Uncovering the Mysteries of Age-Related Macular Degeneration

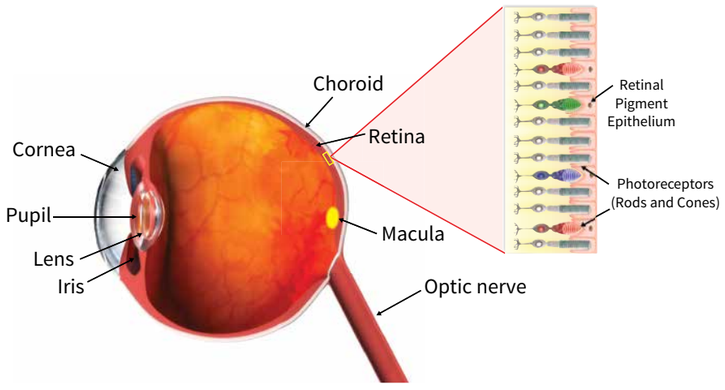
Professor Deborah Ferrington, PhD

UNCOVERING THE MYSTERIES OF AGE-RELATED MACULAR DEGENERATION

Age-related macular degeneration is the leading cause of blindness amongst the elderly population in the developed world. **Professor Deborah Ferrington** and her colleagues at the University of Minnesota are carrying out ground-breaking research on the cellular pathways underlying the pathology of this life altering disease.

The Fight Against Blindness

Age-related macular degeneration (AMD) is a life changing disease which robs people of central vision, limiting their ability to read, drive and even recognise faces. AMD affects over 10 million individuals in the United States and this number is expected to double by 2050 due to the Western world's rapid increase in the ageing population. With limited treatment options available, there is an urgent need to develop new methods for prevention and cure.



AMD leads to vision loss through the destruction of the macula, an oval shaped pigmented area at the centre of the retina. The macula contains a dense concentration of cone cells (which supply high acuity and colour vision). As these cones degenerate, central vision is lost. There are a number of factors which contribute to cone loss, including age, genetic profile and environmental insults such as smoking.

There are two primary forms of AMD. 'Wet' AMD manifests as the abnormal growth of blood vessels into the retina. There are successful treatment options available for individuals with this form of the disease, however only 10% of patients present with 'wet' AMD. The other type is 'dry' or atrophic AMD, which involves changes in and the eventual loss of cells from the retinal pigment epithelium (RPE). The RPE is a monolayer of cells located beneath the neural retina. The RPE maintains retinal health and homeostasis by supporting the function of rod and cone photoreceptors (structures in the eye that respond to light).

AMD leads to the formation of lipoproteinaceous deposits (known as drusen) that form between the RPE and choroid (the pigmented vascular area beneath the RPE). As the disease advances, drusen increases in quantity. Changes to the RPE become evident in the early stages of AMD and because these cells are post-mitotic, which means they are unable to divide, they cannot be replaced if damaged or lost. Without support from the RPE, photoreceptors die and vision loss occurs. There is currently no effective treatment for 'dry' AMD.

This is where the work of Professor Deborah Ferrington comes in. Her laboratory is focused on defining molecular changes that occur in the retina with AMD. 'The ultimate goal of my research is to identify therapeutic targets for treating AMD, which requires a thorough understanding of the disease mechanism,' she explains. Defining the molecular mechanism of AMD is no small task. Because the anatomical structure of the retina is unique to primates, no animal models are available which can faithfully replicate the retinal conditions associated with AMD. Therefore, Professor Ferrington and her team are using human donor tissues to study the disease. They aim to investigate AMD pathogenesis through studying changes in protein expression and in the mitochondria at progressive stages of AMD. 'It was previously thought that one simple

'If we can keep the mitochondria healthy, we may slow the progression to blindness… Because mitochondrial damage occurs before vision loss, early intervention would likely protect or rescue RPE mitochondrial function. This is the best place to start aiming therapies.'

mechanism would cause AMD, but it's not that way at all,' Professor Ferrington says. 'Genes implicated in AMD are clustered in several distinct biochemical pathways. You can develop what looks like the same disease many different ways, which is one of the biggest challenges in finding a treatment.'

Deciphering Damage to Mitochondrial DNA

Over the last decade, Professor Ferrington and her colleagues have been uncovering evidence that mitochondrial dysfunction is associated with AMD pathogenesis. The mitochondria are organelles found in the cell in which respiration and energy production occur. They also contain a small amount of DNA, separate from the DNA found in the nucleus. Mitochondrial DNA (mtDNA) contains 37 genes which are responsible for coding 13 proteins essential for producing energy and all of the machinery required to make those proteins. The remaining ~1800 proteins that reside in the mitochondria are encoded by nuclear DNA, produced outside of the mitochondria, and then transported into the mitochondria.

So, what evidence is there to suggest that mitochondrial damage may play a role in AMD? Mitochondria are a major source of superoxide anions in the cell. These anions generate highly toxic radicals and hydrogen peroxide that can damage the cell by reacting with proteins, DNA and lipids. This oxidative stress may play an important role in disease progression, as suggested by the increased levels of antioxidant enzymes that occur in response to the oxidative stress and protein adducts (generated from carbohydrate and lipid oxidation) found in AMD donor eyes. mtDNA is more susceptible to damage from oxidation than nuclear DNA and when mtDNA is damaged, this may interfere with production of key proteins involved in energy production.

Based on this evidence, the team analysed the sub-set of proteins present in the mitochondria isolated from the RPE. They found that a significant number of affected proteins were subunits of the mitochondrial ATP synthase complex (which is responsible for energy production). These results suggest specific pathological mechanisms involving altered translation of the ATPase subunits encoded by either nuclear DNA or mtDNA as well as defects in the transport of nuclear encoded proteins into the mitochondria. 'Mitochondria are a really good target for therapy,' says Professor Ferrington. 'We see mitochondrial dysfunction in Parkinson's and Alzheimer's diseases, so mitochondria are like an Achilles' heel for several age-related diseases.'

Normal Ageing or AMD?

A 2010 study provided further evidence that AMD development damages mtDNA inside RPE cells. Professor Ferrington and her team evaluated the extent of mtDNA damage in human RPE for donors of different ages but who did not have AMD to determine the effect of ageing and compared those results with damage in donors with AMD. The purpose was to distinguish damage associated with normal ageing from damage due solely to AMD.

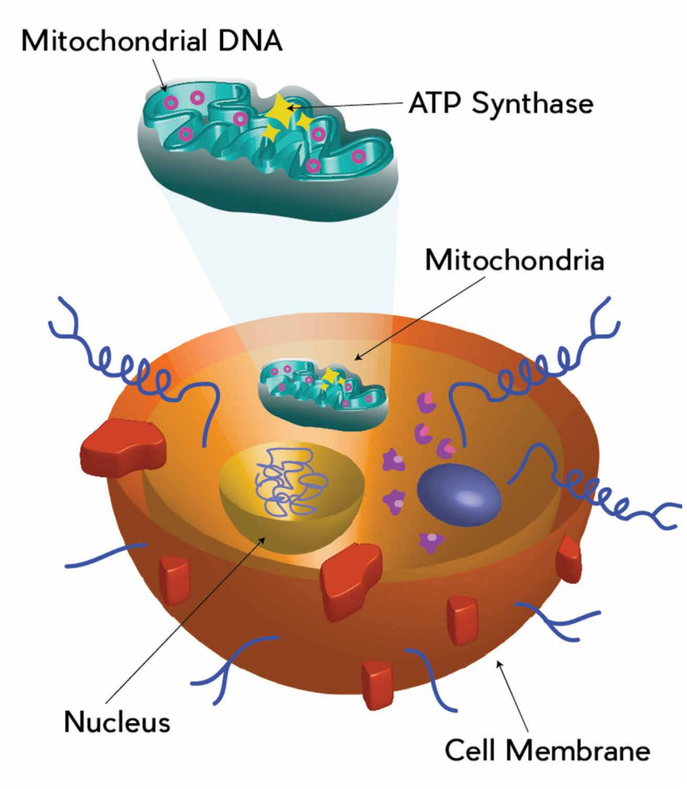
With normal ageing, increased mtDNA damage was limited to a specific region of the mitochondrial genome called the 'common deletion', which is an age-specific modification that had been reported to occur in other post-mitotic cells, such as neurons and skeletal muscle. Conversely, in RPE from donors with AMD, the team observed a significant increase in mtDNA damage throughout the mitochondrial genome. Lesion frequency also increased steadily with disease progression, suggesting a cumulative effect as the disease progressed.

The team also compared lesion frequencies in the mitochondrial and nuclear genomes to determine if mtDNA was more susceptible to damage. In these experiments, AMD tissue showed significant increases in lesion frequency in mtDNA but not in nuclear DNA. This indicates that mtDNA is preferentially damaged with AMD progression.

Identifying Targets for Treatments that Stop Vision Loss

An important consideration for treating AMD is to identify the retinal region (e.g., cell type or geographic area) that should be targeted. To address this point, Professor Ferrington and her colleagues measured the extent and distribution of mtDNA damage in the neural retina (containing glia and neurons, including the photoreceptors) and RPE. They also measured RPE mtDNA damage in the macula and peripheral sections, to test if the macula was selectively damaged in AMD. To gain insight into the potential functional effect of mtDNA damage, small segments of the entire mitochondrial genome were examined to determine whether specific regions are preferentially damaged.

The results showed that mtDNA was limited to the RPE and, as this group had previously shown, lesion frequency increased with disease severity. In contrast, disease stage had no effect on lesions content in the neural retina. 'That was a complete surprise,' Professor Ferrington tells us. 'I had expected that with high damage in RPE the same result would be observed in the neural retina, but that was not so.' The observation



that the retina does not accumulate mtDNA damage with disease progression suggests differences in how AMD manifests in specific tissues. Another important finding was that mtDNA damage was not limited to the macula but was equally abundant in the RPE cells in the peripheral region. This result dispelled a long-held belief that damage only occurs in the macula. Finally, measures of mtDNA damage in small segments of the mitochondrial genome shows that over half of the mitochondrial genome is not significantly damaged in AMD, refining the results of the team's previous reports of genome wide damage to more discrete regions.

These results are important clinically relevant findings – there is now a scientific basis for targeting RPE mitochondria as a treatment strategy. Because this damage occurs before vision loss, early intervention could prevent or at least slow down progression to blindness.

Genetic Risk for Increased mtDNA Damage

Previous genetic analysis of AMD had identified a number of high risk loci associated with the disease. The genes at these loci belong to diverse pathways, suggesting different pathogenic mechanisms lead to the clinical manifestation of the disease and implies that therapies targeting a single pathway will not be effective for all AMD patients. This idea provided the rationale for a 2016 study aimed to determine if individuals with a specific genetic background were at greater risk for mtDNA damage.

Donors were genotyped for several prominent high risk loci associated with AMD and the extent of mtDNA damage was determined in RPE cells. Professor Ferrington and her colleagues found that AMD donors carrying the high-risk allele for the gene complement factor H (CFH) had significantly more mtDNA damage than donors without the gene variant. This supports the hypothesis that the presence of the CFH risk allele makes mtDNA in the RPE more susceptible to damage. CFH is a key regulator of the alternative complement pathway (part of the immune system) that promotes the clearance of debris and dead cells and kills invading pathogens. The role of CFH is to protect host cells from inappropriate complement activation in order to avoid chronic inflammation or damage to healthy cells.

Another discovery was that a small number of healthy donors carrying the risk allele had lower mtDNA damage, suggesting that mitochondrial injury is not a direct consequence of the CFH variant. The team hypothesise that retinal changes associated with disease onset coupled with the presence the gene variant could create cellular conditions conducive for accelerated mitochondrial damage. Overall, this study provides a strong rational for a more personalised approach for treating AMD. Patients harbouring the high-risk allele for CFH may benefit from treatment that stabilise and protect the RPE mitochondria. The potential impact of finding an effective treatment for slowing down AMD progression in this patient subpopulation is immense when considering both their high risk for developing late stage AMD and the high percentage of patients (30–50% of all AMD patients) harbouring this variant.

What Comes Next?

Professor Ferrington and her lab have expanded their focus from discovery science to targeted treatments and are currently testing drugs which might boost mitochondrial function in human donor tissue. Pharmacological treatments such as N-acetyl cysteine are already proving effective in improving mitochondrial function in cultured RPE cells from AMD donors. Her lab has also been making RPE cells using induced pluripotent stem cells (iPSC). They envision using the RPE they create from stem cells to screen potential drug therapies for patients with early AMD or to restore lost RPE cells in patients with advanced disease. For more information, please see this video.

The team will continue to unravel the molecular mechanisms behind ageing. As Professor Ferrington explains, their research is focused on answering: 'What are the cellular changes that occur with ageing? What factors "tip the balance" to pathology? How does the cell respond to disease? How can we protect against pathologic changes? These are the questions that form the core of my research program.'

Meet the researcher

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Professor Deborah Ferrington is Professor and Elaine and Robert Larson Endowed Vision Research Chair in the Department of Ophthalmology and Visual Neurosciences at the University of Minnesota, Twin Cities. After completing her undergraduate degree in Biological Science and Scientific Illustration and a Masters in Education from the University of Pittsburgh, PA, Ferrington went on to receive her PhD in Biochemistry from the University of Kansas, where she also completed a postdoctoral fellowship. She has received several honours over the course of her career, including acting as Executive Board Member for the Ryan Initiative for Macular Research, the Fesler-Lampert Chair in Aging Research and the Elaine and Robert Larson Endowed Vision Research Chair at the University of Minnesota. Professor Ferrington's research aims to develop understanding of ageing at the cellular level witha particular focus on aging retina and age-related macular degeneration. She has successfully secured funding from organisations such as the Foundation Fighting Blindness, American Federation for Aging Research, Arnold and Mabel Beckman Foundation and National Institutes of Health. Professor Ferrington also acts as teacher, adviser and mentor to undergraduate, graduate and medical students and is the Program Director of an NIH-funded training grant that supports the training and education of doctoral and post-doctoral student in the field of aging research.

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