Scientia

TRANSFORMING GLOBAL HEALTH THROUGH SCIENTIFIC DISCOVERY

HIGHLIGHTS:

- A Twin DNA Replication Factory
- Confronting the Challenge of Huntington Disease
- Maternal Diabetes and Adult Morbidity in the Offspring: The TEAM Study at Cincinnati Children's Hospital Medical Center
- Ending the Revolving Door of Emergency Department Visits for Older Adults

EXCLUSIVES:

- UK Biobank
- Neuroscience Ireland

Issue #133

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WELCOME...

As 2020 draws to a close and we look towards 2021, the importance of innovation and progress in science, medicine and healthcare has never felt more urgent or critical. Without doubt, COVID-19 has changed the world, and the pandemic has brought science, medicine and healthcare firmly to the fore. This important issue of Scientia celebrates the vital work of scientists seeking to improve the health and well-being of individuals across the globe and provides testimony to what can be achieved, even in the most challenging of times.

The first section in this edition is devoted to the foundational (or basic) sciences in medicine and healthcare. By understanding the biological processes that underpin life, we also gain insight into those responsible for disease. From exploring how life on earth can grow through the process of DNA replication to understanding how the brain can change and reorganise itself, this exciting journey leads us through a diverse range of research that is laying the foundations for a healthier world.

Our second section highlights the researchers who are working to tackle the challenges presented by diseases and disorders with a focus on medical breakthroughs and scientific advancement. Here, we read of much-needed treatment innovation across both commonly occurring and considerably more rare conditions.

The third section is dedicated to recent advances in the promotion of women's health and well-being. From overcoming the complexities of chronic diseases such as diabetes and breast cancer to difficulties with fertility and menopause, we showcase the work of researchers who are focussed on significantly improving the lives of women across the world.

Our fourth and final section celebrates recent innovations in surgical methods and rehabilitation that are improving patient treatment and care. Here, we can read of vital research into preventing and treating spinal cord injury, the potential uses of novel brain imaging technologies and nanoparticles, and finally, how research collaborations are successfully confronting challenges faced by healthcare systems arising from our increasingly ageing population.

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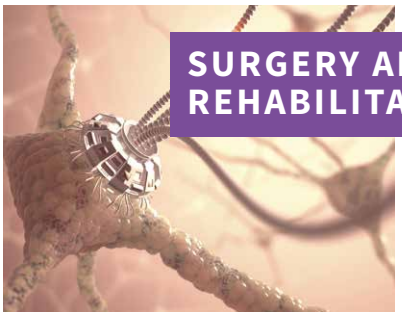

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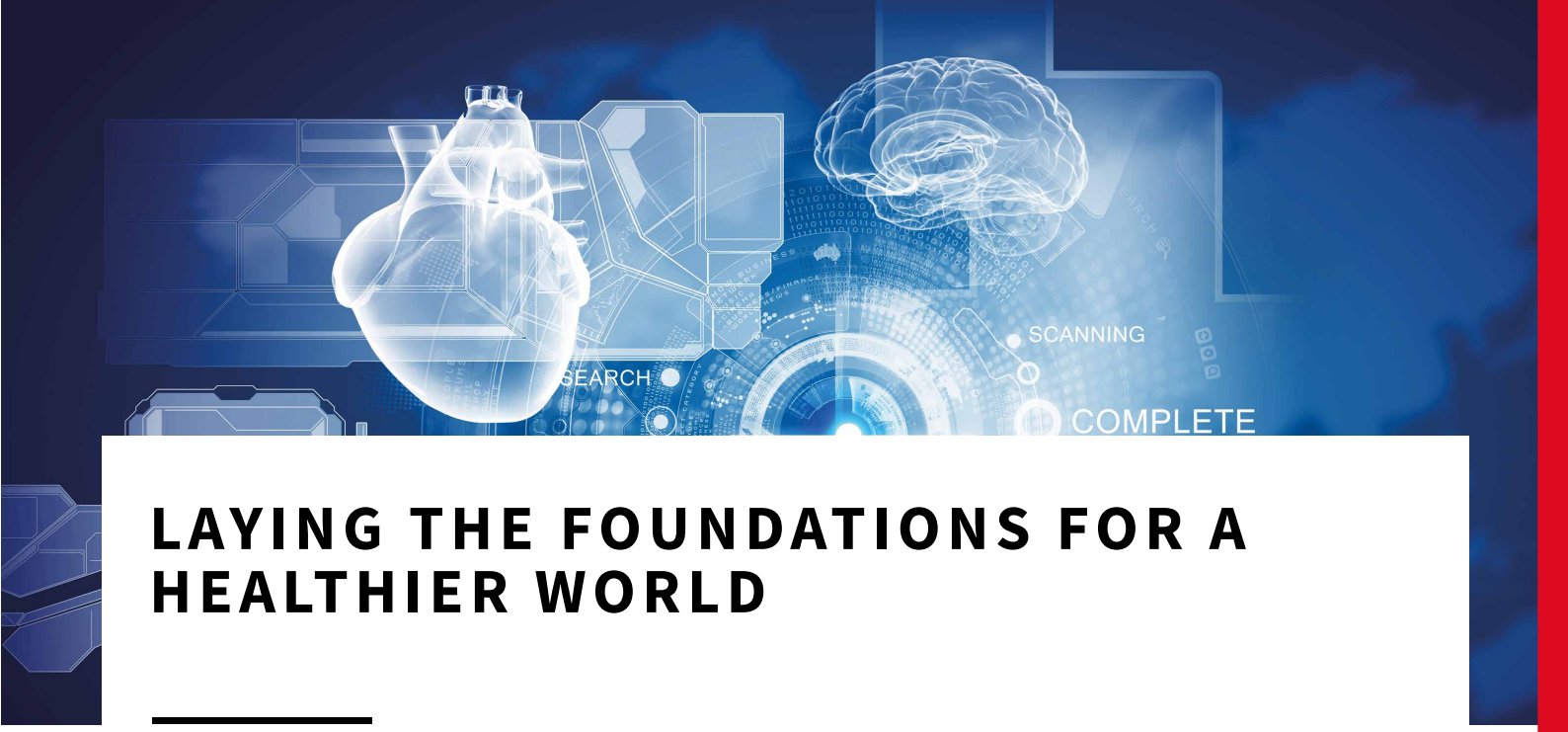


LAYING THE FOUNDATIONS FOR A HEALTHIER WORLD

Researchers in the foundational (or basic) sciences seek to unravel the fascinating complexities of the biological processes underpinning life. Their collective endeavours include the laboratory-based study of molecules, cells and whole organisms. As such, the foundational sciences can also garner a better understanding of the mechanisms underpinning disease and can help identify new targets for treatment. This important issue of Scientia starts by meeting the scientists whose work at laboratory benches is playing a vital role in laying the foundations for a healthier world.

We open this section by meeting Dr Andreas Mayer at the Max Planck Institute for Molecular Genetics in Germany. Dr Mayer has spent more than a decade exploring the mechanisms that control the transcription of DNA to produce RNA and the building blocks of life – proteins. We read how his recent work using newly developed high-resolution genome-wide techniques is elucidating how RNA polymerase II transcription facilitates cells changing from one cell type to another in a process known as cell differentiation.

Remaining on the topic of proteins, we turn Dr Brigitte Pertschy, Dr Ingrid Rössler and Jutta Hafner at the Institute of Molecular Biosciences at the University of Graz. We read how the macromolecules known as ribosomes are responsible for the synthesis of proteins in all living cells, and importantly, how the safe delivery of proteins that comprise the ribosomes is dependent on metaphorical 'private bodyguards'.

Professor Michael O'Donnell, head of the Rockefeller University's DNA replication laboratory, has devoted more than 30 years to understanding how genetic material is copied and reproduced in a process known as DNA replication. His findings represent the exciting beginning of a comprehensive understanding of how replication is organised in the cell.

Cells interact with one another through complex signalling pathways, and furthering our understanding of this is the focus of Professor Kim Dale and her team at the University of Dundee. We read Professor Dale's seminal contributions to understanding how the Notch signalling pathway controls the formation of tissues and organs in the earliest stages of development, and the important implications of this developmental disorders and diseases.

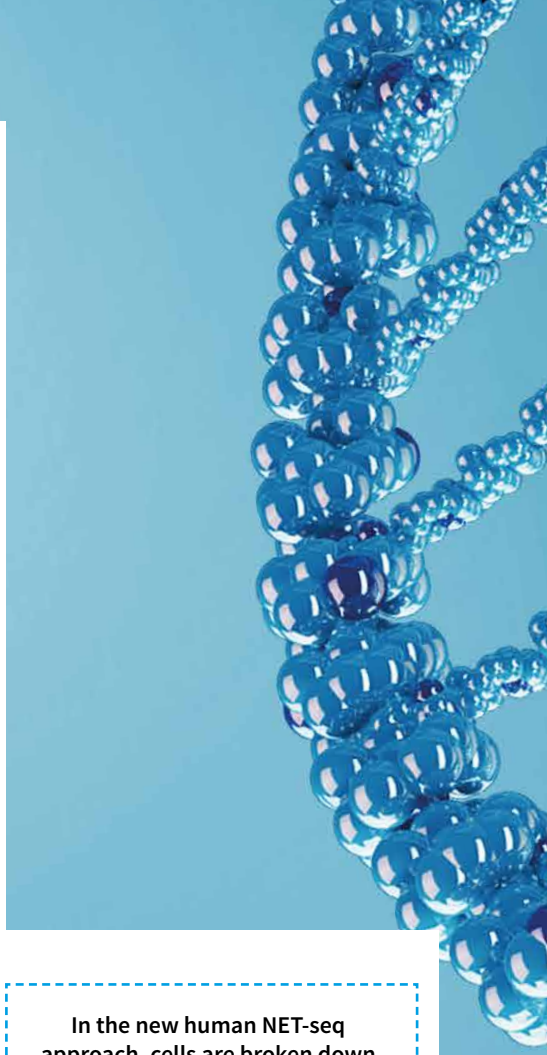
We then turn to the work Dr Elke Glasmacher, Head of Immune and Cell Biology at Roche. We read of her far-reaching research on the molecular mechanisms underlying whether cells are activated or repressed, and her ongoing research programme across disease therapeutic areas at Roche that embraces a range of approaches including single cell sequencing and machine learning.

Nitric Oxide is a molecule produced in our bodies that has a vital role in regulating blood vessels and blood pressure. Dr Alan Schechter at the National Institutes of Health in Bethesda has devoted his remarkable career to better understanding this important compound. We read of his ground-breaking research and, in particular, how this has recently highlighted the relevance of nitric oxide to exercise and sports medicine.

We conclude this section by meeting Dr Nicholas Spitzer from the University of California, San Diego. His work is helping drive forward revolutionary advances in our understanding of how the brain can change and reorganise itself. We read of his research into how and why neurons change their chemical communicator in response to environmental stimuli, and the implications of this for the development of therapeutics for neurological and psychiatric disorders.

GENOME TRANSCRIPTION REGULATION DURING CELL DIFFERENTIATION

The mechanisms that control the transcription of DNA to produce RNA and the building blocks of life, proteins, are a fundamental cellular process in all living organisms. **Dr Andreas Mayer** at the Max Planck Institute for Molecular Genetics in Germany has spent more than a decade unravelling these complex processes. Using newly developed high-resolution genome-wide techniques, his team is discovering the vital role that RNA polymerase II transcription plays in stem cell differentiation, where a cell changes from one cell type to another usually to perform a more specialist function.



Gene Transcription and Translation

Genetic material is stored in the form of DNA in most organisms. In humans, the nucleus of each cell has 3×10^9 (9 zeros) base pairs of DNA, distributed over 23 pairs of chromosomes, and each cell has two copies of the genetic information. This is known collectively as the human genome and contains around 20,000 genes that code for proteins and at least the same number of genes that produce RNA only (non-protein-coding genes).

Converting the genetic information contained in DNA into RNA and proteins is one of the most important and highly regulated tasks performed within cells. Genes are small segments of DNA that provide the code (through combinations of four nucleotides: adenine, cytosine, guanine, and thymine) to synthesise specific RNAs and proteins using two fundamental cellular processes: transcription and translation.

Transcription is the process of producing a strand of RNA, either a messenger RNA (mRNA, protein-coding RNA) or a non-protein-coding RNA, from a DNA template. It comprises three stages: initiation, elongation, and termination. In the initiation stage, the enzyme RNA polymerase II (Pol II) attaches with the help of other proteins, so-called general transcription factors, at the promoter region on each gene. Certain general transcription factors then begin to untwist the two nucleic acid strands of the DNA double helix to allow loading of the DNA template strand into the catalytic centre of Pol II and transcription initiation to occur. During elongation, Pol II creates a new strand of precursor RNA such as a precursor mRNA (pre-mRNA), by adding the complementary nucleotides (with uracil replacing thymine) to the template strand of DNA, which then reforms back into the double helix. Sequences called polyadenylation signals on each gene signal to the Pol II that the RNA transcript is complete.

The pre-mRNA that is released from Pol II can undergo further modification through the addition of molecular 'caps' at one end and a tail to the other end, which stabilise the RNA, and through splicing, remove redundant parts of the code and re-join the remaining

In the new human NET-seq approach, cells are broken down (lysed) and transcribing human RNA polymerases are purified by chromatin fractionation. Next, the 3' ends of the nascent RNA that contain the last nucleotide that was added to the RNA chain prior to cell lysis, are concerted into a sequencing library. With modern, so called 'next-generation sequencing techniques', they can then identify the nucleic-acid sequences as well as the abundance of the ends of these RNA transcripts – and match them to the known cellular DNA sequence to establish the transcripts' and thus RNA polymerases' precise locations. This technique enables researchers to also determine all the points on a cell's genome where transcription activity was busiest, at the time the cell was lysed. Application of the human NET-seq method revealed the fine structure of RNA polymerase transcription and of transcriptional pausing along genes (Figure 2: NET-Seq Tracks).

Box 1: Human NET-Seq Approach. Credit and copyright: Dr Andreas Mayer/ Mayer Laboratory.

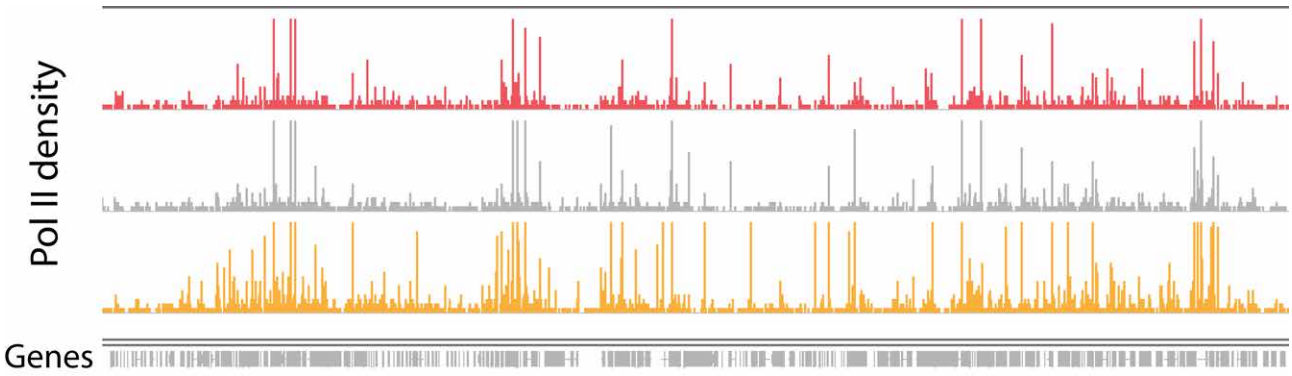


Figure 1: NET-Seq Tracks. Credit and copyright: Dr Andreas Mayer/Mayer Laboratory.

Figure 2: Pervasive Pausing. Credit and copyright: Dr Andreas Mayer/Mayer Laboratory.

pieces back together. The mRNA is then transported from the cell nucleus to the ribosome for translation, where the mRNA provides a template for protein building enzymes to build new proteins.

Cells regulate precisely the transcription of each gene and subsequent production of each required RNA and protein. However, the mechanisms controlling regulation have proven difficult to observe in living cells.

'The Captivating Complexity of Regulatory Mechanisms'

Dr Andreas Mayer, at the Max Planck Institute for Molecular Genetics (MPIMG) in Germany, describes how his interest in the 'captivating complexity of regulatory mechanisms' controlling Pol II transcription began with his PhD research, working in Professor Patrick Cramer's group at Ludwig-Maximilians University, Munich. In a summary of his career to date, he further notes, 'I have now spent more than a decade unravelling the regulatory principles governing RNA polymerase II transcription. This process is fascinating because it underlies nearly all fundamental eukaryotic cell processes.'

In Munich, Dr Mayer began using quantitative genome-wide approaches to discover new general control mechanisms of Pol II transcription in yeast. His work identified the importance of regulatory mechanisms that act downstream of transcription initiation. For decades, it was thought Pol II transcription was predominantly regulated during the initiation phase, but the available technology was not capable of providing accurate location of RNA polymerases that are engaged in transcription in living cells at a single nucleotide level resolution. Dr Mayer notes, 'Knowing the exact locations of transcribing RNA polymerases across the genome is an important step towards understanding how transcription is regulated and misregulated in diseases'.

In 2012, Dr Mayer relocated to Harvard Medical School (HMS) in Boston, Massachusetts, to pursue his goals. He explains, 'To overcome these major technical limitations and discover if post-initiation regulation played a role in mammalian cells, I joined Dr Stirling Churchman's laboratory.' The previous year, Dr Churchman and Dr Jonathan Weissman of the University of California, San Francisco, had developed a new technique for analysing transcription with high precision in yeast, called Native Elongating Transcript Sequencing (NET-Seq). NET-Seq is a high-resolution genome-wide approach, which provides a quantitative measure of newly formed RNA and that allows the genomic localisation of RNA polymerases at nucleotide resolution. However, the original NET-Seq protocol for yeast was not amenable for mammalian cells including human cells.

Dr Mayer continued, 'With my colleagues in Dr Churchman's laboratory I co-developed the human NET-Seq approach (Box 1: Human NET-Seq Approach), allowing us to define the Pol II transcriptional landscape with single-nucleotide resolution (Figure 1: NET-Seq Tracks) and, most importantly, to discover pervasive Pol II transcriptional pausing (Figure 2: Pervasive Pausing). This finding suggested that post-initiation transcription regulation seems to be much more prevalent than anticipated, leading me to wonder how we could identify the potential functions of these events.'

‘I am convinced that bridging the gaps between high-resolution functional genomics, transcription, and cell differentiation will allow important discoveries in the years to come.’

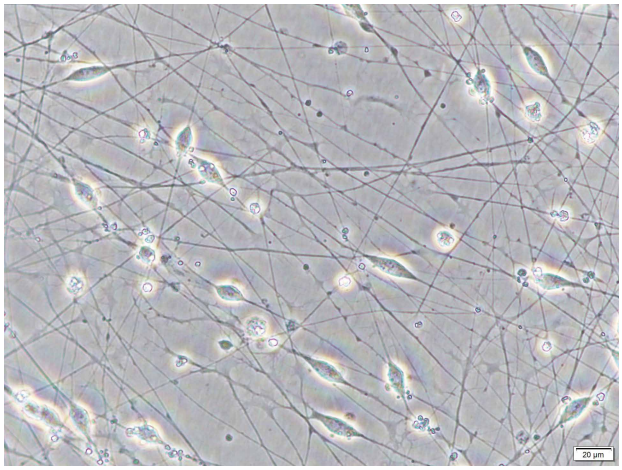


Figure 3. Neuronal Cells. Credit and copyright: Dr Olga Jasnovidova/Mayer Laboratory

The team’s critical finding that previously observed transcriptional pausing *in vivo* was much more prevalent than anticipated also expanded upon the earlier discoveries of widespread transcriptional pausing in the promoter-proximal region of genes in Drosophila and mammalian cells. RNA synthesis was observed to be a discontinuous process, during which phases of productive transcription were frequently interrupted by regular transcriptional pauses of Pol II during elongation and termination (Figure 2: Pervasive pausing). Whilst the causes and consequences of this are still poorly understood, it suggests that post-initiation transcription pausing enables many further additional opportunities for regulating gene expression after Pol II has begun transcribing the gene.

The open and creative working environment at HMS enabled a broad range of researchers from the Departments of Genetics, Genome sciences, and Medicine from HMS, and the University of Washington to collaborate on this work. Dr Mayer describes how he was able to broaden his skills and interests. ‘[The environment] enabled me to interact closely with Professor George Church’s laboratory. I was exposed to a range of cell differentiation systems and taught the practical skills required to handle and manipulate stem and differentiating neuronal cells.’

Pol II Pausing and Cell Differentiation

In 2017, Dr Mayer established an independent research group at the Max Planck Institute for Molecular Genetics (MPIMG) in Berlin, Germany. As he now explains, ‘Starting my own independent Research Group at the MPIMG…has enabled me to continue working on deciphering the regulatory mechanisms of Pol II transcription.’

The Max Planck research group’s primary goal is to reveal the principles that dynamically control and coordinate gene expression, and that drive cell differentiation using neuronal cells. Neuronal cells provide an ideal system to study, due to the extensive transcriptional changes that occur in neurogenesis, as embryonic stem cells differentiate into functional neurons (Figure 3: Neuronal Cells).

Dr Mayer enthusiastically describes the work of his new team. ‘We have successfully adapted a neuronal cell differentiation system, and I am excited that we are poised to use this model to investigate the dynamics of Pol II gene transcription and its function in cell lineage determination.’

Genome-wide approaches have already revealed that Pol II pausing is especially prevalent on genes that control cell development and differentiation, and there is a growing list of pausing regulatory proteins that tightly regulate the process. How the widespread transcriptional pausing controls gene expression and how it affects cell differentiation remains unclear, however.

The Mayer group is addressing these fundamental questions by bringing together a team with a range of interdisciplinary skills, using high-resolution genome-wide approaches, genome engineering techniques, genetics experiments, and bioinformatics tools. The team is also developing new quantitative methods to better investigate the molecular mechanisms that underlie transcription in mammalian cells.

Future Impact

The future work by Dr Mayer and his colleagues will undoubtedly contribute further insights into these unexplored and developing areas of research. The group aims to identify the general principles and dynamics of gene regulation during stem cell differentiation, in order to fully understand the complex and interrelated regulatory logic of genome transcription.

This work has broader implications too, with increasing evidence that misregulation of Pol II transcription and transcriptional pausing has a significant role in a broad range of human diseases and syndromes including cancer, autoimmunity, neurological disorders, diabetes, cardiovascular disease, and obesity. Dr Mayer’s work will address the molecular basis for many of these transcriptional defects and may ultimately contribute to new treatments to prevent or cure these conditions in the future. He concludes, ‘I am convinced that bridging the gaps between high-resolution functional genomics, transcription, and cell differentiation will allow important discoveries in the years to come.’

Meet the researcher

Dr Andreas Mayer
Max Planck Institute for Molecular Genetics
Berlin
Germany

Dr Andreas Mayer received his PhD (summa cum laude) in 2012 from Ludwig-Maximilians University (LMU), Munich, working as part of Professor Patrick Cramer's group studying genome regulation. In the same year, he was awarded the Paula and Richard von Hertwig Award from Helmholtz Zentrum München for interdisciplinary cooperation. In the following 5 years, Dr Mayer joined Dr Stirling Churchman's laboratory at Harvard Medical School in Boston, where he co-developed the human NET-seq approach as part of his specialist study of RNA polymerase II transcription. In 2012, Dr Mayer received an EMBO Long-term Fellowship and between 2013–2016, he was awarded the Human Frontier Science Program Long-term Fellowship. Since 2017, Dr Mayer has led his own independent research group at the Max Planck Institute for Molecular Genetics in Berlin. The group's work aims at bridging the gap between high-resolution functional genomics, genome transcription and stem cell differentiation.

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FURTHER READING

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DEDICATED 'BODYGUARDS' FOR THE SAFE DELIVERY OF ESSENTIAL PROTEINS

Ribosomes are undoubtedly one of the most essential cellular components in life. These macromolecules are responsible for the synthesis of proteins in all living cells. **Dr Brigitte Pertschy**, Dr Ingrid Rössler and Jutta Hafner at the Institute of Molecular Biosciences at the University of Graz, Austria, have discovered that the safe delivery of essential ribosomal proteins that make up the ribosomes is dependant on 'private bodyguards' or 'chaperones'.

Nascent Ribosomal Proteins Journey Across the Cell to the Nucleus

The ribosome is the intricate nano-machinery that translates messenger RNA strands (mRNA) into protein. Our DNA holds the instructions for building every protein needed for our bodies to function. Initially, DNA is transcribed into mRNA, which contains the amino acid sequence of a particular protein. This mRNA strand is processed or translated by the ribosome whereby the amino acids that correspond to the mRNA sequence are recruited and added to a growing peptide chain. The amino acids are linked via peptide bonds and once the full mRNA sequence is translated and the amino acid chain is complete – the protein is now formed and released for use.

Proteins are essential for countless critical functions throughout the body, from cell structure to the regulation of tissues and organs. Ribosomes can be thought of as 'protein-factories' and these extremely important components are made up of ribosomal RNA (rRNA) molecules and ribosomal proteins (r-proteins). The assembly of ribosomes, known as ribosome biosynthesis, is a highly complex, multi-step process and is the specific interest of Dr Brigitte Pertschy and colleagues Dr Ingrid Rössler and Jutta Hafner, from the Institute of Molecular Biosciences at the University of Graz in Austria.

Ribosome synthesis is an important and continuous process. Dr Pertschy describes that a growing cell requires up to 1,000 ribosomes to be synthesised per minute. The r-proteins are produced in the cell cytoplasm by the ribosome itself (that way, the ribosome participates in its own reproduction). From there the r-proteins must travel to the cell nucleus where in a complex maturation process they are joined with the rRNA to form a nascent ribosome. This precursor ribosome further matures and is transported back to the cytoplasm where the mature ribosome performs its function in protein synthesis. During their transit to the nucleus, r-proteins tend to aggregate and become non-functional. Dr Pertschy and her team had been studying the assembly path of r-protein Rps3 when they discovered that certain chaperone proteins could prevent the aggregation of r-proteins. These chaperones accompany the r-proteins on their journey from the cytoplasm to the nucleus and aid their successful incorporation into new ribosomes.

Dr Pertschy and her colleagues explain that there are a number of mechanisms to counteract the aggregation of newly synthesised r-proteins. These include general mechanisms used by many cellular proteins like a general chaperone network that is involved with protecting most new proteins from degradation at the very early stage of their synthesis and proper folding of the proteins. Importins have also been reported as aides in the import of proteins to the cell nucleus as well as in protecting proteins from aggregation.

The team speculated that since r-proteins are produced at extremely high amounts and their correct functioning is so critical for a cell, these general mechanisms acting on most proteins might be insufficient to fully protect r-proteins, and that there must also be more specific mechanisms by which r-proteins are protected until they arrive at their final destination. Further investigations by Dr Pertschy's team revealed that some r-proteins have their own personal chaperones to protect them from aggregation on their journey to the nucleus to join their rRNA counterparts.

Dedicated R-Protein Chaperones to Help Along the Journey

Other research groups, including Dr Pertschy's collaborator, Dieter Kressler and his team at the University of Fribourg's Department of Biology, have also observed the existence of dedicated r-protein chaperones. Dr Pertschy, Dr Rössler and Ms Hafner explain that these dedicated chaperones are able to protect their specific client r-proteins by exploiting different structures and binding mechanisms.

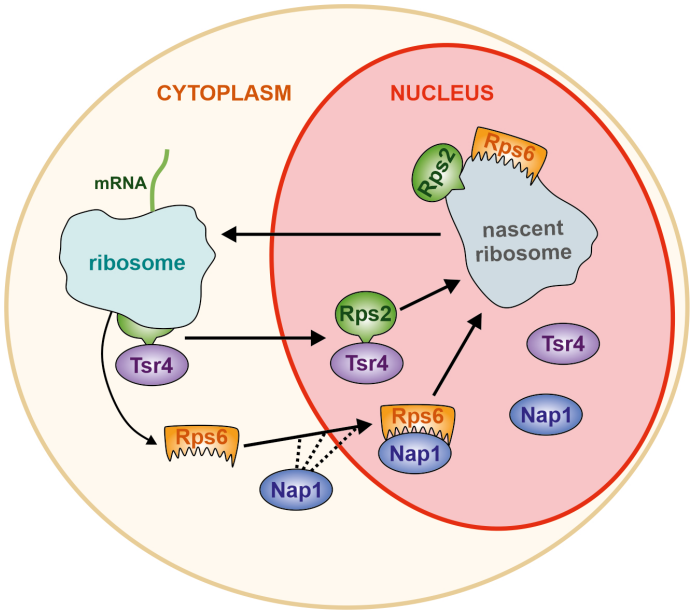
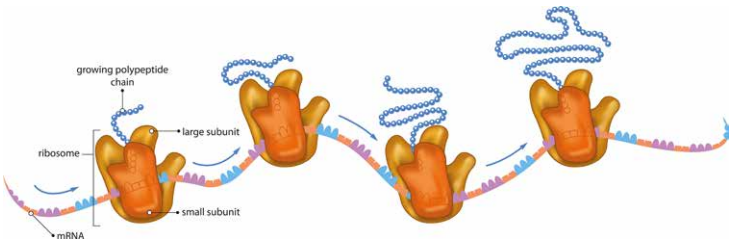


Figure 1: Tsr4 and Nap1, the dedicated chaperones of Rps2 and Rps6. While Tsr4 binds Rps2 already while it is being synthesized by the ribosome, Nap1 binds Rps6 at a later timepoint (after it has been synthesized and released from the ribosome). Whereas Tsr4 binds to a very small region on Rps2, Nap1 requires almost the entire Rps6 protein for binding. After delivery to the nucleus, both ribosomal proteins are incorporated into nascent ribosomes. These undergo a complex maturation process and are transported back to the cytoplasm, where the mature ribosomes can start their job in translation. Credit Brigitte Pertschy.



There are around 80 different r-proteins in eukaryotic cells of which dedicated chaperones of only nine had been identified. Dr Pertschy and colleagues had already studied and reported the importance of these dedicated chaperones and realised that there were many more yet to be discovered. They presumed that the reason why many dedicated r-protein chaperones had remained unidentified was that r-proteins are bound to their dedicated chaperones only during a very short window of time in their lifetime. This means that at a given time point, only a very small fraction of the r-protein is together with its dedicated chaperone, while the majority of the population of this r-protein is bound to its interaction partners within the mature ribosome. With this, the team led by Dr Pertschy set out to establish a method suitable to identify novel dedicated r-protein chaperones among all the other, much more abundant interaction partners of r-proteins. Their rationale was that while these abundant interaction partners are usually common to all r-proteins, dedicated r-protein chaperones should be found only with one or few different r-proteins. Having this in mind, they employed a strategy in which they purified more than 20 different r-proteins and identified all interaction partners by semi-quantitative mass spectrometry. In the subsequent analyses, they ignored all the abundant interaction partners present in all purifications and searched for those interaction partners which were specifically co-enriched with only one, or very few different r-proteins (which were present in the preparations only at low quantities).

The team first investigated if the known chaperones of r-proteins Rps3 and Rps14 were specifically co-enriched in this approach. Results showed that Yar1 had strongly co-enriched with Rps3 and this further confirmed Yar1 is a chaperone dedicated to Rps3. Furthermore, the dedicated chaperone Fap7 was also strongly enriched with its client r-protein Rps14.

The Search for Novel R-Protein Chaperones

Dr Pertschy tells us, 'we are on the one hand exploiting our data to identify additional, so far undiscovered dedicated ribosomal protein chaperones, and on the other hand investigating the cellular function of already identified dedicated ribosomal protein chaperones.' Having demonstrated the direct interaction between some r-proteins and their known dedicated chaperones, Dr Pertschy and her team next focused on analysing the data of two 40S r-proteins for which dedicated chaperones had not yet been identified, Rps6 and Rps2. A protein called Nap1 was, in comparison with all the other r-protein purifications, clearly enriched with Rps6. Nap1 is indicated in DNA repair and as a regulator of cell division. The latest results from Dr Pertschy's team now indicate that Nap1 is also likely to function as an r-protein chaperone for Rps6.

In the Rps2 purification, the team found a protein which was absent from all other r-protein purifications, Tsr4. Tsr4 is known as an acidic protein conserved in eukaryotic cells. Dr Pertschy notes that Tsr4 was previously reported by others to lead to defects in the maturation of the 40S subunit when depleted. However, the nature of its function in ribosome biosynthesis had not been known until now and these new data supported the notion that Tsr4 may be a novel Rps2 chaperone.

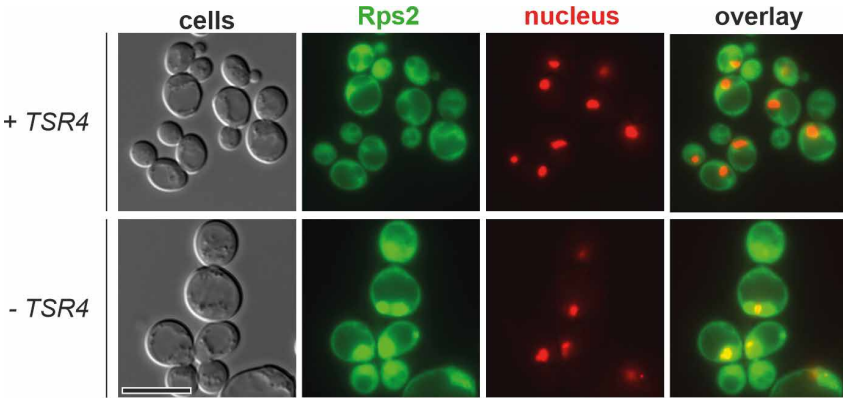


Figure 2: In the presence of Tsr4, Rps2 is mainly present in the cytoplasm, where mature ribosomes are found (no overlap with red nuclear signal). When Tsr4 is absent, Rps2 gets stuck in the nucleus (overlap of green and red signal), likely because it cannot be incorporated into nascent ribosomes and therefore does not travel back into the cytoplasm. Credit Brigitte Pertschy.

Subsequent experiments confirmed the direct interaction between Rps6 and Nap1 and also between Rps2 and Tsr4. It was found that when Rps6 was expressed alone most of the protein was insoluble, whereas when it was co-expressed with Nap1, the r-protein became almost completely soluble. Similarly to Nap1, Tsr4 also increased the solubility of its r-protein client, Rps2. These results suggest that Nap1 and Tsr4 are indeed novel dedicated r-protein chaperones. Through genetic mutation studies, the team also demonstrated the importance of Nap1 and Tsr4 in ribosome biosynthesis, leading to the model that Nap1 and Tsr4 are crucial for the efficient assembly of Rps6 and Rps2 into 40S particles.

Considering that dedicated r-protein chaperones are a very new protein class, there is not much knowledge about how they are functioning. With the thought in mind that a better understanding of the function of Nap1 and Tsr4 may also help to better understand the function of dedicated r-protein chaperones in general, the team sought to further analyse the direct interaction of the newly identified chaperone proteins with their r-protein clients.

When studying in more detail how Nap1 and Rps6 bind, the team found that almost the entire Rps6 protein is contributing to binding to Nap1. On the contrary, for Tsr4 to fully interact with its r-protein Rps2, a very small area of Rps2 is completely sufficient (Figure 1).

Differences were also observed concerning the timing of binding of the dedicated chaperone to its ribosomal protein. Nap1 does not bind Rps6 immediately when it is synthesised, but at a later time point, although it is unclear if that is still in the cytoplasm or after transport of Rps6 into the nucleus. In contrast, Tsr4 binds to Rps2 already during its production in the cytoplasm (Figure 1). The team moreover observed that when Tsr4 is deleted, Rps2 can nevertheless be transported into the nucleus, where it gets 'stuck' and is not assembled into a ribosome, indicating how critical Tsr4 is for Rps2's incorporation into ribosomes (Figure 2).

This study demonstrated that Nap1 and Tsr4, although sharing the function of protecting their r-proteins from aggregation, thereby aiding the efficient execution of ribosome biogenesis, greatly differ with respect to their binding spectrum, binding mechanism and timing of action. This highlights the diversity of the members of the group of dedicated r-protein chaperones, which employ different kinds of mechanisms to reach a similar goal.

Implications That Go Beyond the Cell

The research of Dr Pertschy, Dr Rössler, Jutta Hafner and their colleagues highlights the importance of dedicated chaperones for r-proteins during ribosome biosynthesis. This work paves the way for further studies to identify the yet undiscovered chaperones of the remaining r-proteins and understanding the common mechanisms of this unique class of protective proteins. Dr Pertschy tells us that 'ribosome biosynthesis defects can lead to diseases such as cancer and bone marrow failure,' so a deeper understanding of the intricacies of the process will undoubtedly contribute to a better understanding of certain diseases and the development of potential treatments.

Meet the researcher

Dr Brigitte Pertschy
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Austria

Dr Brigitte Pertschy received her PhD from the Institute for Microbiology at the University of Graz in Austria. Following several years of postdoctoral research at the University of Heidelberg in Germany and the University of Graz, she is currently a research group leader at the Institute of Molecular Biosciences at the University of Graz. Dr Pertschy has focused her research over the years on the ribosome biogenesis pathway including the ribosome assembly path of ribosomal proteins, their nuclear import, and the function of dedicated chaperones of these proteins. Dr Pertschy is a reviewer of several scientific journals including Nature Communications, editorial board member for the journal Microbial Cell and a member of multiple scientific societies.

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FURTHER READING

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A TWIN DNA REPLICATION FACTORY

For life on Earth to grow, its genetic material must be copied and reproduced in a process known as DNA replication. **Professor Michael O'Donnell**, head of the Rockefeller University's DNA replication laboratory, has devoted his over 30-year career to the study of the protein complex that is responsible for just that – the replisome. Recently, Professor O'Donnell and his team uncovered exciting insights into the function of this remarkable piece of molecular machinery.

Behind the Scenes of DNA Replication

From conception, a single fertilised egg cell faces exponential growth in order to become a two trillion cell new-born. During each round of cell division, DNA – copied at a rate of 25 nucleotide units per second – requires extreme accuracy during replication, since any mistake has the potential to be fatal. DNA stores the genetic blueprint of every organism in a series of nucleotide chemical bases known as adenine (A), thymine (T), guanine (G), and cytosine (C). These four letters form a unique genetic code underpinning the characteristics of nearly every life form on Earth.

DNA consists of two complementary, anti-parallel strands that coil around a common axis in the shape of a double helix. A base from one strand pairs with a base from the anti-parallel strand in the following manner: A pairs with T, and G pairs with C. While the order of bases is ever-changing, the manner in which they pair is not. It may seem paradoxical, therefore, that DNA replication occurs so smoothly. Enter the replisome.

'Every time that a cell divides to form two new cells, the DNA instructions for life must be duplicated in a timely and accurate fashion', emphasises Professor Michael O'Donnell from the Rockefeller University, describing what has been the focus of his work for over thirty years. 'This remarkable feat is accomplished by a machinery somewhat like a sewing machine composed of many protein "gears" that function together, referred to as a replisome.'

Professor O'Donnell heads the Rockefeller University's DNA replication laboratory and has devoted his career to understanding the unique architecture of proteins contained within the replisome, by studying both their physical structures and biochemical activities. His work aims to provide new insights into cellular replication, repair, and genetic inheritance.

From Baker's Yeast to Humans

Over a billion years of evolution, the components of the replisome have been largely conserved across the animal, plant and fungi kingdoms. One of the leading protein 'gears' found within is helicase, responsible for separating the two DNA strands. DNA must be unwound for replication to occur, going from a tightly coiled double helix to two straight lines, much like the unzipping of a zip. From baker's yeast to humans,

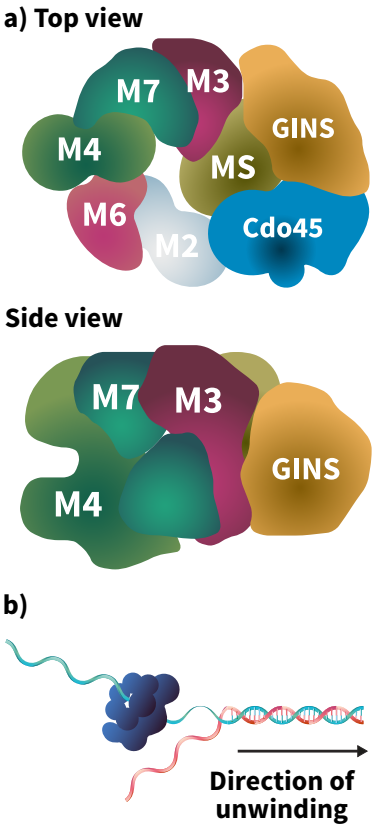

Figure 1: CMG helicase. A) Top and side views of CMG, top, side. B) DNA unwinding is achieved by helicase encircling one strand and excluding the other strand. The helicase motors along the strand it encircles and acts like a wedge to split the DNA duplex into two single-strands.

‘Every time that a cell divides to form two new cells, the DNA instructions for life must be duplicated in a timely and accurate fashion…This remarkable feat is accomplished by a machinery somewhat like a sewing machine composed of many protein “gears” that function together, referred to as a “replisome”’.

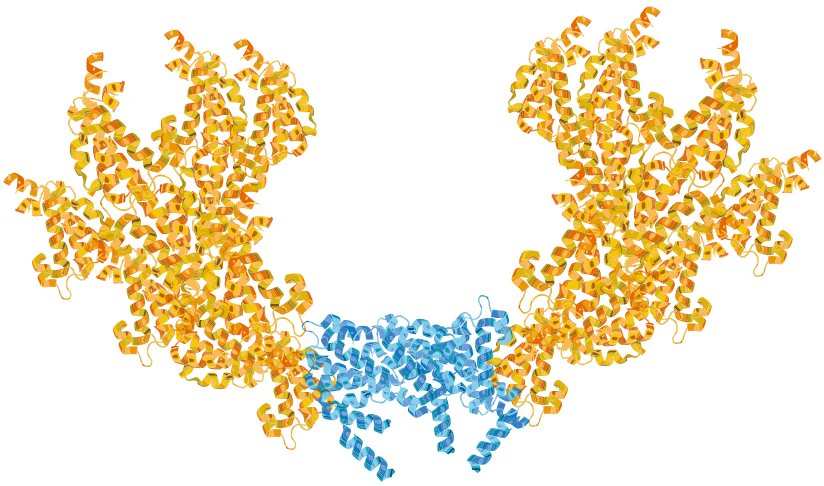

Figure 2. Twin CMGs. The Ctf4 trimer (blue) holds two CMG helicases (orange) in a nearly vertical position relative to the Ctf4 disk. This structure is based on Z Yuan et al, 2019. The Ctf4 trimer organises two sister replisomes and one primase-polymerase alpha into a replication factory core.

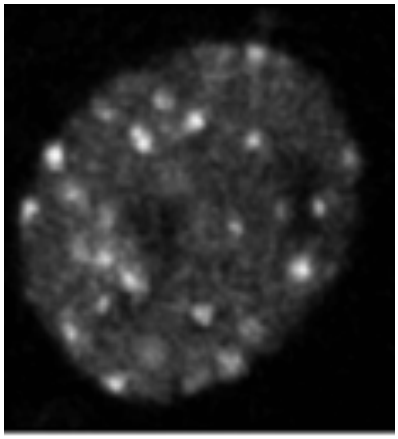

Figure 3: Nuclear foci. PCNA was fused to GFP and imaged in yeast nuclei. Citation: E Kitamura, et al, 2006. Live-cell imaging reveals replication of individual replicons in eukaryotic replication factories, Cell, 125, 1297–1308.

the helicase found within the replisome is an 11-protein assembly called CMG, named for its three components: Cdc45 protein, the MCM2-7 motor ring, and GINS complex.

This unwinding is achieved by CMG encircling one strand of a double-stranded DNA molecule via its MCM ring and excluding the other strand to the outside of the ring. Then, CMG tracks along the encircled strand, acting as a moving wedge to split the DNA duplex apart.

CMG’s other two elements – the Cdc45 protein and GINS complex – are used to attract and capture other protein components of the replisome machine. These include DNA polymerases, used to synthesise DNA by assembling individual nucleotides (building blocks) of DNA; a ring-shaped sliding clamp called PCNA, used to tether DNA polymerases to parent strands of DNA; a clamp loader protein that allows PCNA to clamp around DNA; and a primase, called DNA polymerase α-primase (DNA pol α-primase), which synthesises primers required to initiate DNA replication.

A Factory of Twin Replisomes

DNA replication occurs in the nucleus, or central organelle, of each cell. ‘It has long been known that replication proteins localise to various “spots” in the nucleus that can be observed in the microscope and are referred to as replication foci’, explains Professor O’Donnell. ‘These are the sites of DNA replication. Studies in yeast showed that these spots mainly consist of only two replisomes. Therefore, it would appear that replication occurs in factories of twin replisomes.’

Indeed, Professor O’Donnell and colleagues discovered that a novel player is crucial to the formation of the twin replisome factory: a protein called Ctf4 (Chromosome Transmission Fidelity 4). Ctf4 is a homotrimer, meaning it has three identical subunits, and two of these are used to tightly bind CMG molecules. Nevertheless, the exact structure of this twin CMG-Ctf4 unit remained unknown. This is where Professor O’Donnell teamed up with Dr Huilin Li of the Van Andel Institute in Michigan, an expert in a technique known as cryogenic electron microscopy (cryo-EM) used to examine the high-resolution structures of biomolecules.

With the help of Dr Li, Professor O’Donnell discovered that the mystery CMG-Ctf4 structure was that of two CMG helicases oriented in a head-to-head fashion around one Ctf4 protein. This unique layout explains the observation that DNA foci in yeast contain two replisomes. In humans, however, nuclear foci are bigger but super high-resolution imagery has revealed that each large focus is simply composed of many sub-foci, and each of these sub-foci is a twin replisome. Professor O’Donnell and his team thus uncovered a key finding underpinning the DNA replication factory: twin replisomes are held by a Ctf4 scaffold in a head-to-head fashion.

CMG Unwinds DNA in the N-first Direction

DNA replication is an inherently dynamic process: a helicase must traverse an entire DNA molecule in order to split it into two strands and enable duplication. CMG, like other proteins, has an asymmetric structure, defined by the N- and C-ends of the proteins. The orientation of CMG as it travels on DNA was another mystery that the O'Donnell laboratory was keen to solve. Does it travel N-first or C-first?

The replication factory mechanism occurs as follows: a parental, double-stranded DNA molecule feeds into the system and is separated into two single strands. Of these, one strand threads through CMG, while the other remains outside the CMG ring at the centre of the factory. Professor O'Donnell, with the help of Dr Li, used cryo-EM to enable direct visualisation of this process to investigate CMG's direction of travel. 'We determined the orientation of CMG helicase while it travels on DNA, which was opposite from the orientation that had been assumed by the field for over a decade. This "challenge" has been confirmed by other labs now', explains Professor O'Donnell.

This visualisation showed the fates of each DNA strand. After being threaded through CMG, one DNA strand was duplicated by a polymerase ε protein (DNA pol ε). DNA pol ε, which directly bound CMG, was held in place by the aforementioned PCNA clamp which encircles double-stranded DNA. The duplication of the strand not threaded through CMG, termed the lagging strand, was attended by DNA polymerase δ (DNA pol δ). DNA pol δ replicated the lagging strand thanks to the primers synthesised by DNA pol α-primase while also being held in place by the PCNA sliding clamp.

This cryo-EM visualisation led to another key discovery in solving the replisome puzzle: only one DNA pol α-primase could bind the Ctf4 trimer. Consisting of three subunits, the Ctf4 trimer, therefore, bound two of these to CMG molecules, and the third to DNA pol α-primase, implying that DNA pol α-primase must split its primer forming activity between the two lagging strands. Slowly but surely, Professor O'Donnell was shining a light on the previously hidden intricacies of the replisome.

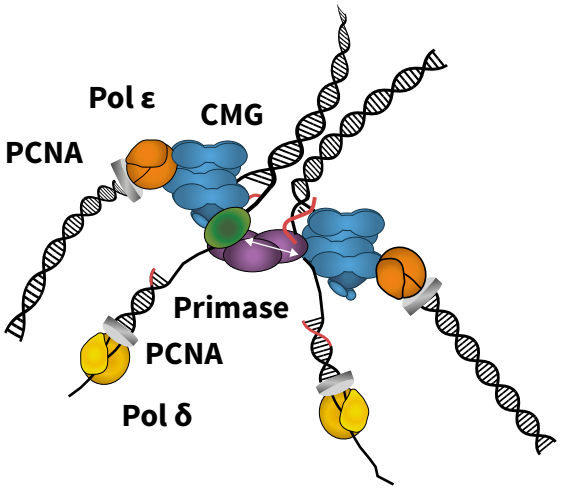

Figure 4. Twin replisome factory. Twin replisomes held by a Ctf4 scaffold. Two CMGs (blue) are held by one Ctf4 trimer (purple). The original parental DNA duplex feeds into each CMG from the centre of the factory and is split at the surface, where one strand goes through CMG, where it is duplicated by Polymerase epsilon (orange)-PCNA clamp (grey) upon its exit from CMG. One flexible primase (green) binds the third Ctf4 subunit and alternates activity between the two strands to form primers (red) that are extended by Polymerase delta (yellow) held to DNA by the PCNA clamp (grey).

An Ensured Inheritance

Perhaps surprisingly, every bodily cell contains the entire selection of DNA or genome. However, only certain genes end up being 'switched on'. Whether or not a gene is expressed depends on epigenetic markers, which, in a nutshell, are chemical tags on DNA. DNA is packaged inside a cell into functional units called nucleosomes which include these markers, ready to determine the function of each cell. Thus, epigenetic inheritance is an essential component of cell division which must be preserved by the replisome. In the model described by Professor O'Donnell, two CMGs bind to one nucleosome, suggesting that the twin replication factory itself facilitates the transfer of nucleosomes to new DNA, ensuring developmental epigenetic inheritance is achieved.

Professor O'Donnell and his team also proposed that the replication factory helps to organise the newly synthesised DNA genome and that the twin model is able to communicate with itself. Professor O'Donnell summarised that 'it is possible the two replisomes communicate their status to one another, such that if one replisome stops due to DNA damage, the other replisome may stop as well'. This communication could be integral for the production of healthy daughter cells, void of rogue DNA damage that could lead to cancer.

Looking to the Future

Professor O'Donnell's laboratory is looking to validate the conclusions drawn here, both in live mammalian cell studies and in bacteria. Presumably, the twin replication factory is applicable to all life forms and provides key information for furthering the study of DNA replication. The enthusiasm that Professor O'Donnell shares for unlocking new secrets of this field is contagious, as he declares 'the current study is only the beginning of a comprehensive understanding of how replication is organised in the cell. We expect that additional proteins, further layers of organisation, and yet to be determined dynamic actions of these proteins, exist in nuclear replication factories.'

Meet the researcher

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Professor Michael O'Donnell received his PhD in biochemistry from the University of Michigan in 1982 and went on to complete a postdoctoral position at Stanford University in 1986. At the close of his postdoctoral studies in 1986 he founded his research group at Cornell Medical College in New York City, which he moved to the Rockefeller University in 1996 where he now heads the Laboratory of DNA Replication. Over his 30-year career, he has achieved an impressive accolade of honours and awards including becoming an investigator with the Howard Hughes Medical Institute in 1990 to the present time, and induction into the United States National Academy of Sciences in 2006. Alongside this, he is an editor and reviewer for several research journals and fundraising bodies. His research focuses on mechanistically understanding how the collection of proteins involved in DNA replication ensures genomic integrity. During the COVID-19 outbreak, Professor O'Donnell and his team are investigating potential vulnerabilities in the reproduction of coronavirus.

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FURTHER READING

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THE ROLE OF NOTCH SIGNALLING WITHIN THE MOLECULAR CLOCK IN THE EARLY DEVELOPMENT OF THE SKELETON

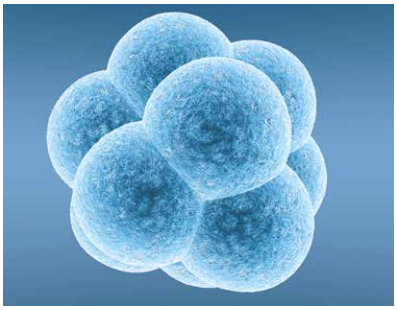
Cells possess the ability to interact with one another through complex signalling pathways. Different signals regulate how cells differentiate, undergoing modifications that ultimately allow them to adopt different cell fates and perform specific functions. The laboratory of **Professor Kim Dale** from the University of Dundee, Scotland, has made seminal contributions to our understanding of how the Notch signalling pathway controls the formation of tissues and organs in the earliest stages of development. Their important research has unveiled new insights into the molecular basis of Notch signalling in the context of normal development which will further our understanding of the molecular basis of developmental disorders and a multitude of diseases correlated with aberrant Notch signalling.

The Formation of Bone Precursors in Vertebrate Embryos

In response to the appropriate signal, stem cells differentiate into highly specialised daughter cells during development in order to construct all of the different tissues and organs of the vertebrate body plan. Notch and other signalling pathways regulate in fine detail the fate of stem cells in the embryo. For example, one particular stem cell called a neuromesodermal stem cell, responds to different cell signalling interactions, to either generate daughters cells that remain as stem cells, or to generate daughters that will go on to lay the foundations of neural or skeletal development.

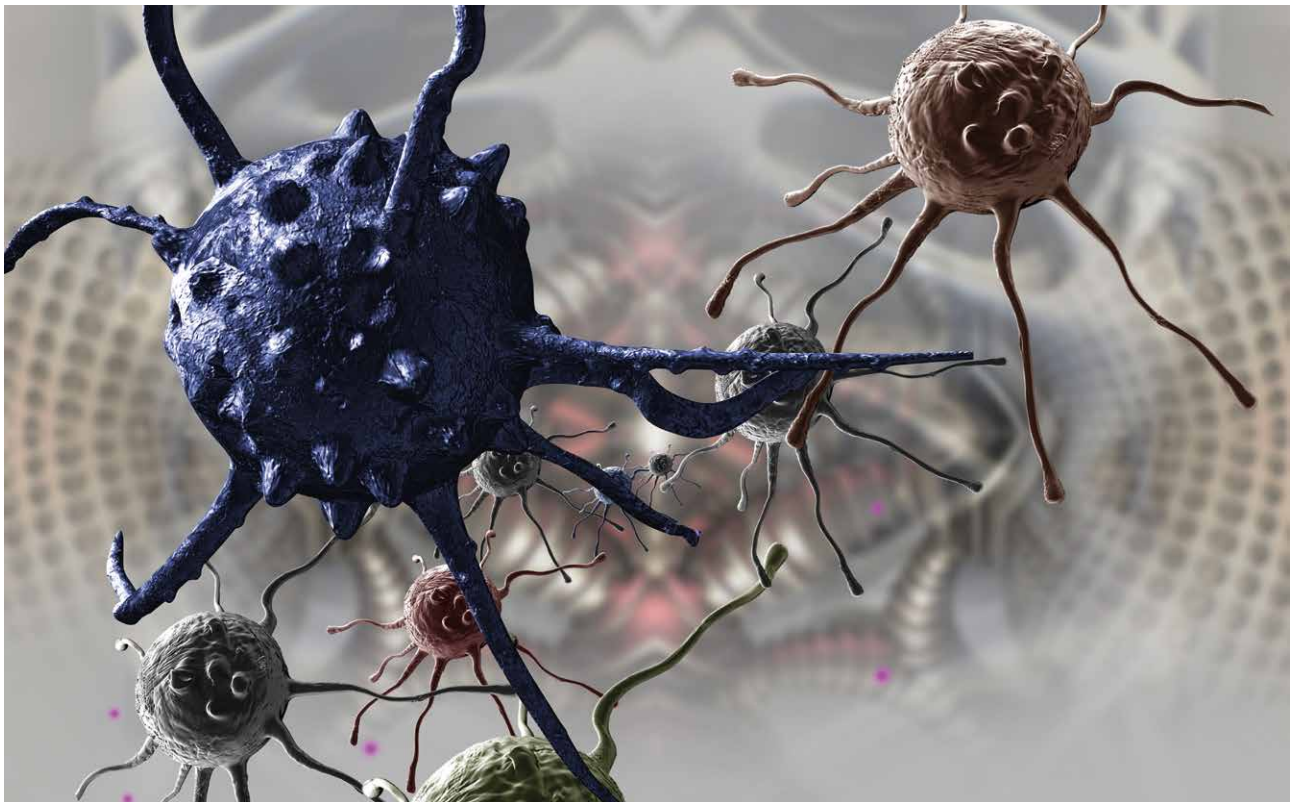
During embryonic skeletal development, segments of tissue are formed that will eventually give rise to the bones of the skeleton; these segments are referred to as somites. The process of somite formation is known as somitogenesis. The team in Professor Kim Dale's laboratory at the University of Dundee are interested in the investigation of the factors that regulate somitogenesis. Professor Dale's laboratory has published several pioneering studies that show that the Notch signalling pathway plays an essential role in the process of somitogenesis.

All vertebrate species share a similar segmented 'body plan' that is established very early in the formation of the embryo. The process of somitogenesis is highly evolutionarily conserved and has been studied since the early 1900s. During this process of



segmentation, a repeated pattern of somites is arranged on either side of the posterior neural tube. These segments are the precursors of the vertebrae, ribs and associated skeletal muscle. Interestingly, the number of somites formed are different for different species.

Somitogenesis is a fine example of tight spatio-temporal regulation, resulting in the formation of three-dimensional



structures in the developing embryo. Somites are formed progressively with a strict species-specific periodicity that is linked to a maturation wavefront that proceeds in an anterior to posterior direction. The advent of molecular biology has allowed researchers to establish that the periodic activation of Notch, in turn, activates several genes that coordinate the timing of the formation of somites.

Despite being so important for somitogenesis and other regulatory mechanisms, the Notch pathway is relatively simple, and its main features are strikingly similar across different species. Notch is divided into two domains, the intracellular domain, facing the inside of the cell, and the extracellular domain, which interacts with everything that surrounds the cell on the outside.

The Notch signalling pathway can be simplified as an interaction between the ligands of a signal-sending cell and the receptors of a signal-receiving cell. Upon ligand-receptor interaction, a molecular 'switch' is activated that eventually liberates the intracellular domain of Notch (NICD). NICD, once free to move towards the nucleus, interacts with the genetic material of the cell, activating several specific genes. The activated genes are ultimately responsible for regulating the stem cell response to the Notch ligand in relation to their survival, growth and the role they take in forming tissues with specific functions.

A Finely Regulated Molecular Clock

Somitogenesis happens in a progressive and tightly regulated fashion. The genes responsible for the exact pace of the segmentation progress can be switched on and off in an oscillatory pattern. This molecular oscillator is known as the segmentation clock. This clock can be visualised through periodic waves of gene expression that occur in the developing embryo with exactly the same frequency observed for somite formation.

Professor Dale contributed to the first pioneering studies that led to the discovery of the segmentation clock. Those studies, conducted in chick embryos, proved that the over-expression of a gene known as *lunatic fringe*, which encodes for a protein that modifies Notch activity, leads to defects in segmentation. Professor Dale and her collaborators, together with other leading laboratories across the world, have since published several studies confirming that the genes making up the 'clock' belong to three main signalling pathways, Notch being one of them.

Professor Dale and her team used a computational model to show that the influence of Notch signalling on regulating the pace of the segmentation clock depends on the stability of its intracellular domain, NICD. According to their model, when the levels of NICD are high, the pace of the segmentation clock slows down. They published a study in 2015 to back up this model with experimental data. The team at Professor Dale's laboratory have used chick and mouse embryos as a model for human segmentation as the process is highly conserved both at the tissue and the molecular level. They showed that when chicken embryos were treated with drugs that interfered with the degradation of NICD, this slowed the segmentation clock as judged by the

formation of fewer but larger somites in a given length of time. Critically, in the same study, the authors showed that the pace of the clock could be reversed back to a faster pace by reducing NICD levels again through treating the chicken embryos with drugs that reduced the production of intracellular levels of NICD.

A Model System for Congenital Scoliosis

Congenital vertebral malformations occur in around 1 every 1,000 humans. In the early stages of somitogenesis, the bone precursor segments are laid down along the axis that runs from the head to the tail of the embryo. As described above, this process is highly conserved across all species of vertebrates. When somitogenesis fails it can lead to several developmental disorders.

Congenital scoliosis is characterised by several malformations of the spine, including defects in the structure of the vertebrae. For many forms of congenital scoliosis, we do not understand the aetiology. However, one particular condition, known as spondylocostal dysostosis (SCD), is linked to mutations in genes belonging to the Notch pathway, highlighting the importance of this pathway to skeletal formation. Moreover, Professor Dale's laboratory has shown that the absence of Notch in mice results in the complete lack of somite formation during segmentation.

The team at Professor Dale's laboratory have used chick and mouse embryos, as well as human-induced pluripotent stem cells, to delve further into the mechanism by which Notch influences this process. They have tested and confirmed their hypothesis that the misregulation of the degradation of the NICD molecule leads to the impaired development of the skeleton by changing the timing of this process, which ultimately will change the number of segments formed.

Going forward Professor Dale's laboratory hopes to build on these findings by manipulating the Notch signalling pathway in cultured chick and mouse embryos and human pluripotent (immature) stem cells to eventually establish a model system for congenital scoliosis. As well as presenting vertebral abnormalities, SCD is characterised by a shortened torso and misaligned ribs. Individuals that are severely affected by SCD may develop life-threatening complications in lung function.

The Role of Notch in Tumorigenesis and Cancer

The cell cycle is a tightly regulated mechanism responsible for cell growth and division. The cell cycle can be understood as a series of interactions with signalling molecules that the cell goes through to copy all its genetic material and eventually divide into two genetically identical daughter cells. When the factors that control the tightly regulated progression through this process malfunction, cells start to divide uncontrollably. Cancer can be one consequence of this uncontrolled cell division that leads to the unregulated growth of tissues known as tumours. Understanding the mechanisms that control cell division is key to the development of effective cancer treatments.

In a paper published in 2019, Professor Dale's laboratory demonstrated that the levels of NICD vary in a manner that is dependent on the presence of proteins involved in regulation of the cell cycle. They showed that the inhibition of CDK-1 and CDK-2, two important cell cycle enzymes, leads to an increase in the levels of NICD, causing a delay in the mouse somitogenesis clock and somite formation. The researchers supported their findings by developing a mathematical model that showed that their experimental observations, made in the mouse embryo and in different cell lines, could be explained in a theoretical framework linking the cell cycle to NICD degradation.

These exciting results highlight the fact that current cancer treatments that target cell cycle progression, need perhaps to be re-evaluated for the impact they may also have on Notch signalling. Indeed, certain cancer patients that develop immunity over time to cancer treatments that rely on cell cycle inhibitors have also exhibited elevated levels of Notch signalling. Thus, these findings may further pave the way towards the development of more effective cancer treatments that target both cell cycle and Notch signalling.

Future Directions

Dr Meijer, one of Professor Dale's team, together with their collaborators are currently trying to identify the missing pieces of the puzzle that makes up the molecular mechanism behind the clock gene oscillations observed during somitogenesis. They aim to achieve this by using molecular probes that will allow them to fully map the complex network of Notch interacting partners and the exact location of key phosphorylation sites on NICD. Once those sites are identified, the team will further investigate how phosphorylation directly affects NICD in vivo while at the same time trying to quantify the impact of altering NICD levels on tissue development.

An exciting and ambitious project that Professor Dale's laboratory is currently working on is to use human stem cells with a fluorescent marker that allows the visualisation of Notch target genes revealing their oscillatory pattern in real time. This approach allows researchers to 'see' the genes that activate the segmentation clock in action, but it also provides an invaluable tool to manipulate the system and thereby identify the currently unknown molecular mechanisms behind this potent oscillatory signalling gene network which will ultimately inform our understanding of disease systems, including several types of cancer, that are linked to Notch. To this end, Professor Dale, Dr Meijer and the team will join forces with a network of high calibre international collaborators who are experts in the field of Notch signalling.

Meet the researchers

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Professor Kim Dale obtained her PhD in developmental neurobiology from the National Institute for Medical Research in London in 1997. Her work on Notch signalling has been instrumental to our understanding of the clock and wavefront model for somite formation and she has been hugely influential in both the Notch signalling and developmental biology fields. Professor Dale started working on the Notch signalling pathway as a postdoctoral researcher in the laboratory of Dr Olivier Pourquie in Marseille, France. The pioneering work of that laboratory has been recognised as one of the milestones in developmental biology of the 20th century by Nature Magazine. Professor Dale established her own group at the University of Dundee as a Royal Society University Research Fellow in 2005. She is now Associate Dean International for the School of Life Sciences at the University of Dundee, where she is also a Professor of Molecular Developmental Biology.

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Dr Hedda Meijer received her PhD in embryology and molecular biology from Utrecht University in the Netherlands, in 2001. Her research interests revolve around the field of embryology and RNA biology. The main focus of her previous research was on the role of translational control, the regulation of poly(A) tail length and RNA stability during oogenesis and embryonic development. Dr Meijer joined the laboratory of Professor Kim Dale in 2019 as a Postdoctoral Fellow and is currently investigating the regulation of Notch signalling in the context of the vertebrate segmentation clock. Before joining the University of Dundee, she held several research positions at the Universities of Nottingham and Cambridge in the field of RNA biology.

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IDENTIFYING MOLECULAR MECHANISMS CONTROLLING IMMUNE CELL FUNCTION

The immune response entails the rapid activation of the immune cells to ensure effective defence from pathogens through the inflammatory pathway, as well as maintain immune homeostasis through the anti-inflammatory pathway. Immune cell activation happens as the result of rapid and severe changes in the expression of the immune-response genes. These depend on regulatory mechanisms controlling the processes of transcription, translation, and modification of these genes to produce functional proteins. **Dr Elke Glasmacher**, Head of Immune and Cell Biology at Roche, researches the important molecular mechanisms underlying how cells are activated or repressed.

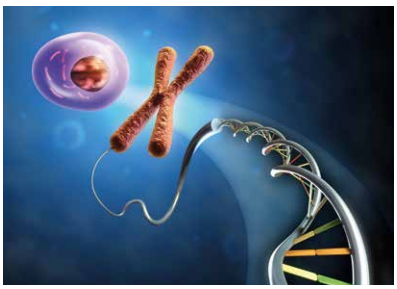
Transcriptional and Posttranscriptional Regulation

Each gene is a piece of DNA containing the information (genetic code) to form a protein inside the cell. A specific DNA sequence is transformed into a specific polypeptide chain that makes the protein. In the journey from DNA to protein, the DNA is first transformed into a complementary molecule called RNA by a process called 'transcription'. Next, this primary RNA molecule undergoes various 'post-transcriptional modifications'. The most notable are: 'splicing', which involves removing of the parts that don't 'code' for the protein and 'capping', a chemical modification that marks the RNA as mature for the cellular machinery to recognise and process for the next stage, the 'translation' into a protein.

Each stage represents an opportunity for the cell to precisely regulate when, how much, and for how long a particular protein will be produced. This fine regulation of gene (or protein) expression is particularly important for the cells of the immune system, as during an immune response there are rapid and severe changes in gene expression to support a rapid response to pathogens. Precise regulation of the immune-response genes is also a prerequisite to sustain immune homeostasis and ensure that the immune system is not aberrantly activated. Defective gene regulation can inhibit both inflammatory and anti-inflammatory responses leading to infections and autoimmune disorders, respectively.

Regulatory Mechanisms in the Immune System

Increasing our knowledge about the regulation of the immune system is important to understand how to better control these processes and how their deregulation can cause disease. As a group leader at the Helmholtz Center (Munich, Germany), then as the Head of Immunobiology at Roche pRED, Large Molecule Research (LMR) (Munich, Germany), and now as Head of Immune and Cell Biology at Roche pRED/LMR, Dr Elke Glasmacher is working to elucidate the key regulatory mechanisms that control immune cell function.



From DNA to Cell

The immune system can be either innate or adaptive. The adaptive immune system provides long-lasting immunity against pathogens with high specificity. The cells that carry out the adaptive immune response are white blood cells known as lymphocytes. There are two main

classes of lymphocytes – B cells and T cells – that mediate antibody responses and cell-mediated immune responses, respectively.

The innate immune system, on the other hand, provides a non-specific response to pathogens and it is mediated by neutrophils, mast cells, basophils, eosinophils, dendritic cells, γδ T cells, and macrophages. Macrophages are among the key first-responders to pathogens and they express a large variety of specific protein receptors on their surface. Upon activation of these receptors, a systemic intracellular immune activation occurs, enabling various defence mechanisms. Thus, macrophages initiate inflammatory responses, including the expression of specific proteins named cytokines. Of these cytokines, interferons (IFNs) are the most inflammatory and rapidly induced. They are named for their ability to 'interfere' with viral replication, thereby protecting cells from virus infections.

Regnase-3: Complimentary Yet Independent Functions

Regnase is an RNA-binding protein with the role of degrading cellular and viral RNA upon certain immune system activation. Dr Glasmacher and her research team analysed Regnase-3 deficient mice, which develop hypertrophic lymph nodes indicative of infection. Regnase-3 deficiency systematically increased IFN signalling, which increased the proportion of immature B and innate immune cells. They found that, unlike Regnase-1, Regnase-3 expression is high specifically in macrophages and is transcriptionally controlled by IFN signalling. Their data consistently demonstrated that Regnase-3 is an RNase is a key regulatory factor in the IFN pathway in tissue macrophages, important to maintain cellular and systemic homeostasis. Although Regnase-3 is, in many ways, a functional complement to Regnase-1, it is also functionally independent due to its effects in macrophages within the IFN pathway.

These results indicate that Regnase-3 may be an important potential therapeutic target for diseases associated with tissue inflammation and deregulation of the IFN pathway, and therefore should inspire future studies to establish the targets and thus define in more detail the molecular mode of action of Regnase-3.

Identifying New Regulatory Elements and Transcription Factor Binding Partners

In the early stages of her research career, Dr Glasmacher studied the transcriptional regulation of T helper 17 (T_H17) cells, a particular type of T lymphocyte. The process of transcription involves the binding of specific proteins known as transcription factors (TFs) to upstream regions of the gene that is going to be transcribed by the enzyme RNA polymerase. TFs often act in synergy and form different complexes with different 'binding partners' recognising different sequence motifs in different cells. This interaction specifies whether transcription of the target gene will be induced or inhibited by the TFs.

TFs play a very important role in transcriptional regulation. Dr Glasmacher was particularly interested in a TF that in B cells forms complexes with a second TF, before recognising specific DNA sequence motif in target genes, leading to 'gene activation'. Binding to the TF co-partner directs the recruitment of this hetero-dimer in the particular gene locus bearing the specific DNA motif. The problem was that T_H17 cells don't produce this particular binding partner, and the mechanism of recognition of the particular DNA motif by the transcription factor was unknown.

Dr Glasmacher's research revealed that, specifically in T_H17 cells, the TF in question partners with a different protein and they both co-operatively bind to a different DNA motif, whose sequence was also identified. Even more importantly, the researchers made the critical finding that one single major complex drives a variety of genes to different extents for specific cells to differentiate.

The Importance of Roquin in Cellular Immune Responses

In addition to the pathways that ensure cellular and systemic responses to pathogens, the anti-inflammatory pathways that ensure immune homeostasis are just as important. Defects in the anti-inflammatory pathways can lead to inflammation caused by non-infectious agents, (such as toxins, chemicals, and mechanical trauma) and even to severe pathologies such as autoimmunity. RNA-regulating factors and posttranscriptional networks are known to play a particularly important role in the interplay between pro- and anti-inflammatory pathways. In the innate immune system, RNA-binding proteins specifically act as sensors, mostly for viruses and are associated with RNA recognition and degradation. RNA-

binding proteins are also implicated in the resolution of cellular immune responses. For example, Roquin-1 and Regnase-1 control cellular immune responses and prevent autoimmunity.

In T cells, Roquin regulates the production of a protein called inducible costimulator (ICOS) by destabilising its mRNA in a process that requires the 3′ untranslated region (3′ UTR) of ICOS mRNA. Loss of Roquin results in loss of this post-transcriptional regulation, stabilisation of ICOS mRNA and therefore, constant ICOS protein production. Consequently, the ICOS protein reaches aberrantly high levels in T cells and, in mice lacking Roquin, this causes autoimmune pheno¬types similar to systemic lupus erythematosus in human patients.

Dr Glasmacher's research demonstrated that Roquin binds directly to ICOS mRNA, showing an intrinsic preference for a previously unrecognised sequence in the 3' untranslated region (3' UTR). Her work also showed that Roquin forms complexes with other factors during this interaction, to confer post-transcriptional repression. Critically, these newly described molecular functions of Roquin may be involved in the prevention of autoimmunity.

A Comprehensive Genome-wide Analysis

Isolated gene studies lack comprehensive analysis between the levels of gene regulation. However, research has now begun to elucidate the complexities of transcriptional and post-transcriptional networks before and after activation.

Notably, Dr Glasmacher's group presented the first comprehensive genome-wide analysis on real-time temporal dynamics of transcription, splicing, and translation during T effector cell activation. Their data suggest a model in which T cells massively change their functional program by regulation of >2,000 genes. Here, changes in transcription and translation are extremely coupled for >90% of genes, and only a few genes show evidence for independent posttranscriptional or translational regulation. This coincides with fluctuations in cotranscriptional splicing rates.

This work by Dr Glasmacher and her group not only revealed the genome-wide course of events during a T helper response but also suggests a model in which rapid de novo recruitment of RNA Pol II dictates changes in transcription, consequently leading to coupled changes in translation. It remains an open question for future research as to how this rapid de novo recruitment is accomplished and whether the temporary reduction in cotranscriptional splicing represents a side effect or an important mechanism at the beginning of cell activation.

Current Work at Roche Innovation Center

In 2017, Dr Glasmacher moved to Roche pRED, Large Molecule Research where she headed the department for Immunobiology that mostly centred on the *in vivo* lead generation process for portfolio projects. Since 2019, she has led the department of Immune and Cell Biology. The department identifies and characterises lead candidates for portfolio projects and aims to mimic the potential mode of action, focussing on primary cellular functional readouts. She additionally leads early research initiatives for the advancement of large molecule platform technologies for all of Roche's disease therapeutic areas.

One project aims to define the molecular mode of action of checkpoint receptor targeting by using engineered antibodies, addressing the question: How does the antibody format affect the checkpoint molecule's mode of action? Here, she establishes high throughput single cell imaging readouts to correlate cellular phenotype and signalling behaviour via machine learning.

The second project is focusing on the identification of the underlying mechanism of immune tolerance in large molecule projects developed for cancer immunotherapy. Single cell sequencing approaches from different *in vivo* cancer models are the foundation and starting point of this project. Here, her background in identifying the gene regulatory mechanisms will help to develop the project further and enable new ideas to overcome such tolerances.

Meet the researcher

Dr Elke Glasmacher
Head of Immune and Cell Biology
Large Molecule Research (LMR)
Roche Innovation Center Munich
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Roche

Dr Elke Glasmacher received her PhD in Immunology and Biochemistry from the Helmholtz Center in Munich, Germany. After a postdoctoral position at Genentech Inc, San Francisco, USA, on the molecular control for the development of autoimmune Th17 cells, she moved back to the Helmholtz Center as a Principal Investigator. Dr Glasmacher established a laboratory at the Helmholtz Center investigating the molecular mechanisms of gene regulation in immune cells. Since 2017, Dr Glasmacher changed to Roche and took over the department of Immune and Cell Biology at Large Molecule Research (LMR) in pRED Munich, Germany. The focus of Dr Glasmacher's research understands the underlying molecular mechanisms that drive immune cell activation programs versus silencing mechanisms. Dr Glasmacher is an internationally recognised researcher with extensive experience in both industry and academia. She has published in prestigious journals, and has been awarded significant funding to support her research endeavours.

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THE ROLE OF NITRATE AND NITRIC OXIDE IN MUSCLE BLOOD FLOW IN EXERCISE

Nitric oxide is an 'A-list' celebrity amongst chemical compounds. Proclaimed 'molecule of the year' in 1992 by the American Association for the Advancement of Science, its physiological importance, discovered in 1985, was recognised in 1998 by the award of a Nobel prize to some of the researchers who had discovered its vital role in regulating blood vessels and blood pressure. Here we look at the pre-eminent work of **Dr Alan N. Schechter** at the National Institutes of Health in Bethesda, Maryland, USA, that is continuing to keep nitric oxide at the front and centre of groundbreaking biological research.

Nitric Oxide

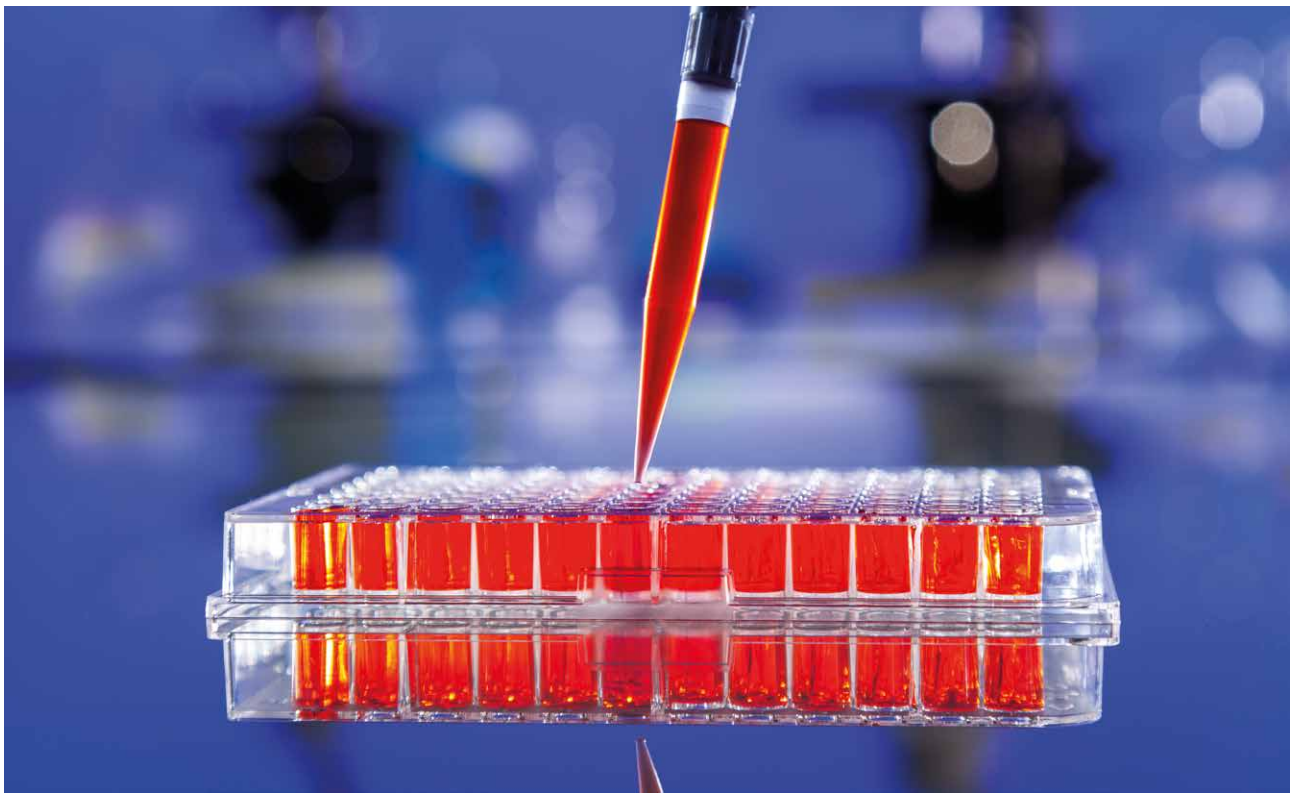
Nitric oxide is a colourless gaseous oxide of nitrogen and is a free radical, meaning that it has an unpaired electron, which is relatively uncommon in chemistry. Historically, nitric oxide was generally considered to be only an air pollutant, a by-product of fossil-fuel combustion. However, research in the late 1980s and early 1990s indicated that nitric oxide has a central role in virtually all cells and, in particular, in animal physiology as well as pathophysiology, the processes associated with disease or injury.

High-profile research on nitric oxide has captured the attention of the scientific world for more than a third of a century, resulting from the pivotal discovery of its role as a cardiovascular signalling molecule, regulating blood pressure and maintaining the health and function of blood vessels. Although nitric oxide is toxic at high concentrations in mammals, including humans, it acts at very low concentrations as a critical signalling or messenger molecule. Its small size enables it to pass easily through cell membranes and walls to carry out various signalling functions. The free radical state of the molecule ensures that it is more reactive than other cellular signalling molecules but limits its chemical lifetime, especially in the presence of oxygen or macromolecules.

Nitric Oxide Metabolism and Transport

Since the early 2000s, there has been a renewed program of research on nitric oxide, some of it emanating from the laboratory of Dr Alan N. Schechter in its Molecular Medicine Branch at the National Institutes of Health (NIH) in Bethesda, Maryland, USA.

Dr Schechter's laboratory has a particular interest in how nitric oxide interacts with haemoglobin, a protein that carries oxygen from the lungs to the rest of the body. Working with a multidisciplinary research team, he has been investigating how nitric oxide is formed and transported by blood, and its potential for use as a pharmacological agent to help deliver treatment (i.e., act as a drug). Dr Schechter believes that using nitric oxide in this way may contribute to the development of therapies for diseases such as sickle cell anaemia and others with impaired blood flow and thus diminished oxygen transportation. One outcome of this work is that Dr Schechter is a co-inventor on a patent at the NIH for the therapeutic uses of nitrite ions (precursors of nitric



oxide), which has been licensed for development by several companies.

Emerging from this line of research, new concepts have been developed in the last two decades on new pathways of formation (as described below) and how these pathways modulate platelet reactivity and blood clotting, and, most recently, how nitrate ions determine muscle function and blood flow.

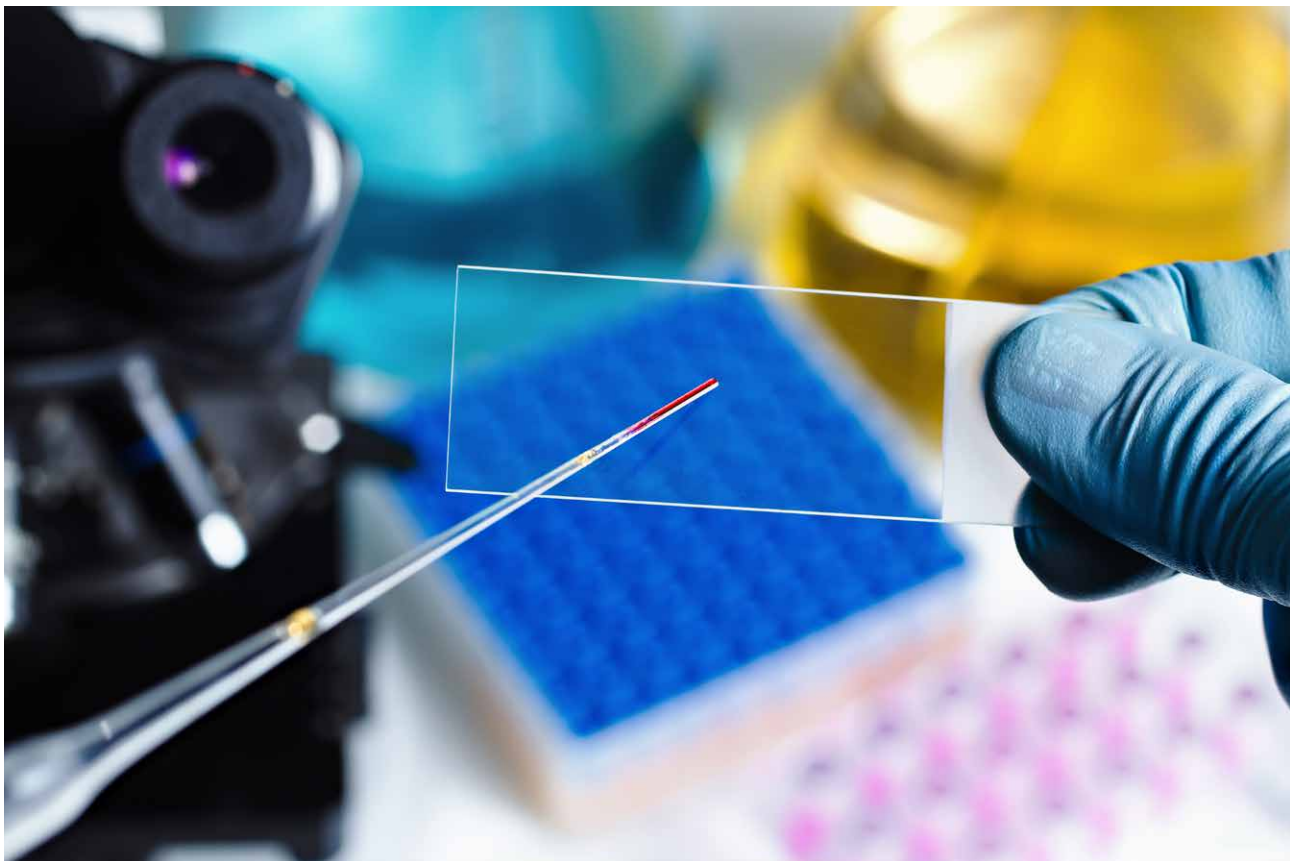
Nitric Oxide Formation

In order to support its broad range of functions and effects in mammals, nitric oxide is produced by tissues in the body in a number of ways. Over the past two decades, Dr Schechter has led a wide-ranging group of researchers to explore these issues in greater depth.

Working closely with Dr Mark Gladwin and many others at the NIH, Dr Schechter has shown, especially under hypoxic conditions, a new pathway of nitric oxide formation in addition to that which occurs when oxygen is freely available in which nitric oxide is mainly produced from the enzymatic conversion of arginine to citrulline by nitric oxide synthase enzymes. Over the past 15 years, these investigators have shown that nitric oxide formation in the blood by the reduction of nitrite ions to nitric oxide by red cell haemoglobin and other proteins and reducing agents, reflects very important sources of nitric oxide production, especially when oxygen is limited. It has been discovered that the cycling of nitric oxide formation and destruction (also by heme proteins such as haemoglobin) is at the heart of the regulation of diverse physiological properties, of which probably the most important is to control the vascular tone of blood vessels and arteries – and thus blood flow.

In their most recent work, conducted with Dr Barbora Piknova in particular, the researchers have shown that in muscle, when oxygen supplies are reduced, such as during exercise, nitric oxide can be supplied by various nonenzymatic reactions, resulting especially from the reduction of the high concentrations of nitrate found in muscle tissues, as well as from nitrite reduction. It is important to notice that this whole reduction cycle from nitrate to nitrite and nitric oxide is innate to mammalian cells that process the entire enzymatic/transporting machinery necessary for maintaining the working cycle, as explained in more detail below.

With the recognition that nitrate and nitrite ions have a vital role in the production of nitric oxide, there has been a much greater focus on the sources and production of nitrites in the body. It was generally thought until recently that the only tissues with nitrate- and nitrite-to-nitric oxide reductase activity (i.e., the nitric oxide cycle) were the blood and some internal organs, predominantly the liver. Nitrate is generally supplied either from the diet or by the oxidation of excess nitric oxide by red cell haemoglobin in the blood. Both ions are also present in foods, especially in green leafy plants, and have long been used to preserve meat products. Dr Schechter and many others in the field believe that although there has long been some uncertainty over their safety in the diet (based on certain animal studies at very high concentrations) that the benefits of these ions in the diet far outweigh any risks.



To learn more, Dr Schechter has recently collaborated with researchers worldwide to investigate the dietary intake of nitrate and nitrite ions, particularly to better understand the metabolism of these nitric oxide precursors in skeletal muscle, a tissue largely unconsidered in this context up to this point. The general physiology of muscle tissue (especially response in exercise), has been widely understood for more than a century, particularly regarding hyperaemia, the rapid 10 to 20-fold (or greater) increase in blood flow into muscle tissue in response to increased metabolic demand for oxygen during heavy exercise. However, the detailed biochemical control of these processes has been much less understood up until now.

In particular, vasodilation or widening of the blood vessels is mediated by the synthesis and release of a range of vasodilatory agents, of which nitric oxide is just one of many (although likely the most potent). These act to relax smooth muscle cells within the walls of large veins, arteries, and arterioles, allowing greater blood flow through them. It has long been speculated that substances produced by muscles affect vasomotor tone. However, none of the potential vasodilators had all the properties necessary until nitric oxide, originally identified as endothelium-derived relaxing factor, was investigated but studies using inhibitors of the nitric oxide synthase enzymes were not consistent with the idea that nitric oxide was the major factor in the control of blood flow to muscle during exercise.

Human Skeletal Muscle is a Reservoir for Nitrate Storage

Dr Schechter's recent studies suggest that skeletal muscle may have developed control of blood flow as a vital function for its nitrate ion reservoir during exercise. In various rodents, and later confirmed in human samples by Dr Schechter's group and several other laboratories, the nitric oxide precursor, nitrate, has been found in far greater quantities in skeletal muscle than in other organs, including blood. A significant proportion of this baseline nitrate storage reservoir was observed to be produced by the nitric oxide synthase enzymes, but it is also significantly boosted from the consumption of nitrates in the diet.

This muscle stored nitrate reservoir is highly accessible via the blood circulatory system and therefore can be easily transported to internal organs (namely the liver, which is the organ with high nitrate reductase activity) to be reduced to nitrite and nitric oxide, as well as direct reduction of nitrate into nitric oxide in the muscle tissue itself. It is notable that although researchers found that the nitrate reductive activity in muscle is significantly less than that of the liver per milligram of tissue, for example, the large total mass of the muscle tissue (as the largest 'organ' in the body) means that even low levels of activity in the muscle ensure that this tissue can be the main site for the production of basal levels of nitrite and nitric oxide.

Continuous amounts, at least at low levels, of nitric oxide are produced in muscle tissue (and probably all tissues and organs) even when at rest, due to its ubiquitous requirements as a signalling molecule, but its production is dramatically

increased during muscle contraction. It has been widely observed that nitrate supplementation, in the form of beetroot juice or sodium/potassium nitrate, can reduce blood pressure and has many other physiological effects such as slightly improving athletic performance. These benefits have been attributed to an increase in the bioavailability of nitric oxide, and it has been further shown that the ingested nitrate is stored within muscle tissues, with a five-fold rise in nitrate and a three-fold rise in nitrite in muscle two hours after the consumption of beetroot juice.

The Role of Sialin and CLC-1

Sialin, a nitrate transporter protein expressed in the salivary glands has been found to be involved in the uptake of nitrate by muscle cells in culture, although as yet its role in muscle storage of nitrate is unclear. However, sialin has been found to be expressed and recruited to the sarcolemmal membrane (the cell membrane of striated muscle fibre cells) and may be involved in the uptake of nitrate from the circulatory system. Research is continuing to clarify the importance of sialin in the suggested mediating role in the storage of nitrate in skeletal muscle, and the muscle's potential function as a whole-body regulator of blood nitrate, controlling the levels circulating in the plasma. CLC-1, an anion transporter specific for skeletal muscle, had been also shown to play a role in nitrate transport into muscle cells. However, the detailed roles of these and, possibly other anion transporters, are still to be elucidated.

In the conversion of stored nitrate to bioactive nitrite and nitric oxide, research on rodents has highlighted the role of the enzyme xanthine oxidoreductase (XOR) and possibly also aldehyde oxidase (AO) in the step-by-step reduction of nitrate to nitrite and nitric oxide in numerous tissues. Both enzymes are expressed in human muscle, and they may be involved in the regulation and conversion of muscle stored nitrate during high-intensity exercise. It has been noted that nitrate levels remain high in resting muscles, but concentrations fall during high-intensity exercise, especially following supplemental nitrate consumption (when muscle nitrate content has been elevated), suggesting that tissues respond to ambient levels of these ions to maximise their physiological effects. These types of effects will be important in working out how to optimize supplementation for achieving physiological effects, including in athletic performance.

Several key strands of research emanating from Dr Schechter's focussed interest in nitric oxide are opening opportunities for a greater understanding of skeletal muscle and the central role of nitrate in exercise and blood flow. These recent studies of nitric oxide formation in muscle have, in particular, caught the attention of investigators in exercise and sports medicine, such as Dr Andrew Jones's group at the University of Exeter in the UK and have resulted in extensive cooperative research related to these fields, as well as one recent scientific symposium at Exeter on this new topic.

'A Remarkable Career of Scientific Accomplishment'

NIDDK Scientific Director Michael Krause recently stated that 'Dr Schechter has had a remarkable career of scientific accomplishment.' Even after 50 years, Dr Alan Schechter continues to be a driving force for an ever-broadening research programme into nitrates, nitrites and nitric oxide, which will have positive consequences for decades to come.

His own particular passion for developing treatments for genetic diseases of haemoglobin, including sickle-cell disease, has led to pioneering clinical research that demonstrates the value of the medication hydroxyurea in treating this disease. More recently, this work has led to a diversification of his team's research into the understanding of the role of haemoglobin in the formation and metabolism of the signalling molecule nitric oxide, and the spin-off (but no less important) research into the critical role of nitrate, nitrite ions and nitric oxide in the regulation of vasodilation, particularly in exercise, linked to its storage in skeletal muscle.

Currently, Dr Schechter is working with several ophthalmologists in also studying nitric oxide formation and function in the mammalian eye. These studies, which have primarily involved analyses of rodent and porcine eyes, have suggested that much of the nitric oxide important for many functions in the eye is also produced by reduction of nitrate ions. Further, they also suggest that the lacrimal glands function in a way analogous to the salivary glands, in using sialin and perhaps other transporters to transfer nitrate from the blood into the tears. In view of the recent use of a nitric oxide donor drug to treat glaucoma, these findings – which will soon be tested in human volunteers – may open up a new area of ocular pharmacology.

Summary

Both directly and indirectly, Dr Schechter's work has led for the first time, to baseline levels of nitrate and nitrite being discovered in far higher concentrations in skeletal muscle than in plasma, and that muscle acts as a reservoir for excess nitrate. When linked to the newly established presence of sialin in skeletal muscle, an active nitrate transporter, this suggests that sialin, together with CLC-1, may be responsible for the increase in muscle nitrate concentration observed following the ingestion of dietary nitrate. The indications are that skeletal muscle acts as a nitrate and nitrite reservoir, functioning to support whole-body nitric oxide homeostasis via a regulated distribution of these ions into the bloodstream. Lastly, the reduction in nitrate concentration following consumption of nitrate occurs following high-intensity exercise, provides the first indication that in skeletal muscle, and perhaps other muscle tissues (or even other organs), that nitric oxide is generated from nitrate stored in the muscles.

Meet the researcher

Dr Alan N. Schechter
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Dr Alan N. Schechter graduated with a BA in zoology from Cornell University in 1959 and completed his MD at Columbia University in 1963. In an eminent and accomplished scientific career spanning more than 60 years, Dr Schechter has worked on protein folding with Dr C. B. Anfinsen, a Nobel Prize winner and has also pioneered the development of treatments for genetic diseases of haemoglobin, including sickle-cell disease. More recently, he has focussed on understanding the role of haemoglobin in the formation and metabolism of the signalling molecule nitric oxide. Dr Schechter is now a Senior Scientist and Chief of the Molecular Medicine Branch of the National Institute of Diabetes and Digestive and Kidney Diseases at the National Institutes of Health in Bethesda, MD. An author on more than 350 scientific papers, Dr Schechter has published in a range of prestigious international journals. Furthermore, Dr Schechter has served on the board of the Foundation for Advanced Education in the Sciences at NIH for almost half-a-century, has helped found and chair the Office of NIH History, and has served on and chaired the Council of the NIH Assembly of Scientists.

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Dr Barbora Piknova

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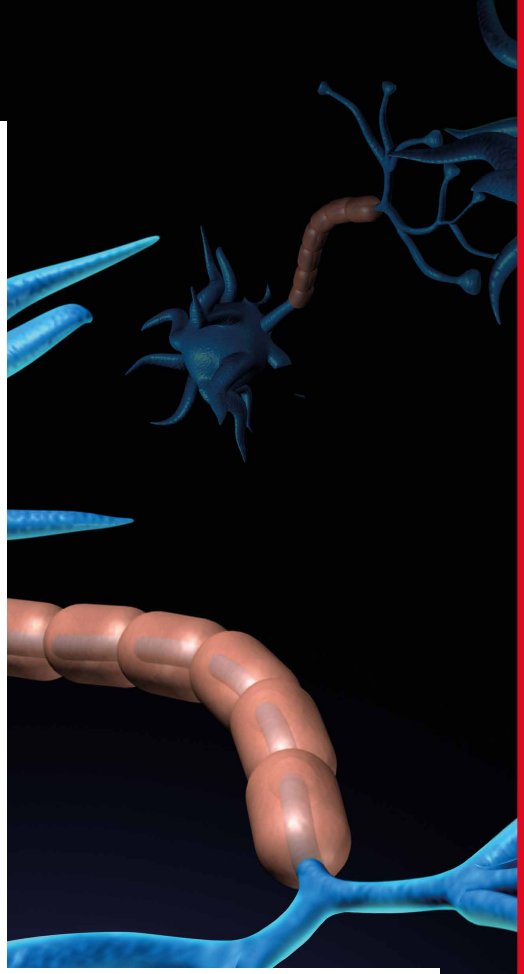
National Institutes of Health

NEUROTRANSMITTER SWITCHING: A NEW FORM OF BRAIN PLASTICITY

The discovery that the brain can change and reorganise itself revolutionised our understanding of neuroscience. Termed neuroplasticity, the field has seen an explosion of interest. An exciting form of plasticity has now been identified, in which neurons change their chemical communicator in response to environmental stimuli. **Dr Nicholas Spitzer** of the University of California, San Diego and his colleagues are looking to understand what causes this, what the effects are, and whether we can harness this exciting new facet of neurobiology to develop novel therapeutics for neurologic and psychiatric disorders.



A Changing Landscape

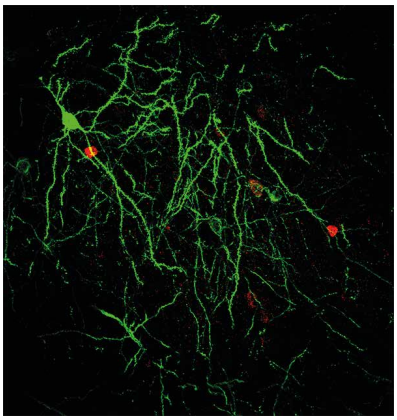
The human brain is the most complex and least well understood organ in the body. It contains almost 100 billion neurons and anywhere between 100 and 1000 trillion connections between them. Neurons in the brain are often identified by the specific chemical communicators they release, known as neurotransmitters. Dopaminergic neurons signal with dopamine and serotoninergic neurons signal with serotonin.

The prevailing view has been that this neurotransmitter identity is fixed. But just as it was previously contended that the adult brain is static and unmalleable, research is pointing towards a very plastic brain, one which can change and adapt over time – including the ability of neurons to switch their neurotransmitter and change their function. Dr Nicholas Spitzer and his colleagues from the University of California, San Diego, have been investigating this process, termed neurotransmitter switching, to find out what causes this switch, where it happens, and what the downstream effects are.

'The discovery of neurotransmitter switching in the adult mammalian brain identified a previously unrecognised form of brain plasticity at the synapse,' says Dr Spitzer. This newfound realm of plasticity opens-up avenues for research in neurological and psychiatric disorders and potential new treatments. His team is looking for links to the chemical imbalances we often see in psychiatric disorders, like depression, and questioning whether neurotransmitter switching may play a role in the development or the treatment of such disorders.

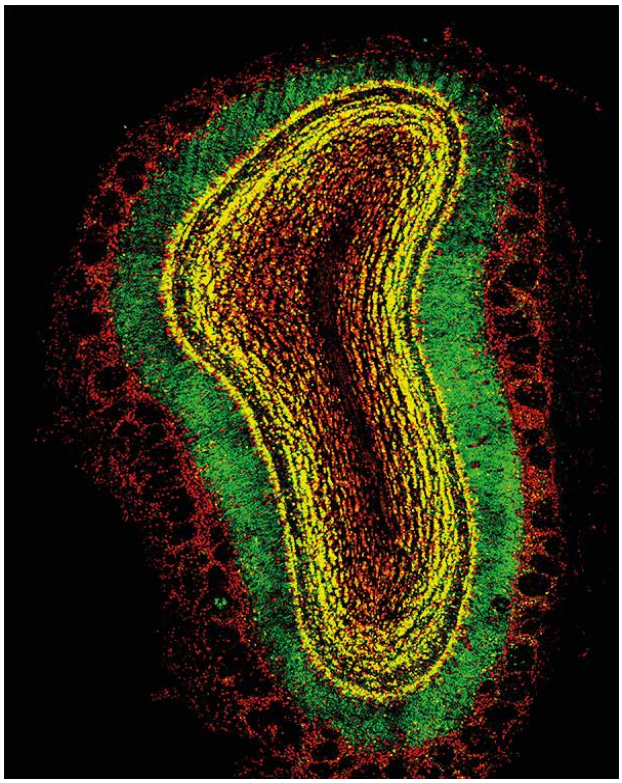
Chemical Conversations

'All our thoughts, emotions, and memories are the result of electrical activity in brain circuits,' says Dr Spitzer; 'these circuits are composed of neurons connected by synapses.' The interaction between neurons at the synapse is crucial in allowing the billions of individual cells to communicate and coordinate with each other. The synapse is the very small space between two neurons and is perfect for quickly exchanging signals.



Strategy to label neurotransmitter switching neurons. *The image shows glutamatergic pyramidal neurons labelled in green. These neurons have been marked using a viral vector strategy that makes them express a green fluorescent protein. The sparse red dots are neurons that express the inhibitory neurotransmitter GABA. When a glutamate-to-GABA neurotransmitter switch occurs, we see the green pyramidal neurons start expressing red and appearing yellow. Credit: Dr Marta Pratelli.*

A single neuron can have synapses with hundreds or thousands of other neurons, allowing extremely complex pathways to form. When a signal in the form of electrical activity reaches the



Strategy for whole-brain investigation of glutamate-to-GABA neurotransmitter switching. *This image shows a coronal section of the olfactory bulb. The mouse from which the image has been taken was genetically modified so that neurons that are or were glutamatergic are labelled by a red fluorescent protein and the cells that can produce GABA at the time of the analysis are labelled in green by a different fluorescent protein. This allows visualisation of green + red = yellow in cells that are or were glutamatergic and now produce GABA.*
Credit: Dr Marta Pratelli.

end of one neuron it must traverse the synapse to get to the next. To do so, a specialised group of chemical compounds called neurotransmitters come into play, which are released from the end of one neuron to cross the synapse and bind to specific receptors on the neuron on the other side.

An important property of neurotransmitters is that they can be divided into two classes: excitatory and inhibitory. As the name suggests, excitatory neurotransmitters will propagate a signal at the synapse and 'carry on the conversation' into the connecting neuron and beyond, while an inhibitory neurotransmitter will halt the signal at that synapse. Glutamate, dopamine and serotonin are some of the most well-known excitatory neurotransmitters, due to their roles in mood regulation, while GABA (gamma aminobutyric acid) is the major inhibitory transmitter in the brain.

Plasticity at the level of the synapse is a well-known concept in the field of neuroscience, and researchers have consistently observed synaptic changes that can either amplify or reduce an incoming signal. Critically, learning and memory take advantage of this property. As Dr Spitzer explains, 'When we learn something, electrical activity causes physical changes in the brain, often referred to as brain plasticity. Much of brain plasticity occurs at the synapse, changing the number of synaptic connections and strengthening or weakening them.' The ability of the brain to change and reorganise allows it to respond to things more effectively in the future.

These changes often involve alterations to the number of receptors present in the synapse. It is only recently that the ability of neurons to change their neurotransmitter has been revealed in the adult mammalian brain. Interestingly, neurotransmitter switching almost always seems to swap an excitatory transmitter out for an inhibitory transmitter or the other way around. These switches determine whether the signal at the synapse will be continued or not and may be responsible for the behavioural changes we see down the line.

Brain Training

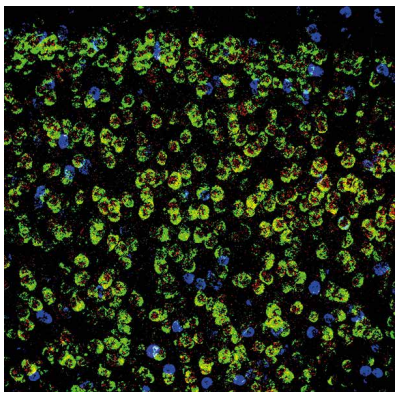
A crucial element of neurotransmitter switching is that it occurs naturally, and probably all the time. Everything we see, hear, and do is a stimulus from the environment which is converted into electrical activity in our brain. If this stimulus is prolonged or repetitive the neurons may switch their neurotransmitter to change the behavioural output.

In a study on motor skill learning, Dr Spitzer's team wanted to examine the effects of sustained wheel-running in mice. What they saw was a subsequent enhancement when learning more difficult motor skills, like balancing on a beam, in the week after running.

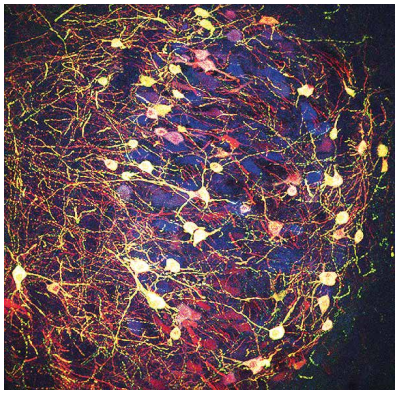
The continuous stimulation from running was enough to cause an uptick in activity in brain areas associated with movement, particularly in an area called the pedunculopontine nucleus which is involved in gait and balance. Using an array of molecular and genetic tests they determined that a large number of the neurons here which normally express acetylcholine, an excitatory transmitter, had switched to expressing GABA, an inhibitory transmitter.

To test the causal connection of the switch to enhanced learning, they tested what would happen if this change were prevented from happening by blocking the enzyme required to synthesise GABA. In these mice, neurotransmitter switching did not occur and their ability to learn new motor skills was not enhanced.

'Sustained running causes transmitter switching in the midbrain of adult mice that facilitates learning of other motor skills', Dr Spitzer explains. But the role of neurotransmitter switching in improving movement control is not where it ends, as he notes, 'Transmitter switching can have either beneficial or detrimental effects.'



Analysis of mRNA expression levels in prefrontal cortex neurons that switch from glutamate to GABA. *The cells in blue are neurons that express a GABAergic marker (GAD1), while green fluorescence permanently labels neurons that are glutamatergic or were glutamatergic in the past. The dot-like red signal shows the expression of a glutamatergic marker (vGluT1). By measuring the number of red dots present, one can determine whether the expression level of the glutamatergic marker (vGluT1) is decreased in switching neurons. Credit: Dr Marta Pratelli.*



Imposing a neurotransmitter switch. *A group of midbrain neurons expressing acetylcholine (blue) are made to express another neurotransmitter, glutamate (green), using adeno-associated virus as a gene delivery platform. The neurons targeted by the virus also express a red fluorescent protein for identification and thus appear a light-yellow colour (green plus red and blue). Credit: Dr Hui-quan Li.*

Light Therapy

One of Dr Spitzer's first discoveries relating to neurotransmitter switching in the adult brain was the effect of changing day lengths on mood. Seasonal affective disorder (SAD) is a form of depression which often recurs when the days get shorter and exposure to sunlight is reduced. Dr Spitzer wanted to know whether this environmental change was triggering unwanted neurotransmitter switching in areas of the brain involved in mood regulation.

He found that in rats, changing the amount of light they were exposed to each day caused dopaminergic neurons to switch to somatostatin expressing neurons. This occurred primarily in an area of the brain which detects light hitting the retina and regulates daily bodily rhythms. Importantly, these rats went on to express more depressive-like and anxious behaviour, spending less time in open spaces and exerting less energy.

In humans, changing day lengths can have similar effects on mood, and post-mortem brain tissue from people who died of natural causes in either summer or winter provides fascinating further insight. Those who died in summer were, at the time of their death, exposed to more sunlight; consequently, they had increased numbers of dopaminergic neurons in the same brain region than those who died in winter. This ties in nicely with current theories of depression which suggest that reduced activity in dopaminergic circuits can have detrimental effects on mood. Unfortunately, stress-induced transmitter switching appears to be more stable than its beneficial counterpart, but that is not to say it cannot be reversed.

Armed with the knowledge that light exposure can manipulate mood, light-based treatments (known as phototherapy) have now been developed to treat SAD which involve sitting in front of a bright light for a few hours per day. Phototherapy may be effective in reducing the symptoms of SAD by reversing or preventing detrimental transmitter switching in the darker months. This new approach to tackling mood disorders benefits from being easy to implement and avoids the many issues associated with standard drug treatments. For periodic mood disorders like SAD, phototherapy seems to tick all the boxes.

The Way Forward

Beyond the compelling results from experiments so far, Dr Spitzer and his colleagues have identified key questions which remain to be answered. These include the cellular location and speed of the switches; the pathways involved in changing from one neurotransmitter to another; whether switching changes as we age; and perhaps most importantly, how this phenomenon could be used to understand human brain disorders and develop new therapies.

A huge hindrance to getting these answers is the tools currently available; future advances in imaging technologies and genetic mouse models may be necessary to allow high-throughput screening of transmitter switching to gain representative results.

The potential for non-invasive behavioural therapy is especially exciting to neurologists and psychiatrists. Based on data so far, we can see how sensory stimuli like exercise and day length can result in behavioural changes. Indeed, there are already myriad studies demonstrating the benefits of light therapy for SAD and showing that sustained exercise therapy can help ameliorate Parkinson's Disease and stroke symptoms.

The role of neurotransmitter switching seems to be linked to many psychiatric conditions we know about. 'It seems natural to want to know whether transmitter switching is generating this chemical imbalance, in autism spectrum disorder, posttraumatic stress disorder and behavioural consequences of drug abuse,' explains Dr Spitzer. If it is, we can focus on developing our understanding of the mechanisms and the behavioural changes it can produce, allowing us to manipulate it for our benefit and enter a new era of treatment for brain-based disorders.

Meet the researcher

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Dr Nicholas C. Spitzer earned his PhD from Harvard University in Neurobiology and went on to work as a postdoctoral fellow at Harvard University and then University College, London. In 1972 he took up a faculty position at the University of California, San Diego, where he now holds the post of Atkinson Family Chair Distinguished Professor of Biological Sciences and Director of the Kavli Institute for Brain and Mind (KIBM). His research is focused on understanding the role of environment in behaviour disorders and advancing study of neurotransmitter switching, a newly appreciated form of neuroplasticity. He is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, and a fellow of the American Association for the Advancement of Science. Dr Spitzer has published prolifically in top scientific journals including Nature and Science, and has received a number of accolades for his research.

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UC San Diego

NEUROSCIENCE IRELAND

Neuroscience Ireland (NI) was established in 2005 as Ireland's National Neuroscience Society, and is a registered charity in the Republic of Ireland. NI has a membership in the region of 200 scientists and clinicians, and represents Ireland on the Governing Council of the Federation of European Neuroscience Societies. In this exclusive interview, we speak with **Professor Áine Kelly**, President of NI, to hear about their vital work driving forward excellence in neuroscience in Ireland and internationally.

To begin, please tell us how Neuroscience Ireland (NI) was established.

Historically, neuroscientists based in Ireland had formed various groups and networks, and organised neuroscience-focussed scientific meetings, but by the early 2000s, it was obvious that to make progress, Ireland needed a single cohesive neuroscience society that was formally constituted. It was also recognised that this society should be affiliated with the Federation of European Neuroscience Societies (FENS). Hence representatives from all major universities in Ireland came together to form the first Council of Neuroscience Ireland (NI), which is celebrating its 15th year as a registered charity and as a FENS member society.

Even though we are a young society, we are building on a long history of Irish contributions to brain science that are perhaps not as well-recognised or celebrated as they should be. Neuroscience Ireland aims to remedy that through an ongoing project run by our former President Dr Richard Roche of NUI Maynooth, the Virtual Museum of Irish Brain Science.

What is the overarching vision at NI? As President, what are your aims for the future?

Our stated mission is to advance research and education in the neurosciences in Ireland, and to represent Irish neuroscience researchers both nationally and internationally. That's quite a wide remit but because of our active membership, we are making continuous progress. Despite chronic underinvestment in neuroscience research, Irish researchers are consistently publishing world-class work in top-tier journals. That's a message that we really want to get out there – not just to showcase what we are already doing, but to signal what we could achieve given the appropriate financial supports.

As President, my main aims for the future are focussed on building networks and relationships nationally and internationally. At home, Neuroscience Ireland is a key element of the neuroscience network in Ireland, through membership of the Irish Brain Council and association with the Neurological Alliance of Ireland and other charities and patient advocacy groups. We also organise a variety of public engagement activities to highlight the important contribution neuroscientists can make to the health and wellbeing of the Irish people.

Neuroscience Ireland is an all-island society that represents members north and south of the border. Recently, we have been delighted to welcome new members from the University of Ulster and Queens University Belfast to our Council and we are hoping to increase and strengthen those north-south links and expand our membership across the island. Our next 'Young Neuroscience Symposium', a biennial scientific meeting focussed on early career

‘Our stated mission is to advance research and education in the neurosciences in Ireland, and to represent Irish neuroscience researchers both nationally and internationally.’

researchers, is being organised by our colleagues based in Northern Ireland and will be held in November. Due to COVID-19, it will be a virtual event but the society hopes to host one of our in-person scientific meetings in Northern Ireland at some time in the future.

Ireland is a small country but we make disproportionately large contributions to science, as in many areas, due to our ability to build strong and lasting working relationships. Just as Ireland sees its future within Europe, Neuroscience Ireland sees membership of FENS as key to its future success. Two Irish members, myself and former President Dr Eilís Dowd of NUI Galway, currently sit on the Governing Council, and we have three members on the FENS’ standing committees. Having a voice at the European level is especially important as we continue to advocate for that important increased investment. We also have a particularly strong relationship with another FENS member society – the British Neuroscience Association (BNA) – with whom we have co-hosted scientific meetings. We want to see this relationship continue to flourish, notwithstanding the complex political and economic climate resulting from Brexit.

Overall, I want to grow the society and to engender within the membership a sense of belonging to a society that represents Ireland’s neuroscience community in all its geographic and disciplinary diversity.

How do you promote education and training for neuroscientists?

I sit on the FENS Committee for Higher Education and Training, so this area is a particular interest of mine. We are fortunate to have a talented pool of young researchers in universities throughout the country. We support these researchers in practical ways, by organising annual scientific meetings where they can share results and connect with each other and with international invitees and speakers. Every second year, this meeting takes the form of the Young Neuroscience Symposium mentioned above, which has a special emphasis on mentorship, networking and career development. We also provide travel bursaries to students and early career members to enable them to attend international conferences. All Neuroscience Ireland members are automatically members of FENS, which opens up huge opportunities for training and career development including the FENS summer schools and winter schools and the CAJAL Advanced Neuroscience Training Programme.

Do you provide funding opportunities for researchers?

We are a small society with limited resources, so we don’t have a budget to fund research directly. Instead, we channel our resources into providing funding for conference attendance to help researchers build their networks. We also provide small grants to help researchers host international conferences in Ireland.

‘There will continue to be huge opportunities to apply knowledge from neuroscience research to different aspects of economic or societal activity…this is where scientific communication and public understanding of science are key.’

In your opinion, what can neuroscience specifically bring to translational research and medicine?

In Ireland alone, over 800,000 people live with neurological conditions such as stroke, epilepsy, Parkinson’s disease, Alzheimer’s disease and multiple sclerosis and the associated costs are estimated to be €3 billion per annum. This is both a personal and a societal crisis, placing an enormous burden on healthcare systems. I think that improved diagnostics and treatments will only come from the type of interdisciplinary collaboration that characterises neuroscience. In Neuroscience Ireland we recognise this by ensuring disciplinary diversity within our Council membership by including clinicians and clinician-scientists, along with researchers working in basic and preclinical neuroscience research.

At the research level, relatively recent technologies like optogenetics have given neuroscientists better tools to explore and attempt to understand fundamental neurophysiological processes and disease processes. While these have the potential for therapeutic use, their invasive nature is an obvious drawback that must be overcome. But importantly, as has always been the case in scientific research, hypotheses, concepts and theoretical frameworks of understanding need to evolve alongside technologies if newly-discovered mechanistic information is to be applied for human benefit.

In recent years, neuroscience has given rise to a range of applied disciplines including neuroeconomics, neuroethics, and neurolaw. What opportunities and challenges do you see on the horizon as a result of this expansion of the application of neuroscience?

Many neuroscientists are very wary of the recent trend for sticking the ‘neuro’ prefix in front of an existing disciplinary name and heralding the arrival of a new discipline. It is not always helpful and can, in fact, be harmful if neuroscience findings are applied in an uninformed, misinformed or premature manner. One clear example here is of the increasing introduction, especially in the USA, of imaging data into the courtroom, where jurors or judges may not have the knowledge to understand the validity of the evidence or the scientific concepts being presented. That being said, there will continue to be huge opportunities to apply knowledge from neuroscience research to different aspects of economic or societal activity. To me, this is where scientific communication and public understanding of science are key. Researchers have a responsibility to communicate their findings to the public directly and in a considered and clear manner. We all have a part to play in ensuring the continuing credibility of scientists and public trust in the scientific enterprise.

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Neuroscience Ireland

TACKLING DISEASE

NEW APPROACHES TO TACKLING DISEASE

The second section of this critical issue of Scientia showcases the important work of researchers striving to overcome the challenges presented by diseases and disorders across the world. We read how the rapid pace of medical breakthroughs and scientific advancements is improving the lives of many, from those living with commonly occurring acquired conditions such cardiovascular disease (CVD) and human immunodeficiency virus (HIV) to more rare genetic conditions such as Prader-Willi syndrome and Huntington disease (HD).

We open this section by meeting Dr Jarrad Scarlett and his research team at the University of Washington and Seattle Children's Hospital. One of the most impactful and costly biomedical challenges is that of Type 2 diabetes (T2D). At the current time, medications for T2D can only delay disease progression and frequently have undesired side effects. We read how Dr Scarlett and his team are working to overcome these difficulties by developing novel pharmaceuticals to target the brain and induce sustained remission of T2D.

We then turn to CVD, the world's leading cause of preventable death. Dr Madhumita Chatterjee (University Hospital Tübingen), Professor Michael Lämmerhofer and Professor Tilman Schäffer (both engaged with the University of Tübingen) are investigating the molecular mechanisms directing the function of tiny blood cells in the formation of blood clots. We read how their work is leading to the development of a new diagnostic tool to determine the risk of thrombosis in patients with CVD and potentially inform the development of preventative therapeutic strategies.

Another major biomedical challenge is that of HIV. It is estimated that 38 million people currently live with HIV infection across the world. Professor Tomáš Hanke (jointly from the University of Oxford, UK, and Kumamoto University, Japan), is working to overcome the challenge of HIV by designing vaccines and coordinating clinical programmes testing advanced vaccine candidates developed by his team.

For chronic or complex conditions such as HIV, managing communication and health data can be a challenge for patients and healthcare professionals alike. Dr Bertrand Lebouché and his team at the McGill University Health Centre are seeking solutions through the development of smartphone-based applications to improve the lives of patients with HIV and those with other conditions.

Although perhaps less well-known than HIV, Legionnaire's disease remains a potentially severe and lethal form of pneumonia, the associated risk of which is increased by the difficulty in identifying different subtypes of the disease. We read how Dr Akihiro Ito at Kurashiki Central Hospital, Japan, is advancing the detection of the different forms of *L. pneumophila* with the aim to facilitating more rapid and efficient diagnosis and thus, more effective and timely treatment.

We then turn to the work of Professor Gordon Carmichael and his team at the University of Connecticut Health Centre on Prader-Willi syndrome. This rare neurodevelopmental disorder is inherited and affects the individual from birth with a wide range of symptoms, including impairments to cognitive function. We read how Professor Carmichael is exploring the affected chromosome 15 region to better understand the pathogenesis of Prader-Willi syndrome, and how his work may advance knowledge about other diseases as well as the development of potential treatments.

Dr James Goldman and Dr Osama Al-Dalahmah, both at Columbia University, USA, are working to better understand the pathology associated with the neurological disorder HD, which is characterised by cognitive impairment among other life-impacting symptoms. We read how Dr Goldman and Dr Al-Dalahmah aim to develop therapeutics that can slow the progression of HD, and ultimately, treat and even prevent it.

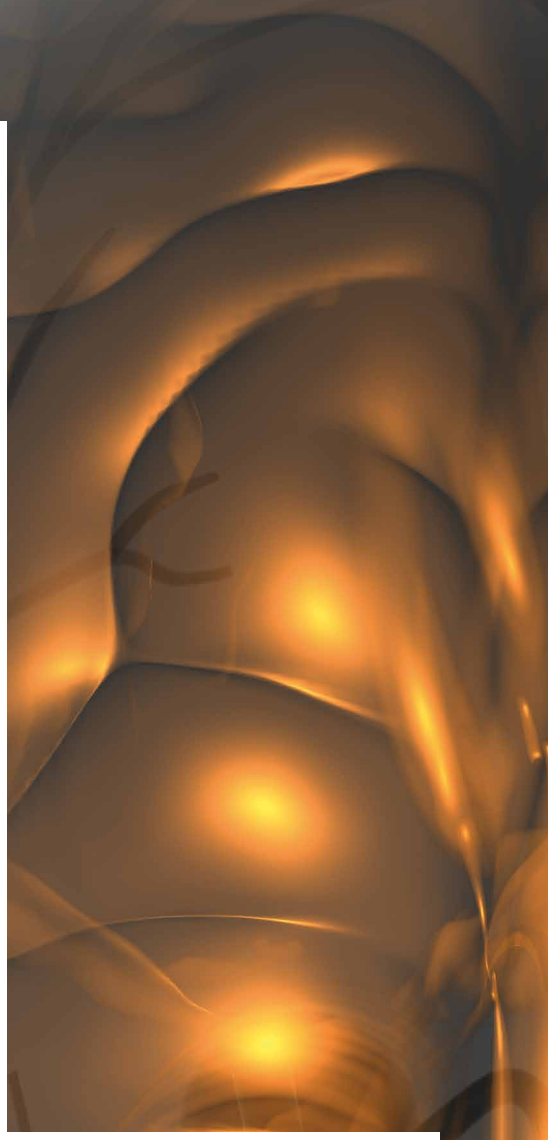
Remaining on the topic of cognitive function, we turn to the work of Professor John Connolly, Director of the ARiEAL Research Centre at McMaster University, Canada, and Co-Founder and Chief Science Officer at VoxNeuro. Professor Connolly has developed an innovative neurotechnology to assess cognitive functioning in individuals that, unlike traditional assessments, does not rely on verbal or behavioural responses. This means cognition can be assessed even in patients who unable to communicate in this way due to impairment arising, for example, from brain injury or disease.

Dr Mary Whitman, at the Boston Children's Hospital, USA, is the final researcher to be featured in this section. We read of her work aiming to better understand the genetic causes and neurological mechanisms underlying eye movement disorders such as strabismus (eye misalignment) and nystagmus (involuntary oscillation of the eyes) to improve treatment and, ultimately, prevent their onset.

We conclude this section by meeting Professor Sir Rory Collins Principal Investigator and Chief Executive of UK Biobank, a large-scale biomedical database and research resource containing genetic, lifestyle and health information from half a million UK participants that is available to researchers across the globe.

TARGETING THE BRAIN IN TYPE 2 DIABETES: THERAPEUTICS TO INDUCE REMISSION

Type 2 diabetes (T2D) is among the most impactful and costly biomedical challenges confronting society. Current treatment regimens for T2D rely upon daily drug dosing and frequent glucose monitoring to normalise blood glucose levels. However, these medications can only delay disease progression and frequently have undesired side effects including hypoglycaemia and weight gain. Growing evidence supports a key role for the brain in glucose homeostasis and diabetes pathogenesis. **Dr Jarrad Scarlett** and his research team at the University of Washington and Seattle Children's Hospital are working on the development of novel pharmaceuticals to target the brain to induce sustained remission of T2D.



Fibroblast Growth Factor 1 and the Brain

Fibroblast Growth Factor 1 (FGF1) is a growth factor and signalling protein that is involved in a wide range of biological processes. While the mechanisms of action of FGF1 in the brain remain to be elucidated, Dr Scarlett and his team have proposed that the brain is capable of initiating full remission of Type 2 diabetes (T2D) via the Fibroblast Growth Factor (FGF) receptor that is expressed on brain glucoregulatory neurocircuits.

To test this proposal, Dr Scarlett and his team compared the effects of central administration of FGF1 through injection into the brain with peripheral administration that was injected into the body. Tests in both mouse and rat models of T2D showed that a single central administration of FGF1 – at a dose only 10% of the peripheral dose – resulted in sustained remission of T2D. The data confirmed that a fall in the level of glucose in the blood resulted from the action of FGF1 binding to receptors specifically located in the brain.

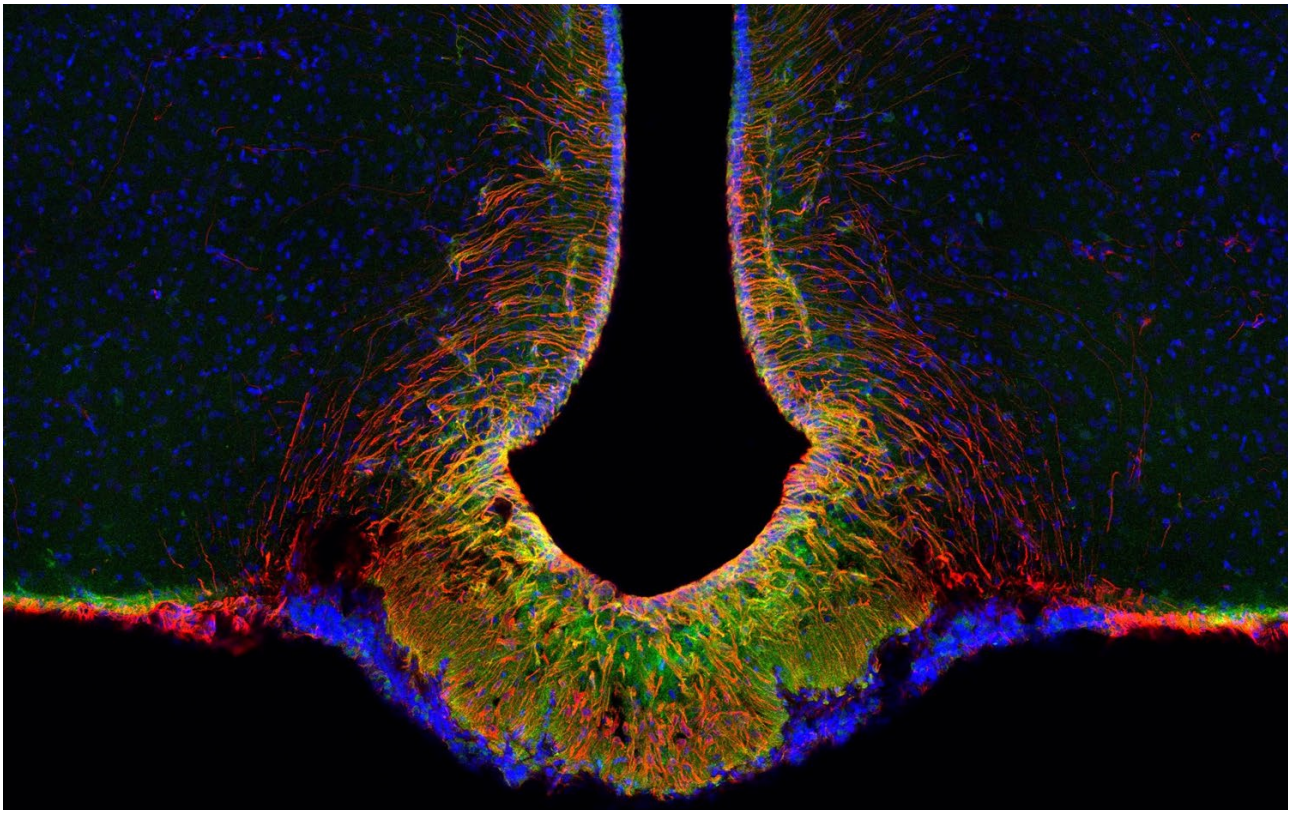
The low dose used in these tests negated the negative side effects associated with the currently prescribed anti-diabetic drugs, namely low blood glucose (hypoglycaemia). As such, the FGF receptors in the brain are believed to be viable targets for pharmacological solutions for diabetes in humans.

Sustained Remission of T2D

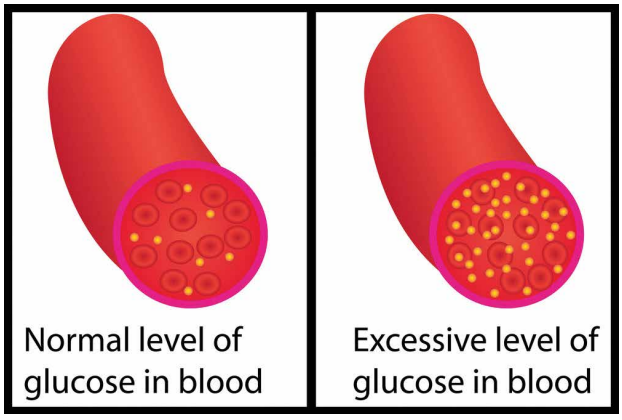
To determine whether this treatment may have longer-term effects and the potential to induce remission in T2D, Dr Scarlett and colleagues tested animals over a period of 18 weeks following a single low dose of FGF1 administered into a lateral cerebral ventricle using an implanted device, known as the ICV route. Blood glucose levels remained at normal levels in both fasted and fed

T2D mice over this extended period. This outcome indicated that sustained remission of hypoglycaemia due to T2D is attainable, at least in the animal models tested.

It is important to note that these changes did not reflect variations



Fibroblast Growth Factor in the hypothalamus. Credit Jarrad Scarlett.



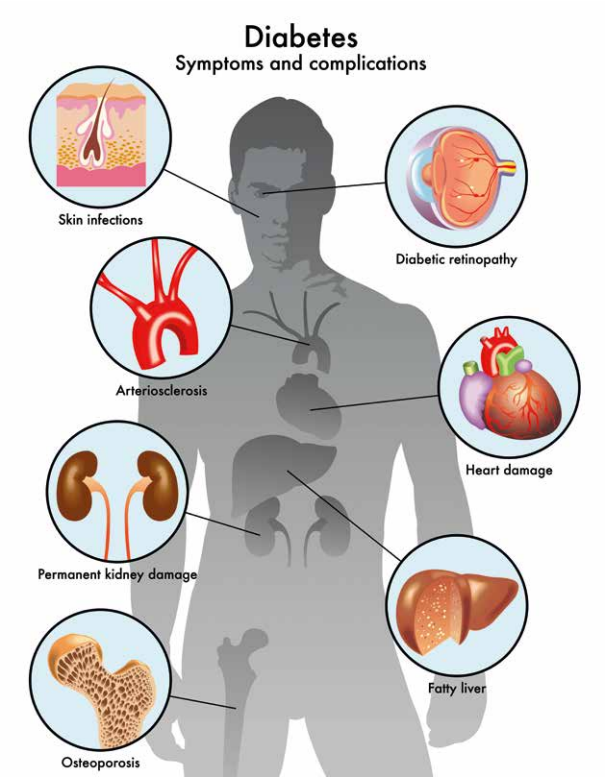
in either insulin or glucagon, the agents responsible for balancing glucose levels in the blood, and thus the mechanism involved is not related to either of these blood glucose control mechanisms. Instead, Dr Scarlett and his team found that central FGF1 action in the brain promoted the sustained lowering of blood glucose by a novel mechanism that doubled the clearance of glucose from the blood in the basal, pre-fed state.

Proposed Mechanism for FGF1 Action in the Brain of T2D Mice

Dr Scarlett and his team propose a mechanism of action that leads to the sustained remission of T2D via an enhanced clearance of glucose from the body. That is, some organs and muscles remove glucose more efficiently from the blood in response to FGF1 action in the brain. Dr Scarlett and his colleagues provide evidence to support this putative mechanism by demonstrating that there is no change to the base level of glucose production by the liver, nor is there any change to glucose tolerance, as denoted by insulin sensitivity, the acute insulin response to glucose, and by insulin-independent glucose disposal. Instead, they found that central FGF1 action produces a significant increase in glucose uptake by skeletal muscle and the liver.

The third ventricle of the brain, in which FGF1-responsive cells are located, is lined with cells known as tanycytes. These cells respond to both blood glucose and FGF1, and Dr Scarlett and his team investigated the importance of these cells in inducing remission of high blood glucose in T2D animal models. To do this, they compared the effects of two different types of FGF, namely, FGF1 and FGF19, and they found that while FGF1 induced remission in T2D mice, FGF19 did not.

In determining the effects of FGFs directly on tanycytes using specific tests for cell activation and expression, Dr Scarlett and colleagues found high levels of activation in response to FGF1 whereas no corresponding effect on the tanycytes was observed on exposure to FGF19. These results support the likelihood of a relationship between the tanycytes of the third brain ventricle and FGF1 in inducing remission in T2D.



Targeting the Brain

Given Dr Scarlett's key focus on translating research findings into useful therapeutics for T2D and other metabolic conditions, the team devised a protocol to determine the optimal brain area to target with the FGF1. Using a rat model of T2D, the team compared two regions of the hypothalamus (a specific area within the brain), and concluded that the arcuate nuclear-median eminence is the desired target area as sustained remission of high blood sugar is delivered on exposure to the FGF1-therapy, while injection of FGF1 into the paraventricular nucleus of the hypothalamus showed no effect with regards to hyperglycaemic remission.

Furthermore, this work demonstrated that a key marker protein, following FGF1 injection, was highly concentrated in glial cells, specifically tanycytes and astrocytes, which surround and support the brain neurons, and which the team has already proposed a putative role for in the initiation of remission of T2D.

Peripheral Changes in Response to Central FGF1 Administration

In humans, following the onset of high blood glucose levels, there is a progressive loss of cell function in the pancreas, which results in lowered insulin production. Recently, using a rat model which closely parallels this key aspect of human T2D, namely, the degradation of cell function in the pancreas, Dr Scarlett and colleagues investigated the peripheral processes that are induced and likely to contribute to the prolonged remission of hyperglycaemia observed when FGF1 is administered to the rat brain.

The team found that hyperglycaemic remission is supported by two processes. First, injection of FGF1 into the rat brain delays the progressive failure of the pancreatic cells that produce insulin and is likely to be a critical aspect for human T2D-remission inducing treatments, and second, by stimulating an increase in uptake of glucose from the blood by the liver.

The Brain, Glucose Management, and Diabetes

While it is clear that the brain has the potential to affect blood glucose levels, it remains unclear as to whether these capabilities are important on a day-to-day basis for glucose control. The influence of the brain is partially managed through fast, highly coordinated adjustments to both insulin sensitivity and secretion.

Dr Scarlett and colleagues propose that alterations in this brain-system, including high levels of blood glucose, contribute to the development of T2D under hypoglycaemic conditions, that is, when blood glucose levels are low. Some researchers have demonstrated the presence of glucose regulatory nerve circuits (neurocircuits) in the brain; however, there is no evidence as yet to indicate that there are similar neurocircuits in normal glucose conditions, only in T2D disease pathology.

Pivotal to this discussion is the finding by Dr Scarlett and colleagues that the brain is capable of reverting diabetic hypoglycaemia to a normalised state, that is, this process is not simply lowering blood sugar but, rather, remodelling dysfunctional glucoregulatory neurocircuits so to restore normoglycemia in a sustained manner.

Future Work

Delivering a pharmacological solution that induces remission of T2D is critical. T2D is a costly condition for health care providers by causing significant damage to patients and perpetuating damage to the pancreatic cells. Furthermore, the currently available drug treatments for T2D have significant side effects, including hypoglycaemia and weight issues.

Understanding of the damage inflicted on both adults and children by T2D has driven Dr Scarlett to focus his efforts on this scientifically controversial and innovative area. His work exploring longer-term remission of T2D, whereby treatment appears to have only minimal side effects, may last weeks or months. Dr Scarlett aims to translate his current work into viable pharmacological treatments for patients. This will necessitate an extensive programme of work including pre-clinical and clinical trials; however, the gains in the treatment of T2D will be many-fold.

Meet the researcher

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Dr Jarrad Scarlett is currently an assistant professor at the University of Washington and an attending paediatric gastroenterologist at Seattle Children's Hospital. After completing his PhD in neuroscience and MD at Oregon Health and Science University in 2009, he subsequently completed a residency in paediatrics and a fellowship in paediatric gastroenterology and hepatology. During his fellowship under the mentorship of Dr Michael Schwartz, Dr Scarlett focused his studies on the mechanisms whereby the brain-centred glucoregulatory system regulates blood glucose in response to neural and hormonal signals. Dr Scarlett has now established an independent research programme within the Diabetes Institute at University of Washington conducting translational research on the pathophysiology of diabetes and metabolic disease. As a ground-breaking physician-scientist, Dr Scarlett has received several young scientist and early career awards and has published multiple high-impact manuscripts.

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UW Medicine
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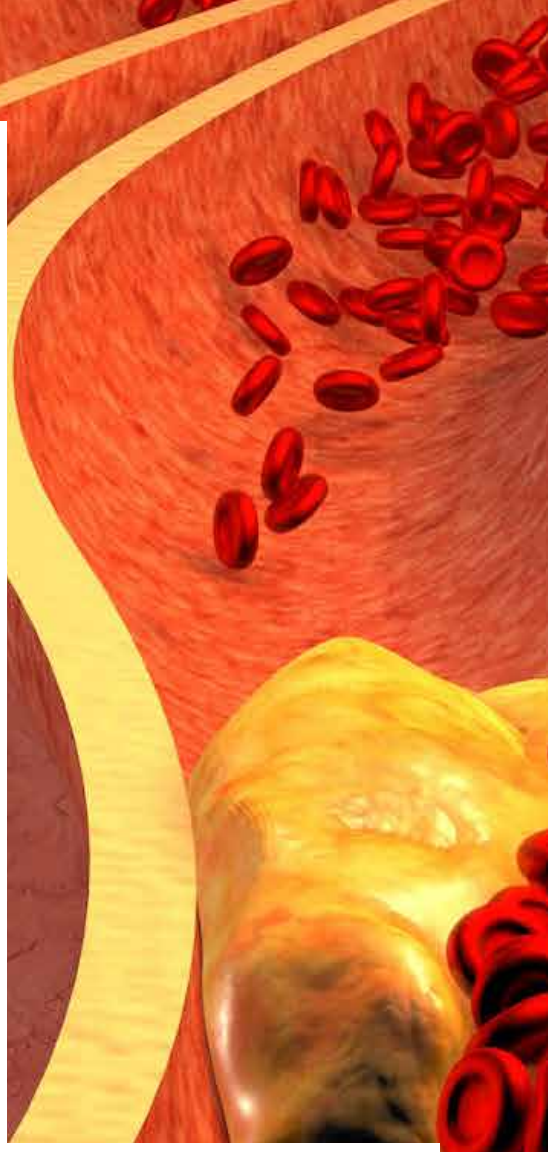
PLATELET LIPIDOMICS: A NOVEL APPROACH TO ASSESSING CARDIOVASCULAR RISK

Cardiovascular disease (CVD) is the world's leading cause of preventable death. A multidisciplinary team of researchers, **Dr Madhumita Chatterjee** (University Hospital Tübingen), **Professor Michael Lämmerhofer** and **Professor Tilman Schäffer** (both engaged with the University of Tübingen) are investigating the previously unrecognised molecular mechanisms that direct the function of tiny blood cells known as platelets in the formation of blood clots or thrombi, a condition that contributes to thrombosis and atherosclerosis. Their work is leading to the development of a new diagnostic tool to determine the risk of thrombosis in patients with CVD and also suggest potential therapeutic strategies to prevent such complications.



CVD – A Global Killer

Cardiovascular disease (CVD) is a broad classification of diseases involving the heart or blood vessels. CVD includes coronary artery disease (CAD) that causes heart attack, angina, sudden cardiac death, and cerebral stroke. CAD is characterised by a reduction in the blood flow to the heart muscle due to atherosclerosis in the blood vessel (coronary artery) supplying the heart.

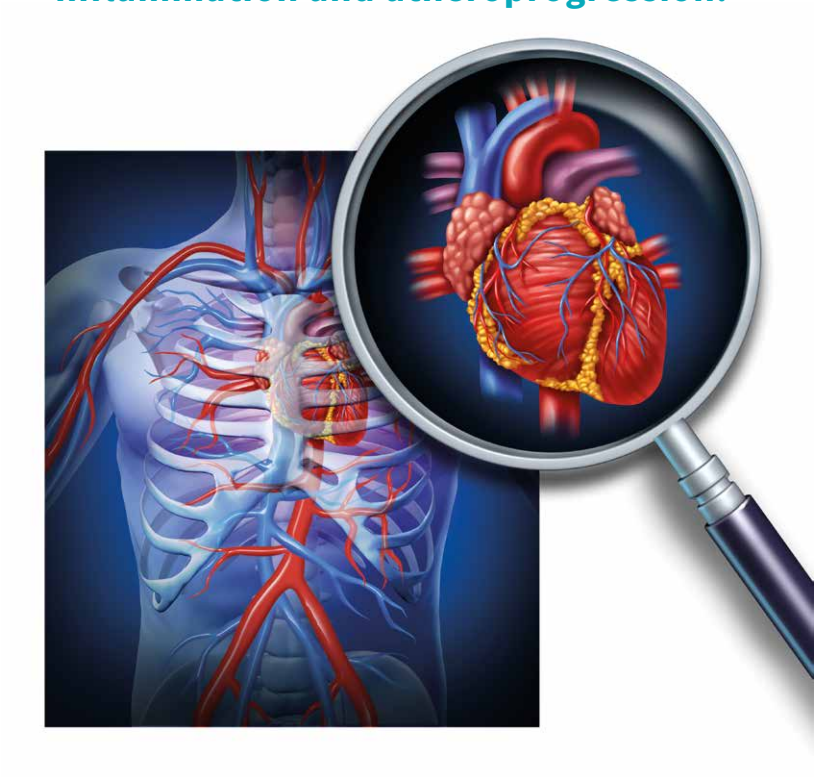
When atherosclerotic plaques rupture or erode, this triggers activation of platelets in the bloodstream leading to thrombus development at that site which is called atherothrombosis. Such thrombotic complications or formation of blood clots can then block the arteries supplying major organs like the heart or brain. The development of atherothrombosis is a complex and highly regulated process involving interaction between the blood vessel wall, platelets, endothelial cells, and plasma coagulation proteins.

There are many risk factors for CVD: age, sex (with males being more susceptible than females), tobacco use, physical inactivity, excessive alcohol consumption, unhealthy diet, obesity, genetic predisposition, raised blood pressure, raised blood sugar, and raised blood cholesterol (hyperlipidaemia).

Hyperlipidaemia refers to abnormally elevated levels of any or all the lipids or lipoproteins in the blood. Lipids (water-insoluble 'fat' molecules) are transported in a protein capsule termed lipoprotein. The size of that capsule, or lipoprotein, determines its density. Traditionally, to measure lipid levels in a patient's blood as an indicator of their CVD risk, the plasma levels of four major lipid categories are tracked – total cholesterol, low density lipoprotein (LDL), high density lipoprotein, and triglycerides. However, such a measure does not do justice to the complexity of the disease.

Ongoing research on platelet lipidomics involving Dr Madhumita Chatterjee (at the University Hospital Tübingen), Professor Michael Lämmerhofer and Professor Tilman Schäffer (both engaged with the University of Tübingen), has resulted in a much deeper understanding of the hidden role that platelets have in the metabolism and transportation of blood lipids and the consequences for the pathophysiology of CVD.

‘Platelets are an active accomplice in vascular inflammation and atheroprogression.’



Platelets, or thrombocytes as they are also known, contain a diverse variety of lipids that play a fundamental role in the regulation of platelet structure, signalling, and activation while promoting thrombosis, as recently reviewed by Dr Chatterjee while discussing her perspectives on the rapidly evolving field of platelet lipidomics, published in the Journal of Thrombosis and Haemostasis. When platelets attach or adhere to the site of blood vessel injury so that they can close the wound, they also deliver the harmful lipids contained within. This may gradually lead to atherosclerosis and increase the risk of subsequent CVD.

The Platelet Lipidome: ‘A Trojan Horse That Advances Undetected and Silently’

Lipidomics is the study of pathways and networks of lipid biogenesis, metabolism, and their functions in biological systems. Such research has been significantly substantiated by recent technical advances in analytical methods, allowing lipidomic profiling of blood plasma and also cellular components in the circulating bloodstream. The term ‘lipidome’ describes the complete lipid profile within a biological system such as plasma or cells.

Although still widely used, it is accepted that the measurement of traditional lipid biomarkers in plasma does not disclose the exact picture of lipid levels within circulating blood cells, which are separated from the blood as plasma samples are prepared in the testing laboratory. This may be one of the reasons why such routine procedures cannot accurately predict cardiovascular risk or clinical outcome in patients with hyperlipidaemia or be used to monitor the therapeutic efficacy of lipid lowering drugs such as statins.

Dr Chatterjee has longstanding expertise in the field of thrombosis and CAD. Professor Lämmerhofer and Professor Schäffer are at the cutting edge of new analytical chemistry and imaging technologies. Together, they are investigating platelet lipidomics and the functional consequences of platelet-lipid association. Professor Lämmerhofer is an expert in analytical chemistry and high-performance liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). His laboratory is engaged in the analysis of samples for the lipidomic profiling of platelets and plasma. Professor Schäffer is an expert in advanced imaging and nanoanalytical methods, examining the mechanistic properties of cells. His laboratory is exploring the physical properties of platelets under hyperlipidemic conditions and how that might influence platelet responsiveness.

In late 2015, working with Professor Lämmerhofer and Professor Schäffer, Dr Chatterjee and the team started performing lipidomic analysis of platelets in CAD patients, those that are at high risk of developing thrombosis and those who were experiencing major cardiovascular complications.

Following the lipidomic analysis of platelets in a small group of CAD patients, the team observed a significant alteration in their platelet lipidome, showing elevated levels of several oxidised phospholipids, triglycerides, cholesteryl esters, acylcarnitines, sphingomyelins, and ceramides. Although most of the patients had a normal plasma cholesterol profile as determined by traditional testing of plasma lipids, and several had already been administered lipid lowering drugs (such as statins), their platelets still contained evidence of potentially harmful atherogenic lipids. These results presented the opportunity to track pathological changes in the circulatory platelet lipidome and pointed to the fascinating prospect that the platelet lipidome might be used to reflect cardiovascular risk.

This study also revealed that platelets are in constant and dynamic interaction with the plasma lipids, and therefore, they carry large quantities of lipids imported from plasma. Some of these lipids are further metabolised internally within platelets to form more reactive and harmful atherogenic lipids. These are not recorded in the routine diagnosis of plasma lipids.

Dr Chatterjee describes the platelet lipidome as 'a Trojan horse that advances undetected or silently' to transfer and deposit atherogenic lipids at the site of blood vessel injury to initiate atherosclerosis. For example, Dr Chatterjee and colleagues have shown that platelets can take up LDL, which is then oxidised to more deleterious oxLDL. They have found increased levels of oxLDL in platelets from CAD patients and particularly in patients suffering from a more severe form of the disease known as ST-elevation myocardial infarction, a serious type of cardiac complication arising from blockage of the artery supplying the heart.

For conceptualising and coordinating this work, published in the reputed and high ranking European Heart Journal in 2017, Dr Chatterjee received the prestigious Uta und Jürgen Breunig-Forschungspreis from German Heart Research Foundation and German Society for Internal Medicine (der Deutsche Herzstiftung und Deutsche Gesellschaft für Innere Medizin).

In 2018, Dr Chatterjee, Professor Lämmerhofer and Professor Schäffer received funding from Deutsche Forschungsgemeinschaft (DFG, German Research Foundation; project no.374031971 – TRR 240) to assess the full impact of all lipids carried in the blood, including plasma lipids and those in peripheral blood cells, such as platelets and monocytes. With their previous vital insights into the platelet lipidome and its implications for CAD, Dr Chatterjee, Professor Lämmerhofer, and Professor Schäffer are now pursuing an extensive clinical investigation with CAD and stroke patients to characterise signature pathologic lipid metabolites in platelets for diagnostic and prognostic purposes. In the future, routine analysis of the platelet lipidome at clinics will aid in assessing the risk of recurrent thrombo-ischaemic complications resulting in heart disease and cerebral stroke, for example.

Therapeutic Potential of a New Anti-Platelet Strategy

Whilst hyperlipidaemia enhances the risk of coronary atherothrombosis, the contribution of platelets to lipid metabolism in CAD remains poorly defined. Platelets also have a decisive role in interacting with immune cells and regulate their inflammatory functions. Thrombus, once formed, becomes a hotspot for further inflammatory processes. Platelets release inflammatory mediators which exaggerate thrombo-inflammation. Furthermore, the pathophysiologically compromised and affected vessel walls in CVD patients trigger circulatory platelet activation and prompt platelet adhesion to the existing atherosclerotic plaques, which facilitates deposition of atherogenic lipids contained in platelets, thereby increasing inflammation and plaque instability.

Therefore, platelets are considered to be an active accomplice in vascular inflammation and atheroprogression, and their pathological attributes need to be controlled by the use of anti-platelet therapies. Dr Chatterjee is currently coordinating research to unravel the molecular mechanisms governing thrombotic and thrombo-inflammatory platelet functions in CVD.

Several anti-platelet drugs are currently available to effectively reduce thrombosis and cardiovascular death. However, they can have serious side effects. Prolonged use of anti-platelet therapies such as aspirin is known to increase the risk of internal bleeding, and so better alternatives are actively being sought by the team in their current research. As Dr Chatterjee explains, 'Our aim is to validate the therapeutic potential of a new anti-platelet strategy to check pathological thrombosis without enhancing bleeding tendency.'

At the core of Dr Chatterjee's research on thrombosis and thrombo-inflammation is understanding the role of G-protein-coupled receptors (GPCRs) – a large family of cell surface proteins that convert a diverse array of extracellular stimuli (such as chemokines and lipids) into intracellular signals, and eventually, regulate the biological response of cells like platelets.

Together with her colleagues at the University Hospital Tübingen, Dr Chatterjee has shown that the surface expression of the GPCR chemokine receptors CXCR4 and CXCR7 on platelets is significantly enhanced in patients with acute coronary syndrome and that the basal expression levels of these receptors influence recovery and prognosis.

The molecular mechanisms and interactions which regulate platelet thrombotic activity are complex and are influenced by an array of surface receptors linked to a network of activatory and inhibitory signalling cascades. However, Dr Chatterjee has identified the GPCR chemokine receptor CXCR7 as an unconventional and novel mechanism that may regulate platelet survival and platelet mediated thrombotic functions, which was published in the journal Circulation Research in 2014. Currently, she is actively engaged in the pre-clinical validation of the therapeutic potential of CXCR7 as an anti-platelet drug target in several animal models of thrombosis and CVD.

On reflecting on her work, Dr Chatterjee concludes, 'As scientists, it is our job to bring new, better diagnostic, prognostic and therapeutic alternatives to clinical practice to improve patient care. However, the translation from basic research to clinical practice is time consuming and requires stringent validation in large clinical cohorts, and possibly in multicenter studies. Nevertheless, we may look forward to a successful clinical implementation of these strategies that will facilitate the recommendation of 'tailor-made', personalised preventative medicines, to individuals at high risk of thrombosis and thereby cardio/cerebrovascular diseases.'

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CRC/TR 240 Platelets –
Molecular, cellular and systemic
functions in health and disease

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USE OF EXPERIMENTAL MEDICINE FOR RATIONAL DEVELOPMENT OF AN EFFECTIVE HIV VACCINE

The UNAIDS estimates that 38 million people currently live with human immunodeficiency virus (HIV) infection. Combination antiretroviral treatment has had great success in saving lives but is also associated with numerous medical and public health challenges. Vaccination remains the best and most cost-effective option for controlling HIV infection across the world. **Professor Tomáš Hanke** jointly from the University of Oxford, UK, and Kumamoto University, Japan, designs vaccines and coordinates clinical programmes testing the most advanced vaccine candidates developed by his team in the UK, Europe, USA and Africa.

End AIDS with Vaccination

Human immunodeficiency virus (HIV) type 1 represents 95% of all HIV infections worldwide and is responsible for the global HIV pandemic. If untreated, HIV-infected patients develop acquired immunodeficiency syndrome – better known as AIDS – that manifests as a progressive failure of their immune system. As a result, patients eventually succumb to opportunistic infections. Combination antiretroviral treatment (cART) has transformed the lives of people living with HIV, and dramatically decreased AIDS-related mortality and onward transmission of HIV.

Unfortunately, the provision of cART to everybody who needs it faces many obstacles particularly in low- and middle-income countries. The cost, complexity of the treatment, necessity of regular monitoring of patients, threat of drug resistance, side effects, social stigma and the use of cART to prevent HIV infections (or pre-exposure prophylaxis), which further stretches the cART supply, make cART a suboptimal therapeutic and an unlikely stand-alone tool to end the HIV epidemic. Therefore, an effective vaccine for both prevention and cure of HIV is urgently needed.

Professor Tomáš Hanke and his team at the Jenner Institute at the University of Oxford, UK, together with their collaborators at the Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan, are studying T cell responses to HIV to inform vaccine development. In addition, Professor Hanke oversees Experimental Medicine trials of his leading T-cell vaccine candidates in healthy and HIV-positive people at several global sites and collaborates with prestigious universities and

organisations such as the International AIDS Vaccine Initiative, IrsiCaixa AIDS Research Institute-HIVACAT in Spain, Imperial College London, the Kenya AIDS Vaccine Initiative-Institute for Clinical Research and National Institute of Allergy and Infectious Diseases. He also co-ordinates the 'Globally Relevant AIDS Vaccine Europe-Africa Trials Partnership' consortium, acronymed GREAT, which builds research capacity and tests vaccine candidates in Eastern and Southern Africa, and is one of the principal investigators of the European AIDS Vaccine Initiative 2020.

Rational Iterative Development

Most of today's HIV vaccine research focuses on antibody-mediated immunity, which neutralises cell-free viruses and typically involves exposing people to the outer HIV spike. However, to achieve HIV control, antibodies may need to be complemented by T-cell responses, the focus of Professor Hanke's research. There is no doubt that T cells contribute in an important way to anti-HIV immunity, whereby CD8 T cells known as 'killer cells' directly kill virus-infected cells, the virus factories, and CD4 T cells or 'helper cells' assist and co-ordinate antibody and T-cell induction. 'The trick is to induce not just any, but protective killer T cells that can slow or stop HIV,' explains Professor Hanke.

The first clinically tested vaccine that Professor Hanke and his colleagues developed was called HIVA. It was derived from an African clade A strain of HIV and was tested in over a dozen clinical trials. Following the field's full appreciation of the HIV's enormous ability to change, Professor Hanke improved his approach by focusing vaccine-elicited T cells on the functionally conserved regions of HIV, which are common to most HIV strains and essential for virus survival. If successful, such a vaccine could be deployed universally in all global regions.

The prototype conserved immunogen was called HIVconsv (to emphasise conserved in addition to consensus sequences) and assembled highly conserved HIV regions into a chimeric protein alternating the global major HIV strains. This vaccine showed encouraging results in initial small clinical trials and informed the design of the second-generation conserved vaccines called HIVconsvX. Notable HIVconsvX improvements include the use of bioinformatics to redefine conserved regions and increase the vaccine match to the global HIV variants by using a so-called 'mosaic' design. The HIVconsvX vaccines entered clinical evaluation in 2019 with further trials in the pipeline.

The Importance of Vaccine Vectors

The quality of vaccine-elicited T-cell responses is strongly influenced by the way HIV immunogens are introduced into the body. The utmost priority is safety and Professor Hanke and his colleagues test all potential vaccine vectors intended for human use in mice and macaques first. The three most promising modalities that progressed in combination to human studies were 1) plasmid DNA, 2) engineered adenovirus of chimpanzee origin, the parent of which causes a common cold-like disease in monkeys, and 3) a poxvirus modified vaccinia virus Ankara (MVA), an attenuated smallpox vaccine used safely in many people during the smallpox eradication campaign. None of these three vaccines is replication-competent and can grow in the vaccinees' body or spread to the environment; they are safe.

The HIVA vaccine was delivered by a combination of DNA and MVA and induced only weak T-cell responses mainly because of the inefficient DNA prime. Induction of T cells by the HIVconsv vaccines was greatly improved

by the addition of the chimpanzee adenovirus. However, the adenovirus-MVA combination without DNA was as good as all the three vectors together and was therefore chosen for further studies.

HIVconsv vaccination-induced strong T cells that recognised multiple sites on the HIV. Vaccine-elicited T cells in HIV-negative volunteers in Nairobi, Kenya, were capable of a broad cross-clade inhibition of HIV under laboratory conditions. The HIVconsv vaccines were also tested in 'kick-and-kill' studies in early treated HIV-positive individuals. During infection, HIV integrates into the host chromosome, stops expressing its proteins ('falls asleep') and becomes invisible to the immune system, but regularly reactivates. This means that to eliminate HIV from the body, all sleeping HIV first needs to be awakened, or 'kicked' before it can be killed by the vaccine-induced killer T cells.

In a small pilot 'kick-and-kick' study in Barcelona, Spain, the HIVconsv vaccines together with an HIV-reactivating drug provided a signal of sustained suppression of HIV replication after stopping cART. Although a marginal result, it was very encouraging and warranted further testing of the 'kick-and-kill' strategy with these vaccines as an HIV cure.

Understanding the Consequences of HIV Variability

A successful vaccine needs to elicit killer T cells capable of reaching HIV-infected cells and killing them to stop virus growth. To be safe and effective, the killer T-cell assault must be sufficiently specific and efficiently target vulnerable parts of the HIV from the very first exposure to the virus. However, HIV is extremely variable and this makes it very good at avoiding the T-cell attack and escaping. There is lots of supporting evidence that people's genetic makeup, the sites on HIV that killer T cells target and HIV escape are the major determinants of how well individuals fight HIV and scientists need to understand these processes in great detail.

Some T-cell responses are better at protecting than others. In the past, attempts to understand which parts of HIV should be targeted for protection often looked at responses to the whole virus and/or full-length virus proteins as units. This blurred the analysis because within each protein there are both stable and variable regions and these are not equally protective. Professor Hanke's strategy exploits the stable and therefore vulnerable parts on HIV proteins.

This idea was supported by studies of Professor Hanke's colleagues at Kumamoto and Tokyo Universities. HIV-infected patients, who never received any cART, controlled HIV better and were healthier (had more CD4 cells in the blood) if they targeted the same regions as used in the vaccine. This is an important observation endorsing this vaccine approach.

The Quest for Improvement

Although Professor Hanke's strategy is rational and, so far, supported by good experimental results, many challenges remain on the road to an effective T-cell vaccine.

To be efficient, T-cell responses must, upon HIV exposure/reactivation, rapidly reach the sites of HIV growth within the patient's body, kill infected cells and produce anti-HIV chemicals, be in sufficient numbers, and recognise multiple vulnerable regions at the same time to make escape difficult. It is plausible that if any one aspect of these T-cell properties is suboptimal, the vaccine may fail.

Professor Hanke and his colleagues study T-cell responses induced by HIV infection and vaccination in order to further refine the vaccine immunogens and their vector delivery. Novel and sometimes small but significant step-by-step improvements are tested in pre-clinical investigations and human trials. 'Iterative improvements are best informed by human data, the only species that ultimately matters,' says Professor Hanke.

Finally, new-born babies, children and adolescents, some of whom have acquired HIV perinatally, that is, via mother-to-child transmission, or babies who are exposed to HIV through mother's milk, remain somewhat unique populations because of their young and, if treated soon after birth, relatively preserved immune system. To date, there have been several hundred HIV vaccine trials in humans, but only a very few tested candidate HIV vaccines in these age groups. Yet, childhood vaccines are the biggest success of vaccinology. Professor Hanke and his colleagues tested the HIVA vaccine in African neonates as the first step towards preventing mother-to-child transmission through breastfeeding and are planning to revisit these age groups using the conserved mosaic vaccines.

Meet the researcher

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Born in Prague, Czechoslovakia, Professor Tomáš Hanke started at Charles University reading Chemistry and completed his BSc in Biochemistry at McMaster University, Canada, followed by an MSc specialised in Herpes simplex virus immunology. He then completed a PhD focused on HIV vaccines at St Andrews University in Scotland, UK. In 1994, he started a postdoctoral fellowship at the University of Oxford where he is now a Professor of Vaccine Immunology at the Jenner Institute. Since 2015, he has taken a part-time appointment at the University of Kumamoto, Japan as Distinguished Professor. This was initially in the International Research Center for Medical Sciences, and since 2019, in the Joint Research Center for Human Retrovirus Infection. Professor Hanke's work is balanced between translational and basic research with the primary goal of contributing to the development of an effective HIV-1 vaccine. He has coordinated a translational programme assessing candidate HIV vaccines at a number of sites in Europe, USA and Africa and built research capacity at several sites in Africa. With numerous international collaborations, Professor Hanke's laboratory innovations aim to stay one step ahead of the clinical testing.

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EMPOWERING PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS AND OTHER PATIENTS USING mHEALTH

The healthcare system can sometimes be puzzling, and even unwelcoming, for people with chronic, complex, or multiple conditions. Many must spend long hours in waiting rooms, try to make sense of complicated scientific information, and book and attend multiple clinic appointments with clinicians from different specialities. Remaining up to date on patients' health data can be a challenge for healthcare professionals as well. Here, we present the critical work of **Dr Bertrand Lebouché** and his team at the McGill University Health Centre, who are seeking solutions in smartphone-based applications relevant to both patients and healthcare professionals.

An App to Navigate the Healthcare Maze

In 2018, a team composed of Laurie Hendren, a patient and researcher at McGill University, and members of the Department of Oncology, Tarek Hijal, John Kildea and Jamil Asselah, implemented a 'patient portal' at the Cedars Cancer Centre of the McGill University Health Centre. This patient portal, named Opal, was designed to address specific difficulties experienced by oncology patients, whose typical treatment requires multiple hospital appointments with clinicians from a variety of specialities. With the need for sometimes rapid care and appraisal, oncology patients and clinicians typically struggle with having access to current or 'real-time' patient health data, particularly when concurrently involved in complex interventions.

Opal was developed as a smartphone app that accords patients immediate and portable access to their medical health record. Patients can consult their appointment schedule, their healthcare professionals' clinical notes, their laboratory test results including ongoing trends and changes, and their radiotherapy treatment plan and current status. In addition, patients are assisted in navigating complex treatment terminology and interventions through personalised educational material tailored to their diagnosis and stage of treatment. Opal reinforces patient-centred care by allowing healthcare professionals to administer patient-reported outcome measures electronically (ePROM) to their patients. Finally, Opal includes a check-in system notifying patients when their physician is ready to see them, reducing the time spent in waiting rooms.

Opal has won several awards and has been proven to successfully empower patients. It provides a practical interface for patients and their healthcare professionals to store, communicate and share information, limiting face-to-face appointments. The use of this technology, known as mobile health (or mHealth), has a strong research track-record for improving engagement in care and health decision-making. Patient users have noted improvements in their perceived self-control and self-efficacy, which are critical factors in encouraging behaviour change and adherence to treatment regimens.

Dr Bertrand Lebouché's team, based at the McGill University Health Center, has integrated principles of patient and stakeholder involvement in their research. Dr Lebouché quickly saw the potential for utilising the Opal app platform for his own work with people living with human immunodeficiency virus (HIV) and hepatitis C virus, building on his work in the previous decade.

mHealth Solutions to Support Behaviour Change

To support HIV patients specifically, Dr Lebouché was looking for ways to better meet the needs, preferences, and specific circumstances of people living with HIV and their healthcare professionals.

HIV treatments dramatically improved since the 1990s. HIV is now treated as a life-long chronic condition. To maintain a near-normal quality and length of life, and to reduce the risk of transmission to sexual partners, people living with HIV need to adhere to their antiretroviral treatment, that is, take their treatment as prescribed. As with most long-term drug regimens, maintaining adherence is often difficult, and only about 60% of people living with HIV take 90% or more of their antiretroviral drugs. The team determined that tools must be developed to support decision-making for patients facing complex situations, and to provide timely and useful data for healthcare providers about patients.

Dr Lebouché's team has been working on better understanding and improving adherence since 2012, while developing patient engagement approaches. In a project entitled the I-Score Study (for Interference Score), they have worked on developing an ePROM, an mHealth tool, for people living with HIV to identify barriers to taking their treatment and report these to their healthcare professionals prior to their clinic appointments. It is expected to enable improved patient-provider dialogue and management of these difficulties. To develop the ePROM, the team conducted a review of qualitative studies on barriers to adherence. They grouped these barriers into six domains and 20 subdomains, showing that they affect virtually all areas of life. The identified domains were very similar to the World Health Organization's core barriers for patients with chronic illness across a range of conditions.

While initiating the I-Score Study, Dr Lebouché's team recruited ten people living with HIV to form an advisory committee, the I-Score Consulting Team. The Consulting Team was invited to provide their perspective and first-hand expertise at each step of the I-Score Study to ensure that the ePROM was responsive to the concerns of people living with HIV. The I-Score Consulting Team is also actively involved in disseminating I-Score research findings to the HIV community, healthcare providers and academics.

The work of Dr Lebouché's team identified deficiencies in the chronic care model that require improvement. Chronic patients generally self-manage significant amounts of their own care (for example, monitoring and taking medications at home), and as identified earlier, often struggle to maintain appropriate levels of self-care. With the growing use of smartphone

technology, including among older generations, mHealth appears to offer a highly flexible approach to promote self-management, guidance, access to health records and improved communication with healthcare providers. It thus has the potential to greatly empower patients to be more involved in medical decision-making about their health.

Integration with Opal

With the successful launch and usage of the Opal app for oncology, Dr Lebouché's team started work to adapt Opal to meet their objectives for people living with HIV and their healthcare professionals. The team administered a survey to 114 people living with HIV on the potential use of Opal. Nearly three-quarters of respondents said they would use Opal, with features such as appointments and educational material being widely appreciated. Understandably, concerns were raised over confidentiality and the wider sharing of personal data.

Meanwhile, the research team has also been working with Sofiane Achiche, Professor at Polytechnique Montreal, on an 'intelligent conversational agent' (ICA), a chatbot named MARVIN (Minimal ARV Interference). They had the idea for MARVIN while considering solutions to barriers identified by the I-Score ePROM that could be addressed with practical information. A multidisciplinary group of patients, physicians, pharmacists and engineers took a user-centred approach to work on the conception of the ICA. Software engineering students from Polytechnique Montréal then built a prototype for early-stage validation by PLHIV testers, leading to a much-improved final version.

An automated AI-based solution, like a chatbot, has the benefit of being available 24 hours a day, is confidential and can send automated reminders. Although still in its early stages, MARVIN will be designed as a retrieval-based ICA trained to have a naturalistic conversation with users via text or voice messaging, to support people living with HIV to overcome some of their adherence barriers. The three areas MARVIN will focus on are guidance for effectively using antiretroviral treatment (e.g., time management, taking it with or without food), dealing with the financial costs of ART, and managing ART when travelling or away from home. A usability study will be conducted with MARVIN in autumn 2020.

Could Opal Be Useful for COVID-19?

With the recent COVID-19 pandemic and its associated social distancing and minimal contact measures, Dr Lebouché and his team proposed that Opal could support COVID-19 patients, especially when they are self-isolating and medical support is limited. In Quebec, about 95% of COVID-19 patients have been required to self-isolate at home and follow strict hygiene rules for a minimum of two weeks, which many reported to be stressful and difficult to follow and maintain. Opal provides a promising tool to accompany patients at home, allowing them to monitor their symptoms and be linked to healthcare services, when needed, including teleconsultations and mental health support for anxiety and depression. Dr Lebouché and his team are currently conducting a pilot study of the Opal app with 50 COVID-19 patients, to daily self-monitor their health status and well-being, and to evaluate its feasibility for this use.

These innovations by Dr Lebouché and his team, combining patient engagement and genuine stakeholder input into the design and development process, illustrate the vast potential for the development of cost-effective tailored apps to improve the health of a wide spectrum of potential patient groups.

Meet the researcher

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Digital App for improving care in persons living with human immunodeficiency virus
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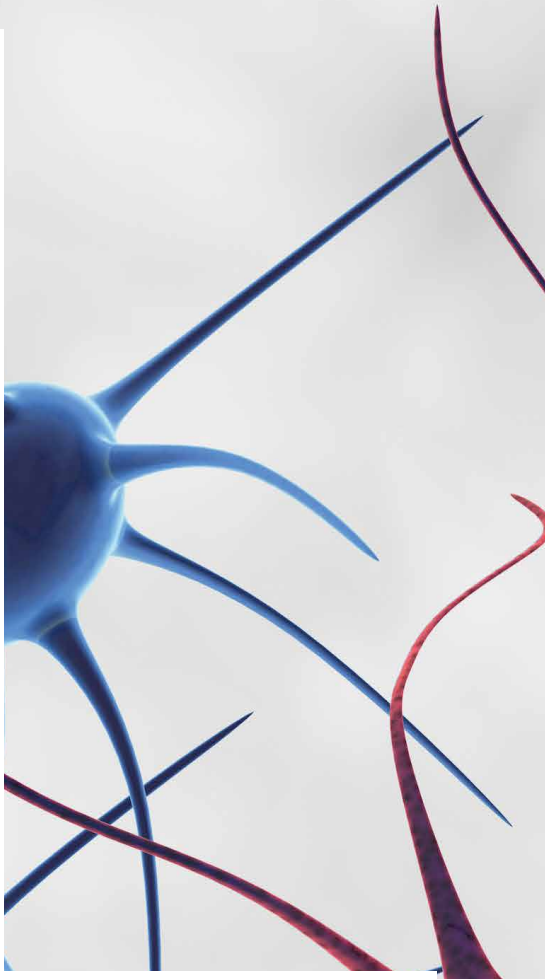
IMPROVING THE DIAGNOSIS OF LEGIONNAIRES' DISEASE

Legionella bacteria are known to cause Legionnaires' disease, a potentially severe and lethal form of pneumonia. The bacteria can be classified into different serogroups forming specific sub-types of *L. pneumophila*. In humans, serogroup 1 is the most common cause of pneumonia, being responsible for approximately 80% of all cases. However, the other subgroups can also cause pneumonia, resulting in a range of outcomes from mild to severe, and these are more difficult to accurately detect. **Dr Akihiro Ito** at Kurashiki Central Hospital, Japan, is advancing the detection of all forms of *L. pneumophila* to facilitate more timely healthcare interventions for Legionnaires' disease.



Approaches to Detecting *L. Pneumophila*

The gold standard approach to the detection of all serogroups of *L. pneumophila* in patients is the culture of respiratory specimens (sputum) to grow and identify the bacteria. Unfortunately, this process is costly and requires a substantial amount of time given the need to grow the cultures, which in some cases can take up to five days. This is problematic as a rapid timeframe is vital in diagnosing Legionnaires' disease, especially given that prompt antibiotic treatment often confers more favourable clinical outcomes.

An alternative and widely used method for the detection of *L. pneumophila* is a urinary antigen test. This test, which detects a protein from *L. pneumophila*, is rapid to perform, with results being available in about 15 minutes rather than within days as required for sputum culture. The difficulty, however, is that it only detects bacteria from serogroup 1, consequently omitting almost 20% of the cases deriving from the other serogroups (that is, non-serogroup 1 bacteria). Furthermore, the urinary antigen test also misses some cases of serogroup 1 *L. pneumophilia*.

The sensitivity of a test is a measure of the ability of the test to correctly identify patients with the disease being tested for (often referred to as the true positive rate). In the case of the urinary analysis test, slightly less than three-quarters of patients infected with *L. pneumophila* are correctly diagnosed. On the other hand, the specificity of this test is 99.1%. Specificity is a measure of the ability of a test to correctly identify patients without the disease – known as the true negative. In other words, when using the urinary analysis test, a positive result would most likely indicate Legionella pneumonia. However, almost one-quarter of cases of Legionnaires' disease are not detected. This total is the combination of cases of serogroup 1 *L. pneumophila* as well as the other serogroups.

L. pneumophila

Six Clinical Signs and Symptoms for Diagnosis

In addition to the urinary analysis diagnostic test, a six-point scoring system consisting of clinical signs and symptoms has been developed as a diagnostic tool. This has been purported to identify both serogroup 1 and non-serogroup 1 cases of *L. pneumophila*.

The assessment is based on the following criteria: the temperature of the patient, the presence of a non-productive cough, and levels outside those specified for four laboratory blood tests, namely, c-reactive protein (a

measure of inflammation in the body), lactate dehydrogenase (a measure of cellular damage), platelet numbers, and sodium levels.

Using these criteria, researchers have found that scores greater than or equal to 5 mean that patients without the condition are likely to be identified given the specificity of 99.0%. However, with a positive predictive value of 17.4%, the diagnostic tool carries a risk of high false positives whereby the patient does not have the disease but nonetheless receives a positive result. More useful is the scoring cut-off of 2 or less, which offers high sensitivity (94.4%) and a high negative predictive value (99.6%), and thus false negatives (whereby the patient has the disease but given the all-clear) are unlikely to occur.

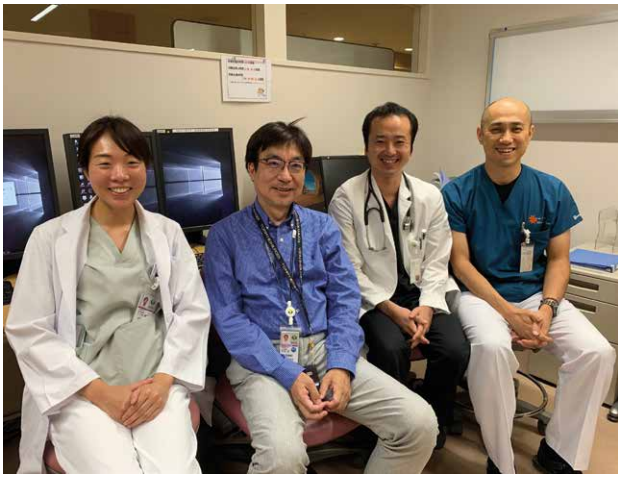
Identifying Non-serogroup 1 *L. Pneumophila*

Dr Akihiro Ito at Kurashiki Central Hospital, Japan, has been working to improve the detection of all cases of *L. pneumophila*. Along with colleagues, Dr Ito conducted a retrospective analysis examining historical cases of over thirty patients with *L. pneumophila* to assess the robustness of the six-point scoring system in predicting cases of non-serogroup 1 compared to serogroup 1 patients.

Of the total of 34 patients, 23 were serogroup 1 and 11 were non-serogroup 1 *L. pneumophila*. The determination of serogroup infection had previously been confirmed by sputum or blood culture analysis. The non-serogroup 1 patients commonly experienced non-specific symptoms, including fever and cough. However, in the laboratory blood analyses, only 54.5% of the non-serogroup 1 patients exhibited an increase in liver enzymes (aspartate aminotransferase or alanine aminotransferase), and none showed lowered blood sodium levels as indicated in the six-point scoring system.

Dr Ito and colleagues reported that cases of non-serogroup 1 *L. pneumophila* varied clinically from mild to severe and that the clinical observations were unremarkable. Applying the cut-off value of greater than or equal to two for the six clinical criteria, they found that the sensitivity for non-serogroup 1 cases was low at only 54.5%, representing just over half of the cases at 6 of 11 patients, and for the serogroup 1 cases, the sensitivity was 95.7%, which equates to the accurate diagnosis of 22 of the 23 serogroup 1 patients.

Expanding the clinical diagnostic criteria to investigate comorbidities, that is, other existing diseases, including diabetes and cardiovascular disease as well as other possible confounding contributors, such as age, history of smoking, and gender, Dr Ito and his team found no significant differences between the serogroup 1 infected patients and the non-serogroup 1 patients. This means that there are no notable markers or indicators which, in combination with the six-point assessment criteria, could reliably signal the presence of an *L. pneumophila* infection of a non-serogroup 1 type.



Dr Akihiro Ito and colleagues

Dr Ito and colleagues concluded from their findings that the six-point diagnostic criteria assessment using the cut-off value of greater than or equal to 2 correctly identifies most serogroup 1 cases. However, they also noted that this approach is completely inadequate for the accurate detection of non-serogroup 1 patients. Dr Ito and colleagues further propose that it is a clinical necessity to rapidly and accurately identify all cases, irrespective of the serogroup causing the infection. A summary of the current status of testing for *L. pneumophila* is as follows:

First, urinary analysis testing fails to detect some serogroup 1 cases of *L. pneumophila* infection and non-serogroup 1 patients are rarely detected by this diagnostic method.

Second, the diagnosis of non-serogroup 1 *L. pneumophila* infected patients by using the clinical six-point scoring system is poor, and Dr Ito's group found that this misses a larger proportion of patients than previously reported. They found a sensitivity of only 54.5% when exclusively focusing on non-serogroup 1 patients.

Dr Ito and colleagues point to the clear and obvious need for the development of a rapid and sensitive method to determine cases that are a result of infection with non-serogroup 1 *L. pneumophila* to enable early and effective intervention.

The New *L. Pneumophila* Urinary Antigen Kit: Ribotest® Legionella

In response to the clinical demand to more accurately detect all forms of *L. pneumophila*, Asahi Kasei Pharma Corporation (https://www.asahikasei-pharma.co.jp/en/) have developed a novel urinary diagnostic assay, which arrived on the Japanese pharmaceutical market early in 2019. Known as Ribotest® Legionella, the assay uses immunochromatography, a method that generates a coloured line to indicate the result and does not necessitate the use of any special equipment. The assay, which takes only 15 minutes to generate a result, identifies key proteins that are present in serogroup 1 *L. pneumophila* and other proteins that are present in serogroups 2 to 15 of *L. pneumophila* bacteria.

It is hoped that the Ribotest® Legionella assay will overcome the major drawbacks to the current existing urinary analysis diagnostic tests kits and the six-point clinical diagnostic framework, and in doing so, allow the rapid and sensitive identification of both serogroup 1 and non-serogroup 1 *L. pneumophila* infections.

Dr Ito and colleagues anticipate the uptake of this new assay in healthcare situations to make a substantial contribution to the diagnosis of Legionnaires' disease, identifying the significant proportion of cases that are currently unidentifiable in a timely manner, and subsequently facilitate the timely intervention with appropriate antibiotic therapies.

To this end, Dr Ito and colleagues have conducted an evaluation of the usefulness of the new rapid urinary antigen kit. Patients have already been recruited and studied, and the results will be published in the near future.

With a large sample size of patients, this prospective observational study represents an ambitious and important clinical trial in this field. The primary outcome is the success of the Ribotest® Legionella assay in diagnosing Legionnaires' disease. The secondary outcome is the extent of agreement between the Ribotest® Legionella assay outcomes with other clinical assessments. Ultimately, this work driving forward early identification and intervention in cases of all *L. pneumophila* infection is likely to lead to improved clinical outcomes for patients, the reduction and even prevention of patient deaths, as well as a reduction in the associated treatment costs of Legionnaires' disease.

Meet the researcher

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Dr Akihiro Ito, Chief of the Department of Respiratory Medicine for the Ohara Healthcare Foundation at Kurashiki Central Hospital, Japan, completed his medical education in 2004 when he graduated from Mie University School of Medicine. Following this, Dr Ito progressed through junior and senior residencies before joining the Kurashiki Central Hospital in 2009. Dr Ito undertook the Assistant Chief of Department role in 2011 and gained leadership of the department in early 2015. The focus of Dr Ito's research is based on assessing the likelihood of identifying cases of *L. pneumophila* using current diagnostic assays and previously confirmed diagnostic criteria. Dr Ito's work explores methodologies with the aim to facilitate the early and accurate diagnosis of the 20% of *L. pneumophila* cases that are undetected by current practices. He has published extensively in his field of expertise and is the recipient of several honours and awards.

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Kurashiki Central Hospital

UNRAVELLING THE BIOLOGY OF snoRNAs IMPLICATED IN PRADER-WILLI SYNDROME

Prader-Willi Syndrome is a rare genetic neurodevelopmental disorder that gives rise to a vast array of symptoms which affect the individual from birth. There is currently no cure for Prader-Willi Syndrome. **Professor Gordon Carmichael** and his team from the Department of Genetics and Genome Sciences at the University of Connecticut Health Centre, USA, believe it is crucial to understand the affected chromosome 15 region to unravel the pathogenesis of Prader-Willi Syndrome and his team are making significant strides towards achieving this goal.

Prader-Willi Syndrome

Prader-Willi Syndrome (PWS) is caused by the loss of function of a specific area of the paternal chromosome 15 – specifically, q11-q13 – due to deletions or mutations of this region. PWS is diagnosed at birth when symptoms include hypotonia (low muscle tone), feeding difficulty, poor growth and delayed development. In childhood, patients develop hypogonadism (a decreased production of sex hormones by the ovaries or testes) and an extreme appetite (hyperphagia) leading to overeating and obesity. Other symptoms include learning disabilities, behavioural problems, sleep abnormalities and underdeveloped genitals.

Adults with PWS are unable to live fully independent lives and the development of obesity due to excessive eating means sufferers are more likely to develop type 2 diabetes, heart failure and respiratory problems. There is no cure for PWS and current treatments are designed only to manage symptoms such as overeating and behavioural problems. Life-expectancy is considered to be around 29 years.

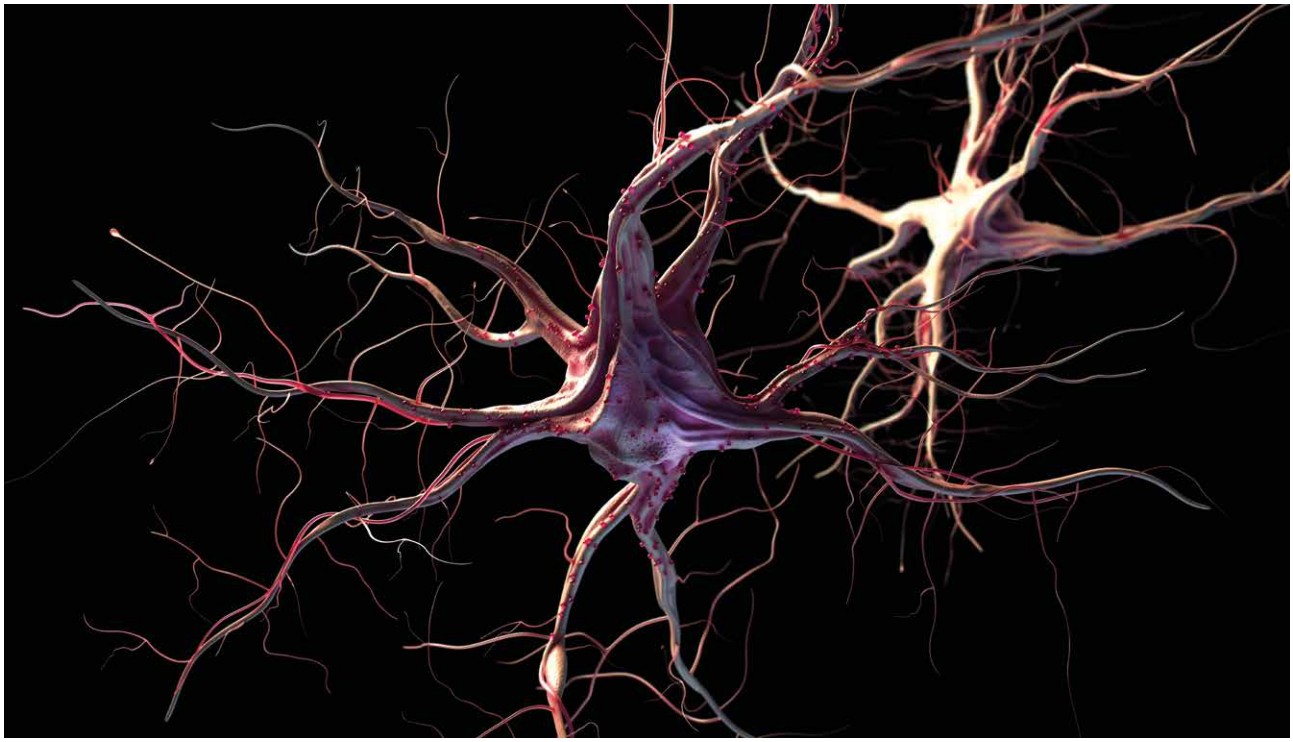
PWS is a complex disorder, the intricacies of which are still unclear. However, understanding of the molecular mechanisms that underlie PWS can potentially change the management and treatment of the disorder and improve the quality of life of sufferers. Professor Gordon Carmichael and his team at the Department of Genetics and Genome Sciences, University of Connecticut Health Centre, are leading the way in characterising the genetic and molecular defects that underlie PWS to help elucidate the mechanisms of this intriguing disease.

The Discovery of New RNAs in PWS Pathogenesis

Professor Carmichael explains that the 15q11-q13 critical region is 'extremely complexed, generating a number of both coding and noncoding RNAs.' Among the important non-coding

RNAs expressed from this region are snoRNAs (small nucleolar RNAs), which are conserved nuclear RNAs between 70–200 nucleotides long and are believed to function in the modification of small nuclear RNAs (snRNAs) or ribosomal RNAs (rRNAs), or may be involved in rRNA processing during ribosome biogenesis. snoRNAs are encoded in the introns of protein-coding genes and several hundred snoRNAs are known to exist.

‘Finding sites of modification will be exciting, but the real challenge that lies ahead is to then figure out how such modifications affect RNA function and are connected to PWS pathology.’



snoRNAs have been reported to be involved in the function and development of the brain. Moreover, those in the chr15q11-q13 region are not expressed from the paternal chromosome of PWS patients or PWS mouse models, indicating a key role of snoRNAs in PWS. While most snoRNAs are excised from debranched introns by a process called exonucleolytic trimming, and then form complexes with specific protein components to form snoRNPs (ribonucleoprotein complexes) and carry out their functions, in a 2012 research paper, Dr Carmichael’s team provided an account of how snoRNAs can also lead to the processing of a new class of long non-coding RNAs.

The many snoRNAs can be divided into two main classes: box C/D snoRNAs and box H/ACA snoRNAs. The box C/D snoRNAs guide 2’-O-methylation and box H/ACA snoRNAs guide pseudouridine modifications. Many researchers agree that snoRNAs are key to PWS. However, the function of the snoRNAs in the PWS critical region and on the function of neurons in the brain is unknown.

Within the 15q11-q13 critical region there lies a cluster of about 30 C/D box snoRNAs – this cluster is called *SNORD116*. *SNORD116* is highly implicated in PWS as all reported deletions and mutations in PWS affect this cluster. ‘The problem with the *SNORD116* snoRNAs’ Professor Carmichael explains, ‘is that they are orphans’ since they do not have complementarity to rRNA or other known RNAs and thus their targets are unknown. Therefore, it is important to identify the targets of the *SNORD116* snoRNAs and their functions to understand their crucial role in PWS.

Developing a 2’-OMe Detection Method to Find Sites Targeted by *SNORD116* snoRNAs

The *SNORD116* cluster of box C/D snoRNAs is highly expressed in the brain and the importance of these types of snoRNAs in PWS pathology is striking. The team already knew that box C/D snoRNAs modify RNAs by 2’-OMe, but strategies to map sites of 2’-OMe on RNA molecules were inefficient.

In 2017, Professor Carmichael and his team developed a new method, RibOxi-seq, to map sites of 2‘-OMe to better understand the targets of the *SNORD116* snoRNAs. Previous methods lacked specificity and relied on negative rather than positive signals. Using next-generation sequencing, the team developed a highly sensitive and accurate method to detect methylation relying on positive signals.

The basic structure of RNA is a five-carbon sugar attached to one of four nitrogenous bases; A, G, U or C, and this

unit of sugar and base is called a nucleotide. An RNA primary structure is made up of a single-stranded chain of nucleotides linked together by phosphodiester bonds. The fifth carbon of the sugar carries an unbound phosphate group and is thus called the 5'end. As the last sugar at the other end of the nucleotide chain has a free hydroxyl (-OH) group at the third carbon, this end is called the 3' end, hence RNA molecules have a 5' and 3' end.

In the RibOxi-seq method, Professor Carmichael's team reported that RNA fragments are generated using random 3'-ends, followed by periodate oxidation (a reaction to split bonds between carbons) of all molecules terminating in the 2',3'-OH groups. In this way, only RNA that harbours 2'-OMe groups at their 3'-ends are intact to be sequenced. Once sequenced, the RNA is aligned to a reference genome in the UCSC Genome Browser and the data is analysed for enrichment. The team tested the RibOxi-seq method to analyse RNA from the human teratoma-derived PA-1 cell line, a cell line that is known to highly express *SNORD116* snoRNAs.

As a result of these experiments, not only was the team able to detect known 2'-OMe sites in model ovarian carcinoma PA-1 cells but also to identify new sites. RibOxi-seq was confirmed to be a highly sensitive and accurate method to detect the modification of RNA by 2'-OMe. The researchers further discussed that there are, however, still some limitations to the method, such as the need for microgram levels of starting material. Also, RNAs that are shorter than 100 base pairs (bp) are more difficult to study, as the fragments needing to be generated would be very small. Professor Carmichael shares with us that it's also 'much harder to map sites in mRNA which is less abundant and very sequence diverse. But we're getting there.' Having initially studied 2'-OMe in PA-1 cells, the team's next goal is to extend these studies to PWS.

Harnessing Neurons as a Model to Study PWS

SNORD116 is expressed at high levels in human stem cells and neurons which make them both ideal cells to study the critical PWS region. Using a human stem cell line, Dr Stormy Chamberlain, one of Dr Carmichael's colleagues, utilised a technology called CRISPR/Cas9 to modify one line of an isogenic pair of stem cells by deleting the paternal PWS critical region. These modified stem cells lacking the critical region, along with their isogenic partners with the critical region still intact, are differentiated into neurons. The team have been performing mRNA-sequencing of extracted RNA from these neurons which will help to identify the differential expression of RNAs between cells with and without *SNORD116*. Alongside these experiments, the team is also using their recently developed RibOxi-seq method to map 2'-OMe sites and identify *SNORD116* snoRNA targets in these cells.

These experiments aim to gain greater insight into the *SNORD116* targets and their role in the pathogenesis of PWS. Professor Carmichael believes 'finding sites of modification will be exciting, but the real challenge that lies ahead is to then figure out how such modifications affect RNA function and are connected to PWS pathology.'

Implications for Other Diseases

As very little is known about the effects of 2'-OMe modification in mRNA, Professor Carmichael believes this work will also lead to a new understanding of snoRNA function in biology. Furthermore, as hundreds of orphan snoRNAs are known to be expressed in the genome, some snoRNAs have also been linked to other diseases and disorders such as cancer. The wider implications of Professor Carmichael's research are the broader understanding of the biology of snoRNAs and 2'-OMe which may lead to a better understanding of other diseases and potentially the development of new treatments in the future.

Meet the researcher

Professor Gordon Carmichael
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Professor Gordon Carmichael is currently a Professor of Genetics and Genome Sciences at the University of Connecticut Health Centre. Professor Carmichael obtained his PhD from Harvard University in 1975 and has a longstanding interest in the molecular mechanisms controlling the function and expression of RNA molecules. At present, his laboratory focuses on the functions of a new class of long noncoding RNA molecules implicated in the pathogenesis of Prader-Willi Syndrome and on small noncoding RNAs expressed from the same genomic locus. During his career, Professor Carmichael was one of the first to use RNA affinity chromatography to purify proteins. He also developed a now widely-used method for RNA gel electrophoresis, and was amongst the first to use synthetic DNA oligonucleotides to produce mutations in viral genes. In addition to these and other achievements, Professor Carmichael also serves as an Editorial Board Member of a number of journals and has been the recipient of numerous honours and awards, representing his outstanding reputation in his field.

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Justin L. Cotney, UConn Health

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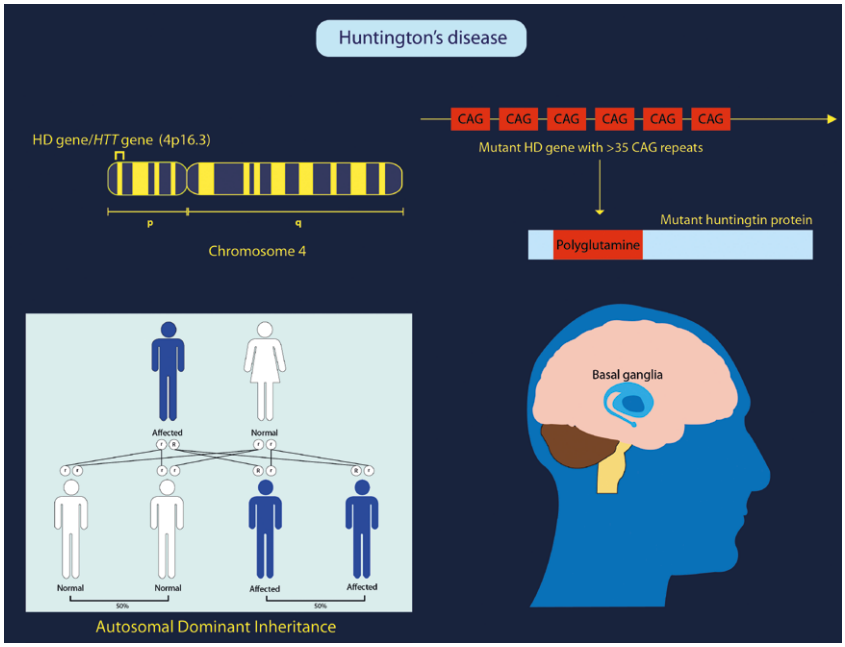
CONFRONTING THE CHALLENGE OF HUNTINGTON DISEASE

Huntington disease (HD) is an inherited and progressive neurological disorder which is currently fatal. **Dr James E. Goldman** and **Dr Osama Al-Dalahmah**, both at Columbia University, USA, are utilising new techniques in molecular biology to better understand the brain pathology associated with HD. Their vision is to develop therapeutics that can slow the progression of the disease, and ultimately, treat and even prevent it.



A Neurodegenerative Disease Without Cure

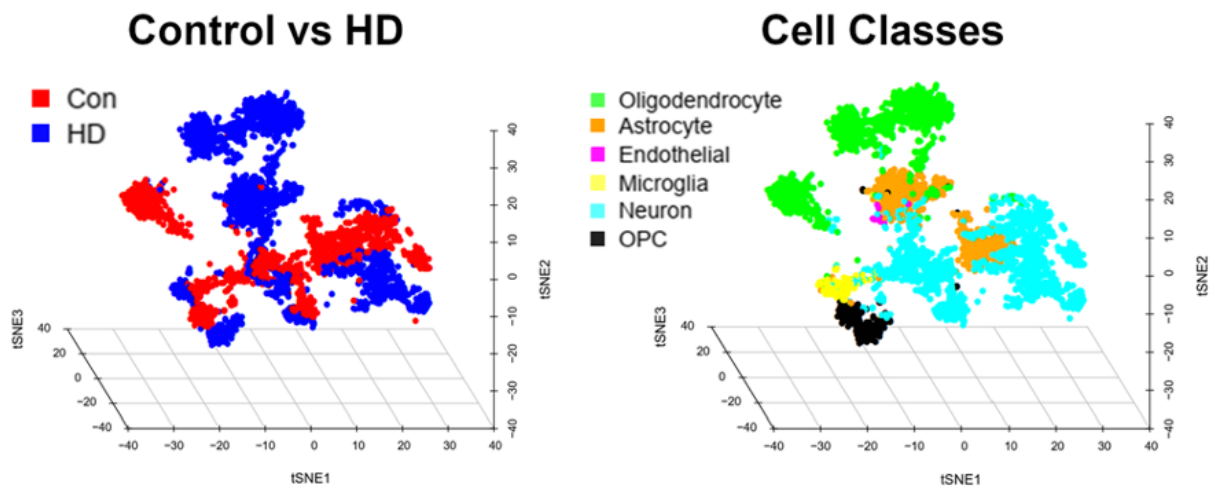
Huntington disease (HD) causes severe and wide-ranging neurological problems. It has a prevalence of between 10 and 14 individuals per every 100,00 in Western populations but much lower rates of prevalence are found in Asian and other non-European groups. Diagnosis of HD is based on a confirmed family history or a positive genetic test along with disturbance to motor function (that is, physical movement).

In the early stages, patients may report emotional difficulties such as depression and anxiety, and cognitive difficulties such as trouble learning new information or making decisions. These difficulties increase with disease progression, along with personality changes. Motor impairments, which typically start as small involuntary or twitching movements, known as chorea (from the ancient Greek word 'choreia,' meaning dancing) also worsen. Thus, as the diseases progresses, patients may experience difficulties with walking, speaking and eventually swallowing, all severely impacting on their physical and psychosocial wellbeing.

Symptoms usually appear in adults aged in their 30s and 40s, and being a neurodegenerative disease that is without a cure, HD is unreservedly fatal. Life expectancy is usually only around 15 years from the first onset of symptoms. In the advanced stages of the disease, patients require total support in all their daily activities. The final cause of death is usually a complication secondary to HD, such as pneumonia, heart failure or infection.

The Genetic Basis of Huntington Disease

HD is an inherited disease, meaning that it is passed down through our deoxyribonucleic acid (DNA), the hereditary material of humans and almost all other organisms. Inside almost all the cells of the body, DNA is contained in a specific part of a cell called the nucleus. Genetic information is stored in DNA in functional units called genes. In the cell, information is transferred from the nucleus (DNA) to the cell machinery that makes the proteins. To do this, the information is copied into ribonucleic acid (RNA)



In the figure above, each dot represents a single cell nucleus. The researchers used the specific sets of RNAs to tell them what types of cells each nucleus belongs to. Note that the dots are clustered so that nuclei of similar cell types, defined by their RNAs, are arranged together. On the right, we display the nuclei corresponding to the different cell types in the brains of Huntington (HD) and non-neurological disease patients, all artificially colour coded. Oligodendrocyte precursor cells are an immature cell type present in the adult brain. On the left, the same set of nuclei are displayed, but here all nuclei from the HD brains are in blue and those from the non-neurological patients' brains in red. Note that nuclei separate into disease vs. non-disease. This means that HD cells produce significant differences in the RNAs from those in non-neurological (CON) brain cells. Understanding these differences is giving us insights into the underlying molecular mechanisms that produce HD and will suggest therapeutic approaches.
Reproduced with permission from Al-Dalahmah et al, Single-nucleus RNA-seq identifies Huntington disease astrocyte states, Acta Neuropathologica Communications, 2020.

inside the nucleus, and then the RNA is transported outside of the nucleus, where it instructs the cell machinery to make proteins.

A fault in the DNA (or gene) is known as a 'mutation' because the protein then encoded by the DNA will be defective and this can result in the development of serious disease, including HD. While many genetic diseases are not passed on to children unless both parents carry the mutation, HD has an autosomal dominant pattern, meaning a child only has to inherit the mutated gene from one parent to develop the disease.

The mutation for HD is found in the HTT gene (also known as the HD gene), which is responsible for making a protein known as huntingtin, which appears particularly important for nerve cells (neurons) in the brain. Even though all of the cells in HD patients carry the HD mutation, the only cells affected appear to be those in the central nervous system, consistent with the neurological effects of the disease. However, within the brain, there is substantial variation in the extent of pathology across different regions.

Obtaining a better understanding of why some regions of the brain are more vulnerable, and others more resilient to HD pathology is critical to the development of new therapies for the disease. Exploring this important question is the focus of Dr James E. Goldman and Dr Osama Al-Dalahmah at Columbia University, USA.

Harnessing New Methodologies to Study Brain Pathology

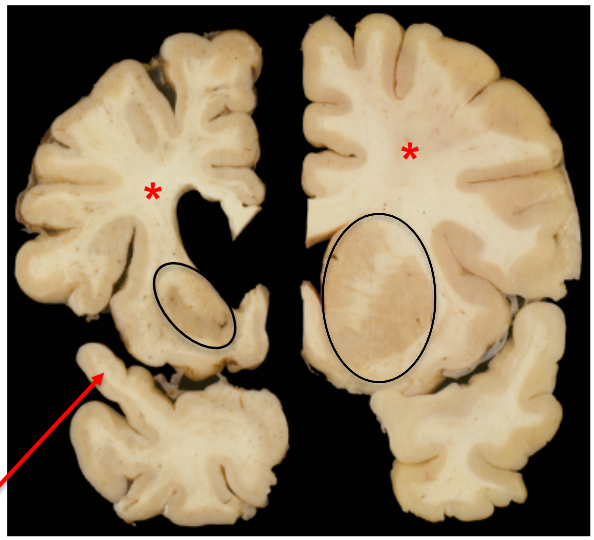
Along with colleagues, Dr Goldman and Dr Al-Dalahmah have very recently used a novel, powerful methodology known as single nucleus RNA sequencing (snRNASeq) to explore how RNA changes in the brains of patients with HD compared to healthy individuals. As this cannot be conducted in patients while they are alive, Dr Goldman and the team used specimens from the New York Brain Bank at Columbia University Medical Center, following patient post-mortem and the removal of the brain for freezing and preservation for research.

The cingulate cortex within the brain is associated with functions ranging from cognition, emotion and behaviour, and has extensive connections to other brain structures. It is often affected in HD, and as such, was the region of particular interest to Dr Goldman and Dr Al Dalahmah. They studied patients with HD at grade III/IV level of disease progression, representing the late intermediate stage and the advanced stage, respectively.

Using snRNASeq, the researchers were able to extract intact nuclei from all of the cells in the frozen brain tissues of patients with HD and healthy individuals. As described above, these nuclei contain RNA that has been copied from the cell's DNA. The RNA was isolated from the nuclei of the individual cells and it was then determined which RNAs were present in each cell. Specifically, this allowed the researchers to identify what genes in the DNA had been copied into the RNAs.

Importantly, not every cell in the human body makes RNAs from every gene, and many genes are, in fact, not copied into RNA across all of the cell types. As Dr Goldman explains, this difference is what makes a cell in the liver different from a cell in the heart, for example.

‘Our studies will allow us to formulate therapies to slow down the progression of Huntington disease.’



This figure shows a slice of one half of a brain from a Huntington disease (HD) patient on the left, and a comparison with a patient without the disease on the right. The HD brain shows a loss of tissue in a region called the basal ganglia (black ovals) and a thinning of the cerebral cortex (one area of cortex is shown by arrow). Note also the loss of white matter (*), which contains the long axon processes by which neurons communicate with other neurons in the brain.

Courtesy of Dr Jean Paul Vonsattel, Columbia University Irving Medical Center

Similarly, there are different cell types in the brain, and each of these copies different genes into RNA. Therefore, it is possible to determine which genes are made into RNAs in the different brain cell types. By comparing these RNAs to those extracted from nuclei of the healthy control individuals, Dr Goldman, Dr Al-Dalahmah and colleagues could see how the RNAs in HD differ as a result of the disease.

They found many changes in the RNAs in HD in all brain cell types, including nerve cells, glial cells, and cells of the immune system. Glial cells are abundant in the brain and two common types are astrocytes and oligodendrocytes. Astrocytes are important in providing critical support for all neurons, while oligodendrocytes make myelin sheaths which wrap around long nerve cell processes called axons, to insulate them and allow very rapid conduction of information.

They found that in HD, while many of the astrocytes appear to try to protect neurons, others appear to be toxic to neurons. The oligodendrocytes are also changed, such that they fail to make myelin properly, meaning that they interfere with the functions of neurons. These findings represent an important step forward in understanding single cell gene expression in HD, and provide a fascinating insight into the role of glial cells, in particular, in HD brain pathology.

Future Work and a Therapeutic Vision

Building on this work, the investigators are now comparing RNAs from patients in different stages of severity of HD and different regions of the HD brain. In particular, they will look for differences in the brains of individuals who have died in the early stages of HD from those in the late stages of the disease. This is critical, given that HD is a long-term, progressive disease.

Targetting RNA and DNA from a therapeutic perspective is described in the literature as the most promising approach we currently have in the efforts to confront and meet the challenge of HD. Dr Goldman reveals his vision that ‘Our studies will allow us to formulate therapies to slow down the progression of Huntington disease.’ Dr Goldman and colleagues are also committed to sharing their data as a resource for all researchers in the field of HD, further driving forward the ultimate goal of treating and, one day, even preventing this currently fatal disease.

Dr Goldman and Dr Al Dalahmah soon will begin to study the brains of patients with Parkinson disease in the same way, isolating cell nuclei and determining what RNAs are expressed in the variety of cell types. They hope to discover the changes that accompany the development of Parkinson disease, a common neurodegenerative disorder that results in tremors, slowness of movement and in some patients, a loss of their cognitive abilities. They anticipate that some of the changes in the Parkinson brain will mirror those in HD, while there may be changes specific to each disease. With other investigators who are pursuing similar strategies in other neurological diseases, they eventually will contribute to an understanding of these diseases at a new and deep molecular level.

Meet the researchers

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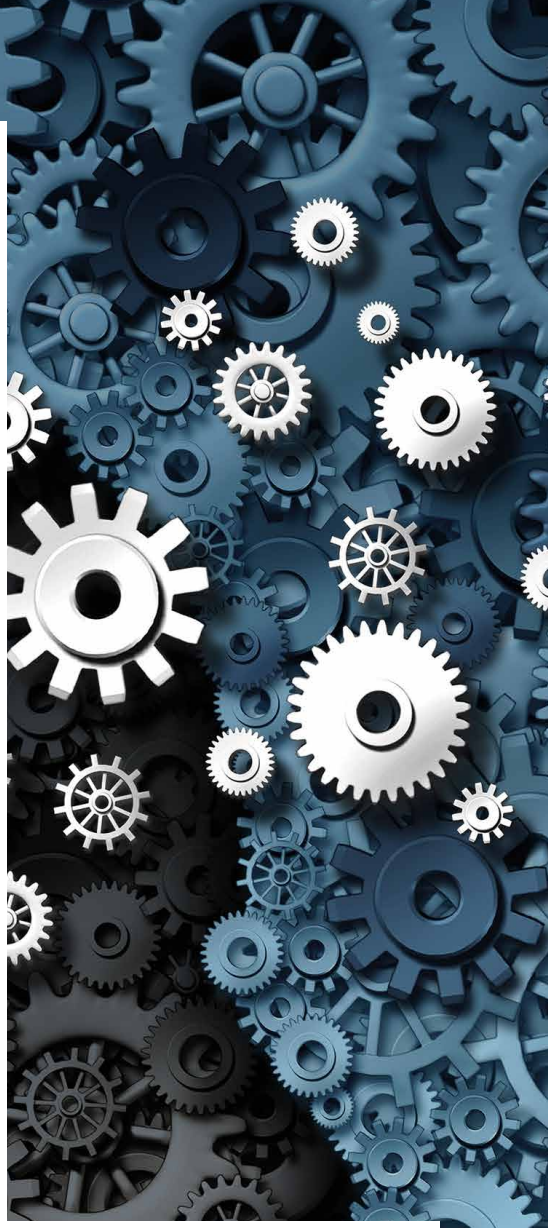
BREAKING THROUGH BARRIERS: ASSESSING COGNITIVE FUNCTION IN PATIENTS UNABLE TO COMMUNICATE AND THOSE WITH INVISIBLE INJURIES

When assessing an individual's cognitive functioning (such as memory and sense of orientation to the environment) traditional strategies have relied on verbal and behavioural responses. But how can this be achieved if a patient is unable to communicate in this way due to impairment arising, for example, from brain injury? **Professor John F. Connolly**, Director of the ARiEAL Research Centre at McMaster University, Canada, and Co-Founder and Chief Science Officer at VoxNeuro, has developed an innovative neurotechnology to assess cognitive functioning in individuals that does not rely on verbal or behavioural responses.



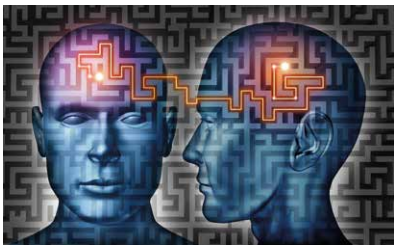
Assessing Cognitive Function

Cognitive function underpins our mental abilities such as memory, orientation, problem-solving, reasoning, comprehension, and attention. Following a mentally debilitating event such as a stroke or brain injury, assessing a patient's cognitive function is essential to their rehabilitation and allows healthcare professionals to determine the best treatment plan. Established tests such as the Peabody Picture Vocabulary Test and the subtests of the Wechsler Adult Intelligence Scale adapted as a neuropsychological instrument are often used to assess patients' language and conceptual functions through verbal and/or behavioural responses. However, when patients are unable to communicate in these ways due to neurological damage, conducting these crucial assessments is either not possible, or even for patients who can engage, can have a high margin of error due to subjectivity both on the part of the patient and the healthcare professional conducting the assessment.

Professor John F. Connolly, Director of the ARiEAL Research Centre at McMaster University, Canada, and Co-Founder and Chief Science Officer at VoxNeuro, has developed a proprietary assessment method that does not rely on the need for verbal or behavioural responses from patients. This means that those patients who had barriers for being assessed accurately for cognitive function can now be assessed in an alternative way – one that is both objective and reliable.

A 'First of its Kind' Case Study

Aphasia is an impairment to speech and/or comprehension resulting from damage to the brain. In the early 1990s,



Professor Connolly and his colleagues tested a new assessment approach in a 21-year-old patient who was characterised as globally aphasic with minimal and unreliable behavioural gestures following a traumatic brain injury (TBI) – in fact, his initial diagnosis was vegetative state.

The researchers used event-related potentials (ERPs), which are brain biomarkers, to measure specific brain response features known as components. These components reflect cognitive processes, commonly thought of as mental abilities – like

‘The goal of my research has always been to use these new methods of assessment to identify those suffering from neuropathological conditions with greater accuracy. These methods are now being used to better inform healthcare professionals charged with treating individuals with these problems.’

Credit: JD Howell, McMaster University.

attention, memory and decision making – that can be assessed even in non-communicative patients. ERPs are derived from electroencephalogram (EEG) recordings of brain responses to stimuli and the components examined have been the subject of many thousands of peer-reviewed papers, as their years of initial discovery range from 1965 to 1980.

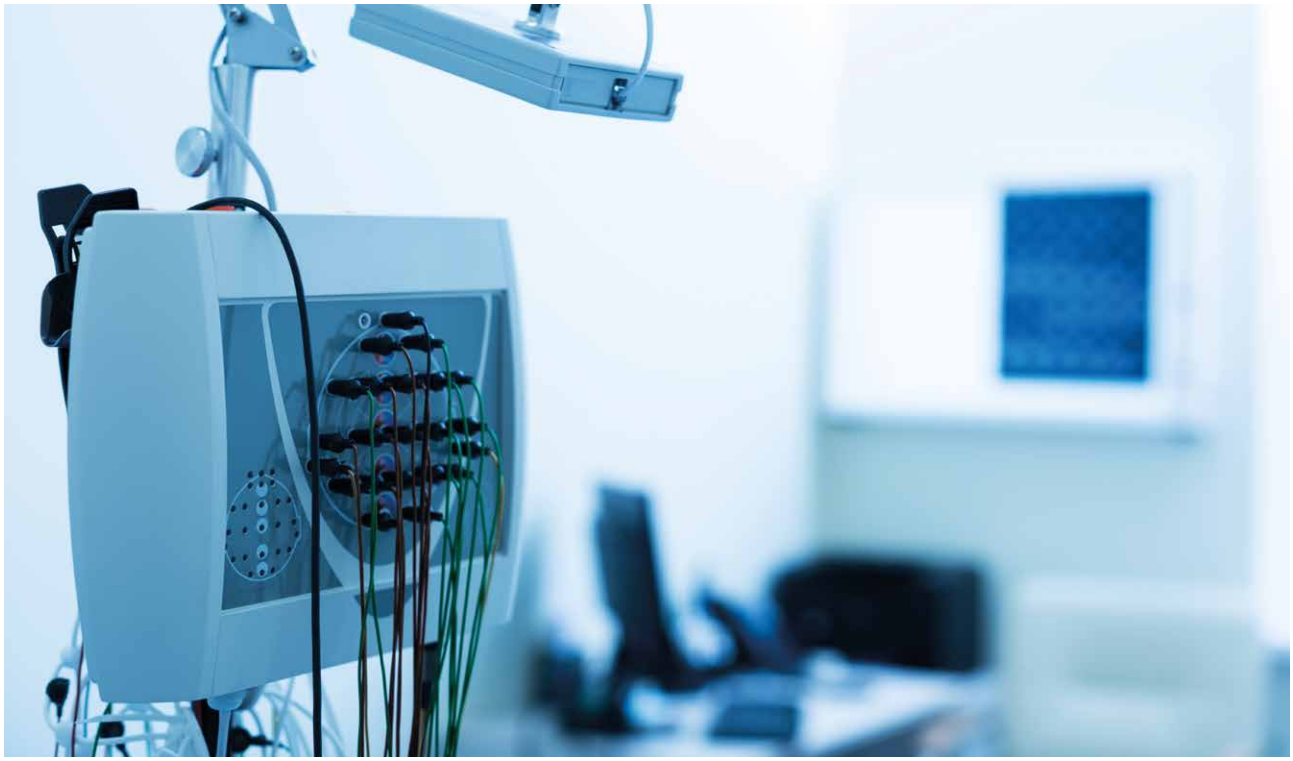
The patient studied by Professor Connolly was severely compromised, and seemingly unresponsive to any aspect of his environment. After 3 weeks of clinical observation, it was concluded that the patient’s progression could not be assessed clinically and thus it was perceived that further intervention would not be beneficial. The patient was scheduled for discharge to a palliative care facility when he was referred for electrophysiological assessment by Professor Connolly through one of his physicians who knew of Professor Connolly’s research.

In this assessment, sentences were presented aurally with half the sentences ending with a congruous word and half with an incongruous word. For example, one congruous sentence used was ‘She could tell he was mad by the tone of his voice’ and an incongruous sentence example is ‘She went to the bakery for a loaf of sink’(with ‘sink’ replacing the congruous word ‘bread’). EEG measurements were examined separately for the sentences with congruous and incongruous terminal words in order to answer the most pressing question at the time – was the patient able to comprehend speech?

Critically, the patient’s ERP responses to spoken sentences ending with incongruous words displayed a differential response compared to sentences ending with congruous words. The researchers found that the patient’s responses were comparable to those of healthy individuals. The same sentences were presented to the patient visually, but the outcomes were abnormal and unreliable. Moreover, in comparison to healthy control participants, the readings were clearly different. The patient’s lack of brain biomarkers for comprehension to visually presented sentences was attributable to a yet-unresolved, injury-related visual abnormality.

Six weeks after the initial electrophysiological assessment by Professor Connolly, the patient was assessed again using traditional behavioural tests for attention, memory and problem-solving. However, the patient’s performance in these tests was poor. This pattern of electrophysiological responses revealing function when behavioural measures could not was the ‘first of its kind in scientific literature’ explains Professor Connolly, as they had demonstrated that the patient understood the meaning of the spoken stimuli through measuring the associated ERPs, that is, the brain biomarkers.

The team concluded that testing both visual and auditory function is important in such patients, as one function may be compromised while the other may remain intact. This work paved the way for Professor Connolly’s innovative use of ERP technology to assess cognitive functions including



receptive vocabulary and semantic comprehension in patients with severe neurological impairment – functions that were once difficult or even impossible to evaluate in such patients using traditional behavioural methods.

Predicting Coma Prognosis

Mismatch negativity (MMN) is an ERP component that can be described as an automatic attentional response to auditory stimuli, reflecting a predictive coding process that is below the level of conscious awareness. The presence of this particular ERP component in the prolonged state of unconsciousness known as coma has been proven to be a strong positive predictor of emergence from coma. However, the utility of the MMN as a clinical tool was compromised by its lack of sensitivity. That is, while those patients who showed the response emerged from coma, many patients who did NOT show the MMN also emerged.

Professor Connolly, along with his colleagues Dr Narges Armanfard (now at McGill University) and Professor James Reilly (McMaster University), tested a new method to detect MMN components from the ERPs of coma patients, in the anticipation of developing a more accurate predictor of coma emergence. They hypothesised that the level of consciousness waxed and waned in coma in ways that could lead to failure to see the response using traditional analysis methods, which involved recording only periodically, rather than for extended periods of time with a finer-grained analysis tool.

Traditionally, the MMN is sought in single test sessions lasting about 20 to 30 minutes. This single occasion testing reduces the likelihood of observing the MMN if consciousness does, in fact, wax and wane in coma and thus may be the cause of the low sensitivity reported in previous studies. Professor Connolly's team proposed tackling this problem with the use of a machine learning (ML) algorithm – a localised feature selector that provided the finer-grained analysis. ML can process high-dimensional clinical data and learn complex patterns that can be too difficult to be recognised by even expert humans.

Professor Connolly and colleagues found that the use of ML accurately detected MMN over a much shorter 2-minute interval and that the MMN appeared and disappeared (the waxing-waning process) when recorded across an extended time period (e.g., 24 hours). It was found that the MMN component could be successfully detected with 92.7% accuracy in the healthy participants. Importantly, a greater similarity between the ERP responses of the coma patients and the healthy participants indicated a more positive prognosis for coma emergence. That is, the two coma patients showed high similarity levels to healthy control participants and both emerged from coma. These results, the team emphasise, are preliminary but indicate the usefulness of this new method in predicting coma emergence and possibly longer-term outcome.

More recently, Professor Connolly and his colleagues (Drs Reilly, Fox-Robichaud, Hamielec, Sonnadara (McMaster University), Tavakoli (McMaster University), Boshra (McMaster University and VoxNeuro), Blain-Moraes (McGill University) and Herrera-Diaz (PhD candidate; McMaster University) have begun a larger trial on the assessment of ERPs in coma to develop an automated procedure for analysing the ERP data with greater accuracy than the current standard clinical approach, and one that is less costly. Data will be collected from 50 individuals in coma, with EEG/ERP data collected for 24 hours at five time

Credit: JD Howell, McMaster University.

points over 30 days to track their progression. Data from 20 healthy adult controls will be used for comparison. This trial is currently on-going as the team works toward a prototype model for use in clinical settings.

ERPs Implicated in the Detection of Concussion

Concussion is a brain injury often labelled as a mild TBI (mTBI). It can cause physical (e.g., headaches and dizziness), emotional (e.g., depression and anxiety) and cognitive (e.g., attention and memory dysfunction) symptoms in the affected individual. ERPs are again implicated as an electrophysiology tool of interest in this condition. Building on the research conducted with non-verbal patients, Professor Connolly, along with colleagues Drs Boshra and Ruiter (McMaster University and VoxNeuro) and Professor Reilly, saw the opportunity to use this assessment method in a patient population for whom behavioural assessments often fail to detect the presence or extent of their injury. Persistent changes in ERPs have been observed in concussed patients, driving the team to explore an expanded version of ML known as deep learning (DL). DL had previously been investigated in other EEG applications, but this study was the first to utilise DL in an EEG/ERP application to mTBI.

The team developed a DL network coined TRauma ODdball Net (TRODNet) that uses a multi-layered architecture to extract information from EEG/ERP data to find signs of concussion from certain response signals in the patient. In this trial, 26 concussed patients and 28 control participants were recruited. The results showed higher accuracy in classifying acute and post-acute mTBI when using EEG/ERP than reported by previous studies using resting-state EEG and quantitative EEG (see doi:10.1038/s41598-019-53751-9).

However, this higher accuracy was only marginally higher than another of the team's studies that reported findings on injury detection decades after the injury had occurred (see Doi:10.1109/TNSRE.2019.2922553). Considering both studies (that is, one dealing with acute/post-acute and the other with detection of injuries incurred decades earlier), the data indicated that some earlier biomarkers such as the MMN reflected irreversible injuries due to concussion found only in chronic deficits from much older injuries.

The researchers discuss the fact that some of the best-reported tools to assess mTBI decline in utility as early as 5 days post-injury. In this study, data collection began on average at 20 days post-injury. Therefore, the team believes that the model's excellent performance may be affected by the time-lapse since injury and they explain that a more stable multi-stage approach to the progression of concussion is needed since effects specific to concussion may only be observable at certain stages after injury and/or recovery.

A subsequent study by the same team proposed three stages of concussion progression as a) acute: the time directly after injury to 4 weeks after, b) post-acute: following the acute phase and c) chronic: decades after injury when effects of concussion have been reported to resurface. The results showed an increase in the functional connectivity of the brain in the acute stage compared with much reduced functional connectivity in the chronic stage, indicating a non-linear time-dependent effect of brain injury.

Professor Connolly describes his research as utilising the best available technologies to enable better assessments of cognitive functioning. He shares with us, 'The goal of my research has always been to use these new methods of assessment to identify those suffering from neuropathological conditions with greater accuracy. These methods are now being used to better inform healthcare professionals charged with treating individuals with these problems.'

This promising new technology can also guide healthcare teams in planning therapeutic interventions for non-communicative patients with the aim of achieving improved recovery outcomes, and arm them with the ability to objectively assess the efficacy of their interventions through repeat assessment. The advances in cognitive assessment made as a result of Professor Connolly's research have been productised by VoxNeuro to complement existing clinical assessment methods, through providing data to reliably inform cognitive interventions and therapies.

While Professor Connolly's research is ongoing in this field to further new discoveries and identify additional clinical applications, his team at VoxNeuro is scaling the technology that has been consistently validated through his research. The Cognitive Health Assessment™ benefits patients and their healthcare providers in need of an objective analysis of brain function, today. This breakthrough assessment is currently clinically available in Ontario, and will continue to be scaled globally in the coming years.

Credit: Steven Kim (Instagram handle: skimphoto_)

Meet the researcher

Professor John F. Connolly
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Professor John F. Connolly received his PhD from King's College London and is currently a Professor and Senator William McMaster Chair in Cognitive Neuroscience at McMaster University, Ontario. Dr Connolly is the Director of the ARiEAL Research Centre at McMaster and is appointed in the Faculties of Engineering, Science and Humanities. Dr Connolly is also the co-founder and Chief Science Officer at VoxNeuro, a company that is commercialising his research and EEG methodologies, that are now clinically available. Professor Connolly's research focuses on the assessment of cognitive functioning in healthy individuals and those with acquired brain injuries. His work has been documented in over 300 peer-reviewed publications and has been supported by funding agencies across the world for almost 40 years. He actively reviews research for a wide range of journals and funding agencies, is a Faculty Affiliate at the Vector Institute of Artificial Intelligence, and is a member of Brain Injury Canada's Scientific Advisory Committee.

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FROM NERVE DEVELOPMENT TO VISION: A BUMPY RIDE

The eye movement disorders strabismus (characterised by eye misalignment) and nystagmus (characterised by involuntary oscillation of the eyes) together affect up to 5% of the population and have a detrimental impact on vision. These disorders also impact on facial appearance and social interaction, which may, in turn, lead to psychological difficulties. **Dr Mary Whitman**, at the Boston Children's Hospital, USA, is working to understand the genetic causes and neurological mechanisms underlying eye movement disorders to improve treatment and, ultimately, prevent their onset.

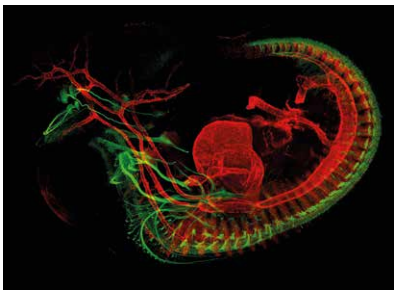
Single-vision Lens Versus Bifocals

Accommodative esotropia is one of the most common types of strabismus observed in children. It is characterised by an inward deviation of the eyes when trying to focus. Early detection is essential for successful improvement and lack of appropriate treatment can result in severe and long-term damage to vision. Children with a specific subtype of accommodative esotropia have traditionally been treated with bifocal glasses to correct both distance and close vision. They are more expensive than single-vision glasses and are often more difficult for young children to get used to.

Dr Mary Whitman at the Boston Children's Hospital, USA, along with her colleagues called this approach to treatment into question. In a paper published in 2016, Dr Whitman reported having retrospectively studied 180 children, comparing the improvement of the esotropia and development of depth perception after treatment with single-vision (only distance correction) or bifocal glasses. She observed similar outcomes in both groups, and a lower risk of surgery in children using single-vision lenses. This study was the first to indicate that single-vision lenses represent the preferable option due to reducing the cost and complexity of care, as well as improving treatment outcomes. On the basis of these findings, the American Academy of Ophthalmology has now recommended single-vision rather than bifocal glasses for the treatment of accommodative esotropia in children.

The Heritable Component of Comitant Strabismus

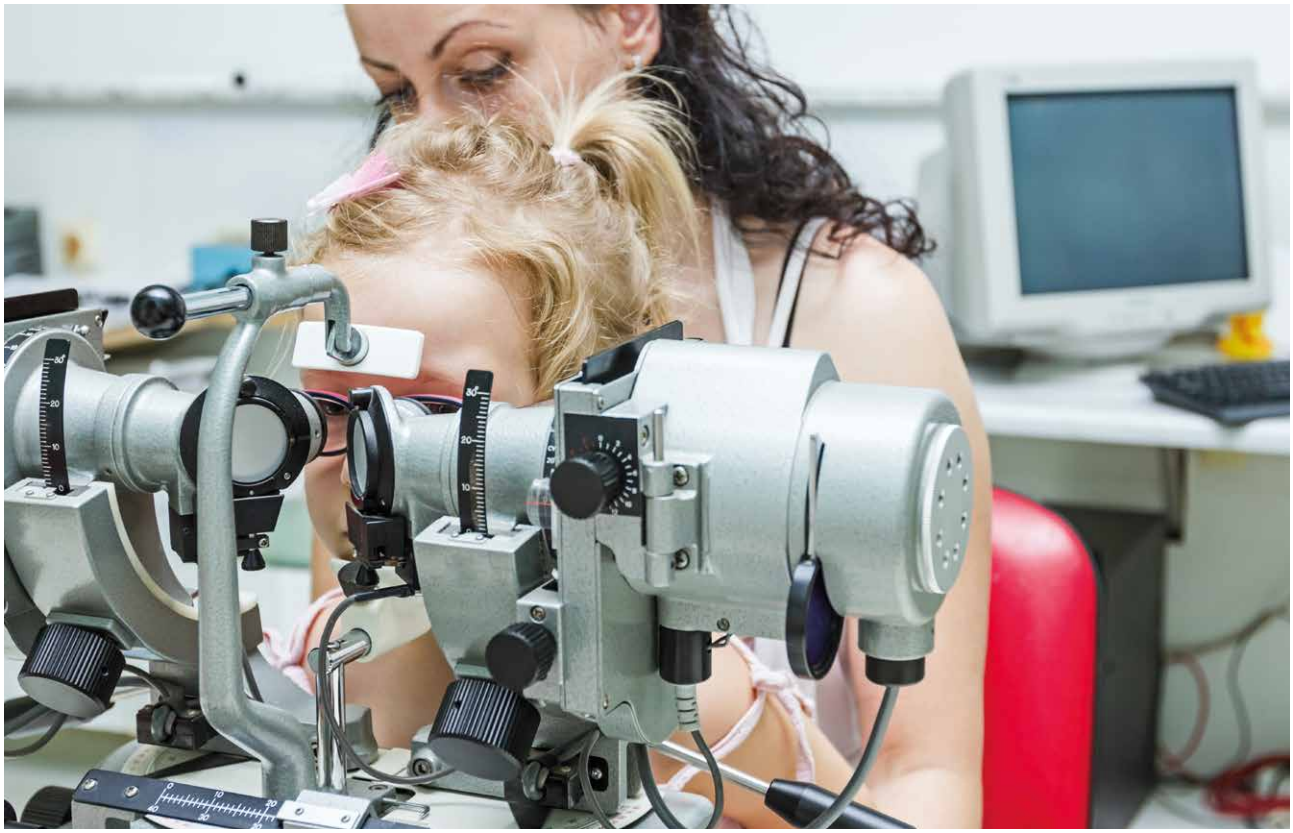
Strabismus alone affects 4% of the population. Noncomitant strabismus occurs where the deviation of the eye depends on the direction of the gaze, whereas comitant strabismus occurs where the deviation remains the same in all directions. Comitant strabismus is also highly associated with psychological difficulties, and the study of its genetic patterns is required to understand its cause and pathophysiology. As early as 400 BC, Hippocrates described the familial



Developing mouse embryo. Motor nerves are in green and muscles are in red. Credit: Mary Whitman.

clustering of comitant strabismus and was the first to propose the possibility of parent-children transmission. Since then, observations of families and twins have confirmed a genetic contribution.

Dr Whitman contributed to an important genome-wide association study published in 2018 that sought to identify the potential associations between comitant esotropia and genetic regions. Such approaches allow scientists to determine which genetic region is most likely to be associated with an observed disease. Genome alterations can be very small variations of the genome (called SNP, standing for single nucleotide



polymorphism), or larger alterations, such as the deletion or insertion of genetic material.

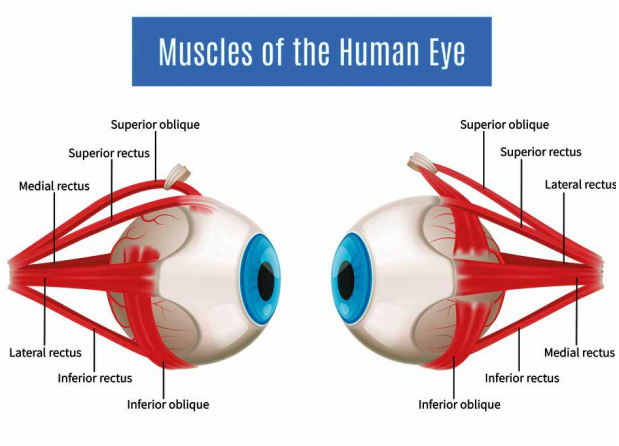
The DNA of large cohorts of people from the United States, the United Kingdom, and Australia was meticulously studied. A significant association between comitant esotropia and an SNP of the gene WRB located on chromosome 21 was identified in this work. The WRB gene is widely expressed in foetal and adult tissues, and codes for a protein playing a critical role in cellular function. Consistent with these findings, it had previously been demonstrated that genetically modified zebrafish lacking the WRB gene are blind and deaf.

Dr Whitman and her colleagues have very recently conducted a genetic association study in which more than 2,000 patients with esotropia were compared with an even larger sample of control participants. The researchers identified that three rare, recurrent DNA duplications (repeated stretches of DNA) increase the risk of esotropia. This important work is a step forward in understanding the pathophysiology of strabismus, although the functional consequences of these duplications, and the genes and regulatory regions involved in them require further exploration.

The Brain Circuitry Behind Sharp Vision

Although a high level of control over eye motion is necessary for sharp vision, little is known about the mechanisms responsible for the development of the ocular motor system. Information has come from the study of ocular congenital cranial dysinnervation disorders (CCDDs). Patients with CCDDs are unable to move one or both eyes in certain directions, and can have involuntary movements of the eyes, the eyelids or the face, sometimes leading to abnormal head or chin positioning. CCDDs were originally thought to be caused by muscle abnormalities, but studies primarily from Dr Elizabeth Engle's group at Boston Children's Hospital have demonstrated that CCDDs are mainly caused by altered muscle innervation rather than muscle abnormality. This represented a breakthrough for researchers in this field and offered new insights into normal development. Whilst some genetic causes of CCDDs have been identified, the development of the complex brain circuitry controlling eye movements remains much of a mystery.

Eye movements are controlled by six extraocular muscles (EOMs) that are innervated by three cranial nerves (CN3, CN4 and CN6), and eyelid opening controlled by a seventh muscle, innervated by CN3. Typically, the neuron axons that are responsible for the transmission of the signal emerge from the brainstem; assembling into a nerve that stretches across the brain to reach the orbit and its corresponding EOM. Unfortunately, this is not a straightforward path and many disruptions can occur along the way, for example, during neuron specification, axon growth, or the trajectory development. Previous studies had already suggested a potential role for EOMs in this process, triggering Dr Whitman's curiosity.



Together with her team and published in 2017, Dr Whitman generated a new line of genetically modified mice that lacked EOMs and expressed fluorescent protein in the ocular motor nerves (responsible for motor action of the eyes and eyelids), allowing scientists to temporally and spatially follow the nerves development in healthy mice embryos and those lacking EOMs. Orbital dissections and imaging techniques provided precise images. Both healthy and mutant embryos had similar ocular motor nerve trajectories from the brainstem to the orbit. However, a difference appeared in their terminal trajectories – nerves from mutant embryos were not able to correctly develop terminal branches to reach the targeted EOM. Embryos lacking EOM showed thin and blunt terminal nerve branches whilst healthy embryo images revealed strong and defined terminal branches.

Most prior studies did not permit the visualisation of the terminal branching, but Dr Whitman highlighted the local role of EOMs in guiding the three ocular motor nerves to finalise their trajectory to the correct EOM. Whether EOMs act through direct contact or are mediated through diffusible cues remained to be determined and motivated Dr Whitman and her team to pursue their research further.

Guiding the Nerves from the Brainstem to the Orbit

Although innervation of the EOMs relies on only three cranial nerves, complex mechanisms allow cranial nerves to reach their designated muscle. Along their trajectories, cranial nerves are guided by several signals that influence their direction. Up until now, studying nerve guidance has been hampered by technical challenges. Traditional in vitro assays remove nerves from their microenvironment which may influence the trajectory of the nerves, and in vivo assays are not appropriate for screening tests.

To overcome these limitations, Dr Whitman and her colleagues developed an embryonic slice culture technique allowing time-lapse imaging of the developing nerve while maintaining an intact microenvironment. Embryos expressing fluorescent protein (GFP-positive) in the oculomotor nerve (CN3) were extracted from the uterine horn and cut into slices containing both the oculomotor nucleus and the orbit. The slices were then grown on cell culture inserts and pictures of the slice were taken every 30 minutes for 3 days. 'The first GFP-positive oculomotor axon reached the orbit/eye over the next 18 to 24 hours, and then began branching to their final targets. We find this basic timing to be recapitulated in slice culture' says Dr Whitman.

In a research paper published in 2018, Dr Whitman and her colleagues studied the CXCR4/CXCL12 signalling influencing oculomotor nerve (CN3) development. CXCR4 is a receptor and CXCL12 its ligand, previous research already identified their role in the nervous system formation, neuron migration, and axon guidance. Adding a CXCR4 inhibitor in slice culture media led CN3 to grow dorsally (toward the back) from the oculomotor nucleus rather than ventrally (toward the front), in the direction of the orbit. These observations were confirmed in vivo, where genetically modified mice lacking CXCR4 in oculomotor neurons showed a misrouting similar to the one observed in the slice culture assay. Furthermore, loss of CXCR4 also caused EOMs to be innervated by aberrant nerves such as CN5, normally not involved in EOMs innervation. 'It is remarkable that the loss of a single guidance factor causes such a profound change in axon trajectory and suggests there may be a repulsive factor ventrally or an attractive factor dorsally that is normally overcome by CXCR4/ CXCL12 signalling. The identity of such a factor is a subject for future study' states Dr Whitman.

Most recently, in a study published in 2019, Dr Whitman studied a consanguineous (meaning closely related) family and linked a unique form of oculomotor synkinesis (involuntary movement of the eyes or eyelids when attempting a different movement) with an alteration of the ACKR3 gene. Interestingly, CXCR4/CXCL12 signalling is also regulated by ACKR3. Upon binding of CXCL12, the receptor ACKR3, a scavenger receptor, internalises and degrades CXCL12. Dr Whitman showed that when ACKR3 is altered, the balance of CXCR4/CXCL12 in the nerve's surrounding is disturbed and leads to similar misrouting of oculomotor nerves observed after CXCR4 loss. Together with CXCR4/CXCL12, ACKR3 is crucial for the proper development of oculomotor nerves.

Dr Whitman remains motivated to discover the developmental mechanisms of the ocular motor system. Always innovative, her research opens doors to ground-breaking medical innovation. Cranial nerves can also regenerate after injuries, aneurysms or tumours. 'As the nerves regenerate, they can form aberrant connections that cause debilitating synkinetic symptoms for patients. Understanding the cues that guide initial cranial nerve trajectories could lead to treatments to prevent or alleviate aberrant regeneration' summarises Dr Whitman.

Meet the researcher

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Dr Mary Whitman obtained her PhD in Neurobiology from Yale University in 2008 rapidly followed by her medical degree from Yale School of Medicine. After she interned at New York University, Dr Whitman became an ophthalmology resident at Colombia University and New York Presbyterian Hospital. She further specialised in pediatric ophthalmology at Boston Children's Hospital in 2014. In addition to being a dedicated ophthalmologist, Dr Whitman is also an Assistant Professor at the renowned Harvard Medical School. Her research focuses on understanding the mechanisms of development of the ocular motor system and the genetic causes of strabismus and ocular congenital cranial dysinnervation disorders. Using genetically modified mice models and complex imaging techniques she is a pioneer researcher in the field. Dr Whitman recently received the Early Career Clinician Scientist Award from the Association for Research in Vision and Ophthalmology.

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UK BIOBANK

UK Biobank is a large-scale biomedical database and research resource containing genetic, lifestyle and health information from half a million UK participants. The database, which is regularly augmented with additional data, is globally accessible to approved researchers and scientists undertaking vital research into the most common and life-threatening diseases. UK Biobank's research resource is a major contributor to the advancement of modern medicine and treatment and has enabled several scientific discoveries that improve human health. In this exclusive interview, we speak with **Professor Sir Rory Collins** FMedSci FRS, Principal Investigator and Chief Executive of UK Biobank, to hear about the achievements to date and the ambitious and unique potential of this exciting project.

The Lancet kit UK Biobank are using in the COVID sero-prevalence study. Credit UK Biobank.

To begin, please tell us how UK Biobank came to fruition.

Understanding the factors that influence the onset and trajectory of common life-threatening and disabling conditions presents a critical challenge to medical science. Prospective cohort studies allow the study of individuals over a period of time, and can assess the exposure of individuals to risk factors before the onset and treatment of disease. The UK Medical Research Council and Wellcome Trust decided that they would fund a large prospective cohort at the turn of the century, and after a lot of discussion about what might be done and how it might be done, I was asked in 2005 to deliver the project.

As Principal Investigator and Chief Executive, what are the aims of UK Biobank and how do you envision these aims will be achieved?

The aims are to allow as many researchers globally as possible to apply their expertise and imagination to the de-identified data from the 500,000 altruistic volunteers in as many different ways as possible in order to generate as much knowledge as possible about the causes of many different conditions, with the ultimate aim being to discover ways to prevent and treat those conditions better.

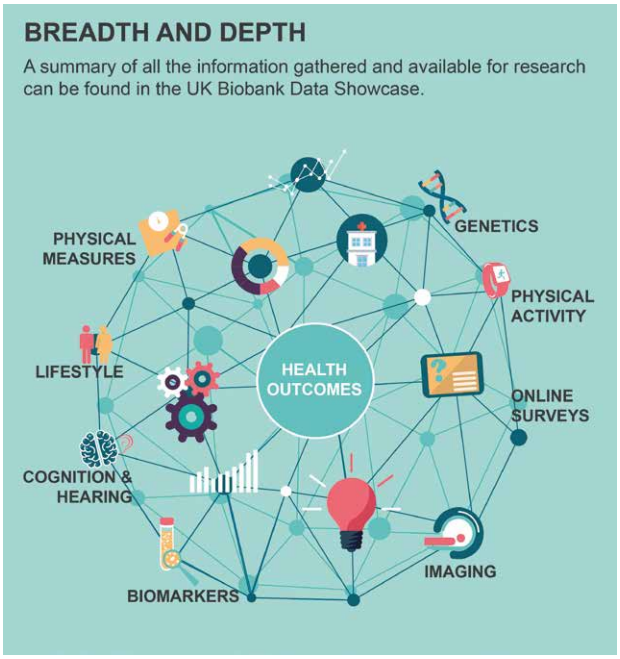
Around 500,000 volunteers have enrolled in the project – what data are being collected from volunteers and how is all this information being used?

At the baseline assessment, all volunteers completed a wide range of measures to ascertain their sociodemographic background, family history and early life exposures, psychosocial and environmental histories, and they also completed in-depth measures of their physical, health and cognitive function. Extensive phenotypic and genotypic information is being collected, including further data obtained from questionnaires, physical measures, sample assays, accelerometry, multimodal imaging, genome-wide genotyping and follow-up assessments conducted over the course of the project for a wide range of health-related outcomes.

What opportunities are available for researchers through UK Biobank?

Uniquely, all of the data (and, indeed, assays of samples) are available to all bona fide researchers (both academic

High technology blood sample processing. Credit UK Biobank.



Credit UK Biobank.

and commercial) worldwide without preferential access for all types of health-related research that is in the public interest. Researchers who fund the enhancement of the resource (e.g., assays of samples) may be given up to a 9-month exclusive access period to use the data that they have generated before those data are made available to all other researchers.

UK Biobank has already led to a wealth of publications in prestigious scientific journals. What do you consider to be UK Biobank's most important findings to date?

The 500,000 participants were only recruited between 2006 and 2010 and, given that a prospective cohort requires long-term follow-up of health outcomes, the most important findings from UK Biobank will really only emerge over the next 5 to 10 years. Having said that, one important 'finding' is that the large investment in UK Biobank made by the Medical Research Council and Wellcome Trust (and others) is already starting to produce dividends in terms of the amount of research that can be conducted rapidly and efficiently by making a very large and very deeply characterised dataset freely available to lots of researchers (as illustrated by the numbers of publications and, increasingly, by their scientific importance). This is a testament to the vision of the funders to invest for the long term and to the altruism of the 500,000 people who volunteered to contribute their information to the resource.

The project is, of course, made possible by the individuals who have volunteered to take part. How are the key findings and implications for health communicated to the general public?

Participants are informed regularly about the progress of the study (and, particularly, about major initiatives), as well as being invited to participate in additional assessments (such as the ongoing project to image 100,000 of the participants which is now half-way completed). They are also encouraged

The vast UK Biobank freezer in Stockport is so large that several double decker buses could fit in it. Credit UK Biobank.

to review detailed updates on UK Biobank's website, which are also available to other members of the public. Individual researchers who generate findings based on UK Biobank also put out their own press releases, which are often picked up by the news media.

In the midst of the current pandemic, a better understanding of health and disease is at the forefront of scientific as well as political agendas. What can UK Biobank offer to the COVID-19 response?

The first thing that we did was to put in place much more frequent updates from health record systems on health outcomes occurring among participants, and extended it to access to primary care records for the first time under emergency legal arrangements specifically for COVID-related research, and fast-tracked approval to use the data for such research. Findings about COVID-19 based on these data are already starting to emerge which we hope will help in its control.

At the request of the Wellcome Trust and UK Government, we have recruited about 10,000 of the volunteers, and extended recruitment to a similar number of their children and grandchildren aged over 18 (in order to include people aged less than 50 years), into a study to track the prevalence of previous infection with SARS-CoV-2 (based on a laboratory test for antibodies) throughout the second half of 2020 in different groups within the UK (e.g., different parts of the country, and in people of different age, gender, ethnicity, and socioeconomic status). The results of that study are being made publicly available, as well as being provided to the Government to help guide policy.

To conclude, if we may, with some personal reflection, what lessons do you feel we may learn from the COVID-19 pandemic in terms of healthcare in the coming decades?

Let reliable evidence really drive the strategies for the prevention of adverse health outcomes, whether that is due to infectious diseases or chronic diseases (e.g., the pandemic, increasingly in the poorer countries of the world, of tobacco-related deaths and disability that is largely ignored by the media… partly because tobacco is not 'new' and partly because the problem is moving away from the richer countries).

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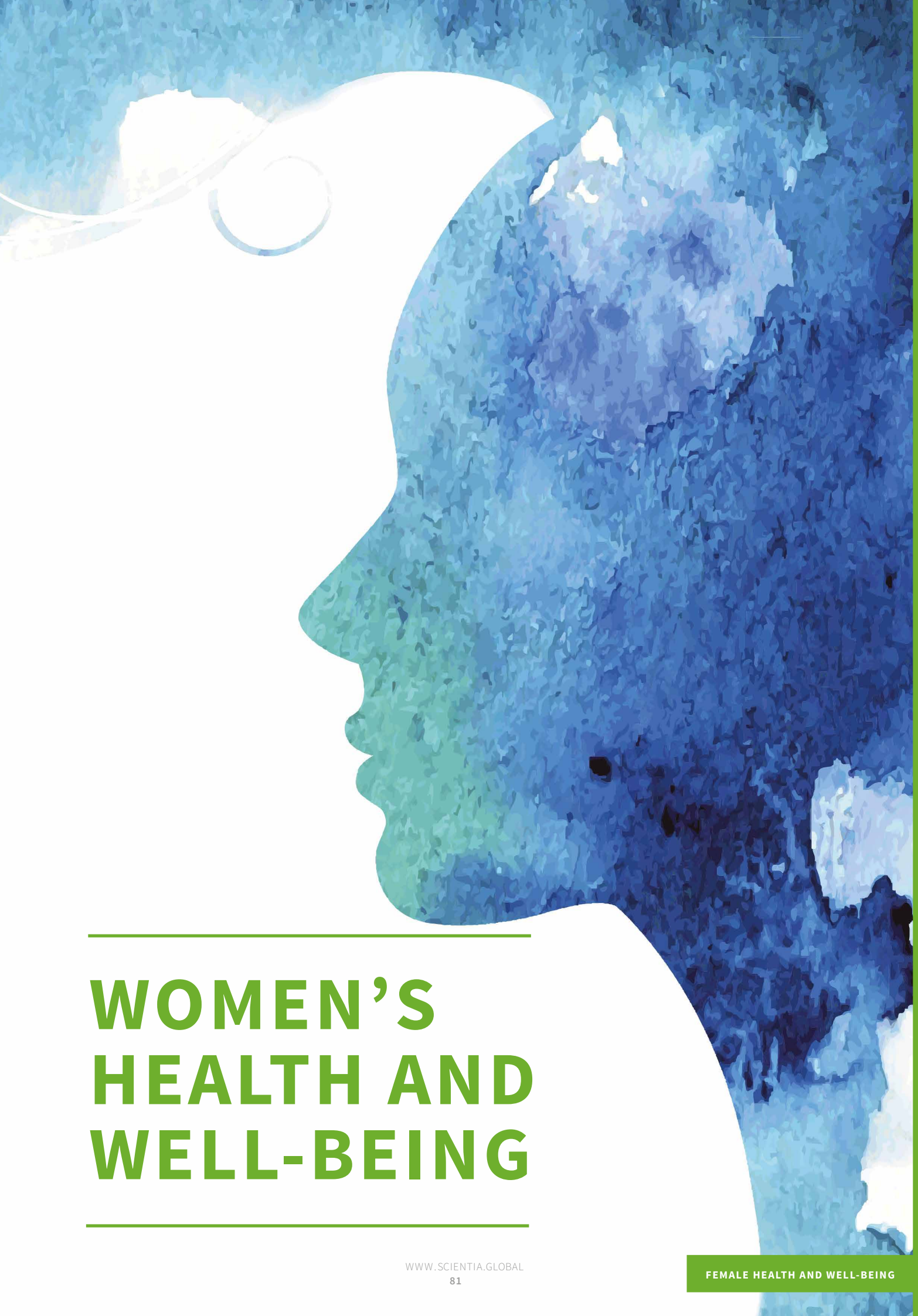
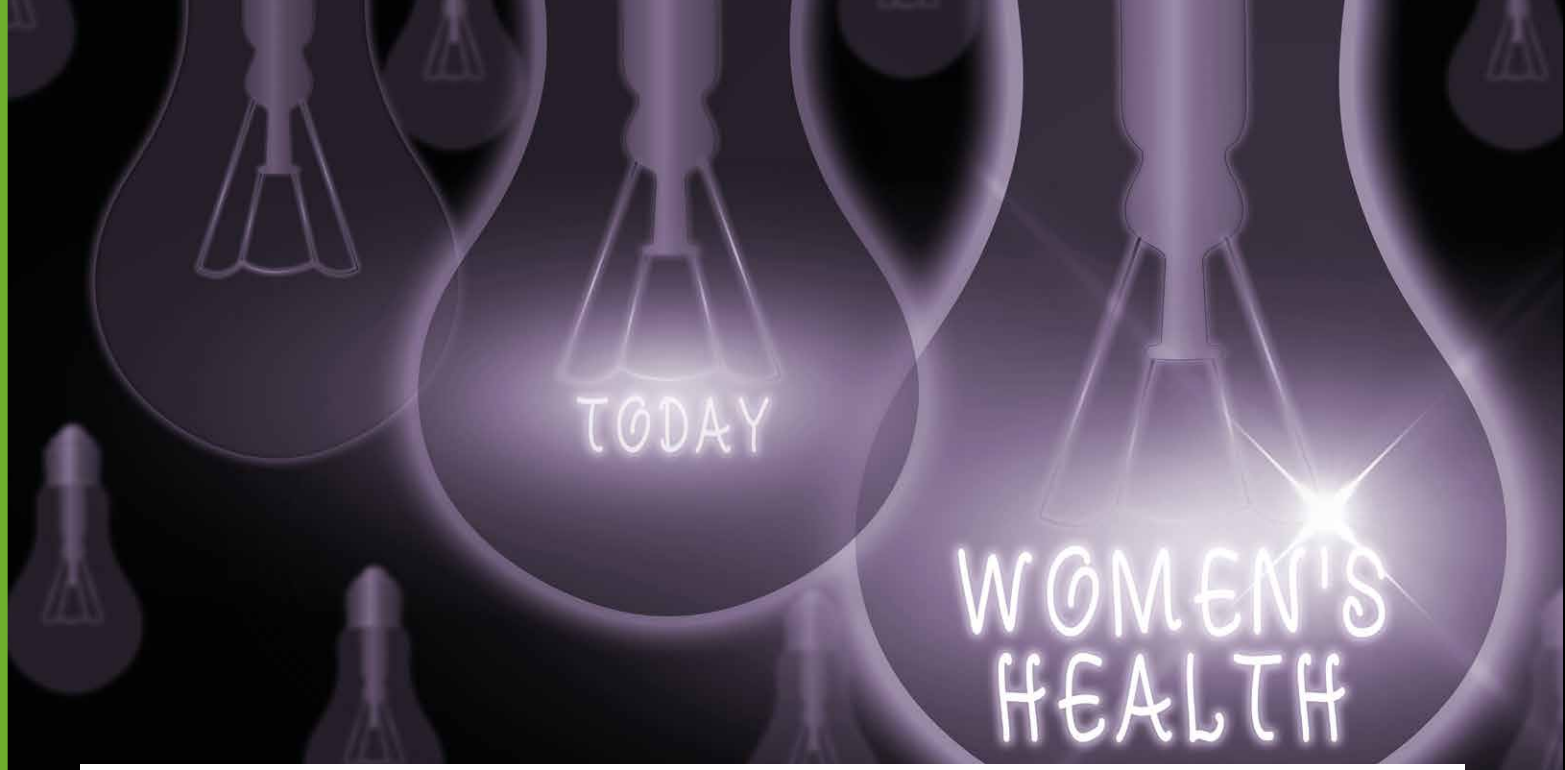
Enabling scientific discoveries that improve human health



WOMEN'S HEALTH AND WELL-BEING



ADVANCES IN PROMOTING WOMEN'S HEALTH AND WELL-BEING

The protection and promotion of women's health and well-being is a priority on the international healthcare agenda. Women can face many unique health concerns relating to menstrual cycles, pregnancy and menopause. Chronic diseases also present a growing challenge to women's health. For example, breast cancer is the most common cancer in the UK – and there are around 150 new cases of breast cancer every day in the UK alone. In this section, we meet the researchers who are dedicated to improving women's health and well-being across the world.

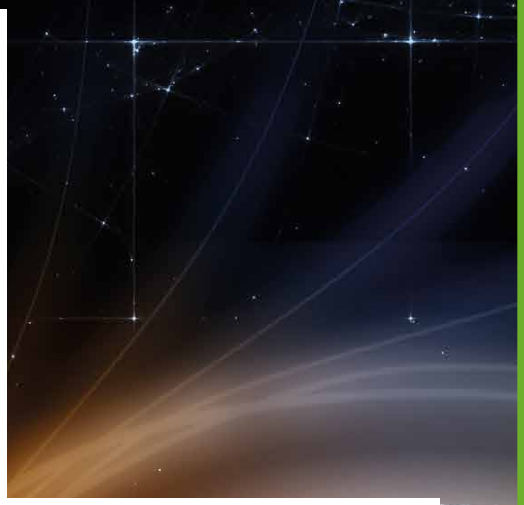
We open with the work of Professor Gary Elkins at Baylor University, Texas, and consider his extensive contribution to science focused on the development of innovative mind-body interventions for symptoms associated with breast cancer and menopause, and women's healthcare more generally. We read how self-administered hypnosis via tele-health technology can overcome hot flushes and sleep disturbances in post-menopausal women and breast cancer survivors.

As one of the most common chronic diseases, most people are aware of the health complications arising from diabetes. However, the impact of maternal diabetes on their children later in life is less understood. Dr Jane Khoury at Cincinnati Children's Hospital Medical Center (Ohio, USA) is leading a research group working to overcome this gap. We read of their ongoing study, 'Level and timing of diabetic hyperglycaemia in utero: The transgenerational effect on adult morbidity' (TEAM study) and how this is improving our understanding of the effects of maternal diabetes during pregnancy, to improve healthcare provision for both mothers and their children.

Dr Jennifer A. Hernandez Gifford and her team from New Mexico State University are investigating the complex process of folliculogenesis, the mechanism through how an ovarian follicle containing an immature egg develops to become ready for the egg's release. We read of the importance of the 'WNT' family of signalling protein molecules and the implications for health, fertility and disease.

Finally, we turn to the work Professors Sara Brucker, Olaf Riess, and Oliver Kohlbacher from the University of Tuebingen. Type I MayerRokitansky-Küster-Hauser (MRKH) syndrome results in the absence of the uterus (womb) and the upper two-thirds of the vagina. Type II MRKH syndrome presents with the same utero-vaginal malformation but also with further difficulties. We read of their elucidation of the molecular pathology of the disorder and their newly developed surgical intervention to overcome the major life-changing aspects of MRKH.

HYPNOSIS: A MIND-BODY SOLUTION FOR HOT FLUSHES AND SLEEP POST-MENOPAUSE AND IN BREAST CANCER SURVIVORS



Professor Gary Elkins at Baylor University, Texas, is a leading expert on complementary and integrative medicine and clinical hypnotherapy. Here, we review his extensive contribution to science in the field of innovative mind-body interventions for symptoms associated with breast cancer and menopause (including the symptoms of hot flushes, sleep, anxiety, quality of life), and women's health care in general.

Credit Gary Elkins

Hypnosis: An Alternative to Pharmacological Treatment?

As Professor of Psychology and Neuroscience and Director of the Mind-Body Medicine Research Laboratory at Baylor University, Waco, Texas, Professor Gary Elkins has, from 2006, led a team of doctoral and post-doctoral researchers exploring the potential applications and benefits of clinical and experimental hypnosis.

One important aspect of Professor Elkins' research focuses on the use of hypnosis in the management of the symptoms of menopause, which can be a common symptom following breast cancer surgery as well as part of normal biological ageing for women. This mind-body approach has significant appeal due to providing an alternative to the traditional pharmaceutical treatment of menopausal symptoms, which is associated with adverse side-effects combined with unsuitability and unreliability for some patients.

Professor Elkins and the Mind-Body Medicine Research Laboratory are currently conducting a multi-site randomised clinical trial of a self-administered hypnosis intervention for hot flushes and sleep disturbances in post-menopausal women and breast cancer survivors. The intervention is delivered using tele-health technology and does not require in-person visits. Recruitment and accrual for this study is on-going at this time.

The Origins of Clinical Hypnosis

Professor Elkins is a leading practitioner and researcher of clinical hypnosis and has been instrumental in establishing its widespread adoption in clinical practice. The Society of Psychological Hypnosis of the American Psychological Association defines hypnotherapy as 'The use of hypnosis in the treatment of a medical or psychological disorder or concern.'

Hypnosis is a psychological state with physiological attributes and involves focused attention. The use of hypnosis can be traced to ancient times, but its modern scientific history began in the late 18th century with Franz Mesmer, a German physician who used hypnosis to treat his patients in Vienna and Paris. Soon afterwards, James Braid, a Scottish physician adapted 'mesmerism' for his medical practice and initially thought the process was similar to sleep, thereby coining the term hypnosis from the Greek word hypnos for sleep. He later came to understand that hypnosis is not sleep, but involves focused attention and suggestions. Since that time, contemporary research has greatly advanced our understanding of hypnosis and its applications in health care.

A Hypnosis Session in Progress. Credit Gary Elkins.

The Problem of Hot Flushes and Sleep

Returning to the work of Professor Elkins, his laboratory has taken a particular interest in the application of hypnosis as an intervention to alleviate symptoms of menopause. The team has conducted two large randomised clinical trials for using hypnosis to manage hot-flushes (or 'hot flashes' in American terminology) and to reduce sleep disturbances in menopause.

The menopause transition is a natural part of ageing and generally happens between the ages of 45 and 55 years of age, as a woman's oestrogen levels and associated fertility decline. In addition, menopausal symptoms can also be induced prematurely by breast cancer surgery or ovarian failure.

The symptoms of menopause are varied and can be severe to the extent that they disable daily functioning for many women. Common symptoms include hot flushes, night sweats, vaginal dryness, difficulty sleeping, low mood, anxiety and difficulty concentrating. Menopausal symptoms may start months or even years before periods stop and can last up to ten years or more after the last period.

Hot flushes are often a spontaneous experience associated with vasodilation (the dilation of blood vessels and drop of blood pressure), accompanied by sweating, skin flushing, fatigue, palpitations and feelings of anxiety, irritability and sometimes panic. Hot flushes are linked to decreased concentrations of oestrogen or gonadotropins but they can also be triggered by external factors such as stress, hot weather, spicy foods, alcohol or caffeine.

Professor Elkins and his team have identified that clinical hypnosis may be a potential treatment for postmenopausal hot flushes and breast cancer survivors who are also affected by this symptom. For the latter group, it is particularly pertinent and offers specific clinical benefits. Women with a history of breast cancer often experience more severe hot flushes, as abrupt chemotherapy can induce premature menopause, and in addition, commonly used oestrogen reducing breast cancer drugs, such as tamoxifen, or discontinued hormone replacement therapy, can also trigger the symptoms. As many as 78% of women receiving chemotherapy for breast cancer and 72% of those taking tamoxifen suffer from hot flushes.

Ovarian failure is usually treated with oestrogen replacement therapy, but oestrogens are associated with increased risk of breast cancer and therefore, must be avoided for breast cancer survivors. Non-hormonal options for treatment of hot flushes is therefore limited.

For these patients, antidepressants, such as paroxetine, venlafaxine and fluoxetine have been shown to have only a modest effect on reducing hot flushes and are not effective for some. In addition, antidepressants, also bring a wide range of potentially unpleasant side-effects, including anxiety, dry mouth, fatigue, sleepiness, and difficulties with concentration. As a result, studies have suggested that many patients discontinue or reduce their medication from the optimum dose. It is therefore apparent that an alternative to the approaches described is very desirable, and hypnosis appears to be a potential solution.

Can Hypnosis Reduce Menopausal Symptoms?

Hypnosis has been used to alleviate a wide range of medical conditions, including acute and chronic pain, irritable bowel syndrome, headaches, anxiety, depression and stress. The work of Professor Elkins' team also suggests that hypnosis may reduce the frequency and severity of hot flushes, principally through 'suggestions for coolness and relaxation, decreasing psychological stress and improving sleep.' There may be considerable similarities between the physical response to hot flushes and the body's stress response (the over-stimulation of the sympathetic nervous system, which regulates vital bodily functions such as heart rate, blood pressure, pupil dilation, body temperature, sweating and digestion). With this potential link in mind, the team started by developing a well-defined hypnosis intervention in the laboratory and then in a small exploratory study, tested the use of hypnosis as a stress management technique to reduce hot flushes in a group of breast cancer survivors.

All participants kept a weekly diary of their hot flushes, followed by a four-week post-treatment diary. Each patient was given a forty-five-minute hypnosis session, four times per week, by a doctoral clinical psychologist, which followed a standardised script. Patients underwent hypnotic induction 'with suggestions for relaxation and mental imagery for coolness,' and were given an audio recording of hypnotic induction to use to practice at home, including instructions for self-hypnosis. The results were very encouraging and achieved the aim of reducing the frequency and severity of hot flushes, with a 70% reduction from the baseline score to the end of the intervention, and comparable or superior to that achieved by the non-hormonal pharmacological treatment such as antidepressants.

This research was repeated two years later, using a larger and more rigorous randomised prospective study and achieved similarly impressive results. A randomised, controlled clinical trial involving 187 post-menopausal women with moderate to severe hot flashes was conducted by Professor Elkins' team. Participants received five weekly sessions of either clinical hypnosis or structured-attention counselling. Hot flushes were measured using both diaries and physiological data. Hot flush scores were determined by daily diaries at weeks 2-6 and week 12. Results demonstrated that flush frequency from baseline to week 12 showed the mean reduction was 80.32% on average for the clinical hypnosis intervention as compared to 15.38% for those that received the structured attention counselling. In addition, results showed significant improvement in the level of daily interference from hot flushes, levels of depression, anxiety and sleep disturbance. The study also showed that patients were positive about their hypnosis experience, with a high level of treatment satisfaction.

Women living with unrelieved hot flushes are known to suffer considerable damaging emotional and physical challenges, often hidden from or treated unsympathetically by others. These undoubtedly add to the consequences of hot flushes, namely, depression, anxiety, sleep disturbance, and decreased quality of life. With the close inter-relationship between the physical and emotional effects of menopause and hot flushes, it is reasonable to hypothesise that the interventions that effectively relieve hot flushes can also improve general mood levels and related factors, such as sleep. This was confirmed by the participants in this study, who reported significant improvement in each of these secondary outcomes.

There are indications that hot flushes may be related to a decrease in the parasympathetic tone. In general terms, the parasympathetic nervous system is part of the autonomous nervous system and acts to slow the heart rate. It is possible that regularly practising clinical hypnosis helps to regulate and increase parasympathetic tone and reduce the hot flushes and related cardiovascular symptoms. However, at the present time, the mechanism of action through which hypnosis reduces hot flushes is not yet fully known. Professor Elkins' research has shown that the effects are not likely to be due to expectancy or placebo effects, but may be related to stress and mind-body interactions. With the demonstrated benefits that hypnotherapy sessions provide, there is no doubt that this relaxing self-care intervention is likely to provide improvements to general well-being as well as stress reduction.

Establishing Hypnosis as a Clinical Alternative

In addition to his work on hot flushes in menopause and breast cancer survivors, Professor Elkins has a pioneering role in the recognition of hypnosis as a valuable treatment for medical and psychological problems.

In 2013, the American Psychological Association, Division of Psychological Hypnosis, appointed Professor Elkins as chair of a task force aiming to create modern and concise definitions of hypnosis, hypnotherapy and hypnotisability to replace previous unsatisfactory and out-dated definitions. He noted that 'The definition of hypnosis is fundamental to scientific inquiry, but the endeavour to define hypnosis from differing theoretical perspectives has given rise to controversy as to the "real" meaning of hypnosis. ...For example, some have defined hypnosis as a "procedure", and at the same time, others have defined it as a "product" of a procedure.'

New, clearer definitions were produced in 2015. The consensus definition of hypnosis is '*a state of consciousness involving focused attention and reduced peripheral awareness characterized by an enhanced capacity for response to suggestion.*' This clearly defines hypnosis as a state of consciousness and that hypnotic induction is a procedure to facilitate the experience of hypnosis. Subsequently, Professor Elkins has been working on improving the tools and training required for clinical hypnosis. Working with other key researchers, he has developed a core curriculum and textbook to raise training standards for health care professionals involved in hypnosis.

After observing the significant individual differences in hypnotisability amongst patients Professor Elkins and his laboratory devised a new, reliable tool to measure someone's hypnotisability. Hypnotisability can be understood as a talent or ability to experience hypnosis and effectively respond to hypnotic suggestions. A new scale was developed to measure individual differences in hypnotic abilities quantifiably, called the Elkins Hypnotizability Scale (EHS). Primarily designed to aid clinical research, the EHS provides an easily administered and relatively rapid tool that takes only about 15-20 minutes to administer.

It is without a doubt that the work of Professor Elkins is making important contributions to the expansion of clinical approaches using non-pharmaceutical, mind-body interventions and solutions for the problems of hot flushes and stress. This approach appears particularly useful for long-term chronic conditions such as pain management and anxiety-related symptoms, and Professor Elkins' work is particularly advancing our understanding and measurement of mind-body therapy (hypnosis) and clinical applications to improve the health care of women.

Meet the researcher

Professor Gary Elkins
Baylor University
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Professor Gary Elkins completed a BA in Psychology in 1975 at Henderson State University (USA) and PhD at Texas A&M University (USA) in 1980. Following the completion of a range of clinical and academic appointments, Dr Elkins is now a Professor at the Department of Psychology and Neuroscience, and Director of the Mind-Body Medicine Research Laboratory at Baylor University. He is also a Medical Associate at Baylor Scott and White Health and Adjunct Professor at the Department of Psychiatry and Behavioral Sciences, Texas A&M Health Science Center College of Medicine. His specialist areas include hypnosis research, sleep, women's health, complementary and integrative medicine. He is the Editor-in-Chief of the *International Journal of Clinical and Experimental Hypnosis*. Dr Elkins is a licensed psychologist and is board certified in Clinical Health Psychology by the American Board of Professional Psychology. As an esteemed clinical researcher, Dr Elkins has published extensively in peer-reviewed journals as well as authoring the popular book *Handbook of Medical and Psychological Hypnosis: Foundations, Applications, and Professional Issues.*

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FURTHER READING

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Baylor University
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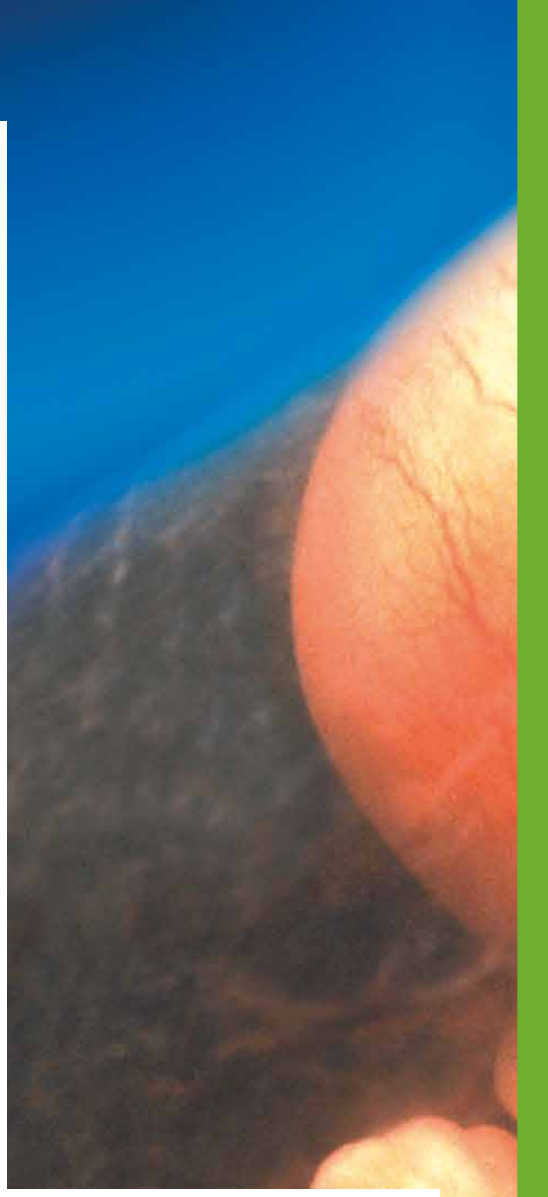
MATERNAL DIABETES AND ADULT MORBIDITY IN THE OFFSPRING: THE TEAM STUDY AT CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER



While most people are aware of the health complications associated with diabetes, the impact of maternal diabetes on their children later in life is less understood. A research group at Cincinnati Children's Hospital Medical Center (Ohio, USA) led by **Dr Jane Khoury** is working to change this. Their ongoing study, 'Level and timing of diabetic hyperglycaemia in utero: The transgenerational effect on adult morbidity' (TEAM study) is driving forward our understanding of the effects of maternal diabetes during pregnancy, to improve healthcare provision for both mothers and their children.

Types of Diabetes

Diabetes is a serious medical condition in which a person's pancreas loses the ability to produce insulin, required to regulate blood sugar levels. Over time, changes in blood sugar levels can cause a variety of complications and health problems.

There are three main types of diabetes – type 1, type 2 (pre-gestational) and gestational. When it comes to managing diabetes, testing blood sugar levels is crucial as uncontrolled blood sugar levels can result in hospitalisation – or even worse consequences.

Type 1 diabetes is often caused by an autoimmune response which incorrectly targets insulin-producing cells (beta cells) in the pancreas and is most commonly diagnosed during childhood. Type 1 diabetes is typically treated with insulin injections or using an insulin pump to prevent hyperglycaemia, high blood sugar levels.

Type 2 diabetes is caused by the body losing its ability to respond to insulin, known as insulin resistance. While the body will still produce insulin, it is not enough to control blood sugar levels. Type 2 diabetics may not require insulin injections but patients may need to take other medications to control their sugar levels.

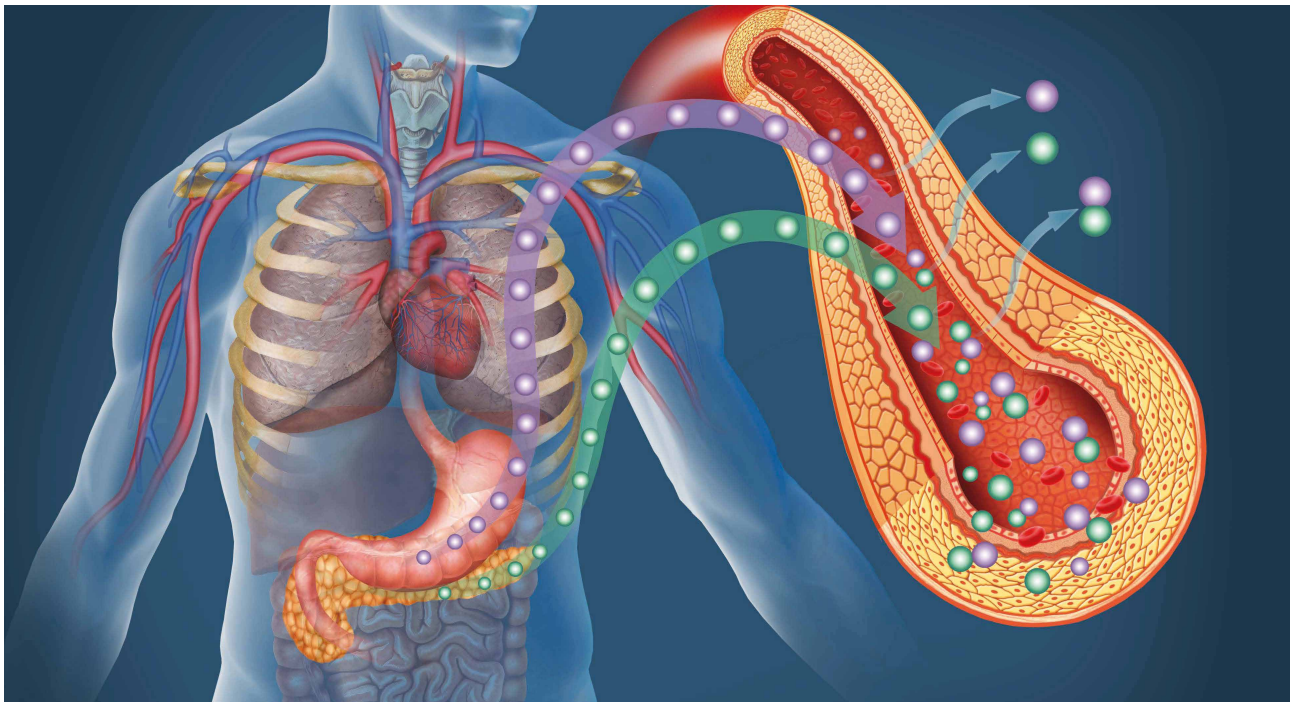
Gestational diabetes develops because the body is unable to produce enough insulin for the mother and the additional needs of pregnancy. This can occur anytime during pregnancy, but is usually diagnosed during the second or third trimester and regresses after giving birth. Gestational diabetes may be treated with dietary modifications or medications as necessary.

Maternal prenatal care is essential for women with pre-gestational diabetes

Understanding Diabetes in Pregnancy

While diabetes in pregnancy can cause immediate and devastating consequences such as miscarriage preeclampsia (characterised by high blood pressure and protein in the urine), preterm delivery, caesarean section delivery, and birthing a large for gestational age infant. or complications during delivery. A further concern is that children born of mothers with diabetes may also develop serious health

This work 'is critical to rapidly and directly determine specific contributions of the intrauterine environment to these more subtle pathophysiologies and preclinical diseases affecting the adult offspring of women with type 1 diabetes during pregnancy.'



problems as they age. The University of Cincinnati and Cincinnati Children's Hospital Medical Center (Ohio, USA) has an established history of research excellence in this field, dating as far back as 1978 with the landmark Diabetes in Pregnancy programme initiated by Dr Harvey Knowles and continued through 1995 under the leadership of Dr Reginald Tsang and Dr Menachem Miodovnik.

Dr Jane Khoury, the principal investigator on the TEAM study, has emphasised that this work 'is critical to rapidly and directly determine specific contributions of the intrauterine environment to these more subtle pathophysiologies and preclinical diseases affecting the adult offspring of women with type 1 diabetes during pregnancy.'

There are approximately 29.1 million people living with diabetes in the USA, 1.25 million of whom have been diagnosed with type 1 diabetes. The number of women of childbearing age with diabetes, in particular type 2 diabetes, is increasing and so it is expected that the number of children exposed to diabetes while in the uterus will also rise. These statistics emphasise the importance of the work of the TEAM research group in extending our understanding of the risk factors that mothers with diabetes and their children are exposed to.

Dr Khoury and the TEAM study researchers plan to identify the effects of maternal high blood sugar and its associated variability throughout the duration of pregnancy on morbidity outcomes in the young adult offspring. Findings will be used to identify the most vulnerable timepoint(s) throughout pregnancy for foetuses so that healthcare professionals and the pregnant women know when it is most important to ensure rigorous and intense monitoring of blood sugar levels. This will help inform timely, targeted clinical treatment strategies and ultimately prevent long-term health conditions in the children of mothers with diabetes.

Metabolic and Cardiovascular Consequences

The TEAM Study aims to examine obesity, insulin resistance, beta cell dysfunction, type 2 diabetes, and kidney function in the offspring of women with pre-gestational diabetes. In addition, early markers of cardiovascular dysfunction are being studied. Using information already collected regarding maternal blood sugar control, the study will pinpoint the most vulnerable periods of pregnancy related to manifestation of the morbidities in the adult offspring. Using novel statistical methods, they will identify the gestational blood sugar profile that predicts the phenotype of offspring at risk for morbidity in adulthood. Only the adult offspring of women who participated in the original Diabetes in Pregnancy Programme are eligible for the TEAM Study.

As a precursor to the TEAM Study, Dr Khoury and colleagues conducted and published a pilot study among 19 offspring of women with type 1 diabetes. They identified an association between blood sugar control during the

Neonatal assessment of weight

mother's pregnancy with blood sugar levels, blood pressure, and weight in the young adult offspring. In addition, they published a manuscript comparing the rate of mothers with type 1 diabetes delivering a large for gestational age baby in the study group (dating from 1978 to 1995), with that of a more contemporary group (2002 to 2008) from the Consortium on Safe Labor dataset provided by the Eunice Kennedy Shriver National Institute of Child Health and Human Development on the Data and Specimen Hub. There was no difference in the rate of large for gestational age (>90th percentile for gestational age, race, and sex) between the two groups, indicating that despite the technological advances in improving diabetes control, the outcome of a large baby has not been resolved.

A previous study led by investigators in the TEAM research group examined the frequency of several adverse health outcomes in four different groups of large for gestational age infants whose mothers had type 1 diabetes (asymmetric large, symmetric large, asymmetric non-large, and symmetric non-large). Where asymmetry is defined as a weight to height ratio, a 'fat' baby. The results showed that asymmetric large for gestational infants had an increased risk of adverse health problems, consistent with other studies in the field. The researchers also found that foetal abdominal growth rate could be used to differentiate between asymmetric and symmetric large for gestational age infants at delivery.

Kidney disease, also known as diabetic nephropathy, is one of the most serious complications of diabetes. It is estimated that approximately 30–40% of all patients with insulin-dependent diabetes succumb to the failure of the kidney to filter waste from the blood. Studies conducted by the University of Cincinnati investigators identified an increased risk of pregnancy complications in mothers and their neonates associated with diabetic nephropathy. Specifically, the children of mothers with diabetic nephropathy are more likely to have poorer health outcomes compared to those without kidney complications; there is a significant increase in premature births, low birth weight babies, and high blood sugar levels in neonates. This work confirmed that children born from mothers with diabetic nephropathy are a high-risk group. 'The identification of offspring at risk, and of the optimal times to initiate potential preventive measures are each critical for improving the health outcomes of these vulnerable foetuses' notes Dr Khoury.

The TEAM researchers are now investigating further the effect of exposure to diabetes on children and their risk of later developing kidney problems in adulthood. They are also assessing the increased effect of perinatal obesity on renal function. Their research will help to pinpoint the gestational period during which infants are particularly vulnerable to the effects of high blood sugar levels in the uterus and how this is linked to specific morbid conditions in the offspring, such as renal and cardiovascular disease, in later life.

Diabetes: Looking Ahead

With the current worldwide obesity epidemic and the associated increase in diabetes, halting further increases in potentially preventable conditions for the next generations is critical. The TEAM study will play an important role in elucidating the effects of diabetes control during gestation on the mother, baby, and offspring as an adult. The study findings will help inform women with pre-gestational diabetes planning on having children and those already expecting how best to protect themselves and their children from adverse health effects.

By helping clinicians identify foetuses at higher risk for life-time morbidities, preventative and timely interventions can be put in place during pregnancy. Dr Khoury explains that their work 'will directly inform clinical management and alert the pregnant woman herself regarding the lifetime consequences for her offspring due to hyperglycaemic excursions during gestation.'

The group has currently recruited over 100 of the offspring of the targeted cohort of women with type 1 diabetes and has already obtained, from the original study, detailed information about each mother's glycaemic control during pregnancy. The current study is expected to run until August 2022 and recruit 250 offspring in total. The findings from the study will help give us a better understanding of the underlying mechanism of how exposure to diabetes during foetal development affects the health outcomes of these offspring later on in life.

Meet the researchers

The Level and Timing of Diabetic Hyperglycaemia In Utero:
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Dr Jane Khoury obtained her PhD in Quantitative Epidemiology from the Department of Environmental and Public health Sciences, University of Cincinnati College of Medicine. As an established biostatistician and quantitative epidemiologist, she has held several research appointments and is currently a Professor in the Division of Biostatistics and Epidemiology with a joint appointment in the Division of Endocrinology at Cincinnati Children's Hospital Medical Center. She also holds a secondary appointment in the Division of Epidemiology, Department of Environmental and Public health Sciences, University of Cincinnati Medical Center. Dr Khoury's research interests centre on diabetes in pregnancy and the long-term effects on mother and offspring, but she also has a long-standing interest in stroke epidemiology, particularly for those with diabetes.

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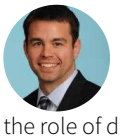
Mekibib Altaye, PhD is a Professor in DBE. His research interest centres on the design and analysis of correlated data focusing on high dimensional data obtained from neuroimaging studies including MRI, fMRI, DTI and MRS.

Katherine Bowers, PhD earned her doctorate in epidemiology from the Johns Hopkins University Bloomberg School of Public Health. She is currently an Associate Professor in DBE with a long-standing interest in how a wide range of exposures during pregnancy affect neurodevelopment.

Shelley Ehrlich, ScD completed her doctorate and post-doctoral training at the Harvard TH Chan School of Public Health and Massachusetts General Hospital. She is a perinatal and environmental epidemiologist in DBE, holding a secondary appointment at the University of Cincinnati College of Medicine in the Department of Environmental and Public health Sciences, Division of Epidemiology. Her research interests include the study of environmental exposures on maternal and child health outcomes.

Resmi Gupta, PhD is a senior biostatistician in DBE. She received her PhD in Biostatistics in August, 2019 from the University of Cincinnati, Department of Environmental and Public health Sciences. Her research focuses on developing prediction models to evaluate the long-term associations between a mother's glycaemic fluctuations and health risks for offspring.



Nicholas Ollberding, PhD is a quantitative epidemiologist and an Associate Professor in DBE. His applied research interests are in investigating the role of diet in the aetiology and progression of chronic disease, and the role of the developing infant intestinal microbiome on growth and early development.

Rhonda Szczesniak (VanDyke), PhD is an Associate Professor in DBE. Her research focuses on the development of dynamic prediction modelling of medical monitoring data; applications include glycemic control in type 1 diabetes in pregnancy, ambulatory blood pressure monitoring and prediction of rapid lung disease progression in cystic fibrosis.

The TEAM study team, from left to right: Mekibib Altaye (co-Investigator), Lisa Tully (Regulatory Specialist), Sang Sam (Clinical Research Coordinator), Amber McKissic (Clinical Research Coordinator), Emily Smith (Clinical Research Coordinator), Shelley Ehrlich (co-Investigator), Katherine Bowers (co-Investigator), Scot Fague (Data Manager), Resmi Gupta (Biostatistician), Jane Khoury (Principal Investigator)

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RELEVANT PUBLICATIONS

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FUNDING

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Diabetes in Pregnancy Program Project Grant. Funded by the National Institutes of Health, Eunice Kennedy Shriver, National Institute of Child Health and Human Development. NIH/NICHD Grant Number HD11725; Tsang, PI.

General Clinical Research Centers' Program, National Center for Research Resources and by an Institutional Clinical and Translational Science Award, NIH/NCRR Grant Number 5UL1RR026314; Heubi, PI

Preventing rapid decline in CF: statistical research career commitment. Funded by the National Institutes of Health, National Heart, Lung and Blood Institute. NIH/NHLBI Grant Number K25 HL125954; Szcznesiak, PI.

The Level and Timing of Diabetic Hyperglycaemia In Utero: The Transgenerational Effect on Adult Morbidity (TEAM Study) Division of Biostatistics and Epidemiology Cincinnati Children's Hospital Medical Center University of Cincinnati College of Medicine Cincinnati, OH USA

Cincinnati Children's™

UNCOVERING NEW SIGNALLING PATHWAYS IN OVARIAN FUNCTION

In mammals, the process by which an ovarian follicle, which contains an immature egg, develops to become ready for the egg's release, is highly complex. Termed folliculogenesis, this mechanism relies on the synchronised input of a range of hormones and signalling pathways. **Dr Jennifer Hernandez Gifford** and her team from New Mexico State University have been investigating the role of the 'WNT' family of signalling protein molecules to further elucidate their involvement in follicle development. The team's research provides novel insights that improve our understanding of the pathways involved, with important implications for health, fertility and disease.

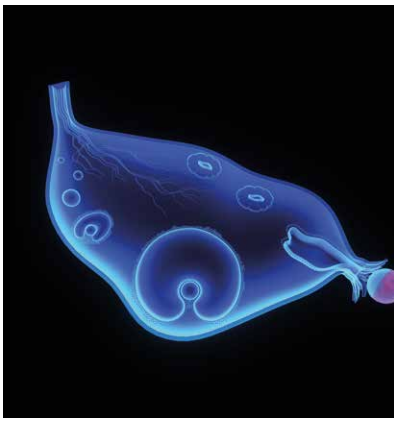
WNT Proteins in Ovarian Function

Women begin puberty with hundreds of thousands of follicles – each with the potential to release an egg at ovulation. During a normal menstrual cycle, one follicle will grow larger until it ruptures at ovulation, releasing the egg. The multifaceted mechanisms behind this process, called folliculogenesis, rely on the synchronised exchange of hormones between the hypothalamus, pituitary, and the ovaries. Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), both members of a group of hormones called 'gonadotropins', are two of the main hormones released by the pituitary.

While the initial stages of follicle development (termed the preantral phase) occur largely in the absence of gonadotropins, the transition from the preantral to preovulatory follicle occurs as a result of increased FSH and LH responsiveness, along with the involvement of numerous other hormones and growth factors. The actions of the gonadotropins are also dependent on other signalling pathways that are active at defined stages of follicular growth.

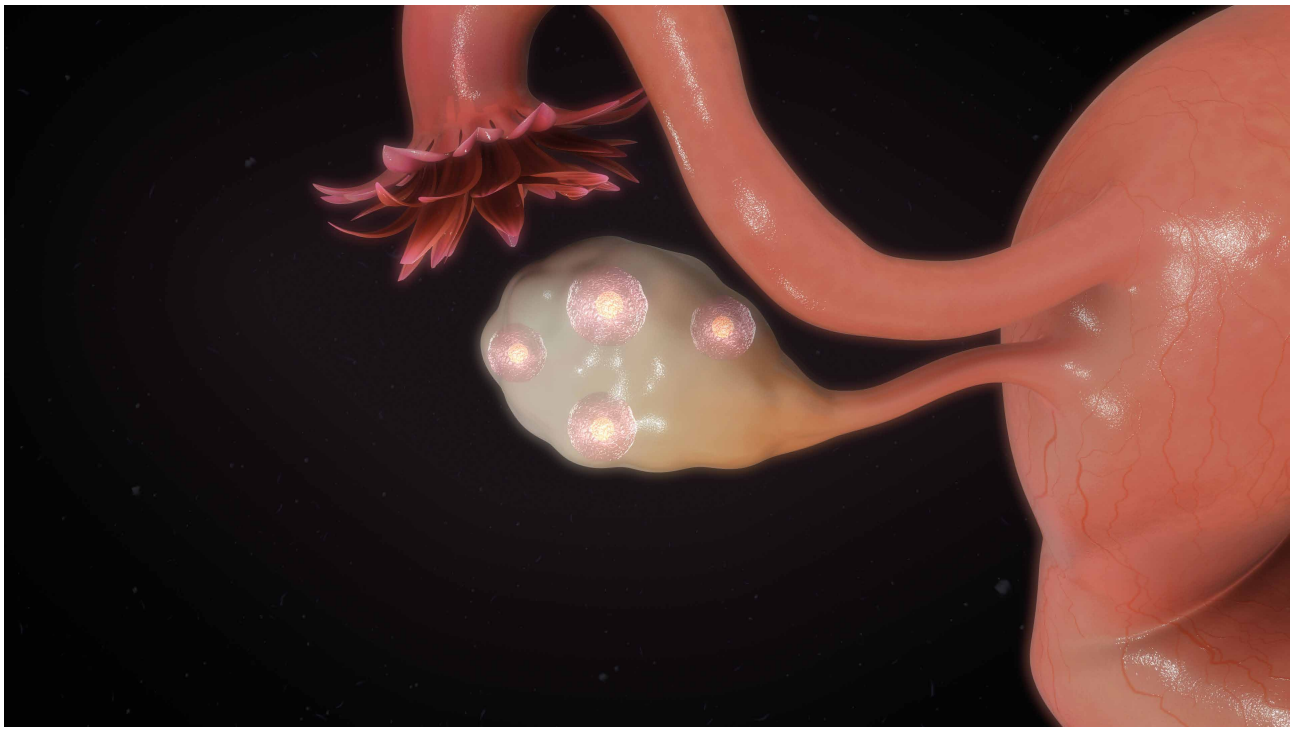
Around 20 years ago, a group of signalling proteins called the 'WNT' family was first identified as being key to the signalling pathways involved in normal ovarian development, and thus suggesting an important role in ovarian function. Prior to this, abnormal WNT signalling had been linked with certain cancers, but not with ovarian development.

In 1999, researchers found that removing the WNT4 gene, which codes for the WNT4 signalling protein, resulted in partial female to male sex reversal in mice, and a paucity of oocytes at birth. Since this initial discovery of the requirement for WNT signalling proteins in the ovary, further functional studies in the adult mammalian ovary have shown a fundamental need for WNT signalling in normal ovarian function and fertility.



Although our understanding of the importance of WNT signalling in folliculogenesis has grown tremendously in recent years, much remains unknown about the broader physiological involvement of WNT signalling in the adult ovary. With this in mind, Dr Jennifer Hernandez Gifford and her team from New Mexico State University have been working to elucidate the mechanisms, modes of action and importance of the WNT family of signalling molecules and downstream components expressed at specific stages of follicle development.

‘Improving our understanding of the mechanisms involved in ovarian follicle development and function will allow us to identify targets of cellular pathways affecting oestrogen levels in health and disease.’



‘To date, our research has provided novel insights into FSH-mediated steroid hormone production and expression of genes important for ovarian maturation,’ explains Dr Hernandez Gifford. ‘Our ongoing research is aimed at further elucidating the signalling pathways involved using large animal models. Improving our understanding of the mechanisms involved in ovarian follicle development and function will allow us to identify targets of cellular pathways affecting oestrogen levels in health and disease.’

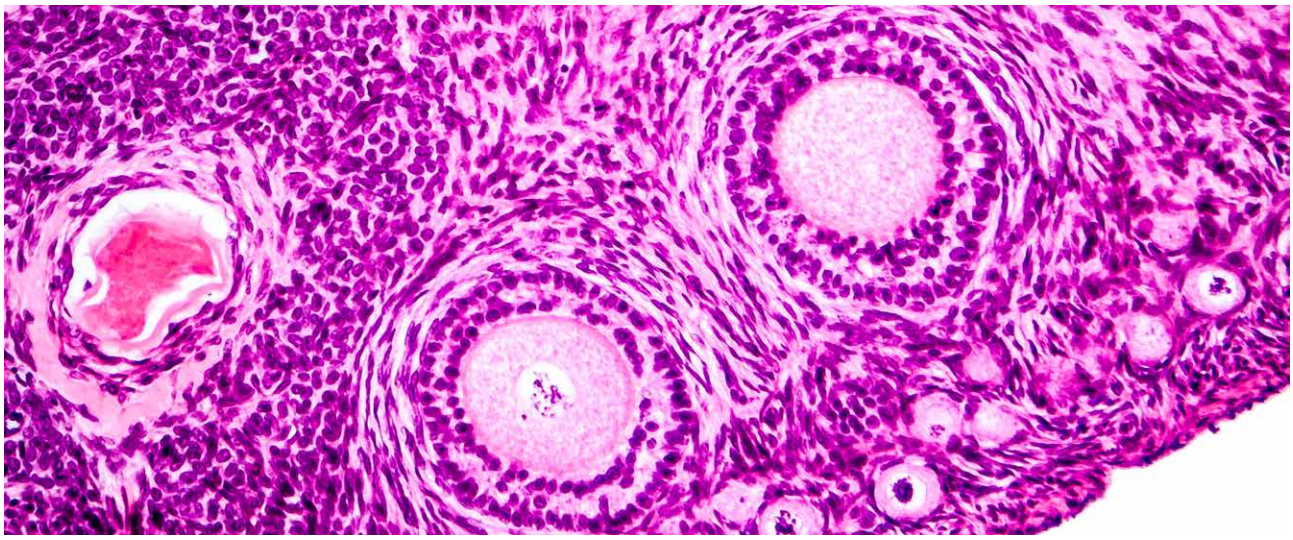
Signalling for Hormone Production

The WNT family of signalling molecules is known to regulate numerous cellular processes, including cell growth, function, differentiation (whereby a cell changes from one type into another) and cell death. Most mammalian genomes comprise 19 WNT genes that code for proteins. The most intensely studied WNT pathway is the ‘canonical WNT signalling cascade’, which regulates levels of an important downstream molecule called beta-catenin (formally designated CTNNB1). Using primary cultures of rat ovarian cells, initial studies by Dr Hernandez Gifford and her team confirmed that CTNNB1 is required for maximal gonadotropin stimulation and ovarian oestrogen production. Although this study, along with several others, began to define a clear role for WNT and CTNNB1 in adult ovarian function, all had been conducted in rodent models. Therefore, Dr Hernandez Gifford and her team were keen to investigate the physiological significance of this signalling pathway in large mammals.

In 2012, the team carried out a study using bovine ovarian cells, which demonstrated for the first time that FSH regulates the CTNNB1 protein and WNT2 gene expression in cattle. This study identified a previously unappreciated role of the WNT signalling pathway in bovine follicular maturation. The team also found that FSH treatment tended to increase the abundance of a protein called AKT, which is known to inactivate members of the CTNNB1 destruction complex.

Based on these results, the team’s next step was to determine whether FSH directly regulates CTNNB1 through the modulation of AKT, or whether it has an indirect effect, by increasing the expression of WNT2, and subsequently activating the canonical WNT pathway.

To investigate the specific contributions of AKT in CTNNB1 accumulation, the team treated bovine ovarian cells with activators of AKT in the presence or absence of FSH. Cells treated with FSH, IGF-1 (Insulin-like Growth Factor-1 – an AKT activator), and both FSH and IGF-1 together exhibited increased CTNNB1 accumulation compared with controls. In contrast, the use of AKT inhibitors suppressed the ability of FSH and the AKT activator to regulate CTNNB1. These findings extended the team’s knowledge regarding how FSH regulates CTNNB1 in bovine ovarian cells, and revealed the importance of AKT-mediated CTNNB1 regulation.



Unexpectedly, this group also demonstrated that canonical WNT signalling actually inhibits FSH stimulation of molecules associated with maturation and differentiation of ovarian follicles. It is therefore likely that FSH regulation of WNT signalling creates a negative feedback loop to ensure that CTNNB1 remains controlled.

Given the temporal expression of FSH, IGF-1 and WNT signalling molecules in the ovary, the team's data suggest that IGF-1 is capable of overriding a negative feedback system set up by WNT signalling on FSH target genes. This ensures that follicle maturation and oestrogen production don't become unregulated, which would have negative effects on fertility.

Although the exact molecular nature of the inhibitory effect remains unclear and requires further examination, this work identifies a new pathway for follicle development through WNT negative feedback. Overall, these results indicate that WNT signalling components not only participate in ovarian hormone production in cattle, but also work in coordination with the pituitary gonadotropin, FSH, and other ovarian molecules such as IGF-1.

Future Benefits for Health and Fertility

The work carried out by Dr Hernandez Gifford and her team paves the way for an improved understanding of the signalling pathways involved in folliculogenesis. The data produced by these studies highlight the importance of WNT molecules in adult ovarian function related to follicle development, hormone production and fertility.

The team's results showing the hormonal regulation of WNT genes at different stages of the oestrus cycle suggest their crucial role in normal ovarian function. Similarly, the finding that FSH requires input from CTNNB1, a lynchpin molecule in canonical WNT signalling, further implicates CTNNB1 in the regulation of follicle maturation.

These findings have important practical applications for our understanding of the molecular processes that may help or hinder fertility. As such, they hold great potential for developing new approaches to improve the success of assisted reproduction.

Furthermore, the team's most recent work highlights even more potentially interesting developments. 'New data have revealed ovarian pathways by which bacterial infection has the potential to alter oestrogen production and subsequent fertility,' says Dr Hernandez Gifford.

A greater understanding of the functions of WNT proteins in folliculogenesis could also help to provide a clearer picture of how this complex family of molecules plays roles in the development of various diseases. In fact, recent research now implicates WNT proteins in an extensive array of health problems in humans, including diabetes, osteoporosis and heart disease, as well as certain cancers.

Inspiring the Next Generation

In addition to her research, Dr Hernandez Gifford is also passionate about inspiring and training the next generation of scientists. 'An exciting additional achievement has come through my involvement in working with both graduate and undergraduate students,' she says.

She makes a point of always involving undergraduate students in her projects, as such experience is vital for ensuring their successful integration into the world of research after they graduate. 'All of the undergraduate students that have worked in my lab have successfully entered schools of medicine, veterinary medicine, dentistry or graduate school,' she says. 'Graduate students from my lab have pursued careers as Extension agents, have been selected for prestigious post-doctoral fellowships, moved to medical facilities to work as research associates, and have accepted faculty positions at community colleges.'

Meet the researcher

Dr Jennifer A. Hernandez Gifford
College of Agricultural, Consumer and Environmental Sciences
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Dr Jennifer Hernandez Gifford is an Associate Professor in the Department of Animal and Range Sciences at New Mexico State University. After completing a MS at New Mexico State University, and a PhD at Washington State University in Animal Science, she conducted postdoctoral research at Washington State University in the School of Molecular Biosciences. In 2009, she joined the faculty at Oklahoma State University and was involved in teaching undergraduate and graduate courses in physiology, endocrinology and biotechnology. Here, she also established a strong research program in the area of ovarian follicle development and steroidogenesis. In 2016, Dr Hernandez Gifford returned to her Alma Mater, New Mexico State University, where she continues her teaching and research program. The long-term goal of her lab's research is to provide fundamental knowledge about the physiological role and mechanism of action of ovarian signalling molecules involved in follicular development, which impact health and disease. Dr Hernandez Gifford is also extremely passionate about mentoring and working with students, and was awarded the prestigious NACTA Educator Award for her work in this area.

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Dr Dennis Hallford (deceased) New Mexico State University

FUNDING

New Mexico Agricultural Experiment Station
Oklahoma Center for the Advancement of Science and Technology
Eunice Kennedy Shriver National Institutes of Child Health and Human Development

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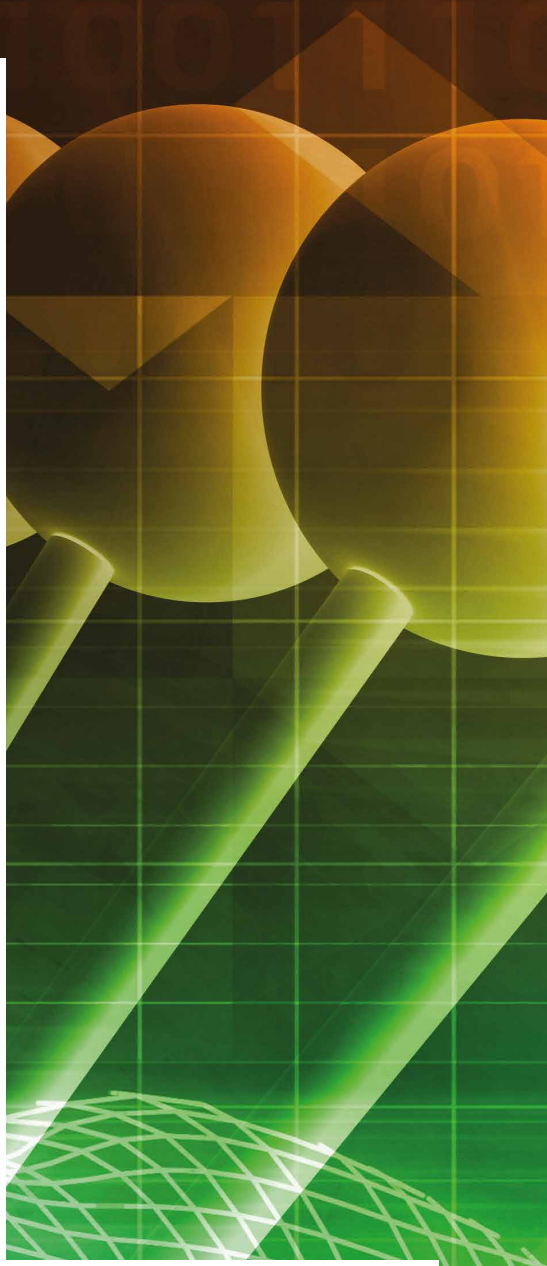
MAYER-ROKITANSKY-KÜSTER-HAUSER SYNDROME: INTERROGATION OF GENETIC PATHOLOGY AND NOVEL SURGICAL INTERVENTION METHODS

In otherwise phenotypical normal females, that is, females with normal ovaries and regular hormone production, Type I Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome results in the absence of the uterus (womb) and the upper two-thirds of the vagina. Type II MRKH syndrome presents with the same utero-vaginal malformation but also further difficulties that can include structural issues within the urological and skeletal systems. **Professors Sara Brucker**, **Olaf Riess**, and **Oliver Kohlbacher** from the University of Tuebingen are elucidating the molecular pathology of the disorder and have developed a surgical intervention to overcome the major life-changing aspects of this condition.



Mayer-Rokitansky-Küster-Hauser (MRKH): Chance or Genetics?

MRKH syndrome is observed in around one out of every 4,500 live female births. Early research into this rare condition concluded that it is a result of a sporadic defect. However, family clusters in which more family members are diagnosed than would typically be expected by chance have also been observed, indicating a possible genetic basis.

Scientists have investigated many genes in search of the cause of MRKH. However, to date, no single factor has been clearly identified and linked with MRKH syndrome. Professors Sara Brucker, Olaf Riess, and Oliver Kohlbacher from the University of Tuebingen propose that the differences in the disease state of each individual may arise from epigenetic sources, which are the observed genetic discrepancies that have occurred as a result of non-genetic influences.

In one study, Professor Brucker and her colleagues noted that the whole genome and methylation comparisons that they conducted on eight pairs of twins (MRKH/normal) showed a deficiency in both oestrogen receptor and specific HOXA genes, the latter of which are the 'master regulators' of embryonic development. This led the team to propose a putative role for either one or both of these in deficiencies in the abnormal development associated with MRKH syndrome.

In addition, in deciphering the pathology of this condition, Professor Brucker and colleagues suggested a putative role for the anti-mullerian hormone promoter, the activation of which may lead to the regression of the Mullerian ducts that typically results in the formation of the uterus and top two-thirds of the vagina during the development of the embryo.

Genetic Mosaicism: Occurrence of Genetic Discrepancies in MRKH Syndrome Females

Continuing their work on the comparison of monozygotic twins, where one twin has MRKH and the other without the condition is the control, the researchers used tissue, blood, and saliva samples to identify two genes

which have known functionality in embryonic development of the uterus and endometrium. Testing the tissues and saliva of the MRKH syndrome individuals showed that the genetic errors were present in the uterine tissue but not in the saliva. Such cases, in which a genetic change is observed only in some tissues and not throughout all the tissues in the body, is known as tissue-specific genetic mosaicism. Somatic mutations, that is, mutations that occur in cells other than sperm and egg (germ cells), resulting in mosaicism may explain the reason why we observe cases of monozygotic twins and explain why the genetic children of MRKH syndrome mothers, via surrogacy, do not have the condition.

Genetic Variants of OXTR and ESRI Genes: Their Link with MRKH

Focusing on two genes that have been demonstrated to have a likely effect in MRKH syndrome, the oxytocin receptor gene (OXTR) and the oestrogen receptor I gene (ESRI), Professor Brucker and colleagues sequenced genes from over 90 patients in an attempt to identify the key variants that are linked to the altered expression of these receptors in MRKH syndrome, specifically, lower oxytocin receptor levels and higher oestrogen receptor levels. In doing so, they identified three variants of OXTR and six variants of ESRI that are likely to be associated with MRKH syndrome.

These findings contribute to the hypothesis that hormone receptor deficiency may be a contributory cause of this syndrome. Professor Brucker and colleagues, however, do recognise that their study was limited due to the small number of participants, and they point to the need for larger studies to support their findings.

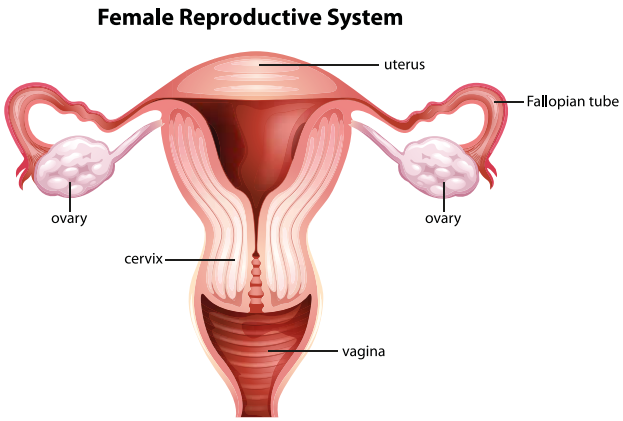
Comparison of methods for Neovaginal Creation

In addition to the focus on elucidating the genetic pathology of MRKH syndrome, Professor Brucker has successfully developed a surgical innovation to create a vagina in female sufferers that enables them to participate in vaginal intercourse. They conducted an initial study to compare the standard method of neovaginal construction with their new and innovative approach. This confirmed that the new method resulted in shorter operative and traction times, a longer neovagina with improved functional results, fewer surgical complications, and no associated technical issues with the procedure.

Professor Brucker and colleagues recommend this method, which is based on stretching the vaginal dimple, the existing part of the vagina in MRKH syndrome females, with a modified traction device which is applied via laparoscopic surgery.

A follow-up study, examining the longer-term outcomes of 240 patients, demonstrated that this innovative methodology results in long term anatomical stability, with all patients noting that the size of the neovaginas remained stable at 11+ months post-operatively. Importantly, even patients who did not wear the vaginal dummy or who did not achieve sexual intercourse retained anatomical stability. Furthermore, over 70% of the patients, a figure comparable with that of the general population, reported long term functional success, measured as an ability to achieve successful, satisfactory coitus.

Investigating the potential negative effects of the treatment in the longer term, the team assessed multiple



parameters, including vaginal prolapse, malignancy, and risk of human papillomavirus infection. The researchers note that patients who failed to follow the necessary protocol, specifically with regards to wearing the vaginal dummy exactly as prescribed, showed some failure, with excessive use of the dummy causing tissue granulation. Meanwhile, failure to use the dummy post-operatively at all resulted, in some cases, in decreased length of the neovagina.

Professor Brucker and colleagues note, that in comparison to non-surgical interventions, such as the use of vaginal dilators, the surgical method produced much more stable results in a more timely fashion. Compared to other surgical methods, like using bowl or skin for a neovagina, there is until now no vaginal prolapse or malignancy or scarring occurred with their method in a very long-term follow-up. The team also highlight the important psychological benefits to the patient of their improved surgical approach.

Surgical Intervention to Enable Pregnancy in MRKH Syndrome Females

Following on from their innovative work on developing a more robust, less problematic neovaginoplasty methodology, Professor Brucker and colleagues have recently presented a review of the processes required to enable an MRKH syndrome female to carry her own biological child. In addition to neovaginoplasty, this also requires uterine transplantation.

Infertility is considered the single most important factor in MRKH syndrome for many women, and with surrogacy illegal in some European and Nordic countries, and many other countries globally, the only option available for these women previously was adoption.

Uterine transplantation can occur from either a live or deceased donor, although a live donation is preferable as pre-operative assessment can ensure suitability and coordination of operations may also be scheduled effectively. Unfortunately, live donor donation carries risks for the donor, including post-operative issues which raise ethical concerns. Furthermore, there may be a psychological risk to the donor if the recipient fails to achieve a pregnancy and live birth following transplantation.

Following extensive animal testing, the first uterine transplants in Sweden showed high success rates, with seven out of the nine patients achieving one or more pregnancies and live birth. Of these seven women, six were MRKH syndrome patients and the seventh had previously undergone surgical removal of her uterus due to cervical cancer. Professor Brucker and her colleagues stressed that there is a substantial amount of monitoring required after the transplantation to ensure the functionality of the transplanted organ is suitable to maintain a pregnancy and live delivery.

Excitingly, by 2019, the collaboration between the Swedish and German scientists had already produced 11 live births from 18 uterine transplants, with the team in Germany having already transplanted four uterus and two live-births. Globally, a team of researchers in Brazil have succeeded in achieving a live birth following a deceased donation, and there is a total of three reported live births that the team is aware of.

Looking to the Future

While Professor Brucker's collaborations on uterine transplantation are on-going and in their early stages, she is still maintaining a focus on investigating the genetic pathology of the disease. Multiple genes are involved in the early stages of urogenital development, and it may be the case that many genes are involved within the same pathway, but at differing levels, which may partially explain the array of levels of severity observed in MRKH syndrome. To untangle this complex scenario, Professor Brucker and colleagues now propose to generate and analyse cases from discordant monozygotic twins, familial cases, and family trios (parents and child) in spontaneous cases of MRKH.

Meet the researchers

Professsor Dr Sara Brucker
Executive Director: Department of Women's Health
Chair: Research Centre for Women's Health
University of Tuebingen
Germany

Professor Dr Sara Brucker works as the Director of the Department of Women's Health and Professor at the University of Tuebingen. Following the completion of her thesis in 1999, she undertook a fellowship in Internal Medicine at the University of Ontario, Canada, and thereafter carried out subsequent residencies in Gynaecology and Obstetrics at her current University. A board certified gynaecologist, Professor Brucker is collaborating on an innovative uterine transplantation project that is designed to enable women suffering from Mayer Rokitansky Küster Hauser (MRKH) syndrome which occurs during development of the embryo that results in the absence of a uterus. Professor Brucker's interests extend to investigating the genetic basis of this condition.

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Professor Dr Oliver Kohlbacher completed his PhD (2001) in Computer Science at Saarland University/ Max Planck Institute for Informatics, Saarbrücken. He is currently a Professor of Applied Bioinformatics and the Director of the Interfaculty Institute for Biomedical Informatics at the University of Tübingen, and Director of the Institute for Translational Bioinformatics at the University Hospital Tübingen. Professor Kohlbacher's team contribute to the management and analysis of the large quantities of data produced in the assessment of the genetic pathology of MRKH syndrome, which aligns with his interest in personalised medicine and the analysis of biological high-throughput data.

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Professor Dr Olaf Riess is a Professor of Human Genetics and Director of the Institute of Human Genetics and Applied Genomics at the University of Tübingen. Since completing his MD in 1990, Professor Riess has had a highly successful research career, leading innovation in the university across a number of departments. In 2014, he was a founding member of the Centre of Personalised Medicine. Professor Riess's work on human genetics and genomics is pivotal in determining the genetic basis of the pathology of MRKH syndrome, and in generating the complex genetic data necessary to investigate the multiple scenarios of its presentation.

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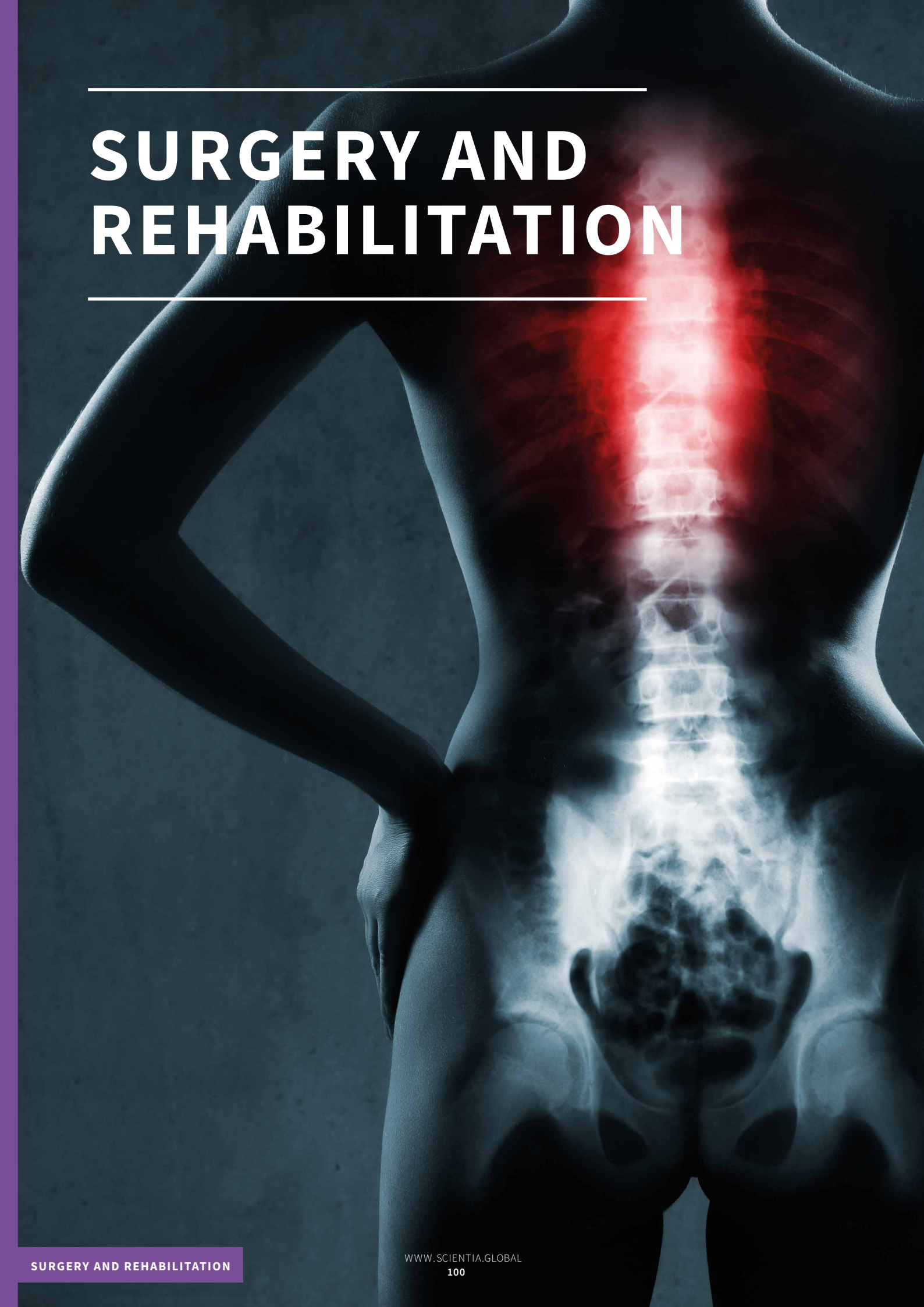
SURGERY AND REHABILITATION



INNOVATION IN SURGERY AND REHABILITATION

This final section of Scientia is dedicated to the researchers who are driving forward innovation in surgical methods and rehabilitation to improve patient treatment and care. From improving surgical planning for patients with brain tumours to revolutionising approaches to the rehabilitation of spinal cord injury, this diverse range of research offers new hope to individuals afflicted by injury or disease.

We open this final section of Scientia by meeting Dr Hamdy Awad at The Ohio State University. We gain insight into Dr Awad's dedication to the development of preclinical animal models to allow a better understanding of the mechanisms of ischaemic spinal cord injury. Dr Awad is also working on the discovery of novel therapeutics to prevent paralysis after aortic aneurysm surgery.

Remaining on the topic of spinal cord injury, we then turn to the work of Professor David Magnuson at the University of Louisville, Kentucky. Using an animal model of spinal cord injury, Professor Magnuson has produced surprising and thought-provoking results. We read how his research is challenging the established clinical beliefs and practices around the ways to best rehabilitate human patients with severe spinal cord injury.

We then consider developments in the field of neurology and neuroscience. Dr Jun Hua at F. M. Kirby Research Center for Functional Brain Imaging at the Kennedy Krieger Institute and Johns Hopkins University, USA, has been pioneering the development of new magnetic resonance imaging techniques to improve pre-surgical planning for neurological patients, such as those with brain tumours. We read how this work could help transform the surgical care of patients across many different neurological disorders.

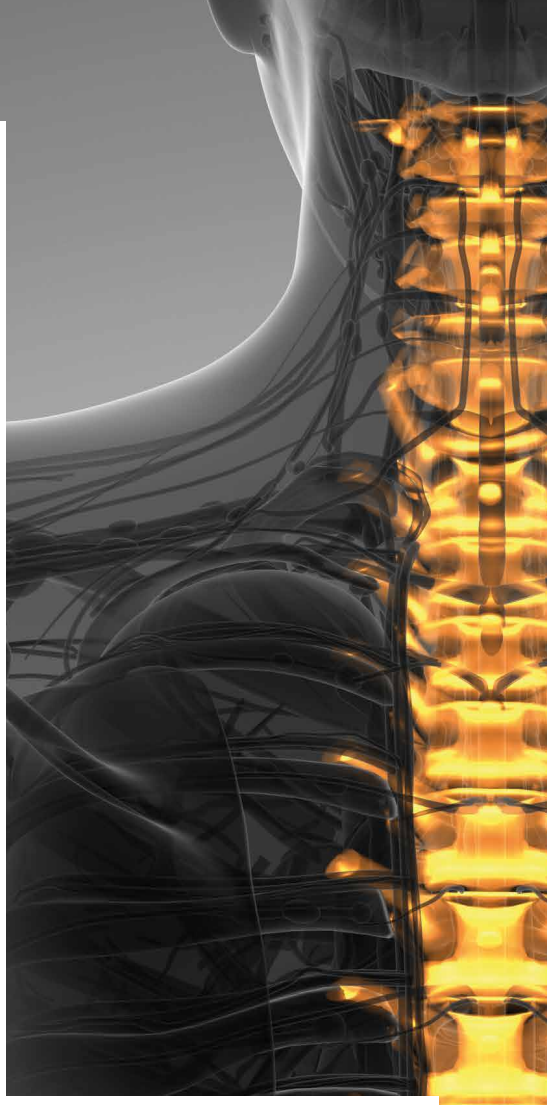
Taking a different approach, Dr Elizabeth Nance at the University of Washington is developing the use of tiny nanoparticles to deliver therapeutic agents to the brain. This offers a novel approach to overcome the delivering drugs directly into the brain due to the highly regulated blood-brain barrier which prevents access to diseased cells. We also read of her current work investigating the potential use of nanoparticles to map tissue structure.

We then turn to the work of Professor Steven E. Wilson at the Cole Eye Institute of the Cleveland Clinic Foundation. Any trauma, such as injury, surgery or infection to the cornea in the eye may result in persistent scarring, as process clinically known as fibrosis. Professor Wilson has identified that defective epithelial basement membrane regeneration plays a central role in this, and we read of the potential relevance of his findings to better understanding the fibrosis that occurs in other organs, such as the lungs and heart.

For individuals who require a wheelchair for mobility, the use of conventional manual pushrim chairs is associated with the risk of shoulder injuries and chronic pain. We meet Steve Green of Green Technologies, Inc, who is overcoming these difficulties through the development and patenting of a wheelchair anti-rollback device that addresses these injury and safety issues with an innovative yet simple mechanism.

We conclude this final section of Scientia by turning to the work of Dr Malcolm Doupe (University of Manitoba) and Dr Frode F. Jacobsen (Western Norway University of Applied Sciences). We read how their long-time research collaboration is effectively helping to overcome the challenges confronted by healthcare systems as the result of our increasingly ageing populations.

ELIMINATING PARALYSIS AFTER AORTIC ANEURYSM SURGERY



Ruptured and dissected aneurysms are medical emergencies that can have fatal consequences. There are two main surgical procedures to repair a ruptured aneurysm: open surgery and endovascular aneurysm repair. Unfortunately, both methods present a risk of developing spinal cord injury and paralysis. In addition, patients who develop paralysis after surgery have a significantly lower survival rate compared to non-paralysed patients. **Dr. Hamdy Awad** at The Ohio State University has spent most of his academic career focusing on the development of preclinical small and large animal models to understand the mechanisms of ischaemic spinal cord injury and discovery of novel therapeutics to prevent paralysis after aortic aneurysm surgery.

Aortic Aneurysm: Incidence and Causes

The aorta acts as the main highway for the transport of oxygen-rich blood around the whole body. To carry out its functions, the aorta is made of strong, elastic walls that enable it to withstand the high pressure of the blood flowing from the heart. An aortic aneurysm is a condition where the walls of the aorta start to weaken and dilate, causing loss of elasticity and increasing the risk of developing ruptures in the aortic wall. Aortic dissection is a condition caused by a tear in the aortic wall, which also leads to rupture.

Aortic aneurysms cause about 12,000 deaths every year in the USA. Several environmental factors increase the risk of aortic aneurysms in humans. The most important factor affecting the integrity of the aortic wall is smoking, which accounts for about three-quarters of all cases of abdominal aortic aneurysms. Other environmental factors include high blood pressure, high cholesterol and atherosclerosis. Rare inherited conditions affecting the connective tissue, such as Marfan syndrome and Ehlers-Danlos syndrome also increase the risk of an aortic aneurysm.

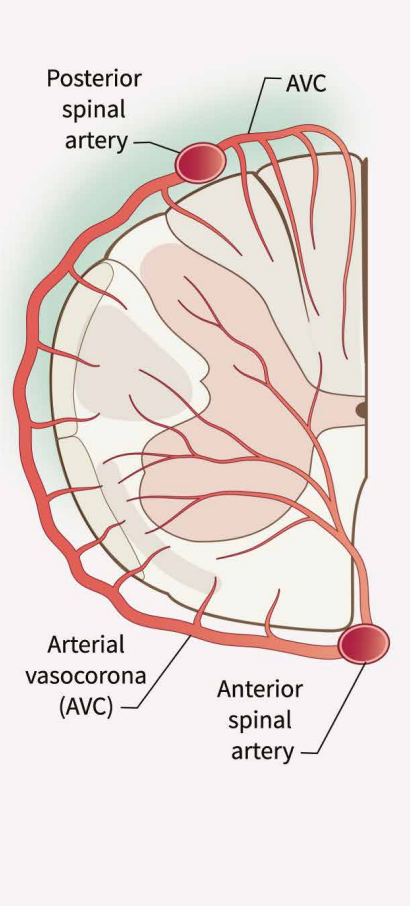
Dr. Hamdy Awad from the Department of Anesthesiology at The Ohio State University Wexner Medical Center has spent most of his clinical and academic career focusing on furthering understanding of the mechanism of ischaemic spinal cord injury and paralysis through the development of preclinical small and large animal models to simulate the clinical paradigm of aortic aneurysm. His aim is to reduce the number of patients who develop paralysis after the surgery, a devastating postoperative complication that is caused by ischaemic injury of the spinal cord.

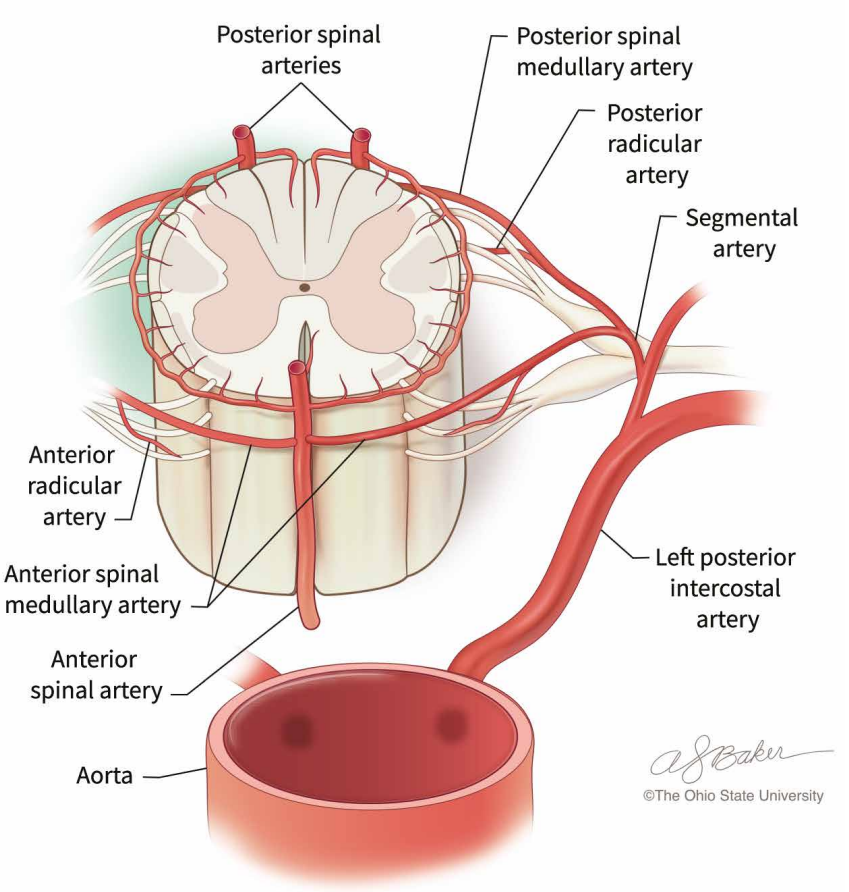
Surgery of the Aorta: Life-Saving Treatment or Russian Roulette?

Acute aortic syndrome includes aortic dissection, intramural haematoma and aortic ulcer. During an aortic dissection, the inner layer of the aorta ruptures. The blood flows through the tear, causing the inner and the middle layer of the aortic wall to separate. If untreated, an aortic dissection can be fatal, because of the risk of internal bleeding.

An intramural haematoma consists of a separation of the inner and middle layers of the aorta, with blood leaking through them. Unlike an aortic dissection, the intramural haematoma does not occur because of a tear in the inner layer of the aorta. Aortic ulcers are formed when atherosclerotic plaques damage and weaken the structure of the inner lining of the aorta, making it prone to dissection.

Despite the large amounts of funding by the National Institutes of Health in





the USA and other institutions around the globe, the research to develop an effective pharmacological therapy for the medical treatment of severe aortic aneurisms and acute aortic syndrome remains inconclusive, and surgery is to date the only effective method to replace a diseased aorta and/or prevent the fatal consequences of a ruptured aneurysm.

There are two main surgical options to repair a diseased aorta prior to or following a ruptured aneurysm: open surgery and the less invasive endovascular aneurysm repair. In both methods, a graft is used to replace the aneurysm. Unfortunately, both procedures also present a risk of developing ischaemic spinal cord injury and paralysis after surgery.

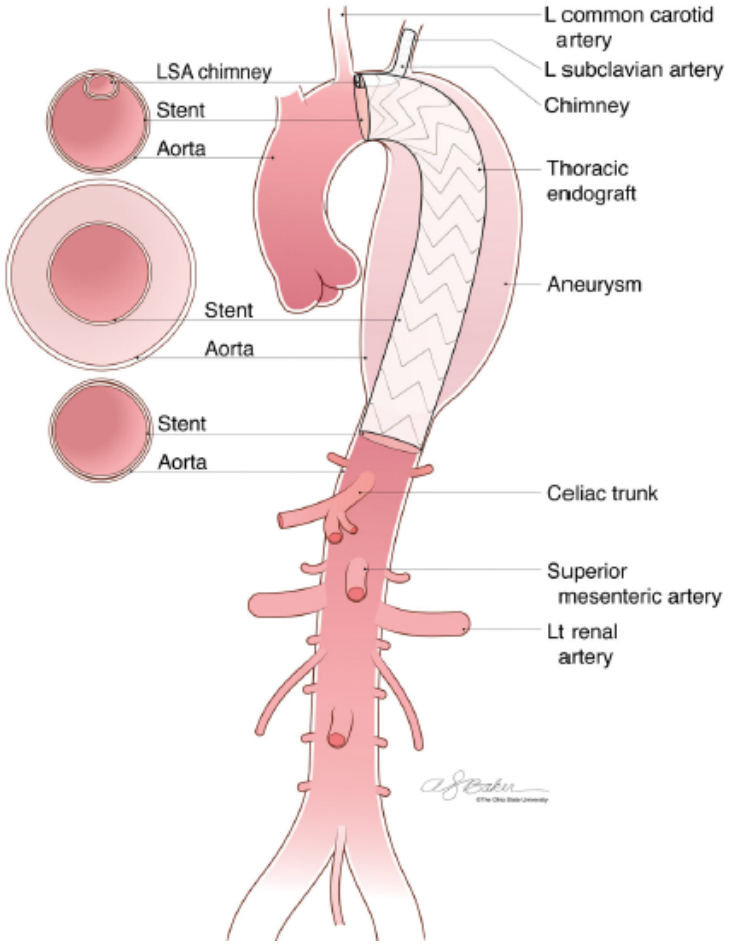
The mechanisms behind these devastating post-surgery complications are not well understood. However, one plausible explanation is that the clamping and unclamping procedures employed in aortic surgery affect the flow of oxygenated blood feeding the spinal cord tissue, resulting in ischaemia and reperfusion injury. Another possibility is that debris from atherosclerotic plaques can become dislodged during the surgical procedure, occluding the blood vessels that supply the spinal cord with blood. Another possibility is the starvation of the spinal cord tissue due to lack of blood flow, which carries substrates such as fatty acids, lactate and oxygen.

Dr. Awad faced the traumatising experience of caring for a patient who survived surgery but later developed ischaemic injury to the spinal cord, followed by paralysis. Dr. Awad was a cardiothoracic anaesthesiologist fellow at the Cleveland Clinic at that time. His patient was an asymptomatic 55-year-old engineer who found out about his thoracic aortic aneurysm after a routine chest X-ray. The patient debated the pros and cons of having surgery versus the risk of developing paralysis. Given the potentially fatal consequences of leaving the aneurysm untreated, he opted for surgery. Unfortunately, the patient woke up from surgery paralysed. Since then, Dr. Awad has made it his mission to find a solution to the 'Russian roulette'-type dilemma faced by cardiovascular surgeons, anaesthesiologists and patients when opting for aneurysm aortic surgery.

Mapping Out a Complex Network of Spinal Cord Vasculature in Normal and Diseased Conditions

The network of vessels feeding the spinal cord with blood is one of the most complex in the human body. To make matters more complicated, researchers can only rely on a very limited range of animal models that are suitable to understand the pathophysiology behind the post-surgery damage to the spinal cord.

Dr. Awad and his team published a book chapter highlighting the significant differences in blood supply to the spinal cord across different species, and described the complexity of identifying a suitable animal model that would allow vascular



Chimney graft technique for left subclavian artery revascularization. A thoracic aortic endograft is excluding a descending thoracic aneurysm and covering the origin of the left subclavian artery (LSA); a chimney graft is inserted in the LSA parallel to the proximal wall of the aortic stent. The top left figure shows a cross section of the aortic arch with the proximal end of the thoracic stent and the LSA chimney graft in place. The bottom left figure shows a cross section of the distal portion of the aortic stent sealing the descending thoracic aorta. LSA: Left subclavian Artery; L: Left
Reproduced with permission from Awad et al, 2017, Spinal cord injury after thoracic endovascular aortic aneurysm repair,
Canadian Journal of Anesthesia.

scientists and surgeons to simulate the ischaemic injury to the spinal cord observed in humans.

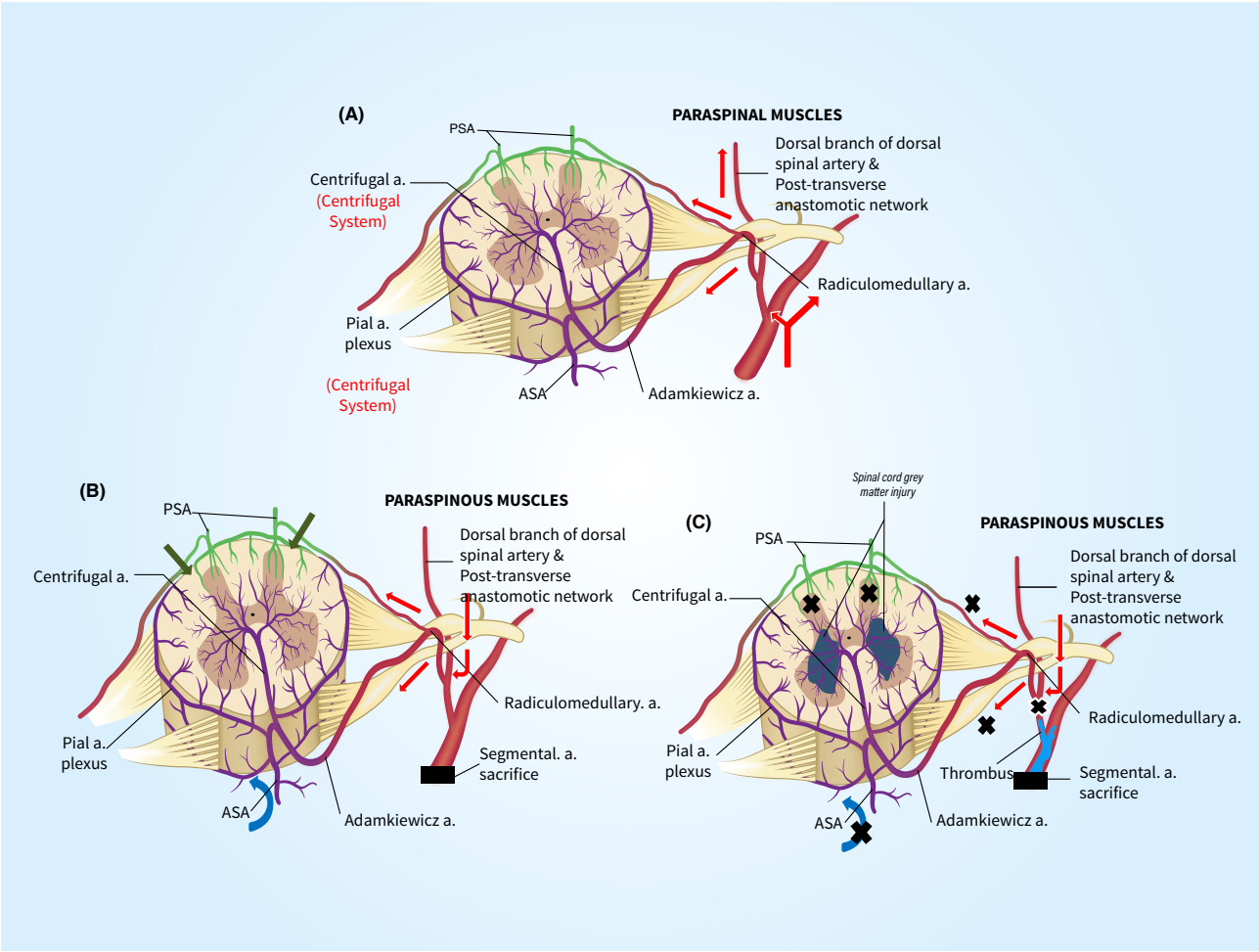
Despite the difficulties, Dr. Awad and his collaborators developed mouse and canine models to shed light on the pathophysiology of the potential ischaemic damage to the spinal cord. The team investigated the effects of cross-clamping the descending aorta on the development of spinal cord injury with delayed paralysis in mice. All mice tested in the study were able to move for up to 24 hours after the procedure, after which they all developed mild or severe paralysis of the hind limbs around 40 hours after aortic cross-clamp. The study also showed that it is not possible to predict beforehand which animals will be severely or mildly affected by the procedure, despite having a similar genetic background and surgical procedure. The two primary factors controlling paralysis were ischaemia time and temperature during aortic cross-clamp.

The team also studied the effect of post aortic surgery inflammation in large animal models on the delayed onset of paralysis. Following the aortic cross-clamping procedure, the animals displayed increased levels of oxidative stress in plasma and the cerebrospinal fluid (CSF) and increased levels of inflammation markers in CSF samples, indicating that the inflammation response is initiated within the central nervous system. Reperfusion injury of the spinal cord caused hind limb paralysis within a three hour interval and this correlated with white blood cell infiltrates three days post-ischaemia. The team used large animals to obtain CSF, which cannot be obtained in the rodent models. They were also able to compare CSF and blood samples from large animal models, as well as from humans, with and without paralysis.

Spinal Cord Protective Strategies During Aortic Aneurysm Surgery

Dr. Awad and his collaborators aim to use their animal models to understand how the pathophysiology of ischaemic injury of the spinal cord differs in the open surgery procedure when compared to the endovascular aneurysm repair approach. Although the risk of developing paralysis is similar in both procedures, endovascular surgery correlates more with the incidence of delayed onset paralysis and less with acute paralysis. This indicates that the two procedures cause different types of damage to the white and gray matter tissue of the spinal cord, and Dr. Awad's team is actively investigating the variables at play behind this difference between open and endovascular repair in the mouse and canine models available.

The team also published a review that evaluated the effectiveness of several spinal cord protective strategies during aortic aneurysm surgery. Among others, the measures adopted to prevent ischaemia include cerebrospinal fluid drainage, the induction of a mild state of hypothermia and optimal blood pressure management. However, to date, there is not a single pharmacologic agent to prevent or protect the spinal cord from ischaemic damage in humans.



Reproduced with permission from Awad et al, 2017, Spinal cord injury after thoracic endovascular aortic aneurysm repair, Canadian Journal of Anesthesia.

There are two methods that the perioperative team utilise to protect the spinal cord from ischaemia: increasing the spinal cord perfusion pressure and drainage of CSF using a spinal drain. The procedure for the insertion of a spinal drain is not devoid of minor and major complications. Drains are usually placed using anatomical landmarks by anaesthesiologists before surgery. The procedure can be technically challenging, often leading to multiple insertion attempts. Fluorescent image-guided spinal drain insertion is an alternative to the conventional blind technique used by anaesthesiologists. It improves the accuracy of correct spinal drain insertion, reducing the potential development of post-surgery complications.

Dr. Awad and his team believe that because of the advent of these endovascular therapies, the overall numbers of patients presenting with thoracoabdominal aortic aneurysm who are now being offered therapy are increasing, potentially increasing the numbers of patients with paralysis who are being seen in health systems. This demands urgency of a solution to the issue of ischaemic spinal cord injury and paralysis in the setting of aortic aneurysm surgery. Dr. Awad and his team have made significant advances towards the understanding of this complex problem that is a major complication in these patients, contributing to both increased costs, significant patient suffering and loss of life.

Future Directions and Biobanking

The team hopes to establish a multicentre blood and cerebrospinal fluid biobank that stores biological samples from patients who have undergone aortic aneurysm surgery in North America and potentially globally, to ascertain what cellular and molecular components are altered in humans that might lead to ischaemic injury of the spinal cord. The human samples will be used to guide the preclinical work performed in animal models and contribute to understanding of the cellular and molecular mechanisms behind ischaemic spinal cord injury and paralysis in the surgical setting. The long-term goal for Dr. Awad's team is to eliminate the onset of paralysis after aortic aneurysm and dissection surgery. Dr. Awad established and personally funded a university fund to achieve the goal. He hopes that additional donations can help fund the research and the recruitment of skilled personnel needed to understand and resolve the problem of ischaemic injury of the spinal cord after surgery of the aorta.

Meet the researcher

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Dr. Hamdy Awad received his medical degree in 1983 at the Zagaziz University, Egypt and specialised in anaesthesiology in 1987 at the same institution. He moved to the USA in the early 1990s and obtained a research fellowship in anaesthesiology at the University of California, San Diego. He then completed his residency training at the University of Texas Southwestern before completing a fellowship in cardiothoracic anaesthesiology at the Cleveland Clinic. In 1998, Dr. Awad moved to The Ohio State University where he is now a tenured associate professor in the Department of Anesthesiology. He has been actively involved in teaching medical students, anaesthesiology residents and cardiothoracic fellows throughout his career. Dr. Awad is also an extremely active clinical researcher and currently holds three USA patents. He has published extensively in prestigious journals and books, reflecting his deeply rooted commitment to developing methods to prevent spinal cord injury following aortic aneurysm repair.

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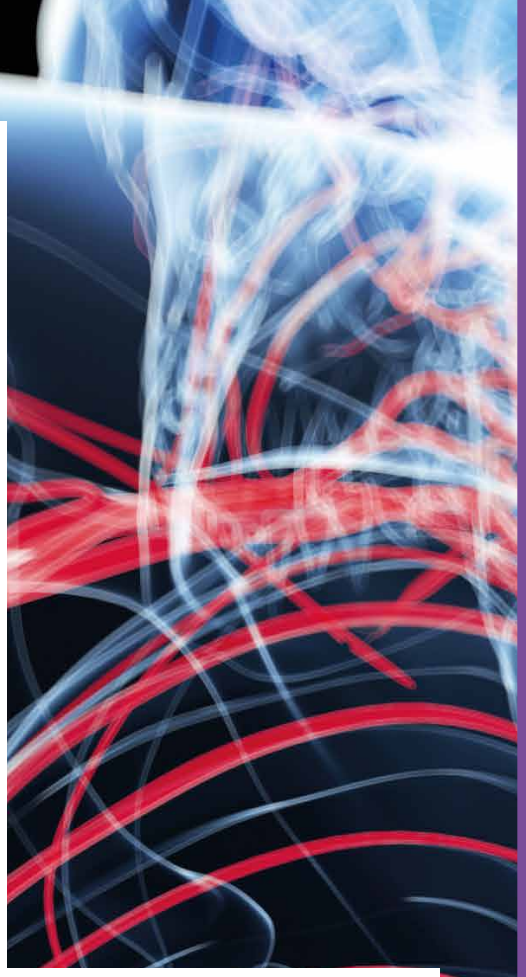
SPINAL CORD INJURY AND RECOVERY IN RATS: INFORMING HUMAN REHABILITATION

Professor David Magnuson, at the University of Louisville, Kentucky, describes himself as 'a CPG guy' and occasionally, more informally as 'a rat guy!' His work on the function of the central pattern generator (CPG) in the rat spinal cord following spinal cord injury, has produced both surprising and thought-provoking results. This research may ultimately challenge the established clinical beliefs and practices around the ways to best rehabilitate human patients with severe spinal cord injury.



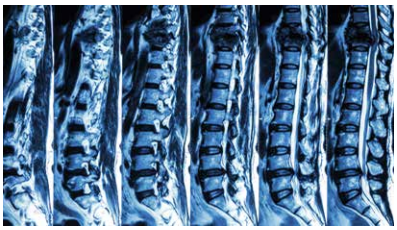
The Impact of Spinal Cord Injury

Severe spinal cord injury (SCI) has dramatic life-changing implications, usually manifested as paralysis below the level of the spinal injury. The full or partial loss of motor or sensory function in the lower extremities is known as paraplegia (from the Ionic Greek 'half-stricken'). The severity of SCI has been quantified by the American Spinal Injury Association, such that ASI Grade A represents the complete loss of sensory function and motor skills below the injury. This ranges to ASI Grade D, where more than half of the muscles below the level of injury can move against gravity.

The rehabilitation of SCI patients is often difficult and challenging for both the patient and those around them. Patients with severe SCI often become reliant on wheelchairs for their mobility, resulting in a lack of active loading and/or weight-bearing in the limbs, and this can lead to muscle wasting and chronic changes in the joints and their supporting structures.

At the same time, neurobiological changes within the spinal cord below the level of injury can lead to the development of spasticity and hypertonia, conditions in which muscles contract uncontrollably, develop tightness and can remain shortened (a resistance to stretching), which affects movement. Over time, this can result in contractures, the permanent tightening and stiffness of muscles, tendons, ligaments, or skin, which can dramatically decrease their range of movement. Critically, hypertonia and contractures/decreased range of motion can dramatically impede the activities of daily life for patients. Even simple things like sitting up in bed to dress, transferring from the bed to wheelchair, wheelchair to toilet, or wheelchair to car, become difficult if, for example, the range of motion at the hip decreases.

Physiotherapy and stretching remain one of the foremost treatments to prevent and treat muscle contractures and spasticity. Therapeutic stretching is known to be effective at reducing contractures following cast-immobilisation and is an essential component of flexibility training for healthy human subjects to improve movement range.

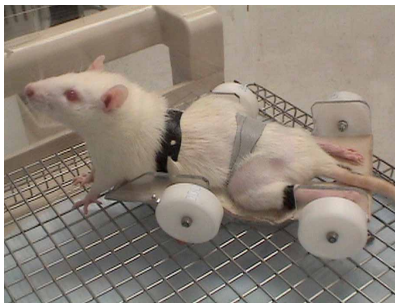


The Importance of Central Pattern Generators

Professor David Magnuson at the University of Louisville, Kentucky, runs an active research laboratory with five PhD students, a technician and laboratory manager, and several bioengineering co-op students. Although originally trained in neuropharmacology and neurophysiology, Professor Magnuson became interested in spinal cord injury after becoming good friends with a colleague living with SCI. He has come to believe that the spinal cord 'has incredible computing power.'

Over the years, Professor Magnuson has focussed his research on the circuitry of the spinal cord and specifically on central pattern generators (CPGs), which are small and autonomous

‘We are not currently maximising the function of existing tissue [remaining in the spinal cord] present after injury.’



neural networks. CPGs create tightly-coupled patterns of neural activity that control rhythmic and routine locomotor behaviours such as walking, swimming, chewing and breathing, without constant input from the higher brain centres. Although acting subconsciously from the brain, CPGs are constantly modulated by afferent stimuli (neural activity being sent to the central nervous system and brain) from sensory neurons in the tissues (e.g., skin, joints and muscles), which respond to changes in the immediate environment. Effectively, the brain instructs the spinal cord to ‘act’, and the Spinal Cord then initiates ‘how’ to implement the action via the CPGs.

Professor Magnuson works with rats because they have demonstrated a strong ability to recover well after SCI rehabilitation, in ways not typically seen in humans. It was noticed that even in rats with severe SCI – that is, less than 10% spared white matter (communication across the injury) – locomotor movements could be rapidly recovered. For the past decade, Professor Magnuson’s laboratory has worked to understand why this is the case.

In 2007–08, during experiments with rats with severe SCI, Professor Magnuson’s team were looking at the rats’ response to rehabilitation. When assessing the rats at 1-week post-SCI, they were placed on the ground and were observed to be effectively paraplegic and dragged their hindlimbs. However, the team were amazed to see that when the rats were placed in two inches of water (which provided 60% body weight support), they actively moved their hind limbs in a stepping motion. This ‘instant movement’ had not required weeks of rehabilitation or retraining of the body. Professor Magnuson stated that ‘it absolutely blew my mind, and in the Magnuson laboratory changed how we viewed this period of retraining [for SCIs].’

The implications were clear. Even after severe SCI, the rats retained sufficient spinal cord circuitry to control movement, independently of the brain. The team tested a shallow water training strategy for rehabilitation, but although they noted rats were more active when group-trained in shallow water, the activity did not improve over-ground stepping. This led the team to conclude that after SCI, the CPG was still working, although the rats did not have the ability for load-bearing and force (propulsion) generation.

In further experiments, it was noted that if shallow water training was delayed until 9 weeks after SCI, untrained rats could not step as well as trained rats in shallow water, suggesting there is a ‘window of opportunity for the CPG’ after which the spinal cord’s plasticity (the ability of the spinal cord and brain to continuously change and adapt after environmental transition or injury) was reduced. The team still wants to know why this occurred.

Rat Rehabilitation Leads to Unexpected Outcomes

In further experiments to test activity-based rehabilitation after SCI, rats were placed in 'wheelchairs' (four-wheeled trolleys) which enabled the rats to pull themselves around the cage with their forelimbs but immobilised their hind limbs. They found that being in a wheelchair worsened recovery and created a number of new problems in the rats, including pressure sores and a loss in the range of movement due to contracture. These are common problems in people that are rarely seen in rats. In an attempt to counter this and mimic human therapy, the rats then underwent stretching therapy. Despite its almost universal use for all patients with spinal cord injuries, stretching has not been studied systematically in animals and not widely studied in human patients.

The stretching protocol had two unexpected and surprising outcomes. Contractures were not prevented by the therapy in rats, and most significantly, the therapy caused a decrease in locomotor function. It should be noted that this decrease was not caused by damage to the muscles. The mechanisms behind this phenomenon are now beginning to be understood through the recent research coming out of Professor Magnuson's laboratory.

The team postulated that 'maladaptive plasticity' may, to some degree, explain their observations. Maladapted plasticity relates to adaptations or changes to the neural system in response to inappropriate afferent signals which result in detrimental outcomes. Professor Magnuson discovered that the stretching protocol in rats stimulated the pain receptors (nociceptors), sensory nerve endings that respond to potentially damaging stimuli (heat, pressure, and so on), creating the sensation of pain if the brain deems the threat as credible. Following SCI, stretching stimulated the nociceptors and, in the absence of brain regulation, appeared to enhance maladaptive sprouting and the impact of C-fibre activation on the CPG circuitry.

To prove that it was the C-fibres that were disrupting locomotive function, the team injected capsaicin into 2-day old rats, which then grew up without the class of C-fibres that sense thermal and chemical pain. Following SCI and stretch therapy, these rats retained much more of their locomotor function. Interestingly, other researchers have also identified C-Fibres as playing a role in neuropathic pain syndromes that are common following SCIs. Maladaptive plasticity of the C-Fibre circuitry, in this case, resulted in the patient experiencing intense pain in response to innocuous stimuli such as a light touch, due to changes in their nerve activation threshold.

Implications for Human Recovery

In his unwavering belief in the 'computational power' of the spinal cord, Professor Magnuson's research has led him to conclude that 'we are not currently maximising the function of existing tissue [remaining in the spinal cord] present after injury.' There are both positive and negative implications of this.

The improvements seen in rat models suggest that there are great opportunities for rehabilitation and mobility, even in severe cases of SCI. However, the findings also challenge the legitimacy of current therapeutic stretching therapy and will require very early intervention (at 1-week post-SCI in rats), which will be a challenge for the treatment of human patients.

A crucial aspect to Professor Magnuson's argument is that activity, particularly in the early stages post-injury (the 'window of opportunity') is a critical factor in maintaining CPG function (as shown by the shallow-water stepping experiment). Conversely, sustained inactivity, through the immobilisation of the limbs (demonstrated by the rat wheelchair) inhibited future locomotor function. Added to this, stretching therapy applied to combat the effects of immobilisation appear to worsen outcomes by enhancing maladaptive plasticity.

Professor Magnuson is clear to explain that, to date, 'we don't yet have the evidence that what is true in rats works in humans'. The application of the findings in a human model holds many practical and ethical challenges. Rats, for example, are active 2 to 4 days post-SCI, but humans clearly require much longer periods of recovery, limiting opportunities for the beneficial 'immediate activity' which appears to be so helpful in rat rehabilitation. The challenge in human treatment will be utilising and maintaining the unforeseen capabilities of the CPG in a clinically acceptable way.

There are important potential avenues now to explore. Professor Magnuson believes that a greater understanding of the CPG circuitry will ultimately help to develop appropriate movement therapy that creates positive 'adaptive' plasticity during rehabilitation. Excitingly, it may even be possible to 'reset' the CPG system to re-open the window of opportunity for retraining the CPG at a later, chronic stage of recovery, significantly opening up the potential for successful intervention.

Meet the researcher

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Professor David Magnuson graduated with a PhD in Neuroscience in 1988 from the University of British Colombia. He then completed a postdoctoral research fellowship in pharmacology at University College, London between 1988–1990, and a postdoctoral research fellowship in physiology at the University of Ottawa between 1990–1992. Between 1992–1995, he was an Assistant Professor in the Department of Physiology, University of Manitoba, and then moved to his present position at the University of Louisville, Kentucky. Between 2000–2010 he was an Associate Professor in the Departments of Neurological Surgery, Anatomical Sciences and Neurobiology, before taking up his current position as Professor, holding an Endowed Chair in Neurological Surgery. Over the past years, Professor Magnuson's research has focused on spinal cord circuitry, the central pattern generator for locomotion, activity-based rehabilitation and cardiovascular/vascular function in rat models of spinal cord injury. He has published extensively in this field.

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INNOVATIONS IN FUNCTIONAL BRAIN IMAGING TO IMPROVE NEUROSURGERY

Dr Jun Hua, Associate Professor at the F. M. Kirby Research Center for Functional Brain Imaging at the Kennedy Krieger Institute and Johns Hopkins University, USA, leads a team focused on developing novel magnetic resonance imaging (MRI) technologies for imaging the structure and function of the brain. Recently, they have been pioneering the development of new MRI techniques that can be used to improve pre-surgical planning for neurological patients and optimise patient outcomes.

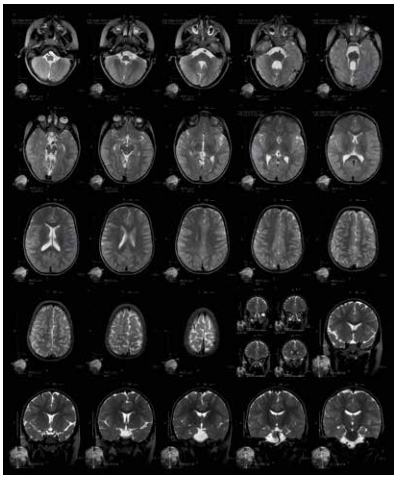
MRI scanning: Looking Inside the Body

Magnetic resonance imaging (MRI) is a powerful imaging technique that has revolutionised the diagnosis and treatment of neurological disease. MRI uses strong magnetic fields and radio waves to precisely image the structures inside the body. MRI scans can distinguish between different types of tissue in the body and are generally used to screen for or monitor soft tissue abnormalities, including tumours, soft tissue injuries, joint injuries, spinal injuries, or damage to organs.

Recent years have seen the introduction of functional magnetic resonance imaging, or functional MRI (fMRI), a technique that allows imaging of the activity of the brain through detecting changes in blood oxygenation and flow. fMRI scans can identify which parts of the brain are associated with different aspects of neurological function such as language, sensory information, and motor function.

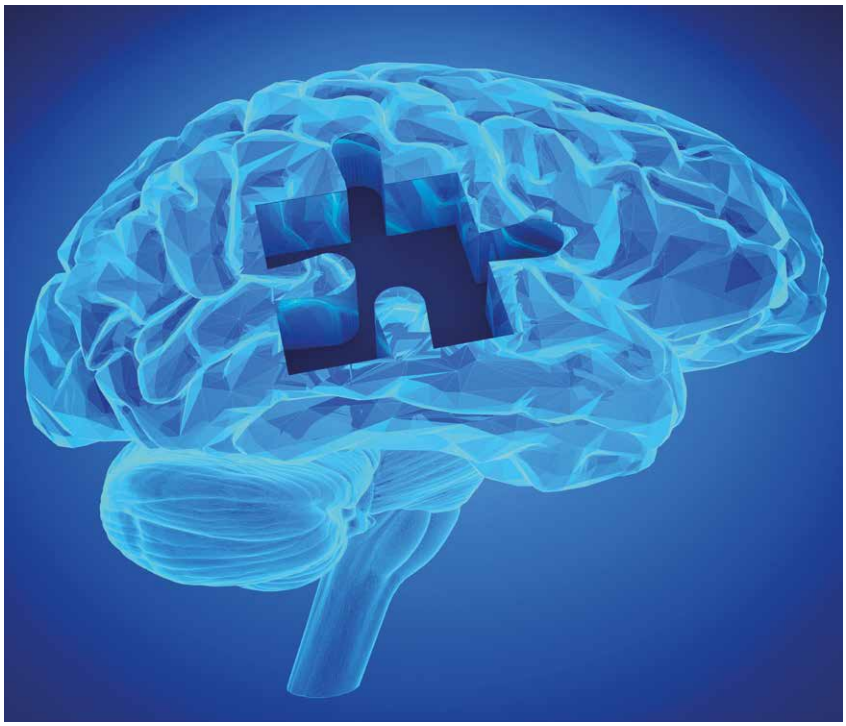
Dr Jun Hua, Associate Professor at the F. M. Kirby Research Center for Functional Brain Imaging at the Kennedy Krieger Institute and the Department of Radiology at Johns Hopkins University, USA, leads a team focused on the development of novel MRI technologies for imaging the structure and function of the brain. His team works on a number of different MRI-based projects, including the development of novel MRI techniques for imaging the microvasculature and metabolism in the brain.

Current work by Dr Hua and the team is focused on understanding the physiological causes of functional MRI signals in healthy individuals and in the brains of patients with neurological conditions such as Huntington's disease, Alzheimer's disease, Parkinson's disease, schizophrenia, and brain tumours. One of the problems that Dr Hua's group has begun to address is how to provide a better imaging tool to aid surgeons during their pre-operative planning.



Blood Oxygen Level-dependent Imaging and the Brain

When surgeons operate to remove a brain tumour, they have to make careful decisions about what tissue to remove. Remove too little, and there is a chance that cancer cells could remain in the brain and regrow. Remove too much, and there is a risk of removing healthy brain tissue and causing loss of brain function in the patient.



Some types of brain tumour, such as glioma, are extremely invasive, and it is sometimes impossible to completely remove it entirely. A balance must be made in excising the maximum amount of tumour while limiting damage to the healthy tissue in order to best prolong and improve the quality of the patient's life.

In order to make these decisions, surgeons are increasingly taking advantage of the information that can be obtained from functional MRI scans. Blood oxygen level-dependent (BOLD) functional magnetic resonance (fMRI) can be used to map the brain pre-operatively to identify which parts of the brain correspond to areas of critical function.

To determine which areas of the brain correspond to which critical functions, patients can be scanned while performing simple tasks that use different parts of the brain. For example, a task such as hand-squeezing will use the part of the patient's brain associated with motor control, and activity in this area will be detectable on the fMRI scans.

However, this approach is not without challenges. Signal dropouts and distortions are well-known issues with current fMRI scanning methods and are a hurdle to using these imaging methods for assisting with surgery. The most often-used method, gradient-echo (GRE) echo planar imaging (EPI), is associated with well-described image distortions. These often occur at areas close to air cavities, including parts of the brain known as the orbitofrontal and temporal lobes. As these areas are associated with language and critical cognitive functions, they are critical areas that need clear imaging in pre-surgical mapping.

Distortions are particularly bad around cavities caused by previous surgical operations. This causes a problem for surgeons attempting to assess patients who have already had an operation to remove a brain tumour. The area around the initial surgical site is most likely to include tissue that needs excising, and most likely to need precise mapping to ensure that healthy tissue is not removed. Other malformations and surgical features, such as cranial implants, haemorrhage, arteriovenous malformations, and MR-compatible metal head implants can also cause disturbances in the fMRI image.

New and Non-Invasive

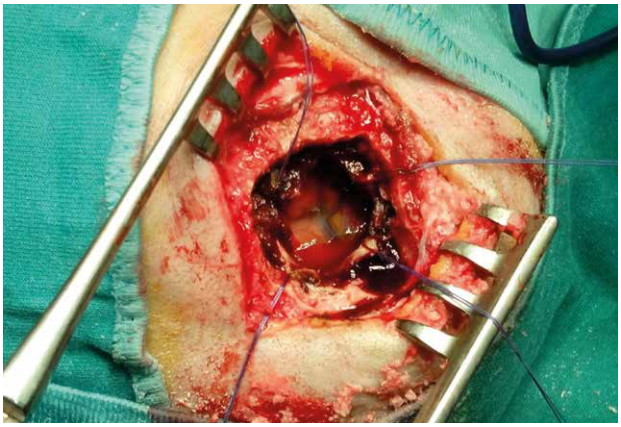
In a 2014 study, Dr Hua and his team demonstrated the utility of a new fMRI technique capable of solving the problem of artefacts in the MRI image. Their study described a newly developed approach, known as T2-prepared (T2prep) BOLD, and showed negligible distortion and signal dropout when used to image the whole brain.

Dr Hua and his group scanned the brains of volunteers undertaking simple tasks designed to use different parts of the brain. These were a visual stimulation task consisting of a blue/yellow flashing checkerboard projected onto the inside of the MRI scanner in front of the volunteer, and a motor task requiring the participant to tap one finger during the flashing period. The team found that their T2prep BOLD technique was able to accurately show areas of activity in the brain with minimal distortions and dropouts.

Having confirmed that they had a more accurate and reliable method for detecting brain activity, Dr Hua and his team then wanted to know if the technique could successfully be used as a pre-operative tool. They conducted a pilot clinical study in four patients with either a brain tumour or a brain lesion caused by epilepsy.

In this study, the group imaged patients using standard imaging techniques such as EPI, and their new T2prep BOLD imaging method. Signal dropouts and distortions were found in the EPI images because of the presence of blood products and air-filled cavities. However, T2prep BOLD imaging produced images with minimal imaging artefacts. The team's method also showed equivalent structural detail to that of existing anatomical brain scans.

The patients also underwent tests of brain function. First, patients undertook a sentence completion test commonly used in pre-surgical language mapping. During the sentence completion test, the patients were scanned with the



existing EPI method, and a T2prep BOLD scan. The team found that in a patient with a tumour very close to an important language area in the brain, activation of this language centre was not detected by EPI imaging whereas activity was visible using T2prep BOLD fMRI. This was also the case for a patient with an epilepsy lesion located in the same area.

These studies demonstrated that the new T2prep BOLD imaging method is capable of mapping brain function extremely close to existing lesions and tumours. This technique could, therefore, constitute a significant step forward in pre-surgery planning, allowing surgeons to determine healthy areas of tissue around brain tumours and then more accurately excise tumours while minimising healthy tissue loss.

Diffusion Tensor Imaging: Visualising Fibre Bundles in the Brain

Diffusion Tensor Imaging (DTI) is a non-invasive imaging method that can visualise the trajectories of fibre bundles in the brain. It provides critical information on the spatial relationship of the fibre tracts to the margins of resectable lesions, which allows neurosurgeons to plan the safest surgical trajectory for lesion resection without damaging eloquent fibre tracts in the vicinity. DTI is frequently used by neurosurgeons together with BOLD fMRI in presurgical brain mapping.

Recently, Dr Hua and his team extended their efforts to the development of novel DTI methods that can provide artefact free images in the presence of metal implants. In a recent study published in Radiology, one of the most prestigious journals in the field, they showed that T2prep BOLD fMRI and diffusion prepared DTI significantly reduced distortion and signal dropout throughout the brain compared to conventional EPI-based methods in healthy individuals wearing metallic orthodontic braces. Thus, diffusion prepared DTI can be applied to presurgical mapping in a way similar to T2prep fMRI, providing an alternative MRI method in the presence of strong susceptibility artefacts.

Next Steps

The current gold-standard method for mapping brain activity is known as electrical stimulation mapping, or ESM. This is performed during an operation to map brain function in the area around a brain lesion. However, this is a highly demanding and invasive technique. While this technique can accurately tell surgeons where language-related brain tissue is located, it requires a longer duration of surgery, runs the risk of inducing seizures, and must be performed in awake patients.

T2prep BOLD imaging technique can therefore not only improve the quality of non-invasive brain activity imaging, but also provide a viable, non-invasive alternative to existing methods. As ESM can also only be used on patients who are awake during their surgery, there is the risk of creating considerable distress, whereas T2prep BOLD can be used on awake or anaesthetised patients, allowing for a much less distressing scanning procedure.

After the success of their initial studies, Dr Hua and the team hope to test their new method in a larger cohort of patients. The next step to validating this technique for clinical use is to compare the T2prep BOLD scan results with those from gold-standard brain mapping techniques such as ESM.

The group believes that once their technique has been validated in a larger patient cohort, the feasibility and usefulness of its use in non-invasive pre-surgical mapping will be significant. This, in turn, will have a positive impact on patient health and surgical outcomes through aiding accurate surgical resections, and also negating the need for distressing and invasive mapping techniques such as ESM.

Finally, the team hopes that T2prep BOLD imaging could also be used for patients with other brain malformations, and plan to recruit patients with malformations that could cause imaging artefacts, including those with surgical cavities, cranial implants, tumours, arteriovenous malformations, internal calcification, and lesions resulting from other causes. If successful in clinical trials, the T2prep BOLD imaging technique could help transform the surgical care of a large number of patients across a wide range of neurological disorders.

Meet the researcher

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Dr Jun Hua received his Master's and Doctoral degrees in biomedical engineering and electrical engineering from Johns Hopkins University. After completing his PhD, Dr Hua embarked on a post-doctoral fellowship in the Department of Radiology at Johns Hopkins, before being promoted to research associate, and then instructor. In 2014, Dr Hua was appointed Assistant Professor at the F.M. Kirby Center for Functional Brain Imaging, where he is now an Associate Professor. Dr Hua's research group is focused on the development of novel MRI technologies that can be used to better image the brain. These technologies include the development of novel MRI methods to measure brain activity, cerebral perfusion, and oxygen metabolism in the healthy brain and in diseases such as Huntington's disease, Alzheimer's disease, Parkinson's disease, schizophrenia, and brain tumours. Dr Hua has been invited to speak at various international conferences. He is an active peer reviewer for a number of prestigious journals and is currently Associate Editor for the journal Neurodegenerative Diseases.

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THE ROLE OF NANOPARTICLES IN NEUROSCIENCE

Dr Elizabeth Nance has an impressive track record. Now a Clare Boothe Luce Assistant Professor of Chemical Engineering at the University of Washington, USA, Dr Nance's work centres around the use of nanoparticles to deliver therapeutic agents to the brain, a seemingly simple operation which is confounded by a highly regulated blood brain barrier which prevents access to the brain and a complex brain environment which prevents access to diseased cells. Her current work also investigates the potential use of nanoparticles to probe tissue environments to map tissue structure, and how tissue structure changes in the presence of a disease.

What Are Nanoparticles?

Dr Elizabeth Nance and her students in the Nance Lab at the University of Washington, USA, integrate a variety of tissue and animal models with imaging, molecular biology, and data science tools to understand changes in the brain in response to injury or disease. One of their main goals is to determine what changes in the brain might impact the ability of a drug to be effective at reaching its target site, and to use engineering to overcome the barriers which stop drugs being effectively delivered to the brain.

One way of enabling drugs to be more effective is through the use of nanotechnology, which involves objects that behave as a whole unit and have at least two dimensions less than 100 nm. The Nance Lab often works with nanoparticles that are spherical in shape and anywhere from 4 nm to 200 nm in size. To put this size scale into context, the thickness of a piece of paper is around 100,000 nm!

Given its small size, a nanoparticle has a relatively large surface area, particularly if it is porous in nature. This surface area, both on the outer surface of the particle and within the particle, provides ample space for a therapeutic agent, such as a drug, to be encapsulated or conjugated. By incorporating a drug into a nanoparticle, its solubility is increased, but it is also protected from degradation or clearance from the body. This allows the drug to circulate around the body for longer, which can improve the bioavailability and effectiveness of the drug.

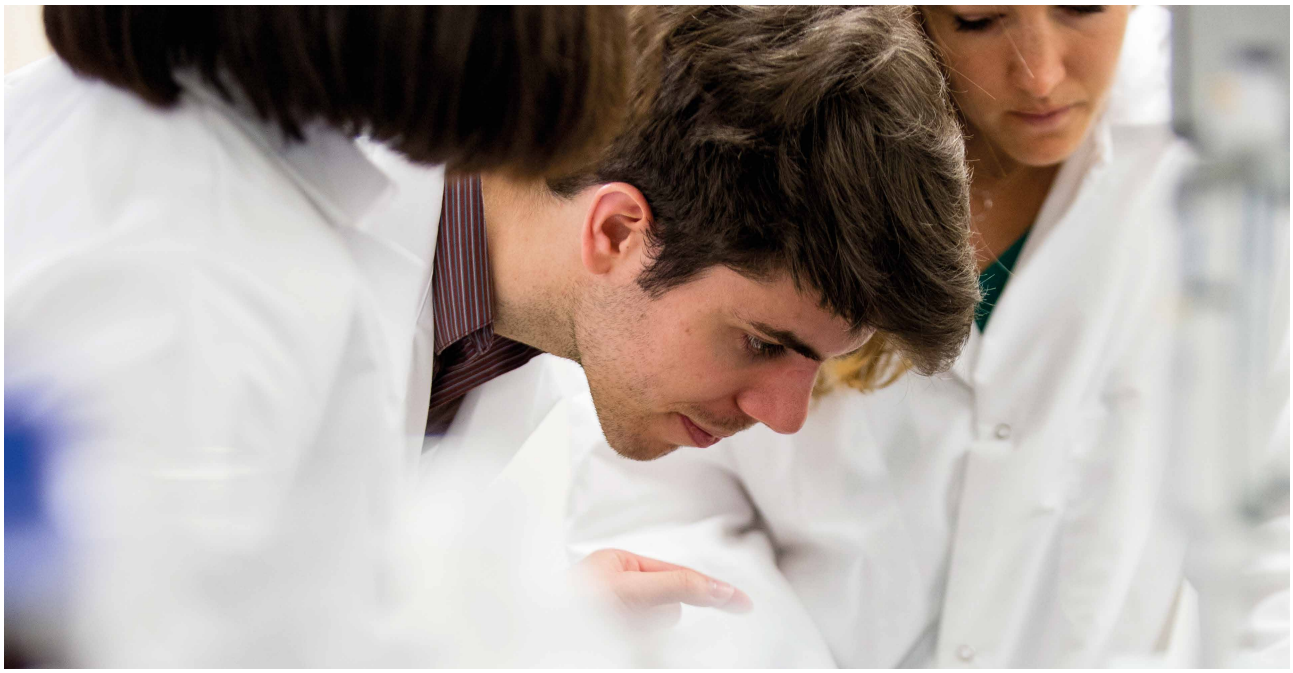
A nanoparticle can also facilitate delivery into a target organ or target cell in a passive manner. For example, adjusting the size and molecular weight of the delivery system containing the drug will alter where in the body the nanoparticles accumulate, as well as the tissue that they are able access.

Nanoparticles can also act in an active manner; by putting specific molecules or surface coatings on a particle, it is more likely to interact with the corresponding receptor on the surface of a target cell or induce interactions with a specific type of cell.

Nanoparticles and Disease

So far, Dr Nance and her team have utilised a wide variety of nanoparticles, including polymer, quantum dot, and dendrimer-based platforms, and have studied their behaviour in the brain, as well as how their behaviour changes in the presence of ongoing injury or disease. However, translating nanotechnology from the laboratory stage to the point at which it can be used as a clinical treatment has proven difficult for the nanotechnology field.

‘With a systems-level approach, there is great potential to leverage resources across the multiple fields working to understand and treat neurological diseases.’



Therefore, Dr Nance and colleagues suggest that a systems-level approach is essential. Dr Nance explains, ‘With a systems-level approach, there is great potential to leverage resources across the multiple fields working to understand and treat neurological diseases.’ This involves collecting information from the field which is associated with understanding brain diseases, and applying that information using technologies in a clinically relevant way. The physiological barriers to drug delivery in the brain must therefore be considered, along with the effects of disease on these barriers, and how nanoparticle technology interacts within the context of the disease in the brain.

Leading on from the hypothesis that tandem evaluation of the disease and the nanotechnology is crucial, Dr Nance’s team found that nanoparticles are very sensitive to the environment and, furthermore, that they behave differently even when small changes occur in this environment. For example, calcium, which is used in signalling pathways between nerve cells, can increase after injury when cells die and release their calcium stores, and this can cause nanoparticles to clump together and become immobilised. This clumping renders the nanoparticles incapable of delivering their therapeutic load. Alternatively, specific cells within the brain become more active due to inflammatory responses caused by disease. Due to their increased activity, these cells may respond to inflammation by readily taking up an increased number of nanoparticles, making them useful targets for drugs that could be used to treat the inflammation.

The Nance Lab has used observations such as these to design nanoparticle-based therapies that can successfully target specific regions and cells within the brain. These therapies have been tested in a variety of disease models which represent aspects of brain diseases that affect new-borns and children, including cerebral palsy, autism, Rett Syndrome, and neonatal brain injury.

Curcumin and Brain Injury in Infants

Hypoxic-ischaemic encephalopathy is a type of brain damage that occurs when an infant’s brain is starved of oxygen and blood flow around the time of birth. It causes permanent brain damage and is one of the top causes of disability-adjusted life years, but there is no known cure. Currently, therapeutic hypothermia is used to treat the disorder and has been shown to improve outcomes in infants starved of oxygen. However, there is still a high level (40–50%) risk of death or disability of those affected, and it is therefore crucial to develop more effective interventions to reduce morbidity and mortality.

One of the main contributing factors to hypoxic-ischemic encephalopathy is inflammation. Therefore, a therapeutic target aim to decrease the dangerous inflammatory responses in the brain. One example of a drug that has been studied in adults is curcumin. Curcumin is a dietary compound (the active component of turmeric) which has been suggested to have anti-inflammatory effects. Dr Nance hypothesised that

‘One of our main goals is to determine what changes in the brain might impact the ability of a drug to be effective at reaching its target site, and engineering to overcome the barriers to effective therapeutic delivery.’

packaging curcumin into nanoparticles 60 nm in diameter and made from a degradable polymer material consisting of repeat units of lactic and glycolic acid may allow effective drug delivery to the brain.

The group at the University of Washington tested this prediction using a rat model of hypoxic-ischemic encephalopathy. They discovered that the curcumin-containing nanoparticles were able to overcome the blood-brain-barrier, which is normally a significant challenge when targeting the brain as substances are unable to cross it. Nanoparticle uptake in the injured brain was observed within 24 hours of administration. They noted that the nanoparticles were localised in the regions of injury. They also reported protective effects of the therapy in the injured brain. Indeed, previous studies have shown that PEG can suppress detrimental free radical production following injury.

The next steps in using nanoparticles to treat brain injury in new-borns will be to evaluate the possible resolution of brain injury over an extended period of time, allowing the long-term impacts of the therapy to be investigated. Dr Nance has identified more promising drug candidates than curcumin, and hopes to explore the mechanism of action of these drugs in new-born brain injury, as well as optimise the dose required for neuroprotection. Her ultimate aim is to investigate the opportunities for the clinical translation of this work.

Other Uses of Nanoparticle Technology

In addition to drug delivery, Dr Nance aims to harness the potential of nanoparticles in other ways. She believes that the diffusion of nanoparticles throughout the brain may present an opportunity to probe and model structural changes, which can be connected to functional aspects of the brain. The precise links between brain structure and function are still being explored, and the behaviour of nanoparticle probes, combined with biological data, could uncover important information about microscopic changes in the brain, that go awry in disease processes.

The project will use excised living brain tissue to validate the approach and to optimise computational and statistical analyses. Dr Nance will then move on to a detailed analysis of nanoparticle diffusion through different microstructures in the brain, for example by studying how nanoparticles behave in the ‘loosely’ associated proteins in the tissue space around all cells verses the ‘structured’ protein areas surrounding specific neurons that enable brain development. Eventually, nanoparticle diffusion data will be collected in diseased tissue and fed into machine learning algorithms with the goal to predict functional level outcomes based on diffusion data from different disease states.

The Future of Nanoparticles

In consideration of future plans, Dr Nance notes ‘One of our main goals is to determine what changes in the brain might impact the ability of a drug to be effective at reaching its target site, and engineering to overcome the barriers to effective therapeutic delivery.’

The long-term goal of the Nance Lab is to transform the way researchers combine data from different fields, for example using machine learning and data science tools to mine advanced biological imaging data. Use of these techniques can also be extrapolated to other organs in the body, opening the door for potential novel therapeutic approaches. This systems-level approach must be dynamic and adaptable, to cope with new understanding and the evolution of the neuroscience and nanotechnology fields.

Finally, in addition to her significant research contributions to neuroscience and nanotechnology, Dr Nance has been working to improve the diversity of the scientific workforce. In 2016, she founded the organisation ‘Women in Chemical Engineering’ at the University of Washington, which now has an additional chapter at the University of Virginia, USA. This organisation wants to change the ways in which women in science, technology, engineering, and mathematics are perceived, and aims to pave the way for future generations of female scientists.

Meet the researcher

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Dr Elizabeth Nance obtained her PhD in Chemical and Biomolecular Engineering from John Hopkins University in 2012. She went on to complete a postdoctoral research position at John Hopkins School of Medicine before joining the University of Washington (UW) in 2015 as the Clare Boothe Luce Assistant Professor of Chemical Engineering, with an adjunct appointment in Radiology. In addition to an extensive list of publications and talks (including as a TEDx invited speaker), she was named the 2018 European Union Horizons 2020 Training Network Inspiring Young Scientist in Nanomedicine, a 2018 Young Innovator in Nanobiotechnology, and was named in the Forbes 30 under 30 in Science in 2015. Dr Nance's research spans several disciplines including engineering, neuroscience, and translational medicine, but she is perhaps best known for her work on nanoparticle applications in the brain, and for founding the Women in Chemical Engineering organisation which aims to educate, empower, and advocate for women chemical engineers.

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WChE

CLEARING THE HAZE: UNDERSTANDING THE PROCESS OF SCARRING FOLLOWING CORNEAL INJURY

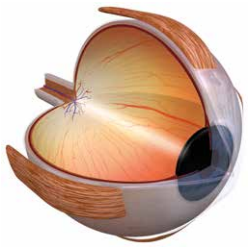
Any injury such as trauma, surgery or infection to the cornea in the eye may result in persistent scarring (clinically referred to as fibrosis) due to the wound healing response. **Professor Steven E. Wilson** at the Cole Eye Institute of the Cleveland Clinic Foundation has identified that defective epithelial basement membrane (EBM) regeneration plays a central role in the development of scar producing myofibroblast cells. Critically, Professor Wilson suggests that the pathophysiological consequences of defective EBM regeneration are also likely to have wider relevance to the fibrosis that occurs in other organs, such as the lungs, heart, kidneys, and skin.

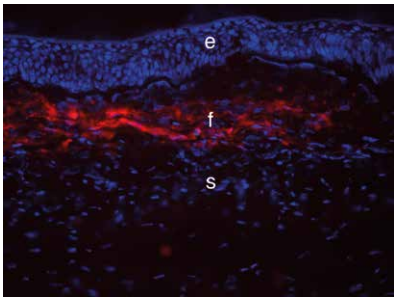
Three Decades of Corneal Research

The cornea is the transparent outer surface of the eye and lies directly in front of the iris and pupil. It acts as a protective structure and is integral to the refraction power of the eye and the precise focusing of light on the retina. Maintaining the cornea's integrity and health is essential if the eye is to function effectively. Unfortunately, trauma and other damage to the cornea can commonly result in fibrosis (also referred to as haze or scarring) that leads to visual impairment caused by the loss of corneal transparency.

Professor Steven E. Wilson at the Cole Eye Institute at the Cleveland Clinic Foundation has spent more than three decades at the frontier of research exploring how injury and the defective regeneration of the cornea's basement membranes may lead to the development of scar-producing cells called myofibroblasts.

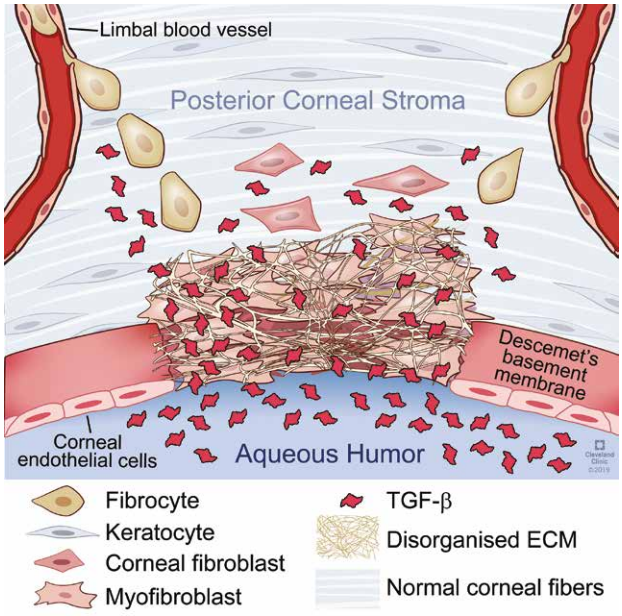
The cornea provides an ideal laboratory model for which to research the mechanisms of fibrosis both within the eye and in other organs due to its easy accessibility and simple physical structure. It comprises a number of layers, including the epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium. The corneal epithelium is an extremely thin, multicellular tissue layer of continually regenerating cells. Beneath the epithelium, the epithelial basement membrane is a highly organised layer of perlecan, nidogen-1, nidogen-2, laminins 511, 521 and 332, and collagen type IV, that attaches the epithelium to the underlying stroma and performs many other critical roles in communications between the epithelium and stroma. The stroma is the thick, transparent, middle layer of the cornea, made up of a regular array of collagen fibres, interwoven with a sparse distribution of keratocytes (resident stromal fibroblastic cells), which





Fibrosis in the rabbit cornea. Credit Steven Wilson.

secrete a highly regular and organised extracellular matrix that includes collagen type I and proteoglycans. Keratocytes also produce large amounts of intracellular crystalline proteins that increase the transparency of the cells.



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Professor Wilson's research has focused specifically on the critical role that keratocytes play in epithelial basement membrane (EBM) repair and function and, in particular, the factors that lead keratocytes and other bone marrow-derived progenitor cells (fibrocytes) to develop into fibrosis-producing myofibroblasts. He is also interested in how keratocytes interact with corneal endothelial cells (that line the posterior surface of the cornea) in the production and maintenance of Descemet's basement membrane — to which endothelial cells adhere. It is these two critical basement membranes that Professor Wilson has identified as determining whether defective tissue regeneration occurs after injury to the cornea, leading to fibrosis.

Defective Epithelial Basement Membrane Regeneration and Myofibroblast Development

In nearly all tissues and organs, fibrosis occurs through the production of myofibroblasts formed from differentiated fibroblast precursors. Critically, these fibroblastic cells are opaque in nature, and they produce a disorganised extracellular matrix within the stroma that disrupts the regular organisation and distribution of collagen fibres that are essential to maintain the transparency of the cornea critical for vision.

In their research, Professor Wilson and his team have pursued the hypothesis that following severe injury, myofibroblast development in the cornea is integrally linked to the defective regeneration of the EBM. Their research has shown that by the timely restoration of the structural and functional integrity of the EBM, fibrosis can be prevented, and corneal transparency maintained. However, delay in the regeneration of the EBM leads to the development of stromal myofibroblasts and fibrosis.

Thus, the corneal EBM plays a critical function and is sandwiched between the basal epithelium and underlying connective tissue of the stroma. Primarily made up of collagen, laminins, the heparin sulfate proteoglycan perlecan and other proteins, it forms a three-dimensional extracellular matrix that acts to maintain the cell's structural form, but also, importantly, acts to regulate the two-way communications between epithelial and keratocyte cells, and the bone marrow-derived cells that invade the cornea after injuries.

One function that appears critical in cellular wound healing and homeostasis (maintaining a relatively stable equilibrium), is the EBM's ability to control cellular functions by binding and mediating the local concentrations of key cytokines, such as transforming growth factor (TGF) β-1 and TGF β-2 and platelet-derived growth factor (PDGF).

In a healthy cornea, the EBM (or more specifically, components in the basement membranes, such as perlecan, nidogen-1, nidogen-2, and collagen type IV), act as barriers to bind and prevent the passage of TGFβ and PDGF from the epithelium, tears and/or aqueous humour (fluid in the anterior chamber behind the cornea) into the central cornea. However, when the EBM 'barrier' is punctured, through injury or trauma, large quantities of these cytokines are able to enter the stroma. After penetrating the stroma, the cytokine growth factors activate keratocytes close to the injury, transforming them into corneal fibroblasts, and ultimately into myofibroblasts if the EBM and/or Descemet's basement membrane doesn't regenerate. Part of the fibroblast's normal function is to contribute key localised components to the EBM during this regenerative healing process.

When the corneal injury is minor, such as in the case of an abrasion, corneal fibroblasts in the stroma may then revert back to the keratocyte phenotype as the wound healing response subsides. However, after severe injuries, when EBM regeneration is defective, sustained exposure to TGFβ-1 and TGFβ-2 triggers corneal fibroblasts to differentiate into myofibroblasts.

Professor Wilson has identified that myofibroblasts are also formed in the cornea from bone-marrow-derived fibrocyte precursors. The myofibroblasts then produce high levels of disorganised extracellular matrix – which along with the fibroblasts themselves, create the opacity seen in the corneal stroma of scarred corneas. Regeneration of the basement membrane(s), which may take months or even years, eventually cuts off the supply of TGFβ-1 and TGFβ-2, which in turn, triggers interleukin-1 (IL-1) from adjacent cells or the myofibroblasts themselves to trigger myofibroblast apoptosis (controlled cell death). Keratocytes can then return back to the fibrotic area of the cornea where the disorganised extracellular matrix materials secreted by the myofibroblasts are removed, and transparency of the cornea is restored.

'The corneal model of basement membrane injury and defective regeneration associated with mature myofibroblast development and persistence [...] likely has a role in the development of fibrosis in other organs where epithelial or parenchymal cell injury is linked to disease.'

The Key Role of Keratocytes

Now that the cause of the stromal scarring and resulting haze was apparent, the next challenge for Professor Wilson and his research team is to determine the underlying aetiology of defective EBM regeneration. He is keen to specifically explore whether a deficiency or abnormality of deposition of the EBM components, perlecan, nidogen-1, nidogen-2, laminins 511, 521 and 332, and collagen type IV, lead to defective EBM regeneration, and ultimately scarring fibrosis.

In earlier experiments, Professor Wilson and colleagues had established that the defective regeneration of the EBM probably occurred due to inadequate numbers of keratocytes in the anterior stroma. This followed from their discovery decades ago that anterior stromal keratocytes underwent significant apoptotic death after corneal epithelial injury, leading to the consequential lack of components such as perlecan and nidogen-2, that the keratocytes produce to regenerate the damaged EBM, or anomalous localisation of these components. They found that in the case of more extensive stromal injury, large numbers of mature myofibroblasts are then formed, which, as we have described, secrete a disordered extracellular matrix that forms a physical tissue barrier. Surviving keratocytes, which undergo mitosis to re-establish their numbers, are thus blocked from getting into close enough proximity to the newly forming EBM to supply and/or organise their essential components and the healing processes are delayed.

Following a minor injury to the EBM, a layer of self-polymerising laminin 511/521 is laid down by the epithelium to begin the regenerative process. However, complete regeneration of the EBM also requires adjoining keratocytes to contribute perlecan, nidogen-1, nidogen-ß2 and perhaps other components to rebuild the lamina lucida and lamina densa layers, which together form the mature EBM that is needed to modulate pro-fibrotic growth factors such as TGFb and PDGF.

Extensive corneal damage, therefore, results in both defective EBM regeneration and the formation and accumulation of myofibroblasts and the disordered extracellular matrix that they produce, and this can persist for many months or even years. A similar process occurs in the posterior cornea if the Descemet's basement membrane is also injured. Eventually, in most scarred corneas, after the injury is eliminated for a long period, recovery begins with small areas of clearing called 'lacunae' that appear in the stromal fibrosis. In these clear areas, normal keratocytes have repopulated the anterior stroma and facilitated the regeneration of mature EBM.

Ultimately, this process facilitates the apoptosis of the underlying myofibroblasts, that will be increasingly deprived of adequate levels of TGFβ and PDGF they need to survive. Over time, the lacunae tend to coalesce and enlarge, as more surrounding EBM regenerate, eventually leading to the restoration of full corneal transparency. In the case of Descemet's basement membrane injuries, little capacity for regeneration of this structure has been noted and corneal transplantation is typically needed to restore transparency.

Keratocyte Apoptosis: A Critical Finding

Professor Wilson's discovery that keratocyte apoptosis occurs in the anterior stroma when there is an injury to the overlying epithelium was a major breakthrough. Prior to his discovery in 1994, keratocytes were thought to be relatively dormant and inactive cells. However, Professor Wilson noted that injury led to the anterior stroma becoming decellularised and that the stroma often did not return to normal cell density as quickly as might be expected after the injury. He argued that keratocytes need to actively migrate within the anterior stroma to repopulate the area and that they need to proliferate in order to restore normal stromal keratocyte density and contribute to healthy EBM regeneration.

The Wider Application of the Corneal Model

The research of Professor Wilson and his team has far-reaching potential beyond having achieved a better understanding of corneal healing. The physiological changes that accompany fibrosis in many organs, such as the lungs, kidneys, and skin, are likely to have many similarities to that identified in corneal injury. Professor Wilson hypothesises that 'The corneal model of basement membrane injury and defective regeneration associated with mature myofibroblast development and persistence, and attributable, at least in some diseases, to insufficient fibroblastic/mesenchymal contributions of EBM components, likely has a role in the development of fibrosis in other organs where epithelial or parenchymal cell injury is linked to disease.' For example, chronic alveolar epithelial cell damage caused by toxins, such as smoke, silica dust or heavy metal dust, could lead to injury and defective regeneration of the underlying basement membrane, anomalous TGFß modulation, myofibroblast development and fibrosis, that parallels the events in corneal fibrosis.

It is, therefore, clear that a greater focus on BM injury and defective BM regeneration in other organs could provide important insights into our greater understanding of the pathophysiology of many fibrotic diseases. Importantly, when a corneal injury is interrupted, the EBM can repair itself and resolve fibrosis. It, therefore, seems probable the fibrosis can be similarly prevented or lessened in other organs, by promoting basement membrane repair.

Meet the researcher

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Professor Steven E. Wilson is the Director of Corneal Research at the Cleveland Clinic Foundation and Professor of Ophthalmology at the Cleveland Clinic School of Medicine, Cleveland, Ohio — posts he has held since 2003. In a widely acclaimed career spanning five decades as a physician and scientist, Professor Wilson is established as a world leader in corneal research. After achieving his MD at the University of California in 1984, he took up an Ophthalmology residency at the Mayo Clinic, Minnesota (1985–88) and then a Fellowship at the LSU Eye Center, New Orleans (1988–1990). He took up a number of professorships between 1990 and 2003, including Professor of Cell Biology, Neurobiology, and Anatomy at the Cleveland Clinic Foundation Health Sciences Center at Ohio State University, and Professor and Chair of the Department of Ophthalmology, University of Washington, Seattle. His most recent honours include the Richard L. Lindstrom Career Award (2013), International Society of Refractive Surgery Recognition Award (2018), and the Barraquer Award from the International Society of Refractive Surgery-American Academy of Ophthalmology (2020).

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FURTHER READING

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Cleveland Clinic

DEVELOPMENT OF NEW ANTI-ROLLBACK DEVICE PROVIDES PAIN RELIEF FOR WHEELCHAIR USERS

It is estimated that in the USA alone, over 2.7 million individuals require a wheelchair for mobility. By far the greatest fraction of manual wheelchair users rely on conventional manual pushrim drive wheelchairs, yet the use of such wheelchairs leads to well-documented shoulder injuries and chronic pain. We read here of how **Steve Green** of Green Technologies, Inc, is tackling this problem through the development and patenting of a wheelchair anti-rollback device that addresses these injury and safety issues with an innovative yet simple mechanism.

Health Challenges Caused by Prolonged Wheelchair Use

Steve Green is a university qualified mechanical engineer and has worked for 40 years in various industries. However, it was a chance invitation to a body builder competition for people with paraplegia that led to his ongoing commitment to the invention of improved devices to allow physically challenged people to better interact with their environments. He explains, 'I am dismayed by the ubiquitous and unnecessary incidence of obstacles to mobility and basic activities of wheelchair users our society allows. As a mechanical engineer and small business entrepreneur, it is my joy and challenge to use my skills and the capabilities of my business to provide systems, devices, and solutions where possible.'

Wheelchairs in the modern form have been around since at least 1760, when John Joseph Merlin invented pushrim propulsion. The Merlin Chair, as it was called, bears a remarkable resemblance to wheelchairs today, despite the intervening 240 years.

The conventional pushrim operated wheelchair is now the most commonly used assistive care mobility device, due to its simplicity, low cost, and intuitive operation. However, there is a downside to these advantages, in that the kinematic design of manual wheelchairs (often referred to as the 'geometry of motion'), leaves much to be desired.

Prolonged manual wheelchair use can lead to pain, repetitive strain injury and muscular damage, especially in the shoulder joints. The pain experienced tends to increase with the time spent using a wheelchair, despite the considerable improvements in wheelchair technology over the past 15 years. Degenerative changes occur from repeated use of the rotator cuff muscles, which can include wear and tear of the cartilage. The position and the repetitive loading of the shoulder joint for propulsion most likely contribute to these changes, along with muscle imbalance due to the mechanics of wheelchair propulsion.

Example of an early wheelchair. Credit Steve Green.

WARD equipped wheelchair. Credit Steve Green.

'I am dismayed by the ubiquitous and unnecessary incidence of obstacles to mobility and basic activities of wheelchair users our society allows. As a mechanical engineer and small business entrepreneur, it is my joy and challenge to use my skills and the capabilities of my business to provide systems, devices, and solutions where possible.'

Wheelchair user on a ramp. Credit Steve Green.

An obvious problem that faces manual wheelchair users is that the world is not flat. Daily challenges include negotiating pavement curbs, street inclines, and building access ramps. For anyone who has experienced using a wheelchair, even a standard ramp with a 1:8 gradient provides significant difficulty. Only if the person propelling the chair has a healthy, strong upper body, is the challenge manageable.

Ascending a ramp with a conventional pushrim wheelchair requires a rapid 'push and grab' propulsion technique, which results in increased forces being repetitively imposed on the arms and shoulders. At the limit of the push stroke, the user is holding their weight, and that of the wheelchair against the force of gravity and physics, which is trying to make the chair roll backward. To propel further up the ramp, the user must release the pushrim and move their hands backward to a new position and grab the pushrim again. This motion must be accomplished very quickly, as the chair immediately rolls backward when they release their grip.

Many wheelchair users have various compounded diagnoses that impact their upper body coordination, and ability to grip. Whilst a person's strength may be adequate, they may have conditions of spasticity and motor control which prevent accomplishing finely controlled arm and hand movements rapidly. It is these specific challenges that inspired Mr Green to seek a new and innovative solution to these long-standing issues.

The Search for an Anti-Rollback Solution

Mr Green is the President of Green Technologies, Inc, a company which combines engineering capabilities with a complete machine shop, enabling him to offer concept design and prototyping services to a varied clientele. Along with new wheelchairs, he is currently designing apparatus for quicker detection of bacteria in food products, a radically improved archery bow for target shooters, and a water hydraulic servo mechanism for prosthetic fingers.

To address the fundamental flaws he noted in wheelchair design and use, Mr Green set out to find a solution that achieved exceptional utility, was safe to use, low cost, low weight and had a conventional appearance. As a result of his research and testing, the company has designed and patented a prototype wheelchair anti-rollback device (WARD), with a simple innovative mechanism which has selectable anti-rollback and freewheeling modes. The mechanism also allows complete manoeuvrability, including backward propelling, while in anti-rollback mode.

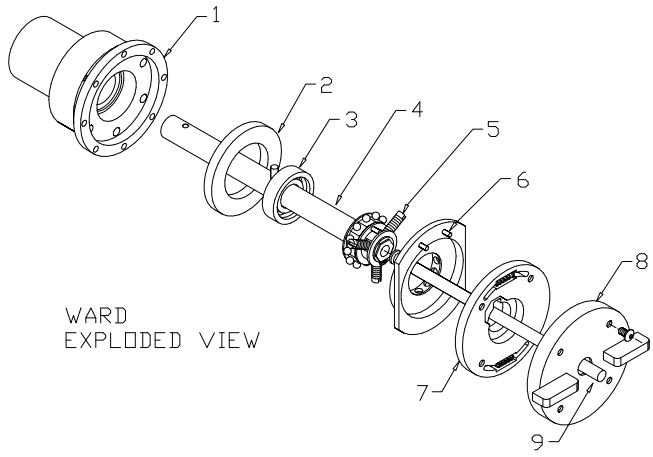
The need for anti-rollback functionality is not new and a search of the United States patent database turns up dozens of patents. The most commonly available solution is a kind of device often called a 'hill holder,' that basically uses a wedge attached to the parking brake lever that jams the wheel to prevent it from rotating backwards. Indeed, many varied anti-rollback mechanisms, wheels, and wheelchairs have been invented, yet none have captured a significant portion of the market, being too expensive or awkward to use.

In comparison testing of some of these devices to identify where improvements could be made, Mr Green determined that a typical 'wedge type' device will add about 20 to 25% to the user's effort to propel the chair forward. The placement of the parking brake lever, which typically actuates hill-holders, often limits the forward push stroke of the handrim.

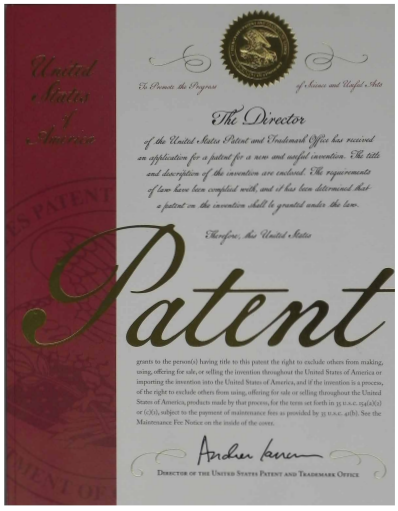
This additional effort while climbing a ramp must be considered in light of the potential user population with weakened strength or stamina. The Wheelchair Anti-Rollback Device (WARD), developed by Green Technologies does not add any extra effort to propelling up ramps, and enables the wheelchair user to rest at any time.

Safety Issues a Concern

A safety issue shared by most anti-rollback mechanisms is that in restraining the propelling wheels from rotating backwards, it increases the potential for a backward rotating 'wheelie' and prevents the user from easily recovering from a wheelie. Clearly, when traversing an incline, the front of the wheelchair is



Credit Steve Green.



higher, increasing the possibility of the wheelchair tipping backwards.

A second common shortcoming of anti-rollback inventions currently available, is that they are very difficult to shift back to 'freewheeling' mode when under the load imposed by an incline. Such a change is required if the manual wheelchair user (MWU) needs to reverse on the ramp before getting to the top, or even change direction to avoid an obstacle. Some hill-holder devices require that the wheelchair get to a level surface to shift, whilst others require that the user let go of the pushrims to operate the wheel lock (parking) brake levers before they can go backwards. If on a ramp, the wheelchair can roll backward, or turn sideways, before the user can grasp the pushrims again, posing a considerable risk to the user.

The inability to shift to neutral under load, and the critical safety issue of wheelie control, has resulted in anti-rollback mechanisms to this point having limited utility and imposing unnecessary risks.

The WARD Concept

The team at Green Technologies is confident that they have simultaneously addressed the safety and utility issues posed by the existing anti-rollback devices. A demonstration of the WARD prototype operating on a ramp can be seen at the Youtube link: http://www.youtube.com/watch?v=LhXtyNhaVww.

There are three core innovative concepts which distinguish the WARD from other available solutions. By incorporating a unique adjustable 'override' clutch in series with an 'anti-rollback' clutch, it allows the user to instantly reverse at any time or under load on a ramp. These clutches operating in concert are the subject of a newly granted US patent. The dual mode anti-rollback clutch can be engaged or disengaged with a simple knob twist. Finally, the unique mode shift compensator spring mechanism that is incorporated into the clutch allows the user to rotate a selector knob from anti-rollback to freewheeling position, even when on a ramp and with the clutch itself locked into anti-rollback position by the load of the wheelchair.

If the MWU wishes to shift out of anti-rollback mode while on a slope, the shift knob will move into the detented freewheeling position, even whilst the clutch components are still 'locked'. Thus, with the mechanism still holding the wheelchair, the MWU can release the pushrims, and use the mode knob to select 'freewheeling'. The MWU can then, unhurriedly grasp the pushrims and make a slight forward propelling motion, which is sufficient to take the gravity load and allow the spring-loaded shift compensator to shift the clutch into freewheeling mode. Since the user's hands are on the pushrims while this happens, there is no loss of control.

The unique adjustable override clutch allows the MWU to propel backward instantly, at any time, even when in anti-rollback mode if this manoeuvre is required. This override clutch is adjustable for holding torque level and is set slightly higher than that required to hold the total weight of the MWU and wheelchair on a standard incline. Thus, adding a slight rearward propelling motion will overcome the override clutch, allowing instant backward propulsion. This is, of course, an important safety feature for controlling wheelies, but is also of great utility in allowing quick directional control movements that are intuitive in the normal manner

The WARD concept development was supported by Phase 1 and Phase 2 SBIR (Small Business Innovation Research) grants from the National Institutes of Health. These grants allowed Mr Green to engage the Human Engineering Research Laboratories of the University of Pittsburgh to comprehensively test WARD enabled wheelchairs under laboratory and daily life conditions. These formal tests demonstrated not only a significant reduction in wheelchair user reported pain while negotiating ramps, but enthusiastic user reactions to the WARD device. The data collected included push dynamics and user outcomes measures allowing correlation of the operation of the device with user reported pain.

Meet the researcher

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Steve Green graduated from the University of Arkansas, USA, as a mechanical engineer. After graduating, he began employment at Fiat-Allis Construction Machinery, Inc, where he designed and prototyped hydraulic steering systems for large earthmoving equipment. Between 1978 and 1994, Steve Green worked for BEI Motion Systems Co, where he designed the Star Selector Servo Sub-System angular encoders for the famous Hubble Space Telescope. Since 1994, Steve Green has been President of Green Technologies, Inc, a concept to production prototype development service. His organisation provides engineering and prototype fabrication services supporting medical and diagnostic device development to start-up businesses, providing specialist services in mechanical design, engineering, and fabrication. Mr Green holds several patents on a machining centre and milling systems design, granted since his formation of Green Technologies, Inc. His inventions have been awarded multiple National Institutes of Health Small Business Innovation Research (SBIR) grants.

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FUNDING

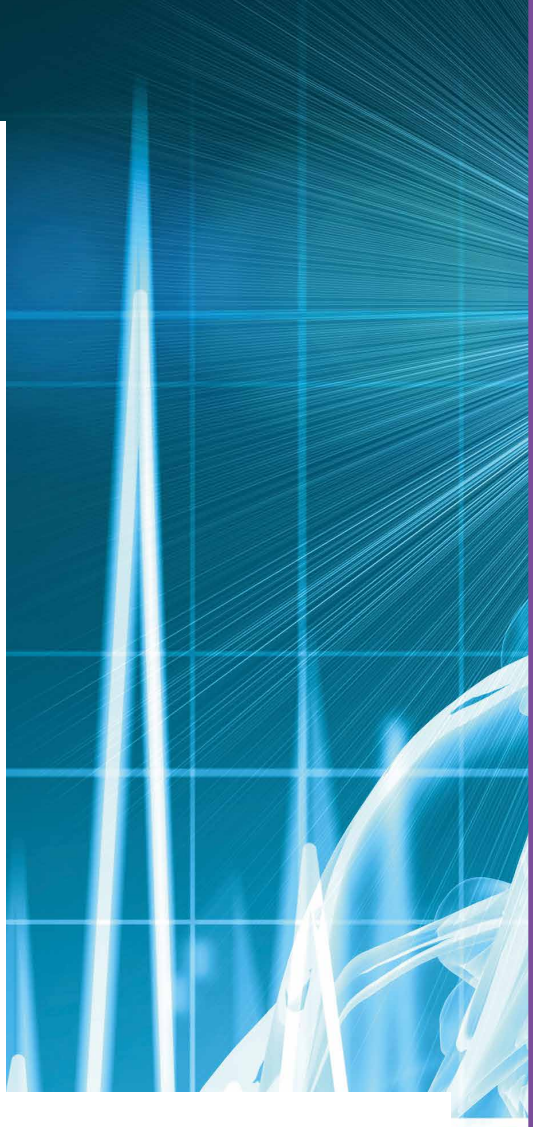
R43AG042664-01; Completed; A Wheelchair Anti-Rollback Device; Principal Investigator: A research project to design, fabricate, and user test a mechanical system that allows improved control of a manual wheelchair on slopes and ramps.

R43HD044271-02; Completed; A Manual Standup Wheelchair; Principal Investigator: A research project to design a standup wheelchair that is totally manual in both propulsion and erection.

HD043516-02; Completed; A Modal Reciprocating Pushrim Drive Wheelchair; Principal Investigator: A research project to design a wheelchair drive that will enable manual pushrim wheelchair propulsion without the need to grab and release the pushrim.

R43HD41272-02; Completed; An Improved Lever Drive Wheelchair; Principal Investigator: A research project to demonstrate the bio-mechanical advantages of an innovative lever drive for manual wheelchairs.

ENDING THE REVOLVING DOOR OF EMERGENCY DEPARTMENT VISITS FOR OLDER ADULTS



'Health is merely the slowest possible rate at which one can die' (Anonymous). Old age faces us all (if we are fortunate), but while we are generally living longer, we are not necessarily living that extended time being healthy. Older adults are now the fastest-growing segment of the population worldwide, and internationally, healthcare systems are scrambling to cope with the new demands this will bring. Here, we review the work of long-time research collaborators **Dr Malcolm Doupe** (University of Manitoba) and **Dr Frode F. Jacobsen** (Western Norway University of Applied Sciences) to address these fundamental issues.

The Challenges of Healthcare for an Ageing Population

With the increase in life expectancy, older people are living longer but often with multiple long-term health conditions. Of these, heart disease, respiratory diseases, cancers, chronic pain, diabetes and neurological conditions are the most common. These conditions are often combined with various disabilities and/or social care challenges such as isolation, unsuitable housing, and a lack of informal and/or community-based day to day support.

As we would expect, some older people are more likely to use healthcare services such as emergency departments (EDs) and hospitalisations. Without proper discharge planning, healthcare planners are well aware that older patients sent back to the community are very likely to have repeat ED visits and unplanned hospitalisations. Internationally, 22% of all ED visits are made by older people and, while they are discharged back to the community about half of the time, this often results in repeat ED visits and/or hospitalisations to deal with unresolved challenges. Both Norway and Canada demonstrate problems typical of this worldwide picture, where older adult ED visits have increased by 70% in the last decade.

Healthcare systems face complex pressures and human resource strains. These systems are also often 'disconnected' from communities leaving gaps in care as older adults transition from EDs or hospitals back home. These gaps in care, coupled with fragmented transition processes, contribute significantly to the everyday challenges that older adults face, thus limiting their ability to live successfully in the community.

Over the past 10 years, internationally collaborative research has been taking place to help improve the healthcare that older adults receive generally. Dr Malcolm Doupe, University of Manitoba, and Dr Frode F. Jacobsen, Western Norway University of Applied Sciences, are leaders in this field of expertise.

A Decade of Collaboration

Dr Jacobsen and Dr Doupe have a long and productive history of academic collaboration, reflecting their strong dedication to the welfare of older people across a range of care settings. In 2010, they were both members of the international 'Re-imagining Long-term Care Project', for the Social Sciences and Humanities Research Council of Canada, as part of their ongoing commitment to improving nursing home care. This has continued through extensive co-published research on international comparisons of healthcare

‘Our long-term goal is to become world-class leaders in developing and testing strategies to improve older adult care transitions from the community to emergency departments [and] hospitals and back.’

standards, nursing practices, models of physician care, and resident choice. Drs Jacobsen and Doupe are also partners on a current Research Council of Norway grant-funded project designed to improve nursing home quality of care. The pair’s close academic relationship has also facilitated closer working links between their academic institutions, the University of Manitoba and Western Norway University of Applied Sciences, which are now instrumental in their ongoing projects.

In 2019, Drs Jacobsen and Doupe began their current research focussed on the emergency department-to-community transition for older people. Their collaboration has led to the successful funding of two key initiatives: *i*NET: An **I**nternational **N**etwork to **E**nhance Older Adult **T**ransitions between Emergency Departments and Communities, and *i*STEP: An **I**nternational **S**tudent **T**raining and **E**xchange **P**roject in Transitional Care.

The Formation of *i*NET

The international *i*NET collaboration is described by Dr Doupe as ‘a leading network of academics and healthcare stakeholders (e.g., decision-makers and providers) from Norway and Canada.’ This new cross-sectoral planning and research group aims to upgrade the quality of education and health services research and provision in both countries, building upon and enhancing existing projects.

A key aspect of *i*NET includes working with two Canadian Universities (namely Alberta and Manitoba) renowned for their international research in older adult care, while ensuring frontline healthcare stakeholders are involved in the research in order to deliver ‘real world’ solutions. Ultimately, the project aims to ‘develop…an innovative and integrated knowledge translation research platform, for continued growth and expansion to additional countries,’ with the goal to become ‘…leaders in developing and testing strategies to improve older adult care transitions from the community to emergency departments [and] hospitals and back.’

*i*NET is planned to operate for 36 months, and in that time, a structured programme of five workshops, international student exchanges and short-course summer schools, and multiple faculty exchanges will be completed. An international team of researchers, ED and community providers, decision-makers, and patients will initially convene to conduct a thorough review and evaluation of international transition practices and to identify promising approaches. Following this, the team will assess how different healthcare policies and structures impact these interventions, and last of all will identify existing administrative data systems that are currently available to define how these transition practices work.

A range of different approaches has been reviewed from international studies, including risk assessment tools

to identify those at potentially higher risk, comprehensive geriatric assessments and follow-ups utilising social workers and healthcare staff, for example. The outcomes, however, have generally been variable, poorly sustained, and have lacked a thorough evaluation to identify where the difficulties lay. Evaluations also have tended to focus only on repeat visits to the ED as the measure, excluding essential factors such as the patient's wants and needs. Health care interventions should also take into account the broader political environment, culture and healthcare system structures when seeking to optimise their long-term effectiveness.

_i_STEP: Preparing Future Academics and Planners

The close academic links forged between the University of Manitoba and Western Norway University of Applied Sciences have been integral to the development of the new *i*STEP collaborative initiative. Drs Jacobsen and Doupe have led the project's developments with colleagues from the major stakeholder organisations involved.

The *i*STEP exchange programme will bring together eight graduate students from Norway and Canada to study and work on the *i*NET project to improve healthcare transitions for older people in each country. *i*STEP will provide students with training on the key *i*NET issues, through planned instructional webinars, opportunities for students to work closely with healthcare planners and providers, and to participate in workshops to review the evidence and plan new approaches. Dr Doupe summarises, 'Through international comparisons, students will learn how culture, legislation, and political context influences healthcare decision making,' ensuring their work will be grounded in 'real-world' understanding.

*i*STEP sets out to fundamentally address a constant challenge for health research, that is flexible and sustainable enough to deliver solutions able to navigate the complexity and inertia integral to healthcare practices and their reform. It is hoped that graduates of the *i*STEP programme will be grounded in the actuality of the healthcare environment and have the appropriate leadership, negotiation and change management skills to implement and evaluate change. The first phase of the project will inevitably be a 'test-bed' to refine the programme's content and methods, but Drs Jacobsen and Doupe are keen for the training programme to continue and expand, even in the absence of future funding.

The Potential for an International Solution

With the unstoppable societal changes of an ageing population now in progression, it is vital that healthcare systems adapt and change to meet the new dynamic changes required to cope with our population needs. The work of Dr Doupe and Dr Jacobsen remains one of the few 'theory into practice' approaches which recognises and truly aims to capture and address the complexities of healthcare that often undermine even the best programmes for change. We can thus hope that the lessons learnt in Norway and Canada can be delivered on a wider, international stage.

Meet the researchers

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Dr Malcolm Doupe is an Associate Professor in the Department of Community Health Sciences, University of Manitoba (UM), Director of the Manitoba Training Programme for Health Services, and a Senior Research Scientist with the Manitoba Centre for Health Policy. He received a doctorate from the Department of Community Health Sciences from the UM in 2004 and has fulfilled various roles at UM including Research Affiliate in the Centre on Ageing (since 2005), Associate Professor in Emergency Medicine (since 2013), and Adjunct Professor in the Departments of Psychology (2013-2018). Dr Doupe was awarded in 2019 the Canadian Over 50s Housing Research Award for Conducting Outstanding Community Health Sciences Research. In 2012–13 he received the CIHR-IHSPR Article of the Year Award, recognising his published research for significantly contributing to the advancement of the field of health services and policy research in Canada.

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Dr Frode F. Jacobsen, a trained medical anthropologist with a background in health studies, has performed fieldwork in Northern Sudan, Indonesia, Jordan, Bolivia, the USA, Canada, Great Britain and Norway. His is presently working comparatively on the organisation of elderly care across Europe and North America. He works as Professor at Western Norway University of Applied Sciences, Professor II at VID Specialized University, Norway, and as Research Director of the Center for Care Research. He received his PhD in anthropology from University of Bergen, Norway, in 1997.

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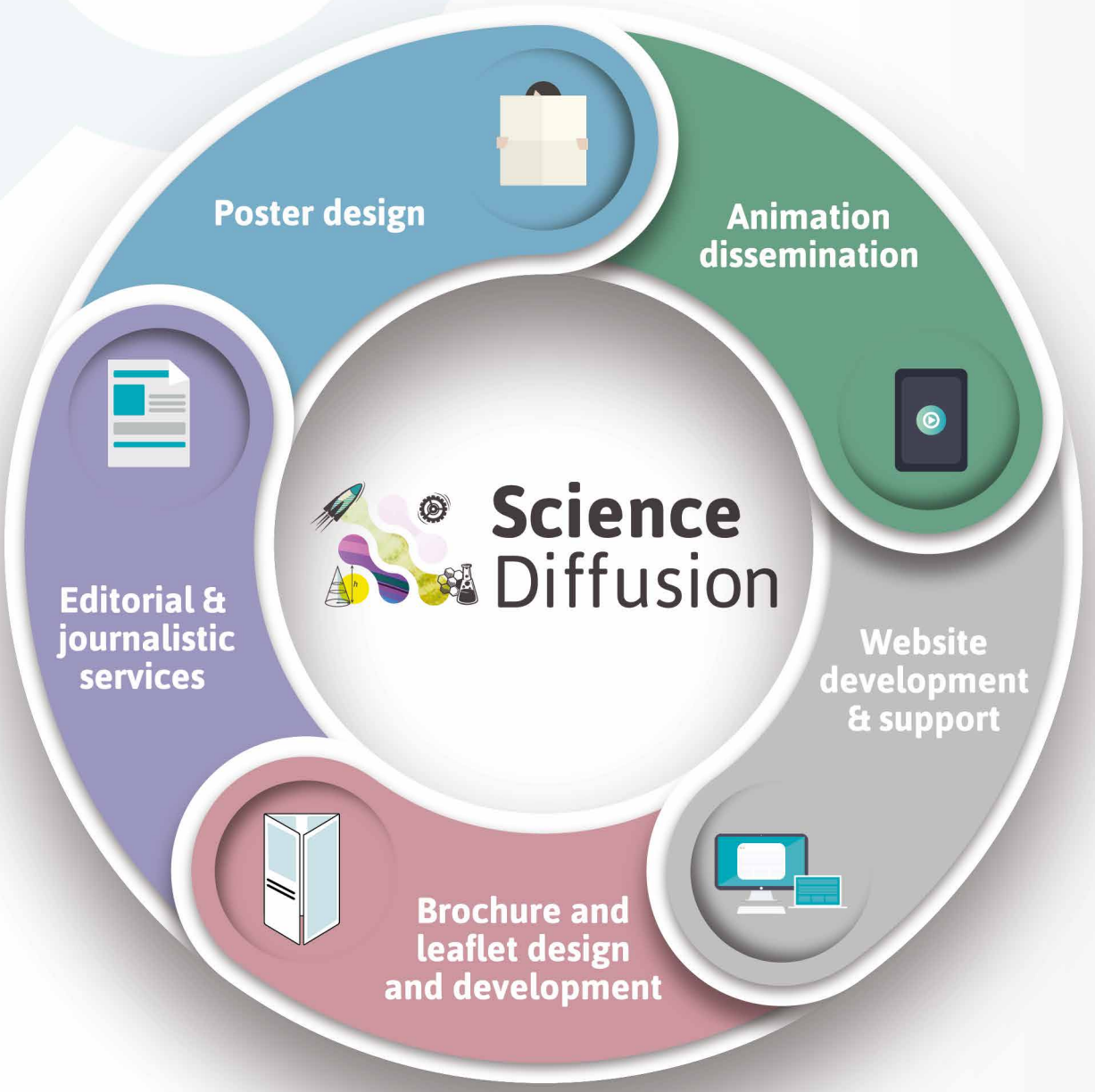

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