concerning both RMD and MHS. Six of these had clinical signs of RMD and carried the CAV3 p.A46T mutation. Out of these, four had pathological IVCT results. Two of these were considered MHEh (equivocal; with a reaction caused by halothane), while two were diagnosed as MHS. Both MHS and MHEh are clinically considered susceptible. Out of the four patients with no signs of RMD and no CAV3 mutation, two were diagnosed as MHS.

The clinical neurological phenotype was similar in RMD patients whether they were found MHS or not. No signs of any muscular hyper-irritability were seen during physical examination of the two individuals with positive IVCT who did not have RMD. EMG had been performed in four individuals. Normal EMG results were seen in two RMD patients considered MHEh (although a slight increase in insertional activity was found in one of these) and in one MHS patient who did not have RMD. The EMG of one MH-negative patient with RMD was interpreted as slightly myopathic.

One of the patients, with RMD considered MHEh had undergone surgery under general anesthesia by MH-triggering agents twice. Also, the mother of this patient, diagnosed with RMD but not tested by IVCT, had undergone surgery with exposure to MH-triggering agents on numerous occasions.

In this family, a total of 23 individuals were diagnosed with RMD. Of the 17 RMD patients not subjected to IVCT, eight had undergone surgery with general anesthesia on one or several occasions. This makes a total of 12 RMD patients who had been anesthetized with MH-triggering agents. In total, at least 18 exposures were identified in the patient’s histories.

No signs of CCD or MmCD were seen in muscle biopsy specimens, obtained from two of these subjects.
Discussion

ADLD

What could we gain in understanding ADLD further? The process of myelination is far from fully understood. A link between myelination and the nuclear envelope could give us useful insights.

A fascinating aspect of this disease is the very specific phenotype – understanding why only central nervous system myelin is involved would be useful in studies of other central myelin disorders as well as peripheral neuropathies. Since lamin B1 is involved in myelin development, it may give us a window into the regeneration and maintenance of myelin – very useful in the search for effective treatment in MS as well as traumatic brain and spinal cord injury.

What particular mechanisms may cause the relatively selective disruption of noradrenergic nerve function? Noradrenergic neurons are postsynaptic, and extend from the sympathetic ganglia to the target organ system. They are unmyelinated, with relatively slow conduction velocity. Defective myelin development or myelin degeneration would not affect the postsynaptic neurons primarily. The preganglionic fibers, however, are myelinated. They extend from the central nervous system to the synapse in the ganglia. The cell bodies of preganglionic fibers are located in the intermediolateral column of the spinal cord from the uppermost thoracic to the upper lumbar segments. Is the extent of atrophy in the spinal cord a sign of selective degeneration of these neurons? Or does ADLD cause disturbances in other systems than only myelin, thus affecting postganglionic fibers directly? Such questions may be addressed by further studies in patients or model systems of ADLD.

There has been some interest in creating useful models for MS by using $LMNB1$ overexpression, but the usefulness of such an approach may be debatable, since the mechanisms of injury seem very different. However, animal or cell culture models using $LMNB1$ over-expression may well be useful for other reasons earlier mentioned.

$LMNB1$ models that have been studied so far include over-expression of the gene in Drosophila eye, leading to a degenerative phenotype. Over-expressing it in neurons or glia is lethal. $LMNB1$ null mice die at birth with bone and lung defects. So far, no model organism has been created with over-expression of $LMNB1$ giving rise to the ADLD phenotype. It is a possible strategy that we hope to pursue. If this can be achieved, not only would
potential treatment studies be possible, but also an easily accessed source for *in vitro* studies of myelination in both the peripheral and central nervous system would be available.

*In vitro* studies would also be greatly facilitated by the generation of iPSC lines, a very attractive option also for its therapeutic implications. Several diseases, both neurological and non-neurological, have thus been modeled so far\textsuperscript{184}.

RMD

The phenotype of RMD may give insights into muscle function ranging wider than only caveolin-3 function. As demonstrated by Ullrich et al\textsuperscript{142}, studies on patient-derived material is both feasible and worthwhile.

The pathways that caveolin-3 interacts with are in many cases associated with more severe diseases, and elucidating them further may help us find treatment options in diseases such as dysferlinopathies (LGMD 2B, Miyoshi myopathy) and Duchenne/Becker muscular dystrophy. The cardiac phenotype of certain *CAV3* mutations and subsequent animal models may enhance knowledge of cardiac-specific muscle pathways and nitric oxide synthase activity\textsuperscript{185,186}. Also, the interactions with the DHPR and RyR may yield further clues into the pathophysiology of MH, still a poorly understood phenomenon. The discordance between MHS and RMD in the family described herein also has some intriguing parallels in earlier literature, where discordance between IVCT results and *RyR1*-mutations within families has been described\textsuperscript{187-189}. This suggests that other mechanisms, genetic or environmental, may play a role in MH. Epigenetic silencing of *RyR1* has been proposed and evaluated, but seems unable to explain all instances of this phenomenon\textsuperscript{190}.

Several animal and cell culture models with *CAV3* mutations have been created, many with relevant phenotypes that have made treatment studies possible. *CAV3* knockout mice reveal a muscular dystrophy phenotype, with changes in the dystrophin complex and t-tubule abnormalities\textsuperscript{191}. A muscular dystrophy phenotype has also been seen in transgenic mice carrying mutations in the *CAV3* gene\textsuperscript{192}. Furthermore, they exhibited hypertrophic cardiomyopathy with increased endothelial nitric oxide synthase activity\textsuperscript{185} *In vitro* studies using antisense oligonucleotides as well as studies using Morpholino oligomers in zebrafish have shown a crucial role of *CAV3* in developmental regulation, muscle differentiation and survival and myoblast fusion\textsuperscript{193,194}. Fascinatingly, *CAV3* over-expression seems to lead to a DMD-like phenotype with down-regulation of dystrophin\textsuperscript{195}.

Understanding the biology of caveolae more deeply could also yield insights into other disease types. For instance, *CAV1*, mutations in which may cause lipodystrophy, has fascinating properties acting both as a tumor suppressor and an oncogene, altered expression patterns found in several cancer
types, including prostate cancer\textsuperscript{196}. It is also up-regulated by infection with human immunodeficiency virus (HIV), causing virus reduction and possibly persistent infection\textsuperscript{197}. 
Enligt socialstyrelsens webbplats (www.sos.se) definieras en ovanlig diagnos som en sjukdom som drabbar färre än 100 personer av en miljon. Man kan då lätt tänka att dessa sjukdomar är obetydliga som försvinner i den stora massan – cancer, till exempel, kommer nästan var fjärde person i Norden att drabbas av!

Att för den skull glömma bort deras existens vore ett konstfel. Det finns tusentals av dessa ovanliga sjukdomar, och sammantaget är flera procent av befolkningen drabbade av dessa åkommor – *det är inte ovanligt med ovanliga sjukdomar!* För vissa av dessa patienter med behandlingsbara sjukdomar kan rätt diagnos vara livräddande, medan det för andra kan underlätta livet enormt. Även om någon bot eller lindrande behandling inte finns i nuläget, kan det vara av mycket stort värde bara att få en specifik diagnos – att få veta vad man är drabbad av.

Genetisk rådgivning kan vara av stort värde för hela familjen, då många av dessa mycket ovanliga sjukdomar är ärftliga. Detta kan betyda att någon av föräldrarna är drabbade, men även om föräldrarna är friska kan barnet drabbas av en ärftlig sjukdom. Hur?


Denna avhandling handlar om två dominant ärftliga sjukdomar, autosomalt dominant leukodystrofi med autonoma symptom (förekrönt ADLD) och rippling muscle disease (RMD).

ADLD är en sjukdom som märks först i vuxen ålder. Den drabbar den vita substansen i hjärnan och ryggmärgen på ett sätt som kan likna multipel skleros. Oftast kommer de första symptomen som märks från det autonoma nervsystemet, som kontrollerar vattenkastning och avföring, förmågan att svettas, blodtryckssfall och även sexuell förmåga. Senare kan patienterna också drabbas av andra symptom, som gångsvårigheter och problem med
finmotorik. Vissa blir svårt sjuka och sängliggande, medan andra inte alls drabbas så hårt och kan jobba fram till pensionen.

Det vi specifikt kunnat visa, är att ryggmärgen hos dessa patienter är väldigt tunn, något som är tydligt även hos personer som ännu inte utvecklat några symptom. Troligtvis är det på detta sätt på grund av en nedbrytande effekt i nervsystemet, som tar sig olika uttryck i olika delar av det. Till exempel är storleken på storhjärnan inte alls lika kraftigt påverkad.

Vi har också kunnat bekräfta tidigare resultat om vari den genetiska defekten hos dessa patienter sitter. De har tre kopior istället för två av en gen som heter Lamin B1, och som tillverkar ett äggviteämne som sitter i det membran som omger cellens kärna. På grund av detta tillverkas för mycket Lamin B1, vilket vi också kunnat visa i vita blodkroppar från patienter. Detta händer alltså i alla kroppens celler, och vi vet inte ännu varför just nervsystemet drabbas av denna mutation.

RMD är en oftast godartad åkommot som tar sig uttryck enbart i muskler. Personer med denna åkommot har mycket lättretliga muskler, som drar sig samman när man slår på dem. Vid lite kraftigare slag får de även en lokal liten muskelsammandragning som kvarstår i cirka tio sekunder. Vissa kan också utlösa osammansändade muskelsammandragningar, det ser ut som vågliknande rörelser under huden (därav namnet “rippling...”). Detta kan orsakas av kroppsegna reaktioner där immunförsvaret angriper kroppens egna proteiner, till exempel i samband med andra sjukdomar som myastenia gravis, men de flesta beskrivna fall har rört sig om den ärfliga formen. Vi har kartlagt en stor släkt med denna åkommot. Ingen person i den släkt vi har undersökt har fått några större besvär, även om vissa kan klaga på lätt morgonstelhet och svårigheter med igångsättning av fysisk aktivitet. Vi har inte heller sett några tecken på att kroppens viktigaste muskel, hjärtat, är påverkad.

Genom att undersöka 39 personer i denna släkt har vi kunnat beskriva symptomen och de väsentliga undersökningarna som är nödvändiga för att kunna ställa denna diagnos i stor detalj. Dessutom har vi studerat ett eventuellt samband mellan denna åkommot och så kallad malign hypertermikänslighet, en livsfarlig överkänslighet mot vanliga narkosmedel som också den är ärflig. Flera personer med RMD har fått besked om att de har denna känslighet, men frågan är om RMD i sig har gjort de tester man använder för malign hypertermikänslighet oanvändbara. Vi har dock kunnat visa att i denna familj finns båda åkommorna, och de samsegregerar inte, det vill säga, en person kan ha den ena åkommot utan att ha den andra och vice versa.

Målet för dessa undersökningar är givetvis framför allt att hitta sätt på vilka vi kan hjälpa personer med dessa sjukdomar, men vi har också förhoppningar om att kunna lära oss mer om kroppens normala funktion, såväl i hjärnan som i muskler, genom att studera dessa patienter och dessa gener i större detalj. På så vis hoppas vi att dessa resultat och de som därpå följer kan vara ännu fler forskare, och i slutändan därmed ännu fler patienter, till gagn.
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