

An eye on genes

Professor Marcela Votruba discusses her investigations into genes associated with mitochondrial plasticity; research that will help to inform understanding of eye disease

PROFESSOR MARCELA VOTRUBA



Firstly, can you give us some insight into the background to this research?

The focus is the role of mitochondrial dynamics in the pathophysiology of human-inherited optic neuropathy and other neurodegenerative disorders. Inherited optic neuropathies are a group of hereditary conditions in which optic nerve dysfunction and optic atrophy arise from loss of retinal ganglion cells (RGC). Patients with inherited optic neuropathies share common clinical features: bilateral, symmetrical, painless reduced visual acuity, colour vision defects, central visual field loss and pallor of the optic disc. The optic neuropathy is generally permanent, may be progressive and is currently irreversible and untreatable. The long-term goal of our research is to understand the disease mechanisms associated with gene defects and to use this to devise new treatments.

We are fundamentally interested in the human disease dominant optic atrophy, which has an incidence of approximately 1:15-20,000. We are studying model systems of RGC loss and inner retinal degeneration, which occurs as a primary phenomenon in these human genetic diseases. Our primary interest is in genes that

are associated with inherited degeneration of the optic nerve. Previous research on the OPA1 and OPA3 genes and human dominant optic atrophies has led us to a strong interest in mitochondrial morphology. OPA1 is known to bind to the inner mitochondrial membrane, and when mutated it is believed that the normal balance of mitochondrial fusion and fission is disturbed and programmed cell death is initiated by the loss of mitochondrial membrane potential and the release of cytochrome c. OPA3, like OPA1, is thought to have a role in mitochondrial membrane dynamics, and when mutated in the human, a complex neurodegenerative disease, comprising optic neuropathy secondary to RGC loss, cataract and neuromuscular degeneration, arises.

Recently, you have engaged in identifying new genetic loci for inherited optic neuropathy. What progress have you made on this front?

We are actively engaged in mapping new genetic loci in families. Genetic linkage studies have revealed that not all families map to known genetic loci. There are clearly still a number of as yet undiscovered genes which may be implicated in inherited optic neuropathies. It is important to find these genes, as this will further our understanding of the pathophysiology of the disease. New genes and proteins may reveal new pathways and cellular and biochemical mechanisms. It will also allow us to help patients and families with genetic and molecular diagnosis, which is really important in order to give an idea of likely future progress of the disease. It is also clinically important to help patients establish their diagnosis in order to give genetic and prognostic counselling. Along these lines we are investigating some exciting new candidate genes.

You have been working with the UK Genetic Testing Network (GTN) to develop a UK approved NHS test. How is this effort progressing?

We received approval in 2007 from the GTN for an OPA1 genetic testing strategy. The GTN Gene dossier for National Health Service (NHS) genetic testing of OPA1 has also been approved for Cardiff. As a result, OPA1 testing at the All Wales Genetic Testing Service has been established and marketed both in the UK and worldwide.

Are there any other prominent partners in your genetic testing efforts?

In the UK genetic testing services are only available as funded tests in the NHS if they have been assessed by the GTN and approved. I have also been working with ASPER Biotech, based in Estonia, on developing a new chip-based screening test for human OPA1 mutation detection for the community. Internationally available OPA1 testing at ASPER Biotech was established and validated in 2006.

How do you see the future panning out for the field of vision and eye research?

I am involved in mentoring, both as a mentor and as a mentee, and I would hope that in the future I could inspire young budding vision researchers, both clinical and non-clinical, to work on eye disease. I am aware that the career structure in academia for young researchers is precarious and I feel that it is important that we build stronger research networks in vision research and that we talk to industry to evolve partnerships for training and career development. We will need the next generation of vision researchers to take forward the new opportunities in the post-genomic era for the benefit for our patients.



Early detection of degenerative eye disease

A **Medical Research Council** funded project is exploring mitochondrial shaping proteins in models of optic neuropathy, delivering exciting opportunities for the future development of ophthalmic care

AS THE WORLD'S population rapidly ages there is increasing pressure on the medical industry to develop improved medication and care for the elderly. The diseases of older generations can be particularly distressing; Alzheimer's, dementias and chronic neurodegenerative diseases all result in a vastly decreased quality of life in the later years of people's lives. Some recent research has identified that dendritic spines, which are small membranous protrusions projecting from a neuron's dendrite, play a significant role in the ageing process. It is also becoming increasingly clear that mitochondrial fusion and fission are critical for normal dendritic spine formation in the central nervous system generally. There is now evidence emerging that loss of dendritic spines, which transmit electrical signals from neuron to neuron, is a feature of many of the classic and common degenerative diseases.

A group of eye and vision experts based at the School of Optometry and Vision Sciences at Cardiff University are investigating the key role genes play in controlling normal mitochondrial plasticity and the impact this has on diseases associated with ageing. Professor Marcela Votruba, who is leading the research funded by the Medical Research Council, is excited about the implications of their studies. She observes that mitochondrial shaping proteins are fundamental to the control of normal cellular physiology, especially in neural tissue, and that they play a

significant role in the pathophysiology of human-inherited optic neuropathy. "Relative expression levels of pro-fusion and pro-fission genes and proteins may be fundamental to mitochondrial morphology in different tissues during development, and this may underlie the basis for tissue specific disease pathophysiology." In a bid to advance understanding of these processes, Votruba's work investigates how these genes affect ageing of the eye in health and disease.

DISSEMINATING THROUGH EVER

There are some key goals this research project is hoping to achieve, particularly to address some of the fundamental issues in degenerative eye diseases. The main aims are to identify evidence for new fusion and fission genes, and to explore both if any are involved in inherited optic neuropathy and also the spatio-temporal expression profile for mitochondrial shaping genes and proteins in the normal developing and adult mammalian eye. The research team are generating murine models, either by conventional transgenesis or by utilising *in vivo* knockdown in the eye, and through this work aim to comprehensively characterise these mutant effects and interactions.

Votruba is an active member of the European Association for Vision and Eye Research (EVER), which is focused on encouraging research and the dissemination of knowledge about eye and vision through the exchange of information. Funding for projects such as Votruba's is vital to help give patients any hope of future treatment. With over 750 members from 67 countries around the world, EVER provides the perfect opportunity to share research and it is through this collaboration, and others like it, that Votruba hopes to be able to disseminate her team's findings.



FIGURE 2.

Colour fundus photograph of left optic disc from a patient with autosomal dominant optic atrophy due to mutation in the OPA1 gene. The visual acuity was 6/24. Note the pale appearance of the optic nerve head.

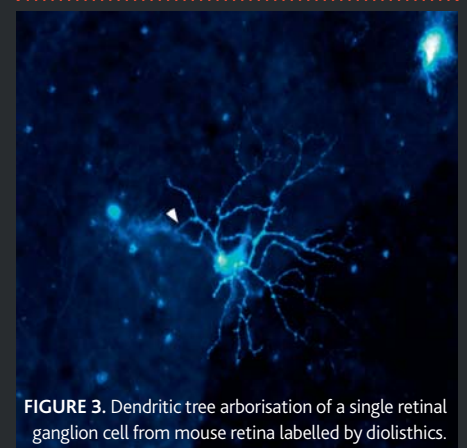


FIGURE 3. Dendritic tree arborisation of a single retinal ganglion cell from mouse retina labelled by diolistics.

RESCUING INTACT RETINAL CELLS

It has become increasingly apparent through this study and others that a collection of mitochondrial shaping proteins function together to maintain the dynamic control of mitochondrial morphology. Votruba describes how such proteins include pro-fusion GTPases, such as mitofusin (Mfn)1 and 2, and pro-fission GTPases, such as dynamic related protein 1 (Drp1) and Fis1. OPA1 and Mfn1 work synergistically to regulate mitochondrial fusion. It is a synergistic relationship because OPA1 is unable to promote mitochondrial fusion in the absence of Mfn1, and Mfn1 cannot induce mitochondrial elongation in the absence of OPA1. Thus, acting as anti-apoptotic GTPases, OPA1 and Mfn1 may

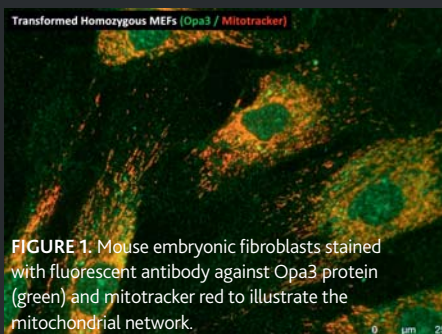


FIGURE 1. Mouse embryonic fibroblasts stained with fluorescent antibody against Opa3 protein (green) and mitotracker red to illustrate the mitochondrial network.

INTELLIGENCE

MITOCHONDRIAL SHAPING PROTEINS IN MODELS OF OPTIC NEUROPATHY

OBJECTIVES

Professor Votruba's work focuses on mitochondrial plasticity and the OPA1 gene, the generation of animal models of mitochondrial fusion – fission defects and new therapeutic targets for retinal ganglion cell loss, inner retinal degeneration and repair. In 2007 her laboratory published a mouse mutant modelling human optic atrophy, carrying a mutation in Opa1.

KEY COLLABORATORS

International:

Professor Anne A Knowlton, MD, University of California, Davis, USA • **Professor Peter Nuernberg, PhD**, Cologne Centre for Genomics, University of Cologne, Germany

National:

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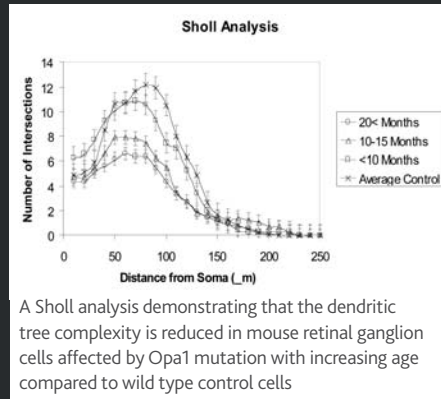
www.cf.ac.uk/optom/staff/votruba.html

PROFESSOR VOTRUBA is an Honorary Consultant in Ophthalmology at the University Hospital of Wales and Professor at Cardiff University School of Optometry & Vision Sciences. She obtained a PhD from University College London and has held Wellcome Trust and MRC Clinician Scientist Fellowships. She is a former Consultant at Moorfields Eye Hospital.



VOTRUBA LAB JUNE 2011: From left to right, top row: Amanda Mui, Peter Williams, bottom row: Elaine Taylor, Caroline Waters, Rebecca Thirgood and Marcela Votruba.

protect the cell from spontaneous apoptosis. However, explains Votruba, there remain as yet unidentified proteins acting in this balance and it is clear that there are complex potential interactions. This is all part of the exploratory work needed to develop some sound and robust findings from the research.



A Sholl analysis demonstrating that the dendritic tree complexity is reduced in mouse retinal ganglion cells affected by Opa1 mutation with increasing age compared to wild type control cells

Being able to take laboratory investigations and findings and converting this into successful practical applications for real life patients is the ultimate goal of this research initiative. Votruba's group has recently been successful in documenting the presence of retinal ganglion cell dendritic tree retraction, pruning and loss of synaptic connectivity, as a marker of early disease state in the OPA1 mutant mouse retina. This has opened up the door to new thinking about the pathophysiology of optic atrophy. "For the first time we have shown that retinal ganglion cells may lose their functional connections and yet remain in situ for long periods," says Votruba.

The other implication of our findings is that we may be able to detect the very earliest changes associated with disease status before there are overt and irreversible signs of vision loss

She believes this highlights the fact that cell soma loss is the end-stage of the disease and that there may be a significant period before this when there are still intact cells present, and that these cells could still be rescued. This is of critical importance because it is always going to be much harder to replace lost retinal ganglion cells than to rescue the living ones that are still in place, as the complexity of their wiring and projections make replacement challenging. The outcome of this latest research has the potential to change our understanding of genetic degenerative diseases. "The other implication of our findings is that we may be able to detect the very earliest changes associated with disease status before there are overt and irreversible signs of vision loss," Votruba asserts. From her perspective this has significant consequences for earlier diagnosis than what is currently possible, thus also making therapeutic intervention much more likely to succeed.

ONE SMALL STEP

Sometimes just one seemingly trivial progression inside the laboratory can end up being one of the most notable successes of an entire research project. In the case of this particular project, cloning the new disease gene OPA1 in 2001 after six years of research was a great moment. Votruba finds it hard to believe just how much this means even now and how incredible it is that their team was involved in such successful investigations. More recently, she is very proud of their new approach to unravelling the effects of OPA1 defects at the retinal ganglion cell synapse and dendrites. The apparent loss of synaptic connectivity, and hence dysfunction, are exciting new avenues for further exploration and Votruba is confident that this just may provide the eye and vision world with some new diagnostic opportunities: "This work may also lead to a deeper understanding of the role that mitochondrial dynamics plays in the retina and future therapeutic targets". An active area of interest for future investigation is glaucoma and the role that mitochondrial dysfunction may have in the pathology of this common blinding disease. It would seem that their work is far from over, much to the benefit of the world's blind.

