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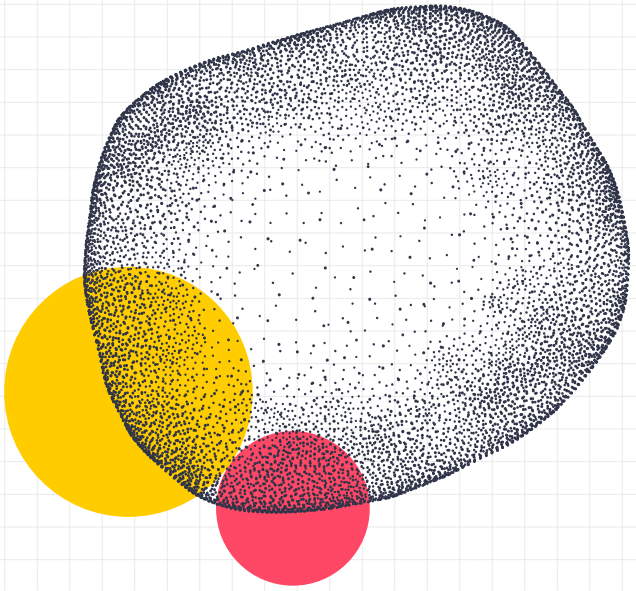

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WELCOME...

Welcome to the latest issue of Scientia! In this inspiring instalment of the latest advances in science and research, we focus on the vital work of researchers whose common goal is to make the world healthier and happier.

Globally, human life expectancy has more than doubled in the past two centuries, and in many ways, life is easier than ever before. When the COVID-19 pandemic struck in 2020, we were provided with a sudden and stark reminder of the fragility of life, the vulnerability of our healthcare systems, and the potential for major disease outbreaks to change all aspects of life as we know it. At the same time, we also face other urgent, global challenges to human health, including cancer, heart disease, and the increasing prevalence of mental health conditions.

Our first section is dedicated to critical advances in medicine and health. We can read about the latest developments in understanding the multifaceted causes of cancer as well as innovative approaches to optimising treatment for this devastating disease. We can also read of important new insights into human immunodeficiency virus and autoimmune diseases, and key developments in cardiology, urology and other clinical fields.

Our second section focuses on pioneering research in psychology and neuroscience. Here, we can read about the mechanisms underlying neurodegenerative diseases such as Alzheimer's disease and cognitive decline, as well as how this is paving the way for the development of new therapeutics. We can also read about the development of effective Interventions to improve mental health in rural communities and in prisons, and a range of other approaches levelled at untangling the complexities of the human mind and behaviour.

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HEALTH & MEDICINE

THERE'S SOMETHING IN THE WATER: *N*-NITROSODIMETHYLAMINE

N-nitrosodimethylamine (NDMA for short) is a worryingly prevalent, potentially potent carcinogen found in food, water, cigarettes and medical drugs. Researchers at the Massachusetts Institute of Technology, USA, are working to better understand what NDMA does and how cells can defend themselves from its effects, with important implications for public health.

A Once Little-known Carcinogen

Millions of people worldwide have been exposed to what was once a little-known carcinogen. *N*-nitrosodimethylamine (or NDMA) is becoming a household name as a result of nearly a dozen recalls on critical medications that were contaminated with NDMA and taken by people on a daily basis. Valsartan (for blood pressure), Metformin (for diabetes), and Zantac (for gastric reflux) have all been recalled due to the presence of NDMA, leaving people both concerned and without critical medicines, and costing billions of dollars to the pharmaceutical industry.

NDMA has also been found in drinking water consumed by millions of people as a result of chemical reactions that occur when water containing organic materials is treated via chloramination. In fact, a survey by the United States Environmental Protection Agency revealed that as many as 10% of municipal water sources contain NDMA.

Currently, the impact of NDMA exposure is uncertain and depends on many factors, including how long people were exposed, at what age, at what level, and whether or not they are genetically predisposed to be susceptible to NDMA's carcinogenic potential.

Professor Bevin Engelward is the Director of the MIT Superfund Research Program (SRP) and she is joined by Professors Desiree Plata and John Essigmann, who serve as Deputy-Directors. The MIT SRP team is currently studying NDMA. While the team cannot yet answer the question of 'how much NDMA is too much?', they are making important advances toward understanding genes that likely impact susceptibility to cancer. They also provide convincing reasons to believe that exposure during development is more problematic than exposure as an adult.

What Does *N*-nitrosodimethylamine Do?

A key issue for the MIT SRP team is to determine what exactly NDMA does, and to identify how cells can defend themselves from its effects.

For a small stretch of DNA – let's say 10 base pairs reading ATCGTATATG – there are 97 carbon atoms, 73 nitrogen atoms and 20 oxygen atoms. Professor Engelward explains that adding just one additional carbon to a place it does not belong can create a 'DNA lesion' with dire biological consequences. The problem is that an extra carbon (a 'methyl' group) at certain sites on DNA bases like adenine can 'jam up' DNA polymerases, which replicate DNA, causing them to get stuck.

If there is one critical thing that a cell has to do every time it divides, it is to accurately replicate the entire genome. If there are 3-methyladenines in the way, polymerases can bumble over the damage, creating a risk of putting in the wrong nucleotide, and thus causing a mutation. While one mutation in the context of the 6 billion base

pairs may seem trivial, it all depends on where it happens. A mutation in a gene called p53, for example, puts cells one step closer to cancer. Given that NDMA is very good at creating 3-methyladenine lesions, it is no surprise that it can cause cancer.

Work led by Dr. Jennifer Kay with support from Mr. Joshua Corrigan, Dr. Amanda Armijo, and other members of the MIT SRP team, has demonstrated for the first time that 3-methyladenine lesions caused by NDMA are indeed carcinogenic in animal models. By genetically engineering mice that are not able to remove 3-methyladenine, the team has demonstrated that these mice are highly prone to NDMA-induced mutations and cancer.

The enzyme responsible for repairing 3-methyladenine is called AAG. This enzyme scans and hops along the DNA searching for 3-methyladenine lesions (and other kinds of damage). When it finds a lesion, it swivels the damaged base into its active site and chops it off. While this is a good thing, since it gets rid of the 3-methyladenine problem, it also creates a new problem: a missing base.

As you can imagine, not having a base is a problem, since the cell won't know how to read that piece of DNA. But there's an even more serious problem. To resolve the empty site where the damaged base was removed, the cell needs to cut the DNA backbone so that replication machinery can get in and fill in the missing information. While this is generally a good thing, it can be a problem if there are too many repair patches. Professor Engelward provides the analogy of having a bump in the road. The bump itself is a problem, but digging out the bump and repaving the road is also a problem until everything is fixed.

This is where the story gets even more interesting. Dr. Kay and the MIT SRP team used mice that have very high levels of AAG to see what would happen when they were exposed to NDMA. Not surprisingly, the mice had fewer instances of cancer, since there were lower levels of 3-methyladenine and therefore lower levels of mutations that could promote cancer. However, the mice had a new problem: cells were dying. A lot of cell death means that the damaged tissues cannot function properly and in some cases, the mice could not survive. But how does this all relate to the problem of people being exposed to NDMA?

The Consequences of Human Exposure

The biggest challenge in discerning the risks of NDMA exposure is that we really don't know what happens to people who are exposed to relatively low levels of NDMA for a long period of time. Professor Engelward notes that, if we were to take an educated guess, we would hypothesize that exposure *in utero* would be more problematic than exposure as an adult, because there needs to be a lot of cell division to go from one fertilized egg to the trillion cells that are necessary to create a baby. Every time a cell divides, there is a risk of a mutation. If you now layer onto that risk the additional DNA damage caused by exposure to NDMA, there is certainly a reason to be concerned that NDMA could promote mutations that eventually give rise to childhood cancer.

Remarkably, an epidemiological study (the study of the distribution and determinants of disease) showed a connection between NDMA exposure *in utero* and an increased risk of cancer in children. This came about because of a Superfund site located in Wilmington, Massachusetts, where waste products reacted with one another to create extremely high levels of NDMA that eventually made their way into the town's drinking water wells. The tragedy that ensued was intolerable. Nearly two dozen children in the town with a population of under 20,000 got cancer, and some of them did not survive.

The people of Wilmington have been struggling for years to find out what was causing cancer in their children. A study by the Massachusetts Department of Public Health that pointed to an association between NDMA exposure *in utero* and childhood cancer provided some relief since it validated the townspeople's beliefs, but at the same time, it caused a resurfacing of extreme grief. Professor Engelward explains that while there is nothing that can be done about the past, the people of Wilmington want to make sure that nobody else has to suffer the way that they did.

It is important to recognise that this is just one study. In most cases, researchers compile data from multiple epidemiological studies in order to gain confidence in the results. Clearly, much more work needs to be done to piece together the missing information on the extent to which NDMA in drinking water is a risk for cancer.

In pursuit of that goal, Dr. Robert Croy and Professor John Essigmann, who are members of the MIT SRP team, have developed a novel way to determine the biological effects of NDMA in drinking water. They developed a sensitive method to measure the products of DNA damage by NDMA (e.g., O^{6}-methylguanine, 3-methyladenine and 7-methylguanine) in the genomes of animals that have been given small amounts of NDMA in drinking water over several weeks. At least one of these DNA-derived products, O^{6}-methylguanine, is a widely known biomarker of genetic damage by environmental chemicals. Their work meshes seamlessly with the additional genetic studies, and the development of sensors to detect NDMA, going on as other parts of the MIT SRP.

Critical Next Steps

When asked about her next steps, Professor Engelward explains, 'We see a path forward with two elements. The first thing is that we want to understand what happens when animals are exposed to NDMA in drinking water, the way that people are exposed'. She further elaborates, 'In our earlier studies, mice received a high dose of NDMA, much higher than what people experience. We are now setting off to do long-term drinking water studies with much more realistic levels.'

Professor Engelward and the team's ongoing work including DNA repair-deficient mice in their studies is important because AAG levels vary from person to person, and people with low AAG may be at increased risk. When thinking about the risk of exposure in the population, it is critical to consider the risk of those who are most vulnerable. As her earlier research pointed to AAG as a susceptibility factor, Professor Engelward now aims to find out if it in fact defends against cancer caused by contaminated drinking water.

There is much work to be done by researchers, and a lot of work to be done by people in the pharmaceutical industry to make sure that people's drugs are free from NDMA. Professor Engelward reminds us that 'The good news is that we have a lot of knowledge about NDMA and we have ideas about its potential biological effects; now it is time to do the hard work and get the research done.'

For people who already have been exposed, there are steps that can be taken. On this, Professor Engelward concludes, 'All of the good advice that you get from your nurse or doctor about exercise, eating right, and avoiding exposure to chemicals, is good advice, and can go a long way toward offsetting any possible increased risk of cancer caused by NDMA.'

With a team of highly talented students and more senior researchers, as well as support from the National Institute of Environmental Health's Superfund Research Program, we can be confident that the MIT SRP will soon present a much better understanding of the risks that NDMA poses and the implications for public health.

Meet the researchers

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Professor Bevin Page Engelward graduated from Yale University and then received her doctoral degree from the Harvard School of Public Health. In 1997, she became an Assistant Professor at the Massachusetts Institute of Technology (MIT). She is now a Professor in the Department of Biological Engineering and the Director of the MIT Superfund Research Program. With an overarching commitment to improving public health, Professor Engelward's research focuses on gene-environment interactions that modulate disease susceptibility through the development of novel tools for studying exogenously induced genetic changes in animals and human cells.

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Dr. Jennifer Kay received her bachelor's degree from the University of Pittsburgh and went on to complete her PhD and postdoctoral training in Professor Bevin Engelward's laboratory in the Biological Engineering Department at MIT. As part of the SRP, Dr. Kay helped lead research projects using mice genetically engineered to have different DNA repair capacities and reporter genes for measuring mutations. She also served as the MIT SRP Research Translation Core director, bringing MIT SRP research advances to community groups, government agencies, other academics, and the general public. Dr. Kay is now a Research Scientist at Silent Spring Institute, studying environmental exposures that can lead to breast cancer. Dr. Kay's goals are to understand what chemicals do to cause cancer so that people can be better protected from potentially dangerous exposure.

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DYSREGULATED INTRACELLULAR PH CAN ENABLE DIFFERENT DISEASES

The pH inside our cells is constantly changing, but also carefully controlled within certain limits. Dynamic intracellular pH (pHi) is essential for normal cell behaviours, but when it becomes dysregulated, it can enable an array of diseases from cancer to Alzheimer's. **Dr Diane Barber** from the University of California San Francisco has carried out extensive research into how normal pHi dynamics regulate cell behaviours and the impact that dysregulated pHi can have in different diseases.

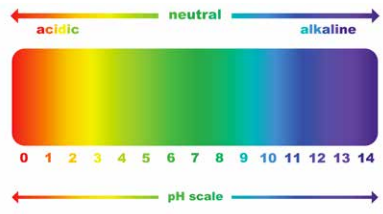
Understanding pH in Cells

The pH of a substance is a measure of how acidic or alkaline it is, or in other words, the concentration of hydrogen ions (H+) it contains. A solution with a higher concentration of hydrogen ions is acidic and has a lower pH, one that has a value of less than 7. The pH scale is measured between 0 and 14, so an alkaline solution is one with a value of 8 and above. Neutral solutions are those with a pH of 7 – such as water, for example.

In cells, pH generally ranges between 7.0 to 7.6 but is dynamic, meaning that it can fluctuate, and controlling these pH changes is important for normal cell behaviours. During disease processes, this balance can be disturbed. For example, compared to the pHi of normal cells, cancer cells generally have a higher dynamic intracellular pH (pHi) and brain cells with neurodegenerative diseases have a lower pHi. It is important to understand how and why this happens as well as what the implications are if we are to better understand human pathologies. This is the focus of the research of Dr Diane Barber from the University of California in San Francisco. Her studies have led to fascinating new discoveries into the inner working of our cells and what can go very wrong.

Protonation and Deprotonation as Posttranslational Modifications

Proteins are created using the coding structures of our DNA. Through processes called transcription and translation, genes are read by cell machinery and used as a template to bring together amino acids to create a required protein. Once this new protein has been released into the cell to carry out its functions, it will often be subject to posttranslational modifications. These alterations are important to regulate the stability and activity of the protein as well as its binding affinity (how well it attaches to other molecules). Common examples of posttranscriptional modifications are phosphorylation, whereby a phosphoryl group (PO3-) is added, and ubiquitination, which is the addition of a protein called ubiquitin.



But work from Dr Barber and her collaborator Dr Matthew Jacobson, a computational protein biophysicist at the University of California San Francisco, compellingly shows that protonation and deprotonation should be classed as a posttranslational modification. They showed that fluctuations of the pH within cells (intracellular) or outside of them (extracellular) can change the charge of an amino acid side chain, and with protonation or deprotonation, alter the structure and function of that protein. Whilst other posttranslational modifications need an enzyme to facilitate the reaction, adding or removing a proton does not and it is very quick as well as easily reversible.

Drs Barber and Jacobson discovered that this type of modification is achieved on certain pH-sensing

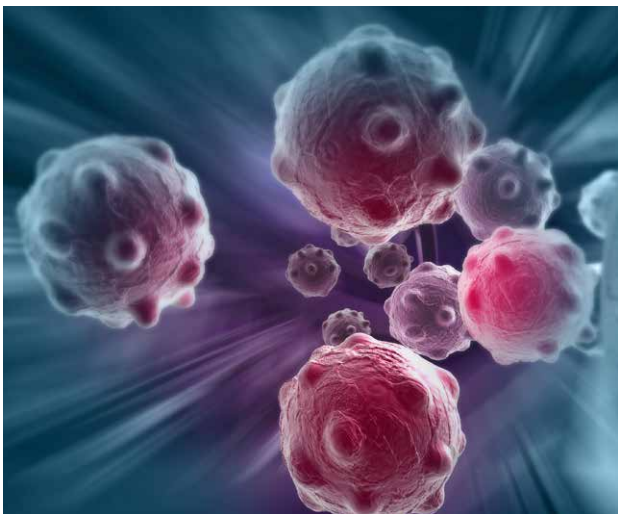
proteins via a small number of sites which they called pH sensors. Subsequently, Dr Barber investigated the structural mechanisms of these proton posttranslational modifications and the consequences that modified pH-sensing proteins have when regulating cellular processes. According to Dr Barber, 'Determining protonation and deprotonation, however, is more challenging compared with other types of posttranslational modifications because it is not detected by mass spectrometry or antibodies, nor is it catalysed by an enzyme.'

Nevertheless, she found that the pHi is highly dynamic and this is essential for many normal cell behaviours such as cell migration, metabolism and cell cycle progression. However, they are also implicated in many pathological cellular events, including cancer and tumorigenesis. 'We now know that pHi is dysregulated in many diseases. For example, compared with normal cells, pHi is constitutively increased in most cancers regardless of tissue origin or gene mutations. In contrast, pHi is constitutively decreased in neurodegenerative disorders, including Alzheimer's disease and frontotemporal dementia' explains Dr Barber. Because of this, engineering methods to target and control proton posttranslational modification could be a promising avenue to explore in the development of novel therapeutics.

pH Dysregulation in Cancer

The issue of pHi dysregulation –specifically, increased pHi in cancers – is a current focus of Dr Barber's group. A relatively high internal pH and low external pH are already known markers of a cancerous cell and it has been shown in in vitro cell models to increase cell division, cell migration, and limit cell death (known as apoptosis). However, this had not been thoroughly investigated *in vivo* – in a living organism. Therefore, Dr Barber and her team used a type of fly that is a common animal model called Drosophila melanogaster in their study.

Interestingly, they showed that increasing pH was sufficient to induce dysplasia, which is an abnormal development of cells that can lead to cancer. This was seen in the absence of an activated oncogene, a mutated gene that sets off reactions that result in a tumour. The newly raised internal pH of the cells enabled cancer cell behaviours like dividing faster and growing into surrounding tissues. But promisingly, the study



also demonstrated how these cancer cells might be overcome. They revealed that preventing hydrogen ions from leaving cells having cancer-causing oncogenic Raf or Ras mutations caused acidosis and this man-made increased acidity had the ability to kill the cancer cells. Dubbed synthetic lethality, Dr Barber believes that utilising it alongside activated oncogene expression could be a therapeutic method of preventing cancer progression whilst limiting side effects.

Characterising Amino Acid Mutation Signatures

In a separate series of studies that explored other ways that a higher pHi might enable cancer, the Barber group turned their attention to cancer-causing mutations in proteins that change amino acids to be more sensitive to pH regulation. Specifically, they found that if a mutation caused an amino acid change from an arginine, which is always protonated, to a histidine, which can be protonated at lower pH and deprotonated at higher pH, it conferred pH sensing that enabled the higher pHi of cancer cells to enhance the function of oncogenes and impair the function of tumour suppressor proteins.

From this work on what they call charge-changing mutations, with Dr Jacobson and Dr Ryan Hernandez (also at the University of California San Francisco), Dr Barber used machine learning and computational analysis to create a newly defined classification of cancer subtypes. In general, cancer is thought of as a group of different diseases, differentiated by distinct mutations and tissue origins, which are commonly used to classify cancer subtypes. In contrast, the alternative classification proposed by Dr Barber and her colleagues is based on amino acid mutation signatures.

When a genetic mutation occurs, it means that part of the DNA has been spontaneously altered in some way. Mutations can be a base substitution, a deletion or an insertion. Because the genes provide the coding for which amino acids to bring together to form a protein strand, an alteration in the DNA can result in incorrect amino acids being added to the protein – an amino acid mutation.

When Dr Barber and her colleagues examined 29 different cancers for amino acid mutation signatures, their analysis revealed some clear and exciting results. Certain cancer types have mutation signatures with high instances of an arginine amino acid changing to a histidine, others are dominated by glutamic acid to lysine mutations, and some have very complex signatures characterised by multiple amino acid mutations.

Using an open-source catalogue called the COSMIC somatic mutation database, the team validated their results from samples all over the world. Through this investigation, they identified which amino acid mutations are common among specific cancer in addition to which mutations frequently appear together. What made their analysis particularly significant is their finding that some cancers previously classified as disparate, such as brain medulloblastoma and pancreatic cancers share a high occurrence of arginine to histidine mutations. Additionally, previously considered disparate cancers such as melanoma and bladder cancer share a high occurrence of glutamic to lysine mutations.

Importantly, charge changing mutations do not only occur in cancer, but in fact, they appear in a vast range of diseases. Muscular dystrophy, Alzheimer's disease, heart septal defects, cystic fibrosis and many more, are all implicated by charge changing mutations. Dysregulated pHi is also associated with many of these diseases, which opens new directions for testing effects on how charge changing mutations confer disease pathologies.

Through her dedicated work, Dr Barber has greatly improved our knowledge and understanding of the role that pHi plays in an array of diseases. By delving deeper into charge changing mutations, she has provided insight into how dysregulated pHi dynamics can enable some diseases and where new therapeutics investigations might be directed in the future.

Meet the researcher

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Dr Diane Barber received her BSc in Biological Science and her MSc in Physiology from the University of California Davis, followed by her PhD in Anatomy from the University of California Los Angeles. She is currently an Endowed Professor and Chair in the Department of Cell and Tissue Biology at the University of California San Francisco. Dr Barber's pioneering research has earned her many awards, including election as a Fellow to the American Association for the Advancement of Science in recognition of her distinguished contributions to science. Her research focuses on studying the dynamic nature of intracellular pH and the effect this has on cell behaviours, including those that lead to cancer.

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CHEMOTHERAPY- AND CARCINOGEN-INDUCED CELL DEBRIS INITIATES CANCER RECURRENCE

Chemotherapy, one of the mainstays of cancer treatment, can unfortunately act as a double-edged sword. While achieving the intended aim of killing cancerous cells, it also generates an accumulation of cell debris, which in turn, promotes tumour growth by stimulating inflammation in the tumour microenvironment. **Dr Dipak Panigrahy** and his colleagues from Harvard Medical School, USA, have conducted several studies in mice showing that targeting the tumour cell debris-mediated surge of proinflammatory and protumourigenic factors provides a strategy for enhancing the efficacy of chemotherapy.

The Double-Edged Sword of Chemotherapy

With advances in genomics and drug discovery, chemotherapy is the frontline treatment for cancer now more than ever before. However, accumulating evidence from various animal models of the disease suggests that rather than simply killing the cancerous cells, chemotherapy can also initiate the recurrence of cancerous tumours. Unfortunately, the mechanisms behind this double-edged sword are still poorly understood. Working to resolve important questions about this critical issue is Dr Dipak Panigrahy, along with his colleagues at Harvard Medical School, USA.

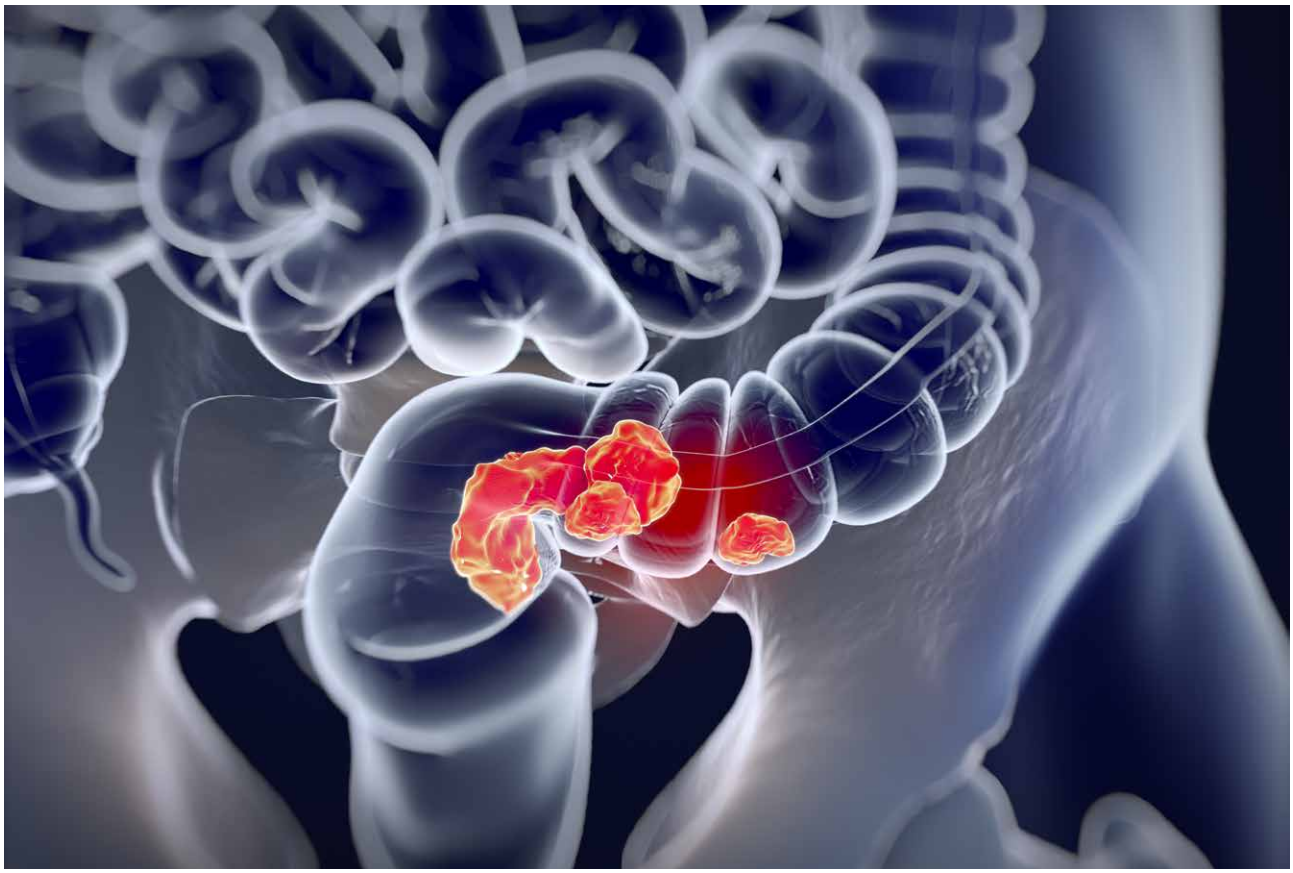
Apoptosis is the process of programmed cell death, and this may trigger escape from tumour dormancy by causing a cellular stress response linked to inflammation. Dr Panigrahy and his colleagues argue that increased levels of spontaneous apoptotic cell death in the tumours of cancer patients are associated with poor prognosis in several cancer types.

5-fluorouracil (5-FU) is a chemotherapeutic drug used to treat colorectal cancer. It reduces tumour mass by causing cell death, creating tumour cell debris in the form of apoptotic cells and cell fragments. Observing that apoptotic tumour cells can stimulate specialised cells known as macrophages and the production of proinflammatory cytokines, Dr Panigrahy and his colleagues proposed that 5-FU may be a source of tumour growth stimulation. In 2019, the Panigrahy laboratory published an important study that clearly demonstrated that 5-FU generates cellular debris that causes tumour cells and host macrophages to release a tumour factor known as osteopontin (OPN).

In clinical settings, OPN expression is linked to poor 5-year survival in many cancer types. OPN is a well-characterised factor that has been linked to cancer progression and angiogenesis, which is the growth of new blood vessels that tumours need to grow. Conventional chemotherapy may contribute to tumour progression and

relapse via cell debris, suggesting that treating the tumour-promoting activity of cell debris is critical for the prevention of tumour recurrence.

In the 2019 study, Dr Panigrahy examined the cytotoxic activity of 5-FU in mice that were previously inoculated with colorectal cancer cells. As predicted, the researchers observed increased cell death in tumours that were treated with 5-FU compared with size-matched control tumours. Furthermore, the study confirmed that systemic 5-FU treatment and tumour cell debris increase OPN levels and that debris-stimulated tumour growth is mediated by enhanced tumour angiogenesis. The most important finding, however, was that pharmacologic and genetic ablation of OPN inhibited debris-stimulated tumour growth. Dr Panigrahy and his colleagues



demonstrated that a combination of neutralising antibodies to inhibit OPN and continued treatment of 5-FU dramatically inhibited tumour growth.

Chemotherapy-generated Debris and Ovarian Cancer Resurgence

Epithelial ovarian cancer, a major cause of death in women worldwide, is characterised by a high tumour recurrence, which can occur in up to 70% of patients. To ascertain whether chemotherapy-generated debris is biologically relevant in ovarian cancer, via a similar mechanism initiated by 5-FU and mediated by OPN in colorectal cancer, Dr Panigrahy and his colleagues treated mouse and human cell lines with cytotoxic platinum- or taxane-based chemotherapeutic agents used for treating ovarian cancer. As a consequence of the treatment, the colleagues observed a surge of proinflammatory cytokines and bioactive lipid molecules known as eicosanoids, released by macrophages, in the tumour microenvironment. The findings of this study were published in 2019 in the prestigious journal, the Proceedings of the National Academy of Sciences (PNAS).

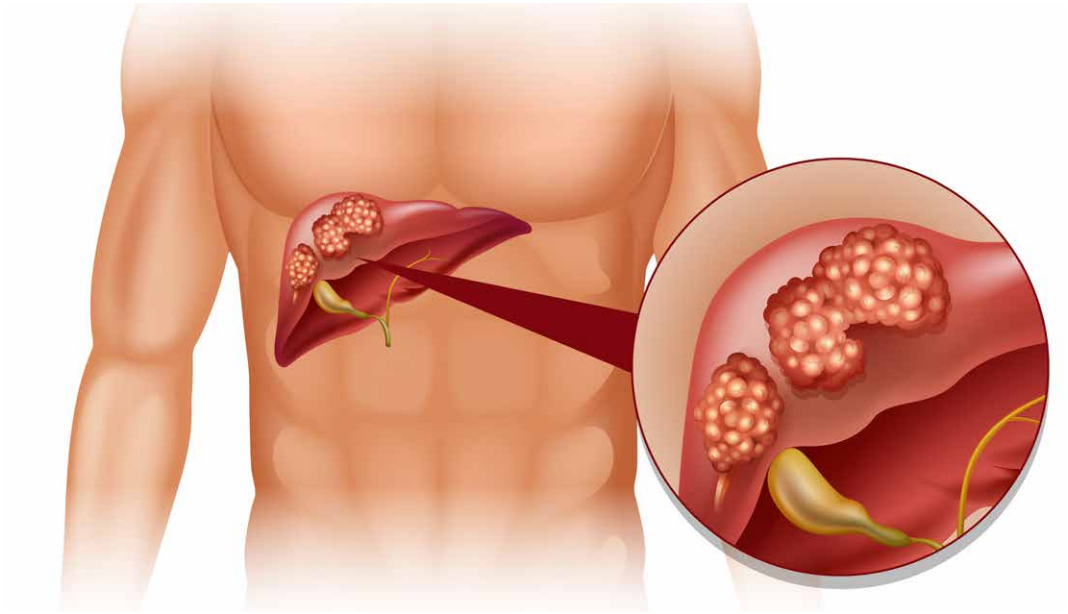
The research team also observed that the presence of debris alone without macrophages in the culture medium resulted in minimal to undetectable levels of cytokines, confirming that the release of lipid mediators and cytokines is macrophage-dependent. The PNAS study showed that the combined pharmacological inhibition of the cyclooxygenase-2 (COX-2) and soluble epoxide hydrolase (sEH) pathways prevented the surge of both cytokines and lipid mediators by macrophages.

These results confirmed that ovarian cancer patients may benefit from the suppression of eicosanoid and cytokine mediators, protecting the body from a therapy-induced debris-mediated cytotoxic and tumourigenic response.

Aspirin-triggered Mediators as Optimal Chemopreventive Agents

Many studies suggest that the nonsteroidal anti-inflammatory drug (NSAID) aspirin is potent in counteracting the formation of tumours. Despite numerous reports confirming its beneficial properties in cancer prevention, the biochemical mechanisms behind this unique antitumour activity of aspirin compared with other NSAIDs remain poorly understood. Cyclooxygenase (COX)-1 and COX-2 are key targets of aspirin and are involved in the biosynthesis of proinflammatory lipids, such as prostaglandins. Dr Panigrahy and his colleagues published another study in 2019 showing that aspirin not only blocks the biosynthesis of prostaglandins, but also stimulates the endogenous production of anti-inflammatory mediators termed ‘aspirin-triggered specialised pro-resolving mediators’ (AT-SPMs), such as ‘aspirin-triggered resolvins’ (AT-RvDs) and ‘aspirin-triggered lipoxins’ (AT-LXs).

The research team demonstrated that treatment of mice with AT-RvDs or AT-LXs inhibited primary tumour growth by enhancing macrophage removal of tumour cell debris and inhibiting the production of macrophage-secreted proinflammatory cytokines. Following the publication of the 2019 study, AT-SPMs, including resolvins, have been considered



in clinical studies for their tumour-preventing activity. Dr Panigrahy and his colleagues have shown that, given the risks associated with chronic low-dose aspirin intake, mediators such as aspirin-triggered resolvins and other AT-SPMs may be a more desirable therapeutic option, since they display more potent antitumour activity and are devoid of aspirin-related toxicity.

Resolvins Enhance Cancer Therapy by Clearing Cell Debris

As demonstrated in many studies by the Panigrahy team, dead and dying tumour cells greatly affect the tumour microenvironment. This leaves the medical profession with a dilemma between treating tumours with chemotherapy and minimising the effects of debris-induced tumour progression. Resolving this dilemma is paramount to preventing tumour recurrence after therapy.

In a study published in 2017, Dr Panigrahy and his colleagues demonstrated that apoptotic debris stimulates tumour growth through the action of phosphatidylserine (PS), a modified amino acid that is present on the surface of apoptotic cells. The study showed that blocking PS in the debris with a recombinant protein or an anti-PS neutralising antibody significantly inhibited debris-stimulated tumour growth in a dose-dependent manner.

The 2017 study adds further evidence in support of using specialised pro-resolving mediators, such as resolvins, to clear apoptotic debris. The novel approach alongside chemotherapy would greatly prevent tumour recurrence and enhance the benefits of cancer therapy. The observations were supported by the results of a 2019 study in which Dr Panigrahy and his colleagues demonstrated that the resolution of inflammation via resolvins, before surgery, inhibited the formation of new tumour growth, inducing robust anticancer T cell immunity in mice affected by Lewis lung carcinoma.

Resolution of Inflammation Halts Liver Cancer Progression

Building on the observations published in previous years, Dr Panigrahy and his colleagues recently published a new study on the effects of inflammation on the changes to the tumour microenvironment triggered by cytokine and eicosanoid storms during hepatocellular carcinoma (HCC). Aflatoxin B1 (AFB1), a mycotoxin produced by Aspergillus fungi, may play a causative role in 4.6 to 28.2% of all HCC cases worldwide. Aflatoxin-induced HCC is most prevalent in developing countries due to the regular consumption of food contaminated with aflatoxins.

HCC is associated with excessive production of proinflammatory cytokines, including TNF-α and IL-6, which lead to apoptotic cell death in multiple cell types. AFB1 can also negatively impact macrophages by impairing their ability to remove cell debris. By causing excessive production of oxidative stress, proinflammatory cytokines start a cascade that leads to DNA damage and new tumour growth, correlating with poor patient survival. Dr Panigrahy and his colleagues demonstrated that tumour cells killed by AFB1 stimulate primary HCC growth when co-injected in mice with a nontumourigenic inoculum of tumour cells and that the malignant growth is dependent on a macrophage-derived eicosanoid and cytokine storm that also involves mediators that promote the formation of new blood vessels.

Dr Panigrahy and colleagues demonstrated that dual COX-2/sEH inhibitors can be administered during and immediately after periods of high exposure to aflatoxins, resulting in a physiological switch from a pattern of inflammation to the resolution of inflammation. By targeting the debris-mediated eicosanoid and cytokine storm, via clearance of tumour cell debris, dual COX-2/sEH inhibition may provide an effective strategy for the prevention of AFB1-induced hepatocellular carcinoma.

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Dr Dipak Panigrahy obtained his MD in 1994 from Boston University School of Medicine, Boston, MA. During his postdoctoral studies, Dr Panigrahy specialised in Vascular Biology and Surgery and is now Assistant Professor of Pathology at Harvard Medical School. The Panigrahy Laboratory studies a class of lipid autacoid mediators known as eicosanoids and their pathophysiologic roles in the development of cancer. This translates into an ongoing collaboration with industry to study eicosanoid modulating drugs in experimental cancer models. As an outstanding clinician-scientist, Dr Panigrahy has written numerous publications on cancer treatment, has chaired numerous international conferences and symposia on cancer biology, and sat on the editorial boards of several prestigious scientific journals.

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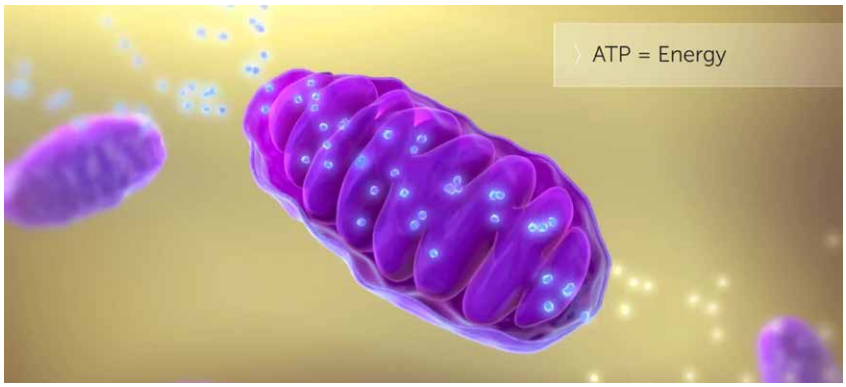
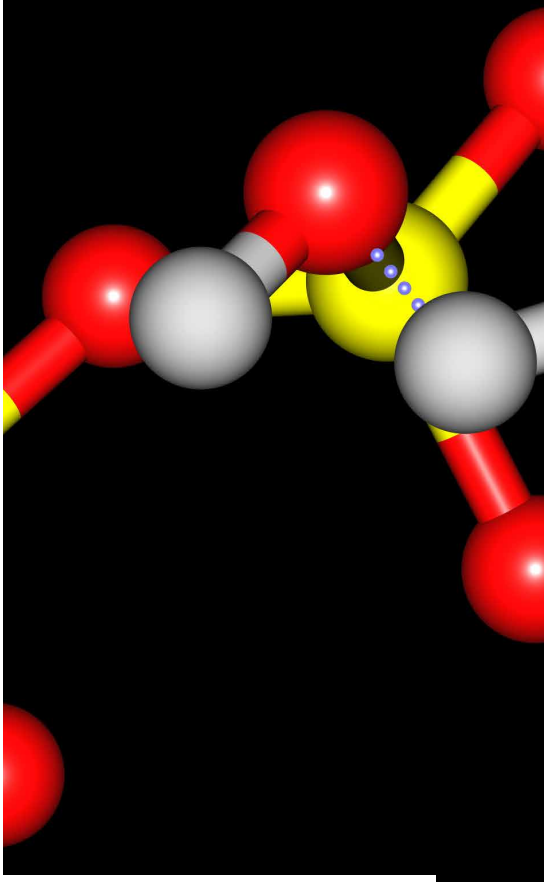
p38γ
It's More than Just a Kinase

Kinases take energy from adenosine triphosphate molecules to fuel other molecules in performing vital biological processes. **Dr Xu Hannah Zhang** at City of Hope, Los Angeles, has worked with colleagues to better understand the p38 family of kinases, and in particular, how the p38γ isoform plays a role in cancer. Her work has shown – for the first time – that p38γ is much more than just a kinase, and her recent studies point to new avenues in the search for cutaneous T-cell lymphoma therapeutics.





The Vital Work of Kinases

Vital biological processes such as cell growth and division (cell proliferation) and cell death (apoptosis) are fueled by chemical energy. In our bodies, adenosine triphosphate (ATP) carries chemical energy from the food we eat. Kinases then transfer this energy in the form of phosphates from the ATP molecules and add it to other molecules (such as sugars and other proteins) in a process known as phosphorylation.

In humans, the p38 mitogen-activated protein kinases (MAPK) family of kinases consists of four isoforms (also known as variants): p38α, p38β, p38γ and p38δ. Each isoform performs unique functions at different developmental stages in the lifespan, and while p38α and p38β are found throughout the body, p38γ and p38δ are only found in specific tissues.

p38γ: Links to Cancer

Our understanding of p38γ and p38δ is much less extensive than that of p38α and p38β. However, we do know that p38γ is associated with the spread of a diverse range of cancers (including colon, prostate, oesophagal, breast and liver cancers) and also cutaneous T-cell lymphoma, a rare form of cancer that begins in the white blood cells known as T cells and affects the skin, the body's largest organ.

Dr Xu Hannah Zhang at City of Hope and her colleagues, under the leadership of Dr Steven T Rosen, Provost and Chief Scientific Officer of the institute, recently explored the role of p38γ in cutaneous T-cell lymphoma – and uncovered some novel and important findings in the process.

To Bind or Not to Bind?

Binding sites (so-called 'pockets') are the parts of a protein that allow them to accommodate via affinity the smaller, incoming molecules. As p38γ shares its ATP-binding site with other kinases, Dr Zhang and her colleagues also studied a non-ATP-binding site to help them to identify any specific effects of p38γ. They were particularly interested in a hydrophobic (water-repelling) non-ATP site capable of attracting lipid-like small molecules, such as those required to target p38γ for the cure of the diseases such as cutaneous T-cell lymphoma.

In the field of bioinformatics, molecular docking is one of the most commonly used virtual screening methods supporting drug discovery and can be used to investigate interactions between small molecules and proteins. This is

the approach used by Dr Zhang and her colleagues to examine all 270,000 compounds currently available in the National Institute of Cancer Development Therapeutics Program library and assess the extent to which each would bind to the non-ATP site.

The 80 drugs identified as most effective in binding to the non-ATP site were investigated further using virtual screening to determine their potential toxicity to cutaneous T-cell lymphoma cells. Of these, Dr Zhang and her colleagues selected two small molecules: CSH71 and CSH18 (note that CSH18CN is a modified form of CSH18 to increase more specific binding). The researcers then confirmed the effects of both small molecules in the laboratory, using real samples.

As expected, both CSH71 and CSH18CN were toxic to cutaneous T-cell lymphoma cells. The effects of small molecule CSH71 were dose-dependent, but critically, at higher doses, CSH71 was found to bind to the ATP-binding site of p38γ and also the non-ATP site. This observation lead Dr Zhang and her colleagues to make the novel report that p38γ also functions as a non-kinase in T malignant cells, serving to drive cell proliferation. In contrast, normal healthy blood cells were spared because their p38γ is silent (not expressed).

Therapeutic and Other Implications

Dr Zhang and her colleagues propose that these new insights into how drugs can bind to the ATP-binding site and non-ATP binding site of p38γ will lead to treatment innovation in cutaneous T-cell lymphoma. Specifically, she proposes that targeting the non-ATP binding site will be a particularly fruitful avenue of exploration. It is also worth noting that her findings also validate the use of relatively new computational screening techniques in drug discovery with nuclear magnetic resonance Spectroscopy.

An Intriguing Idea

As a final aside, Dr Zhang notes that CSH71 treatment impacts olfactory receptors, which give rise to our sense of smell. The compound CSH18, as also studied by the researchers, impacts olfactory receptors but in a different collection to CSH71. This currently unpublished data from Dr Zhang lends support to the intriguing idea that each compound may trigger a unique 'fingerprint' for T cells to react to chemotaxis as part of its immune defence mechanism. She suggests that our current understanding of olfactory receptors may require revision, and further work to unpick how olfactory receptors interact with other cells and are regulated is now warranted.

Meet the researcher

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Dr Hannah Zhang completed her PhD in Biochemistry and Molecular Biology at Peking Union Medical College in China and then completed postdoctoral fellowships at Mount Sinai Hospital and Albert Einstein School of Medicine. She remained in New York to take up research appointments at Mount Sinai Hospital and Weill Cornell Medical College and was later appointed Assistant Research Professor at the prestigious City of Hope in Los Angeles. In 2022, Dr Zhang was appointed to her current position of Associate Research Professor. Her academic record of publications and funding is testimony to her extensive research experience and expertise in molecular biology, cell biology and immunology, and also her thorough knowledge of the molecular mechanisms of human disease.

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KNOWLEDGE IS POWER: IMPROVING OUTCOMES IN OVARIAN CANCER

Ovarian cancer is the second most common gynecological cancer and has the highest mortality rate of all female reproductive cancers in the United States. A lack of early detection, typically aggressive progression, and rapid development of resistance to chemotherapy are key contributing factors to the mortality rate. **Dr. Dong-Joo (Ellen) Cheon** and her team at Albany Medical College are working to determine the role of key players in the resistance to chemotherapy treatments and examining how best we can target these therapeutically to improve survival.

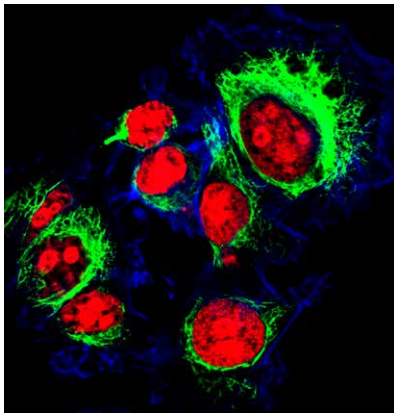
The Knowledge Gap in Ovarian Cancer

Each year, over 19,000 women are diagnosed with ovarian cancer in the United States. Of these, only 35% will go on to survive beyond the 10-year mark despite research advances. Unfortunately, there are several reasons why ovarian cancer remains such a serious threat.

Ovarian cancer is an aggressive disease that is further complicated by the same symptoms appearing as part of other gynecological issues. As a result, it is often not diagnosed until patients are at the later stages of the disease, when the tumor has spread to other organs. Treatment options include surgery and chemotherapy, either alone or in combination.

Chemotherapy is one of the key treatments for patients at all stages of ovarian cancer, with 54% of patients undergoing curative or palliative chemotherapy as part of their primary cancer treatment. Platinum-based drugs (cisplatin, carboplatin) are the most commonly used chemotherapy for ovarian cancer treatment. They work by causing damage to the DNA in cells and subsequently inducing cell death. Although patients are initially sensitive to platinum chemotherapy, about 70% of patients experience cancer recurrence, and many develop resistance to platinum therapy. As such, uncovering mechanisms that allow patients to become resistant to platinum therapy is a key area of research in the mission to improve survival outcomes for patients, particularly those with more advanced disease.

Dr. Dong-Joo (Ellen) Cheon is an Associate Professor of Regenerative and Cancer Cell Biology at Albany Medical College in New York. Her team's research focuses on understanding how patients can develop resistance to platinum chemotherapy over time. Conducting detailed analysis of clinical samples and validating their findings in a pre-clinical setting, they work to highlight key genes and pathways that are involved in promoting platinum resistance. These key genes are then explored as targets for the development of therapies and can help clinicians create a more personalized approach to treatment.



Plugging the Gaps

As the genetic expression found in a cell dictates its protein expression and therefore, its function, examining the gene expression profiles of patient tumors can give researchers an insight into changes that are driving the cells to become more cancerous and resistant to therapies. The genetic profile of different cancer types can also tell us a lot about how a patient may respond to treatment and how the tumor growth will progress over time.

With an increased understanding of a patient's genetic profile comes the opportunity to tailor treatment plans to be most effective against the key

therapeutic targets for that tumor. As such, much of the work in cancer research in recent years has moved towards improving our understanding of what drives the growth of tumors and their resistance to therapies. This involves an in-depth look into both the genetic and protein expression that occurs in different cancer types and in different patient groups, e.g., responders to treatments vs those who do not respond efficiently.

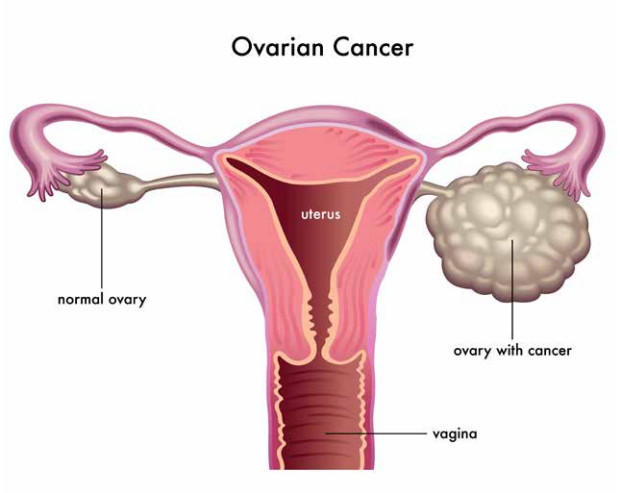
Currently, there is no fully validated or clinically applied test to guide treatment decisions in ovarian cancer. Although several research groups have used gene expression data to develop signatures that predict clinical outcomes in ovarian cancer, the different gene signatures described to date exhibit little overlap and lack the correlation to poor outcomes. Consequently, there is not only a critical need for markers that can assess the risk of poor survival in patients with ovarian cancer, but also for a better understanding of the mechanisms that are involved in tumor progression which can be targeted with novel treatment strategies.

Noting this important gap in the research literature, Dr. Cheon and her team set about analyzing patient genetic data from three different datasets (the Cancer Genome Atlas, GSE26712 dataset, and GSE51088 dataset) to identify common gene expression patterns across ovarian cancer patients. Their results showed 61 genes that were present in at least two out of three of the datasets, of which ten genes were expressed across all three. This group of ten genes was then taken as their 'genetic signature' for poor prognosis as they found high expression of these genes indicated worse overall survival.

Interestingly, the team found that most of the ten genes are known to produce stiff collagen matrices around cancer cells, highlighting the impact of the structural properties of cancer cells on patient survival.

Collagen Type XI Alpha 1 (COL11A1) was found to be the most significantly expressed gene out of the ten highlighted by the researchers. COL11A1 is mostly expressed by a subset of cancer-associated fibroblast cells adjacent to tumor cells, and a small number of cancer cells as well. COL11A1 has previously been shown to have increased expression in several other cancer types, including lung, pancreas, and colorectal cancers, with its high expression often being associated with poor survival, resistance to chemotherapy, and recurrence of the tumor.

Based on the results of their genetic analysis and previous work done in other cancer types, Dr. Cheon and her colleagues set out to validate that COL11A1 played a significant role in tumor progression. They found that removal of COL11A1 expression via genetic modification of cancer cells reduced tumor growth and decreased cell migration, invasion, and tumor progression, supporting the idea of its importance in tumor development. The team also discovered that COL11A1 makes cancer cells more resistant to cisplatin, supporting the clinical observation that COL11A1 is one of the top upregulated genes among patients who are resistant to platinum therapy than those who are sensitive. With these results, the team concluded that the role of COL11A1 made it a promising marker to target therapeutically but they wanted to understand in more detail what made COL11A1 so key for these processes in the tumor.



Delving Deeper into Discovery

Dr. Cheon and her team particularly examined the question of how COL11A1 makes ovarian cancer cells more resistant to platinum therapy. They analyzed proteins upregulated by COL11A1 and discovered that several key proteins of fatty acid oxidation (FAO) are upregulated by COL11A1. FAO is the metabolic process by which fatty acids enter the cell and are broken down to produce energy, reducing power, and biomolecules. This form of metabolism is common in cancers as rapidly proliferating cells rely on large amounts of fatty acids to support various biological processes including membrane formation and signaling. However, it is not fully understood which signals are produced by COL11A1 to regulate this metabolic switching, despite aberrant fatty acid metabolism being implicated in driving malignancy and chemotherapy resistance in several cancers. Therefore, Dr. Cheon and her team set about uncovering the pathways that lead from COL11A1 to metabolic switching and how this is linked to cisplatin resistance.

The team discovered that COL11A1 binds to the cell surface molecule and activates downstream signaling to increase FAO in ovarian cancer cells, making them more resistant to cisplatin. To try and work out the underlying mechanisms, they examined the activation of proteins downstream of COL11A1 and found that heat shock protein 27 (HSP27) activation correlated with levels of COL11A1 expression, suggesting a link between the two. Interestingly, HSP27 is a protein that has previously been implicated in cancer cell survival and resistance to chemotherapies across many cancer types. In ovarian cancer, several studies have established a relationship between this protein and poor patient survival.

In 2021, Dr. Cheon and her team investigated if there is a link between COL11A1/HSP27 expression and platinum resistance. They confirmed that COL11A1 increases HSP27 expression, and inhibiting the function of HSP27 caused re-sensitization of cells to cisplatin, suggesting that HSP27 mediates COL11A1-induced cisplatin resistance in ovarian cancer cells. The team also found that when HSP27 function is inhibited cancer cells upregulate FAO to survive during chemotherapy treatment without HSP27. When both HSP27 and FAO are inhibited, cancer cells show dramatic cell death after cisplatin treatment, suggesting that COL11A1 activates two parallel pathways-FAO and HSP27- to make cancer cells resistant to cisplatin. However, how exactly HSP27 inhibition upregulates FAO and how effective dual inhibition of HSP27 and FAO is in pre-clinical models remain to be determined.

Looking to the future

The work of Dr. Cheon has led to the important discovery that ovarian cancer patients who express high levels of the protein COL11A1 show poor survival outcomes and have a higher risk of developing chemotherapy resistance. Delving deeper into the reasons behind this, she has shown that COL11A1 promotes cisplatin resistance by increasing FAO in ovarian cancer cells. Using inhibitors to target this process, Dr. Cheon has shown that COL11A1^{high} cisplatin-resistant ovarian cancer cells can be effectively killed by FAO inhibitors in combination with HSP27 inhibitors. As such, these results provide a novel biomarker-guided targeted therapy for cisplatin-resistant ovarian cancer.

Although the link between COL11A1, HSP27, and cancer cell chemotherapy resistance has now been clearly established, understanding the full picture of how this occurs is a challenge that Dr. Cheon now faces. A key area of research will be improving our understanding of metabolism, in particular the metabolism of fats, within cancer cells and the environment surrounding the tumor. A particular interest is in the area of lipid droplets, a type of organelle made up of fats and proteins that help to regulate fat-based metabolism within ovarian cancer cells. Understanding how these organelles are formed and the proteins found within them will help the team pick apart the metabolic processes that allow ovarian cancer cells to aggressively grow. Dr. Cheon hopes that this will lead to a greater understanding of the regulation of platinum resistance within ovarian cancer cells and provide potential targets for the future development of therapies.

Meet the researcher

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Dr. Dong-Joo (Ellen) Cheon received her PhD in Genes and Development from The University of Texas Health Science Centre at Houston and MD Anderson Cancer Center. After a postdoctoral posting at Cedars-Sinai Medical Center as part of the Women's Cancer Program, Dr. Cheon went on to become an Assistant Professor at the Department of Regenerative and Cancer Cell Biology at Albany Medical College. Here, her research has been focused on the molecular mechanisms of resistance to chemotherapy in ovarian cancer with a particular spotlight on improving our understanding of the role of a novel collagen subtype COL11A1 in chemoresistance and its related signaling pathways. Among other accolades and achievements, Dr. Cheon is an active member of several professional societies and a keen mentor of students both inside her laboratory and in the local community.

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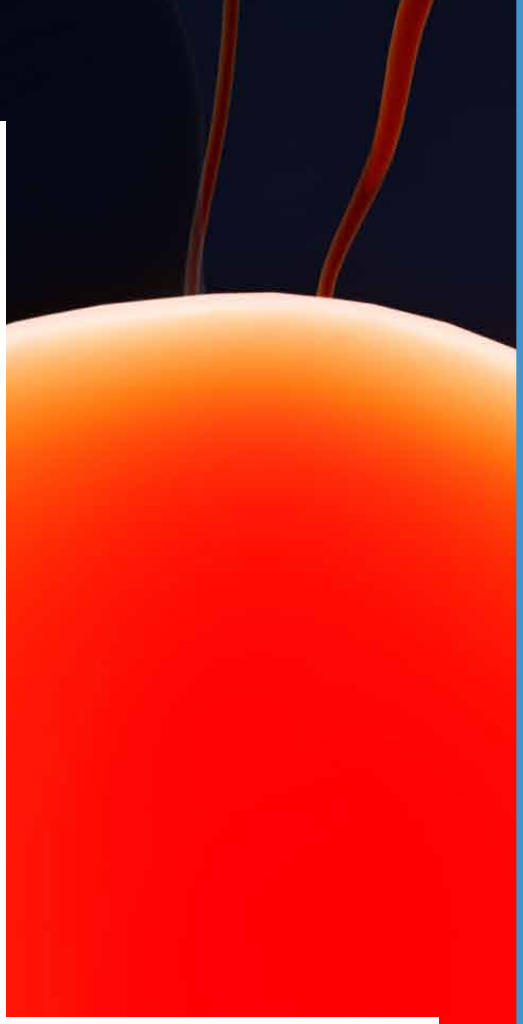
PROSTATE CANCER: THE IMPORTANCE OF NUTRITION IN PREVENTION AND TREATMENT

Prostate cancer is a leading cause of illness and death in men around the world, and to date, no prevention strategies have been discovered. **Dr Anait S. Levenson** and a team of cancer researchers from Long Island University in the United States of America are working to advance our understanding of how and why prostate cancer develops. Their important work also demonstrates how compounds found in foods such as grapes and blueberries may help prevent the development and progression of cancer.



Understanding Prostate Cancer

Cancer is a leading cause of death worldwide. In the United States of America, 1,898,160 new cancer cases and 608,570 cancer deaths were projected for 2021 but the reality for prostate cancer was even starker – an alarming increase was seen for both new cases and deaths.

There is currently no treatment available to prevent this type of cancer and 30% of men with risk factors placing them under active surveillance for prostate cancer develop more aggressive disease requiring intensive treatment such as surgery and hormone therapy (chemical castration), which have devastating effects on the individual and their quality of life.

As a result, scientists and healthcare professionals are dedicated to further understanding the pathways leading to prostate cancer and discovering new ways to prevent this disease. Dr Anait S. Levenson from Long Island University in the United States of America is one of the scientists driving a new understanding of the impact of diet on prostate cancer progression and prevention.

Understanding the underlying pathways of cancer progression is key to being able to optimise diagnostics, improve treatment and develop new medicines. Dr Levenson and her team are particularly interested in the molecular and genetic mechanisms of prostate cancer development and progression. To date, the team's research has focussed on the MTA1 protein, an epigenetic molecule which is highly expressed in several types of cancer, including prostate cancer.

It is now known that MTA1 is involved in multiple stages of prostate cancer including inflammation, tumour growth and invasion. In addition to regulating survival pathways through various epigenetic modulations, MTA1 can impact molecules called microRNAs (miRNAs for short) which control multiple cancer development pathways, including enhancing cancer progression and the ability of the cancer cells to spread (a process known as metastasis). Dr Levenson and her team highlighted the role of MTA1 in prostate cancer progression by studying mice genetically modified to overproduce MTA1. Their research showed that mice with higher levels of MTA1 were more likely to develop cancerous prostate cells than mice with normal MTA1 levels.

Diet and Cancer Prevention

Although the link between a person's diet and cancer risk is complicated, data have linked a diet high in dairy and fats with an increased risk of prostate cancer. More recently, diets high in certain fruits, vegetables, and soy foods have been associated with a decreased risk of prostate cancer.

As prostate cancer is an age-related and slow-growing disease likely influenced by nutrition, the use of diet for prevention and as an intervention alongside conventional treatment has huge potential. Nutrition-based intervention is a particularly exciting prospect given that there are currently no other preventative actions or treatment options available.

Most modern medicines are based on naturally occurring compounds.

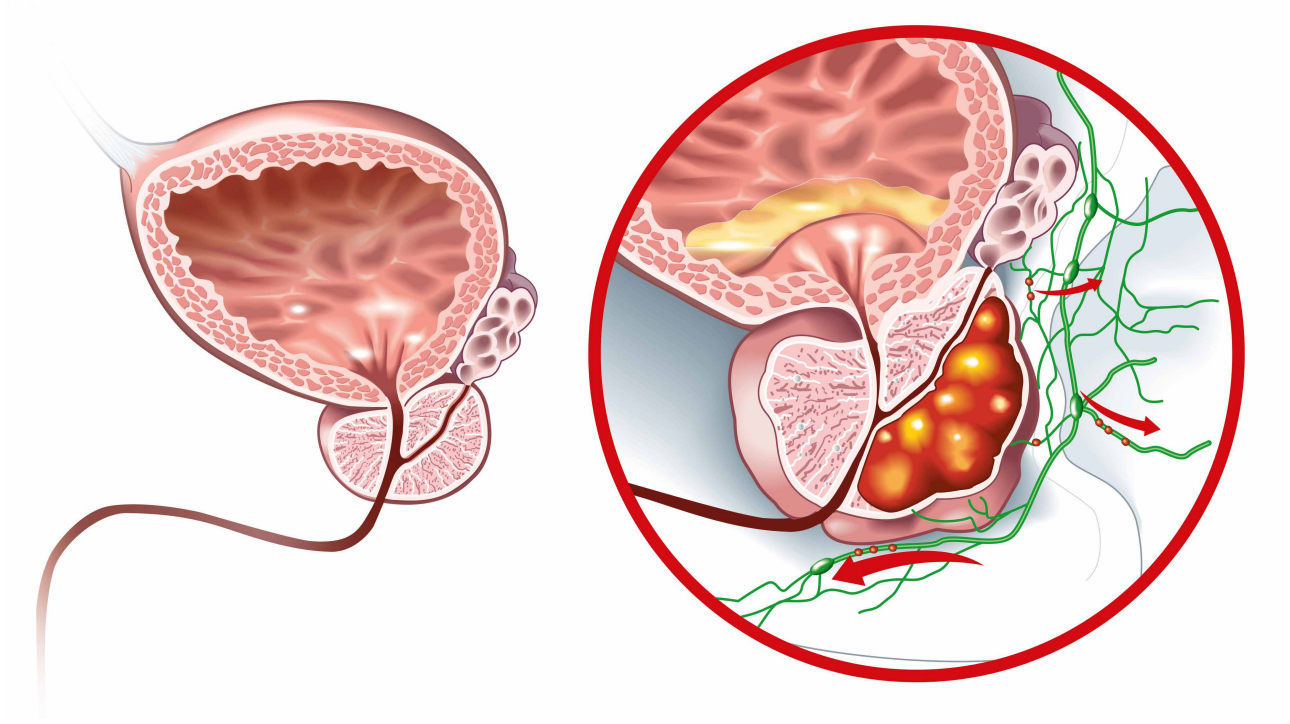


Illustration of healthy prostate and cancerous prostate

Grapes and blueberries contain high levels of stilbenes

Currently, scientists around the world are investigating the effects of diets high in these compounds as well as how these natural compounds can be modified to make more effective medicines.

Dr Levenson's team is currently investigating the preventive and therapeutic potential of naturally occurring compounds called polyphenols, which are found in plant-based foods such as fruits, vegetables, tea and dark chocolate. Previous studies have linked diets high in these compounds with a lower risk of developing cancer.

Importantly, Dr Levenson's research has focused on a type of polyphenol produced by certain plants when under environmental stress. These polyphenols, called stilbenes, are known to have antioxidant, anti-inflammatory, antimicrobial and anti-tumour properties. There are over 400 types of natural stilbenes which can be found in foods such as grapes (including wine), blueberries and peanuts.

Dr Levenson's team is currently working on resveratrol (which comes from grapes and is probably the most well-known stilbene), pterostilbene (which comes from blueberries), and gnetin C (which comes from the melinjo plant, commonly used in Indonesian food).

While the exact mechanisms underlying the beneficial effects of stilbenes are not yet completely understood, Dr Levenson's team has shown how stilbenes can protect against prostate cancer by targeting MTA1 and altering miRNAs, leading to tumour-suppressing genes being turned on and thus, cancer progression and inflammation pathways being slowed down.

Stilbenes in Practice

In 2013–15, Dr Levenson's team treated prostate cancer cells in the lab with resveratrol (a polyphenol found in grapes and wine) and pterostilbene (a polyphenol found in blueberries). They discovered that these compounds suppressed the growth rate of tumour cells and that pterostilbene was more potent than resveratrol in inhibiting MTA1.

Following on from this, the team treated mice which were genetically modified to overproduce MTA1 with a pterostilbene-supplemented diet. They found that the mice with higher levels of MTA1 were more likely to develop cancerous prostate cells than mice with normal MTA1 levels. They also discovered that the mice who had eaten a pterostilbene-supplemented diet had

reduced levels of MTA1, and certain miRNAs, and subsequently, showed fewer cancerous prostate cells.

In 2020, the team added grape powder (which contains both resveratrol and pterostilbene) to the diet of mice who were genetically predisposed to developing prostate cancer. They found that the mice who were treated with grape powder had lower levels of tumour-causing miRNAs and that these mice had less abnormal prostate cell growth.

Less is known about gnetin C, a resveratrol dimer, most commonly found in the melinjo plant, which is part of Indonesian cuisine. In an impressive series of experiments, Dr Levenson's team treated mice with prostate overexpressing MTA1 with gnetin C, resveratrol, and pterostilbene, and found that gnetin C was much more efficient at slowing tumour progression than either of the other two compounds.

By analysing the prostate tissue, the team could see that gnetin C slowed the progression of prostate cancer by reducing cell division, inflammation, and the formation of new blood vessels and by inducing cell death (apoptosis). These studies highlight the role that MTA1 and miRNAs play in prostate cancer development and demonstrate that dietary stilbenes can reduce prostate cancer progression.

Dr Levenson's findings have exciting implications for the future of prostate cancer prevention and treatment. The team's latest findings show how including more stilbene-containing foods in our diets may help protect both the general population and 'at risk' patients from prostate cancer. In a recent publication, Dr Levenson stated 'A substantial portion of prostate cancer cases could be prevented by applying effective "prostate cancer-specific diets" that contain bioactive dietary polyphenols and micronutrients'.

The Future of Stilbenes

Whilst there have not yet been any human trials on the effect of stilbenes on prostate cancer development, the future of these compounds is bright as the evidence increases to support their use to prevent and treat cancer. These natural dietary compounds have been shown to protect against prostate cancer in mice, and remarkably, they have been shown to make drug-resistant cancer cells in the laboratory sensitive to treatment again. Whilst the significant amounts of stilbenes in foods such as blueberries and grapes may help to prevent cancer from developing, the ability to modify these natural substances into potent anticancer drugs is a thrilling development in the quest to provide better outcomes for cancer patients.

Meet the researcher

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Dr Anait S. Levenson obtained her medical degree from the Second Moscow State Medical Institute and then went on to receive a PhD in Clinical Immunology from the Institute of Tuberculosis, Moscow. Dr Levenson's interests lie in cancer research and pharmacology. Her research group focuses on understanding the molecular and genetic pathways leading to prostate cancer progression and metastasis, as well as investigating the potential of natural dietary compounds known as stilbenes for cancer prevention and treatment. Dr Levenson is an internationally recognised leader in nutritional cancer chemoprevention, and she has published over 60 journal articles and seven book chapters. She is an active member of several leading professional societies including the American Association for Cancer Research, the New York Academy of Sciences, and the American Council for Medicinally Active Plants where she served as President for two years (2020–2022).

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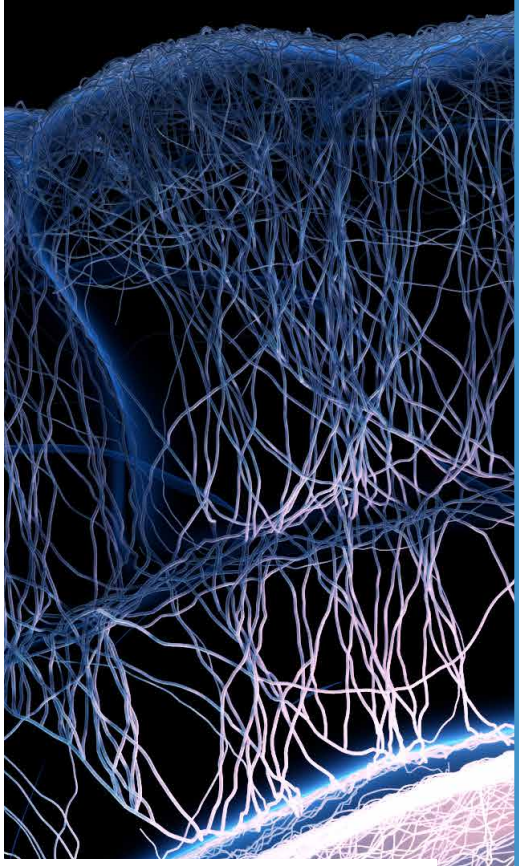
TACKLING AGGRESSIVE BRAIN CANCER WITH MICRORNA AND NANOPARTICLES



Glioblastoma multiforme is an aggressive and life-threatening form of brain cancer. Although some treatments are available to provide comfort and prolong life, it remains an incurable and devastating disease. With the goal of advancing diagnostics and treatments for glioblastoma, **Dr Hernando Lopez-Bertoni** is carrying out exciting research at the Johns Hopkins University School of Medicine. Taking on board the cancer stem cell hypothesis, Dr Lopez-Bertoni has made fascinating discoveries into how miRNA genetic material can be utilised and how it could be delivered to the brain via nanoparticles.

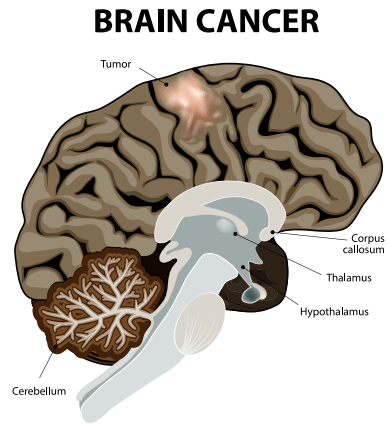
Glioblastoma: An Aggressive Brain Cancer

Glioblastoma multiforme is an aggressive, fast-growing and fast-spreading type of brain cancer. Although brain cancers as a whole are relatively uncommon, glioblastoma are the most common type of malignant brain tumour in adults. Usually starting in the largest area of the brain – the cerebrum – star-shaped cells called astrocytes build up to form glioblastoma. As they create their own blood supply, it is easy for these tumours to grow and invade adjacent brain tissue.

The rapidly growing nature of glioblastoma and the resulting pressure on the brain means that the first symptoms that appear are often constant headaches, difficulty thinking and speaking, blurry vision and unusual changes in mood or personality. Once a patient sees a doctor with these issues, they should be sent for an MRI or CT scan so any masses in the brain can be found.

Unfortunately, there is no complete cure for glioblastoma and it can be very difficult to treat, but the symptoms can be eased and patients can be put into periods of remission with certain therapies. Surgery is the initial option, whereby a surgeon tries to take out as much of the tumour as possible without damaging important structures of the brain. This is near impossible in some areas, meaning not all of the tumour can be removed. Radiation therapy and sometimes chemotherapy can be used to try to kill the leftover tumour cells or tumour pieces after surgery.

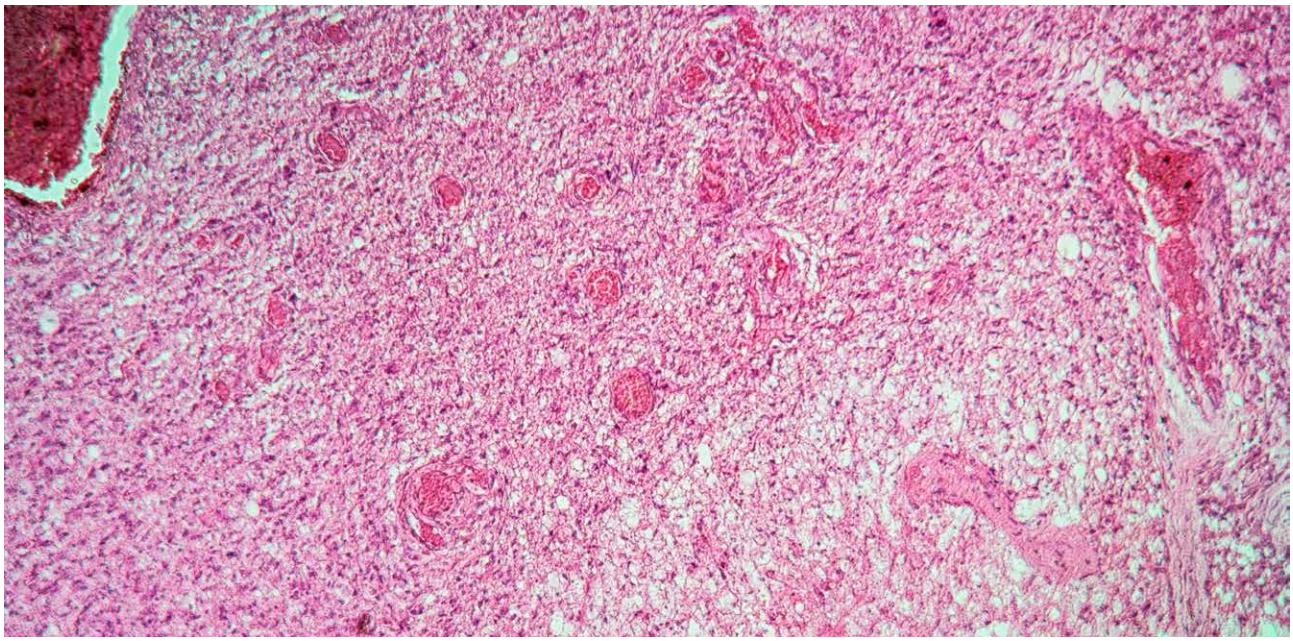
Other, innovative treatments are sometimes available. A method called wafer therapy involves implanting a biodegradable disc into tumour left over after surgery, which then slowly releases chemotherapy. Another method is called electric field therapy, which targets tumour cells with an electric field via electrodes on the scalp. However, the main goal of all treatments for this brain cancer is to slow down and control the tumour growth and to allow a patient to be comfortable; sadly only 5% of patients survive more than five years after diagnosis.



The Cancer Stem Cell Hypothesis

Although the outlook of glioblastoma seems bleak, ongoing clinical trials are in progress testing novel and innovative therapies. These treatments are the result of extensive, dedicated research of scientists all over the world. One such researcher is Dr Hernando Lopez-Bertoni at Johns Hopkins University School of Medicine in Baltimore, Maryland. Within the Lopez-Bertoni Lab in the Department of Neurology, Dr Lopez-Bertoni and his team, along with collaborators, investigate the underlying, molecular mechanisms behind brain tumours. They hope that

‘We want to expand upon our current experimental model and conceptual paradigms and identify miRNAs and tumour suppressive pathways capable of inhibiting GBM stem-like tumour-propagating phenotype and apply that knowledge to develop new therapeutic approaches.’



Diseased tissue from a tumour in the brain

their work will be incredibly useful for designing new, advanced and targeted therapies for the disease.

An underlying theory to Dr Lopez-Bertoni’s work is the Cancer Stem Cell Hypothesis. This theory suggests that the cells that make up tumours can be found at different levels of maturity (or differentiation), similar to normal, healthy tissue. Therefore, there is only a relatively small number of multipotent neoplastic stem-like cells that can multiply to form tumours. In more simple terms, these are cells that can divide into lots of different types of cells (like stem cells) to form an abnormal growth (a neoplasm). If this is the case, then eradicating the original cells that cause cancer, the ‘cancer stem cells’, could be an effective method of treating tumours and preventing them from returning.

Research has shown that these cancer stem cells are in fact present in most cancer types and they are largely responsible for tumour re-growth. Due to the adaptable, or plastic, nature of these cells they can divide to alter their growth rate and survival mechanisms to resist therapy and encourage tumour growth. This is why they are a primary target for Dr Lopez-Bertoni, who studies how these cells grow and divide in order to exploit their vulnerabilities and control their spread. His work involves delving into the genetic and molecular levels of the cell.

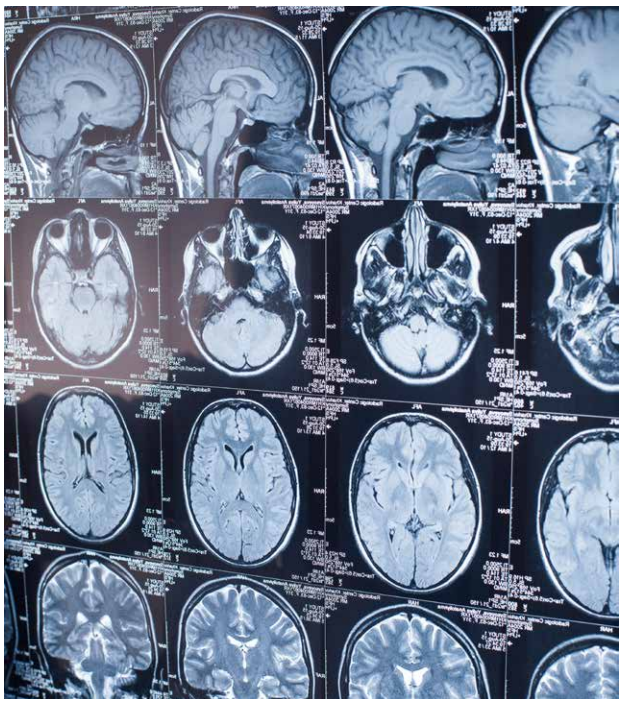
MicroRNAs and Tumour Suppressive Pathways

One of the tiny components of interest to Dr Lopez-Bertoni are microRNAs (miRNAs). These are short, single-stranded lengths of genetic material that do not code for anything but do play an important part in regulating gene expression. This means that they help to decide which genes are decoded to form proteins that make up the structure and the function of the cell. Importantly, the disrupted function of miRNAs has been proven to be a large causative factor in multiple different diseases, including cancer.

Research from Dr Lopez-Bertoni demonstrated that this is because the miRNAs regulate huge networks of gene expression. But at the same time, the process of making the miRNAs themselves can be altered which also impacts how the system works. According to Dr Lopez-Bertoni, ‘We want to expand upon our current experimental model and conceptual paradigms and identify miRNAs and tumour suppressive pathways capable of inhibiting GBM stem-like tumour-propagating phenotype and apply that knowledge to develop new therapeutic approaches’.

Studies from his lab have given evidence that encouraging miRNAs that suppress tumours or inhibiting those that facilitate tumour growth can be an effective method of normalising dysregulated molecular networks. For example, in a recent study, Dr Lopez-Bertoni newly identified a molecular mechanism that leads to glioblastoma.

This mechanism involves two transcription factors that also regulate which genes are allowed to be decoded into proteins, including those that code for miRNA strands. These transcription factors, called Oct4 and Sox2, repress specific miRNAs, which he found leads to the development of cancer cells in



the brain. Dr Lopez-Bertoni also discovered that reintroducing the repressed miRNAs into the stem-like glioblastoma cells, prevented them from acting like cancer stem cells. This was seen through a much-reduced ability to spread and a longer survival time in their animal model.

These exciting results reveal a promising approach to treating tumours. Dr Lopez-Bertoni believes that by identifying regulators of the molecular mechanisms within cancer cells, they can be targeted to create more effective therapies for glioblastoma.

A Small But Effective Delivery

Even if effective targets for glioblastoma can be identified, it is not always simple to get to them with drugs. This is because our brains are a highly protected organ – the skull, cushioning fluid and layers of special membranes all play a part. Within these membranes is what is known as the blood-brain barrier, which is a boundary between circulating blood and the brain itself. This barrier is highly selective, so it only permits the necessary nutrients and compounds through, thereby blocking toxic substances from affecting the brain.

While this is an essential function, it can create an issue for drug delivery. Many drugs would be too large or not allowed through the blood-brain barrier, so inventive solutions are needed to transport therapies into tumour-affected brains. This was the work of Dr Lopz-Bertoni in collaboration with Dr Jordan Green, also at the Johns Hopkins School of Medicine. In a pioneering experiment, they made tiny packets of the miRNA Dr Lopez-Bertoni had studied, wrapped in a biodegradable plastic similar to surgical sutures. These packets were so tiny they were 1,000 times smaller than the width of a human hair, which meant that they were small enough to pass through a blood-brain barrier.

Once the packets reach a brain cancer cell, they engulf it and it breaks apart to release the miRNA inside. As a result, the therapy is administered right into a glioblastoma, where it can take action against it.

Positive Results for MicoRNA and Nanoparticles

This was clearly seen in their tests, in which they implanted human glioblastoma cells into mice and waited until they had established tumours. Half of the mice were given medicine that contained tiny packets of active miRNA and the other half contained inactive miRNA. The mice that received the active miRNA lived significantly longer on average than the others and either had very small or no brain tumours at all.

Dr Lopez-Bertoni believes one reason this method could be so effective is that miRNAs can target multiple gene networks, in contrast to many other genetic medicines that only target one gene. By giving them direct access to the target cells and dysregulated genetic systems with nanotechnology, they could be a hugely promising solution to glioblastoma. In the future, he hopes to look further into how the blood-brain barrier could be overcome with ultrasound technology to enhance the uptake of nanoparticles.

Although Dr Lopez-Bertoni has already made significant contributions to his field of neurology, he is continuing to innovate and build on his work. He shows that studying the cellular mechanisms that cause glioblastoma can lead to inspiring new insights into how even currently difficult cancers to treat, may be less threatening in the years to come.

Meet the researcher

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Dr Hernando Lopez-Bertoni achieved his BSc in Biology/Microbiology/Pre-Med degrees at Kansas State University before moving to the University of Nebraska to complete a PhD in Cancer Biology. He then went to Johns Hopkins University School of Medicine in Baltimore, Maryland, to carry out his postdoctoral training in cancer stem cell and brain tumour biology. This is where he currently serves as an Assistant Professor, based in the Department of Neurology, conducting research into brain tumour biology and investigating new ways to treat brain cancer. His work focuses on understanding the molecular mechanisms behind brain tumour formation and how this might be used to develop new diagnostics and treatments.

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American Brain Tumor Association

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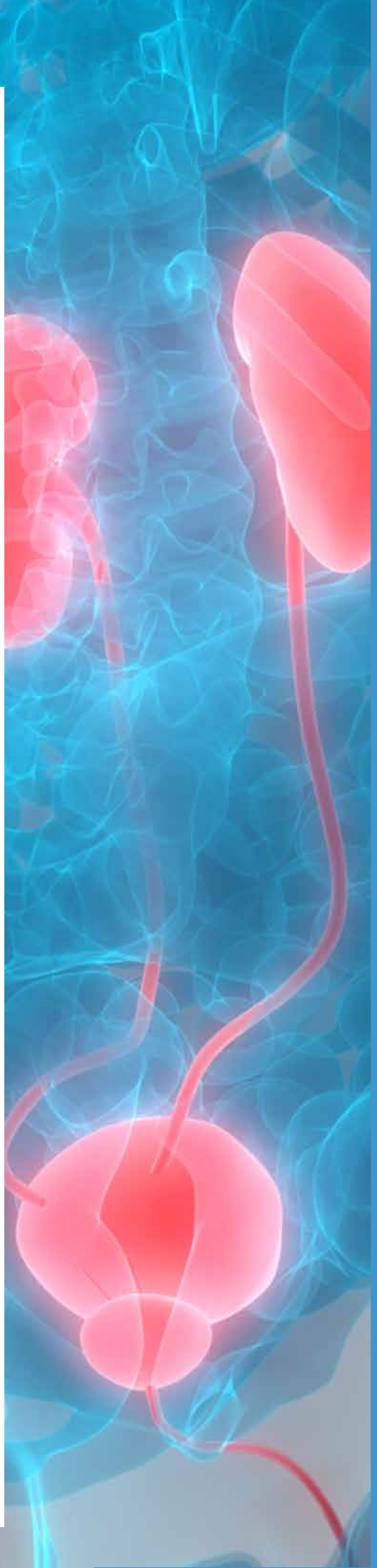
IMPROVING OUTCOMES IN PATIENTS WITH COMPLEX UROLOGY CONDITIONS: THE ERN EUROGEN EXPERIENCE

Patients with rare diseases and complex conditions pose unique challenges for clinicians, largely due to limited exposure to their associated anomalies. To overcome clinical obstacles, the European Commission launched a new Cross Border Health Innovation involving European experts in urology who have formed a European Reference Network (ERN) to facilitate knowledge sharing and skill development amongst healthcare providers. Recently, **Ms Loes Oomen** and colleagues in the Department of Urology at the Radboudumc Amalia Children's Hospital in The Netherlands have reviewed the clinical activity and procedures across this newly established network and identified potential areas for improvement.

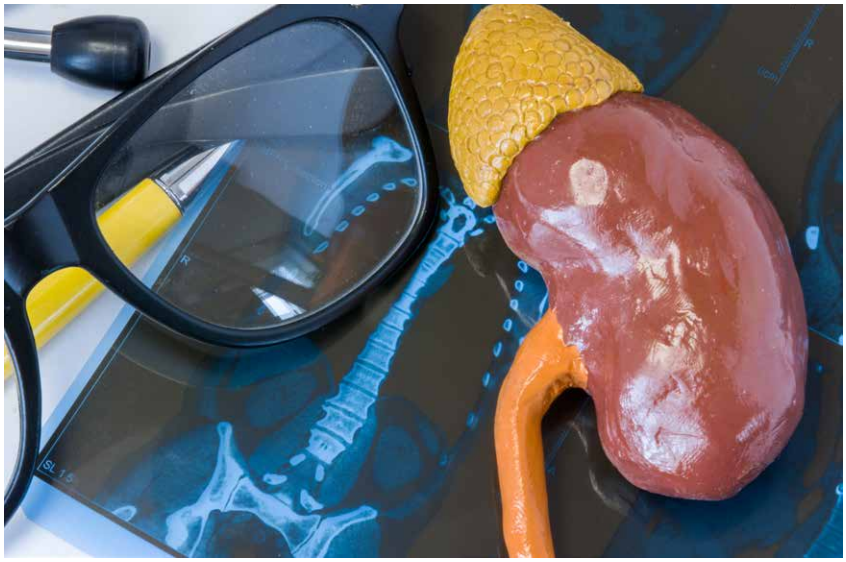


The Need for Knowledge Sharing

Clinicians face challenges in diagnosing and treating patients with rare and complex conditions due to limited knowledge, information, and exposure to uncommon symptoms and disease mechanisms. Urology is centred around the function of the urinary tract (kidneys, ureters, bladder and urethra) and the reproductive and related organs such as the rectum. As with many other medical specialities, urologists may encounter unusual variations of common ailments during their practice.

With this in mind, and following discussions with patients, politicians and healthcare specialists, the European Commission (EC) identified a need for so-called European Reference Networks (ERNs) – which are essentially pools of experts who work closely together with patient representatives to offer innovations in advice and practical solutions regarding complicated versions of specific diseases. A group of leading European urology practitioners formed ERN eUROGEN to garner wide-ranging expertise regarding rare urological diseases and complex conditions and share knowledge with healthcare providers across Europe.

Ms Loes Oomen and colleagues in the Department of Urology at the Radboudumc Amalia Children's Hospital in The Netherlands have presented their findings following a comprehensive overview of ERN eUROGEN's clinical practices since its inception, and have identified specific challenges around data collection and definitions of disease, which have contributed to discrepancies in the documentation of information using the continuous monitoring system developed by the EC.



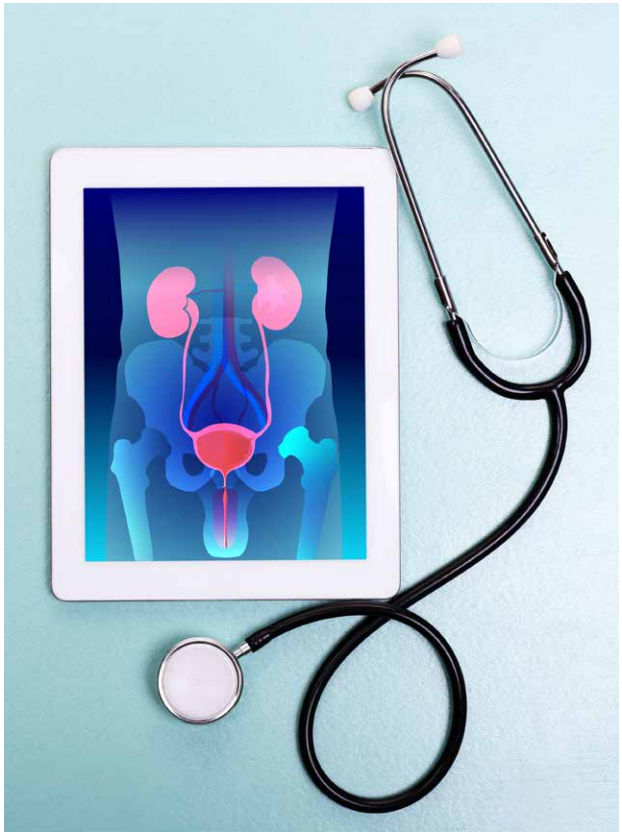
Establishing the ERN eUROGEN Network

Following a stringent application process, the newly formed ERN eUROGEN became operational in 2017 boasting an initial membership of 29 centres across 11 EU Member States. At present, ERN eUROGEN comprises 57 Healthcare Provider Full Members and Affiliated Partners from 20 EU Member States. With a collective mission to reduce healthcare inequalities across Europe, the main aims of the network include facilitating the exchange of clinical information through a centralised patient management system, the production of evidence-based clinical practice guidelines, the development of clinical decision support tools, and a longstanding patient registry for supportive evidence.

Additionally, ERN eUROGEN promotes education, research, and the generation and sharing of evidence using an innovative disease registry. To simplify the network structure as much as possible, diseases were allocated to one of three categories, each with an overarching description of the contents and with a different team of experts managing the group. The categories were separated into congenital anomalies, functional conditions requiring specialised surgery, and rare urogenital tumours.

Improving the ERN eUROGEN Network

Ms Oomen and the team started by analysing data on the total number of patients requiring long-term care, the total number of new patients, and the total complex surgeries performed each year across all pre-determined disease categories at the 29 initially enrolled centres between 2013 and 2019. They noted that effective monitoring of clinical activity benefits from a standardised terminology system to



reflect the diagnosis and treatment of rare diseases, which are defined as clinical conditions affecting ≥1 in 2000 individuals and involving ≥2 organ systems.

Along with the International Classification of Diseases database maintained by the World Health Organisation, the EC created a new coding system specifically for rare diseases, and in combination, these formed unique diagnostic codes to be used by all members of ERN eUROGEN to classify rare and complex urological conditions using Orphanet codes (when applicable) and ICD10 codes.

During the specified period there were variations in the number of patients and surgical procedures, although overall, >122,000 patients with rare and complex urological diseases required long-term care, and clinical activity increased year-on-year in all centres. There were notable differences in the clinical activity in each category, with some displaying minor decreases and others showing increases of ≥300%.

Reliable data analysis was found to be hindered by inaccurate coding and record keeping, with some clinics relying on transcribing details from paper files, thus increasing the likelihood of errors. The use of some disease codes was also found to be inconsistent, resulting in further inaccuracies. Whilst the issues arising regarding patient numbers during the application process have since been rectified and further validation of the disease identification codes completed, there remains a need to ensure that all members of ERN eUROGEN agree on disease terminology to maximise data quality and promote meaningful analysis.

Care, Share, Cure

To facilitate easier data extraction and more precise analysis, it is important to achieve further clarity in disease classification and standardise coding systems, thereby reducing variability. This will not only validate the findings but also aid in the ongoing development of ERN eUROGEN and contribute to a more accurate representation of the European population and beyond. Ultimately, this will help achieve the overarching goal of improving treatment coverage and promoting health equality.

This insightful study demonstrates how ERN eUROGEN (www.eurogen-ern.eu) has made great strides in gathering novel urological knowledge from a swathe of respected clinicians, analysing data from many thousands of patients with rare and complex conditions.

With Ms Oomen's ever-expanding expertise, and a lauded scientific community working to ensure that high levels of proficiency are maintained within the network, this impressive EU Health program for patients with a rare disease or complex condition can only flourish. The generation of top-quality data, the streamlined extraction and analysis of information, and the creation of a centralised patient registry will further assist in attaining the vital understanding necessary to achieve ERN eUROGEN's ultimate aim to 'Share. Care. Cure.' (where possible), whilst promoting equal access to healthcare for generations to come.

Meet the researcher

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Loes Oomen attained both her Bachelor and Master in Medicine degrees from Radboud University and is currently studying for a PhD in Paediatric Urology whilst working as a Clinical Specialist in the European Reference Network eUROGEN. Whilst Ms Oomen's research to date has primarily centred around paediatric kidney transplantation, her current work with eUROGEN is focused on improving knowledge exchange and expertise around rare and complex urological conditions to optimise patient outcomes and reduce global health inequalities. Ms Oomen is a staunch advocate for human rights and has experience in project management, data collection and analysis. Additionally, she has contributed substantially to several publications in highly regarded scientific journals and has been employed in diverse patient-facing roles as an adjunct to her postgraduate academic career.

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KIDNEY DISEASE AND URINARY TRACT CANCER: HOW A TRADITIONAL MEDICINAL PLANT CAUSES SERIOUS HEALTH ISSUES

This is a recounting of a scientific investigation into a mysterious but potentially deadly disease that first came to light principally in the Balkan states, and then several Asian countries. It manifests itself first as a nephropathy resulting in the destruction of kidney tissue often followed by a cancer of the upper urothelial tract. **Professors Arthur Grollman** and **Francis Johnson** at Stony Brook University have achieved a revolutionary understanding of the molecular biology, epidemiology and root cause of both diseases. Their findings have critical implications for medical and scientific communities, as well as the general public.

A Rare Kidney Disease Endemic to the Balkans

Within the region of the Balkans, there is an unusual disease known as Balkan endemic nephropathy (BEN). People who live in this southeast area of Europe have an unusually high risk of developing chronic renal (kidney) disease. It progresses slowly so that only those aged 20 years old show symptoms, but once it manifests itself, the outcome can be disastrous. Accompanying these symptoms is an excess of protein in the urine (proteinuria), which goes unnoticed until sickness occurs and certain clinical tests are done.

Often, people suffering from BEN will develop tubulointerstitial fibrosis, which is the accumulation of collagen and other protein fibres in the spaces between the cell membranes of the kidneys' tubules. Additionally, in the advanced stages of the disease, the kidneys shrink so dramatically that they are only 2–3 cm across, compared to a healthy 10–12 cm. Eventually, this leads to renal failure and if renal dialysis is not duly administered, BEN is universally fatal. Adding even further to the burden of this disease, half of the people who have BEN also develop cancer. These tend to be urothelial carcinomas of the upper urinary tract (UUC).

One of the most unusual aspects of BEN is its specific and unchanging geographic distribution. It was first described by a scientist in 1956 after physicians in certain areas of Bulgaria had noticed an abnormally high prevalence of kidney disease. Following a large study of residents, it was seen that people in specific villages were at very high risk. But more than that, it could be seen running in families and even within households. Health science experts gathered through the World Health Organization and eventually named and classified Balkan endemic nephropathy as a new disease.

However, it was not until several years later that theories for the cause of BEN were propounded, studied and confirmed. This has been the work of Professors Arthur Grollman and Francis Johnson in the Renaissance School of Medicine at Stony Brook University in New York, USA. Together, they have conducted ground-breaking research into the aetiology (cause) of BEN, and in the process, discovered a vital link with a similar disease in Asia.

Finding the Acidic Culprit of BEN

Professor Grollman's initial interest was sparked by a study of a group of healthy

Traditional Chinese medicines

Belgian women who had developed chronic kidney disease with many requiring dialysis, transplantation and cancer care. The factor linking them together was a slimming regimen they all followed involving a mixture of Chinese herbs and the disease was dubbed Chinese Herbs Nephropathy (CHN). One of these plants was *Aristolochia clematitis (A. clematitis)*. Studies had shown that when the aristolochic acid within this plant is ingested, it is metabolised into a product that eventually causes cancer.

Picking up on the similarities between BEN and CHN, Professor Grollman and his team set out to investigate the potential links between *A. clematitis* and nephropathy. In Croatia, they found that the plant commonly grew in crop fields, as it was considered a harmless weed. Subsequently, traditional wheat-harvesting and milling methods allowed the seeds of *A. clematitis* to end up in the locally baked bread. The researchers concluded that when a person ingests aristolochic acid through their diet, and if they have a pre-existing genetic sensitivity, they are at high risk of nephropathy and UUC. Importantly, they determined that BEN and CHN are actually the same disease, which they called Aristolochic Acid Nephropathy (AAN).

The next step in understanding AAN was to clarify how aristolochic acid interacts with the body physiologically and why this leads to severe health issues. To do this, Professors Grollman and Johnson needed to draw on their chemical-biology expertise.

The DNA Mutations That Lead to BEN

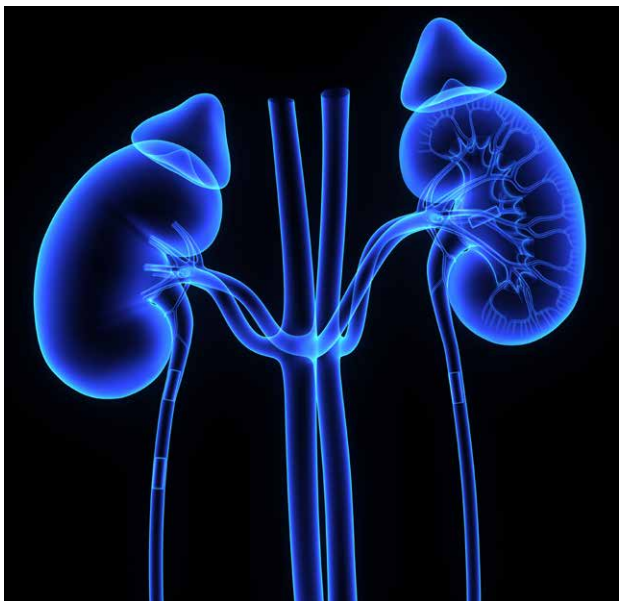
Metabolism of aristolochic acid results in a reactive intermediate which reacts with DNA to create an adduct. The latter is the name for a basic unit of DNA that is attached to a carcinogenic chemical, and this particular adduct is referred to as AL-DNA. It provides a significant connection between exposure to and ingestion of aristolochic acid and subsequent kidney disease and cancer.

During Professor Grollman and Professor Johnson's investigations, they discovered unusually high levels of AL-DNA adducts in the tissue of patients residing in areas where nephropathy and UUC were endemic. Building on this observation, the researchers explored how these adducts arise and what their consequences are, on a molecular and genetic level.

Professors Grollman and Johnson synthesised AL-DNA adducts and introduced them into cells to see how they would be processed by the cell's machinery. Although this damage to the DNA was mostly corrected by the cells themselves (a normal cellular cancer-preventative measure), some was still processed for replication. The new strands that were coded for by the damaged DNA frequently contained A to T transversion, a point mutation on DNA whereby an arginine base is incorrectly replaced by a tyrosine base.

Critically, the research team discovered that this mutation often takes place on the gene, *TP53*. This is a tumour-suppressor gene and the protein that is synthesised from it plays a vital role in preventing the development of cancer. In healthy cells, it achieves this by preventing the uncontrolled cell division characteristic of cancer, but when it is mutated or missing, it cannot fulfil its duties.

These are fascinating findings because they demonstrated that aristolochic acid is the disease-causing agent for both endemic neuropathy and UUC and uncovered unique biomarkers of the



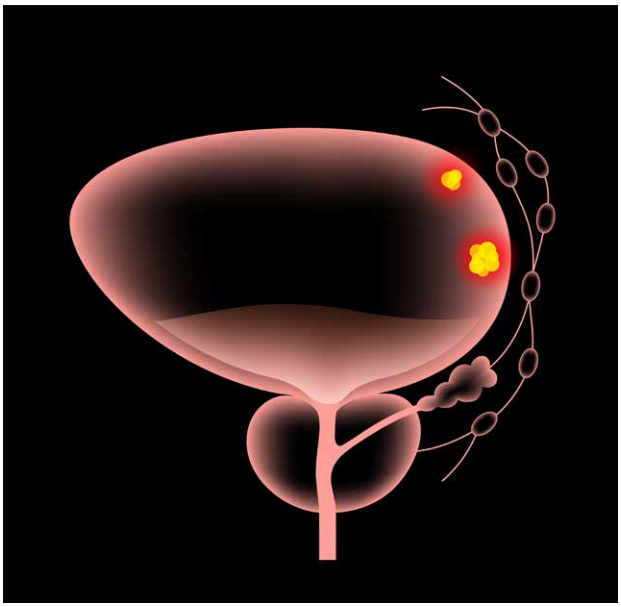


Illustration of the kidneys

disease. Biomarkers are biological measures or characteristics that can reveal the state of a disease. In this case, the markers are the AL-DNA adducts in the kidneys and the specific *TP53* mutations induced by the metabolic products of aristolochic acid and which can be measured.

Traditional Herbal Remedies Could Have Serious Health Consequences

Professors Grollman and Johnson then utilised their new biomarkers to understand how widespread globally the nephropathy and UUC caused by aristolochic acid actually was. *Aristolochia* plants have been used in traditional Chinese medicines for thousands of years, with most people being unaware of their toxicity. To investigate whether this was causing an unrecognised global health issue, the researchers selected the country with the highest incidence of chronic kidney disease and UUC worldwide for further studies – namely, Taiwan.

Aristolochia herbal medicines are commonly used in Taiwan (and elsewhere in Asia), leading to an estimated one-third of the country's population ingesting large amounts of aristolochic acid. They began a study to ascertain whether *Aristolochia* in these traditional medicines was indeed the culprit for the unusually high prevalence of kidney disease. When the researchers tested patients with UUC, they discovered over half of those with *TP53* mutations showed the biomarker A to T transversion in almost identical patterns to the residents in the Balkans. Many of the patients also had AL-DNA adducts in their kidneys.

This groundbreaking research conclusively revealed that the extensive exposure to aristolochic acid in Taiwan had led to a significant proportion of the nephropathies and UUC identified there, solidifying the evidence that BEN, CHN and AAN are all the same ailment. Professor Grollman notes that while this finding is an important one, it is also a worrying one because the damage done to the kidneys and renal DNA by aristolochic acid is irreversible. As these effects only physically present themselves decades after initial ingestion of the herb, banning it in herbal medicines will not have an impact on the incidence of AAN and UUC for at least 20 years, but should prevent further cases from arising beyond this time period.

The extent of *Aristolochia* herbal medicine usage in mainland China is similar to that in Taiwan, and Taiwan formerly imported the herb from there. Professor Grollman estimates that the thousands of tonnes of *Aristolochia* produced in China each year could be sufficient to cause disease in 300 million people residing within the country.

However, Taiwan is not the only country that imports the traditional medicine, and around 10% of the *Aristolochia* produced in China is exported around the globe to Chinese ex-patriate populations, including that in the USA. The continuation of using the herb as a remedy is putting hundreds of thousands of people across the world at risk of chronic renal disease and upper urothelial cancer.

The research of Professors Grollman and Johnson has clearly shown that the ingestion of aristolochic acid, whether intentionally or otherwise, has serious global health implications. Their work has demonstrated that using epidemiological approaches coupled with chemical biology methods can produce novel and crucial findings for health. They continue to build on their research as they study which genes make a person more susceptible to aristolochic acid-induced diseases, how biomarkers can be put to use to predict those most likely to become ill, and how their work can now be utilised for global health interventions.

Meet the researchers

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Dr Arthur Grollman received his BA in Chemistry at the University of California and his MD from the Johns Hopkins School of Medicine. Following further clinical training at Johns Hopkins, he conducted research at the National Institutes of Health, then joined the faculty at the Albert Einstein College of Medicine. Dr Grollman was founding Chairman of the Department of Pharmacological Sciences in the Renaissance School of Medicine at Stony Brook University, serving simultaneously as Attending Physician at the Stony Brook University Hospital. He holds the titles of Distinguished Professor of Pharmacological Sciences, Glick Professor of Experimental Medicine, and Director of the Leo and Judy Zickler Laboratory of Chemical Biology.

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Stony Brook University

HUMAN IMMUNODEFICIENCY VIRUS CO-MORBIDITIES: HOW LIPID HOMEOSTASIS ALTERATIONS LEAD TO CARDIOVASCULAR AND NEUROLOGICAL DISORDERS

Although human immunodeficiency virus (HIV) is still prevalent worldwide, life-saving antiretroviral drugs can now prevent an infection from progressing into acquired immunodeficiency syndrome (AIDS). Nevertheless, people who are HIV-positive are still at increased risk of developing neurological disorders and cardiovascular diseases, known as co-morbidities. **Professor Michael Bukrinsky** from the George Washington University in Washington DC works to understand the underlying biological mechanisms that lead to these disorders. His research has produced interesting results that demonstrate the role of altered lipid (cholesterol) homeostasis in HIV-infected cells and how this comes to pass.

Uncovering Causes and Proposing Solutions

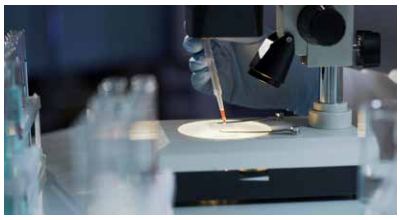
Over 37 million people worldwide are infected with human immunodeficiency virus (HIV), and an estimated 1.7 million of these are children. The virus replicates by entering immune cells, particularly CD4+ T cells and macrophages, and multiplying inside them. Once new HIV virions have been produced, they burst out of the host cell to repeat replication, which kills the cell in the process. Due to viral reproduction in the immune cells, the immune systems of people infected with HIV are greatly impacted and they become susceptible to additional diseases.

HIV is spread via certain bodily fluids and can, therefore, be transmitted during unprotected sex, sharing needles and during pregnancy or breastfeeding. Although initial infection may result in a few weeks of flu-like symptoms, many people usually will not present any symptoms for a number of years. However, if it is left untreated, HIV leads to acquired immunodeficiency syndrome (AIDS), whereby the immune system is so damaged and unable to repair itself, that previously minor infections and illnesses become life-threatening.

Thankfully, treatments have been found that allow those living with HIV infection to live long, healthy lives. These treatments come in the form of antiretroviral drugs, which prevent the virus from infecting new T cells and macrophages and in turn, allow the immune system to repair itself. Eventually, taking these tablets every day results in an undetectable viral load, meaning that the amount of HIV in the body is so low that it cannot be detected by a test and the risk of transmission is minimal.

Although impressive progress has been made to improve the lives of those living with HIV/AIDS, research has shown that they still have a high risk of co-morbidities. These are ailments that are not caused by a damaged immune system, but by other, HIV-associated, yet less clear mechanisms. For example, people who are HIV-positive are more likely to have high blood pressure and high cholesterol, develop heart disease, kidney disease, neurological issues and cancer.

Uncovering the reasons behind and solutions to these troubling HIV co-morbidities is Professor Michael Bukrinsky from the Department of



Microbiology, Immunology and Tropical Medicine at the George Washington University in Washington DC. In collaboration with scientists across the globe, Professor Bukrinsky is working to elucidate the molecular mechanisms behind these comorbidities so that they can be targeted with therapies.

Lipid Homeostasis in HIV-infected Macrophages

The role of cholesterol in cardiovascular diseases has been well established. However, prior to Dr Bukrinsky's studies, there has been little investigation into the impact HIV has on cholesterol homeostasis. Cholesterol is an essential lipid (or fat) in the body that aids in the synthesis of cell membranes, hormones and vitamins. But when there is an imbalance between the high-density lipoprotein (often called 'good' HDL) and low-density lipoprotein ('bad' LDL) forms, the risk of cardiovascular diseases increases. An excess of LDL in the bloodstream also frequently leads to

Professor Bukrinsky's group during an informal lunch meeting

atherosclerosis, which is a thick deposit of cholesterol, fat and calcium within an artery. As this deposit (known as a plaque) grows, the artery narrows and a blood clot trapped in the small space can result in a heart attack or stroke.

Professor Bukrinsky and his colleagues carried out research to discover why this is so common in HIV patients. His team included Professor Dmitri Sviridov at the Baker Heart and Diabetes Institute, Dr Zahedi Mujawar (Astra Zeneca, a former graduate student), and Professor Michael Fitzgerald at Harvard Medical School. They discovered that the virus inhibits a cholesterol transporter protein in macrophages – a type of white blood cell in the immune system. This transporter is called ATP-binding cassette transporter A1 (ABCA1) and when it is prevented from functioning normally, cholesterol cannot leave the macrophage cell. The inhibition of cholesterol efflux is a condition previously documented as causing high atherosclerosis risk.

The team ascertained that in HIV-infected cells, this inhibition event was regulated by an HIV protein called Nef, and further investigation found that two biological mechanisms were responsible. First, Nef binds to and down-regulates ABCA1, meaning that it reduces its abundance – and thus, its ability to facilitate cholesterol efflux. Second, Nef interacts with another cellular protein, calnexin, which regulates glycosylation and maturation of ABCA1. As a result, ABCA1 cannot mature and is sent to the degradation pathway as a cellular defence reaction to the accumulation of immature proteins. Together, these mechanisms cause the reduction of ABCA1 and prevent the transfer of cholesterol to apolipoprotein A-I, a major component of HDL cholesterol. These processes result in an imbalance of blood lipoprotein cholesterol.

Studies by Dr Brichacek in Professor Bukrinsky's group further demonstrated that the described above effects of Nef are also reproduced by treating cells with Nef-containing extracellular vesicles (EVs). These vesicles are produced by HIV-infected cells and persist in infected individuals even when the virus is undetectable. The reason for this is that the antiretroviral drugs used to treat infected patients prevent new infections, but do not block low-level production of Nef in infected cells. Circulation of these Nef-containing EVs may be the reason for the persistence of co-morbidities in successfully treated patients with undetectable HIV load.

Professor Bukrinsky's studies also showed that HIV-infected and Nef-EV-treated macrophages accumulated a significant amount of lipids within them. This gave them a resemblance to foam cells, a type of macrophage that sticks to blood vessel walls, internalising LDL and facilitating atherosclerosis formation. In addition, cholesterol enrichment leads to changes in the cellular membrane, increasing the size and number of cholesterol-rich islets called 'lipid rafts'. These lipid rafts are places where cellular receptors sensing and responding to inflammation accumulate, making cholesterol-rich macrophages overresponsive to inflammatory stimuli. This mechanism may underlie another important cause of atherosclerosis in HIV-infected individuals, persistent inflammation. When Professor Bukrinsky stimulated cholesterol efflux from HIV-infected macrophages, the HIV viruses that were produced inside were much less infectious. This shows that the virus requires a certain, high cholesterol level within its host cell to maximise its replication ability, and HIV achieves this by preventing cholesterol efflux.

These exciting results revealed brand-new mechanisms by which HIV Nef regulates intracellular lipid homeostasis within HIV-infected cells and cells encountering Nef-containing EVs. In this way, Professor Bukrinsky's work showed how HIV infection of macrophages contributes to the co-morbidity of atherosclerosis and its associated risks. A subsequent study with Dr Ruth Hunegnaw (National Institutes of Health, a former graduate student) and Professor Alexei Adzhubei (Engelhardt Institute of Molecular Biology) and Amol Kulkarni (Howard University) revealed that specific interactions with Nef could be blocked and therefore potentially targeted with drugs to prevent HIV from impacting cholesterol homeostasis and thus, reduce the risk of atherosclerosis.

Neurodegeneration in HIV

Professor Bukrinsky's group has further studied lipid rafts, the solid structures in the plasma membrane and the barrier between a cell and its surroundings. These rafts are full of cholesterol molecules and help to mediate interactions with the cell's environment. Whilst these interactions are usually beneficial, pathogens can take advantage of lipid rafts and use and even modify them to facilitate their own replication. Lipid raft therapy can be implemented as a treatment for viral infections by reducing unwanted modifications of lipid rafts or preventing viral interactions with these regions, and Professor Bukrinsky suggests this may even be an effective treatment for COVID-19.
Similar to atherosclerosis, lipid raft structures are key players in HIV-associated neurocognitive disorders (HAND), which are very frequent even in HIV patients receiving treatment. HAND is often seen as behavioural changes, declining cognitive function

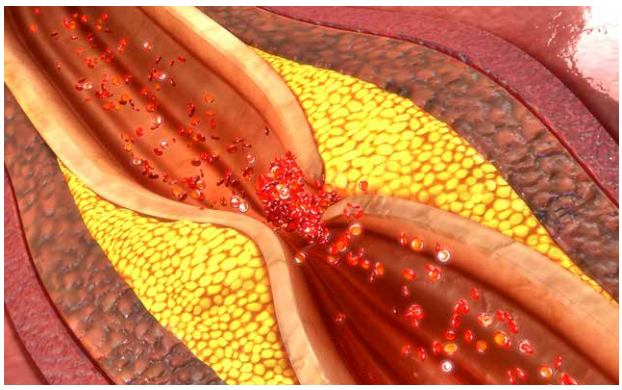


Illustration of coronary artery plaque

and motor impairment, and Professor Bukrinsky studied the role of Nef in HAND.

His studies showed that the Nef protein secreted from HIV-infected cells in EVs was rapidly internalised by neural and glial cells, and as seen in previous experiments, reduced ABCA1 abundance and cholesterol efflux, and also increased the number of modified lipid rafts in the plasma membrane.

Nef EVs also caused the increase and redistribution of amyloid precursor protein (APP) and Tau to lipid rafts, both of which are associated with brain disorders. With the addition of the activation of inflammatory pathways, Professor Bukrinsky observed consequent neuronal functional impairment which was reversed when the lipid rafts were disrupted. These findings were supported by evidence from brain tissue of deceased HIV patients with HAND, which showed lower levels of ABCA1 and more lipid rafts compared to HIV-negative brain tissue. Additionally, an abundance of Nef in the HIV-positive tissue correlated with APP and Tau levels. Finally, Jessica Schenck, a graduate student in Bukrinsky's laboratory, demonstrated that Nef-carrying EVs cause demyelination of the brain. Myelin forms cholesterol-rich sheaths of neurons required for neuronal functions, and cholesterol transport is essential for their maintenance.

As a result, Professor Bukrinsky concluded that the cholesterol homeostasis alterations arising from the presence of Nef in the brains of HIV-infected people may contribute to neurodegeneration and HAND. Understanding the underlying mechanisms of disease facilitates the development of therapies to target them, and Professor Bukrinsky's research suggests that treatments preventing Nef-induced alterations of cholesterol homeostasis and/or normalising lipid raft structure may reverse HAND.

Discovering HIV-Specific Memory

The most recent discovery by Larisa Dubrovsky, a scientist in Professor Bukrinsky's group demonstrated that cells treated with Nef EVs acquire a memory of this encounter. This memory is maintained by epigenetic (not involving genetic material) changes that regulate the expression of pro-inflammatory and lipid metabolism genes. As a result, cells, and in particular macrophages, become overresponsive to inflammatory stimuli.

This mechanism supplements and potentiates the mechanisms described above. The length of this memory is extended by the possibility that Nef EVs affect progenitor cells in the bone marrow, thus potentially impacting cells produced from these progenitors for a very long time. This finding adds another complication to be considered in the efforts to cure HIV infection. Indeed, besides silencing HIV, either by eliminating it from the body or by permanently blocking the virus from producing proteins and nucleic acids, care should be taken to clear the memory formed during the encounter with the virus or Nef EVs. Professor Bukrinsky's studies support the development of approaches aimed at reversing or blocking the establishment of this memory.

Discovering and Blocking a Pro-inflammatory Protein

While Professor Bukrinsky has devoted much of his research to HIV and its co-morbidities, his laboratory explores other avenues of research as well. These include obtaining a better understanding of inflammatory diseases, the biological interactions that cause them and how they can also be targeted by drugs. Studies with Professors Yurchenko (University of Ostrava) and Sherry (Feinstein Institutes) identified that extracellular proteins called cyclophilins and their receptor on immune cells called CD147 regulate various inflammatory disorders such as acute lung inflammation, cardiovascular disease and rheumatoid arthritis. CD147- mediated signalling by cyclophilins guide immune cells to the sites of inflammation.

Therefore, Professor Bukrinsky and his colleagues believed that targeting these interactions would be a promising possibility for anti-inflammatory therapeutics. Together with Professors Stephanie Constant (National Institutes of Health) and Gunter Fischer (Max Planck Research Unit) he developed and patented an exciting new drug that blocks the action of extracellular cyclophilin A. They hope that this could be used to alleviate the symptoms of both acute and chronic inflammation and mitigate the subsequent risks associated with it.

Meet the researcher

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Professor Michael Bukrinsky received his first degree in Biochemistry from the Second Moscow State Medical Institute before completing a PhD at the Institute of Molecular Biology in the USSR Academy of Sciences. Professor Bukrinsky has served in many teaching and research roles throughout his career and currently works at the George Washington University School of Medicine and Health Sciences in Washington DC. Here, he is a Professor in the Department of Microbiology, Immunology & Tropical Medicine and also Professor in Biochemistry and Molecular Medicine. His studies investigate successfully treated HIV cases that still result in persistent co-morbidities, and have been widely recognised with prestigious awards.

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BRINGING AN END TO THE HIV EPIDEMIC THROUGH UNIVERSAL TESTING AND TREATMENT

Worldwide, an estimated 38 million people are living with HIV. Many are still unaware of their status and so are not linked to care and treatment which can prevent them from passing HIV onto their partners and infants and keep them healthy. Dr Richard Hayes from the London School of Hygiene and Tropical Medicine in the UK has led the HIV Prevention Trials Network's 071 (PopART) trial over the past ten years. This dedicated international collaboration of experts has successfully demonstrated how universal testing and treatment can reduce new HIV infections in Zambia, South Africa and beyond.

HIV/AIDS and Effective Treatment

HIV is a viral infection transmitted through unprotected sex. It can also be passed on from a mother to child through giving birth and breastfeeding, as well as through sharing needles with an HIV-positive person.

HIV infects cells of the immune system. Without treatment with antiretroviral therapy (ART), HIV ultimately causes severe immune damage leading to life-threatening infections and cancers. However, life-saving ART has now dramatically transformed survival for people living with HIV and limits the virus in their bodies to levels below which HIV cannot be passed on to sexual partners or infants.

However, the only way to ensure all people living with HIV start ART and remain healthy while also reducing the risk of passing their infection to their partners and infants is to know their HIV status. This requires regular and accurate HIV testing amongst all sexually active populations. When an individual receives a positive HIV result, it is recommended that they start taking ART. This combination of drugs blocks HIV replication, preventing further damage to the immune system.

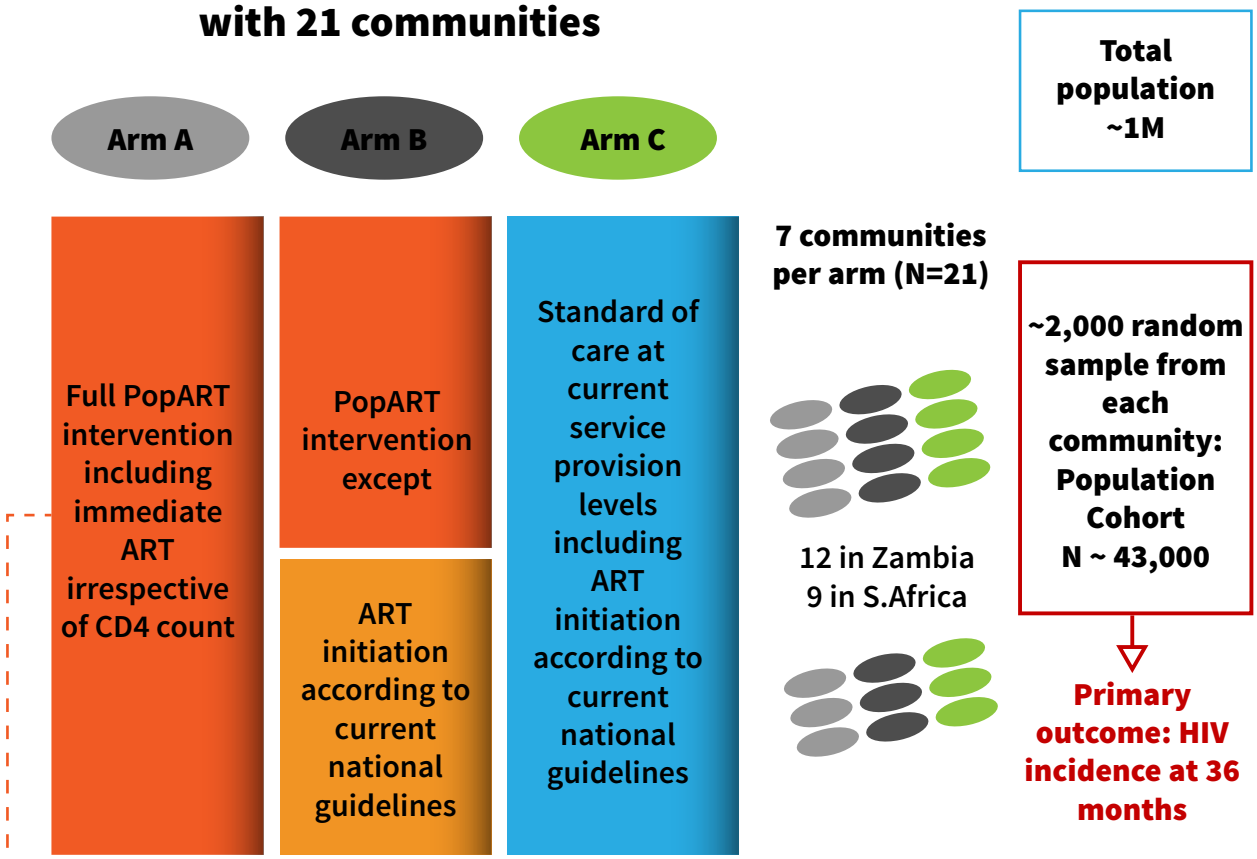
Working Together to End the HIV/AIDS Epidemic

Although new HIV infections have gradually decreased over the last 10 years, it remains a major global health issue, with around 38 million people living with HIV worldwide – 1.7 million of whom are children below 14 years. Since the beginning of the epidemic in the 1980s, over 30 million people have lost their lives to AIDS-related causes.

Among the worst impacted are those living in Eastern and Southern Africa, where almost 21 million people live with HIV (more than half of all HIV cases globally). With the aim of ending the AIDS epidemic, in 2014 the Joint United

Credit: Kim Cloete.

3 arm cluster-randomised trial with 21 communities



PopART intervention package

- Annual rounds of Home Based Voluntary HIV Testing by Community HIV-care providers (CHiPs)
- Health promotion, Active Referral and/ or Retention in Care support by CHiPs for the following:
  - Voluntary Medical Male Circumcision (VMMC) for HIV negative men
  - Prevention of Mother to Child Transmission (PMCT) for HIV positive women
  - HIV treatment and care for all HIV positive individuals
  - Promotion of sexual health and TB services
  - Condom provision

ART irrespective of CD4 count (immune status) provided at the local health centre in Arm A
Arms B and C transitioned to universal ART from 2016 in accordance with changes to national guidelines

Nations Programme on HIV/AIDS (UNAIDS) set the 90-90-90 targets – that is, for 90% of people with HIV to know their HIV status, for 90% of those diagnosed to receive sustained ART and for 90% of people on ART to show viral suppression by 2020. Going forward from 2020 to 2030, these targets are now set even higher at 95-95-95.

As part of working towards these ambitious goals, the HIV Prevention Trials Network (HPTN) conducted the HPTN 071 (PopART) trial, a collaborative study into universal testing and treatment for HIV. This study was led by Dr Richard Hayes from the London School of Hygiene and Tropical Medicine along with Dr Sarah Fidler at Imperial College, Dr Helen Ayles at the Zambia AIDS Related Tuberculosis Project (Zambia) and Dr Nulda Beyers and Dr Peter Bock at the Desmond Tutu TB Centre at Stellenbosch University in South Africa. Multitudes of organisations across several countries and research disciplines were also involved in this effort to find a solution to the global health issue of HIV. From local health centres in Zambia and South Africa to huge USA funding agencies and academic institutions in the UK, a truly collaborative effort was initiated.

Universal Testing and Treatment

An important strategy proposed to reduce new HIV infections is the application of universal testing and treatment. According to Dr Hayes, 'The idea is simple: that everyone in the community should be offered a regular HIV test so that all community members know their HIV status. And that all those who are diagnosed HIV-positive should be linked to care and encouraged to start ART immediately.' If a high enough proportion of HIV-positive people are aware of their status and are treated with ART to become virally suppressed, new infections among a given community should be significantly reduced.

The PopART trial tested whether the strategy of universal testing and treatment would be possible in low-income settings and if so, whether it would have an impact on reducing new HIV infections. A total of 21 communities in South Africa and Zambia with around 50,000 residents in each and an overall HIV prevalence of around 15% were chosen to take part. Each community was assigned to one of the three arms of the study.

In Arm A, annual, voluntary HIV testing was provided by community health workers who went directly to the doors of everyone in the community. Those who tested positive were linked to care for immediate, free ART at their local health centre. Those who tested negative were directed to different proven HIV prevention measures such as condoms and voluntary male circumcision. In Arm B, communities followed the same strategy, but HIV treatment was given according to respective national guidelines. Here, some guidelines specified ART could only be started once the immune cells reached a certain threshold of damage. Finally, Arm C acted as a control and these communities received HIV testing and prevention services according to their local standard of care and treatment according to national guidelines.

At four time points over the course of the study (which ran from 2013 to 2018), members of the study team visited a random group of participants who lived in the PopART communities and who had given their consent to contribute to the trial outcome measures. At each visit, these participants provided questionnaire data and completed a blood test for HIV infection. The HIV results of the blood tests from these study participants informed the researchers about the impact of the different community approaches in each of the study arms on HIV incidence.

The Impact of PopART

Critically, the researchers found that utilising the universal testing and treatment strategy allowed them to meet the UNAIDS 90-90-90 targets, meaning the intervention presented a feasible method at the community level. By the end of the study, their interventions had reduced the overall rate of new HIV infections in the communities by 20%. Even more impressively, Dr Hayes describes how, 'mathematical models fitted to the data from our trial estimated that if the intervention were to be kept in place over a longer time period, the impact would increase over time, eventually reaching a reduction of around 50%.'

However, the team did notice that certain groups were more difficult to find and engage in the intervention. More specifically, younger people aged between 15 and 24 years, and especially young men, had the lowest ART coverage by the end of the study. This important finding led the researchers to recommend that special efforts should be made in future work to reach these groups to ensure better coverage and uptake of the intervention.

Having confirmed that universal testing and treatment can reduce the number of new HIV infections in Zambia and South Africa when delivered to large urban and peri-urban communities, the researchers wanted to know how much money this intervention costs. An economic evaluation of the findings determined that the PopART home-based intervention package cost between $5.10–$6.80 in Zambia and between $6.40–$8.20 in South Africa per person per year. This was deemed to be cost-effective in the long term if sustained.

Overall, findings were strongly in support of the use of door-to-door universal testing and treatment, suggesting this is a feasible and community-accepted intervention. Many people were appreciative that they did not have to wait in queues for testing and did not have to face judgement from their community through being able to take the tests in the privacy of their own homes. Clearly, broad coverage and high uptake can be achieved if the whole community is reached and interventions are sustained. When this happens, the team proposes that new HIV cases will continue to drop.

The PopART study team concluded that '…this universal testing and treatment approach could be delivered effectively at community level, and over three years it reached the UNAIDS targets for knowledge of HIV status, ART coverage and HIV viral suppression, and significantly reduced the number of new HIV infections.'

This dedicated work by the study team has made vital strides forward in improving the quality of life and longevity of life for people living with HIV. If these approaches are implemented on a wider scale, we could be much closer to finally bringing an end to the devastating HIV epidemic.

Meet the researcher

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Established in 2000, the HIV Prevention Trials Network (HPTN) is a worldwide clinical trials collaboration bringing together investigators, ethicists, members of the community and other partners to develop and test the safety and efficacy of interventions designed to prevent the transmission of HIV. The HPTN 071 (PopART) trial was led by Dr Richard Hayes at the London School of Hygiene and Tropical Medicine (UK) in collaboration with Dr Sarah Fidler at Imperial College (also in the UK), Dr Helen Ayles at the Zambia AIDS Related Tuberculosis Project (Zambia) and finally, Dr Nulda Beyers and Dr Peter Bock at the Desmond Tutu TB Centre at Stellenbosch University in South Africa.

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National Institute of Mental Health

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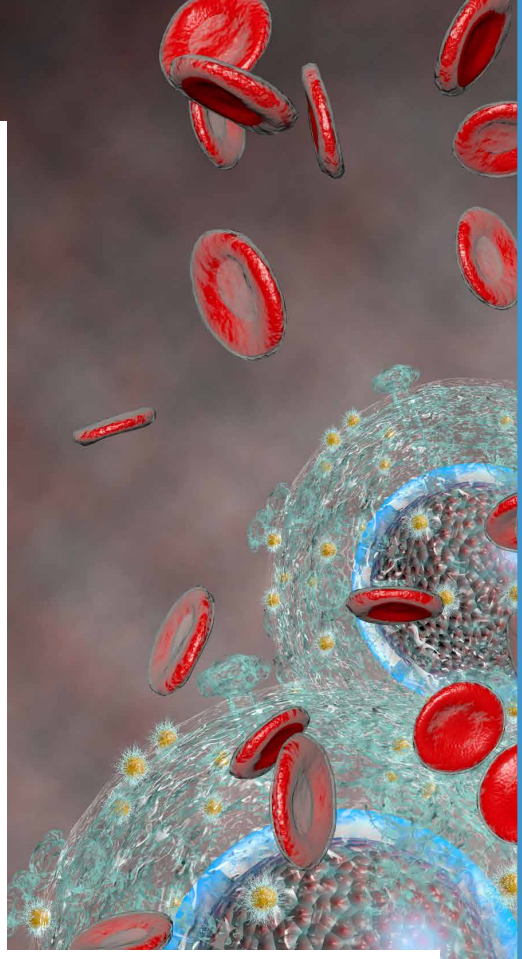
LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

International Statistics & Epidemiology Group

SHINING NEW LIGHT ON HUMAN IMMUNODEFICIENCY VIRUS ASSEMBLY MECHANISMS

People living with human immunodeficiency virus (HIV) now have very effective treatment options to allow them to live long lives but the need for new and improved therapeutics remains. **Dr Delphine Muriaux** from Le Centre national de la recherche scientifique (CNRS) in Montpellier, France, researches HIV infection and replication utilising advanced state-of-the-art microscopy. This super-resolution imaging has led to new findings on the importance of the HIV-1 Gag proteins and the cellular host co-factor IRSp53, a membrane curving protein, and how they interact with host cell membranes.



Human Immunodeficiency Virus: Risks and Replication

Human immunodeficiency virus (HIV) is a pathogen that infects and destroys cells in the immune system. Because it is found in certain bodily fluids, HIV can be passed on through sex, sharing needles and pregnancy or breastfeeding. Initially, an infection may present with flu-like symptoms for a couple of weeks and then seemingly disappear for years. However, if left untreated, HIV eventually leads to acquired immune deficiency syndrome (AIDS), where the immune system becomes increasingly damaged and minor additional infections can prove severe or even fatal.

Diagnosing HIV can be achieved through a blood or saliva test and fortunately, there are now very effective treatments for those who are HIV positive. Antiretroviral drugs are taken every day to prevent the virus from replicating so that the immune system can repair itself. Thanks to these lifesaving therapeutics and other interventions, new HIV infections have been dropping since 1997, but there are still an estimated 37.7 million people living with the virus worldwide.

In order to continue to improve available therapeutics and quality of care for HIV patients, it is important to study the virus and all its complex activities. Dr Delphine Muriaux has built a 'Membrane Domains and Viral Domains' team of dedicated scientists to do just this at the Institut de Recherche en Infectiologie de Montpellier (IRIM). This institute sits within Le Centre national de la recherche scientifique (CNRS) in Montpellier, France.

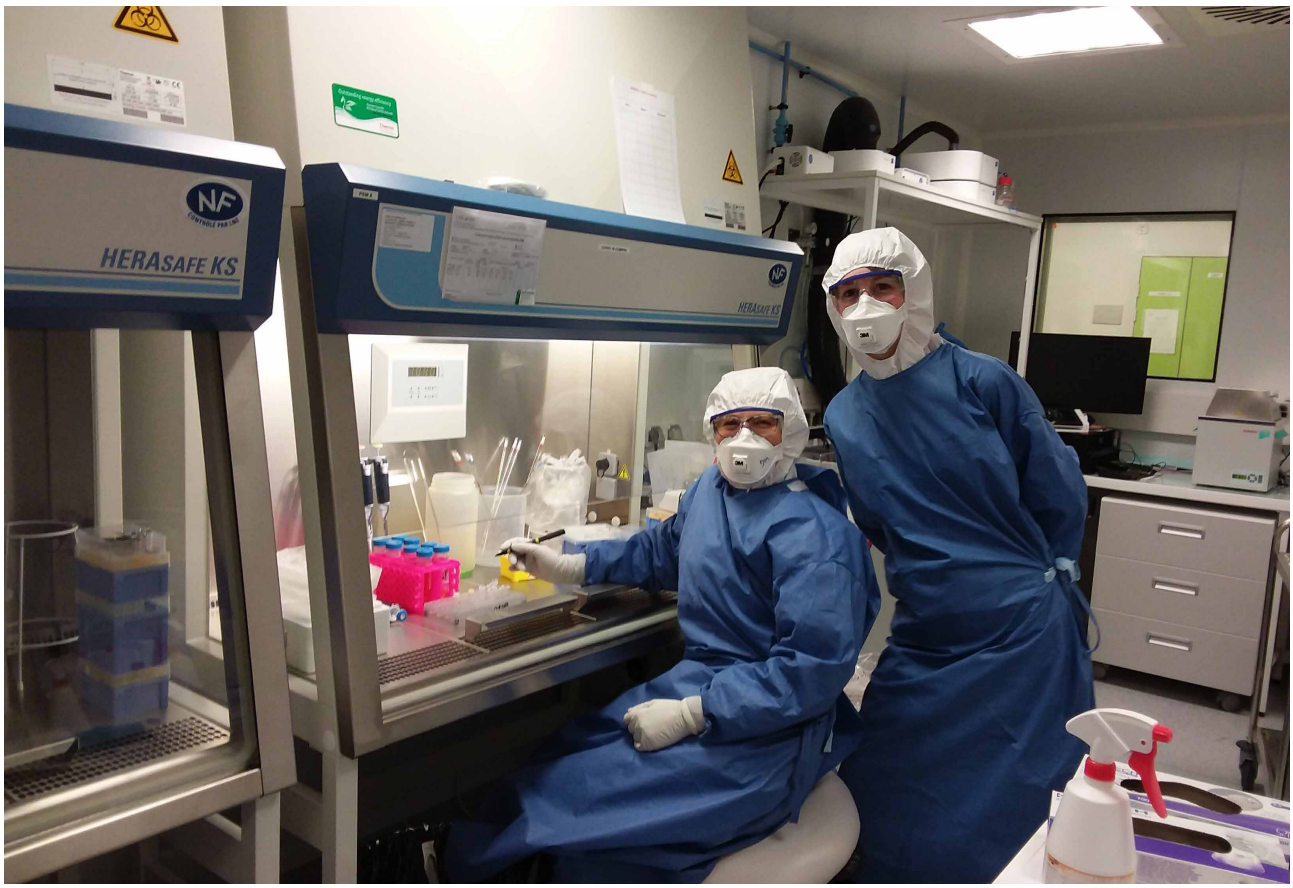
The mechanisms by which HIV infects cells are extensively researched, but there remains much to discover about its molecular activities. The virus invades immune cells called CD4+ T helper cells, which are a type of white blood cell that help to activate other aspects of the immune system as well as suppress immune reactions where necessary. HIV can also infect types of white blood cells called macrophages and dendritic cells.

To enter a target host cell, a glycoprotein on the HIV particle binds to a receptor on the CD4+ cell. These two structures have a strong affinity (attraction) to each other. Once attached, a viral

nucleocapsid containing its genetic material and certain enzymes enters the cell. These enzymes including reverse transcriptase, protease and viral integrase, are used to synthesise the new genome and integrate viral DNA into the host genome. From there, the host's own DNA replication machinery is used to produce HIV proteins to be transported to the cytoplasm and assembled at the inner leaflet of the plasma membrane. The new HIV particle then assembles at the membrane and buds off to repeat the process, killing the host immune cell. This is why a patient's immune system can be devastated by an uncontrolled HIV infection.

Shining New Light on Viral Assembly

Although this process of viral assembly has been understood for decades,



Dr Muriaux in the bioSafety level 3 laboratory at CEMIPAI CNRS Montpellier. Credit. Delphine Muriaux.

the timeframe in which it occurs has only more recently been laid out. This is thanks to advancements in technology that allow scientists to observe biological activities on a molecular level, in real-time. Previous techniques were limited by the diffraction of light, which minimises the resolution possibilities for a microscope image. The diffraction barrier prevents a microscope from distinguishing between two objects that are less than half the wavelength of light used apart from each other. These objects can be singular molecules, which means that the molecular details of an HIV assembling within a host cell could not be viewed.

New solutions have overcome this barrier to enable super-resolution imaging of endless biological occurrences and structures. These visualisations often utilise microscopic molecules called fluorophores or fluorescent tags, which absorb specific wavelengths of light and emit a longer wavelength which is seen as colour. They can be designed to attach to specific biological structures so that different structures can be clearly observed in different colours. Super-resolution microscopy either involves selective deactivation of fluorophores or single-molecule detection and localisation.

In addition to the improvements in fluorescent tags, super-resolution microscopy has assisted new research from Dr Muriaux. After co-authoring a review on these advanced techniques and the results they have facilitated, she went on to utilise them in her own studies of the assembly of HIV in CD4 T cell plasma membrane. She believes that elucidating invasion and replication mechanisms on a never-before-seen molecular level will allow the entire infection process to be seen in a new light.

Using Super-resolution Microscopy

One important aspect of HIV infection that Dr Muriaux could see in more detail was the activity of a protein called Gag. It is the main structural protein of HIV and other retroviruses – responsible for the assembly of viral particles. When HIV particles have replicated and budded from the host cell, the composition of their outer lipid membrane is very different from the plasma membrane of the host cell. It contains a notably higher concentration of types of phospholipids called phosphoinositides, including one major called phosphatidylinositol 4,5-bisphosphate lipid, or PI(4,5)P2 for short, as well as some cholesterol. The lipids (fats) in the HIV membrane are also highly ordered and densely packed.

These unusual differences led Dr Muriaux to question how the structures of an HIV particle such as Gag interact with a host plasma membrane to create such a specific environment. Is Gag targeted at existing lipid domains in the plasma membrane or does it somehow create these lipid compositions to help new viruses form? Dr Muriaux, with the help of Dr Cyril Favard from CNRS in Montpellier, France, and Drs Jakub Chojnacki and Christian Eggeling from the University of Oxford (UK),

investigated these questions using super-resolution microscopy in living CD4+ T cells that were either infected with HIV or were not infected but were still expressing Gag. To visualise the movement of the lipids, they were tagged with fluorophores, as were the Gag proteins.

Tracking these different molecules in HIV-infected cells revealed that the virus immobilises PI(4,5)P2 and cholesterol but does allow sphingomyelins to move throughout the host cell. The cells that were not infected but expressed Gag showed the same characteristics, restricting the movement of the lipids at HIV assembly sites. This means that Dr Muriaux, her team and collaborators demonstrated that HIV-1 utilises its Gag proteins to selectively trap PI(4,5)P2 and cholesterol in host cells to produce their own specialised lipid membrane for particle assembly.

Facilitating Human Immunodeficiency Virus Budding

Dr Muriaux continued to investigate HIV Gag and plasma membrane deformation in a subsequent study. This time, she and her colleagues focused on membrane curvature, which is the bending of the membrane to accommodate the shape of organelles or biological processes. These can include endocytosis (taking an outside particle into a cell) and vesiculation (exiting the cell via formation, integration with the membrane and release of a vesicle). These mechanisms are important for HIV assembly and release. Previously, scientists thought that Gag was the sole driver of plasma membrane curvature for HIV particle formation, but Dr Muriaux has revealed that there is a more detailed mechanism.

She studied an I-BAR protein, IRSp53, which binds to membranes in a PI(4,5) P2-dependent manner with high affinity to generate a negative membrane curvature, meaning the membrane protrudes outwards. She chose IRSp53 due to her previous observations that it is implicated in a signalling pathway that promotes the release of HIV particles. These factors made it a prime candidate for being important in HIV Gag assembly and viral bud formation. She utilised single-molecule localisation microscopy to observe IRSp53 and its activities within cells.

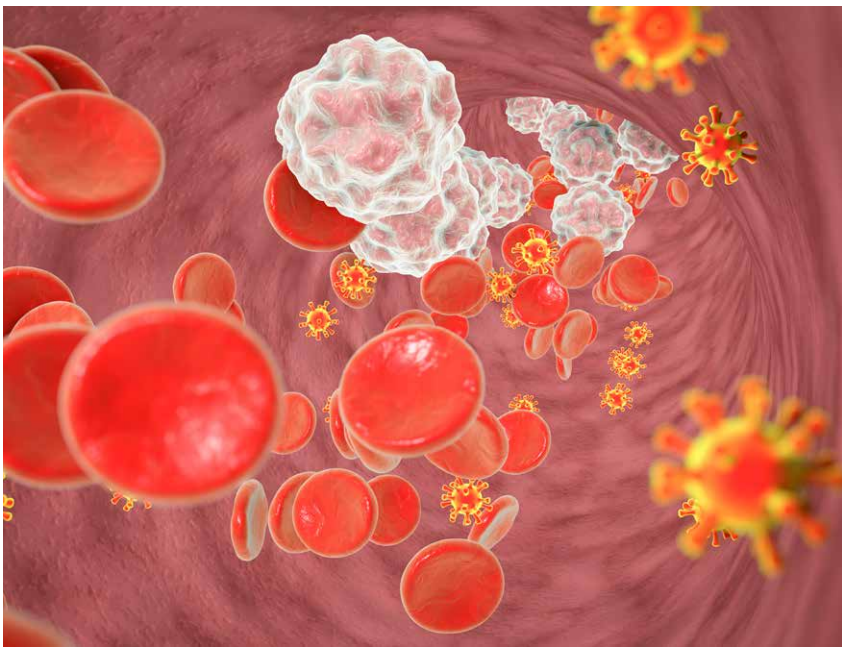
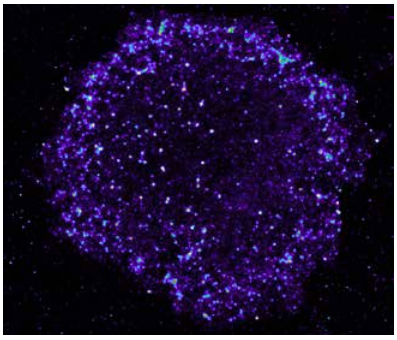


Illustration of HIV in blood

The results from these observations revealed that IRSp53 forms a complex (a bound structure) with Gag within cells, next to the cell plasma membrane. Therefore, through its membrane-bending abilities and interactions with Gag, IRSp53 is an essential factor for optimal HIV particle formation and replication. Proper and efficient HIV membrane curvature and complete assembly at the cell plasma membrane require IRSp53 and Gag to work together.

The importance of IRSp53 in viral budding was also demonstrated in a test whereby the gene that produces the IRSp53 protein was knocked down, meaning much less of the protein is produced. This resulted in a decrease in overall viral particle production and HIV budding was stopped halfway through the process. By discovering and documenting the replication mechanisms of HIV, scientists can better understand where and how to target them with drugs to prevent the virus from reproducing.



T cells infected by HIV. Each dot is an HIV assembly platform at the cell surface of the infected CD4+ T lymphocytes. Each dot surrounding the cell is a single virus. Credit. MDVA research team, IRIM Montpellier, France.

The research of Dr Muriaux, with her team at CNRS and collaboration with national and international academia laboratories as well as biotechnology companies, has allowed us to understand HIV infection in a new, much more detailed way. Her work is on track to inform the development of novel therapeutics for HIV and also pandemic viruses such as influenza viruses and coronaviruses.

Meet the researcher

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Dr Delphine Muriaux completed her PhD in molecular and cellular biology from Pierre et Marie Curie University in Paris before postdoctoral training in virology at the National Cancer Institute (National Institutes of Health) in the USA. As a Research Director, she went on to establish a team at the Institut de Recherche en Infectiologie de Montpellier (IRIM) within Le Centre national de la recherche scientifique (CNRS) in France. Dr Muriaux and her colleagues investigate membrane domains and viral assembly, focusing on the molecular and cellular mechanisms of virus replication. She is also the head of the CEMIPAI laboratory which assists academics and private companies in antiviral drug screening and nano-biological object detection and characterisation using super-resolution microscopes in a level 3 biosafety laboratory. She also trains students by providing workshops in scientific national schools and mentoring PhD students from the University of Montpellier Woman in Science Mentoring programme.

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ANR (Agence National de Recherche)

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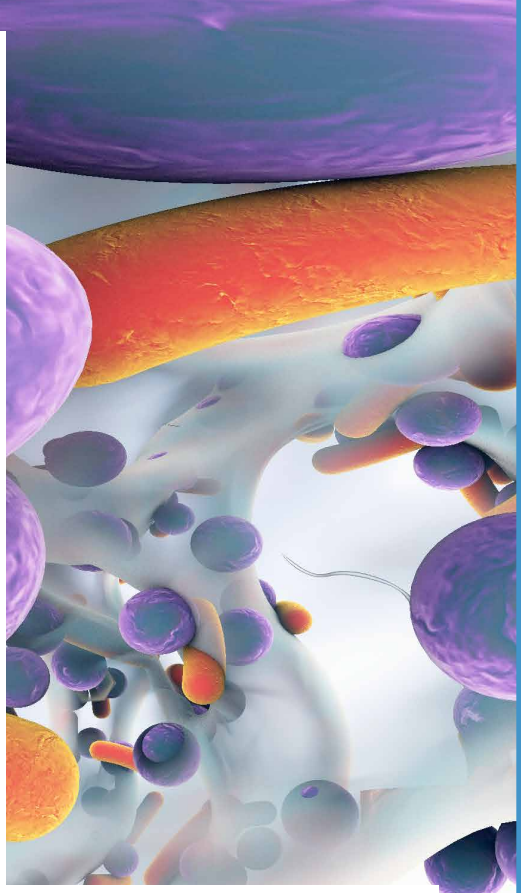
A SIMPLE POINT-OF-CARE TEST TO HELP COMBAT ANTIBIOTIC RESISTANCE

As the strains of bacteria that are not killed by antibiotics proliferate, increasing numbers of people are at risk of severe illness and even death. **Dr Rogier Hopstaken** from Star-shl Diagnostic Centres in the Netherlands has shown that a simple, yet effective technique may be the answer to antibiotic over-prescription. A C-reactive protein test at primary points of care can indicate whether a patient with a respiratory tract infection has a severe (bacterial) infection and thus, whether antibiotics are required. This test may be our best tool yet to help combat antibiotic resistance in primary care.



The Threat of Antibiotic Resistance

In 1928, Alexander Fleming's discovery of the Penicillin antibiotic revolutionised healthcare and has saved countless lives since. Antibiotics can shut down a bacterial infection by preventing bacterial reproduction or by killing the bacteria via various mechanisms. Whilst antibiotics are an essential tool for treating disease, their effectiveness in preventing severe illness and death is under threat. Antibiotic resistance, also known as bacterial antimicrobial resistance (AMR), is now one of the biggest dangers to public and global health.

When a bacterial infection is treated with antibiotics, certain bacteria in a patient may hold genetic mutations that allow them to evade detection or destruction by the drugs. These bacteria are described as 'resistant' to the antibiotics and they may eventually die or be killed off by the immune system or, they may continue to proliferate. In this case, the infection can become more severe and spread further, requiring the administration of different and stronger antibiotics to overcome it. Worryingly, these strains of antibiotic-resistant bacteria can infect other people and create wide-reaching problems.

There is a direct correlation between the amounts of antibiotics consumed and the growth of AMR. It is without a doubt that the more AMR spreads, the lower the efficacy of antibiotics and as a result, complications and deaths due to AMR increase. Around 4.95 million deaths were related to antibiotic resistance in 2019 (with sub-Saharan Africa feeling the greatest burden of this, followed by South Asia). Nearly 80% of deaths due to antibiotic resistance are due to bloodstream infections, intra-abdominal infections and lower respiratory tract infections – the latter was associated with 1.5 million deaths in 2019. Cases of life-threatening bacterial antimicrobial resistance are on the rise and there are a number of reasons for this.

One contributing factor is the use of antibiotics in livestock as a preventative measure and to improve yields. When resistant bacterial strains evolve in these animals, there is strong evidence to suggest that they can be passed on to

humans through their meat. Another important contributor is the extensive over-prescription of antibiotics to patients all over the world. There remains a strong belief that antibiotics will cure everything, and antibiotics remain cheap and easily available. These factors are compounded by a lack of public and professional knowledge regarding effective antibiotic use and the need for conservative treatment. Another factor is that in low and middle-income countries in particular, lack of access to laboratory microbiology testing often impairs decision-making regarding appropriate antibiotic use.

Around the globe, scientists and clinicians are working to better understand the progression of bacterial antimicrobial resistance and how to address it. One of these researchers is Dr Rogier Hopstaken, who is a

General Practitioner (GP) and innovation specialist at Star-shl Diagnostic Centres in the Netherlands. Around 80% of all antibiotics for humans are prescribed in primary care. Focus on better antibiotic stewardship in this particular setting is rare, however. Through his investigations, Dr Hopstaken has discovered that most antibiotics are prescribed in a primary care setting (such as a GP's practice), and for respiratory tract infections. Yet 70% of these infections are caused by viruses and are, therefore, completely untreatable with antibiotics. Most importantly, regardless of the cause, non-severe infections do not require treatment with antibiotics. Dr Hopstaken has made it his life's mission to evidence and make known an effective new method of antibiotic prescribing to finally minimise the burden of antibiotic resistance. His extensive research allows us to ask and answer key questions about this worldwide dilemma.

Making Decisions About Antibiotic Prescribing

Antibiotics for respiratory tract infections are mostly prescribed based on personal beliefs, perceived patient expectations, patient desire, and findings from patient history taking and physical examination. Whilst some symptoms can be objective and measurable (such as an abnormal body temperature, blood pressure and respiratory rate), others are not (such as chest auscultation, pain, fatigue and gut feeling). This leaves room for misdiagnosis, particularly in presumed pneumonia cases. The mostly self-limiting acute bronchitis cases are often unnecessarily judged to be pneumonia, leading to large-scale overuse of antibiotics. In contrast, secondary care (such as referral to a hospital) often has the resources to allow a deeper investigation to take place before diagnosis, with the added benefits of immunology, microbiology and radiology, for example.

To address this unnecessary, precautionary prescribing of antibiotics, Dr Hopstaken is pushing forward a technique called C-reactive protein point-of-care testing (CRP POCT). Point-of-care testing in family practice is used to inform clinical decision-making, a process through which the patient and physician agree upon the most appropriate way forward.

CRP is an acute-phase protein that is produced by the liver in response to inflammation. By testing CRP levels, physicians can determine whether a respiratory tract infection is severe, and thus, whether antibiotics are required. Dr Hopstaken and his team have shown that CRP is by far the best predictor of pneumonia, performing much better than any symptom or sign, and even better than the combination of all symptoms and signs that help to diagnose pneumonia properly.

Clinical Evidence for C-reactive Protein Point-of-care Testing

Through work conducted over a number of years, Dr Hopstaken's team has shown how useful CRP POCT can be for reducing unneeded antibiotic use. In two randomised controlled studies, his team has shown that proper introduction of CRP POCT resulted in a 30% reduction of unneeded antibiotic prescriptions. The addition of improved communication skills added considerably to this effect. In their randomised controlled trial published in the British Medical Journal in 2009, the participants were adult patients visiting their GP with acute cough. In their more recent randomised controlled trial, published in 2021 (also in the British Medical Journal), participants were vulnerable elderly individuals with lower respiratory tract infections living in nursing homes. The same, spectacular result was achieved.

Since these studies were performed in a country with one of the lowest antibiotic prescribing rates in the world (the Netherlands), this approach holds much promise globally. Dr Hopstaken, therefore, focuses now on implementing CRP POCT for those countries with much higher antibiotic prescribing rates, and with much higher AMR rates as a consequence, already leading to many unneeded complications and casualties.

Barriers to Implementation

Whilst CRP POCT is very effective, widespread implementation is primarily hindered by funding availability. Although Dr Hopstaken's team has shown that CRP POCT is cost-effective at a certain willingness to pay or invest, implementation has been processed in the Scandinavian countries, Switzerland and the Netherlands only. If cost-effectiveness studies had taken into account the longer-term benefits of preventing the negative consequences of AMR at the individual, local institution and societal levels, both nationally and globally, the positive impact of implementing CRP POCT economically would have been much larger. Additionally, health systems would have to understand and budget for the fact that the paybacks from CRP POCT may take place in different areas of healthcare from where primary testing takes place. Considerations must also be made to prevent over-testing or overreliance on testing, by integrating POCT effectively into existing systems.

Another important barrier is the existing gap between the traditional hospital laboratory world and primary care. Quality-assured POCT takes place outside the walls of the classical laboratory and effective implementation requires more intensive collaboration than is current practice. Silo-budgeting, lack of multi-disciplinary guidelines, and the lack of time or motivation may currently prevent successful implementation.

International Experience in C-reactive Protein Point-of-care Testing

Since Dr Hopstaken's studies, there have been numerous other positive studies on CRP POCT in various countries, although it has not yet resulted in the implementation of this effective strategy. In Scandinavian countries, the experiences with CRP POCT are ample, but were not always accompanied by proper guidance when introduced. In the Netherlands, Dr Hopstaken has put a lot of effort to bring various stakeholders together that are needed for long-lasting POCT success. This has resulted in guidelines on when and how to use CRP POCT, and multidisciplinary guidelines on quality aspects and how to collaborate as primary care physicians and laboratory professionals. Evidence suggests that no significant over-testing has occurred in the Netherlands in the past decade after introducing CRP POCT in routine care. In Sweden, some evidence of over-testing with CRP exists, possibly because of the lack of guidance in the early years of introduction.

The existing POCT systems in the Netherlands provide a useful model for how other countries could implement testing. All their testing resources, staff training and quality management are provided by the same organisations that carry out the central laboratory testing. Government reimbursement is usually provided to the supporting laboratory, rather than to the GP. In return, the GP can test for free and has no significant administrative and logistic burden. In addition, Dr Hopstaken has co-authored guidelines for his and other countries to use. Combining POCT implementation results from Australia and the Netherlands reveals that when GPs adapt and integrate testing into their practice, it improves their efficiency.

What Are the Next Steps?

Dr Hopstaken explains, 'Together with many other researchers in Europe, we have gathered so much evidence of the added value of CRP POCT in diagnosing pneumonia, and for better antibiotic stewardship. We have also proven that care professionals and patients are extremely satisfied with CRP POCT and our intervention strategy to communicate better with patients on the topic of lower respiratory tract infection, illness aspects, antibiotics and AMR.' He further notes, 'We have shown a possible best case of implementation of those in the Netherlands. But if we want to have an impact on AMR, we need to bring this across the border and collaborate globally. We need to involve all crucial stakeholders, including policymakers and the diagnostic industry, to find solutions to existing barriers to implementation of CRP POCT.'

Most importantly, Dr Hopstaken wants to improve the diagnostic and communication processes used by physicians so that more patients with pneumonia get the antibiotics they need and those with minor illnesses are prescribed them less frequently. To achieve this, he argues that a support model must also be created for GPs to facilitate integration, and ensure the quality and monitoring of testing and antibiotic prescribing. Finally, Dr Hopstaken believes the funding or reimbursement of POCT should be seen as a wider investment in better healthcare as a whole, with the caveat that perversive incentives for testing should not be created. Although CRP POCT and better communication styles will play an important role in reducing antibiotic resistance, it should be seen as one intervention in a multi-step process. As such, behavioural and regulatory processes for antibiotic use should also be enforced.

Through his extensive research into CRP POCT, Dr Hopstaken has shown that this manner of antibiotic stewardship could pave the way to finally addressing antibiotic resistance, starting with general practice where most antibiotics are prescribed, and then beyond.

Meet the researcher

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Dr Rogier Hopstaken is a General Practitioner in Hapert in the Netherlands and also works as an innovation specialist at Star-shl Diagnostic Centres in Etten-Leur/Rotterdam. Much of his research centres around point-of-care testing (POCT) and in collaboration with a number of universities, he studies the value of POCT, and how proven tests can be effectively implemented. He has a particular interest in lower respiratory tract infections, antimicrobial resistance, and C-reactive protein POCT. Dr Hopstaken is the principal author of the Dutch guideline on POCT in general practice, and is chairing the Special Interest Group of POCT of the World Organization of Family Doctors (WONCA).

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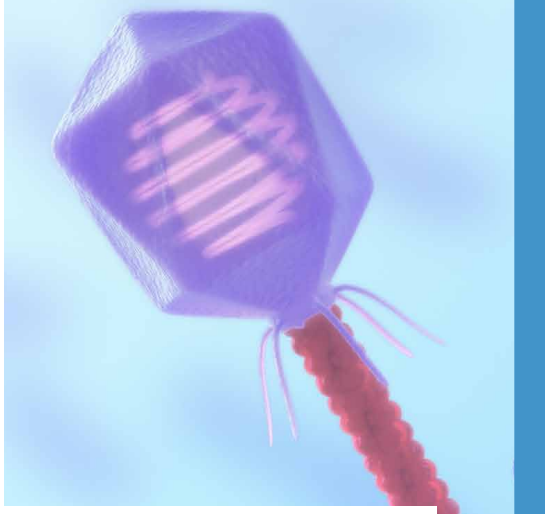
BACTERIOPHAGE HUNTING: SEARCHING FOR THE TINY VIRUSES THAT KILL HARMFUL BACTERIA

Bacteriophages are tiny viruses that infect and kill bacteria but are harmless to humans. With the world facing devastating antibiotic resistance in the coming years, they may be our best hope for treating bacterial disease. There are believed to be 10^31 bacteriophages in the world, and most of them have not yet been identified. **Dr Kristin Parent** from Michigan State University is working on exciting, collaborative projects hunting for bacteriophages in their natural environment. She has made huge strides in elucidating the mysterious and important world of bacteriophages.



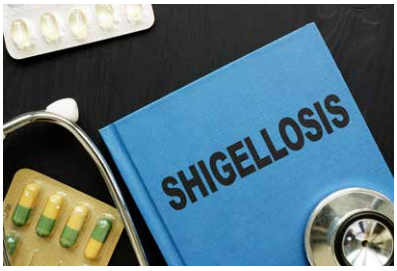
Shigella Bacteria and Bacterial Infection

Shigellosis is a nasty infection of the *Shigella* bacteria with over 164 million cases each year leading to 1.1 million deaths. Sadly, many of these deaths are children, as those under the age of 5 are most likely to catch a *Shigella* infection. This is partly due to the method of transmission – accidentally ingesting the bacteria residing in the stool of an infected person. Often this occurs in childcare settings if carers don't thoroughly wash their hands after toilet training, for example. Swimming and drinking contaminated water or eating food by an infected person can also pass along *Shigella*.

The symptoms of shigellosis include fever, nausea, stomach pain and diarrhoea which often contains blood. These issues usually last for around a week, but they can result in secondary complications such as dehydration, seizures and blood infections. Whilst antibiotics are sometimes given for shigellosis, many strains are now resistant so treating the diarrhoea and consequent dehydration is often the priority until the infection runs its course.

Shigellosis is the most common form of dysentery; other types are caused by *Salmonella* and *E. coli*, among other bacteria. There are four different species of *Shigella* called S. boydii, S. dysenteriae, S. flexneri, and S. sonnei. S. flexneri is the most common type and is often associated with low-income countries, however, cases of S. sonnei, which tends to be found in high-income countries, are increasing. This is due to the low dose of bacteria needed to cause illness and the ever-increasing antibiotic resistance of the bacteria.

Antibiotic resistance is a huge and growing issue globally, for many different bacteria. The misuse of antibiotics in humans and animals has resulted in bacterial strains that are not killed by conventional antibiotics. When these new strains are passed on, so is the antibiotic resistance and the infections they cause are much harder



to treat and mortality rates become higher. A well-known example of this is the superbug methicillin-resistant Staphylococcus aureus, better known as MRSA, a type of bacteria that is now resistant to most common antibiotics and is becoming resistant to even the last-resort antibiotics.

Bacteriophages and Combating Antibiotic Resistance

Researchers are working to combat this global health threat by innovating alternatives, or supplements, to antibiotics. One of these approaches utilises bacteriophages, the tiny viruses that are harmless to humans but instead, infect and kill bacteria. They do this by attaching to a bacterium,

Students learning how to apply science in the real world. Credit: Charles Bittle.

injecting them with their genome, replicating and multiplying inside and then bursting out to destroy the bacteria and repeat the process.

Excitingly, bacteriophages have already successfully been used to treat some antibiotic-resistant strains of bacteria. However, different bacteriophages infect only specific bacteria, so specific bacteriophages need to be found to treat different bacterial infections. Even though one of the first bacteriophages was identified in 1917 and was found to cure S. dysenteriae, very few studies have subsequently been conducted on *Shigella* bacteriophages. Previously, there were only around 35 of them logged on public databases, while over 400 Escherichia and *Salmonella* bacteriophages are known.

This is one reason that Dr Kristin Parent from Michigan State University in the USA decided to study this area. In addition, in 2016, her university's state of Michigan experienced the worst outbreak of shigellosis in 30 years, bringing to light the importance and relevance of anti-microbial work. In a collaborative effort, Dr Parent set out to discover and characterise *Shigella* bacteriophages in her area so that they might be used in novel therapeutics.

The Discovery of 16 New *Shigella* Bacteriophages

With an estimated 10^31 bacteriophages on the planet – more than every other organism on earth combined – there were many to be found. As part of a course, Dr Parent enlisted the help of microbiology graduate students at Michigan State University who were asked to develop hypotheses for promising locations surrounding the university in which to hunt bacteriophages. They took samples from a variety of places including from river sediment downstream from a wastewater treatment plant, from pond and river water and even from water at the bottom of university bathroom hand dryers.

These samples were filtered to remove the native bacteria residing in them. The leftover filtrate (the sample without the bacteria but hopefully with bacteriophages) was then used to inoculate a number of different Enterobacteria. This is the family of bacteria that tends to be found in the digestive tract and the one to which *Shigella*, *Salmonella* and *E. coli* belong. In microbiology, inoculation refers to introducing micro-organisms into a medium that facilitates cell growth. For this experiment, this meant adding bacteriophages in the filtrate to the culture of Enterobacteria.

After the team had allowed the bacteria to grow and therefore, the bacteriophages that infect them to multiply, they isolated the bacteriophages from the bacteria cells to examine them. Interestingly, most of the bacteriophages that they found were on *Shigella* bacteria, specifically, S. flexneri, and all of them were extracted from samples that came from rivers. The bacteriophages were then purified and placed onto agar plates with additional types of Enterobacteria and left overnight. This method allowed Dr Parent to observe which bacteriophages infect which bacteria, because clear spots on the plate indicate that the specified bacteria has been killed by the added bacteriophage.

Overall, the team discovered 16 new bacteriophages that infect *Shigella* and that six of these can infect more than one species of the bacteria. Having

identified these novel bacteriophages, Dr Parent then named each of them; for example, those that infected S. flexneri were given the prefix Sf and then a number. These were exciting findings for Dr Parent’s initial bacteriophage hunting project and she continued to build on the work.

Shigella Bacteriophages: Unexpectedly Abundant in the Environment

From these early but critical first steps, Dr Parent’s research subsequent research was funded by the prestigious National Science Foundation CAREER award. She involved graduate students in addition to students at Lincoln Southwest High School in Nebraska and their instructors, Kevin Schrad, Charles Bittle and Peter Stone.

This partnership with LSW high school students was an extension of Dr Parent’s original microbiology graduate assignment for discovering phages in aquatic environments. For a few days, these high school students became field scientists trained in collecting samples through direct instruction by Dr Parent and her colleagues. Dr Parent’s desire to cultivate the next generation of problem solvers extended beyond her graduate students and into the hands of 9th and 10th graders.

This study took place in Nebraska, which is located far from Michigan and is typically warmer and drier as well as having a different main water source and a unique salt marsh. These factors all mean that Nebraska gives rise to different environments for bacteriophages to live in and so, the possibility of unearthing even more new species.

Each group in Dr Parent’s study followed the same processes for bacteriophage hunting as before with the goal of identifying new S. flexneri phages in the environment and comparing them to other Enterobacteria bacteriophages. Through this collaborative research, Dr Parent and her colleagues identified around 100 new bacteriophages each year, including many *Shigella* bacteriophages, even though there had been no recent shigellosis outbreak in Nebraska as was the case in Michigan. As a result, they concluded that *Shigella* bacteriophages are unexpectedly abundant in the environment and subsequent molecular studies of the bacteriophages helped them to better understand their inner workings and structure.

According to Dr Parent, ‘this is both scientific outreach and work that directly fuels my research laboratory. Our constantly expanding library of phage types will allow us to gather an unprecedented wealth of knowledge of phage interactions with enteric bacteria.’

According to the Nebraska teachers involved, ‘students have first-hand experience with a highly advanced and relevant scientific endeavour with real-world implication. The gravity of the students’ contributions to Dr Parent’s work becomes notable when the Parent lab meets virtually with them to share the electron microscopy results in real-time.’

Investigating Bacteriophages in their Microbiome

Currently, Dr Parent is researching how viruses like bacteriophages infect hosts via host recognition and transfer of their genetic material across their cell membranes. Often, viruses recognise a specific receptor on their host that facilitates infection and so, if this receptor is altered or deleted, they are better protected. However, Dr Parent found that the S. flexneri bacteriophage, Sf6 can recognise multiple receptors and therefore, overcome *Shigella* defence mechanisms.

She is utilising this knowledge to investigate how the structure of Sf6 allows it to do this but also expanding her research into more complex environments. The relationship of bacteriophages with bacteria means that they can drive the evolution of their microbiome, as the bacteria try to adapt to evade the destructive effects of the tiny viruses. Dr Parent is studying the mechanisms of this phage-mediated evolution within complex microbial networks, which has scarcely been attempted before.

Summing up her fascinating research, Dr Parent says that, ‘we have made a ground-breaking discovery that *Shigella* phages are highly abundant in the environment. In addition, we have found some highly novel isolates that have different genome sizes, shapes, and structures compared to other previously identified bacteriophages that infect bacteria such as *Salmonella* and *E. coli*.’ Her work has opened exciting new avenues to explore in the world of bacteriophages and antibiotic resistance and will be incredibly useful for more research to come, as well as capturing the minds of budding scientists at Lincoln Southwest High School.

Meet the researcher

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Dr Kristin Parent received her BSc in Molecular and Cell Biology from the University of Connecticut where she also went on to complete a PhD in Biochemistry. She conducted postdoctoral work at the University of San Diego and then took up the position of Assistant Professor at Michigan State University, where she is now Associate Professor. In addition to these achievements, Dr Parent independently established and is the Director of the RTSF Cryo-EM facility at the university and carries out her research here. Her work focuses on discovering bacteriophages in the natural environment and studying the underlying mechanisms of viral infection via host recognition and transfer of genetic material across cell membranes.

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- Kendal Tinney
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FUNDING

National Science Foundation CAREER Award (1750125)

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MICHIGAN STATE UNIVERSITY

DEVELOPING INNOVATIVE AND EFFECTIVE NON-SURGICAL THERAPIES FOR SCOLIOSIS

Scoliosis, the curving of the spine, is a relatively common condition that develops in early adolescence. Whilst there are surgical options to neutralise the curve, there is no solid evidence for its long-term impact. **Dr Hans-Rudolf Weiss** is an expert in scoliosis treatments and has dedicated his research to improving the care of scoliosis patients. Through his back brace innovations and novel exercise therapies, he has developed ground-breaking new standards of care for patients. Additionally, through his Schroth Best Practice program, he is educating a new generation of doctors and physiotherapists on his non-surgical methods.

Scoliosis: Symptoms and Treatments

Scoliosis is a curving of the spine that usually appears in young adolescence and can become more severe as a child grows. As the spine curve becomes more prominent, the space within the chest is reduced, and this can impact the function of the lungs and create breathing difficulties in a small proportion of patients. Other scoliosis symptoms include uneven shoulders and waist, one shoulder blade or hip appearing more prominent than the other and one side of the rib cage being obviously pushed forward. This is because the condition usually causes rotation of the spine in addition to the curve.

The exact causes of scoliosis are unclear but as it sometimes runs in families, it may be a hereditary disease. However, some types form as a result of birth defects, injuries and neuromuscular conditions like cerebral palsy. X-ray or magnetic resonance imaging scans can be used to definitively diagnose scoliosis and to determine the severity of the spinal curve. For some children, the condition may be mild enough to not require treatment but others will need help to straighten their spine in order to prevent severe back pain and other complications in the future.

Some children are provided with a back brace, which, according to current standards and qualities, usually does not reverse the curve but can stop it from getting worse and is worn until they stop growing. For severe scoliosis, surgery may be performed. Spinal fusion surgery involves connecting some of the spinal bones (vertebrae) and using a metal rod to keep the pieces still to allow them to fuse. Alternatively, in a procedure called vertebral body tethering, screws and a flexible cord is attached to the spine which can be tightened to straighten the spine.

Investigating What We (Think We) Know

Although these treatments are frequently used for scoliosis patients, the long-term effectiveness of surgery is not well-evidenced and more research is needed to improve care. Dr Hans-Rudolf Weiss is an orthopaedic surgeon, physical medicine practitioner and chiropractor in addition to serving as Senior Consultant at Koob Scolitech GmbH in Neu Bamberg, Germany. In his research, he leads an innovative team to build upon our understanding of scoliosis treatments and to develop exciting new treatment methods.

Credit Hans-Rudolf Weiss

Dr Weiss is the grandson of Katharina Schroth, who developed the 'three-dimensional scoliosis treatment according to Schroth' which utilises patient-customised exercises to de-rotate, elongate and stabilise the spine. Through this method, the spine is eased

Credit Hans-Rudolf Weiss

into a more neutral position in its three-dimensional plane. This renowned family background inspired Dr Weiss to build upon existing knowledge and push forward the field of scoliosis care.

Along with Professor Tugba Kuru Colak, Dr Weiss put together a special issue of the South African Journal of Physiotherapy, which covers their broad and in-depth range of scoliosis research. It includes topics such as assessing the reliability of established deformity classification methods and looking into the effect of specific styles of back braces. The issue also has an explorative study into using vision-based augmented reality to educate physiotherapy students on the spine and its possible deformities. It was shown to be a great success, with students demonstrating a significantly higher understanding of spinal anatomy, function and pathologies than control groups.

Another important paper in this issue is the critical appraisal conducted by Dr Weiss into what evidence is available to demonstrate the long-term effectiveness of scoliosis surgery. He carried out a comprehensive review of the available scientific literature surrounding spinal surgery and discovered that there is no evidence to support the claim that surgery is the best treatment option. This means that long-term variables and possible complications are unknown and therefore, doctors recommending surgery should carefully consider each patient's case and risks. In addition, though, Dr Weiss says that a wider systematic review of spinal surgery patients is necessary to be able to confidently recommend it as a safe and effective treatment.

Even methods that are considered 'conservative' and less invasive (like back braces) are not fully backed by evidence. Back braces can be physically uncomfortable and even painful, which may lead to psychological discomfort and distress. Additionally, methods of brace application and selection are not standardised so patients may not be receiving equitable care. This is in part due to an incomplete consolidation of the scientific literature that could provide a more solid grounding for patient care. According to Dr Weiss, 'too many contemporaries advertise their own methods without any scientific proof and ultimately cause their patients more disappointment than the joy of a successful treatment.'

As a result, in a narrative review of the literature for the special issue of the South African Journal of Physiotherapy, Dr Weiss found a wide range of success rates for the wide range of different brace applications. Nonetheless, he concluded there is sufficient evidence that back braces can stop curvature progression, improve spine and trunk appearance, and reduce the number of patients needing surgery. He subsequently provided recommendations for proper brace application and maintenance using appropriate information from each individual patient and a standardised computer-aided design. If implemented correctly, this should result in a less stressful and more safe treatment experience, with the best possible patient outcomes. Results should reflect a considerable proportion of patients demonstrating improved curves and significantly improved clinical results (cosmetics).

Credit Hans-Rudolf Weiss

The Schroth Best Practice Program

Dr Weiss sought to find additional solutions to what he felt were insufficient treatment options, and describes how 'confronted again and again with frightened, disappointed and misinformed children, adolescents and their parents, I felt the desire to change something'. So, he improved upon existing guidance and built a guidebook for patients and family members to help them help themselves, explaining that 'these experiences became the driving force behind my efforts to significantly improve non-surgical treatment measures and to make evidence-based information available to patients.' The guidelines include a therapy for patients to learn postural awareness, thus helping them avoid behaviour that exacerbates the curving of their spines.

In addition to this therapy, Dr Weiss innovated a new back brace concept to overcome the issues that he had experienced as a doctor. This brace adopts a Chêneau style, which is asymmetric to accommodate the curved spine. It also utilises computer-aided design, which removes the need for traditional plaster casting and provides a highly patient-specific brace design. A patient's trunk is scanned in 3D and their clinical measurements are input to the computer to create a brace model that is a perfect fit. Dr Weiss named this the Gensingen brace after the place in which it was designed and it has proven to be a success. Patients have described it as comfortable to wear, spine corrections have been achieved and they have contributed to a wider-spread improvement in treatment standards, especially for kyphosis and chronic back pain.

Collating his substantial research and progressive ideas, Dr Weiss created the Schroth Best Practice Program in 2010. This brings together his guidelines for scoliosis patients, his modernised exercise treatment and his novel brace designs to form a state-of-the-art teaching program through the Schroth Best Practice Academy. Dr Weiss is a Senior Instructor and Consultant at the Academy, founded in 2010 from small beginnings as a non-profit organisation to become the basis for the training of thousands of international therapists working in the treatment of patients with spinal deformities and making modern treatment techniques available worldwide.

The impact of this original approach can be seen in another paper included in the special issue of the South African Journal of Physiotherapy. In this case report, a young girl had developed severe scoliosis and was at high risk for her condition to significantly progress. Normally, she may have been referred for surgery, but in this case, her parental figures declined. Instead, she received a Genisngen brace and intensive treatment according to the Schroth Best Practice program. After 18 months, her treatment had prevented the curve of her spine from worsening and she maintained an improved cosmetic result. By turning down surgery, the patient also avoided the risk of a stiff spinal deformity that can happen as a result.

Although Dr Weiss says more long-term research is needed to corroborate these promising results, he believes this type of high-impact, conservative treatment should be implemented before surgery is considered. In this way, both known and unknown future complications may be avoided.

Dr Weiss's extensive experience and research have led to ground-breaking new methods including the introduction of extracorporeal shockwave therapy to the treatment of scoliosis, and a new exercise programme aiming at the mobilisation of the spinal cord which might be tethered in certain forms of scoliosis. With positive results and an ongoing, thriving education program, his work has and will continue to change the landscape for what is the best practice for scoliosis patients and their care. This is sure to make a demonstrably positive impact in the lives of those developing and living with spinal irregularities, and their families, all around the world.

Meet the researcher

Dr Hans-Rudolf Weiss
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Germany

Dr Hans-Rudolf Weiss studied for his medical doctorate across Germany in Regensburg, Mainz and Frankfurt. His medical work includes serving as an orthopaedic surgeon, a physical medicine practitioner and also a chiropractor. He is the former Medical Director of the Katharina Schroth Spinal Deformities Rehabilitation Center in Bad Sobernheim, Germany and also of the Spinal Deformities Rehabilitation Clinic in Gensingen. Currently, Dr Weiss serves as a Senior Consultant for Koob Scolitech GmbH in Neu Bamberg where he oversees quality management of computer-aided brace designs for his innovative spinal braces created for patients around the world. Additionally, he is Senior Instructor and Senior Consultant for the Schroth Best Practice Academy.

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PIONEERING UNDERSTANDING OF CELL MEMBRANE COMPONENTS

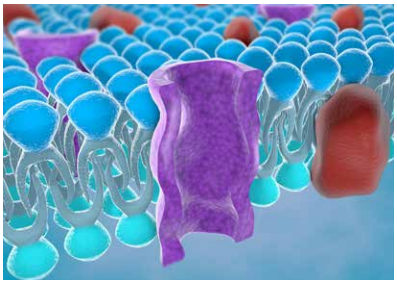
Cell membranes are critical for cellular life. The effective extraction of proteins and lipids from cell membranes is a necessity for research, but traditional methods may damage the membrane components and limit the accuracy of data. **Dr Youzhong Guo** at Virginia Commonwealth University has recently developed a revolutionary method for the extraction of membrane components in the format of native cell membrane nanoparticles to allow in-depth structural studies of membrane proteins whilst preserving functionality and limiting damage to vital mechanisms. This exciting work is driving forward the understanding of the structure, function and protein-lipid interactions of membrane protein.

Membrane Proteins: Critical for Cellular Life

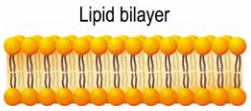
Cell membranes are an essential constituent of living organisms. Not only do cell membranes protect and organise cells, but they underpin a range of vital functions ensuring survival across species. For example, in humans, cell membranes in the brain are responsible for memory and consciousness, underscoring their importance to life itself. Given the critical role of cell membranes, it is unsurprising that research in this field is captivating to the scientific community and beyond, as evidenced by the history of Nobel prizes awarded to researchers elucidating the crucial and fascinating ways in which cell membranes work.

Research into the components of membranes, and the interactions between these components, provides critical insight into their structure and function. The two fundamental elements of cell membranes are proteins and lipids, which form a diverse and complex system connecting the membrane to the wider cell. Additionally, native lipid environments are vital in the maintenance of protein structure and function.

Traditionally, detergents have been used in the extraction of membrane proteins and lipids. However, these can induce structural damage and alter functionality which may hinder meaningful research. Protein-lipid interactions are critical in many biological systems, including targeted drug delivery and the development of vaccines – key concerns in medical science. Effective study of these interactions is dependent upon the presence of membrane proteins. The destruction of cell membranes by detergents may result in the removal of protein-associated lipid molecules, thus methods that successfully solubilise cell membrane proteins whilst retaining the lipid components are needed. Extraction of membrane proteins into lipoprotein particles using membrane-active polymers offers a possible substitute for detergent-based procedures and is now emerging as an important, viable alternative in the study of membrane protein function and structure.



Structure of plasma membrane of cell



Revolutionising the Extraction of Membrane Proteins in Native Lipid Environments

Recently, Dr Youzhong Guo and his team at Virginia Commonwealth University's Department of Medicinal Chemistry in Richmond, USA, have revolutionised the extraction of cell membrane components using detergent-free processes and developed a unique technique to produce native cell membrane nanoparticles (NCMN).

In addition to the novel membrane co-polymer system invented by Dr Guo, a

series of complementary linked libraries have been developed, comprising a comprehensive polymer library, a library of tailored preparation and extraction protocols, and a library of analysis protocols. This system has resulted in the production of detailed high-resolution structural models of various membrane proteins complex from bacteria, fungi, plants and animals and humans, contributing to the knowledge of protein-lipid interactions and allowing the overall functionality of cell membranes to be elucidated.

Discovery of a Structurally Preserved Lipid Bilayer

In order to accurately determine the structure of a membrane-embedded multidrug exporter, and to investigate its mechanism of active transport, Dr Guo and colleagues employed their ground-breaking detergent-free extraction protocol to prepare proteins for biochemical analysis, resulting in the discovery of a distinct lipid bilayer within the exporter structure.

Following extraction, the membrane proteins were purified and snap-frozen for analysis using electron microscopy. A 3D reconstruction of the resulting images revealed that each structural unit exists in one of three states, namely, ready for binding (loose), substrate-bound (tight) or substrate-released (open). Further analysis revealed that the transmembrane region was surrounded by a disordered lipid belt, and the central cavity of the structure contained an organised lipid bilayer with a hexagonal pattern and triangular double-layered shape. The layers were comprised of an inner and outer leaflet with distinct molecular patterns; the inner leaflet contained tightly packed lipid molecules with straight tails, whereas those in the outer leaflet were more loosely packed with curvier tails. Furthermore, several specific protein-lipid interactions were identified within the central cavity, including via protrusions and bonds.

It is recognised that, as proteins undergo conformational changes, lipid bilayers can adapt due to their fluid nature. Dr Guo proposed that the central lipid bilayer structure has an important role in the mechanism of action of multidrug exporters by acting as a mediator of these conformational changes and promoting drug extrusion via the transmembrane transporter, as well as providing structural support. The optimal environment for membrane proteins is most certainly within the native membrane, and the completely detergent-free extraction method developed by Dr Guo does appear to facilitate the preservation of this environment, which confers several advantages over other methods.

Characterisation of Membrane Protein Channels and Assembly of Nanodevices

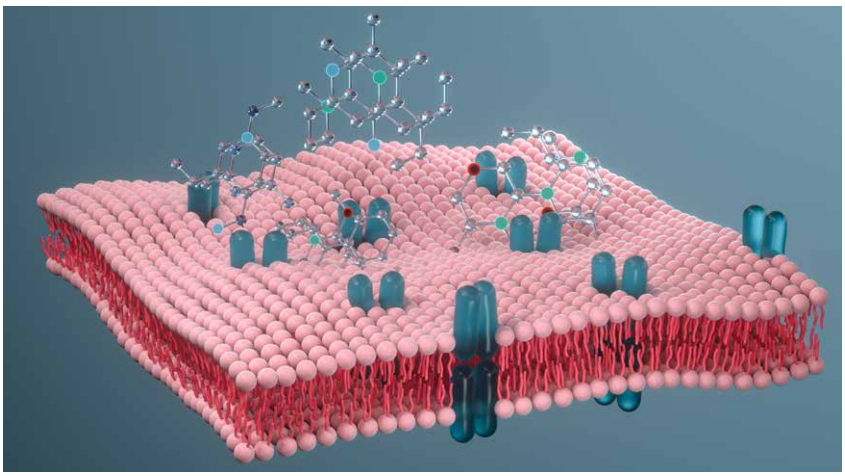
A particularly challenging aspect of membrane protein research includes the characterisation and reconstitution of integral components such as channels and transporters, especially when protein-degrading detergents are used in the process of extraction. Regardless of whether the membrane protein structure is maintained, functionality is often compromised, and reconstitution of the proteins into proteoliposomes is required if functional characteristics are to be determined. Proteoliposomes mimic the cell membrane environment, which not only allows the study of membrane protein structure and function, but also analysis of the mechanisms of drug delivery devices.

Using the previously described NCMN system, Dr Guo and colleagues studied a number of integrated membrane protein channels which aid in the rapid expulsion of molecules from within the cell. The researchers found that NCMN particles can be used to directly reconstitute the channels into liposomes, suggesting that this method may be a feasible option for the routine production of functional channel proteins for use in membrane research.

Channels were constructed to optimise protein expression and function, transformed into cells of interest, and grown in culture, followed by induction of protein expression, further culture and cell harvesting. For the preparation of nanoparticles, the resulting cell membrane proteins were mixed with NCMN buffer and NCMN polymer to achieve a pre-determined protein concentration. Following purification of the sample, analysis using electron microscopy and reconstitution into proteoliposomes was possible.

For electron microscopy image acquisition, an aliquot of sample was absorbed onto a copper grid, followed by a series of drying and washing steps. Images were captured and recorded using a camera attached to the microscope. Analysis of the images confirmed the high quality of the resulting nanoparticles. The reconstitution of proteins using NCMN polymers commenced with the drying of the lipid solution, followed by rehydration of the lipids in specific buffers or sucrose solution to form liposomes. NCMN protein particles were then added for reconstitution according to experimental requirements. The reconstitution of functional channels using rehydration with buffers was successful with some polymers, reinforcing the notion that NCMN particles are a viable option for the study of reconstituted membrane channels. Additionally, in contrast to detergent-based methods, channel particles remained stable when refrigerated and retained functional viability for several months.

Next, Dr Guo and his team proceeded to determine whether modified reconstituted channels might be assembled into functional nanodevices which represent gated membrane-embedded valves with the ability to deliver specific substances into cells residing in low-pH environments, such as within inflamed or malignant tissue. The simplified reconstitution protocol described above was utilised, with the addition of a fluorescent dye to the mixture. The fluorescent signal of the solution was monitored for 5 minutes, the pH was lowered, and the fluorescent signal monitored for a further 30 minutes. The release of dye from the nanodevices was confirmed with an 80% increase in fluorescent signal over the monitoring period, indicating that functionality was indeed preserved following assembly. This has the potential to significantly advance the development of bespoke drug delivery nanodevices.



A Comparison of Membrane Protein Extraction Systems

Recently, Dr Guo conducted a concise but thorough mini-review evaluating a selection of the available methods for the detergent-based and detergent-free extraction of membrane components. A comparison of the techniques with regard to the extent of protein and lipid survival and successful reconstruction was made. Interestingly, the most commonly used detergent-free extraction methods did not fare better than detergent-based methods regarding the preservation of native cell membrane lipids, or when determining membrane structure. In contrast, the NCMN system developed by Dr Guo and his team demonstrated superior preservation of native cell membrane lipids associated with the membrane proteins and produced particles of sufficient quality to perform high-resolution structural analysis.

Building on Success: The Wider Context

Detergents have indisputably contributed to advances in the study of membrane proteins. However, the associated destruction of the lipid bilayer may have implications for both structural and functional analysis. Research by Dr Guo revealing the structure of the lipid bilayer within a multidrug exporter and its interaction with the surrounding environment has driven forward the understanding of mechanisms of transport across cell membranes, while the continual expansion of the NCMN polymer library has enabled many more new membrane proteins and complexes to be investigated and has permitted the unique properties of a wide variety of membrane proteins to be accommodated. Using the NCMN system in the reconstitution of proteoliposomes is a relatively straightforward process and applicable to a variety of integral membrane constituents, representing a powerful new tool in nanobiotechnology.

Detergent-free extraction methods confer significant advantages over detergent-based methods in terms of maintaining stability during longer-term storage and retention of functionality upon reconstitution. Whilst the implementation of detergent-free protocols for extracting cell membrane proteins and lipids is still relatively recent, there is much potential surrounding the development and application of the methods employed.

The research carried out by Dr Guo and his team is revolutionising the understanding of membrane protein biology and there is plenty more to come. Dr Guo has a plethora of manuscripts awaiting publication detailing the ongoing work of his team. This exciting area of biological research may be in its infancy but holds much promise for the future of cell membrane studies. Dr Guo and his laboratory will undoubtedly be at the forefront with their pioneering technologies and insights.

Meet the researcher

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Dr Youzhong Guo is an Assistant Professor and also a member of the Institute for Structure Biology, Drug Discovery and Development at Virginia Commonwealth University. Having been awarded his PhD by the University of Texas at Austin in 2010, Dr Guo took up a postdoctoral position at Columbia University before accepting his current position. Dr Guo has authored and co-authored more than 20 published papers, several of which appear in highly acclaimed peer-reviewed journals, including Science, *PNAS and Nature Communications.* As an expert in the structure biological of membrane proteins, Dr Guo's ground-breaking work in the development of novel native cell membrane nanoparticles system has resulted in patented technologies, with several more pending. He is the recipient of several high-profile project grants and has presented his research at conferences all over the world. In addition to his extensive teaching and mentoring duties at Virginia Commonwealth University, Dr Guo is the organiser of the International SMALP Conference and Co-Director of SMALP.NET. He is also a founder of NCMN Bio.

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VIRGINIA COMMONWEALTH UNIVERSITY

CAN EATING GREEN VEGETABLES IMPROVE EXERCISE PERFORMANCE?

Recent work in humans and rodents suggests that consuming nutrients rich in nitrate improves exercise performance, although scientists do not fully understand the mechanisms behind this phenomenon. **Dr. Rosa Keller** from Oregon State University in the USA, worked with colleagues to investigate the effects of nitrate on muscle function. Unconventionally, the scientists decided to study the effect of nitrate in zebrafish. They observed that keeping fish in nitrate-containing water increased their ability to swim for extensive periods. Analysing chemical changes in the treated animals provided major novel insights into the inner workings of energy use during exercise.

The Need for Energy

Any movement by a human or animal muscle requires energy. Yet, few people are familiar with the fuel muscle cells use to allow them to contract. The immediate source of energy is not sugar or fats but the universal fuel of life on Earth, adenosine triphosphate – ATP for short. As muscle fibres utilise this stored energy, ATP breaks down. We use a lot of this stored energy daily – think about having to replace your own body weight in ATP in 24 hours!

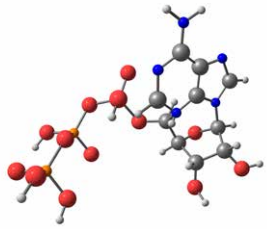
This, of course, is not practical. Instead of 'eating' large quantities of ATP, our cells remake it using the energy stored in food we all recognise: sugars and fats. However, there is a catch here. While the breakdown of ATP is instantaneous, rebuilding it by 'burning' sugars and fats involves a complex process and is relatively slow. There is another key difference between the two processes: while the use of ATP does not require oxygen, its rebuilding does. This is why we breathe more heavily during physical activity.

Supply and Demand

The chemical synthesis of ATP does not use nitrates, so why would nitrates affect exercise performance? The oxygen and nutrients needed to replenish this critical energy store come from the blood. When we exercise, we do not use all muscles equally, so blood and the oxygen within it, need to reach those muscles working particularly hard during a given activity. This is where nitrates play a role. Released from the active muscles nitrates make blood vessels dilate, ensuring adequate nutrient supply to areas of high demand. Dr. Keller from Oregon State University in the USA was intrigued to find out why nitrate improves exercise performance.

A Gym for Fish

Working with collaborators, Dr. Keller devised a set of experiments to study the effect of nitrates in zebrafish. This included keeping the fish in normal water and putting them through rigorous exercise while measuring their biological behaviour. After establishing



this baseline performance, the animals were kept in water containing nitrate for 21 days. After this 'treatment', the exercise challenge was repeated, measuring the performance of the same fish.

Using zebrafish in exercise research is a relatively new development, as scientists are just discovering that the responses of fish and human muscles to exercise training show some striking similarities. To study the composition of the muscles, a tiny sample was taken from the animals before the exercise, after the completion of an exhausting swim against a rapid current of water, and at a final post-exercise time point. Then the fish were treated with the nitrate, repeated the exercise and the same samples were taken again.

Chemical Changes

Dr. Keller and her colleagues analysed the chemical composition of the collected muscle samples using a sophisticated method called mass spectrometry, allowing them to detect hundreds of chemicals simultaneously. This way, the team could assess the concentration of not just ATP but also various sugars, fats, and protein components and their breakdown products.

By comparing the amount of these chemical compounds before, during, and after the exercise – with and without nitrate treatment – the scientists were able to build a picture of how muscles changed their behaviour in response to nitrates. In addition, the scientists also analysed whether the response of the muscles involved changes in what genes were active before and after keeping the fish in nitrate-containing water.

More Efficient Swimmers

As Dr. Keller explains, it is possible to measure the level of oxygen in the water while the fish are swimming. Analysing this data indicated an unexpected finding. The distance, time, and speed at which the fish swam were identical before and after nitrate treatment. Yet, the nitrate-treated fish used less oxygen during the exercise challenge, suggesting that their muscles somehow became more efficient at using energy.

When the scientists looked at the chemical composition of the muscles it became apparent that the treatment increased the amount of stored ATP, sugars, and fats inside the muscles, making the animals better prepared for exercise. In addition, genes associated with energy production from sugars and fats were also more active after treatment.

An Exciting Theory

Nonetheless, the swim the fish completed was very strenuous, being both fast and long, so the better performance of the nitrate-treated fish could not be explained by the increased amount of ATP and other nutrients stored in their muscles. Simply put, those stores would have been depleted during the 40 minutes of exercise. Thus, additional mechanisms must have been at work to make the nitrate-treated muscles more efficient.

One possible explanation is that the higher nitrate concentration allowed the treated animals to dilate their arteries more, providing more efficient nutrient supply to their muscles via improved blood flow. However, Dr. Keller has a more interesting theory. She believes that the higher ATP content in nitrate-treated muscles does not act simply as an energy source, but also changes the structure of the muscle, increasing the amount of liquid surrounding muscle fibres. According to Dr. Keller, this better hydration may be the mechanism that allows muscle fibres to work more efficiently. Exploring this theory will be the next important challenge for the team.

Meet the researcher

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Dr Keller graduated from Oregon State University with a B.S. degree in Anthropology. After a change in interests, she also gained a B.S. degree in Nutrition from the School of Biological and Population Health Sciences in 2017, during which she was awarded the Best Student of the Year prize. The next step in her scientific training was the completion of a PhD in Nutrition at Oregon State University, where she was working as a graduate research assistant, conducting experimental work using advanced techniques in metabolomics and molecular biology. Dr Keller currently works as a dietetic intern at the University of California San Francisco Nutrition and Food Services, where she looks after the dietetic needs of kidney, lung, bone marrow, and liver transplant patients and children requiring extensive nutritional support.

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A PARADOX EXPLAINED: WHY A SUPER SELECTIVE ß1-BLOCKER WORKS IN ACUTE HEART FAILURE

ß1-adrenoceptors are found in the heart where they bind neurotransmitters/hormones such as noradrenaline and adrenaline. The binding of these ß1-adrenoceptors agonists activates a response in the heart muscle that helps regulate the heart's beat and contractile force. Drugs that block the action of these receptors are an established treatment for those suffering from left ventricular dysfunction due to chronic heart failure. However, their use in the acute setting is controversial. **Professor Thomas Feuerstein** of the University Hospital Freiburg in Germany and **Dr Günther Krumpl** of the Medical Research Network in Vienna are challenging these sceptical attitudes through mathematical modelling.

A Global Problem

Heart failure affects at least 26 million people worldwide and the prevalence is increasing. Predominantly seen in those over 65 years old, the incidence increases with age and is more likely to be seen in those with conditions such as diabetes and obesity, which can lead to narrowed arteries and high blood pressure. As such, heart failure is characterised by the reduced ability of the heart to pump and/or fill with blood, commonly leading to the pooling of blood in the left ventricle and the patient suffering from fatigue, shortness of breath, nausea, rapid weight gain because of fluid retention and chest pain.

Heart failure can present as either acute or chronic, and treatments are tailored accordingly. Insertion of a pacemaker to restore and correct the heart rhythm, heart surgery to improve blood flow and correct valve defects, or medication are the most used options, alongside recommended lifestyle changes such as keeping to a healthy weight and diet, limiting alcohol consumption, quitting smoking and keeping active.

Treatment for Heart Failure

One of the mainstay treatments for cardiovascular issues is the administration of ß-adrenoceptor blockers. Approximately 30 million adults in the United States use these beta-blockers for cardiovascular conditions such as angina, heart failure, atrial fibrillation, heart attacks and high blood pressure. These drugs target ß-adrenoceptors (ß-AR) which are found on the surface of cells and are subcategorised as ß1, ß2 and ß3 receptors, depending on their function.

In healthy cardiovascular cells, ß1-receptors represent 75–80% of all beta receptors while ß2-receptors represent only 15–18%. But when the heart starts to fail the receptors reach a 50/50 balance. These receptors work together to mediate increases in heart chronotropy (rate), inotropy (power of contraction), dromotropy (rate of electrical impulse) and bathmotropy (influence of a stimulus on excitability). Therefore, blocking these receptors can have several benefits for patients with chronic heart failure, including a reduction in heart rate and arrhythmia (abnormal heart rhythm) management.

Though initial studies in patients with reduced cardiac function raised concerns about the functionality of beta-blockers as a treatment for those with contractile problems, a series of low-dose studies with several different beta-blockers found that ß1-AR blockade can improve contractility and prolong survival when administered long-term. But despite their acceptance for use in chronic treatment, their benefits are not currently acknowledged in relation to acute heart failure. This is in part due to their potential to inhibit the effects of other drugs that are used to help treat patients experiencing acute heart failure. It may be thought that ß-blockers inhibit the effects of positive inotropic agents used intravenously for the treatment in the acute situation. With regards to unwanted side effects of usual ß-blockers, their longer duration of action may also lead to significant difficulties in acute situations.

Professor Thomas Feuerstein of University Hospital Freiburg in Germany and Dr Günther Krumpl of the Medical Research Network in Vienna argue that all the arguments raised in support of the use of beta-blockers in chronic treatment also point to their useful applicability in the acute context. They argue that what needs to be determined is the most appropriate dosage, type of beta-blocker and method of delivery.

Earlier studies have already shown that extremely short-acting beta-blockers can provide significant advantages in patients with acute heart failure by helping to preserve blood pressure. This was true despite the absence of a convincing rationale for the use of short-acting beta-blockers which are assumed to act in a negative inotropic fashion

(i.e. they decrease the force of heart contractions at rather low concentrations of the endogenous agonists, noradrenaline and adrenaline, at theß$_1$-AR in the heart). However, their negative inotropic effect may be reversed with high concentrations of endogenous agonists. The condition of highly elevated concentrations of endogenous agonists is typical for heart failure.

Currently, Landiolol is the most effectiveß$_1$-blocker given intravenously for acute heart failure and is now used alongside so-called positive inotropic agents to increase the force of heart contractions in intensive care patients.

Building on this work, Professor Feuerstein and Dr Krumpl set out to provide a rationale as to why aß$_1$--blocker might not act as a negative inotropic agent but have a positive inotropic effect in the acute setting.

A Mathematical Approach to Understanding the Effects of Beta-blockers

Understanding the mode of action for beta-blockers is key for elucidating their impact at different doses. Critically,ß$_1$-AR have a receptor reserve, meaning that only a fraction of the existing receptor population, i.e., 50% in our case, needs to be activated to produce the maximum response. Also,ß$_1$-AR occur as receptors with two identical subunits (protomers) for binding. Binding only one of these subunits is sufficient to induce the maximum response from the receptor dimer. Subsequently, if another agent binds to the second subunit, this can be negatively influenced by the binding to the first protomer, decreasing the affinity for the agent to bind to the second subunit. Note that this negative influence is only true forß$_1$-AR agonists, like noradrenaline, not for antagonists, like Landiolol.

Basing their work on 'receptor theory' of the interaction between an agonist or antagonist and a receptor, Professor Feuerstein and Dr Krumpl mathematically modelled the impact of agonist and antagonist binding toß$_1$-AR in the real world. The Binomial Distribution describes the occupancy of dimeric ß$_1$-AR best: This distribution reflects the number of successes, i.e., binding to one or two protomer(s), in a sequence of n independent experiments, each asking a 'yes–no' question, where 'yes' means 'bound protomer' and 'no' means 'unbound protomer'. The number n of independent binding events is two in our case (a dimer is composed of two protomers).

It is important to note that the above-mentioned negative influence, i.e., that agonist binding to the first protomer decreases the affinity for the same agent or another agonist to bind to the second subunit, is not compatible with the independence of the two binding processes in a single homodimer. This breach of the binomial principle of independence of individual experiments through the biologically most meaningful interaction of two protomers belonging together had to be modelled. Obviously, such an interaction may protect the heart muscle from harmful overstimulation due to massively elevated endogenous noradrenaline and adrenaline.

The researchers assumed that, when all homodimers are doubly bound, this state is just about compatible with the basic pumping capacity of the heart. However, the basic pumping capacity does not represent the maximum pumping capacity of the heart, since a protomer bound by a first agonist decreases the binding of a second agonist to the partner protomer, thereby diminishing inotropy. Freeing up only one subunit via a beta-blocker molecule will then improve the inotropic condition at one dimeric receptor, as it ensures the receptor is no longer activated by two stimuli. The benefits of this displacement will prevail if the addition of beta-blocker molecules results in more receptors bound with one beta-blocker and one agonist, versus receptors bound with two beta-blocker molecules.

Working on this principle, the researchers were able to show that highly selective and potent, short-acting beta-blockers such as Landiolol, when administered with other inotropes, can recruit positive inotropy in acute heart failure when used at rather low doses. Taking this a step further, they translated their model to demonstrate the optimum dose for Landiolol to maximise positive inotropy. They found that their predicted optimum dose is within the recommended range for the treatment of acute tachycardic atrial fibrillation (irregular and rapid heartbeat), which fits with having a positive inotropic influence on patients suffering from heart failure.

It is important to remember, however, that the dose will have to be adapted for clinical treatment according to the level of cardiac dysfunction seen in the patient and that the ideal dose range will vary according to the elevated levels of endogenous catecholamines.

Challenging Sceptic Attitudes Through Data

Overall, Professor Feuerstein and Dr Krumpl's approach demonstrated that during the co-administration ofß$_1$-receptor agonists and antagonists (the latter stop the response of the receptor upon activation by an agonist), the antagonist may, based on the behaviours laid out in receptor theory, dose-dependently induce a positive inotropic effect in patients with acute heart failure. As such, the negative inotropic effect that has been previously demonstrated with higher doses can be seen to be converted to positive inotropy at moderate to low concentrations of ß$_1$ beta-blockers.

In the case of Landiolol, a super-selective ß$_1$ beta-blocker with 4 minutes half-life and specific dose recommendations for patients with left ventricular dysfunction, support the individual handling in the clinical setting. Professor Feuerstein and Dr Krumpl are confident that their work, along with the already existing clinical evidence, presents a strong case towards erasing sceptic attitudes towards the use of this ß$_1$-AR antagonist in patients with acute left ventricular dysfunction.

Meet the researcher

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Professor Thomas Feuerstein studied medicine at the University of Freiburg in Germany and then completed mandatory military service as a staff physician, followed by postdoctoral research at the State Psychiatric Hospital Reichenau and the Neurological University Hospital Freiburg. Having discovered a strong interest in neuro- and psychopharmacology, Professor Feuerstein transferred to the Institute of Pharmacology at the University of Freiburg and specialised first in pharmacology and toxicology. On returning to the Neurological University Hospital, he completed his training as a neurologist and psychiatrist and then qualified for the state doctorate (Habilitation) in 1988. After being awarded the Constance Medical Sponsorship in 1989, he worked as Head of Department, Clinical Research CNS, at the pharmaceutical firm Goedecke AG/Parke-Davis/ Pfizer (Freiburg and Ann Arbor, Michigan) until 1994 when he was appointed Professor and Head of the Section of Clinical Neuropharmacology, Neurocenter of the University Hospital Freiburg. There he continued to pursue his research interests in local anti-epileptic therapies and endogenous anti-seizure mechanisms, especially with regard to the meaning of the transporter-mediated selective GABA release.

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UNDERSTANDING HOW RECEPTORS AND GENETIC MUTATIONS PROMOTE HYPERTENSION

Hypertension (high blood pressure) is a common health issue in adults that can lead to numerous co-morbidities. For hypertension to develop, a pathway called the renin-angiotensin-aldosterone system needs to be activated, and a vital part of this system involves the angiotensin 1 receptor. **Dr Sudhir Jain** from New York Medical College studies this receptor and how specific groups of genetic mutations, known as haplotypes, promote hypertension under different conditions.

Lifestyle Factors and Biological Mechanisms

Hypertension, also known as high blood pressure, is an increasingly common condition. Astonishingly, an estimated one-half of adults in the USA have hypertension. Blood pressure is measured in millimetres of mercury (mmHg) representing systolic pressure in the arteries when the heart beats versus diastolic pressure in the arteries when the heart rests between beats. A healthy blood pressure reading for systolic versus diastolic pressure should be between 90/60 mmHg and 120/80 mmHg whilst a measurement of 140/90 mmHg or higher tends to indicate hypertension.

Many factors can increase a person's risk of high blood pressure including but not limited to being overweight, smoking, being sleep deprived, and being over 65 years old. Because hypertension increases the strain on blood vessels as well as organs such as the heart, kidneys, eyes and brain, it can result in serious co-morbidities. Heart disease, attack or failure may occur, and strokes, kidney disease and vascular dementia are also potential risks.

Fortunately, introducing healthy lifestyle changes can bring down high blood pressure and prevent it from becoming a problem in the first place. Increasing exercise, reducing salt, alcohol and caffeine intake, increasing fruit and vegetable consumption, and stopping smoking are all key interventions. There are also a number of drugs, usually in the form of pills, that clinicians use to try to control hypertension. ACE inhibitors, calcium channel blockers, diuretics, beta-blockers, and angiotensin-II receptor blockers, are all frequently prescribed to patients.

In particular, angiotensin II receptor blockers target the AT1-receptor, thus directly blocking the vasoconstrictive effect of its ligand angiotensin II, which is a part of the renin-angiotensin-aldosterone system that regulates blood pressure. When angiotensin-II is activated, it creates vasoconstriction (the narrowing of blood vessels) which causes elevated blood pressure. It also stimulates the release of another hormone called aldosterone which encourages sodium retention within the kidneys, increasing the overall blood volume and therefore, increasing blood pressure.

The angiotensin-II activation for these processes to take place comes via receptors called angiotensin 1 receptors (AT1R for short). This receptor and the biological activities it promotes are the focus of Dr Sudhir Jain's research at New York Medical College in the USA.

Utilising Mice Models

Dr Jain studies how genetic differences (more formally and precisely referred to as genetic heterogeneity) impact illness, specifically diseases of the cardiovascular-renal systems – those of the heart and blood vessels and the kidneys. Every human's genetic makeup is unique and this is in part due to genetic mutations; some of these are harmless but some result in a higher risk of disease. This is because our genes are read by cellular machinery to produce proteins and a consequent

wrong amount or wrong types of protein can cause issues. One of the most common mutations in humans are called single nucleotide polymorphisms (SNPs – pronounced 'snips'). These are characterised by a single nucleotide, a DNA building block, being altered within the genetic sequence. For example, a cytosine nucleotide being swapped for a guanine nucleotide.

Dr Jain researches SNPs within the renin-angiotensin-aldosterone system because they can give insight into how and why hypertension develops. In turn, this will help him to identify novel molecular targets within the system which tailored drugs for hypertension and its co-morbidities could be aimed towards.

To this end, Dr Jain and his colleagues have genetically engineered mice models with the human angiotensinogen gene (the precursor to angiotensin-II) and human AT1R gene variants. These mice are used to investigate how SNPs that are reported in the human population may result in altered regulation of the renin-angiotensin-aldosterone system and predisposition to hypertension.

Inducing SNPs in Angiotensin 1 Receptor Genes

One of Dr Jain's studies built on the evidence that increased AT1R expression contributes to the onset of high blood pressure. An increase in a receptor's expression can be due to SNPs appearing on the promoter region of the gene. This is a section of DNA adjacent to the gene of interest to which the DNA transcription machinery will bind and initiate the process of protein synthesis. Therefore, Dr Jain chose a promoter region on the AT1R gene, and its common SNPs, to study in relation to hypertension.

He discovered two separate sets of four specific SNPs that always occur together and named them haplotype-I and haplotype-II; a haplotype is the name for a set of DNA variations. Previous research had revealed that haplotype-I results in increased promoter activity and is associated with hypertension within the Caucasian population. The mice that Dr Jain and his team engineered had either haplotype-I or II in their AT1R genes. They discovered that, in comparison to mice with haplotype-II, those with haplotype-I had higher levels of the mRNA that would go on to aid the synthesis of AT1R proteins. They also showed an increased expression of inflammatory markers, high oxidative stress and higher blood pressure.

Impact of a High-fat Diet

A subsequent study by Dr Jain progressed this research further to look into how diet may impact the expression of the AT1R gene. His initial hypothesis was that a high-fat diet would increase AT1R gene expression via alterations in the transcriptional environment, dependent on the haplotype present. As a result of inflammation and oxidative stress, he predicted that hypertension would occur in haplotype-I mice. Metabolic syndrome, a combination of obesity and high blood pressure, is a known consequence of an overactive renin-angiotensin-aldosterone system. Therefore, Dr Jain set out to understand how diet-induced metabolic syndrome impacts the cellular environment for transcription and its effects on AT1R expression.

Once again, he used engineered mice with haplotype I or II, and fed them a high-fat diet for 20 weeks. After this time, their blood pressure and tissue samples were analysed. In line with his previous results, Dr Jain found that haplotype-I mice

showed an increased expression of the AT1R gene, an increase in inflammatory molecules and suppression of antioxidant defences, compared to haplotype-II mice. Although the high-fat diet increased blood pressure in both sets of mice, these factors resulted in more severe hypertension in the haplotype-I mice. This suggests that the presence of the haplotype-I mutations predisposes the mice, and likely humans as well, to hypertension, especially under poor diet conditions.

Older Age Is Also a Risk Factor

This time focusing on a different risk factor for hypertension, Dr Jain carried out a study on the effect of age on AT1R expression. As we age, many of our biological processes alter in some way, so Dr Jain hypothesised that this would be another factor that changed the transcriptional environment for the regulation of AT1R gene expression. And once again, he believed that the haplotype present in the AT1R gene would impact the result of this.

The mice he and the team used for this experiment were either adults aged 10–12 weeks old or aged 20–34 months old. Haplotype I and II mice were present in both of these groups. The results were as he predicted. Aged (i.e., older) mice with haplotype-I displayed increased AT1R expression with higher blood pressure, in addition to suppressed antioxidant defences and anti-ageing molecules. These factors were coupled with elevated inflammatory molecule levels and increased insulin resistance (which can lead to diabetes).

Through these three studies, Dr Jain has revealed vital information that has pushed forward our understanding of hypertension. The results from his mice model experiments strongly suggest that humans who hold a haplotype-I mutation in their AT1R genes are much more likely to experience high blood pressure than those without. In addition, consuming a high-fat diet or being older is likely to increase the risk of hypertension more for these people than others.

Continuing to Investigate Hypertension

Although Dr Jain has already uncovered exciting new findings, he continues to research this important receptor and how different physiological conditions affect its role in hypertension. For example, a recent study examined the role of ageing and high blood pressure in lung pathologies. Perhaps unsurprisingly, aged haplotype-I mice showed worsened lung damage and high mortality after infection.

Another study looked at the impact of a typically Westernised high-fat diet on mice, revealing an increase in blood pressure for both haplotypes I and II mice, but more severely in haplotype-I. Consequent biological changes also resulted in kidney damage and even kidney failure. In his most recent paper, Dr Jain demonstrated how this westernised diet also has negative consequences for the heart in haplotype I and II mice.

Dr Jain has also completed a detailed gene expression analysis of the RNA sequencing data from the heart, kidney, and lung tissues of transgenic mice. This was completed using Ingenuity Pathways Analysis software from Qiagen, which is a web-based bioinformatics application that allows researchers to upload data analysis results from high-throughput experiments such as microarray and next-generation sequencing for functional analysis, integration and further understanding.

Recent work with collaborator Dr Marcello Rotta has extended Dr Jain's contribution to our understanding of heart pathophysiology. In one study, the researchers used an in vivo approach to explore electrical recovery and diastolic function at the cellular level, substantiating the role of sodium channel subunits. In another study, the researchers explored the alterations in heart rhythm dynamics associated with myocardial infarction in rodents, confirming the validity of this approach in researching the development and manifestation of cardiovascular conditions in humans.

Through his dedicated research, Dr Jain continues to explore the genetic markers regulated by diet, ageing and lifestyle to prevent high blood pressure and its associated health issues. By utilising a range of scientific approaches and methodologies as described here, his work is pioneering a better understanding of heart pathophysiology for the benefit of human health.

Meet the researcher

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Dr Sudhir Jain received his BSc in Zoology, Chemistry and Environmental Science from Delhi University in India before completing an MSc in Zoology at Sagar University. He then went on to achieve his PhD in Life Sciences from Devi Ahilya University in Indore before moving to the USA to serve in various research and teaching roles at New York Medical College. Currently, Dr Jain is an Associate Professor in the Department of Pathology, Microbiology and Immunology. His published work in genetic heterogeneity in pathological conditions associated with cardiovascular-renal systems has earned him a number of awards.

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Myocyte Repolarization and Cardiac Dysfunction with Age
2018-2023: Collaborator (PI: Marcello Rota): R01, National Institute of Aging

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NEW YORK MEDICAL COLLEGE
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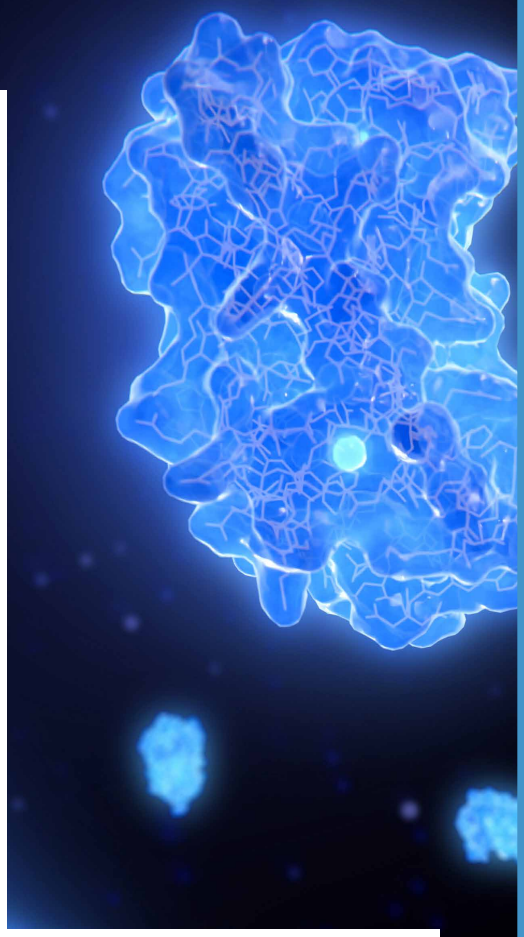
DECIPHERING NOVEL CYTOKINE SECRETION MECHANISMS

Following exposure to injury or infection, the body elicits a counteractive immune response which involves many different cell types and processes. Cytokines are substances secreted by cells which play a pivotal role in the regulation of this response. **Professor Paige Lacy** and colleagues in the Department of Medicine at the University of Alberta in Edmonton, Canada, have conducted extensive research into the exact mechanisms underpinning the regulation of cytokine release during the immune response with a particular focus on airway inflammatory disorders.



Cytokines and the Immune Response

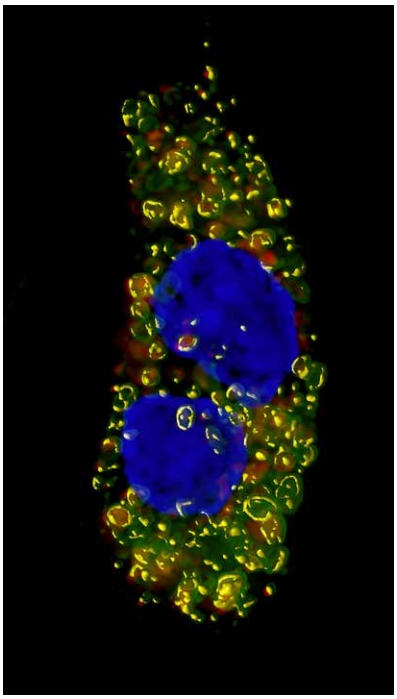
Cytokines are intercellular messengers and purveyors of soluble regulatory signals which mediate the body's response to injury due to pathogens, allergens and injury. Whilst cytokine release has been extensively studied, the precise mechanisms controlling this process are not yet fully understood, although it is acknowledged that the immune response is multifaceted and involves a complex system of different cell types, transport machinery and molecular pathways.

Over the past two decades, Professor Paige Lacy and her team in the Department of Medicine at the University of Alberta in Edmonton, Canada, have been investigating exactly how cytokines are synthesised, packaged, trafficked and released in response to external stimuli which cause cellular damage. Their work has identified many enzymes, membrane proteins, and receptors involved in these processes, and has revealed novel mechanisms of action in the regulation of the immune response in allergic airway inflammation and asthma.

Initially, Professor Lacy and colleagues reviewed published evidence to identify knowledge gaps relating to the mechanisms of cytokine release from immune cells. They explained that cytokine secretion by a range of cell types is a fundamental aspect of the immune response, and greatly influences the body's reaction to stimuli.

Cytokine Secretion Pathways

Many different cells secrete cytokines, including epithelial cells, eosinophils, and macrophages. Epithelial cells are omnipresent, forming thin layers of lining tissue throughout the body, and are among the first cells to release cytokines in response to harmful signals. Epithelial cells work closely with the immune system by sending cues to initiate appropriate physiological reactions. Innate immune cells, such as macrophages and eosinophils, are naturally occurring cells which rapidly mobilise to the site of injury or infection, and can generate a wide range of cytokines. Collectively, these cells control pathogen invasion by recognising threats and producing toxic substances which kill harmful invaders, although the mechanisms relating



Credit Paige Lacy

to the movement of cytokines from epithelial cells prior to release remain to be fully determined.

Cytokines may be secreted via classical or non-classical pathways, which are defined depending upon the specific mode of action, and several pathways have been identified in specific immune

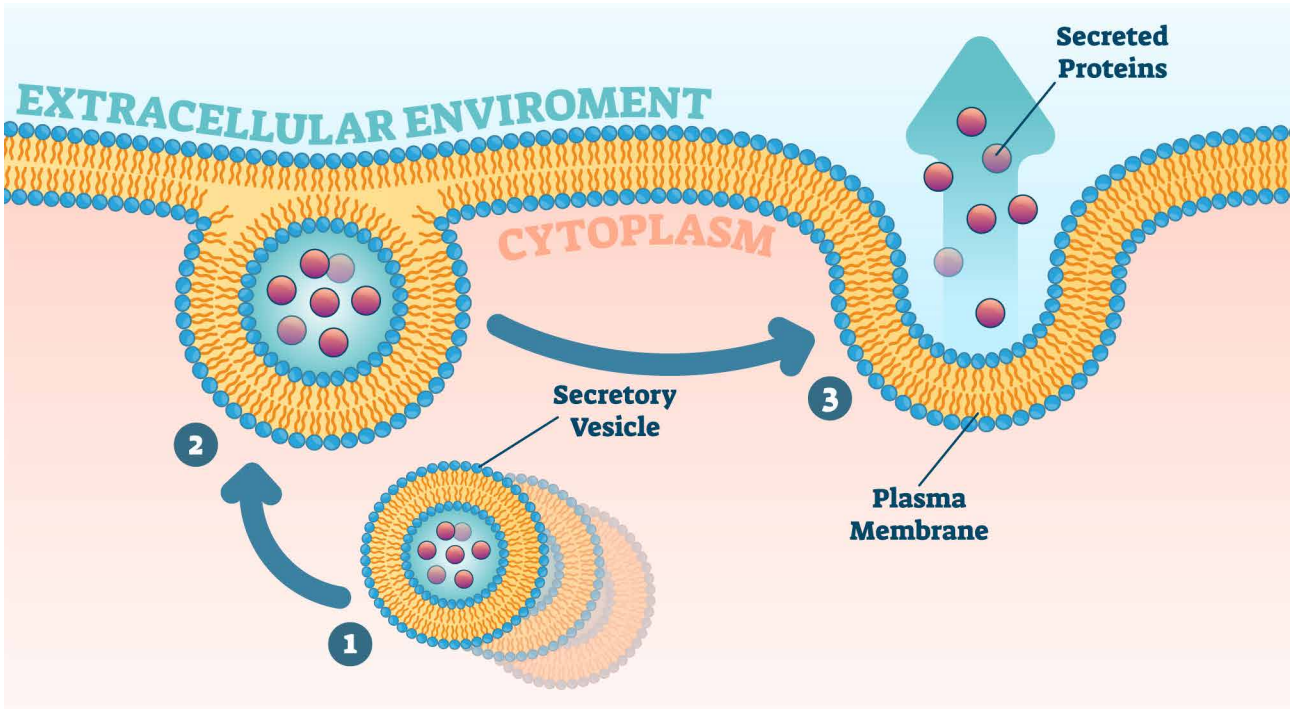


Illustration of cytokine secretion

cells. A major purpose of each pathway is to selectively regulate temporal cytokine release and to suitably terminate the response when necessary.

Most cytokine secretion, including from eosinophils, is via the classical pathway which is characterised by the packing and storage of cytokines in secretory granules within the cell prior to receptor-induced regulated release facilitated by membrane fusion. Alternatively, such as in macrophages, cytokines may be released immediately following synthesis which can ensue in a polarised manner. Furthermore, the process of cytokine secretion is customisable depending upon the required cell-specific immune function.

Eosinophils are highly granulated white blood cells which increase in abundance during an allergic response, and are capable of synthesising, storing, and secreting up to 35 different cytokines. Eosinophils have been observed in the airways of around half of asthma sufferers and may contribute to tissue damage. Cytokine secretion from eosinophils occurs predominantly via so-called piecemeal degranulation, where cytokines are recruited from larger storage granules and transported to the cell membrane in smaller vesicles, and degranulation may also occur via classical or compound exocytosis, as well as by lysis in dying cells.

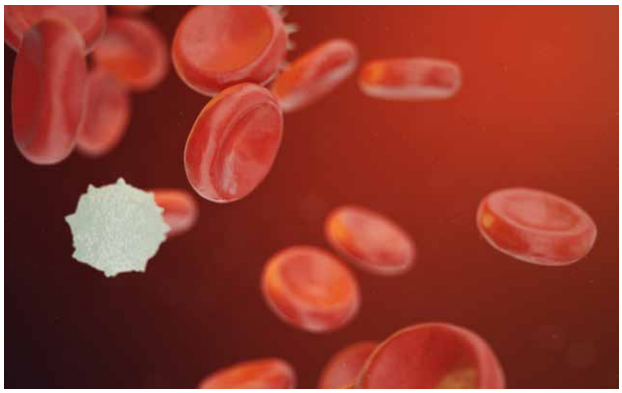
Macrophages are derived from white blood cells predominantly found on mucosal surfaces including the airways and have a key role in wound healing and tissue repair. Cytokines within macrophages are continuously transported to the cell surface in preparation for release when these cells are activated.

Professor Lacy and her team summarised that the movement of cytokines through immune cells is dependent upon membrane-bound or cytoplasmic enzymes, proteins, trafficking molecules, and intracellular membrane receptors, which mediate transport, facilitate membrane fusion, and regulate secretion. Initiation of the secretion pathway occurs within minutes of encountering an agonist. However, the specific roles of certain enzymes and proteins and the importance of cellular structural rearrangements in cytokine release from innate immune cells had not yet been elucidated.

Determining Cytokine Secretion Mechanisms

Given the paucity of available information, Professor Lacy and colleagues attempted to determine which molecules regulate eosinophil degranulation within the context of airway inflammation. To do this, the team built upon an earlier study in which they discovered that asthma patients exhibited a higher expression and activity of a specific enzyme (Rab27a) within their eosinophils, and that this likely contributed to the physiological traits typically observed in asthma. Human and mouse eosinophils were isolated and subjected to various laboratory techniques to determine the presence, subcellular localisation, and polarisation of the selected molecule.

They found that the molecule selectively redistributed when the cells were stimulated with an agonist, which suggested that eosinophil cytokine release indeed occurred in a manner that was dependent on Rab27a. Further studies in eosinophils demonstrated a role for other intracellular enzymes and receptor proteins (Cdk5, VAMP-7). Professor Lacy and her team confirmed these findings by repeating the experiments in strains of mice which had been genetically modified to



eliminate the proteins necessary for normal eosinophil function and observed that degranulation responses became defective. This research identified for the first time a direct role for specific regulatory molecules in inducing and controlling eosinophil degranulation in allergic inflammation and asthma.

Following these encouraging insights, Professor Lacy and her team endeavoured to further classify the proteins involved in the degranulation of eosinophils, since stimuli-induced activation of eosinophils is recognised as an exacerbating factor in the airway hyperresponsiveness associated with asthma. The researchers selected a vesicle-associated membrane protein (VAMP-7) which had previously been identified as an essential component of degranulation and confirmed that it was present in mouse eosinophils using fluorescence microscopy. Upon stimulation, isolated eosinophils translocate to gather at the edge of the cell, preparing for the release of granular contents.

Furthermore, genetically modified mice models lacking the chosen protein and mimicking allergic airway inflammation demonstrated a reduced incidence of degranulation and fewer cell-damaging products being released, suggesting a dysfunction in the eosinophil activation process, and confirming that eosinophil degranulation contributes to airway hyperresponsiveness. The fact that degranulation was not entirely abolished may be attributed to the small proportion of eosinophils that release their granular contents upon cell lysis, a recognised occurrence in allergic responses which depends on different signalling mechanisms. The researchers concluded that airway inflammation is at least partly mediated by degranulating eosinophils which can directly exacerbate the condition.

A Role for Cellular Structural Changes

To further investigate the mechanisms of cytokine secretion, Professor Lacy and colleagues proceeded to evaluate the intracellular storage sites of selected cytokines in eosinophils, and the pathways involved in their release. First, the team isolated human eosinophils from allergic or asthmatic participants and stimulated them to initiate cytokine trafficking. Fluorescence microscopy revealed altered eosinophil morphology and spatiotemporal increases in the levels of some cytokines following stimulation. Thereafter, the team deduced that membrane recycling pathways are likely employed by eosinophils to transport certain cytokines to the cell membrane for release, providing further insight into trafficking mechanisms within eosinophils.

This finding echoed that of earlier research conducted by Professor Lacy and her team investigating the mechanisms of cytokine secretion in macrophages, during which they reported that newly synthesised cytokines were also trafficked via a membrane recycling pathway. Delving further, the team continued to explore the mechanisms underlying the trafficking pathways and establish which mediators may be involved in the associated cellular structural changes. Using fluorescence microscopy techniques, dramatic alterations in the shape of cells were observed in the presence of a specific enzyme (Rac1), which was also associated with increased release of a selected cytokine and found to be vital for the final trafficking step prior to secretion within activated macrophages. Indeed, inhibition of the selected enzyme did not prevent cytokine synthesis in macrophages but did reduce transport and secretion, thus confirming, for the first time, its essential role in cytokine trafficking via the membrane recycling pathway.

Implications for Future Study

Understanding the various intercellular pathways involved in cytokine secretion is crucial for increasing our knowledge of cellular function in innate immunity and the associated ramifications for disease. Further studies surrounding the interrelationships of the regulatory pathways involved in the transport and secretion of cytokines and pro-inflammatory mediators may help to determine the underlying mechanisms unique to individual cell types. This is particularly prudent in the study of the mechanisms of cytokine release via non-classical pathways, since the theories surrounding this remain controversial.

More in-depth research to assess the secretion pathways of a wider range of cytokines and different cell types will undoubtedly prove highly beneficial in elucidating the underlying mechanisms of these phenomena. Using increasingly refined models of disease states has enabled Professor Lacy, her team, and collaborators to elicit the role of specific proteins in the degranulation of eosinophils in allergic inflammation using sophisticated gene targeting strategies. It is probable that complex multimodal mechanisms are involved in airway hyperresponsiveness involving synergistic product relationships, and experiments to decipher the contribution of individual components of eosinophil degranulation in asthmatic inflammation are warranted.

Perhaps most encouragingly, there is scope for exciting collaborations between scientific and clinical teams to apply the knowledge gained regarding the mechanisms of cytokine release in inflammatory disorders to the development of novel therapeutic targets.

Meet the researcher

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Professor Paige Lacy received her PhD in cell physiology from Wellington School of Medicine at the University of Otago, New Zealand. Following her inaugural postdoctoral fellowship, Professor Lacy held several assistant professor roles before attaining full professorship in her current role at the University of Alberta. The primary focus of Professor Lacy's research is to elucidate the molecular and cellular mechanisms involved in inflammatory responses, and in particular, the release of cytokines by innate immune cells. In addition to her plethora of accolades, Professor Lacy has led several research groups and education programmes in her capacity as former director of Alberta Respiratory Centre and has been the recipient of many coveted awards in recognition of her outstanding contributions to allergy research. Professor Lacy is a member of the prestigious Collegium Internationale Allergologicum, as well as numerous professional societies, and has published more than 126 peer-reviewed articles to date.

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UNIVERSITY OF ALBERTA

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TARGETED DRUG DELIVERY: FROM SCIENCE FICTION TO REALITY

Most human diseases are localised in terms of their location but currently, injected or orally administered drugs are evenly distributed all over the body and thus, act indiscriminately. The targeted delivery of medication to the exact site where it is needed is a common theme in science fiction but thanks to **Professor Richard Klemke** and his team at the University of California San Diego's Moores Cancer Center, this fantasy may soon become a reality.

The Fiction

Most of us will be familiar with the recurring scenario in science fiction: the protagonist, struck down with some fatal disease or injury, is saved miraculously by an ingenious drone programmed to navigate through the blood vessels, arrive at the site of the problem, and release the necessary drug just in time. Our hero survives another day. But is there any element of truth in this popular storyline that holds such promise for the future of medicine?

The Reality

The reality is that our ability to deliver drugs to precisely where they are needed in the body is very limited. The main problem is that most diseases are localised. Cancer, for example, usually affects only one site. Arthritis, most infections, diseases of the nervous system, and, in fact, just about any disease you can think of, will involve either one site or a limited number of select locations. Yet, in the absence of a better option, drugs are administered systemically. Irrespective of whether taken orally, injected into the blood, into a muscle or under the skin, medication is absorbed, circulates in the blood, and is deposited indiscriminately, everywhere in the body.

This approach has two significant shortcomings. First, a very large proportion of the drug ends up in places where it is not needed. This is not only wasteful but can be outright damaging for the patient. Take cancer again as an example. In an ideal world, we would want all cancer-killing chemicals concentrated within the tumour to provide the best chance of a cure because when the drug goes everywhere, it also affects other tissues. Problematically, cancer-killing chemicals in the bone marrow can switch off the production of red blood cells, causing anaemia. Elsewhere, damage to cells lining the gut can cause nausea and weight loss. An unwanted effect in the skin is hair loss. Other vital organs, the kidneys, the heart muscle, nerves, and hormone-producing organs can all be affected by indiscriminate drug distribution, causing severe side effects.

For these reasons, physicians try to delicately balance the dose of medication to ensure that it is suitably effective while also, minimising

unwanted side effects. Unfortunately, such compromises limit our ability to treat many serious diseases effectively. Although researchers have tried several methods to overcome these problems (for example, utilising nanotechnology and microscopic fat droplet-mediated drug delivery), the results have usually been poor.

Starting with Mesenchymal Stem Cells

Professor Richard Klemke at the University of California San Diego's Moores Cancer Center is a world leader in this exciting field. He explains that the starting point for his work was a fairly simple observation regarding mesenchymal stem cells (MSCs) involved in tissue regeneration. For example, when we cut a finger, MSCs

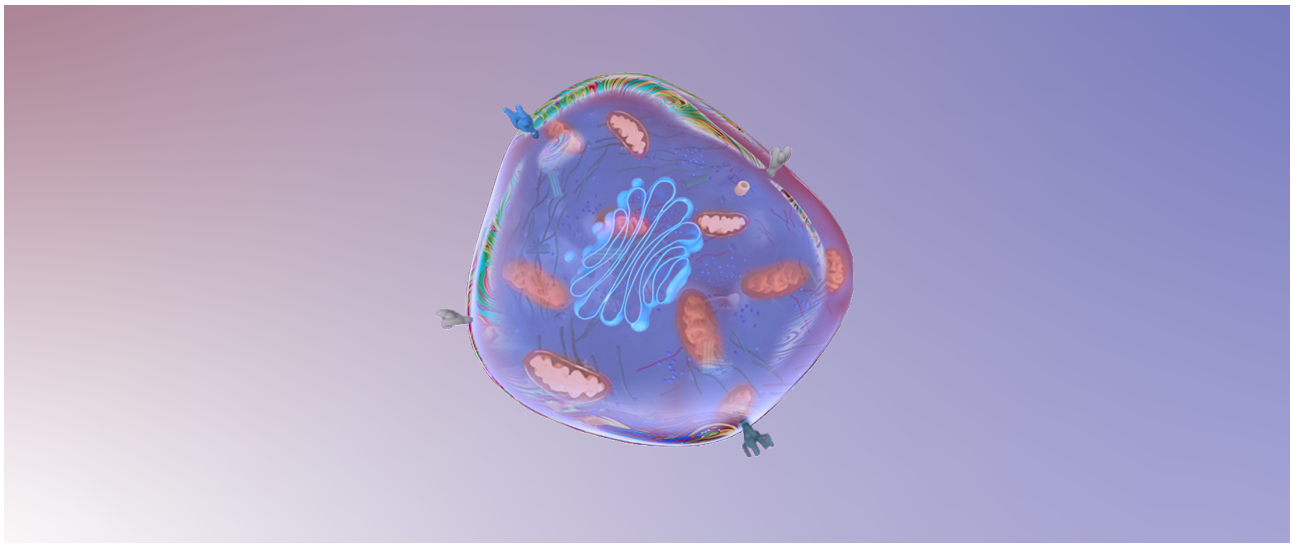

Illustration of a CargocyteTM. Reproduced with permission from Richard Klemke.

move to the site of the injury and repair the damage by turning into cells that are needed locally and releasing beneficial substances.

MSCs caught Professor Klemke's attention because here, he noted, is a cell that can move, can find its way to an injury, and can release large amounts of chemicals on the spot. Was there a way to utilise these MSCs for the targeted delivery of specific drugs?

Theoretically, it all seemed possible although there were considerable challenges to be overcome. For a start, MSCs repair damage by turning into other cells that are lost during the initial injury and uncontrollably produce unwanted factors. This ability is not only unnecessary for the purposes of targeted drug delivery but is also potentially damaging. As such, the accumulation and long-term survival of such cells and their secreted products could alter structures or cause other harm.

The genetic programme necessary for these unwanted effects is coded in the nucleus of the MSCs. Professor Klemke saw that the solution to several problems associated with using nucleated cells as therapeutic delivery devices was simply to remove the dangerous DNA from the cell. By removing the entire nucleus through a process known as cell enucleation, he reasoned that the cell would be rendered safe with a lower potential for carcinogenicity. Cell enucleation would also block new gene transcription, preventing the induction of potentially unwanted products after the cell was introduced into the body. Finally, the enucleated cell would have a defined life span which would make drug delivery more controlled and predictable.

But key questions remained, including the issue of how long the cells would survive after enucleation, whether they would still perform the critical migration functions necessary for locating and physically moving into disease tissues, and whether they would produce and deliver a defined therapeutic at the site of disease as intended. But, if the enucleated cells could survive and perform as hoped, then possibly, they might act like delivery drones dedicated to bringing defined therapeutics to diseased tissues in a much safer, controlled, and predictable manner than previously possible.

Professor Klemke's team developed the necessary technology to achieve this by optimising the conditions for gently removing the nucleus from the cell. The resulting structures could no longer be considered cells, as they couldn't divide, survive for long, or develop independently. Due to their intended use, the team decided to call these nucleus-free, cargo-carrying cell-derived particles Cargocytes™. The removal of the nucleus meant that most of the regulatory concerns and risks associated with cell therapy would no longer apply, making it easier to gain regulatory approval for their use in the future.

Professor Klemke and the team undertook rigorous functional testing to determine the therapeutic utility of the enucleated cells using animal systems – and this worked pretty well. But excitingly, the researchers knew they could substantially improve this process even further by introducing a GPS-like system to guide cells to diseased tissues using gene and cell engineering technologies.

The Guidance System

While Cargocytes™ have the necessary machinery to move, their use for drug delivery required a way to guide them to the location where they are needed. The biology behind this is complex. Imagine that there is an infection in your right earlobe, while Cargocytes™ intended to treat this infection are swimming around in your blood, inside blood vessels. How would a Cargocyte™ 'know' where to come out and deposit its content?

Cancer on healthy tissue.

What helps is that there is a signal coming from the infected earlobe, alerting other cells to the problem. Biologists refer to these signals as chemokines. The first thing the team needed to do was to provide the Cargocyte™ with the ability to detect these signals. Molecules with this sensory function are called chemokine receptors. Perhaps it is easiest to imagine these as antennae on the Cargocyte™ surface, allowing it to detect the presence of a chemokine.

Once the Cargocyte™ reaches the location where the chemokine signal is the strongest, there needs to be a mechanism that prevents it from being swept along with the blood flow. Rather, it needs to stop and then move out of the blood vessels passing near the diseased tissue. There are two types of molecules involved in this process: adhesion molecules and selectins. Adhesion molecules can be thought of as 'hands' holding onto selectins, microscopic 'grab handles'. Adhesion molecules attaching themselves to selectins allow the Cargocyte™ to stop and 'climb' in the required direction.

During initial experiments, Professor Klemke and his team were able to insert three different chemokine receptors – 'antenna' – onto the surface of Cargocytes™. They also showed that these structures were functional, in other words, that the modified Cargocytes™ detected and responded to chemokine guidance signals, adhered to diseased blood vessels, and moved out of the vascular system into the diseased tissue

These experiments were first attempted in Petri dishes but encouraged by their early success, Professor Klemke and the team then conducted experiments in mice. Here, one ear of the animals was deliberately irritated, causing a mild inflammation. When unmodified Cargocytes™ were injected into these animals, they did not leave the bloodstream. However, Cargocytes™ modified with the right chemokine receptors and adhesion molecule rapidly accumulated in the inflamed ear, while practically none were seen in the healthy normal ear on the other side or other organs in the body. Guiding Cargocytes™ to the right location was now a very real possibility.

Loading the Cargo

Finally, Professor Klemke and his team introduced a third modification in which they inserted a purified mRNA that made the Cargocytes™ produce IL-10, a strong anti-inflammatory molecule. When these triple-modified Cargocytes™ were injected into mice with one inflamed ear, their homing to the site of the inflammation worked as expected.

Furthermore, due to the action of IL-10, the swelling of the inflamed ear resolved rapidly. An even more rigorous preclinical test of the triple-modified Cargocytes™ involved tests in animals with inflammation of the pancreas. Sitting deep behind the stomach and hence difficult to approach even during surgery, the pancreas produces digestive juices and insulin. The inflammation of this vital organ is a common life-threatening problem in humans. Experiments demonstrated that triple-modified Cargocytes™ found their way to the damaged gland and the anti-inflammatory effect of IL-10 significantly reduced further damage to the pancreas in the treated mice. Furthermore, the team did not find any evidence of adverse effects associated with the administration of Cargocytes™, instilling further optimism.

Not Perfect – Yet…

These results are extremely encouraging. They show that Cargocytes™ represent a novel and highly promising way to selectively deliver drugs to specific locations. This possibility will open up important new therapeutic approaches although challenges remain. First of all, production and manufacturing methods need to be scaled for clinical applications and the safety of Cargocytes™ needs to be rigorously tested before clinical use. Professor Klemke's team will also need to show that by changing the chemokine receptors and adhesion molecules, they can direct Cargocytes™ to other locations, for example, to a tumour.

This dedicated work means that Professor Klemke is well on the way to making science fiction a reality. In the not-too-distant future, these tiny little biological structures may be able to improve the medical treatment of millions of people.

Meet the researcher

Professor Richard L. Klemke
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Professor Richard Klemke earned his PhD in Cell and Developmental Biology from Texas Tech University Health Sciences Center in the USA. After working as a research scientist at Scripps Research (previously known as The Scripps Research Institute), he joined the University of California San Diego's Moores Cancer Center as Professor of Pathology in 2006, where he is now Professor of Pathology and Cancer Biology. Over the past 20 years, Professor Klemke has been a highly active teacher, mentor and lecturer in the academic realm, and also a valued consultant in the biotech and pharmaceutical industries. His laboratory has received numerous federal and private awards to support his development of novel therapeutics for cancer and investigations into the critical mechanisms that drive cancer progression and cancer immunity. Unsurprisingly, Professor Klemke's pioneering work has attracted international recognition, and he continues to provide new insights into the underlying processes of how malignant tumours develop, induce a blood supply, and avoid immune recognition.

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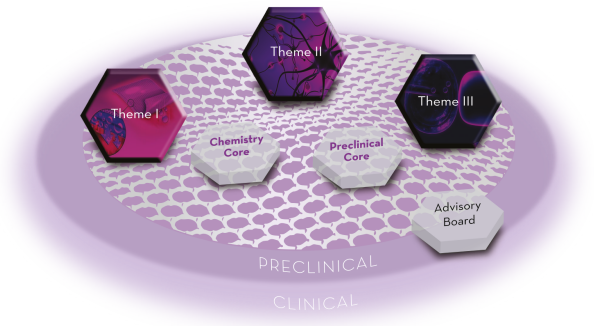
UC San Diego

CLINICAL TRANSLATION OF INNOVATIVE GRAPHENE-BASED THERAPEUTICS

Two-dimensional materials, like graphene, have enriched the world of nanoscience and shown great potential for a variety of different application areas. In medicine, the use of graphene is being developed and adapted to offer novel solutions in addressing clinical challenges. The *2D-Health* research programme, headed by **Professor Kostas Kostarelos**, is a multi-disciplinary research effort looking to engineer and refine graphene-based technologies that can be clinically utilised. Based mainly at the University of Manchester, work at *2D-Health* is split into three Themes addressing needs in wound care and orthopaedic surgery, cell therapeutics and cancer immunotherapy. This exciting work is aiming to offer novel solutions in healthcare whilst deepening our knowledge of two-dimensional materials interacting with the living body.



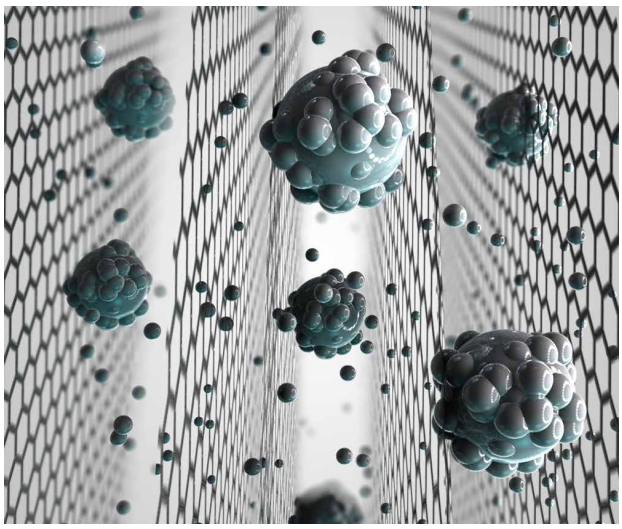
Work at 2D-Health is split into three Themes.

2-Dimensional Ideas for Novel Therapeutics

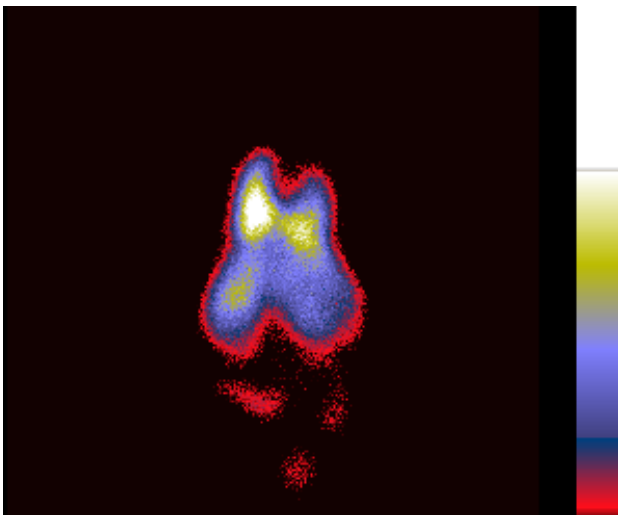
The world of healthcare technology innovation is exciting and rapidly expanding. With so many avenues to explore, the possibilities for new therapeutics seem never-ending. One such avenue is the use of one-atom-thick, or two-dimensional (2D) materials. Members of this large family of flat crystals include hexagonal boron nitride, transition metal dichalcogenides and graphene. They can be used for all sorts of useful technologies, thanks to their variety of properties. Depending on the material, they can be either stiff or flexible, conductive or insulating, making the family a rich material template for manipulation.

2D-Health is the name of a multidisciplinary programme of research led by nanomedicine expert, Professor Kostas Kostarelos. The assembled team of experienced researchers has attempted to expand our knowledge of how the versatile material, graphene, can be used in medicine. The University of Manchester provides a base for this group of scientists who are all experts in their respective fields, to investigate how 2D materials can be used in future therapies. As population growth and demand on the UK's National Health Service (NHS) increases, the system is coming under increasing financial pressure. *2D-Health* believes that cultivating new therapies with innovative technology will significantly aid the NHS by offering safer and more effective treatments whilst reducing overall cost.

Funded by the UKRI Engineering and Physical Sciences Research Council, the programme is divided into three Themes underpinned by two Cores. The Chemistry Core aims to fabricate and modify graphene-based two-dimensional (G2D) materials, so they are more functional for use in the biomedical work of the programme. The Pre-Clinical Core has a similar facilitating role but focuses on how G2D materials can be developed and used as pharmacological platforms for the development of therapeutics in specific disease models.



Schematic image of ion sieving through angstrom-size capillaries in graphene-based membranes. Credit: Rahul Nair, The University of Manchester



Tagged graphene flakes are being tracked in the body of living animals using imaging cameras, as in the tomography shown above. Green/white signal indicates higher amounts of graphene flakes localising in different tissues (in this image, the kidney). Credit: Nanomedicine Lab, The University of Manchester.

The *2D-Health* consortium has built strong partnerships with two Industrial Champions that allow the team to focus on research that can be translated into clinically relevant solutions in the future. Theme I is navigated through the advice of the global medical device company, Smith & Nephew, and Theme III is linked with the global biopharmaceutical company, AstraZeneca.

The project is overseen by an Advisory Board made up of external scientists, philosophers, ethicists and regulatory experts. Chaired by Professor Bernadette Bensaude-Vincent from Sorbonne University (Paris, France), they fulfil multiple roles from reviewing the progress and direction of the programme to advising on strategy, ethics and outreach.

Graphene Oxide Membranes for Surgical Wound Healing (Theme I)

Led by Professor Rahul Raveendran Nair, Theme I explores how G2D membranes can be used in the development of advanced implants and dressings for surgical and topical wounds. The goal is to create a film that protects it from infection and monitors its occurrence whilst speeding up healing. There are four characteristics the team wants to incorporate into such a 'smart' material. Sensing mechanisms built into the membrane would allow a clinician to understand how well their patients' wounds are healing and whether an infection is looming. If necessary, the membrane could be able to release therapeutic molecules as and when they are needed, for healing or pain relief. The material should be electroconductive to boost its efficacy and lastly, its selective permeability should be controllable. This means that the amount and types of molecules (for example, water and ions) that are permitted to pass in and out of the membrane, are regulated to allow for favourable biological responses in the tissue.

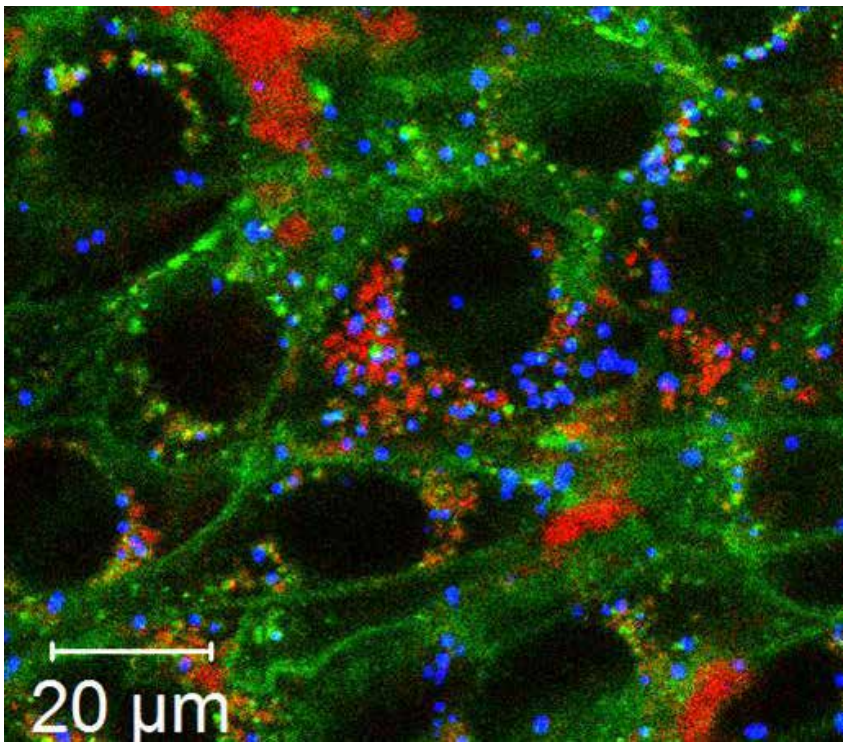
The potential applications for G2D smart membranes are staggering. Orthopaedic wounds, which are those of the musculoskeletal system (bones, muscles and nerves), perhaps due to diabetes complications, might be more easily treated with this technology.

Exfoliated 2-Dimensional Sheets in Cell Therapeutics (Theme II)

Theme II is led by Professor Cinzia Casiraghi. She and her team investigate how inks made from G2D material could be used to track where cells administered during cell therapy have travelled to. Cell therapy, also known as cell transplantation, involves administering healthy stem cells (cells that can develop into lots of different cell types) to patients via injection or implantation. It can be used to restore damaged or dysfunctional tissue that has resulted from a multitude of diseases, such as cancer and cardiovascular disease.

However, it is currently very difficult to track the whereabouts of cells once they have been administered in the body. Resolving this issue would be extremely helpful for clinicians in gauging whether their patient's treatment was given accurately and monitoring how long the stem cells have remained in the body. Consequently, clinicians could decide whether the patient has healed well or if they require further treatment.

The innovative solution that Dr Casiraghi is developing involves tagging a G2D ink label to the cell populations and using one of a variety of tracking signals to locate them in the body. These signals include near-infrared imaging, magnetic, photoluminescence or radiological signals. Already, the team have created some G2D inks that are in the safety-testing stages, with positive preliminary results suggesting they are safe for use on human cells.



Cancer cell internalisation of graphene oxide sheets (red signal) for therapeutic purposes. Credit: Nanomedicine Lab, The University of Manchester.

Graphene Oxide Transporters for Cancer Immunomodulation (Theme III)

The last theme in the programme, Theme III, is headed by Professor Andrew MacDonald. This element of the project delves into cancer immunotherapy. Using your body's own immune system to combat cancer is a relatively new, but promising method of treatment.

The goal of this Theme's work is to use specially engineered graphene oxide sheets as a means of presenting biomolecules capable of modulating the immune system. Antigen-presenting cells (APCs) are found in tissues involved in the immune response, like the spleen, or in areas of the body that require protection from the outside world, like the lining of the nose. APCs process the antigens and then present them on their own. Different types of T cells recognise these antigens via their receptors and in this way get 'activated' to fight against the foreign body they are primed against. If the antigen is from a virus or a cancer cell, the type of T cell it attracts is a cytotoxic T cell. Therefore, if the engineered graphene oxide complexes present effectively the cancer cell antigens, cytotoxic T cells can be induced to fight against the tumour.

The thin graphene oxide sheets used tend to target the spleen, so are a great way to deliver molecules specifically to that area. However, this is just one of the reasons the team is developing this technology as a nanocarrier. It also has a large surface area on which to carry desired molecules

Thanks to their partnership with AstraZeneca, Theme III could be clinically translated to cancer therapeutics in the future.

Clinical Safety and Oncology Collaborations with Edinburgh and Cambridge

Due to the exciting prospects of G2D materials, many other organisations and scientists are studying their potential capabilities and effects. The team at *2D-Health* have formed collaborations with other universities to enhance their capacity to achieve impact.

In collaboration with the University of Edinburgh, the team has conducted research on the consequences of inhaling different nanoparticles, including graphene oxide. They are investigating whether a group of fats called eicosanoids drive the deterioration of lung and cardiovascular diseases after graphene inhalation. The research will culminate in the first-in-human clinical study of its kind and is led by Dr Mark Miller. It is anticipated that by using the findings from this work, *2D-Health* will contribute to the determination of the possible risks and limitations associated with human exposure to 2D materials.

The University of Cambridge is the second academic institution that *2D-Health* has been partnering with. The collaborative effort is investigating the design and fabrication of electrically conductive technologies to manage cancers that are notoriously aggressive and difficult to treat. Both the Theme II and Theme III teams of the core *2D-Health* consortium are actively collaborating with the University of Cambridge research teams to achieve the project's goals.

An Exciting future for Graphene-based Therapeutics

The *2D-Health* teams have made impressive strides in bettering our knowledge and understanding of graphene-based 2D materials and their use in medical applications. Although the research programme is attempting to address very different biomedical and clinical application spaces, the exploration of graphene provides a prototypical fundamental effort on how to design and develop 2D materials with great promise for the delivery of innovative and effective therapeutics in the near future.

Meet the Researchers

2D-Health

University of Manchester

UK

Professor Kostas Kostarelos

Director of *2D-Health*

Professor Kostas Kostarelos obtained his BSc in Chemistry from the University of Leeds, then went on to complete a Diploma and PhD in Chemical Engineering at Imperial College London. Currently, Professor Kostarelos holds a Professorship in Nanomedicine at the University of Manchester and is a Severo Ochoa Distinguished Professor at the Catalan Institute of Nanoscience & Nanotechnology in Barcelona, Spain.

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Professor Rahul Raveendran Nair

Theme I lead for *2D-Health*

Dr Rahul Raveendran Nair achieved his PhD in Physics from the University of Manchester in 2010. He has been awarded many accolades during his career and is now a Professor of Materials Physics. His research focuses on 2D materials-based membranes and how they can be applied for use in the real world.

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Professor Cinzia Casiraghi

Theme II lead for *2D-Health*

Professor Cinzia Casiraghi completed her BSc and MSc in Nuclear Engineering at the Politecnico di Milano in Italy. She went on to achieve her PhD and then completed a PhD in Electrical Engineering at Cambridge University. Professor Casiraghi now holds a Professorship in Nanoscience at the University of Manchester, whilst carrying out research into biomedical applications of printed electronics using 2D inks.

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Professor Andrew MacDonald

Theme III lead for *2D-Health*

Professor Andrew MacDonald holds a BSc in Parasitology from the University of Glasgow and a PhD in Immunoparasitology from the University of Edinburgh. Whilst fulfilling his role as Professor of Immunology at the Manchester Collaborative Centre for Inflammation Research, he also conducts research into immune cell activation and function in allergies, infection and cancer.

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Dr Marianne Ashford

Dr Marianne Ashford is a Senior Principal Scientist from AstraZeneca, working closely with Theme III in her role as an Industrial Champion.

HOW ROBOTS ARE HELPING US UNDERSTAND KNEE INJURIES

Knee injuries can be notoriously complex. In recent years, many studies have attempted to investigate a potential link between the geometry of the knee and the risk of injury to a ligament called the ACL (anterior cruciate ligament). **Dr Alan Barhorst** from the University of Lousiana at Lafayette, alongside colleague **Mr Ross Wilson**, enlisted the help of two robots to perform a biomechanical study of this phenomenon. Their findings provide valuable insight into our vulnerability to ACL injuries.

The Problem with Knee Injuries

In our bodies, the ACL – known in full as the anterior cruciate ligament – provides a thick sheath of connective tissue that not only stabilises our knee but prevents our shinbone (tibia) from sliding in front of our thighbone (femur).

The ACL is a crucial area of study for several reasons. First, the American Board of Orthopaedic Surgeons reports ACL surgery to be one of the most common orthopaedic (bone or muscle) surgeries performed worldwide. Second, ACL injuries are very common in sports that involve a lot of force (e.g., jumping, contact, rapid acceleration and pivot movements). While the posterior, medial and lateral cruciate ligaments also work hard to support the knee, they can usually be rehabilitated with non-surgical treatments such as physiotherapy.

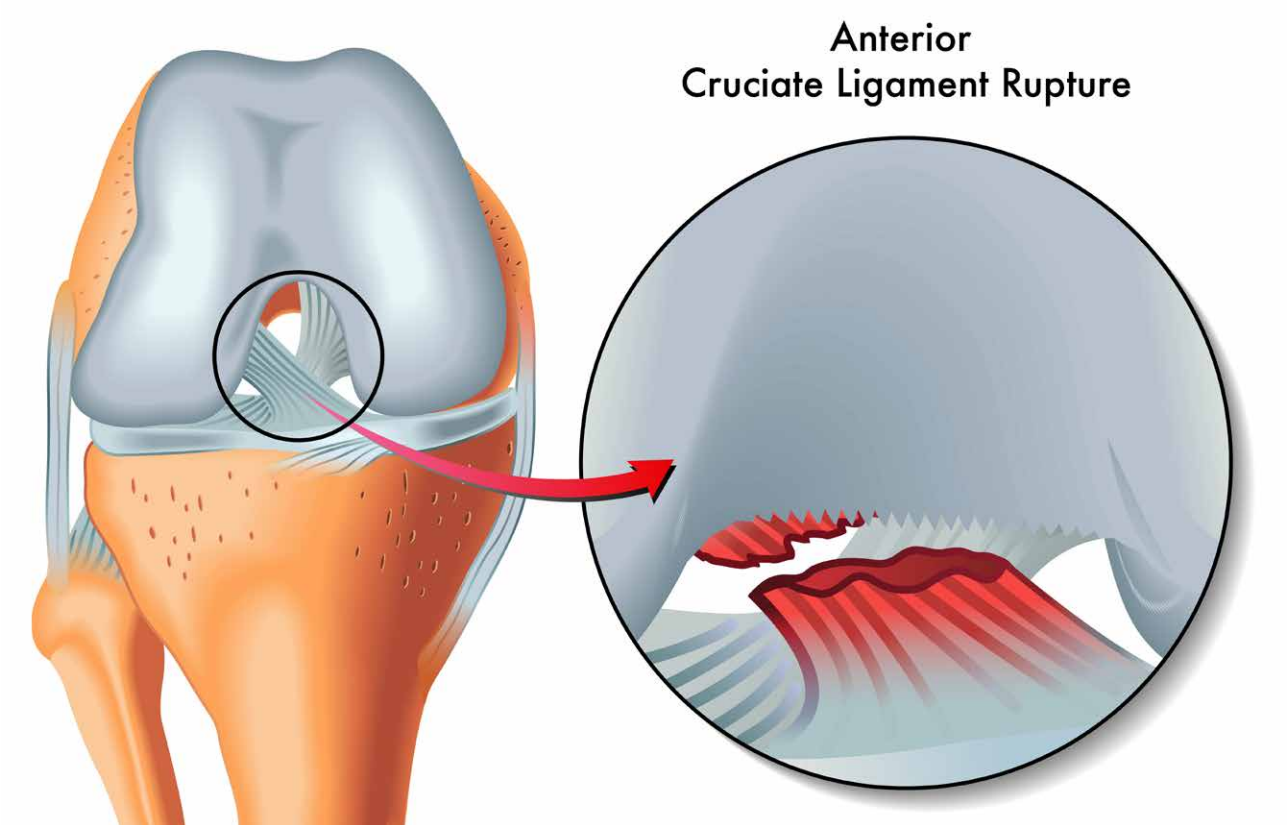
However, if you've ever skied, played rugby, or football, run, or are generally very active, you might have experienced ACL pain. That's because the ACL plays a vital role in supporting and stabilising the knee in all activities, and when subjected to more stress or force than it is able to handle, it becomes injured. And that's a problem because ACL injuries often require surgery. Dr Alan Barhorst from the University of Louisiana at Lafayette and his colleague, Mr Ross Wilson, are working to better understand how and why such injuries occur in the first place.

Taking it up a Notch

More specifically, Dr Barhorst and Mr Wilson wanted to know if a smaller-sized intercondylar notch might make us more likely to suffer from injuries to our ACL.

But what exactly is an intercondylar notch? It is called 'intercondylar' because it is a deep space that sits between (inter) the two condyles (or rounded ends) of our thighbone (where the thighbone attaches to the knee). It stabilises the knee and also provides the attachment site for the ACL and posterior cruciate ligaments. These ligaments play a vital role in connecting our thighbone to our shinbone.

Dr Barhorst and Mr Wilson looked specifically at whether having a smaller intercondylar notch would be more



likely to cause a lateral impingement (causing a transverse load) of the ACL, and whether any observed impingement could be a cause of injury to the ACL.

The Robots Begin

To explore this question, the researchers utilised two robotic manipulators. These robots directed the knee joints of multiple cadavers (corpses), in order to simulate a range of complex biomechanical movements. The use of a two-robot system was necessary because it allowed observation of how the femur and tibia work together across a vast range of movements.

The three female and three male cadavers used were non-osteoarthritic (i.e., without arthritis), and under the age of seventy years. Biomechanical testing allowed Dr Barhorst and Mr Wilson to observe the rate of impingement in these knees, and to also observe if these impingements caused injuries. The experimental data pointed to impingements in five out of the six cadaver knees but these impingements were not a cause of injuries. This suggests that such impingements may actually be a regular and normal occurrence in healthy knees.

Male and Female Differences

Interestingly, a lower elasticity was reported in the ACL of women. That could potentially mean a lower ability to withstand force and a greater potential for injury of the ACL. Dr Barhorst and Mr Wilson also observed impingement to be more frequent in females, who also exhibited smaller intercondylar notch sizes.

Future Directions and Clinical Implications

Dr Barhorst and Mr Wilson have extended our understanding of ACL injury, providing valuable experimental data. They recommended the use of larger sample sizes, continued testing and 3D anatomical measurements in future studies. Their work ultimately contributes to a fascinating field that helps us to understand, treat, and prevent, knee injuries far more effectively. And this is great news for all of the sports people and fitness fans out there who love to stay active but injury free.

Meet the researcher

Dr Alan Andrew Barhorst
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Dr Barhorst graduated from Texas A&M University in 1984 and 1989 respectively, with a BS degree and then an MS degree in Mechanical Engineering. Dr Barhost went on to complete his studies at his alma mater, being awarded a PhD in Mechanical Engineering in 1991. He is currently Professor and Head of the Mechanical Engineering Department, Alumni Association & LEQSF (Louisiana Education Quality Support Fund) at the University of Louisiana at Lafayette. In addition, Dr Barhost is the inaugural co-editor of the Springer academic journal Data Enabled Discovery and Applications. Elected a Fellow of the American Society of Mechanical Engineers in 2013, Dr Barhost was later awarded a J. Tinsley Oden Faculty Fellowship at the University of Texas at Austin in 2016. His research interests include biomechanics, control system design, dynamics, design, fluid-structure interaction and healthcare engineering.

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FUNDING

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FURTHER READING

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UNIVERSITY of LOUISIANA LAFAYETTE

LEARNING FROM FISH HOW TO RE-BUILD THE BRAIN IN OLDER AGE

Worldwide, people are living longer lives. One outcome of this is that the prevalence of neurodegenerative diseases whereby the cells in the brain stop working or even die, is also increasing. Based in KU Leuven's Department of Biology, Belgium, **Professor Lieve Moons** has been working to better understand how the central nervous system can regrow and repair, with a particular focus on ageing. Her work has important implications for identifying new therapeutic targets for neurorepair in elderly humans.

Neurodegeneration: An Increasing Burden

Neurodegenerative diseases, including those leading to dementia such as Alzheimer's disease, affect millions of people worldwide. Neurodegenerative diseases are progressive, meaning that they worsen over time and spontaneous recovery does not occur. As disease progression continues, a person's independence and quality of life are sadly diminished.

Approximately 50 million people live with dementia globally. As generations live for longer, this burden is expected to increase to 152 million by the year 2050, creating an unprecedented urgency to develop new therapies which seek to treat, and ultimately prevent, central nervous system (CNS) diseases.

////Subheading: Using Animal Models to Discover New Treatment Targets

Efforts to discover new targets for CNS repair in so-called neuro(re)generative research, make use of animal models in the laboratory that retain the ability to regenerate after birth and into adulthood. Studying these animals allows scientists to pin down the cellular and molecular mechanisms underlying regeneration in the hope of extrapolating from these findings to inform drug discovery in humans.

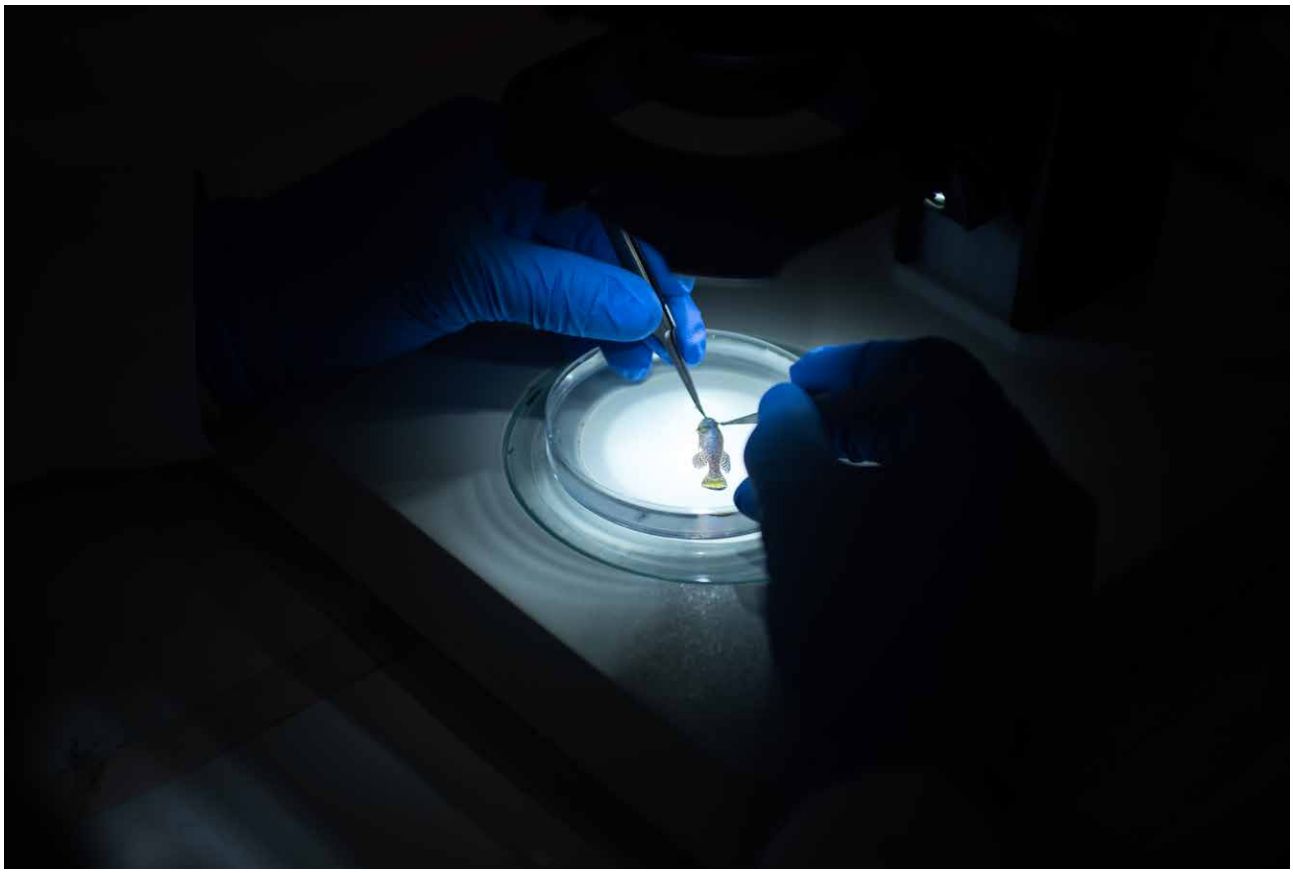
Professor Lieve Moons is head of the Neural Circuit Development and Regeneration (NCDR) Research Group at KU Leuven. The group aims to improve understanding of how ageing affects regeneration in the CNS.

More than 8 years ago, Professor Moons and her team started working with the well-characterised zebrafish model to explore age-related and injury-induced vision loss and how this functionally recovers. Zebrafish are well-studied in biomedical research, and scientists have sequenced their entire genome to show that they share 70% of their coding genes with humans, and these genes likely have a shared cellular function between the two species. Zebrafish are relatively cheap to house and quick to breed new generations and have been incredibly useful across research disciplines.

Zebrafish are particularly useful in regenerative medicine due to their ability to rejuvenate lost tissue (such as fins, heart and brain) following damage.

When model organisms possess the ability to regenerate, insight into the specific factors involved can inform scientists on the underlying processes and help them develop techniques to repair tissue damage resulting from brain injury and neurodegenerative disease, for example.

We know ageing is a risk factor for the development of neurodegenerative conditions, but surprisingly, few research laboratories focus on the impact of ageing on regenerative potential. This is largely due to the impracticality of the 3–5-year lifespan of zebrafish, which creates a barrier to studying old age in the laboratory. The NCDR team, situated in the Biology Department of KU Leuven, found that injury-induced regrowth slows



CREDIT: Luca Masin

down in older zebrafish but is not lost at older age. These fish, unlike mammals, can regain vision well into their golden years, meaning that their application to understanding similar processes in aged humans is unfortunately limited.

Professor Moons and the team noticed that ecologists in the department were using African turquoise killifish, one of the shortest-lived vertebrates with regenerative powers, in their ecotoxicology research. Killifish have short lifespans in the wild where they reside in seasonal ponds and only live up to 6 months in captivity. They display ageing hallmarks like those seen in humans, including impaired protein self-regulation, damage to specialised parts of cells called organelles, and a reduced genesis of new cells in the CNS. These similarities make killifish an ideal model to study ageing in the context of neuro(re)generation. This led to the birth of the Leuven KillAge Consortium, in collaboration with several other laboratories from the Animal Physiology and Neurobiology division at KU Leuven, in 2018.

Translating Killifish Findings to Mammalian Brain Injury and Recovery

Professor Moons and the team are particularly interested in filling the gap in neuro(re)generative research by identifying the structural, genetic, and functional changes caused by growing old. Killifish share 60% of their coding genes with humans, allowing the team to investigate why these regenerative powers are locked away in the mammalian brain – and perhaps find a way to harness the body's ability to self-repair.

Before long, Professor Moons and her colleagues made an important discovery. While killifish are very similar to their zebrafish relatives at a young age, as they get older, they switch to being more mammalian-like – critically, lacking the power of injury-induced regeneration. This means that the killifish model has countless implications for studying diseases affecting older people.

To explore how killifish regrow parts of the CNS to recover from injury, the team uses an optic nerve crush (ONC) injury model whereby anaesthetised fish have their optic nerve crushed by forceps. The fish are allowed to recover before undergoing vision tests to measure visual acuity and functional recovery.

The similarities between killifish and humans will help scientists to establish better treatments for diseases that affect the retina and optic nerve that form part of the CNS (e.g., glaucoma), and many other neurodegenerative diseases. As the retina-brain axis is more easily accessible than the brain or spinal cord, it is a practical choice for conducting this sort of regenerative research.

Inside-Out: The Effects of Ageing

Using killifish, Professor Moons and her team of dedicated

researchers are working to define the neuron-intrinsic (within the nerve cell) and extrinsic (from other cells surrounding the neurons) factors responsible for the observed reduced regenerative capacity during ageing. Using the ONC injury model in killifish of various life stages, the team revealed that the loss of visual recovery in aged animals is due to a combination of cell-intrinsic and extrinsic processes.

The team focuses on the retinal ganglion cells, the only retinal nerve cells that carry information from the retina to the brain through long cables, called axons, that run into the optic nerve. Upon ONC, part of the injured ganglion cells survive, they regrow an axon and re-establish their connections with the neurons in the brain, more specifically in the optic tectum, which leads to functional recovery of the circuit required for vision.

On examination of the retinal ganglion cells, the team observed a reduced survival and a decrease in the expression levels of genes that are important for axonal regrowth. Furthermore, the nerve cells, as well as their neighbouring cells in the retina, optic nerve and brain, start to express more cell cycle inhibitors and therefore, there is a higher incidence of cellular senescence in aged killifish. Cellular senescence occurs when a cell ages and permanently stops dividing (meaning the cell cycle is stopped) but it does not die. Instead, it remains senescent and continues to release chemicals to the environment, which can trigger inflammation.

As such, the immediate environment external to the nerve cells becomes increasingly toxic at older age, in the retina as well as in the optic nerve and brain. This toxic environment is in part caused by inflammation and excess production of cytokines and reactive oxygen species, which are important cell signalling molecules for normal biological processes. However, in the contexts of ageing and disease, having too many cytokines and reactive oxygen

species can cause damage to cells, proteins and DNA.

This toxic environment impairs regeneration. Excitingly, Professor Moons' team is the first to demonstrate the emergence of a long-term scar at the optic nerve lesion site, caused by non-neuronal glia cells, in an animal model capable of regeneration. Scar tissue is not the same as normal tissue because it has less elasticity, which can lead to local tightness and a limited ability of the axons to cross this mechanical barrier and regrow towards the brain. This scar tissue may help explain the severe and permanent impairment that occurs for the retinal ganglion cells leaving them unable to renew the lost connections required for functional recovery of vision.

Professor Moons' team has demonstrated that a combination of an increased number of dying cells and the reduced axonal regrowth of surviving cells in the retina of aged fish post-injury contribute to the observed decline in regenerative capacity. The team also reports that the defective repair of damaged synapses, which are the junctions between neurons essential for their communication, can diminish the functional repair of vision.

Professor Moons' research sheds light on the post-injury changes inside older cells which contribute to cell and environment toxicity, and so the cycle ensues. Sick cells cause environmental toxicity, which causes more cells to become sick. But in a chicken-and-egg situation, it is important to understand what comes first in the bid to develop targeted therapies which may halt this downward health spiral.

The Future for Neuroprotection and Regeneration

Professor Moons' and her team have provided the field of regenerative medicine with the critical knowledge that killifish completely lose their regenerative ability in older age. With killifish becoming mammalian-like upon ageing, they form the ideal model to further unravel the mechanisms underlying de- and regeneration in the ageing CNS, and to continue the search for the genes and pathways that gatekeep spontaneous repair or even rejuvenation from humans.

Notably, Professor Moons now aims to validate new identified targets arising from their killifish research in mammalian models. To this end, the team has an ONC mouse model available that can be employed to work closer towards the clinical translation of their important findings to date.

Developing effective therapies that promote the establishment of novel neuronal connections to replace those damaged by injury or disease and thus restore communication, would revolutionise modern medicine. The possibility that the secret to protecting neural circuits in age-related disease could be locked within a tropical fish a little larger than a paperclip, is nothing short of amazing.

Meet the researcher

Professor Lieve Moons
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Professor Lieve Moons received her PhD in Biology from the Katholieke Universiteit (KU) Leuven, Belgium. After undertaking several roles working across neuroendocrinology, immunological biotechnology, and molecular and vascular biology, she worked as a Senior Scientist at the Flemish Interuniversity Institute for Biotechnology, before returning to KU Leuven as an Assistant, Associate, and now, Full Professor. Professor Moons is now also Head of the Neural Circuit Development and Regeneration Research Group at KU Leuven. With a particular interest in the impact of ageing, she uses the killifish model to study the underlying mechanisms of axonal regeneration, supported by an impressive portfolio of academic and industrial funding.

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THE COMMUNITY ASSESSMENT OF FREEWAY EXPOSURE AND HEALTH STUDIES: MINIMISING EXPOSURE TO TRAFFIC-RELATED AIR POLLUTION

People who live close to busy roads and highways are exposed to high levels of traffic-related air pollution. This puts them at risk of significant health difficulties such as high blood pressure, heart attacks and cancer. The Community Assessment of Freeway Exposure and Health Studies led by **Dr Doug Brugge** from the University of Connecticut represent community-engaged research into the biological impact of high exposure to pollution and importantly, possible solutions to this. This work has shown that high-efficiency particulate arrestance filters are one promising intervention for minimising exposure to pollution and thus improving health.

A Serious Health Issue Worldwide

Traffic-related air pollution is a concern for the health of people living in urban areas all over the world. In the USA, exposure to air pollution is associated with 100,000–200,000 deaths annually This pollution comes in the form of gases and particulate matter of many sizes and compositions suspended in the air.

These pollutants can have serious health implications, and where people live and how long they stay there impacts a person's exposure and their likelihood of associated illness. The air around high traffic areas can contain two to three times higher concentrations of polluting gases and particulates than other sites, and even inside cars during commuting, concentrations are high.

Ambient airborne particulate matter is one of the top five causes of morbidity and mortality globally. To improve the health of the people living in cities and traffic-heavy areas, it is vital to study local traffic pollution, which has been studied much less than regional pollution in order to understand the impact it has and to reduce exposure and health consequences.

This is the focus of a group of researchers and community partners led by Dr Doug Brugge at the University of Connecticut, USA. Starting in 2008, the original Community Assessment of Freeway Exposure and Health (CAFEH) Study investigated levels of pollution in Boston communities near high traffic volume highways. CAFEH now provides an umbrella for several ongoing research projects that collaborate with affected communities. This work is providing insights into how highway traffic pollution affects nearby communities.

Air Filtration to Lower Pollution Particle Concentration

'I have played a leading role in a series of community-based participatory research studies and implementation projects in the Boston area for about 15 years' explains CAFEH Principal Investigator and Steering Committee Co-chair, Dr Brugge.

Particles emitted from vehicle exhaust pipes generally make up around 30% of fine particulate matter and are regulated in the USA. Concentrations are consistent over tens to hundreds of kilometres making them a regional pollutant. But a particular concern is the high concentration of the smaller ultrafine particles commonly found in traffic-heavy areas of cities. These concentrations vary a great deal over tens to hundreds of metres and remain unregulated.

Air purifier.

Ultrafine particles represent an emerging health burden, considering there is evidence to suggest poor health indicators and cardiovascular disease risks are elevated by their presence. 'We started out studying the effects of traffic-related ultrafine particles on health, focusing on biomarkers of cardiovascular risk... (and) we were among the first to report associations of ultrafine particles with biological health measures and continue to study these associations. However, we rapidly shifted from studying the problem to also exploring options to reduce exposure', explains Dr Brugge.

These explorations lead them to air purifiers, which have become increasingly popular due to the COVID-19 pandemic. These machines have the ability to reduce the concentrations of particulate matter indoors, including traffic-related particles, allergens and even viruses. They could be a promising tool although their efficacy is not well established for residential exposure to traffic particles. Therefore, the CAFEH project team conducted a pilot study that recruited low-income Puerto Ricans across 23 households in Boston and Chelsea in Massachusetts. Reducing ultrafine particles in low-income households can be especially difficult due to a lack of mechanical ventilation in their homes.

Participants were given high-efficiency particulate arrestance (HEPA) filtration devices for their homes for three weeks and a sham device that had no filtration capabilities for a separate three weeks (with the order of the intervention/sham intervention randomised and participants blinded as to which they had). Particle number concentrations were measured continuously over the six weeks. The results revealed that when HEPA filtration was in effect, the participants' homes had 50–85% lower concentrations of particles than with sham filtration. However, the researchers did not find a statistically significant reduction in blood inflammatory biomarkers in these participants.

A number of explanations could account for this finding, including the amount of time that participants spent in rooms with the filters and serious underlying health conditions that might mask the benefits of reduced exposure to particulate matter. Therefore, the team concluded that whilst HEPA filtration does lower ultrafine particle concentration, there was a need for an improved study design to better understand the impact of filtration on health indicators.

Controlled Air Filtration Study

Dr Brugge and the CAFEH project team next developed a methodologically more rigorous study, which they conducted under highly controlled conditions. This research focused on the effect that traffic-related air pollution has on short-term changes in blood pressure, the elevation of which is a risk factor for cardiovascular disease. A total of 77 participants each spent two hours in a room near an interstate highway on three separate occasions. A HEPA filter and the opening/closing of windows and doors were used to control the concentration of particles and therefore, the exposure the participants experienced. In a randomised order, participants were exposed to low, medium and high levels of traffic pollution, with a one-week 'washout' period in between each test to allow valid comparisons between the different study conditions.

Systolic blood pressure (the larger blood pressure number, which represents the pressure in the heart during contraction) and diastolic blood pressure (the smaller number, which is the pressure in the arteries between beats/at rest) were measured every 10 minutes. These readings showed that systolic blood

pressure increase over the two hours but that the amount of increase was lower with lower exposures. According to Dr Brugge, this study from his team provides 'strong causal evidence that air purifiers could both reduce ultrafine concentrations indoors near a highway and positively affect blood pressure.'

Investigating Air Filtration in the Real World

Although this controlled exposure study was incredibly useful in demonstrating that near highway pollution affects health and that air purifiers could be a useful tool to reduce risk, it did not reflect real-world conditions. To understand the effectiveness of air filtration in the real world, Dr Brugge and the CAFEH project team have set out to study 200 adults who live near a highway in Somerville, Massachusetts. This study is still ongoing and expected to continue for 2–3 years.

Households in this study have been given two HEPA filters so that they could have them running in both the living and bedrooms. Either real or sham filtration is used for one month, followed by a month-long washout period and then the alternate configuration for another month. Half of the participants begin with sham filtration followed by true filtration to ensure participants were blinded as to which version they had. Unlike the previous study, this one allows participants to open windows, cook food, spend time in rooms without the filter and generally go about their lives.

This investigation is ongoing and Dr Brugge is keen to see the outcomes in due course. The primary health outcomes include systolic blood pressure and the secondary outcomes include diastolic and central blood pressure. Blood levels of C-reactive protein, which is an indicator of inflammation, are also being measured, as well as D-dimer, a blood clotting factor. Throughout the study, other factors will be monitored and used to evaluate the success or failure of the air filtration intervention. These include indoor and outdoor monitoring of particulate pollution levels, size and composition of the particulates in the air, tracking the amount of time spent in the room with the filter and in addition, interviews to gain qualitative feedback from participants.

Once all these elements and the health outcomes have been assessed and analysed, the CAFEH project team will be able to determine whether the daily habits of people impact the potential benefits of filtration shown in the previous study. If successful, this promising research could provide evidence for an effective intervention to reduce the exposure to traffic-related air pollution in people who live near busy roads. In turn, this could improve the cardiovascular and overall health of these residents.

Utilising the knowledge built up through ongoing research, Dr Brugge and colleague Sharon Ron (Metropolitan Area Planning Council) wrote a white paper to inform policy for near roadway exposure to ultrafine particles. Their paper outlines what is known about the health risks of exposure to traffic-related particulate matter and why it should be addressed. Their recommendations cover high-efficiency air filtration, noise barriers, building away from highways and many others.

With considerable work already completed and even more underway, Dr Brugge and the CAFEH project team remain committed to influencing health outcomes related to local traffic pollution at a policy level, which will, ultimately, be critical for the large-scale protection of health. By expanding our understanding of this underappreciated type of pollution, the CAFEH project team is also raising awareness about this invisible, odourless toxic pollution that comes from motor vehicles.

CREDIT: Kevin Jackson

Shir Ginzburg, Chermaine Mason, Amy Law, Doug Brugge, Misha Eliasziw, Wig Zamore, Teresa Vazquez-Dodero.

Meet the researchers

The Community Assessment of Freeway Exposure and Health Study (CAFEH)

Dr Doug Brugge
University of Connecticut
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USA

The Community Assessment of Freeway Exposure and Health Study (CAFEH) is led by Dr Doug Brugge, who is Principal Investigator for most of the CAFEH studies. He is Co-chair of CAFEH Steering Committee, in addition to Chair and Professor in the Department of Public Health Sciences at the University of Connecticut School of Medicine. CAFEH serves as the larger umbrella for five related community-based participatory research air pollution studies. These projects have full participation of the community partners in all aspects of the science including: developing the proposal, leading the study, and collecting, analysing and interpreting the data. The CAFEH project teams include staff (oversight, management, field, clinic, research, graduate students and student interns) and additional research collaborators. By improving understanding of how air pollution from traffic and highways affects people's health, the team is developing approaches to mitigate this exposure at the individual and policy and practice levels.

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CAFEH

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Environmental Protection Agency
Kresge Foundation

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IDENTIFYING, UNDERSTANDING AND MANAGING TREATMENT-RELATED RISKS OF MEDICINES PRESCRIBED TO CHILDREN

The relatively new field of paediatric pharmacovigilance aims to improve the clinical care of children by understanding and appropriately managing the risks of medicines administered to this group of patients. **Dr Beate Aurich** is an established expert in this field, and with colleagues, has published an article on the practical aspects of paediatric pharmacovigilance. She notes that the assessment of the benefit-risk balance of available treatment options should be based on multidisciplinary efforts and include both children and their families.

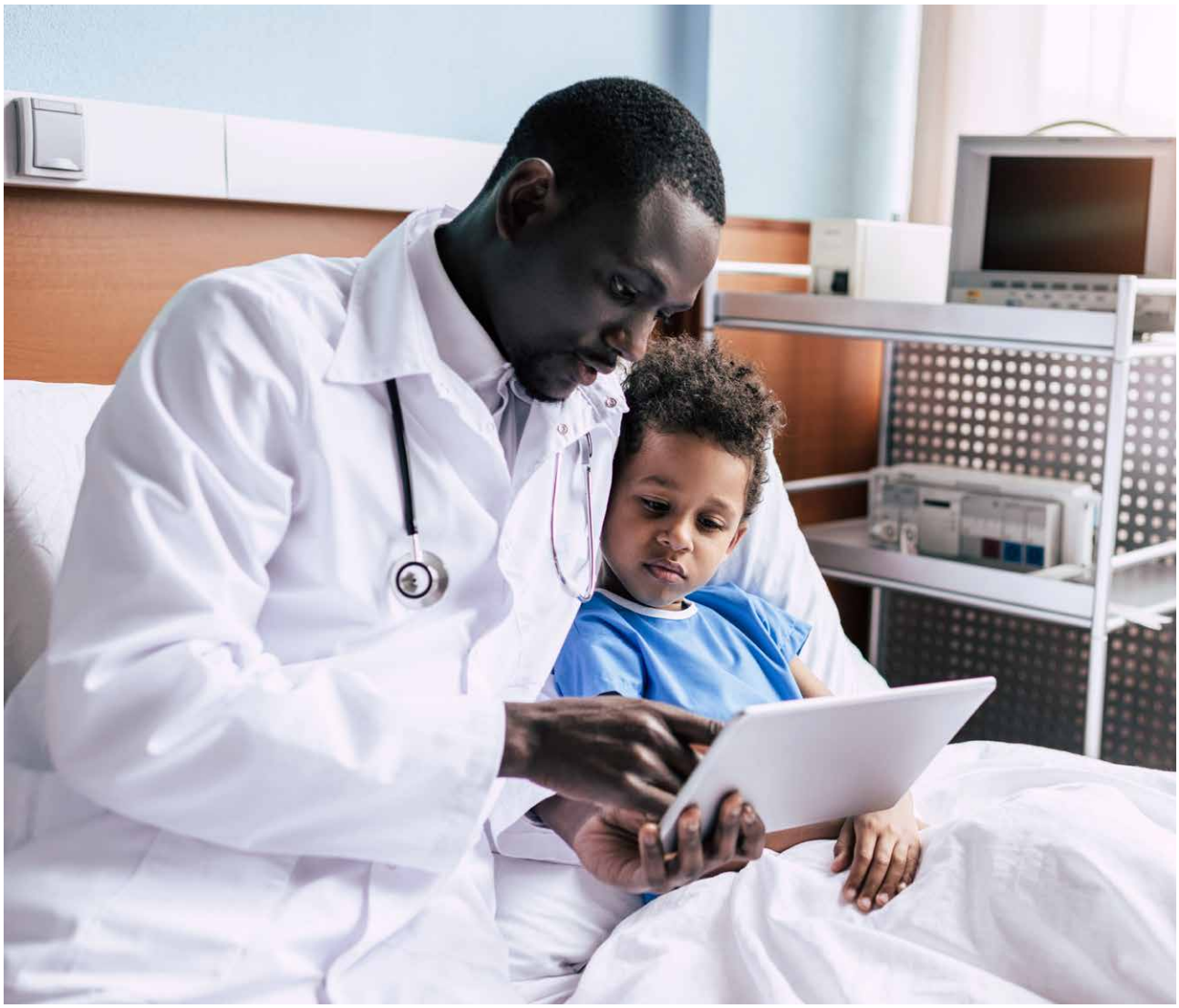
The Importance of Paediatric Pharmacovigilance

Research into the risks of medicines is called pharmacovigilance. It helps to identify, understand and reduce treatment-related risks – so-called adverse drug reactions or ADRs. Pharmacovigilance is a relatively young speciality that began to emerge in the 1930s following several major drug safety disasters.

Pharmacovigilance is a continuous process of monitoring treatment-related risks throughout the life cycle of medicinal products worldwide. It starts at the time of pre-clinical studies, i.e., before a new product is administered for the first time in humans. It is continued as long as there are still patients taking the medicine. To better understand certain long-term risks, pharmacovigilance may even be continued after a drug has been taken off the market.

Paediatric pharmacovigilance combines the understanding of general pharmacovigilance with the special requirements of children and how ADRs may affect the growing body. The overall aim is to help parents, children and healthcare professionals to make evidence-based treatment decisions by informing them about the possible risks of medicines and how these can be managed.

Dr Beate Aurich is a pharmacovigilance and drug safety consultant with extensive experience. One of her peer-reviewed publications, 'c4c: Paediatric pharmacovigilance: Methodological



considerations in research and development of medicines for children – A c4c expert group white paper', was written in collaboration with colleagues from the conect4children (c4c) network. This white paper describes some of the points to consider with regards to the detection and proactive management of treatment-related risks in paediatric studies and paediatric clinical practice.

Identifying Treatment-related Risks in Children

Treatment-related risks in children are often different compared to adults. They can also vary between different paediatric age groups, for example, babies and adolescents. These differences can be due to factors such as age-related changes in the body's metabolism, comorbidities (additional illnesses) and comedications (additional medicines). A further challenge is a higher risk of medication errors because formulations are frequently not adapted to children's needs. Medicines administered to children may need to be prepared from drugs intended for adults, for example, by crushing a tablet and this can be a source of errors. The identification of treatment-related risks in children is based on the age-group-specific safety specification.

The safety specification summarises the scientific evidence for known and potential treatment-related risks and which safety data is currently missing. The analysis of existing data may include, for example, safety data from pre-clinical studies, modelling and simulation, pharmacology, interventional and non-interventional studies, registries, safety and electronic health care databases and the literature (e.g., drug class effects, information from health authorities). The safety data is then combined with the specifics of children including, for example, paediatric pharmacology, how ADRs present clinically in children, the risk for medication errors, possible interactions with the child's nutrition and other medicines, and the challenges of collecting and analysing safety data in children.

Managing Treatment-related Risks in Children

The risk management of medicines administered to children has similarities to risk management in other domains of our daily life. For example, car seat-belt systems are adapted to the age and size of a child. This is based on data from real and simulated accidents and the understanding of the effects of the laws of physics on a child's body. However, many medicines prescribed to children have very limited child-specific

information on treatment-related risks. The management of treatment-related risks uses standard and additional risk management tools. The product's package insert (label) is used for standard risk management. However, this is often difficult to understand, even for adults, and frequently not adapted to the needs of children. Informing children and parents about any important updates, in particular for medicines which are taken regularly, is a challenge. Additional risk management strategies may, for example, include informed assent/consent by the prescribing doctor or targeted testing for certain ADRs (e.g., liver function, genetic risk factors).

The Importance of Patient involvement

Paediatric pharmacovigilance and risk management require a multidisciplinary effort. Dr Aurich proposes that 'the inclusion of children and their families for the effective management of treatment-related risks should be standard practice for paediatric pharmacovigilance and risk management activities'.

Proactive paediatric pharmacovigilance and risk management is reducing the frequency and severity of ADRs in children, including fatal ADRs. Research into developmental pharmacology and high-quality child-specific electronic health care data will play an important role in preventing and managing ADRs in children. Current efforts on developing open access, multilingual information on treatment-related risks in children and evidence-based dosing of medicines will contribute to improved treatment outcomes for children across the globe.

Meet the researcher

Dr Beate Aurich

Pharmacovigilance and Drug Safety Consultant

Dr Beate Aurich obtained her medical degree in 1996 from Humboldt University, Berlin, Germany. In 2001, she obtained the Membership of the Royal College of Paediatrics and Child Health (MRCPCH), London, UK. Between 2002 and 2006, she worked at the Drug Safety Research Unit in Southampton, UK, as a Medical Research Fellow. She obtained a medical doctorate in pharmacoepidemiology at Portsmouth University (UK) in 2006. From 2006 to 2015, she was the Director of Global Pharmacovigilance and Drug Safety at GlaxoSmithKline and Novartis, leading the Global Pharmacovigilance Team for marketed drugs and drugs in clinical development. Between 2015 and 2021, she worked as a Project Leader in the Department of Paediatric Clinical Pharmacology at Robert Debré Hospital in Paris, France.

She has over 20 years of experience in pharmacovigilance, pharmacoepidemiology and risk management in academic research and the pharmaceutical industry. This includes the detection and assessment of safety signals, risk minimisation, safety profiling, the review of individual case safety reports and aggregate safety data and writing risk management plans. She is also experienced in reviewing protocols, informed consent forms and paediatric investigation plans, planning safety data analyses, advising on the Medical Dictionary for Regulatory Activities (MedDRA) coding and contributing to regulatory documents (e.g., clinical safety summaries, study reports, development safety update reports, periodic safety update reports).

Since February 2020, she is a member of the conect4children (c4c) Expert Group for Paediatric Pharmacovigilance, writing articles on paediatric pharmacovigilance, teaching paediatric pharmacovigilance and drug safety and advising CDISC on the particularities of capturing clinical trial data in children. Dr Aurich is currently working as a pharmacovigilance and drug safety consultant in adult and paediatric research.

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A NEW TEST TO IDENTIFY CHRONIC KIDNEY DISEASE – A COSTLY AND SILENT KILLER

Chronic kidney disease is a common but irreversible condition with an increasing worldwide prevalence. The significant patient morbidity and mortality are accompanied by an unmet clinical need for more effective testing methods to identify affected patients and patients at high risk at the early stages of the disease, before it becomes irreversible. **Dr. Aaron Carrithers** and **Dr. Stephen Carrithers** at PrognostX Health have developed a new test to help reduce the number of patients progressing to late-stage chronic kidney disease and end-stage renal disease, aiming to improve human health and reduce the financial burdens on our pressured healthcare systems.

Chronic Kidney Disease: A Global Epidemic

Your kidneys help to remove waste products from the body, maintain balanced electrolyte levels, and regulate blood pressure. Chronic kidney disease (CKD) is a condition characterised by a gradual loss of kidney function. One way of assessing this is to calculate the 'eGFR' which is the estimated glomerular filtration rate of the kidneys. This is based on a patient's serum creatinine level (which shows how well your body is performing its job of filtering waste from the blood) and currently requires the patient's age, sex and race.

Using eGFR, a stage can be assigned to reflect the severity of disease in patients. Stage 1 indicates that the damage to kidneys is very mild, whereas Stage 5 is assigned to patients whose kidneys have lost almost all of their function and typically requires life-sustaining dialysis treatments three times per week. This is known as end-stage renal disease (ESRD), which leads to many more added medical complications, increased expenses to both the patient and the healthcare system, decreased quality of life, and a high likelihood of early death. However, Stage 3 CKD is considered the point at which the disease is irreversible and due to the subtle and underlying symptoms associated with this disease stage, over 90% of individuals are unaware of their chronicity. Thus, progression to dialysis and ESRD is likely imminent without early intervention.

Kidney disease is currently recognised as a global epidemic affecting nearly 850 million people worldwide, and approximately 700 million of these patients have CKD. Many are undiagnosed, untreated, and unaware of their irreversible condition. As such, CKD is associated with significant patient morbidity and mortality, placing a huge financial burden on already struggling healthcare systems.

Hypertension, diabetes and acute kidney injury (including patients with kidney conditions resulting from COVID-19 infection) are the major predisposing conditions for developing CKD, accounting for more than three in every four new cases. Adverse outcomes include an increased risk of developing cardiovascular and cardiorenal disease, Alzheimer's disease, depression, and progression to end-stage renal disease, leading the patient to require 2x–3x weekly dialysis treatment and/or kidney transplantation.

A Silent Killer

CKD is clinically diagnosed in about one in seven people – but many people are unaware that they have it. Among adults with CKD, the percentage aware of their disease state was fewer than 10% for patients in Stage 3 and fewer than 60% in Stage 4. CKD is therefore often referred to as a 'silent killer', being generally asymptomatic until very the late stages of illness.

This means that there are large numbers of people with later-stage CKD who remain undiagnosed and untreated, resulting in a considerably shorter lifespan than if it had been detected earlier. Poor rates in diagnostic efficacy and low patient awareness of CKD are secondary to the inherent limitations of the current diagnostic standard of care. Indeed, current methods require multiple tests and take no less than three months to even diagnose a patient with CKD, and for many patients, the process could take one year. Furthermore, due to this untimely diagnostic method, about 60% of patients are lost to follow-up, which contributes to the rising incidence of dialysis and ESRD. Despite efforts made to raise diagnostic efficiency and patient awareness, substantial improvements have yet to be seen.

Novel and Effective Interventions: Need for Timely Diagnosis

Novel medications have been shown to effectively slow CKD progression and help prevent end-stage renal failure, and have received both U.S. (Food and Drug Administration) and European approval for patient use. These critical advances point to the urgent need for accurate and early diagnostic tests if we are to identify patients with the disease and thus ensure timely treatment. This is especially critical during the early stages of CKD among high-risk groups, such as those with type 2 diabetes, when the disease is often asymptomatic.

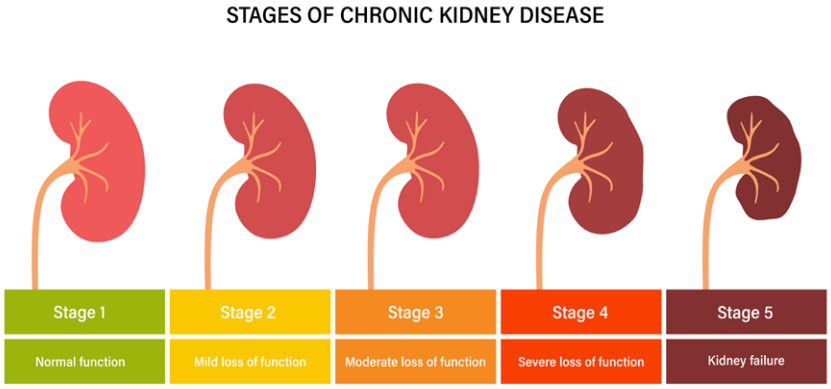
To address this need, researchers Drs. Stephen Carrithers and Aaron Carrithers at PrognostX Health in the USA have developed a new, simple and specific assay that detects early CKD with just one single test in high-risk patients.

Current Diagnostic Standard of Care and Associated Challenges

The current diagnostic standard of care is complicated, not least because it requires at least 90 to 150 days of follow-up visits to the clinic and the repeat laboratory testing of a patient's samples. Patients who attend follow-up appointments for confirmation of diagnosis are at an unprecedented low, meaning that many are remaining undiagnosed.

The current laboratory tests are not suitably specific in identifying patients with CKD, and so many patients are missed in the first round of testing. For example, the laboratory reporting of eGFR alone does not result in optimal clinical diagnoses. Another type of test can be used to assess glomerular integrity, but is only helpful for picking up the very early stages of CKD in some patients but must be re-evaluated on a routine basis for confirmation of diagnosis.

Current tests are also unable to accurately discern acute kidney disease patients from those with chronic kidney conditions. This means that patients are only being diagnosed at a much later stage after multiple rounds of testing when the disease has progressed further and has reached a more advanced stage. This ultimately results in a larger number of patients who have progressed to dialysis and end-stage renal disease. It is also currently difficult to identify high-risk patients, such as those



with diabetes or hypertension who might have developed early-stage CKD but are unaware of their condition.

A New Approach: ConfirmCKD!

The new test – 'ConfirmCKD!' – was developed by Drs. Carrithers and Carrithers. It is a simple type of immunoassay test which can be performed quickly and easily in the laboratory using a blood sample obtained from a patient. The test itself takes advantage of specific biomarkers that circulate in the bloodstream in the very early stages of CKD – before a patient or high-risk individual has any symptoms and is still in a stage in which the disease is most likely reversible. Biomarkers are molecules that indicate normal or abnormal processes taking place in your body and elevated levels of a particular biomarker can be a sign of an underlying condition or disease. In this case, the test looks for the presence of a particular biomarker, the gene product(s) expressed by renal Guca2b.

These gene products, specifically the C-terminal region of the expressed and secreted prohormone from Guca2b, have been shown to be involved in the physiological regulation of salt levels in the body. The mature Guca2b gene product levels in the blood are normally low in humans but when certain kidney-related diseases or mechanisms of chronic kidney dysfunction occur in the body, the level of this biomarker can increase significantly, prior to losing significant function of the kidney. This rise can therefore be detected and used to identify patients who have CKD.

Drs. Aaron and Stephen Carrithers have undertaken validation of the test and determined the cut-off value for diagnosing CKD in a patient. In this way, a rise in the serum or blood can be used as a simple and effective way to diagnose and provide treatment in early CKD, and also to monitor the disease progression of kidney dysfunction in a particular patient. Furthermore, the test can be used to predict the outcome of a treatment program for kidney disease and/or dysfunction without interference from other common diseases or cancers that are generally associated with CKD patients.

This new test has been demonstrated to be convenient, quick and easy to use, and can be readily incorporated into any hospital or testing laboratory facility using the equipment that is typically available. Another advantage of this immunoassay is that it can be converted easily into other test formats, such as a lateral flow device, which could potentially be used by patients in their homes.

Gathering Vital Clinical Evidence

The initial and adjudicated validation studies conducted by Drs. Carrithers and Carrithers in 384 patients demonstrated a remarkable accuracy of the new assay in correctly identifying those who had CKD from just one test. Stages 3 to 5 of CKD were diagnosed and staged with a higher than 97% accuracy from patients whose renal function was within normal limits.

Testing produced very few false positives (in which a test incorrectly indicates the presence of a disease) and even fewer false negatives (in which a test result incorrectly indicates the absence of a disease when it is present). The assay has several significant advantages over the current standard-of-care protocols, in particular, the removal of the need for patients to undergo months of repeat testing. Furthermore, the researchers demonstrated that the results of their new assay were not affected by other concurrent pathologies or medications, nor by confounding patient variables such as age, gender or race.

ConfirmCKD! has undergone further development, and now functions as a simple and effective qualitative 'positive/negative', 'treat/no treat' result, with an accuracy of over 97% for single-assessment identification of eGFR-defined CKD (i.e., patients with stages 3a, 3b, and 4). Drs. Aaron and Stephen Carrithers have adapted their testing approach to work most effectively in tandem with the recently approved pharmaceutical agents that are indicated for the treatment of stages 3a, 3b, and 4 CKD. The combination of novel pharmaceutical agents along with the application of better testing methods promises to lead to a substantial improvement in the detection and treatment of CKD.

Implications and Important Benefits

This simple-to-use and effective test offers the potential to reduce the worldwide burden of CKD and overcome the significant difficulties associated with the current standard of care approach. By helping to identify patients who are at risk of developing ESRD, the individual cost to patients and their families should be considerably reduced, as well as the financial burdens on health systems worldwide.

Aaron, Stephen and Brennan (from left to right)

PrognostX Health
Kentucky
USA

PrognostX Health is an in vitro clinical diagnostics development biotechnology company that develops innovative tests for the early identification and treatment of chronic diseases, with an emphasis on addressing the unmet clinical need and gap in testing modalities for chronic kidney disease (CKD), so that high-risk patients such as those with diabetes, hypertension and acute kidney injury can be treated before the disease becomes irreversible. Initially formed as AmDx PrognostX Inc, the unique family dynamic of The Carrithers Group – consisting of Dr. Stephen Carrithers, Dr. Aaron Carrithers and Dr. Brennan Carrithers – assembled their individual skill sets and experiences to form a new venture in which promising but under-developed early-stage technologies in renal care and diabetes health could be developed, validated, and brought to those unaware of their underlying conditions to address the rising incidence of CKD, dialysis and end-stage renal disease. Now doing business as PrognostX Health, the Carrithers are leveraging their experience and global partnerships to help provide better personalised care for those at high risk of (or with) CKD.

COLLABORATORS AND PARTNERS

Dr. Brennan Carrithers, MD, MBA, MSc – New York University, Department of Psychiatry, and Board Member and Advisor for Health Insurance Implementation & Reimbursement at PrognostX Health
Ms. Tien Bui, BA – Chief Business Development Officer and Director of Strategic Partnerships at PrognostX Health
Dr. Eugene Krentsel, PhD – XLerateHealth, CSO and Vice President for Strategic Partnerships and Alliance, and President/Founder of innoVEK, LLC.
Research Use Only (RUO) ELISA kits available only through Ethos Biosciences (Newtown Square, PA)

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KY Innovation, Office of Entrepreneurship, Kentucky Cabinet for Economic Development and Kentucky Science and Technology Corporation

Meet the researchers

Dr. Aaron L. Carrithers, MD
Vice President and Chief Medical Officer, Principal Investigator

Dr. Aaron Carrithers is a physician-scientist and clinical translational entrepreneur who has leveraged his expertise and experience to evaluate, develop and commercialise clinical diagnostics. He graduated from the University of Kentucky College of Medicine in 2018 with his MD followed by a postdoctoral fellowship in Translational Medicine and Biotechnology whilst working at Sequela, a biotechnology company based in Kentucky, where he later became Chief Scientific Officer (CSO) prior to co-founding PrognostX Health. While undertaking his postdoctoral research he also became director of Clinical Operations at Lagrange Scientific, a company addressing kidney cancer through multi-omics technologies and big data algorithm development, a position he still holds. He is currently Vice President, Chief Medical Officer, and Principal Investigator at PrognostX Health.

Dr. Stephen L. Carrithers, PhD
President and Chief Executive Officer
Director of Innovation

Dr. Stephen Carrithers is currently the President and Chief Executive Officer as well as Director of Innovation of PrognostX Health. He received his PhD in Biochemistry and Molecular Biology from the University of Louisville School of Medicine in Kentucky. During his National Institutes of Health Postdoctoral Fellowship in Clinical Pharmacology at Thomas Jefferson University in Philadelphia, he co-founded his first biotechnology company which resulted in licensed products within the clinical diagnostics and therapeutics space for colon cancer and IBS-C. Dr. Carrithers joined the Division of Infectious Disease as Assistant Professor at the University of Kentucky Chandler Medical Center with a joint academic position in the Department of Physiology, Renal Division. During his academic tenure, he co-founded two more biotechnology companies. Over his extensive career, which has spanned more than 30 years, he has researched, explored, developed and commercialised various clinical diagnostics specifically looking at diabetes, diseases and cancers of the kidney. Recent work is also exploring the neurodegenerative sequelae of diabetes and CKD.

‘Getting One Step Ahead of Disease’

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PSYCHOLOGY & NEUROSCIENCE

SINGING AND SPEAKING: THE COMPLEX INTERTWINING OF MUSIC AND LANGUAGE

Dr Francesca Lawson from Brigham Young University has spent the last few decades researching the relationship between music and language. She has looked beyond Western culture and conducted many studies in the People's Republic of China, focusing on music- and speech-based performance. Dr Lawson's most recent work demonstrates how musicality is important in underpinning our communication, highlighting that when attitudes align, speech becomes rhythmically and musically coordinated.

Researching Speech and Song

Over the last few decades, Dr Francesca Lawson from Brigham Young University has studied the relationship between music and language. As a musician herself, she is keen to understand how our two most fundamental forms of communication relate to each other. Dr Lawson believes that studying speech and song in different cultures helps to enrich our knowledge in this field. As she explains, 'Musicality – the biological phenomenon that undergirds both music and language – is one of the most overlooked areas of human communication because it happens so quickly that we are unable to detect it.'

Dr Lawson's takes a pioneering approach to her research by looking beyond the boundaries of Western music. Since the mid-80s, she has undertaken a wealth of research in the People's Republic of China, with a particular focus on how the Chinese understand and articulate music and language in vocal performances.

The Chinese language is well suited to this type of research because it is tonal, meaning that that the tone or pitch of a syllable determines its meaning and distinguishes words. This can make singing complex, as the melody may conflict with the linguistic tone of the lyrics. Chinese culture features many different types of performances that incorporate both speech and song, including rapid poem recitals and long storytelling ballads. Dr Lawson has recently taken a more empirical approach to her research, utilising acoustic analysis software. Without this, she would have been unable to detect some of the nuanced interplays between speech and language in Chinese performances.

Biology versus Technology

There are two opposing perspectives around whether music represents a biological adaption or a technology and scholars from different disciplines disagree about its origin. The biological argument claims that using music within communication has adapted or evolved as part of natural selection to benefit human functioning. An example of this is the engagement of rhythmic and musical interactions between a mother and child, which may have helped improve maternal success and

infant survival. The opposing argument is that music is a transformative technology, meaning it is something that humans have invented to improve their experience or quality of life but is not an adaptive trait in terms of our survival.

Dr Lawson's research into northern Chinese shuochang or 'speaking-singing', supports the notion that musical communication might be an adaptive trait that has evolved for our benefit as it appears to display some characteristics consistent with communicative musicality. In addition, she believes it is also an example of transformative technology. Dr Lawson suggests the desire to technologise music and language may also come from a biological predisposition for pleasure.

The Complexities of Acoustic Analysis

Research in relation to music and speech can be a complex process, particularly the analysis. The traditional approach is for a researcher to listen to recordings and make transcriptions that compare elements such as melody, tone, embellishment and pace. Dr Lawson has taken a proactive approach to investigating if technology could help to advance research in the field of ethnomusicology. In some of her recent research, Dr Lawson has employed the use of acoustic performance software. One of its first uses was to help compare vocal signature or singing style within Chinese narrative performances.

In the early part of the twentieth century, narrative performances were one of the most prominent forms of entertainment in China. The performers were originally male but are now predominately female. This shift was likely due to the emergence of feminist movements and the founding of the new Chinese Republic. It was also the catalyst for the first female singer to establish her own Chinese singing school, something previously only done by men.

To determine if unique traits in music are passed on to subsequent generations, Dr Lawson compared recordings from two prominent singing schools. One was originally founded by a woman and the other by a man (although there are no longer many male singers).

Acoustic analysis software was used to compare the pitch and duration across a number of recordings, while also undertaking some more traditional analysis. Dr Lawson found there were many similarities between the male and female teachers from the two different schools, despite the fact that they taught different singing styles. Even more surprisingly, the students – all of whom were female – showed greater differences compared with their teachers than observed between the two teachers themselves. Dr Lawson speculates that this is an indicator of students wanting to establish vocal distinctness from their tutors in a newly emerging, competitive field of primarily female performers.

Despite this unexpected outcome, this study successfully demonstrated the effectiveness of using acoustic analysis software in this field of research. Importantly, Dr Lawson concluded that the software was able to detect nuances that are unattainable through traditional transcription.

The Role of Musicality in Communication

An important aspect of Dr Lawson's research is how musicality underpins our ability to communicate verbally through speech and song. Researchers have previously focused on the way we move, talk and gesticulate but until more recently had not looked at music as an explanation for our patterns of speech and language. Linguists and sociologists have studied infant-mother relationships and acknowledged musicality as a form of communication. Scientific scholars tend to look at the

biology behind musicality. In humanities, the research is often focused on cultural differences in creating and performing music.

Dr Lawson hopes that finding commonality between the musicality of speech and song could help to enrich our knowledge about both music and language, and so further encourage healthy debate. Previous research undertaken at the University of Cambridge has paved the way for this by revealing that in certain contexts, speech and song involve common processes. This demonstrates an attitudinal alignment between speakers.

Chinese Crosstalk

Dr Lawson has undertaken further research using Chinese musical performance to demonstrate how musicality underpins our communication by looking at Chinese crosstalk. Chinese xiangsheng or crosstalk is a comedic dialogue that is part of the shuochang ('speaking-singing') tradition in north China. It is a short vocal performance involving interaction between speech and song. Traditional crosstalk consists of four parts culminating in a punchline that typically catches the audience by surprise. Although appearing unrehearsed, it is usually scripted. While crosstalk is presentational in its style, it elicits loud and enthusiastic vocal responses from the audience, creating a mix of both presentation and participatory dialogue and interaction.

To explore this, Dr Lawson used a YouTube video of a popular crosstalk performance called 'A Carefree Life'. This involves two famous performers; Guo, the 'joke-cracker' who is nonsensical and ridiculous, showing disregard for Chinese elite culture and Yu, the 'joke setter or straight man'. They battle against each other to perform a notoriously high-pitched Beijing opera aria, which results in some comedic musical performances. Their routine receives a rapturous response from audiences and unlike Western performances, the audience is much more likely to get involved vocally.

Analysis of the performance was undertaken using acoustic software. Dr Lawson concluded that crosstalk does a good job of demonstrating that music and language are two essential components of the human communicative repertoire. While speech is more dominant, the songs are integral to the performance. She notes that musicality is particularly apparent in the way the actors relate to each other in both spoken and musical forms throughout the performance. The back and forth between performers and audience members provides additional musicality. This appeared to be most prominent at the end of the sung elements, the more emotional parts of the performance.

These findings raise the interesting question of whether a strong emotional connection might increase musicality. Dr Lawson believes this requires further investigation but

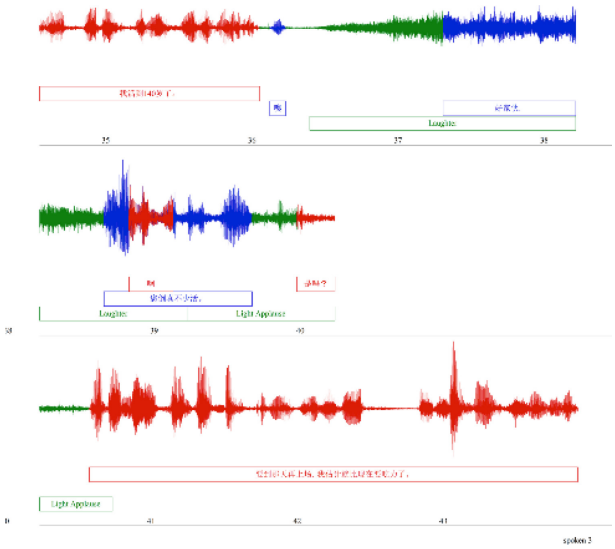

Illustration of audience laughter (green) at Guo (red) in the top line contributes to Yu's response (blue), which, in turn, contributes to more audience laughter at the beginning of the second line. Reproduced under the Creative Commons CC-BY-NC license from FRS Lawson, et al., When Audiences Become Performers and Speech Becomes Music: New Tools to Analyze Speech, Song, and Participation in Chinese Crosstalk, Music & Science, 2020.

maintains that her study demonstrates that music and language are relevant to scholars from both scientific and humanistic backgrounds.

Audience-Performer Dynamics

A further aim of Dr Lawson's research was to understand the dynamics between the audience and the performer in speech and song. Her research into Chinese crosstalk uncovered that the responses from the audience often attempted to match the pitch of the previous utterance. As expected, the actors Guo and Yu matched pitches during their aria performances as well as at other times. In addition, the audience also attempted to match pitches with the performers, who in turn attempted to match their pitch. This happened spontaneously, particularly through the final scenes.

When the audience's attitudes aligned with the performers, their speech became rhythmically and musically coordinated. Dr Lawson proposes that 'pitch mutuality' is an appropriate term to describe the interaction between the performers and the audience. She believes this work helps to demonstrate that speech and music are not separate domains. This pitch mutuality reinforces the relationship between speech and music, and between both performers and audience members. It seems that when intertwined, music and speech are an integral foundation for our communication. Dr Lawson's research demonstrates the strength of this inter-relationship but also the importance of looking beyond Western culture to enrich our understanding of music cognition.

Meet the researcher

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Dr Francesca Lawson is the Marshall Professor and a Fellow at the Humanities Center at Brigham Young University. She received her undergraduate degree in harp performance from Brigham Young University, a master's degree in ethnomusicology from the University of California at Los Angeles and a PhD in ethnomusicology from the University of Washington in Seattle. Formerly the Humanities Professor of Ethnomusicology, she is currently the Section Head of Interdisciplinary Humanities in the Department of Comparative Arts and Letters at Brigham Young University. Dr Lawson has undertaken a wealth of research focused on the inter-relationships of language and music in Chinese narrative arts. Her ongoing research interests include the interplay between music and language, the cultural and biological implications of the gendered voice, and the divergence between cultural and neurological perspectives on music and consciousness. She continues to pursue research that addresses the boundaries between music and science.

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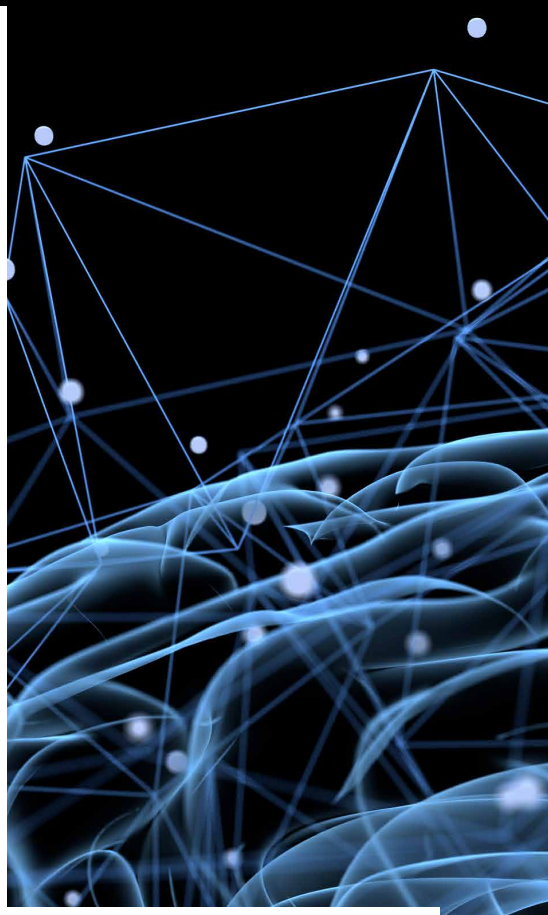
INNOVATION IN PROMOTING THE RECOVERY OF LANGUAGE AFTER STROKE



Stroke can impair a person's ability to communicate, resulting in a disorder known as aphasia. To facilitate recovery, scientists must understand how language is processed normally as well as how a stroke may impact the language system in the brain. **Dr Cynthia K. Thompson**, Ralph and Jean Sundin Professor of Communication Science and Professor of Neurology at Northwestern University, has been researching normal and disordered language for over thirty years. Her focus is on understanding and supporting the recovery of language processes when the brain has been damaged.

The Complexity of Language and the Brain

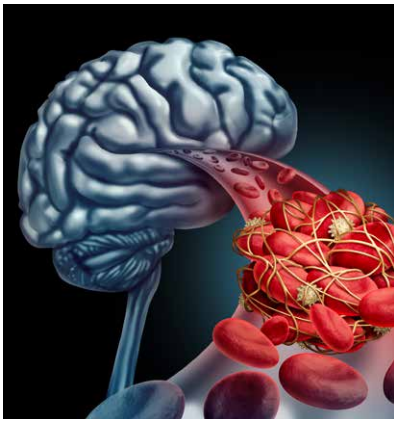
The uniquely human capacity for language resides in the most complex organ in the body, the brain. While significant insights have been gained in ascertaining the neurobiology of language, the inherent complexity of the brain, as well as language and linguistic processes, means much is still to be achieved. Damage to the brain can occur through a wide range of different diseases and injuries, and can have severe consequences for the language capabilities of an individual.

Aphasia Caused by Stroke Damage

Aphasia is a language disorder that results from brain damage. A major cause of aphasia is stroke, which affects over 15 million people across the world. Stroke is a life-threatening condition, in which blood supply to areas of the brain is cut off or significantly reduced. Reduced blood supply to regions of the brain that support language processing renders those regions unable to process language normally. It is estimated that around one-third of stroke survivors are left with aphasia, which affects the ability to understand and formulate language. This often has wide-reaching effects on the ability to communicate, which impacts social independence and the quality of life and increases the likelihood of social isolation.

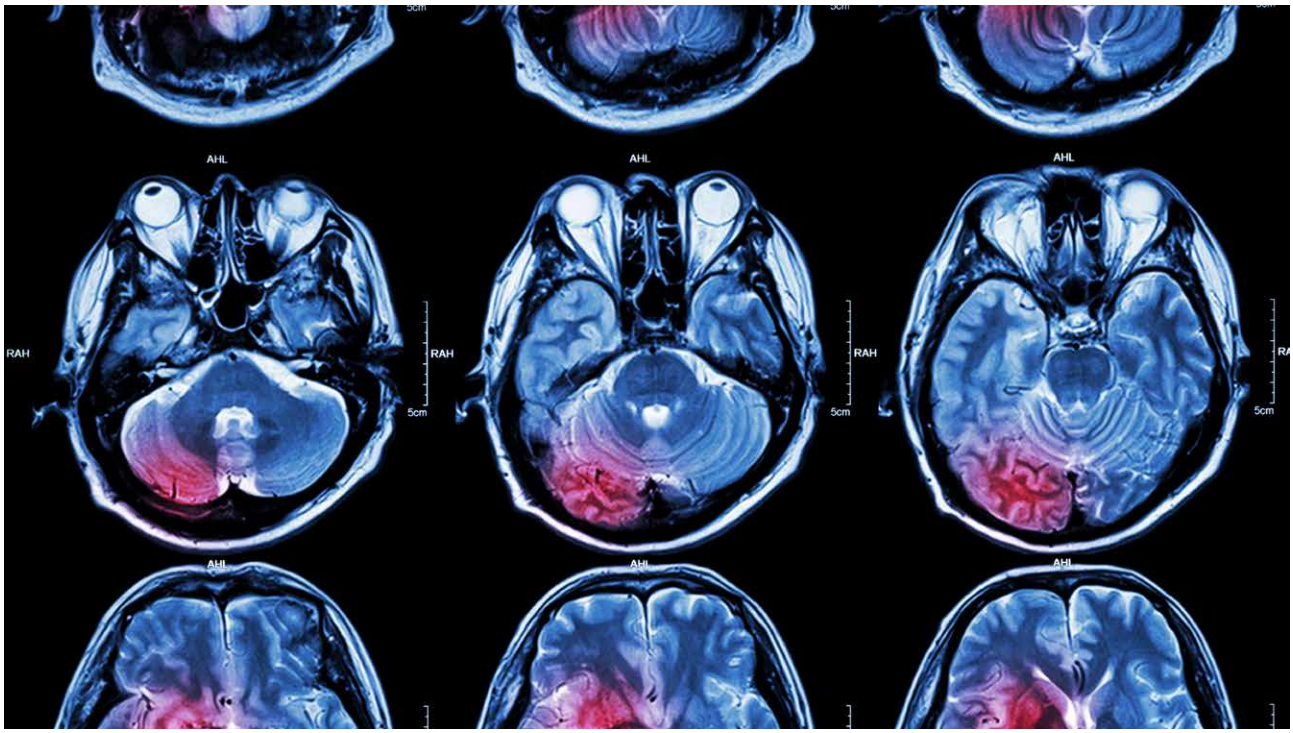
Dr Cynthia K. Thompson at Northwestern University works to understand which areas of the brain are responsible for language processing – in particular, sentence processing – in healthy individuals to aid rehabilitation and recovery in those with deficits. By mapping the neural structures of sentence comprehension and production in healthy volunteers, her work is providing vital clues as to how networks may be rebuilt, potentially in different areas of the brain – a phenomenon known as 'brain reorganisation'.

Many previous studies have used neuroimaging techniques to record the complexity of neural activity across multiple brain regions when language is processed. Dr Thompson and her



colleagues published a review of previous studies observing the neural activity of people recovering from aphasia and healthy individuals. They found some important differences between the two groups. Healthy individuals typically displayed activity primarily in the left hemisphere of the brain when processing complex sentences whereas those recovering from aphasia were found to display greater activity in the right hemisphere. This observation supports Dr Thompson's conviction that right hemisphere brain tissue may be recruited to support sentence processing in people with aphasia.

‘If we can find the parts of the brain that are the most likely candidates to be recruited for recovery, then the next step is to push those parts of the language network of the brain using non-invasive neurostimulation.’



In further research, Dr Thompson has effectively demonstrated that language functions can indeed be regained with training. Dr Thompson also maintains that it isn’t just those who have recently suffered a stroke that can recover language skills. She has shown that patients can demonstrate significant brain reorganisation many years poststroke, allowing the return of at least some previous language skills and functioning. Dr Thompson explains her approach as follows: ‘If we can find the parts of the brain that are the most likely candidates to be recruited for recovery, then the next step is to push those parts of the language network of the brain using non-invasive neurostimulation.’

Neurocognitive Mechanisms Leading to Agrammatism

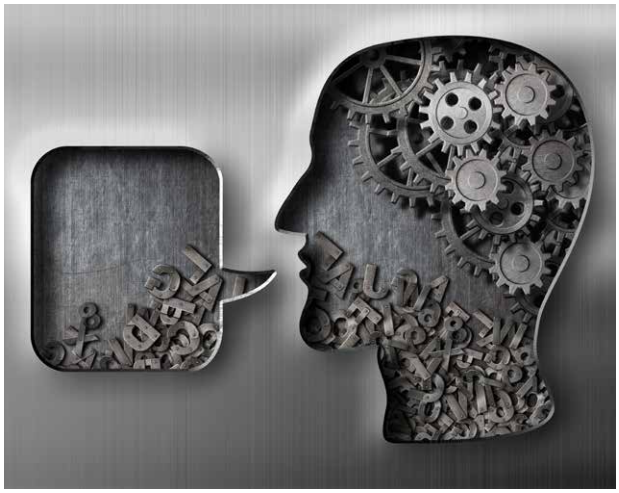
Dr Thompson and her team investigate the neurocognitive mechanisms that lead to a specific deficit known as agrammatism, a type of aphasia that affects grammatical aspects of language processing. Neurocognition refers to the study of cognitive functions that are closely linked to specific brain areas or pathways.

Dr Thompson and colleagues found that agrammatism is caused by damage to frontal and temporal lobe regions of the brain as well as the subcortical white matter fibres which link them (known as the dorsal pathway) in the left hemisphere, which is associated with language processing in the majority of humans. More specifically, these regions play an important role in using and understanding verbs and verb-argument structure within speech, which are crucial for the production and comprehension of grammatical sentences. This suggests that damage to regions within the dorsal language system leads to sentence processing errors.

The Success in the Rehabilitation of Language

In addition to her focus on the neurological impact of stroke, Dr Thompson has directed much of her research towards developing and improving treatments. She is the creator of a linguistic therapy called the Treatment of Underlying Forms (TUF), which helps to improve sentence comprehension and processing in people with aphasia. The treatment is based on Dr Thompson’s theory – The Complexity Account of Treatment Efficacy (CATE) – that using complex rather than basic sentence structures during rehabilitation improves recovery. She explains, ‘Previously, the common practice in language rehabilitation therapies was to train only simple sentences. We have found that training people using linguistically complex sentences results in greater improvement on both simple and complex sentences.’

Recently, Dr Thompson and her colleagues demonstrated that TUF can improve the real-time processing of sentences in individuals with aphasia. In this study, participants with aphasia received a 12-week programme of TUF which focused on the production and comprehension of complex sentences. Before and after the training, eye movements were tracked whilst performing a sentence and picture matching task and brain processing was mapped using functional magnetic resonance imaging (fMRI). After treatment, participants with aphasia who received treatment were more



likely to respond correctly when processing complex and basic sentences. Furthermore, they showed eye movements comparable to those of healthy individuals, and recruited brain tissue primarily within the right hemisphere of the brain. Interestingly, the right hemisphere regions recruited in recovery were also recruited by healthy people or were opposite to left hemisphere regions that are typically engaged for sentence processing.

These findings provide further evidence for the neural reorganisation of language. They also complement earlier research indicating a positive impact of TUF rehabilitation by demonstrating the emergence of more normal sentence comprehension and production processes following treatment.

The Influence of Language and Brain Variables on Recovery

Another important component of Dr Thompson's work is the identification of neurobehavioural markers of recovery which can be used to inform treatment planning and prognosis. Recovery of language abilities after a stroke typically depends on multiple factors. For example, a patient's specific language impairment, how severe the impairment is, and factors related to the stroke lesion can all influence the response to therapy and recovery.

For example, smaller lesions, which cause less damage within the brain, appear to be associated with a better prognosis, presumably because the more brain tissue left intact, the easier it is for language functions to be remapped. Conversely, larger lesions are assumed to lead to a broader range of language skills being disrupted.

Dr Thompson believes that the location of the lesion is also fundamental, arguing that the location of the lesion site may play a more important role than its size when predicting the possible extent of language recovery. Her team used voxel-based lesion symptom mapping to establish the relationship between lesion location and the ability to produce and comprehend sentences. They found that lesions in the posterior temporal lobe were associated with the ability to process complex sentences. Although a limited number of studies have been conducted to date, this adds evidence to suggest that the site of the damage influences how language may be impacted and the possible level of recovery.

Resting state functional magnetic resonance imaging (rsfMRI) allows us to see what is happening inside the brain when it is not engaged in an explicit task (hence 'resting'). rsfMRI has been found to be particularly relevant to the study of aphasia, where patients can be distinguished from healthy control participants through differences in the functional connectivity of their resting networks. Dr Thompson and her colleagues recently demonstrated that predictive models of individual response to therapy incorporating rsfMRI connectivity are more powerful than those relying solely on anatomical (e.g., location of lesion) or behavioural (e.g., initial severity of language impairment) measures.

In a further study, Dr Thompson and her colleagues incorporated the assessment of white matter integrity (the deep pathways of the brain that connect the cortical regions) into the development of predictive models of patient response to treatment in post-stroke aphasia. Again, rsfMRI connectivity was identified as being particularly useful in predicting patient response, with further benefits gained with the inclusion of white matter integrity.

Dr Thompson believes that improving predictive models of response to treatment and recovery by including a wider range of brain-based assessments will lead us closer towards the goal of personalised treatments for individuals with post-stroke aphasia.

Neural Reorganisation Following Treatment: Neuroplasticity

The recovery of language, even in the case of left hemisphere damage, is possible due to neuroplasticity, in which neural networks within the brain reorganise to make new connections and regain function. Dr Thompson has provided direct evidence of the right hemisphere adapting to rebuild language processes – the opposite side of the brain to the damage causing aphasia – with exciting implications for stroke treatment and recovery.

Dr Thompson and her team have recently focused on understanding where specifically in the right hemisphere these neural changes resulting from treatment for aphasia take place. Understanding the specific locations where functions rebuild can highlight where stimulation should be focused during rehabilitation.

The wealth of research that has been undertaken by Dr Thompson gives continued hope to those who have suffered aphasia as a result of stroke or brain damage. Her ongoing aim is to provide further evidence to enhance rehabilitation techniques and support recovery.

Meet the researcher

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Dr Cynthia K. Thompson has a background in Psychology, Linguistics, and Speech and Language Pathology, receiving degrees from the University of Oregon (BA, MS) and the University of Kansas (PhD). She joined the faculty at Northwestern University's Department of Science Communications and Disorders in 1992 where she was appointed the Ralph and Jean Sundin Professor in Communications Science in 2009. The focus of her research is language recovery in people with brain damage, and her work informs the treatment of individuals who have suffered strokes and other brain injuries. Dr Thompson's research has been supported by major grants consecutively since 1992, funded primarily by the National Institutes of Health.

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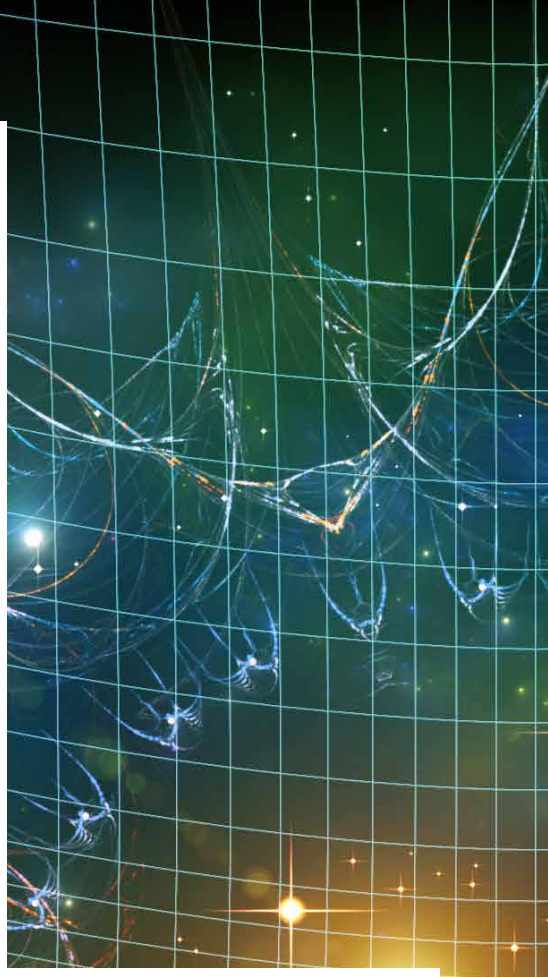
MOVING BEYOND 'ONE-SIZE-FITS-ALL' BRAIN TRAINING SOLUTIONS

Brain training allows us to improve our cognition in the same way that gym workouts improve our physical health. The ultimate goal is transferable learning, which improves performance in real-world activities beyond the original training tasks. **Dr Susanne M. Jaeggi**, **Dr Anja Pahor** and **Dr Aaron R. Seitz** from the University of California Irvine and Riverside, are collectively driving forward exciting advances in brain training, as well as addressing the controversy surrounding its effectiveness and limitations. Above all, they aim to understand the key ingredients for creating successful interventions.



What is Brain Training?

Brain training is regarded as the mental equivalent of going to the gym. Instead of improving our physical fitness, it aims to strengthen the brain systems that support cognitive performance. Just as we would spend time on the treadmill to maintain our cardiovascular health, brain training can enhance cognitive functions such as motor control, memory, and attention.

There are many different approaches to brain training, depending on what the learner wishes to accomplish. Some are educational, targeting reading or math; others may focus on a specific work-based skill. Dr Susanne M. Jaeggi, Dr Anja Pahor and Dr Aaron R. Seitz, all from the University of California Irvine and Riverside, agree that brain training programmes must be designed appropriately to improve cognition and that beneficial effects should be unlikely to arise by chance. To demonstrate this, they compare brain training to the act of going to the gym with the intent of improving fitness. This differs from playing a sport such as basketball, where the focus is typically entertainment and skill development, and any improvement to fitness is a secondary, albeit beneficial factor.

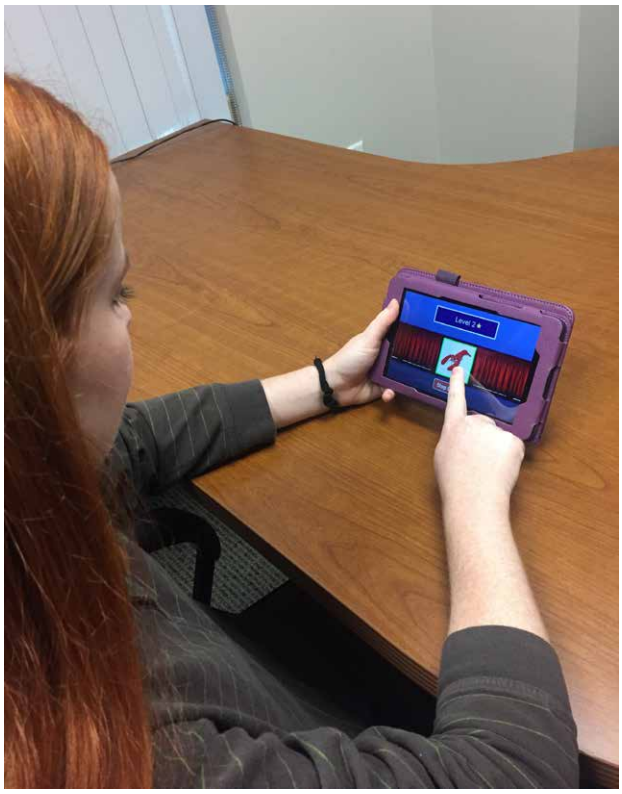
An important aim of brain training is 'transfer', which is an improvement in individuals' abilities beyond the trained task. The desired outcome is that transfer is evident across real-world activities, not just those that feature within the training. For example, the focus may be improving the storage of memories but the skills learnt could also help someone to undertake non-trained tasks such as understanding complex information or decision-making. There are two different levels of transfer. Near transfer is when learning enhances activities that are similar to the training tasks. Far transfer occurs when learning is utilised in different contexts beyond the training context. This is the ideal outcome as it brings more benefits to everyday life.

Everyone can potentially benefit from brain training, but it is often developed to help individuals overcome difficulties or to develop superior abilities. Brain training has proved useful amongst the ageing population to sustain declining

Courtesy of the UCI School of Education

cognitive function, particularly in those at risk for dementia. People with impaired cognitive abilities arising from brain injury, developmental issues and even addiction often utilise these techniques in their rehabilitation. A less well-known usage is the training of specialists. This can range from bolstering the learning of surgeons in complex medical tasks, to boosting academic performance, to enhancing expert performance in sports. For example, Dr Seitz has shown that vision training can improve on-field performance in baseball.

‘Our goal is to avoid a one-size-fits-all approach. Instead, we want to advance a new model based upon the premise that people are diverse in their cognitive strengths and needs, and therefore require the type of interventions that would serve them best.’



Courtesy of the UCI Working Memory & Plasticity Lab.

Addressing the Limited Evidence for Success

Brain training has become a billion-dollar business over the last decade. As Dr Pahor states ‘The possibility of improving core cognitive functions is alluring, creating a high demand for brain training programs.’ Despite the rapid growth in this sector, scientists remain cautious about the lack of evidence to support claims of success. One of the main controversies surrounds how the effectiveness of brain training programs are measured. Often participants are tested using very similar tasks to those used within the training. This makes it unclear whether learning can improve performance in different activities and real-world settings. Another limitation is the small groups of participants used in this research which makes it difficult to detect the individual differences that influence the key outcomes.

Nonetheless, the researchers believe that flawed methodologies do not mean we should rule out the positive effects of brain training. Instead, it is important to consider what these research studies were aiming to achieve. If the focus was the impact upon brain processes, then measures will not be set up to adequately assess the effectiveness of transfer to real-world settings. Drs Jaeggi, Pahor and Seitz emphasise that more research, using robust testing measures, is required to fully elucidate the benefits of specific brain training approaches, and critically, identify for whom these benefits can be seen.

The Effect of Multisensory Learning

The design principles that underpin brain training can have a significant impact on its effectiveness. Drs Jaeggi, Seitz and their research team have investigated the factors that may enhance program design. They theorised that features from the field of perceptual learning, in which learners use information from their experiences across multiple senses, could have a positive impact. Stimulating multiple senses throughout brain training could help to replicate real-life learning. When experiencing new situations, we can use vision, hearing, touch and even smell and taste to facilitate learning.

In 2020, Drs Jaeggi, Pahor, Seitz and their research team tested the impact of multisensory brain training. They demonstrated that combining auditory and visual learning enhanced abilities on untrained tasks. Participants trained using visuals alone, or alternating between audio and visual, did not perform as well as those completing the combined auditory and visual learning. This shows that using multiple senses helps to recreate realistic learning experiences and thus increasing the potential for broader transfer. Furthermore, these findings may be useful in designing brain training approaches for people who have specific sensory deficits.

Gamification of Training

Brain training programs often incorporate game-like features to enhance participant motivation. Much evidence already exists demonstrating that off-the-shelf videos games can improve players’ perceptual and cognitive abilities. However, scientists are concerned that few of the successful principles found in regular video games are applied to commercially available brain training apps and products. The success of video games is not a random phenomenon – very carefully crafted levels, challenges and settings reduce player frustration and create a fun experience.

Clearly, adding simple graphics and sounds to regular cognitive tests does not have the same impact as utilising the more effective principles from video gaming. Without proper design, the introduction of gamification to brain training could reduce the effectiveness or even become a distraction. There is no doubt that gaming features can be a powerful motivator but when designing brain training there must be careful consideration given to which elements will enhance transferable learning. This is an important aim of ongoing work by Drs Jaeggi, Pahor and Seitz.

Courtesy of the UCR Brain Game Center.

The Impact of Individual Differences

Drs Jaeggi, Pahor and Seitz strongly believe that in order to enhance brain training we must acknowledge the many individual factors that come into play. Training can be impacted by age, general cognitive abilities and even attitudes. They argue that more adaptable programs are required to counteract or even capitalise on those individual differences.

Recently, Dr Jaeggi and her research team reviewed a number of scientific studies to highlight personal factors that impact brain training effectiveness. For example, it has been shown that those with room to improve show the most benefits. We know that cognition declines with age; hence, many brain training programs target older adults. The researchers noted that despite an emphasis on comparing the young and the old, there are very few studies capturing the effects across middle age. The research team also suggest that ceiling effects lead to doubt whether testing measures are sensitive enough to capture change, especially at high levels of performance.

Dr Jaeggi and her research colleagues have also uncovered attitudinal factors that may influence the effectiveness of brain training. An individual's belief that their own intelligence can be improved has been shown to positively impact their ability to apply learning from brain training. Placebo effects shown in a control group highlighted this discovery. Unsurprisingly, motivation also played a key role. People who perceived they had some weaknesses in their memory or cognition were more likely to engage with training and see positive results. Despite the belief that they needed brain training, these participants did not actually show poorer baseline performance than other participants. Furthermore, those who found the training demanding were less likely to complete the intervention, and if they did, were less likely to benefit.

Not a 'One-Size-Fits-All' Approach

Individual differences highlight that there is no 'one-size-fits-all' approach in terms of brain training. People come from diverse backgrounds and have different experiences that may influence how they respond to training. There are varying needs and motivations for pursuing brain training, which dictate the kind of programs that are most suitable.

Drs Jaeggi, Pahor and Seitz are currently undertaking a large-scale citizen science study that aims to assess the impact of individual differences. They hope to provide robust evidence in order to argue against a 'one-size-fits-all' approach. The online study aims to recruit 30,000 participants and use a standard set of measures to assess multiple training approaches. By evaluating such large numbers and focusing on individual needs, the researchers will identify the individuals for whom brain training can provide the most benefits and the reasons why.

In line with the drive towards personalised medicine, it is clear that the important question now is not whether brain training works, but how to determine which type of training is right for the individual and their circumstances. Drs Jaeggi, Pahor and Seitz explain further that 'Our goal is to avoid a one-size-fits-all approach. Instead, we want to advance a new model based upon the premise that people are diverse in their cognitive strengths and needs, and therefore require the type of interventions that would serve them best.'

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Dr Susanne Jaeggi received PhDs in Cognitive Psychology and Neuroscience, as well as an advanced 'Habilitation' degree in Psychology from the University of Bern in Switzerland. She went on to become a Postdoctoral Fellow in Cognition and Cognitive Neuroscience at the University of Michigan. Currently, Dr Jaeggi is a Professor in Education and Cognitive Science at the University of California, Irvine. She studies working memory and related cognitive functions across the lifespan. Her major work focuses on the development of cognitive interventions and the investigation of how these may generalise to untrained non-cognitive abilities.

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Dr Anja Pahor received her PhD in Behavioral and Cognitive Neuroscience from the University of Maribor in Slovenia and conducted postdoctoral work at the University of California, Riverside. She is a member of the UC Irvine Working Memory and Plasticity Lab, where she works as a Project Scientist, and the UC Riverside Brain Game Center for Mental Fitness and Well-being. Her research interests involve understanding the mechanisms underlying higher-level cognitive functions, particularly memory and reasoning. She is currently working on several multi-site research projects involving the assessment and training of executive functions in children and adults.

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Dr Aaron R. Seitz received his PhD in Cognitive and Neural Systems from Boston University, and went on to conduct postdoctoral work in the Department of Neurobiology at Harvard Medical School in Neuroscience. He is now a Professor of Psychology and the Director of the UCR Brain Game Center for Mental Fitness and Well-being at the University of California, Riverside. Dr Seitz has a long-term interest in mechanisms of brain plasticity and transfer of learning. He uses psychophysical, physiological, brain imaging, psychopharmacological, genetic and computational approaches to understand mechanisms of human perception, attention, learning and memory.

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NOVEL BIOMARKERS AND PROMISING THERAPEUTIC TARGETS IN ALZHEIMER'S DISEASE

Alzheimer's disease is a form of dementia that affects tens of millions of people globally. Although we can modestly improve the quality of life of patients, there is currently no cure, largely because the underlying biological mechanisms of the disease are poorly defined. Understanding the abnormal molecular characteristics of Alzheimer's disease is the focus of **Dr. Erin Norris's** research at The Rockefeller University. By studying the dysfunction of the plasma contact system, which may result in abnormal coagulation and inflammation that may lead to Alzheimer's disease, Dr. Norris and her colleagues have uncovered novel biomarkers, paving the way for exciting new therapeutics.

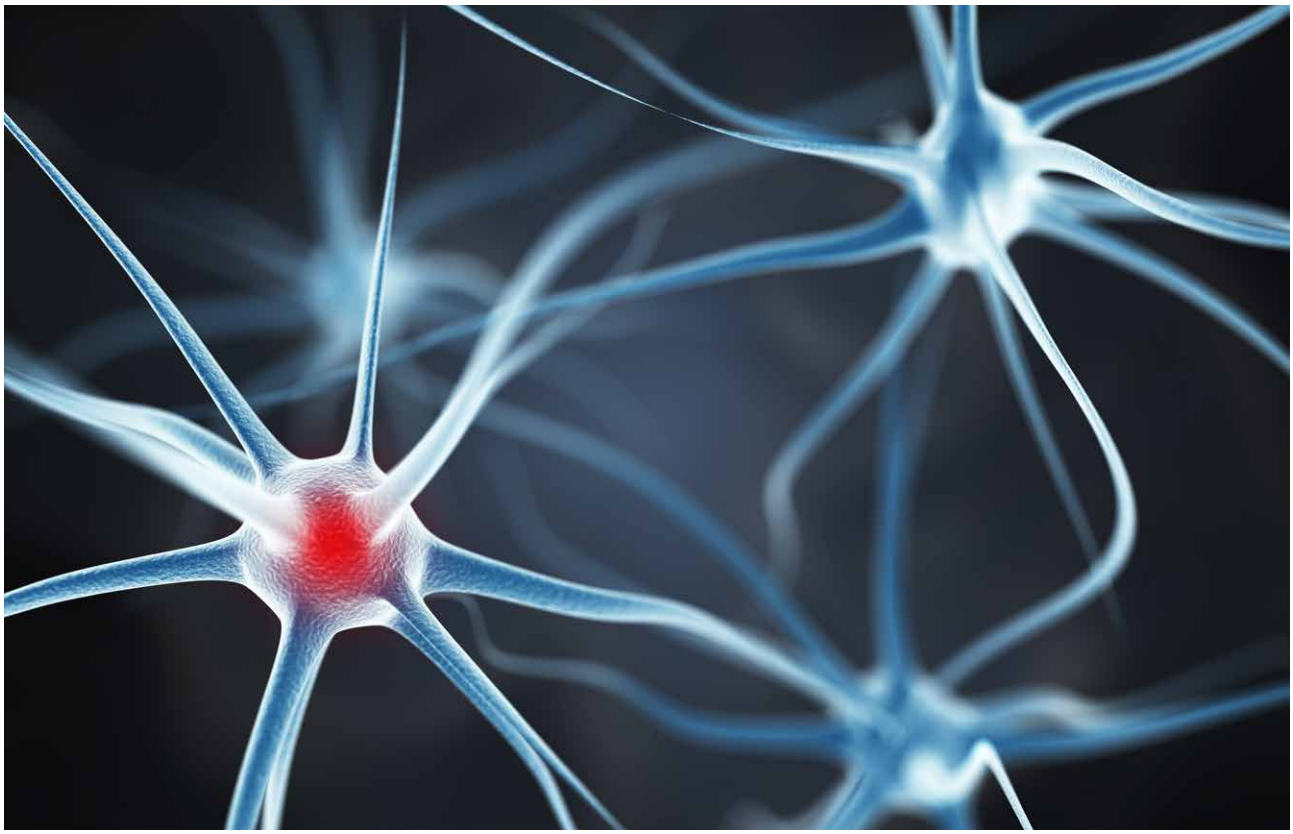
A Common but Challenging Disease

Alzheimer's disease is the most common form of dementia, which is an umbrella term encompassing a variety of diseases characterised by a progressive decline in cognitive functions such as memory, language, attention and reasoning. The symptoms often initially present as minor problems with memory that progress into confusion, issues with speech and movement, alterations in personality like aggression, and even hallucinations. Risk factors for Alzheimer's disease include a family history of the condition, older age, lifestyle factors associated with cardiovascular disease and untreated depression.

While people do not tend to pass away from the illness itself, many patients experience a reduced appetite and their diminished cognitive ability can result in difficulty swallowing, causing inhalation of food and frequent chest infections. Currently, there is no cure but certain drugs can alleviate some of the symptoms. Psychological treatments such as cognitive stimulation therapy can try to help prevent the further deterioration of memory and language. Changing the home environment in which a person with Alzheimer's disease resides can also allow them to live more independently by simplifying day-to-day tasks.

Research across the globe has been dedicated to finding and targeting the molecular irregularities that cause Alzheimer's disease. Although the exact causes are not well-defined, it is understood that when proteins (the building blocks of all cells) in the brain act abnormally, the function of brain cells known as neurons is disrupted. The subsequent series of toxic events results in damage to neurons. The connections that facilitate brain activity are also disrupted, which eventually causes the neurons to die, leading to a decline in cognitive function.

The proteins responsible for these destructive events are called beta-amyloid and tau. Beta-amyloid is a fragment of a very large protein called amyloid precursor protein. When these fragments aggregate they disrupt cell-to-cell communication and continue to cluster with other cellular debris into large deposits called amyloid plaques. These plaques can then also lead to chronic inflammation in the brain, which is toxic to neurons. Tau proteins are important for the internal support of neurons and help to carry essential nutrients and materials. However, in Alzheimer's disease, tau proteins become atypically organised to create neurofibrillary tangles which disrupt the transport system.



Targeting the Plasma Contact System

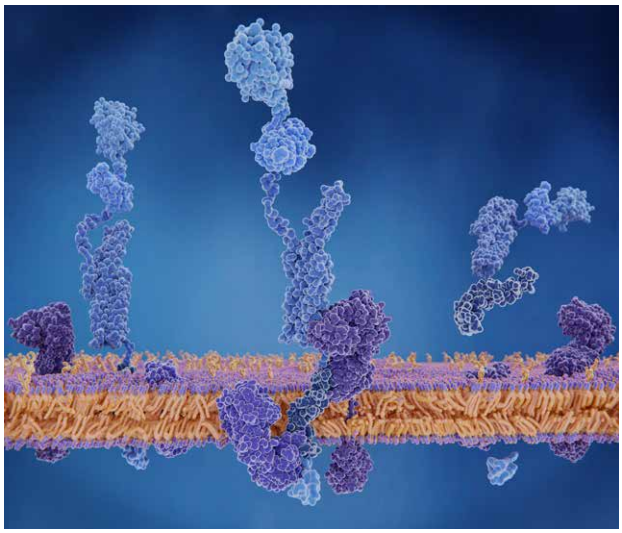
These pathologies of Alzheimer's disease have been extensively studied, but an issue that has been less explored is vascular dysfunction – problems in blood vessels and blood circulation. Many patients experience a reduced blood flow in the brain known as hypoperfusion, damage to brain blood vessels and disruption to normal mechanisms that stop bleeding and repair damage (haemostasis). Vascular disorders such as obesity, hypertension and diabetes are also common in those with Alzheimer's. Dr. Erin Norris and her colleagues from The Rockefeller University in New York are working to understand the links between Alzheimer's and vascular dysfunction and the biological processes behind them.

The vascular system is responsible for coagulation, which is the process of liquid blood changing into a solid state, or blood clot, to repair injuries. There are two mechanisms by which this occurs – the first is extrinsic clotting. When an injury or trauma occurs outside of the blood vessel (a cut, for example), this pathway is activated to heal the damage. The other is intrinsic clotting, which occurs when there is trauma to the blood vessel itself or the tissues surrounding it. These two mechanisms merge together to form blood clots via the cleavage (cutting up) of fibrinogen proteins into smaller fibrin proteins using an enzyme called thrombin.

Research from Dr. Norris and her scientific team has revealed that the intrinsic clotting pathway in patients with Alzheimer's is disrupted, whilst the extrinsic system is left intact. In one study, Dr. Norris and her colleagues investigated the plasma contact system within the intrinsic pathway. This system is initiated by a protein called coagulation factor XII (FXII) being activated into its active form (FXIIa), which then triggers two arms of the process. One arm is the clotting pathway that utilises factor XI (FXI) to produce thrombin and blood clots, while the other is an inflammatory pathway that uses prekallikrein (PK) to produce the pro-inflammatory peptide, bradykinin.

Bradykinin is cleaved out of high molecular weight kininogen (HK) by the activated form of PK (or PKa). HK is important for both arms of the system because it forms complexes with both FXI and PK allowing for their efficient activation by FXIIa. Due to this dual function of HK, reducing or inhibiting the cleavage of HK can prevent unwanted blood clots and inflammation, as shown in some of Dr. Norris's experiments. To prevent HK cleavage, they depleted the amount of FXII in Alzheimer's model mice, which in turn prevented the function of HK. The mice showed reduced neuroinflammation and neurodegeneration and performed better cognitively compared to untreated mice.

These exciting results demonstrate a potential novel focus for Alzheimer's therapies. As contact system activation may play a part in the pathology of Alzheimer's, blocking it could slow disease progression. Dr. Norris suggests HK as a prime target in the quest to prevent inflammation as well as abnormal coagulation, and her team has already discovered a way of inhibiting the protein's cleavage. Her method utilises an antibody that binds to HK and prevents its cleavage and thus its ability to generate bradykinin. Whilst the antibody is being tested, she studies other abnormal molecular occurrences in Alzheimer's patients.



Defining Biomarkers for Alzheimer's Disease

The overactivation of the intrinsic pathway in Alzheimer's could be due to the unusual build-up of beta-amyloid protein. Beta-amyloid can bind to FXII and lead to activation of the plasma contact system, resulting in a high amount of cleaved HK and bradykinin in the plasma of Alzheimer's patients as well as mouse models. While not all Alzheimer's patients have vascular dysfunction and contact system activation, being able to identify those patients suffering from this condition would be advantageous as there are already FDA-approved treatments. Any method to slow the progression of the disease in even a subset of patients would be beneficial. Therefore, cleaved HK could be categorised as a biomarker, and could be a useful means of diagnosing Alzheimer's patients with vascular dysfunction.

In order to create a diagnostic tool, Dr. Norris's laboratory team set out to generate and screen for antibodies that could specifically recognise both the cleaved and uncleaved versions of HK. Once these were identified, they used a method called enzyme-linked immunosorbent assay to screen for these proteins in human plasma. The tests revealed decreased uncleaved HK and increased cleaved HK in those plasma samples from Alzheimer's patients, and these results correlated with dementia and levels of beta-amyloid plaque in the brain. This study provides solid evidence that cleaved HK in plasma could be used as a novel biomarker for the diagnosis of Alzheimer's disease with a vascular pathophysiological component.

Dr. Norris continued to explore this avenue in a subsequent study that centred around mild cognitive impairment (MCI). This disorder is a transitional stage between the normal cognitive decline that comes with ageing and the commencement of mild dementia. Patients with MCI tend to adequately carry out daily tasks but have some deficits in cognitive function such as short-term memory retrieval. Approximately 10–15% of MCI patients convert to an Alzheimer's diagnosis per year.

Dr. Norris's team examined the plasma of MCI patients for evidence of contact system activation. Aligning with their previous results, those living with MCI who experienced poor memory recall had increased kallikrein activity and subsequently, higher levels of cleaved HK. This finding further supported her and her team's hypothesis that contact system dysfunction is a biomarker for Alzheimer's and its related dementias as it can contribute to its pathogenesis.

Implementing Novel Biomarkers

A further study by Dr. Norris' group showed significantly elevated levels of bradykinin in the plasma of Alzheimer's patients compared to healthy participants. Increased levels of bradykinin are due to increased contact system activation and cleavage of HK. Furthermore, those with a more profound increase in bradykinin displayed more severe cognitive impairment. These results strongly suggest that cleaved HK and bradykinin are involved in disease pathogenesis. Moreover, since bradykinin is a potent inflammatory molecule, it could be responsible for much of the neuroinflammation found in Alzheimer's patients. Interestingly, post-mortem examination of Alzheimer's patients' brain tissue showed that bradykinin co-deposits with beta-amyloid plaques.

Dr. Norris and her colleagues at The Rockefeller University have uncovered multiple, yet linked, biomarkers for Alzheimer's disease, and they are continuing their research into how they can be targeted to develop novel and effective therapeutics. They are particularly focused on an anti-HK antibody called 3E8 which binds very strongly to HK to inhibit the activation of the contact system by beta-amyloid to prevent abnormal clotting as well as inflammation. They believe this antibody could be a promising method for slowing or preventing cognitive decline to improve the quality of life of many MCI and Alzheimer's disease patients in the future.

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Dr. Erin Norris completed her BA in Biology with a Minor in Chemistry at Franklin & Marshall College in Pennsylvania. She then went on to earn a PhD in Pharmacological Sciences at the University of Pennsylvania and completed her postdoctoral training at The Rockefeller University. She has undertaken numerous lecturing and research roles, as well as scientific journal editing positions. Currently, Dr. Norris is a Research Associate Professor in the Laboratory of Neurobiology & Genetics at The Rockefeller University in New York. She carries out research investigating the role of vascular dysfunction in Alzheimer's disease pathophysiology and how it can be used to develop novel biomarkers and therapeutics. Supported by significant and prestigious funding, Dr. Norris has published over 50 peer-reviewed articles to date and is a popular invited speaker at international scientific meetings and conferences.

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DISCOVERY OF NEUROTROPHIC FACTOR-α1 REVEALS NEW TREATMENT STRATEGIES FOR STRESS-INDUCED NEURODEGENERATIVE DISEASES AND DEPRESSION

Stress produces numerous negative effects on the human body. Lying deep within the brain, one particularly sensitive area is the hippocampus, where chronic exposure to stress hormones can lead to the degeneration and death of neurons. Thankfully, the brain holds defence mechanisms that block some of these negative effects. Deciphering these mechanisms with the aim of better treating neurodegenerative diseases and depression is **Dr Y. Peng Loh** from the Eunice Kennedy Shriver National Institute of Child Health and Human Development in the USA.

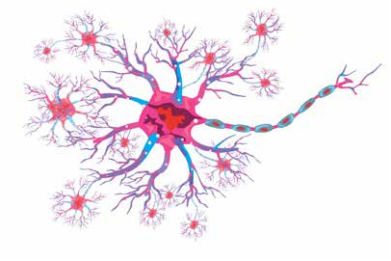
Stress on the Brain

Stress is a feeling familiar to us all, and results in the body releasing a cascade of hormones that cause physiological changes. These changes are important survival mechanisms; a quickened heart rate and reduced gastrointestinal activity help prepare the body for a fight-or-flight response. However, the modern age brings with it chronic stress, something the human body is not fully prepared for.

Continual activation of the stress response can lead to a number of health problems, including high blood pressure, heart attacks, and alterations to the brain that contribute to mental health issues. Fortunately, protection mechanisms are in place to prevent some damage, including damage to the stress-sensitive brain, although these are not always effective. One specific area of the brain, the hippocampus, is particularly at risk of the destructive effects of the stress hormones called glucocorticoids.

The hippocampus is a complex structure deep within the brain and it plays a major role in emotion, learning and memory, including spatial memory. As a result, when Alzheimer's disease and other forms of dementia develop, the damage is often first seen in this area. This manifests as short-term memory loss and disorientation that worsens over time. A shrunken hippocampus is sometimes observed in patients with untreated depression, and it is implicated in this disease as well.

Investigating how the brain protects itself from stress-induced damage and how this knowledge can be used to develop treatments for dementia and depression is the goal of Dr Y. Peng Loh and her team from the Section on Cellular Neurobiology at the Eunice Kennedy Shriver National Institute of Child Health and Human Development within the National Institutes of Health, USA.



The Role of Neurotrophic Factor-α1

The early focus of Dr Loh's work was an enzyme called carboxypeptidase E. Enzymes are proteins that catalyse biochemical reactions. Importantly, this enzyme is involved in the production of amino acid chains called neuropeptides which regulate brain activity and also the production of peptide hormones, which are involved in the body's many different responses to its environment.

Peptide hormones are also important in helping neuron cells develop and connect with surrounding neurons for brain activity. These types of molecules are known as trophic factors or trophins. They are secreted outside the cell and bind to a cell receptor which then transduces a signal directing the cell

to modify its function. In 2013, Dr Loh discovered that carboxypeptidase E is also a trophic factor that works independently of its enzyme function, leading her to coin the new name of neurotrophic factor-α1 (or NF-α1 for short). NF-α1 has been found to have profound effects in protecting hippocampal cells from oxidative stress-induced cell death. Not only has Dr Loh found that NF-α1 is important for neurodevelopment, but it is also implicated in cancer development, found in significant quantities in tumours accompanied by strong evidence it promotes tumour growth.

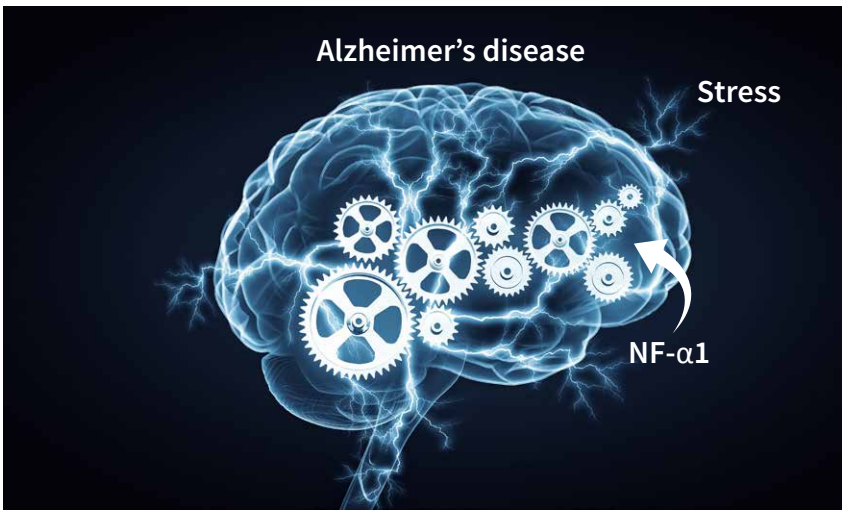
Clearly, the role of NF-α1 in the brain is varied and complex, and the full range of its functions is not yet well understood. Dr Loh is shining a light on a previously unknown task of this important enzyme – that of a trophin, working to prevent neuronal degeneration and death when the brain is faced with severe stress.

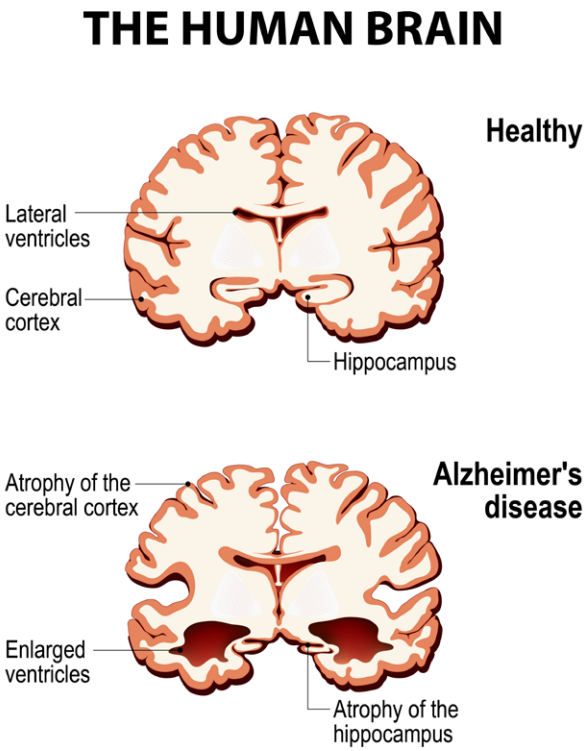
More Important Than Previously Thought

Trophic factors are proteins that promote cell growth and viability. For example, brain-derived neurotrophic factor (BDNF) is protective against stress and when it is reduced, neurodegeneration is observed, as seen in post-mortem examinations of Alzheimer's patients. As such, BDNF is needed for the survival, proliferation and protection of neurons in the brain.

However, Dr Loh found that the known trophic factors did not account for the full neuroprotection of CA3 pyramidal neurons in the highly stress-sensitive hippocampus. Intrigued by this, she turned her attention to NF-α1, which is produced in high quantities by the CA3 neurons. In these groundbreaking experiments, Dr Loh used mice that were engineered to be unable to produce NF-α1.

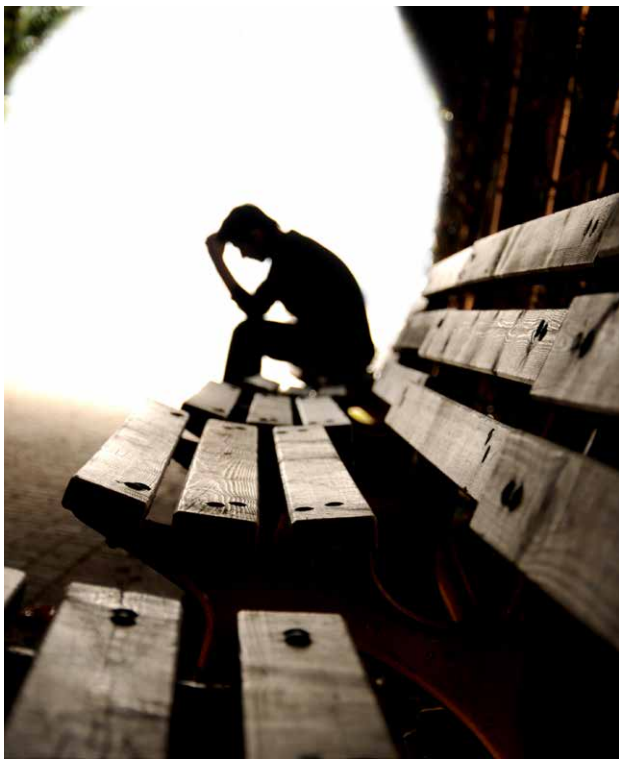
At three weeks old, the mice were subjected to social and physical stress. Even though the mice had sufficient





BDNF, this stress led to complete CA3 neuron degeneration. According to Dr Loh, this shows that NF-α1 is essential for neuroprotection during stress, as when it is missing, neurodegeneration clearly occurs. Even more importantly, she showed for the first time that NF-α1 is more critical to neuroprotection than BDNF.

In the same study, Dr Loh studied a different set of mice that were engineered not to make normal NF-α1 but a form that could not act as an enzyme for its already known functions. After stress, these mice showed no neurodegeneration and showed normal learning and memory, unlike the mice with no NF-α1 at all. This means that the protective function and activities of NF-α1 are separate from its functions as an enzyme. Protection against stress-induced degeneration protects against cognitive decline which can present as depression. Dr Loh has further solidified NF-α1 as an important protective factor by elucidating how it works on a molecular level.



The Molecular Mechanisms of NF-α1

When investigating a biological pathway such that of NF-α1 in protecting neurons, it is important to understand the minute details if the right molecules and reactions are to be targeted when the research is eventually used to innovate new therapies. For the NF-α1 pathway, such therapies may in the future include new gene therapy approaches, antidepressants or even drugs to treat Alzheimer's disease or depression.

Dr Loh also looked into how NF-α1 works to prevent stress from destroying the cells of the hippocampus. She did this using hippocampus cells from mice and found that glucocorticoids released during stress increase NF-α1 production. NF-α1 binds specifically and strongly to these types of cells (or more precisely, to receptors on their surface). Recently, she identified the receptor to which NF-α1 binds – HTR1E, a serotonin receptor in human neurons.

When human neurons grown in Petri dishes were engineered to not express the HTR1E receptors, added NF-α1 was unable to protect these cells from dying when subjected to induced oxidative and neurotoxic stress. Once NF-α1 has bound to the receptors, a pathway called the ERK pathway is activated and the cell is signalled to increase energy metabolism. It also increases the amount of mitochondrial pro-survival protein, BCL2, both of which boost the neuron's ability to survive.

Another mechanism that Dr Loh and her colleagues investigated in a different study is the effect of NF-α1 on a signalling protein called fibroblast growth factor 2 (FGF2). This protein is involved in the survival and replication of fibroblasts, the cells that create structure in connective tissue and aid in wound healing. This study also used mice, some of which were subjected to short-term chronic stress and others to long-term chronic stress.

The hippocampus of the mice that experienced short-term stress showed high levels of NF-α1 which was accompanied by high levels of FGF2. They hypothesised that as a direct result of these elevated levels, these mice had high neurogenesis and exhibited no depressive-like behaviours. However, the mice with long-term stress had low levels of NF-α1, FGF2 and neurogenesis and consequently, they showed clear signs of depressive behaviour. This shows that NF-α1 is protective, but prolonged stress can still cause damage to the brain.

Other mice that had been engineered to not produce NF-α1 had decreased FGF2 and neurogenesis within their hippocampus whilst also showing depressive-like behaviours. This supported the idea that NF-α1 regulates the abundance of FGF2 in the hippocampus and so, depression. In addition, once these mice were treated with FGF2, their depression symptoms were reversed.

Additional studies have demonstrated that when hippocampal neuron cells are grown in Petri dishes and treated with NF-α1, this directly upregulates FGF2 expression (more of it is made). Therefore, Dr Loh explains, when mice are subjected to short-term stress, the hippocampus makes more NF-α1 and becomes more active and has a key part to play in preventing depression through upregulating FGF2 for neurogenesis.

The Potential for New Antidepressants

To understand these findings even further, Dr Loh studied a drug that is known to have antidepressant qualities for both mice and humans – rosiglitazone (Rosi). Interestingly, the drug increased the activity of both NF-α1 and FGF2 in the hippocampus, resulting in increased neurogenesis. This provides exciting insight into the potential use of Rosi, or other drugs that act on this pathway, as antidepressants.

Dr Loh believes that NF-α1 and the neurogenesis pathways associated with it are the future for promising new therapies for depression. The proven effectiveness of targeting these and mitochondrial metabolic pathways to prevent neuron death and degeneration is a real indicator of their promise. But with that in mind, she notes that additional NF-α1 -activated signalling pathways may be involved in neurogenesis and neuroprotection should also be investigated.

Dr Loh is continuing to drive progress in her field through exploring different potential therapeutic targets and innovating novel therapies for depression and neurodegenerative diseases such as Alzheimer's disease.

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Dr Y. Peng Loh achieved her BSc in Biochemistry from University College Dublin in Ireland, followed by her PhD in Molecular Biology from the University of Pennsylvania in the USA. She carried out postdoctoral work both at the National Institutes of Health in the USA and the Max Planck Institute in Germany, and has received multiple honours and awards for her work. Currently, Dr Loh serves as the Senior Investigator and Head of the Section on Cellular Neurobiology Eunice Kennedy Shriver National Institute of Child Health and Human Development at the National Institutes of Health. Her research has led to exciting developments in our understanding of stress-induced neurodegeneration and depression, and the neuroprotective and anti-depressant mechanisms mediated by the new neurotrophic factor discovered in her laboratory, now named NF-α1.

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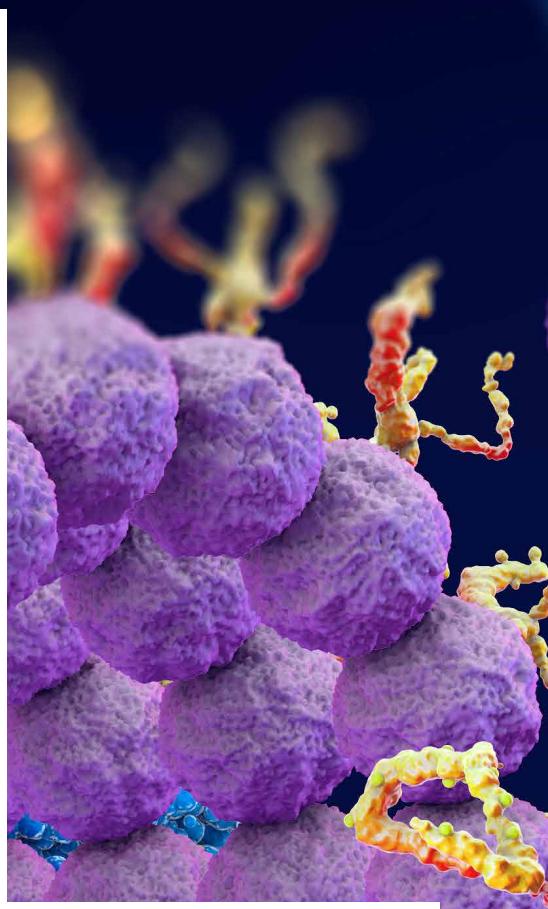
MISFOLDING OF BRAIN PROTEINS TRIGGERING NEURODEGENERATIVE DISEASES

Our DNA codes for proteins that are essential for the normal structure and function of our cells, tissues and organs. These proteins are folded in specific ways to facilitate these functions, but in disease states, this folding can go wrong. **Dr. David Westaway** from the University of Alberta in Canada investigates how and why protein misfolding occurs and how strains of misfolded proteins result in neurodegenerative diseases like dementia. His research is paving the way for novel therapies for these currently incurable and devasting conditions.



The Structure and Function of Proteins in Our Cells

Proteins are large and complex molecules that are found in every cell of the human body. They have an extensive number of different roles, ranging from making up structures within cells to carrying out specific functional roles or regulating other processes for normal body tissue and organ function. One example of a structural protein is called actin; the actin protein gives structure and support to cells and allows proper body movement. Antibodies that protect our bodies from infection are functional proteins, whereas enzymes are regulatory proteins that that help to carry out vital chemical reactions such as turning food into energy.

Each different protein is coded for by specific sequences of DNA, known as genes. Once cell machinery has deciphered these genes and determined which proteins are required, it joins together the relevant amino acids in the correct order to make these proteins. There are 20 different amino acids that can be stitched together in different orders and amounts to create a vast number of protein possibilities. This order of amino acids is known as a protein's primary structure.

For proteins to function correctly, the secondary and tertiary structures, as well as the primary structure of the protein must be exact. The secondary structure is the local interactions between smaller sections of the protein whilst the tertiary structure is the overall, three-dimensional folding and structure of the protein. These three-dimensional shapes and their associated rigidity – or sometimes, aspects of their flexibility – are what allow a protein to perform its duties and keep cells healthy.

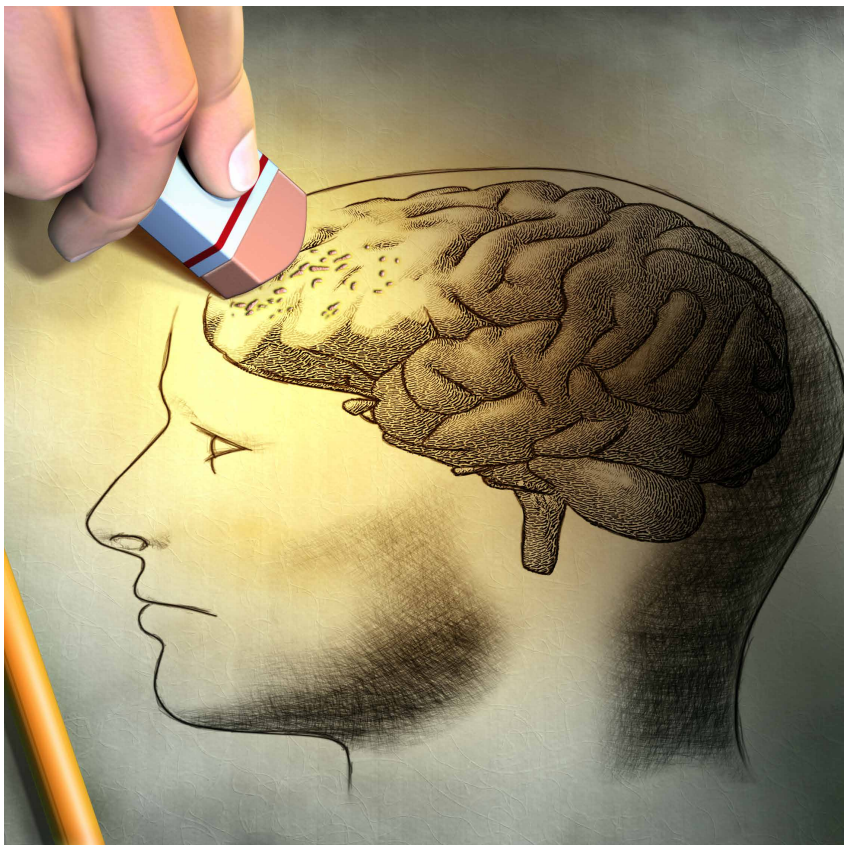
What Happens When Protein Folding Goes Wrong in the Brain?

Unfortunately, protein folding does not always go according to plan. In neurodegenerative brain diseases, a small group of proteins can be incorrectly folded and then cluster together. These clusters of proteins

become sufficiently large that they can easily be seen with a simple microscope, and are observed to lie inside or next to brain cells.

To make matters worse, once these clusters have started to form, they can catalyse their own formation by taking new, normally folded proteins and disrupting their structure to be misfolded. In this process, the normal proteins are known as substrates and the misfolded proteins are collectively known as products.

Often, the result of a catalytic misfolding process is a neurodegenerative disease whereby brain function continues to decline over time. This loss of brain function can be associated with several different symptoms including



memory loss, difficulty of movement, trouble speaking and unpredictable moods. Profound loss of reasoning and memory function is synonymous with the umbrella term dementia. One of the most common types of dementia is Alzheimer's disease, which is well-known to affect a brain structure called the hippocampus while frontotemporal dementia produces degeneration nearer to the front of the brain. Apart from rare cases where substrate proteins are predisposed to misfold by a mutation, dementias are associated with older age and there is evidence that different lifestyle factors can either advance or stave off their development.

Unfortunately, around one-sixth of people over the age of 80 have dementia and the World Health Organization estimates 50 million people live with it worldwide. Whilst there are some medications available to ease the symptoms of dementia, the first generation of curatives is proving controversial and so, where possible, alternative treatments may be suggested. This might include cognitive stimulation therapy to improve brain function or cognitive rehabilitation to facilitate everyday tasks. Nevertheless, due to erosion of memory, intellect and normal behaviours, neurodegenerative diseases remain a difficult issue for many people and their families. Understanding the underlying events through research is an important step towards progressing different mechanism-based therapeutics.

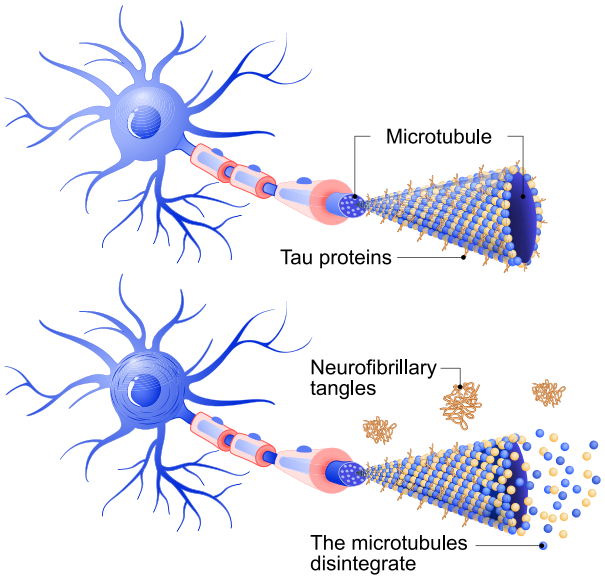
Studying the Molecular Origins of Neurodegenerative Diseases

Unravelling the reasons for, mechanisms behind and solutions to neurodegenerative disease processes is Dr. David Westaway from the Centre for Prions and Protein Folding Diseases at the University of Alberta in Canada. He describes how the series of steps leading to the assembly of misfolded proteins is complex and the outcomes for any given misfolded protein are diverse. As a result, different types of protein clusters can form in the brain called 'strains' – and superficially similar to viral strains we are now all familiar with because they have certain predictable properties. Different misfolded protein strains can react differently to medications too; whereas one strain may diminish and respond well to a certain treatment in petri-dish experiments, another strain may partially or completely evade treatment.

According to Dr. Westaway, 'My laboratory focuses on these issues and events for two proteins of interest – the cellular prion protein and the protein tau'. The cellular prion protein is found on the surface of cells in many different tissues and organs but especially in the central and peripheral nervous systems. As the brain is a part of the central nervous system, when the cellular surface protein becomes misfolded and builds up it causes neurodegenerative diseases. These are collectively referred to as prion diseases. Tau proteins are also found in abundance in the central nervous system and neurons, and abnormal accumulations of tau are associated with Alzheimer's and Pick's disease, which are also neurodegenerative syndromes. The collective name for tau diseases is tauopathies.

Misfolding of the Cellular Prion Protein

Before scientists were aware of different strains of neurodegenerative disease proteins, it was generally believed that



one type of amino acid chain could only result in one type of three-dimensional folding and one type of misfolding. This perspective has now been superseded and Dr. Westaway explains that there is 'another layer of complexity – the misfolding outcomes for a protein can be diverse and can change depending on which part of the molecule is being considered.'

This has previously been studied and documented in a protein called amyloid precursor protein in relation to Alzheimer's disease. Following on from this, Dr. Westaway and his team have looked into cellular prion protein and how it is involved in disease development. They have studied mutant types of the cellular prion protein that are predisposed to misfolding and inevitably cause disease.

A mutant protein has single or multiple amino acids replacing those that are standard, resulting in an abnormal protein. This is due to genes that are themselves coded abnormally or alternatively, it is due to a mistake in protein quality control somewhere along the production line in cells. Dr. Westaway is interested in finding and developing chemicals and drugs that could control misfolding events due to mutant proteins. This could lead to exciting developments in therapeutics for multiple neurodegenerative diseases.

Strains of the Tau Protein Lead to Different types of Dementias

Dr. Westaway has also moved forward our understanding of changes in tau in relation to different types of tauopathy. Describing his work, Dr. Westaway notes, 'for the protein tau, using susceptible mice made by genetic engineering and a chemical test of misfolding, we have shown that one type of substrate molecule can create a collection of different misfolded variants; this work was carried out with our collaborator Jiri G. Safar, MD. As elapsed time increased beyond two-thirds of the natural lifespan of these mice, this mixture of variants evolved into two or three clearly recognisable strains of misfolded tau protein.'

This means that he was able to demonstrate one protein misfolding into multiple strains in a real-world example. In animal models of disease (e.g., mice), disease progression is sometimes deliberately accelerated to make experiments quicker, not to mention less expensive. However, this can mean that the processes that result in different tau strains are difficult to detect. Therefore, Dr. Westaway utilised a 'slow' model of neurodegenerative disease and this allowed him to study the evolution of events that lead up to strain formation.

The team found that, in a human context that shares the same type of tau protein as the mouse disease model, the different tau protein strains were associated with different types of dementia that affect the frontal lobes of the brain (collectively known as frontotemporal dementias). Here, the suspicion was – and has now been verified by chemical analyses of human and animal model samples – that the differences that could be seen in patients behaviours originated from slightly different forms of abnormal tau.

'My laboratory is tracking down the chemical processes and cells which participate in the evolution of toxic misfolded forms of the tau protein', says Dr. Westaway. If these processes can be thoroughly defined, they can be targeted by novel therapies for the disease. Blocking the formation and diversification of these unwanted strains through drugs could be a promising method of preventing and blocking neurodegenerative diseases.

Continuing the Vital Work: New Funding

Recently, Dr. Westaway and his colleagues in the Centre for Prions and Protein Folding Diseases, in the Neuroscience and Mental Health Institute and in the Department of Physics at the University of Alberta, have secured novel federal, provincial and for-profit funding of an incredible $9.5 million. This backing came thanks to an investment initiative of over half a billion dollars led by Canada's Prime Minister, Justin Trudeau. The goal of distributing this funding is to sustain innovative, scientific research and discovery within the country. This exciting financial support will help Dr. Westaway and his colleagues carry on advanced research into neurodegenerative diseases.

Already, Dr. Westaway and his team have made impressive strides into deepening our understanding of protein folding and misfolding and how they impact health and disease. By elucidating the processes that result in protein strains and consequent neurodegenerative disease, he is beginning to point towards possible new therapies for the future. This provides important hope for the millions of people suffering from dementia worldwide.

Meet the researcher

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Dr. David Westaway completed his BSc in Biochemistry at the University of Sussex in the UK, and went on to achieve a PhD in the Biochemistry Department of Imperial College at the University of London. Having received multiple awards in his field and filled numerous academic positions, Dr. Westaway is now a Professor in the Department of Medicine (Neurology) at the University of Alberta in Canada. He is also the Director of the Centre for Prions and Protein Folding Disease at the University of Alberta, which is where he carries out his research into what happens when protein folding goes wrong in our cells.

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UNIVERSITY OF ALBERTA

MAPPING THE BRAIN'S NEURONAL NETWORKS TO UNDERSTAND BEHAVIOUR

The human brain is wonderfully complex. Billions of neuron cells connect in unique ways to create networks that determine each individual's brain function and consequent behaviour. Given the expanse and complexity of these networks, it is not surprising that they are not yet fully mapped out or understood. Bringing innovative new and exciting ideas to this field of neurobiology is **Dr Martin Schwarz** from the Institute for Experimental Epileptology and Cognition Research and the Life & Brain Center at the University of Bonn Medical Center in Germany.

Neurons: The 'Talking' Cells in the Brain

Our brains are made up of a staggering 86 billion neuron cells connecting and working together to keep our minds alive. Neurons are one of the two types of cells in the nervous system, the others are glial cells. They have a cell body that holds the nucleus with its genetic material and controls the activities of the cell. From this body, extensions called dendrites branch out to talk with adjacent axons, the long tail of the cell that transmits messages.

These messages are sent and received in the form of chemicals called neurotransmitters, such as dopamine, serotonin and adrenaline, among hundreds of others. Neurotransmitters are sent across the tiny spaces, known as synapses, between the dendrites and axons to deliver information across the brain to the necessary areas. Neurons can be sensory, motor or another type called interneurons. Sensory neurons carry information from the sensory organs, like the nose and eyes, to the brain whilst motor neurons control voluntary muscle movement like speaking by sending messages from the brain to the muscles.

Because there are so many different types of neurons, each with its own set of abilities, and because each person has a distinctive network of them, the minds of humans are unique. The number and types of neuronal connections in the brain determine how a person thinks, feels and behaves, making them an essential part of the body. However, due to the complex and extensive nature of these neuronal networks, our understanding of how they all fit and work together is not yet complete.

As a result, there is also a gap in our knowledge in the way in which these circuits impact behaviour. Working to fill this knowledge gap is Dr Martin Schwarz from the Institute for Experimental Epileptology and Cognition Research and the Life & Brain Center at the University of Bonn Medical Center in Germany. Dr Schwarz explains that 'a prerequisite to understanding these "brain algorithms" is to understand a circuits' hardware – in other words: who is synapsing with whom, how often and how does this concerted activity then result in a specific behaviour.'

To achieve these insights, Dr Schwarz is developing, employing and combining novel methods to determine how neuronal structure and function are linked to complex behaviour. Importantly, the success of this requires active collaboration with researchers from other disciplines, providing testimony to the power of inter- and multi-disciplinary research in driving scientific advancement.

Mapping and Manipulating Neuronal Networks with Artificial Intelligence

Dr Schwarz and his team develop and utilise clever technologies to help map and characterise the neuronal circuits involved in the continuous processing of information. One such technology is an artificial intelligence system called DeepLabStream that recognises the behaviour of mice and facilitates the labelling of the neuronal networks that are actively involved in that behaviour.

"This experimental strategy has the potential to untangle previously unknown causal relationships between brain activity and behaviour" Jens Schweihoff.

In an experimental setup using DeepLabStream, Dr Schwarz and his co-worker Jens Schweihoff placed mice in a box and allowed them to move freely. Whenever the mouse looked in a specific direction, the behaviour was detected by DeepLabStream and the involved neuronal circuits were labelled for subsequent analysis.

For this, Dr Schwarz and his team made use of a set of proteins brought into the brain, called Cal-Light, that can be used to label neurons that are active during specific durations by shining a light into the brain. In combination with DeepLabStream, the scientists could use the system to capture neuronal networks during selected behaviour. This effectively enabled them to determine which neuronal networks were active during specific behaviours in real-time.

This technique also allows researchers to manipulate neuronal activity during any type of behaviour – such as learning and memory – in a short-term manner. This is an alternative to longer-lasting and behaviour-altering manipulations such as creating brain lesions which often result in long-term changes in the brain that make it difficult to relate behaviour back to neuronal networks.

These are exciting firsts for the field and Dr Schwarz believes they have promising implications. Thanks to the DeepLabStream system's improved accuracy, Cal-Light can now be utilised effectively in a broader range of experiments and reduce variability across the board. The software is open source so scientists across the globe can contribute to improving our understanding of neurobiology.

Visualising Transplant Connections with the Rabies Virus

As part of another study, Dr Schwarz and his co-workers visualised the underlying biology of the brain in a different way. When a person sustains a brain injury or develops a brain disease, their neuronal cells may die and thus be lost. To combat this issue, researchers, including Dr Schwarz, are innovating methods of replacing these cells through transplantation. For example, in the neurodegenerative disorder Parkinson's disease, it would be extremely useful to reintroduce the neurons that produce and transmit the dopamine that is lost.

However, until now, it has been difficult to ascertain whether transplanted neurons actually reconnect with those around them to produce an operational neuronal network and restore normal function. Dr Schwarz's team, together with the team of Prof Oliver Brüstle, used an unusual source in their quest to identify a new technique for seeing implanted cells within the brain in high resolution.

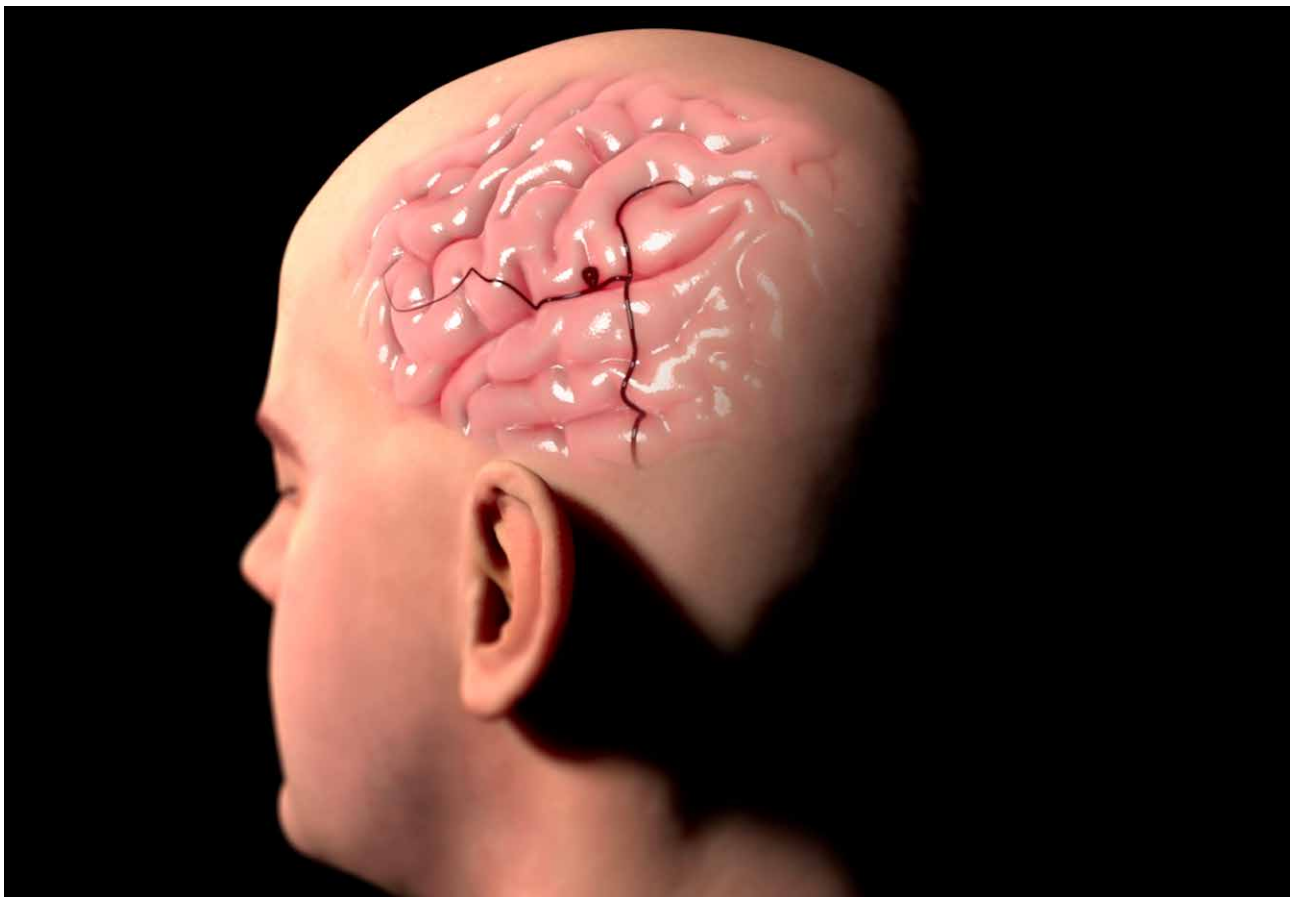
The researchers used genetically engineered rabies viruses that were no longer harmful but still travelled via their usual method of spreading backwards through the nervous system, across the synapses. Attached to each virus was a fluorescent protein that allowed the team to observe a green glow under a microscope when a transplanted neuron was connected to an existing one.

In the study, Dr Schwarz and his co-workers transplanted human stem cells into two brain regions of mice, which matured into neurons. They were then infected with the modified rabies virus and the brains underwent a procedure that made them as transparent as glass so any fluorescence can be seen throughout the entire brain. The relevant section of the brain is scanned with a light-sheet microscope to create a three-dimensional map of the connected transplant and recipient neurons.

Using this exciting technique, the team was able to reveal that their transplants were incorporated into precisely the right neuronal circuits in the target regions. This is an exciting result because it holds the potential to guide clinical studies in the future.

Light Sheet Fluorescence Expansion Microscopy

A significant portion of Dr Schwarz's work aims to improve current technologies to expand the boundaries of research. The previously mentioned Cal-Light technique needs a type of light-sheet fluorescence microscopy – one of the technologies that Dr Schwarz's group studies and improves. For example, his team together with the team of Prof Ulrich Kubitscheck developed a light-sheet fluorescence microscopy method that allowed the evaluation of large sections of brain tissue at very high resolutions previously unseen.



To achieve this, they used a technique known as tissue expansion, in which the brain sample is placed within an expandable polymer and evenly expanded to a multitude of its original size. When combined with light-sheet fluorescence microscopy, it allows even the tiny synapses between the neurons to be seen fluorescing under a microscope. For this reason, this method is known as light-sheet fluorescence expansion microscopy. These novel ways of visualising brain tissue and the neuronal circuits within will help researchers to continue to build up a map of these complex networks and link them to behaviour.

The Power of Smell

Of particular interest to Dr Schwarz is the behavioural response elicited by smells. At the bottom of the brain is a bilateral structure called the horizontal diagonal band (HDB). Although it plays an important role in the olfactory (habituation) system, the HDB does not directly receive olfactory input. Instead, it indirectly recognises when odour information is incoming and regulates odour sensation within a primary olfactory processing region called the olfactory bulb.

Dr Schwarz and his team investigated this neuronal circuit in relation to olfactory habituation – the behaviour of repetitive, irrelevant odour stimuli being ignored to allow more processing of significant odour input. His studies showed that when the HDB is inhibited, olfactory habituation and the ability to distinguish different odours are completely prevented. This demonstrates a causal link between the two regions and the research team hypothesised that the neuronal circuits that actively control olfactory habituation and discrimination reside within the HDB. Moving forward, Dr Schwarz and his co-workers aim to map these networks using the Cal-Light system and determine the functional circuits of distinct odours to dissect odour specific neuronal ensembles.

Speaking about the entirety of his work to date, Dr Schwarz explains, ‘Our long-term goal is to anatomically dissect and functionally characterise neuronal network components to better understand how erroneous neuronal wiring finally translates into specific behavioural deficits.’

Dr Schwarz has already pushed forwards our understanding of neuronal networks. He concludes by noting that future technical developments in this field will certainly require the utilisation of artificial intelligence-based systems to facilitate a more precise and less error-prone analysis of behavioural algorithms and their neuronal implementation in health and disease.

Meet the researcher

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Dr Martin Schwarz received his doctor rerum naturalium (equivalent to a PhD) from the University of Vienna in cooperation with the Max Planck Institute for Biophysical Chemistry in Göttingen in Germany. Over the course of his career, Dr Schwarz has held a range of positions at prestigious German institutions (Max-Planck Institute of Medical Research in Heidelberg) and currently serves as the Principal Investigator of the Functional Neuroconnectomics group at the Institute of Experimental Epileptology and Cognition Research and is also affiliated with the Life & Brain Center at the University of Bonn Medical Center in Germany. His work focuses on the development of cutting-edge methods to facilitate the identification and characterisation of neuronal networks within the brain to better understand the correlation between neuronal activity and behaviour.

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FUNDING

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UNDERSTANDING THE MALLEABILITY OF EMOTIONAL MEMORIES

Dr Vanessa van Ast from the University of Amsterdam in the Netherlands is driving forward understanding of how and why our emotional memories change over time. As well as elucidating how our memories of specific events and emotions influence behaviour, her most recent work is unveiling the impact that different contexts may have on the storage and recall of memories.

How Emotional Memories Can Be Altered

We have all doubted our memory on occasion, especially when trying to recall specific details of a past event. But when it comes to reflecting on how we felt during that event, we are likely to be much more confident about remembering the associated emotions correctly. Nonetheless, our emotional episodic memories, which link our feelings to specific events, can change over time. Dr Vanessa van Ast from the University of Amsterdam has spent much of her career investigating how and why these alterations occur. This work is particularly important, not least because replaying or reconstructing emotional events can lead to mental health issues, including anxiety and depression. Conversely, successful psychotherapy outcomes also require retrieval-induced reductions in memory emotionality.

Eliciting Psychophysical Responses While Remembering

Our behaviour is often guided by past experiences, and specific episodic memories of the past can be used as a tool to harness such learning and direct our future actions. These memories are particularly powerful in dictating the extent to which we wish to repeat or avoid certain behaviours. For example, if we have experienced joy in the past, we are likely to be motivated to repeat the associated behaviour or event again. Similarly, if we have felt sadness or fear, this would likely influence us to avoid similar situations in future.

It remains relatively uncertain whether we elicit appropriate psychophysiological responses when revisiting emotional episodic memories. Psychophysiological responses occur when there is an interrelationship between the mind and the body, and they can help us to prepare our behaviour for an upcoming situation. One example is that of heart rate increases when we experience fear – this response helps us respond quickly to any potential threats. Thus, psychophysiological responses correspond to elicited emotion, and stronger elicited emotions during memory retrieval may serve as more powerful drives in motivating subsequent behaviour.

For these reasons, Dr van Ast worked with colleagues and PhD student Sascha Duken to determine whether reliving emotional episodic memories

is associated with psychophysiological responding. In one study, young adults were shown movie clips classified as positive, negative, or neutral. One day later, they were asked to recall the clips and revisit these emotions. During the recollection, psychophysiological responses were measured through the facial responses of smiling or frowning, showing that recalling the positive movie clips led to more smiling than the neutral or negative movies. Conversely, study participants were more likely to frown when recollecting the negative rather than the positive movie clips (although this did not differ in comparison to the neutral clips).

These observations confirm that recollection of emotional episodes can indeed elicit corresponding emotional psychophysiological responses. The research team had further theorised that the emotional intensity of the original experience would affect the level of physiological response. For example, they predicted that if people had experienced a movie clip as very positive, they would smile more when remembering the clip in comparison to clips that were experienced as somewhat less positive. The results were unable to confirm this theory, but rather, suggest that memory retrieval is not a direct representation of the prior experience, suggesting that memory is, at least partly, reconstructive.

Dr van Ast nonetheless emphasises the importance of using psychophysiological measures when exploring emotional episodic memory: previous studies have relied heavily on self-report as the sole measure of emotional responses, but they are prone to experimental biases such as expectancy and demand effects and require conscious awareness. Psychophysiological measures, in contrast, index more automatic and unconscious emotional responses. For this reason, the present study paves the way to further investigate not only how emotional memories are expressed, but also how they may be changed in health and disease.

Understanding the Causes of Memory Distortions in Depression

A number of factors can interfere with our memories. Many of these are external and driven by cues relating to when our memories are formed or retrieved. However, in some instances, the content of autobiographical memories can become distorted. Our autobiographical memories provide a mental timeline of key events in our lives, and we relive moments of joy and sadness that can guide our future behaviours. In people suffering from dysphoria (more commonly known as low mood), memories can become distorted such that situations that were positive or neutral become associated with negative feelings. This can lead to altered mood, increased stress, and worsening of depression.

Dr van Ast and her research team including Sascha Duken, are currently working to understand the cause of these memory distortions. Currently, two main opposing theories attempt to explain memory impairments in dysphoria. The first is that of overgeneral memory bias, which emphasises individual differences in accessing specific episodic details of positive and negative autobiographical memories. This theory states that people suffering from dysphoria tend to recall general memories that lack episodic detail and that they struggle to bring to mind specific times and events. The negative bias theory offers an alternative perspective and suggests that people who suffer from dysphoria have negative world views that bias their perception and interpretation of information. Therefore, they are more likely to retrieve negative information.

Both theories propose that there is a critical link between retrieved memories and emotional responses. Dr van Ast and her colleagues therefore also incorporate the assessment of emotional responses into their experimental protocol (such as using facial responses like in the previous study), as few previous studies to date have focused on this. This study protocol is accepted in principle as a registered report, which is the gold standard in open science for minimising publication research biases in hypothesis-driven research – which are unfortunately all too common. This work will undoubtedly provide valuable insight into emotional memory that may ultimately, support the development of therapeutic interventions for disorders such as clinical depression.

How Threat Learning May Affect Relational Memory

To enhance our learning experiences and predict future behaviours, our memories update regularly as new information is added to help us navigate the complex world we live in. This enhances our relational memory, which involves combining pieces of information from different events, to help us function in new situations. Although relational memory has been the subject of much recent research, often in efforts to unpick its underlying neural mechanisms, Dr van Ast and her colleagues noted that few studies have looked at emotional memories.

To address this gap, one of Dr van Ast's most recent studies focused on understanding the impact of emotional experiences – specifically, those relating to threatening events – on the subsequent ability to make inferences among these memories. Dr van Ast's PhD student Olivier de Vries and the research team hypothesised

that relational memories would be different in strength if they involved a memory of a threatening event. From an evolutionary point of view, it would be adaptive if such relational memories were strengthened because of their importance to survival. However, previous research had shown that emotion often weakens memory for associations within distinct events, and the same could be true for associations across several events.

Dr van Ast and the team found that when information from a previous threatening event needs to be recombined with another neutral memory, it actually weakens this process, whereas the neutral memory is strengthened. She proposes that our brains may have a mechanism that prevents the integration of negatively charged emotional events with unrelated, pre-existing memories. Ultimately, this may suggest that the brains of healthy individuals prevent the linkage of safe memories to threatening memories as a safeguard against overgeneralised fear and anxiety.

The Impact of Contextual Similarity

Dr van Ast and her colleagues noted that little research has focused on understanding the impact of spatial context on memory malleability. They believe that environmental conditions in which events occur can influence how similar memories are stored and recalled. However, there is an ongoing debate around whether memories of similar events can strengthen each other or conversely, lead to interference.

Over the last century, the most widely accepted theory states that creating memories in a similar spatial context at separate times can cause interference, meaning that memories might be recalled incorrectly or even be forgotten. More recently, contemporary memory integration theory suggests that the opposite may occur. Dr van Ast, her postdoctoral researcher Wouter Cox, and their colleagues have recently undertaken research aiming to critically test the classic theory and see whether different environmental conditions may in fact be more likely to provide interference, than when there is consistency in a person's surroundings.

The research team undertook a series of experiments that manipulated and controlled environments when specific events occurred. They found that memories strengthen each other's retrieval when they happen within the same spatial context. Conversely, they found that recall is more likely to be impaired when events occur across different environments. These observations are in line with integration theory.

However, when contextual cues are provided during recall, these patterns change and, in some cases, even seem to reverse. This finding reconciles classic interference theory and integration theory. Dr van Ast believes these results highlight the importance of spatial context during episodic memory encoding and retrieval, and this insight may help develop

strategies that optimise memory retrieval, for instance, in educational settings. More generally, contrary to a more common conception that memories are fixed in how they function (like a file drawer), the findings underscore the malleability of our episodic memories.

The Need for a New Framework

Further deciphering the conditions that initiate the integration of emotional memories can also have far-reaching consequences for understanding why our emotional memories are malleable and can modify over time. Even though many psychologists agree that it is upon memory reactivation that our existing memories become changed, most earlier studies (like the previous one) have focused on non-emotional and simple stimuli, therefore so far, no framework exists to explain how integration affects evoked emotions of real-life and complex memories.

Such a framework could hold great clinical relevance for emotional disorders caused by dysfunctional memories (e.g., posttraumatic stress disorder), since successful psychotherapy outcomes may require memory integration. However, even though the insights regarding the role of contexts are an important starting point, why memory retrieval does not always lead to integration and the consequences of these processes for evoked emotions, are unknown. This undermines the effectiveness of psychotherapies that rely upon retrieval-induced integration.

In the future, Dr van Ast and her colleagues are keen to uncover more about the mechanisms behind the process of integration, its consequences for evoked emotions, and ultimately, mental health disorders that are rooted in dysfunctional emotional memories. 'It is important to further develop and employ experimental approaches in the lab that are capable of indexing (mal)adaptive properties of personally relevant and real-life memories, since this is essential to bridge core insights from fundamental research and application in clinical science ("bench to bedside"). Such an approach will pave the way to employ this knowledge in effective interventions for emotional memory disorders', concludes Dr van Ast.

Meet the researcher

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Dr Vanessa van Ast is currently an Assistant Professor in the Clinical Psychology Department at the University of Amsterdam in the Netherlands, where she also completed her PhD. As part of her postdoctoral training at the Donders Institute for Brain, Cognition and Behaviour (at Radboud University Nijmegen), Dr van Ast helped to design and set up a large ongoing prospective study investigating the neurobiology of human defensive reactions and their role in the development of posttraumatic stress in police recruits. Since a well-functioning memory system is at the core of adaptive behaviour, Dr van Ast's recent research focuses on the neuroendocrinological, physiological and psycho-emotional mechanisms of memory formation and change. Debilitating emotional disorders such as posttraumatic stress disorder are thought to originate from dysfunctional memories eliciting disproportionate emotional responses. She has therefore specifically focused on explaining clinical phenomena such as overgeneralised fearful memories and intrusive memories. Dr van Ast utilises a wide array of methods and techniques in her research, including behavioural experimentation, psychophysiological assessment and advanced statistics.

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FUNDING

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REGULATION AND LOSS OF NEUROPROTECTION IN VIRAL AND AUTOIMMUNE DISEASES OF THE CENTRAL NERVOUS SYSTEM

Viral and autoimmune diseases of the central nervous system (CNS) are often characterised by the onset of inflammation leading to neurological dysfunction, including impairment to memory and other cognitive domains. **Dr Robyn S. Klein** at the Washington University School of Medicine in St. Louis, leads a team that specialises in neuroinflammatory diseases of the CNS. In recent years, they have investigated the regulation of blood-brain barrier permeability in autoimmune diseases and viral infections with pathogens such as the West Nile virus.

Blood-brain Barrier Permeability in Autoimmune and Viral Diseases

Dr Robyn S. Klein is an internationally recognised expert in the field of inflammatory diseases of the central nervous system (CNS). In recent years, her laboratory has been focusing on the molecular mechanisms behind inflammation and how they regulate blood-brain barrier (BBB) permeability in viral and autoimmune diseases.

Dr Klein's team has developed in vitro (outside the living organism) models of the BBB to investigate what mechanisms allow pathogens like the West Nile Virus (WNV) and white blood cells released during autoimmune disease to gain access to the CNS, causing encephalitis. The team has also used *in vivo* (within the living animal) models of autoimmune and WNV encephalitis to identify the molecular basis of persistent inflammation.

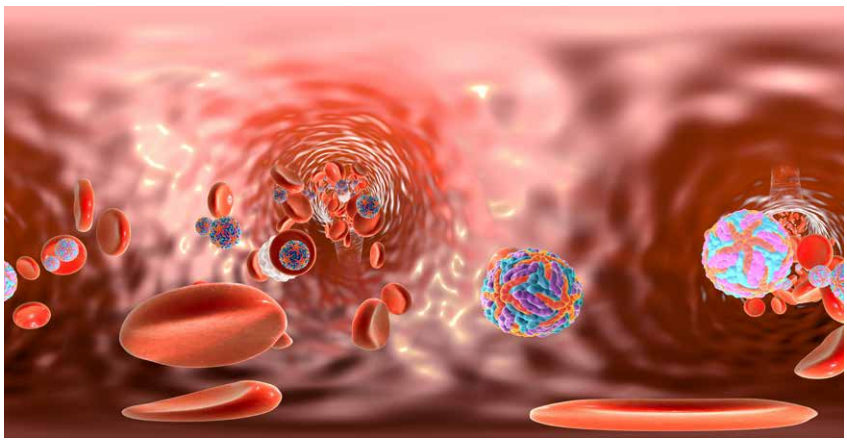
The BBB is composed of microvascular endothelial cells joined by tight junctions, the latter consisting of regulatory proteins that control cellular permeability. Viruses may cross the BBB through several routes, including direct infection of the microvascular endothelial cells of the brain and infiltration of infected peripheral white blood cells. Viral trafficking across the BBB is also possible as a result of increased permeability of the endothelium, which in turn can be a consequence of viral attack mechanisms or the overexpression of inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) and interleukin-1 (IL-1).

The Link Between Cytokine Signalling and Neuroinflammation

In a study published in 2014, Dr Klein and her collaborators highlighted how WNV, a mosquito-borne pathogen that causes viral encephalitis, triggers the expression of TNF-α and IL-1 to cause a cascade of reactions that limit

viral replication, facilitate antigen presentation for recognition by the immune cells, and direct the trafficking of antiviral white blood cells. In the same study, they showed that despite limiting viral replication in the CNS, TNF-α and IL-1 signalling can also contribute to neuropathology by directly enhancing mice BBB permeability *in vivo* (that is, within the living organism) and indirectly facilitating the trafficking of WNV across the endothelium.

In contrast with the BBB disruption caused by the above-mentioned cytokines, the 2014 study showed that a different cytokine, known as type I interferon (IFN), promoted and stabilised BBB function instead. This



Zika virus in blood.

finding was supported by experiments *in vivo*, since mice with impaired type I IFN responses exhibited enhanced BBB permeability. In the same study, the researchers demonstrated that promoting Type-I IFN signalling in vitro resulted in an enhancement of the barrier function over baseline conditions rescuing Il-1 and TNF- α-mediated barrier dysregulation, suggesting that CNS Type-I IFN may be able to reverse BBB permeability. These findings were followed by the discoveries that type III interferon and the TAM receptor Merk can each synergise with type I interferon in maintaining BBB integrity during neurotropic viral infections.

Synaptic Loss During NWV Infection: The Role of Astrocytes

Although inflammation is necessary for CNS infection clearance, immune molecules can trigger a cascade of events that lead to neurological dysfunction. Patients surviving WNV encephalitis show high rates of memory impairment and cognitive impairment. More specifically, detriments to neurons in the hippocampus, which is essential for visual and spatial (visuospatial) processing – the ability to identify visual and spatial relationships among objects – and visuospatial memory, are commonly found.

In 2016, Dr Klein and her colleagues published a study demonstrating their development of an effective model of WNV-induced spatial memory impairment. Critically, this allowed them to identify that the virus induces elimination of presynaptic terminals without loss of hippocampal neurons or volume, pointing to this as a potential mechanism underlying such deficits in patients recovering from WNV infection.

The Klein laboratory later published a study in 2017 confirming that IL-1 alters the proliferation and differentiation of neural progenitor cells, which cause an increase in the production of astrocytes and a decrease in the production of neurons. Astrocytes are star-shaped cells that surround and outnumber neurons in the CNS. They have an important role in the maintenance of the BBB, bridging the neural tissue to the vascular tissue and secreting factors that regulate the inflammatory cascade during infection.

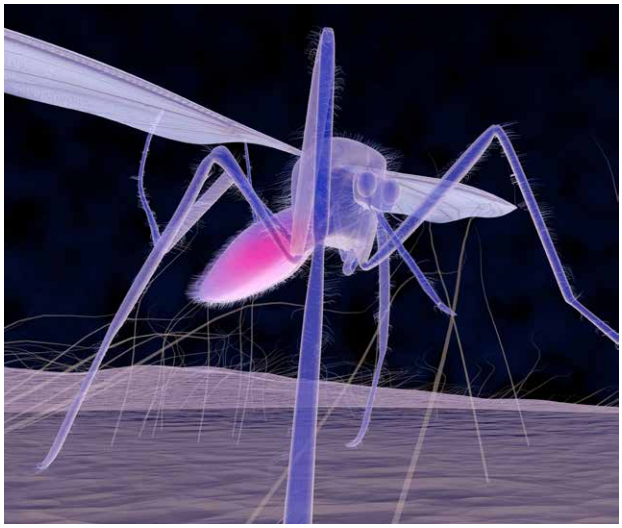
It is plausible that pro-inflammatory astrocytes may contribute to the start of a vicious circle following infection with NWV, by triggering the recruitment of IL-1, which in turn continues to inhibit neurogenesis, causing the memory impairment and loss of hippocampal synapses observed during WNV neuroinvasive disease. This was confirmed by *in vivo* results showing that, following infection with WNV, genetically modified mice lacking the ability to express IL-1 exhibit a significant recovery in the number of synapses and in their cognitive abilities in comparison with wild type animals as found in nature.

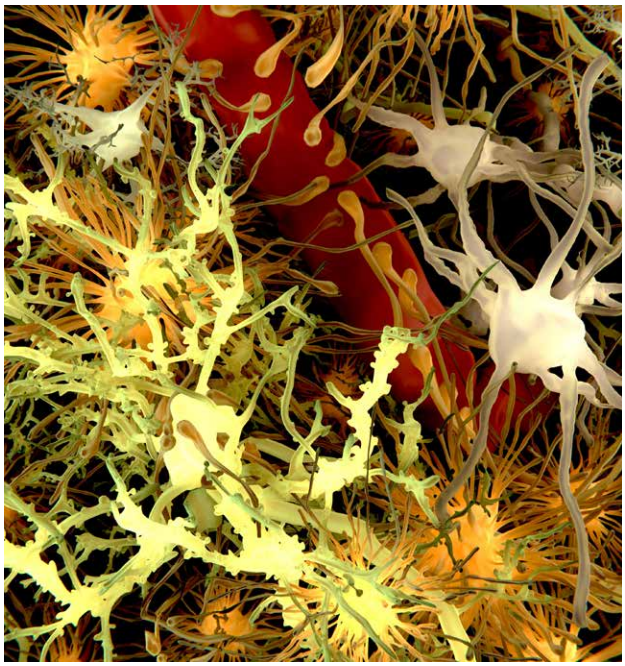
Interferon-γ Regulates Neuron Loss During WNV and Zika Virus Infection

The recently emerged flavivirus Zika Virus (ZIKV) can cause several neurological complications in adults, including encephalopathy, meningomyelitis and encephalomyelitis, which can affect memory and cognition with unknown long-term outcomes. Recovery from infection with flaviviruses is associated with reduced learning abilities, suggesting that there might be an underlying mechanism of cognitive impairment that correlates with a broader category of memory disorders.

Activation of microglia and astrocytes in the brain during acute infection promotes the recruitment of antiviral T cells, via a cytokine-mediated mechanism. T-cell cytokines such as IFN-γ (not to be confused with the Type-I IFN discussed above) may also influence microglial biology, as observed in brain tissues of patients with multiple sclerosis (MS), Parkinson's disease and Alzheimer's disease.

Dr Klein and her team reported in a paper published in 2019 that antiviral T cells persist within the hippocampus after recovery from flaviviral infection in two *in vivo* animal models of WNV and ZIKV. The presence of T cells is associated with the development of specific learning defects through microglial activation and loss of synapses formation. The two pathogens, however, caused slightly different outcomes; while WNV was responsible for the elimination of presynaptic termini, ZIKV promoted the loss of neuronal nuclei and postsynaptic termini. As further confirmation of this, animals deficient in CD8+ T cells or IFN-γ signalling in microglia demonstrated protection against synapse elimination following WNV infection and decreased neuronal apoptosis with synapse recovery following ZIKV infection.





Three types of brain cells. Red: astrocytes, green: pyramidal neurons, blue: microglia cells

Type I-Interferon Protects the BBB in Viral Equine Encephalitis

Venezuelan and western equine encephalitis viruses (VEEV and WEEV, respectively) and eastern equine encephalitis virus (EEEV) are emerging infectious diseases in the Americas, causing several major outbreaks in the human and horse population during the past few decades. Shortly after infection, these viruses can infect the CNS, resulting in severe long-term neurological deficits or death.

Following peripheral infection with alphaviruses, VEEV and WEEV are trafficked at the BBB into the CNS. The transmigration occurs via a caveolin-1 (Cav-1)-mediated mechanism across an intact BBB, which can be halted by Type I-IFN.

In vivo examination of early viral entry in Cav-1-deficient mice confirmed the presence of significantly lower VEEV and WEEV viral burdens in the brain in comparison to similarly infected wild-type animals, confirming that Cav-1 signalling allows alphaviruses to enter the CNS and that type-I IFN limits this process at the BBB.

Mechanisms of Neuroinflammation in Multiple Sclerosis

The autoimmune disease MS and one of its animal models, experimental autoimmune encephalomyelitis (EAE) are characterised by a pronounced T cell autoimmune response against the myelin sheath that protects the nerves in the CNS, leading to motor and sensory function loss. Dr Klein and her team, following previous reports that IL-1, TNFα and other cytokines have critical roles in MS and EAE (including the upregulation of vascular adhesion molecules at the BBB), decided to delve deeper into understanding the molecular mechanisms that facilitate immune cell entry into the CNS during neuroinflammation.

The team investigated cytokine signalling in different regions of the CNS, following induction of EAE in mice, and reported the findings of the study in a paper published in 2020. The study identified a 'regionality' in the way cytokines direct T cells towards different parts of the CNS.

Using *in vivo* models of spinal cord versus brain stem trafficking of myelin-specific T cells, the researchers confirmed that different cytokines differentially regulate astrocyte expression of the adhesion molecules VCAM-1 and CXCR7 in these locations. While the IN-17 cytokine preferentially upregulated VCAM-1 on brain stem astrocytes, IFNγ, modulated the expression of CXCR7 chemokine, facilitating T cell entry into the spinal cord. In the absence of IFNγ, brain stem astrocytes upregulated VCAM-1 expression and astrocyte-specific deletion of VCAM-1 reduced the severity of EAE, suggesting IFNγ may partially limit T cell entry into the brain stem during EAE by inhibiting the expression of VCAM-1 on astrocytes.

Next Steps

It is clear that astrocytes play important roles in neurological diseases at multiple stages of pathogenesis and repair. Astrocytes also critically impact recovery in several models of neurodegeneration, and fragmented mitochondria released by damaged neurons may be removed by neighbouring astrocytes.

There is emerging evidence to suggest that altered astrocyte function may contribute to Parkinson's disease, but the underlying mechanisms have not been fully elucidated. The Klein laboratory is continuing to investigate the roles played by cytokine signalling and astrocytes in CNS autoimmunity, to provide new insights for the development of CNS-targeted therapies.

Meet the researcher

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Dr Robyn Sue Klein obtained her MD and PhD in Neuroscience in 1993 from the Albert Einstein College of Medicine, New York. After completing clinical training in Internal Medicine, Infectious Diseases and a postdoctoral fellowship in Immunology at Harvard Medical School, she joined Washington University School of Medicine, St. Louis, where she is currently the Robert E. and Louise F. Dunn Professor of Medical Sciences, Director of the Center for Neuroimmunology and Neuroinfectious Diseases, and Professor of Medicine, Pathology & Immunology, and Neurosciences. She has received numerous awards, the most recent being the Distinguished Educator Award, Washington University School of Medicine, St. Louis, and Chair of the NIH Study Section, Clinical Neuroimmunology & Brain Tumors. Dr Klein, who has co-authored more than 100 peer-reviewed publications, was in 2021 appointed as Co-Editor-in-Chief for the prestigious Journal of Neuroimmunology.

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THE LINKS BETWEEN STRESS, SIGNALLING AND EXCESSIVE ALCOHOL CONSUMPTION

While alcohol is often consumed to help us relieve stress and relax, excessive consumption can negatively impact the way that our brains process and cope with stress, leading to further difficulties. **Dr Lara Hwa** from the Department of Psychology and Neuroscience at Baylor University is investigating the link between external stressors and stress signalling in the brain to understand how these processes govern excessive drinking.

The Link Between Alcohol and Stress

Alcohol abuse has a tremendous toll on society, with impacts ranging from increased healthcare costs and crime to losses in workplace productivity. With respect to healthcare, long-term drinking has been shown to have several detrimental effects on our health, including negatively affecting systems in our brain that help us to process and cope with stress. Alcohol consumption and stress have long been linked and it is thought that we drink alcohol to relieve stress and relax. Yet, despite this long-held theory, we still do not fully understand the link between the nervous system and this behaviour.

Stress has been shown to drive social drinking. It can also be responsible for relapse in people with alcohol use disorders. Chronic alcohol drinking also causes stress by impacting the stress systems in the brain which can, in turn, govern excessive drinking. One theory is that the progression from high alcohol intake to alcohol dependence may be driven by repeated cycles of heavy drinking followed by deprivation. This area of research is particularly important for understanding the link between psychological mechanisms and the underlying neurobiology of relapse behaviour. By understanding this link, researchers may be able to identify therapeutic targets that will improve the treatment of addiction.

Dr Lara Hwa at Baylor University uses a variety of experimental techniques to investigate how external stressors and endogenous stress signalling in the brain govern excessive drinking. This important research is elucidating how stress and the availability of alcohol interact with brain mechanisms, and will help us to design better treatment plans for alcohol use disorders.

Episodic Drinking Increases Alcohol Consumption

Excessive alcohol use and binge drinking have been shown to cause an increased risk for a variety of health problems including injuries, violence, liver diseases and cancer. As such, research has focused on the links between binge drinking and increased alcohol consumption. Previous research has shown that intermittent, or limited, access to alcohol ultimately leads to an increase in consumption.

In a study published in 2011, Dr Hwa and her colleagues explored escalated drinking behaviour in adult C57BL/6J mice given intermittent access to alcohol. C57BL/6J mice are a laboratory strain of mice that are widely used in research as models of human disease. These mice also exhibit high alcohol intake compared to other strains, making them conceivable models on which to examine how intermittent access to alcohol can affect alcohol consumption. Dr Hwa found that mice that were given intermittent access to alcohol drank more in a two-hour 'binge' period and across the 24-hour day than those that were given consistent, continuous access to alcohol, even when fresh water was available.

In addition to looking at intermittent access, another avenue that has been explored is the alcohol deprivation

'The connection between stress and alcohol use is highly complex. On one hand, there is the idea of having a drink to "steady the nerves". On the other hand, different responses to stress often accompany heavy drinking, as seen in alcohol use disorder. We are continuing to investigate whether stress causes excessive drinking, or vice versa.'

effect, whereby periods of alcohol access are alternated with periods of deprivation in weekly cycles. Dr Hwa showed that some mice that were given intermittent access to alcohol demonstrated symptoms of withdrawal, indicating a potential parallel to the withdrawal symptoms suffered by human patients with alcoholism.

Reducing Intermittent Alcohol Consumption

As research in this area develops, several pharmacotherapies have been explored that aim to target specific areas of the brain to reduce intermittent alcohol drinking. For example, naltrexone is an opioid antagonist that works to block opioids from binding to receptors in the brain. This drug has also been shown to reduce alcohol drinking both during intermittent and continuous access by up to 20%.

The discovery of compounds like naltrexone and the identification of their beneficial effects suggest that certain receptors in the brain play a role in excessive alcohol consumption and that these receptors are good targets for therapeutic interventions. In addition, it has been shown that using a combination of these therapies could be useful in further reducing intermittent alcohol drinking.

Dr Hwa built on this previous research to investigate the use of two such therapeutic interventions, naltrexone and a corticotropin-releasing factor type-1 receptor (CRF-R1) antagonist, to see if they act independently or dependently in a certain area of the brain known as the dorsal raphe nucleus to reduce intermittent alcohol drinking in mice. The results demonstrated that both therapeutic interventions reduced intermittent alcohol consumption in mice when administered independently and when given together. However, the two compounds did not additively suppress alcohol drinking, suggesting that both act via a common mechanism in reducing alcohol intake.

The CRF-R1 is a binding site for corticotropin-releasing factor (CRF) which is a neuropeptide involved in the endocrine stress response within the body. CRF initiates this stress response which results in a whole suite of reactions that lead to an increase in stress hormones, such as corticosterone which help to combat stress through initiating several changes within the body. Not only has CRF been shown to be involved in the stress response, but it has also been linked to heavy alcohol drinking. Previous studies have demonstrated that social defeat and subordination stress in mice and monkeys can lead to increased alcohol consumption when compared to non-stressed or more highly ranking individuals.

In a second study also published in 2016, Dr Hwa and colleagues investigated the link between social stress and increased alcohol consumption. This study explored the mechanistic link between social stress and drinking and the role that CRF receptors – namely CRF-R1 – play in this relationship, again using mice as a model.

The researchers demonstrated that stress increased voluntary alcohol drinking in mice that had a history of social defeat. Social defeat is a type of social stress that is chronic and is characterised by hostile interactions. This type of stress is identifiable in both humans and animals, and is capable of causing significant changes in behaviour, brain functioning, neurotransmitter and hormone levels

as well as health. In this study, mice that experienced brief episodes of defeat stress for ten days consumed more alcohol and preferred more alcohol than non-stressed mice.

The research team also showed that treating mice with a CRF-R1 antagonist which blocked CRF from binding to its receptor was an effective treatment for reducing alcohol intake in both stressed and non-stressed mice that were given intermittent access to alcohol. Interestingly, this treatment did not reduce intake in mice given continuous access to alcohol. Together, these results show that both intermittent alcohol availability and the stress experienced can influence the brain stress systems. CRF-R1 may be a neural target that becomes adapted in long-term heavy drinkers, but not as much in social drinkers. Thus, these experiments and others imply that administering a CRF-R1 antagonist may be a viable treatment to help balance the dysregulation of stress and reward in alcohol use disorders.

Long-term Links Between Alcohol and Stress

The next step in looking at the link between alcohol consumption and stress signalling is to understand how drinking alcohol impacts the ability of the brain to cope with stress in the long term. Maladaptive responses to stress have long been associated with alcohol consumption and are the hallmark of alcohol use disorders. Alcohol has been shown to alter the hormonal balance as well as the way the body perceives and responds to stress but only limited research has looked at the underlying mechanisms behind this.

The neuropeptide prodynorphin (Pdyn) and its receptor is a molecule that is linked to another stress system that operates in the brain which has been studied with relation to mood and alcohol disorders. To look at the link between alcohol and abnormal stress responses, a recent study by Dr Hwa published in 2020 explored whether stress signalling in the brain linked to Pdyn regulates altered stress responses after long-term alcohol drinking. To do this, the researchers used mice that had been subjected to six weeks of intermittent alcohol access and exposed them to a stressor, which in this case was the scent of a predator, an odour isolated from fox faeces.

The results demonstrated that the signalling initiated by Pdyn and its receptor in the brain disrupts stress-related behavioural responses following heavy alcohol drinking. It appears that this is because stressed mice with a history of alcohol drinking had increased activity in a part of their brain known as the corticolimbic system, specifically from the prefrontal cortex to the bed nucleus of the stria terminalis. This system, among others, is responsible for processing a broad range of behavioural and cognitive functions, including decision-making and emotional regulation.

These important results provide further insight into the links between these systems in the brain and how neuropeptide signalling can be altered by stress and alcohol consumption. As Dr Hwa explains, 'The connection between stress and alcohol use is highly complex. On one hand, there is the idea of having a drink to "steady the nerves". On the other hand, different responses to stress often accompany heavy drinking, as seen in alcohol use disorder. We are continuing to investigate whether stress causes excessive drinking, or vice versa.'

Developing Future Therapeutic Interventions

Dr Hwa's research highlights the links between stress, coping mechanisms and excessive alcohol consumption. Her work has suggested that responses to stress both precede heavy alcohol drinking as well as become changed as a consequence of it. Using preclinical models, this research has identified several targets for therapeutic interventions that could help reduce alcohol consumption as well as enhancing the stress coping mechanisms in people with alcohol use disorders.

Meet the researcher

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In 2015, Dr Lara Hwa received her PhD in Experimental Psychology from Tufts University in the USA. Following this, she completed her postdoctoral fellowship at the Bowles Center for Alcohol Studies at the University of North Carolina School of Medicine. In January 2021, Dr Hwa moved to Baylor University's Department of Psychology and Neuroscience where she was appointed as an Assistant Professor. Dr Hwa's research focuses on the cells and circuits underlying how stress interacts with long-term alcohol drinking, aiming to answer fundamental questions in behavioural neuroscience. In addition to her multiple research accolades, Dr Hwa has also been formally recognised for her outstanding mentorship of young researchers.

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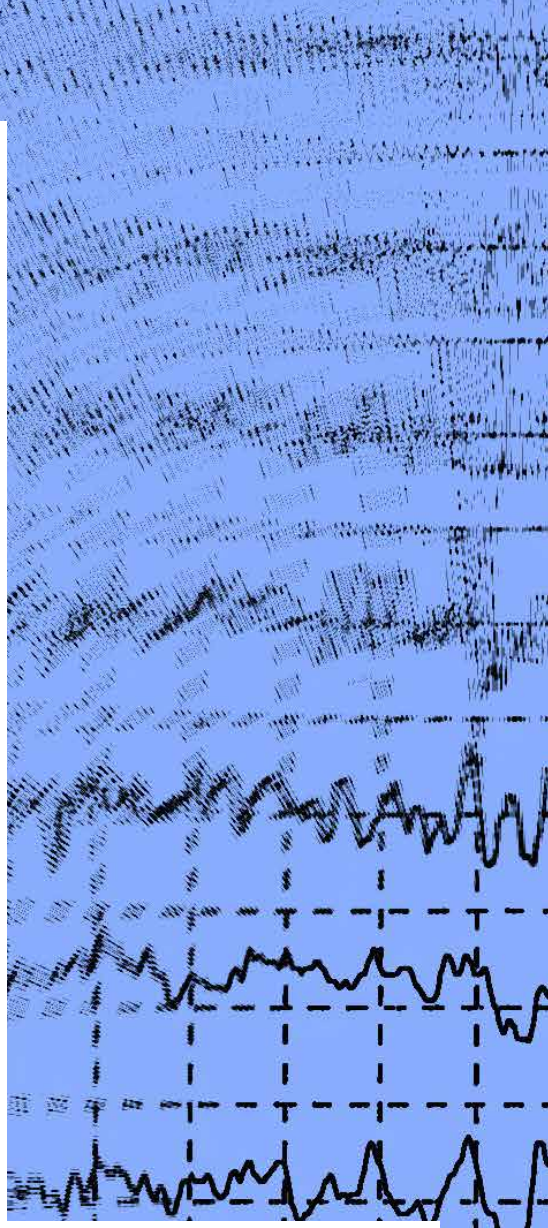
MAKING MAGNETIC RESONANCE IMAGING EXAMINATIONS SAFER FOR PATIENTS WITH DEEP BRAIN STIMULATION IMPLANTS

Deep brain stimulation (DBS) is an increasingly popular treatment for abnormal brain circuits found in epilepsy, obsessive-compulsive disorder, Parkinson's disease and other conditions. Magnetic resonance imaging (MRI) examinations are part of the medical workup to implant DBS devices correctly, and can be used after the procedure to assess potential complications, provide long-term follow-up or evaluate new disease. At present, however, MRI of patients with DBS implants may introduce a significant risk of heating brain tissue. **Dr Simon Graham**, at the Sunnybrook Research Institute in Toronto, investigates how MRI can be optimised to keep DBS patients safe.



Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is used to create images of the soft tissues inside the body. Detailed MRI examinations are now an essential tool in medicine, assisting in the diagnosis and treatment of many ailments such as strokes, infections and tumours.

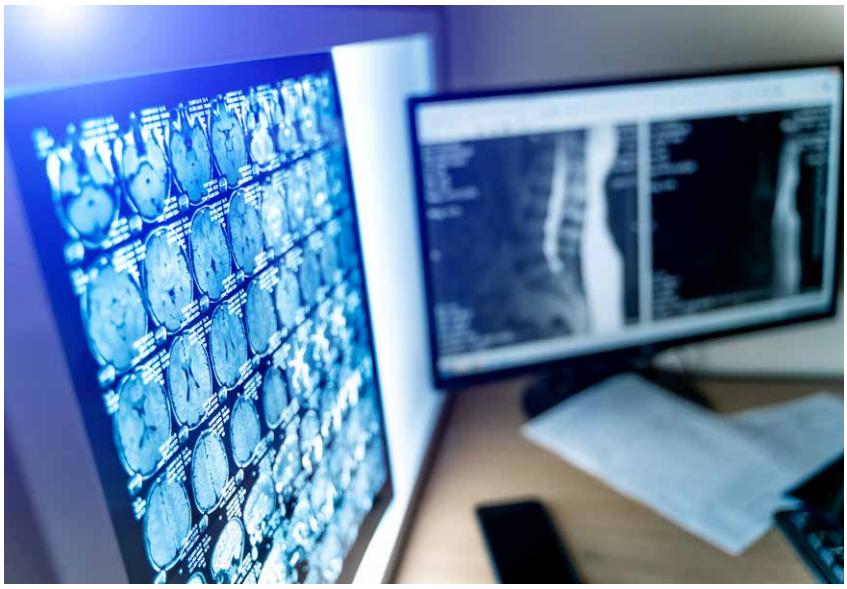
Powerful magnets are the main component of MRI machines. When a patient lies inside, the magnet pulls on the magnetic fields of protons in all of the hydrogen atoms in the patient, forcing the protons towards magnetic alignment. This alignment is then knocked out of order by transmitting short bursts of radiofrequency (RF) electromagnetic waves into the patient. Once the RF transmission stops, the protons return to their previous alignment and release RF signals which are then read by the MRI machine.

During imaging, other magnetic fields that vary linearly with position, and aspects of the RF transmission are manipulated so that the received MRI signals are 'spatially encoded'. Physicians are then able to distinguish between normal tissues, and between normal and diseased tissues, based on MRI signal location and signal strength.

The MRI signals are primarily received by water and fat molecules and have different signal strengths in different tissues for many reasons. Variations in tissue water and fat content play a role; another contributing factor is that protons realign at varying speeds according to the different molecular and microstructural environments in each type of tissue. Because MRI signals have diverse properties, many different types of images can be created for various medical purposes.

Functional Magnetic Resonance Imaging

As just mentioned, there are many different imaging techniques in the 'MRI portfolio'. Beyond images of anatomy, a technique called functional magnetic resonance imaging (fMRI) looks into the function of neural tissue in the brain and spinal cord. When a region of the brain becomes active during mental processing, increased blood flow, blood volume and blood oxygenation are supplied to the region as part of supporting the energy demands of



cells. Most fMRI exploits this effect by measuring blood oxygen level-dependent (BOLD) signals, to produce colour-coded images that depict the strength of activity across the brain.

When performed during an appropriate behavioural task (or increasingly, when patients are at rest and thinking of nothing in particular) fMRI can be used to determine sites of activation supporting brain functions such as memory, movement and language. These data can then be taken into account before brain surgery, for example, to give a surgeon guidance on the areas that should be avoided to minimise behavioural side effects.

Deep Brain Stimulation

Implanting electrodes into the brain may seem extreme, but in fact, it is an increasingly common approach when other treatment options, like drugs, do not work or become ineffective for various diseases. Deep brain stimulation (DBS) involves neurosurgery to implant a thin wire lead tipped with electrodes into the brain. Alternatively, two wires can be inserted to target similar structures on each side of the brain.

To ensure that the electrodes are placed correctly and precisely, patients undergo MRI before their operation so that the surgeons can see the patient's specific brain structure and identify the treatment targets. Incredibly, patients are usually not fully sedated when the DBS device is implanted. A local anaesthetic may be given to block pain during the opening of the skin and drilling through the skull. However, the brain contains no nerve endings and pain cannot be felt there, enabling most patients to remain awake so that the surgeon can monitor their behavioural signs during insertion of the lead and electrodes. Afterwards, the remaining extent of the lead (or leads) is then run under the skin and attached to a battery-powered pulse generator implanted near the collarbone.

After successful surgery, electrical impulses are delivered to the brain to regulate the abnormal neural activity resulting from disease. As the pulse generator is adjusted by remote control, the patient and their doctor can manage and program it easily from outside the body. The amount and timings of stimulation depend on the patient's condition – DBS may only be necessary during the day, for example, or it may be constant.

A person may try DBS for very many reasons. Parkinson's disease, epilepsy, obsessive-compulsive disorder, and a common movement disorder called essential tremor are some of the most common conditions that are treated by DBS, but its usefulness for other diseases is being investigated too. For example, DBS holds early promise for Tourette syndrome, addiction, major depression and multiple sclerosis.

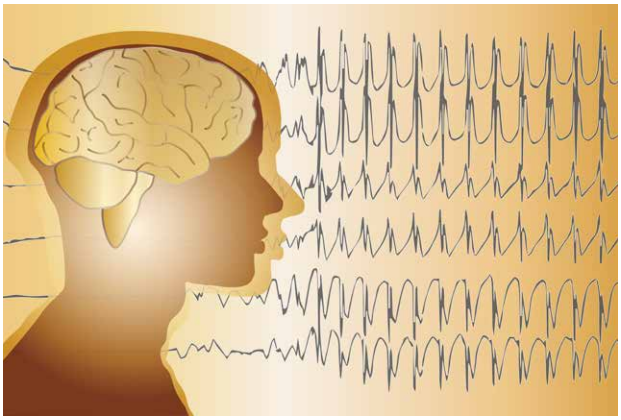
However, there is a specific issue that arises when DBS and MRI are combined, and this is the focus of Dr Simon Graham's research at the Sunnybrook Research Institute in Toronto.

The Risk of Magnetic Resonance Imaging after Deep Brain Stimulation

An MRI examination is completed before a DBS operation but is also useful afterwards. This is because any side effects of the treatment can be examined, the implant location can be checked, and a patient's health can be monitored over the long term. Functional MRI may also be useful to assess the patient's brain activity and see whether it becomes more normal, or as part of research trials to investigate more about how DBS treatments work, toward optimising DBS device technology. Together with fMRI, another technique called diffusion weighted imaging may be useful to identify how different brain regions are connected – to verify the neural network under treatment, and whether other networks are being stimulated in error, potentially with behavioural side effects.

A more powerful MRI machine is becoming increasingly available, producing better quality images than before. It generates a magnetic field of 3 Tesla (T), which means that it is twice as strong as previous machines that operated at 1.5 T. These 3T MRI machines are great for pre-operative imaging of DBS patients because the electrodes can be placed even more accurately.

However, MRI is a possible safety risk for patients with DBS implants, especially when 3T machines are used. The main concern is that current may be induced in the metal leads of DBS devices during the RF transmission portion of MRI. The leads carry this unwanted current to the electrodes, which then potentially heat and damage the brain tissue



surrounding the wire. Temperature increases from 1 degree Celsius to as high as 46 degrees have been found, depending on various factors including magnetic field strength and the type of 'coil' hardware used for RF transmission. Damage to tissue in the brain could have serious consequences, so this heat effect presents too big of a risk for most patients to receive an MRI following a DBS implant.

While there are some exceptions where specific 'MRI-conditional' DBS devices can be imaged, these are under very conservative conditions that reduce image quality and DBS capabilities during imaging. Dr Graham and his team are investigating how to overcome this state of affairs, and uncover how MRI can be carried out more safely for patients with DBS implants.

Tailoring Radiofrequency Transmission

Two main avenues for improving safety have been explored by researchers: revising the design and materials of DBS devices, and modifying RF transmission. Dr Graham investigates the latter in his work, using a technique called parallel radiofrequency transmission (pTx). Instead of a single coil surrounding the patient for RF transmission, pTx utilises multiple coils that can be independently coordinated and powered. As a result, the distribution and strength of the RF magnetic fields can be controlled to maintain image quality, while also manipulating the RF electric field to minimise localised power deposition and tissue heating from the DBS device.

Dr Graham's team wanted to confirm the efficacy of using pTx during MRI in their initial study published in 2015, and they found that it did, in fact, reduce localised power deposition and tissue heating, due to a 95–99% reduction in the local RF electric field. The team used electromagnetic simulations for their tests. They studied how the number of RF transmitter coil elements affected homogeneity (the ability to generate a uniform image) while suppressing localised heating in a uniform, cylindrical, tissue-equivalent medium containing an implanted wire. Two, four and eight elements were tested and the team noted that although more investigations would be needed, the results with four elements were very promising.

Building on the Findings

Further studies in 2017 and 2019 allowed Dr Graham and his team to continue developing how pTx can be incorporated in MRI to give the best outcomes for DBS patients. Preliminary studies had used DBS leads with extremely simplified trajectories. In real-world scenarios, however, the leads can have complex geometries. Excess lead length is often placed in overlapping loops at the surface of the skull, and the lead configuration ultimately differs from person to person due to various factors including the shape of the head, the type of disease and the clinical symptoms displayed. The variability in DBS lead trajectories should not be overlooked, as it can have a major influence on the extent that tissue heating occurs near the electrodes. Therefore, Dr Graham and his team wanted to research heat-reduction methods such as pTx in realistic patient-specific situations. They also improved on their previous simulations to make the study more relevant to the real world. For example, they introduced insulated wires with conductive 'electrode' ends, rather than perfectly conducting wires, and validated simulation results using custom-built four-element pTx hardware that was much more advanced.

The improved variables and calculations gave promising results. Localised power deposition around the electrode was impressively reduced by 94% when using four-element pTx and 97% when using eight-element pTx. When their optimised results were tested experimentally on a realistic model, called a head phantom, they generated good-quality images with four-element pTx while finding almost no temperature rise surrounding the tip of the wire.

Promising Results for Real-world Applications

Dr Graham and his team of dedicated researchers have delved into the topic of parallel RF transmission and provided optimised data that can be used in the future to minimise temperature rises in the brains of DBS patients receiving MRI. His work will contribute towards drastically reducing the risks for people receiving this treatment, and may prevent life-altering brain injuries. Combined with his additional work on brain activity, disease and MRI investigations, Dr Graham continues to make innovative contributions to his field.

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MENTAL HEALTH FIRST AID: BRIDGING THE GAP BETWEEN RURAL COMMUNITIES AND ACCESS TO CARE

In the USA, poor mental health and opioid addiction are prominent and widespread. With a lack of understanding and resources in many rural areas in Texas, many people facing mental health and addiction challenges do not know where to turn. **Dr Lakshmi Mahadevan** at Texas A&M AgriLife Extension Service is helping to train up rural communities in Mental Health First Aid so that they can provide better care for those in need.

The Chemical Balance of Mental Health

Mental health is becoming an increasingly widely and openly discussed topic. Looking after mental wellbeing is an essential part of our overall health, yet it is still too often overlooked and stigmatised. Many people are unaware of who or where to turn to when in need, or even that they may require help. This often prevents people from seeking medical treatment and tragically, many succumb to their mental illness.

In the USA, suicide is the 10th leading cause of death and was responsible for more than 47,500 deaths in 2019 according to the Centres for Disease Control and Prevention, and one in every five adults lives with some form of mental illness (see figures published by the National Alliance on Mental Illness in 2019). Depression is one of the most common mental illnesses and many people will experience it at some point in their life. This is because it can be triggered by stressful or traumatic events in life such as losing a loved one or a job or giving birth (known as postpartum depression). However, depression can also be genetic and run in families, but also, there may be no obvious cause at all. This is the same for another common mental health issue, anxiety. This disorder presents as worries and fears that can even result in physical manifestations such as heart palpitations and nausea.

In both disorders, whatever the root cause, there is an imbalance of brain chemicals called neurotransmitters. These important chemicals help to control and regulate all sorts of cognitive functions like mood, emotions, concentration and memory by sending signals throughout the brain. Therefore, when neurotransmitters are not working properly, these functions are disrupted and different mental health issues can arise. The specific issue that arises depends on which neurotransmitters are affected. For example, people with depression tend to have too little of the neurotransmitters known as dopamine, norepinephrine and serotonin.

Attempting to balance an individual's brain chemistry is one of the primary targets for mental illness treatments. This may be achieved through talking treatments such as cognitive behavioural therapy, medications and improving behavioural habits like exercising or reducing any existing alcohol or drug consumption.

Targeting Opioid Misuse in Rural USA

Drug and alcohol use is a real problem amongst many coping with mental health challenges. In the USA, around a quarter of adults who have an opioid use disorder also have a substantial mental illness. The opioid crisis in the USA has been a burgeoning public health issue for years and in 2019, unintentional drug overdose-related deaths remained high. Of these deaths, 72.9% were attributed to opioids and this figure continues to rise.

In the same year, over 21.2 million people over 12 years of age required but did not receive treatment for substance disuse. According to the Substance Abuse and Mental Health Administration, over half of adults with a mental illness either do not look for or receive the treatment they need. During 2017–2019, the annual average prevalence of past-year mental health service use among those with any mental illness in Texas was 39.5% (or 1.4 million), lower than both the regional average (41.2%) and the national average (43.6%). Clearly, there is a real need for appropriate and effective health interventions to improve the care of those suffering from mental illness and opioid addiction.

This is the goal of Dr Lakshmi Mahadevan and her colleagues who have developed the Mental Health First Aid in Rural Texas (MHFA_RTX) programme through the AgriLife Extension Service at Texas A&M System. This project, funded by the US Department of Agriculture, aims to reduce opioid misuse and improve mental health service seeking behaviours in the rural counties of Texas.

The programme has an established set of objectives to help them achieve this goal. First and foremost, Dr Mahadevan and her team wish to raise awareness of opioid misuse and its related risky behaviours in multiple rural Texas counties. Through Mental Health First Aid (MHFA) training, they will also increase and improve mental health literacy (knowledge and understanding) of adults who interact with young people or other adults in rural communities. Trainees will also be provided with overdose reversal administration (NARCAN) kits in order to reduce harm, prevent overdose and increase education on this difficult issue. Education is a key component of MHFA_RTX and the team want to teach communities how to come together to address the opioid problems in their specific areas.

Mental Health First Aid Training

Although first aid is a well-understood concept, it may be unfamiliar for many to associate it with mental health. However, it is an essential element of the MHFA_RTX programme. There are a number of key aspects to MHFA that help people to safely identify and address a potential mental health challenge or substance use disorder. This is achieved through an action plan that can be taught and learned through proper training. Dr Mahadevan and colleagues are certified by the National Council for Mental Wellbeing to provide such MHFA training for adults residing or providing services within rural Texas.

Trainees learn how to spot the signs and symptoms of mental health challenges in the people around them. They are specially trained on the symptoms of anxiety, depression, psychosis, substance abuse, self-harm and suicidal behaviours and the available treatment options. However, it is important for them to understand that they are not creating a diagnosis, but rather, understanding that there may be an issue and what that issue may be. They are also taught that mental illness can be treated and when help is sought earlier rather than late recovery is possible, but that stigma, poor access, cost and fear often deter people from seeking professional help. Trainees of the MHFA programme are shown how to be patient, calm, non-judgemental and reassuring with those experiencing a mental health challenge or crisis. This is important so that they can give timely MHFA to prevent serious self-injury.

Those learning MHFA are given the knowledge and tools to administer effective aid to a person struggling with their mental health and potentially substance abuse, which has the capacity to save lives. The programme emphasises to trainees that identifying and responding to early signs of mental illness and substance use can allow them to point the person they have identified to the appropriate professional or interim self-care. In turn, MHFA has the potential to reduce the burden of these disorders on their sufferers whilst reducing the stigma and hesitation around discussing them. Consequently, the work by Dr Mahadevan could improve the understanding of mental health and its related concerns to allow for better outcomes.

Accomplishments of the Programme

One group that received training from the MHFA-RTX programme was the Military Families Learning Network, but other areas in Texas have also benefited. After completing the initial stages of the programme, Dr Mahadevan surveyed the participants, who showed promising signs of development. All of them reported that they had increased their knowledge of mental health and all received certification as a mental health first aider. Each participant was also successfully provided with opioid overdose prevention and reversal kits.

The trainees were also well educated on how to collaborate with their communities by creating First Responders Advisory Groups (FRAG) and Recovery Orientated Systems Coalition (ROSC). FRAGs are composed of local law enforcement personnel, district attorneys, emergency workers, pharmacological experts and other individuals who can help. They come together to improve how opioid episodes are dealt with in an emergency, how outcomes can be bettered and have productive discussions with law enforcement. ROSCs are a committee of local people representing a community's available resources – community health centres, medical personnel, and so on. They can meet to discuss and act on improving the prevention, rehabilitation and recovery of opioid users.

Dr Mahadevan believes that bringing together and educating rural communities in this way is making and will continue to make a real impact on their health and wellbeing.

The work of Dr Mahadevan and her team has made a significant impact on these rural communities who were lacking the knowledge and training of how to deliver proper mental health and drug care to their citizens. With these newly trained Mental Health First Aiders (133 in total), and with more surely to come, those who need help in these communities will now be safer, better understood and provide 'hope with facts'.

Meet the researcher

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Dr Lakshmi Mahadevan received her BA in Psychology at the Sophia College for Women in Mumbai, India and then her MA in Special Education from the Women's Christian College in Chennai. She went on to achieve her PhD in Career Development Education from the Department of Education Psychology at Texas A&M University in College Station, Texas in the USA. During her career, Dr Mahadevan has filled multiple roles within this university, including Programme Coordinator and Associate Professor. She is currently an Associate Professor and Extension Specialist for Special Populations in the Texas A&M AgriLife Extension Service. This is also where Dr Mahadevan carries out her work into reducing opioid misuse and providing Mental Health First Aid in rural communities in Texas.

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START NOW: AN EFFECTIVE MENTAL HEALTH INTERVENTION

Dr. Robert Trestman, at the Carilion Clinic and Virginia Tech Carilion School of Medicine, has co-developed START NOW, a successful group psychotherapy intervention specifically targeting mental health issues in prisoners. It combines elements of cognitive behavioral therapy to form skills-based learning. Furthermore, START NOW is easily accessible, cost-effective, and designed for use in settings with limited resources. Due to its success within correctional institutions, START NOW is being adapted for use in fields such as adolescent conduct issues and opioid misuse.

Development of START NOW

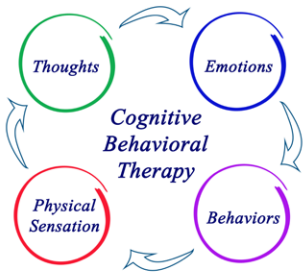
Mental health issues affect people across all walks of life but the prevalence amongst prisoners is particularly high. It is estimated that as many as 50% of prisoners in the USA have issues with impulse control or other mental health problems. Meanwhile, in Connecticut, almost two in every three newly detained prisoners have been found to have at least one long-term psychiatric illness. This can increase the threat of violence towards people within the correctional environment, as well as out in the community.

There is a lack of evidence-based mental health interventions that have been developed specifically for use in correctional settings. Cognitive behavioral therapy (CBT) has shown some effectiveness in reducing reoffending but although different types of CBT treatments have been trialed, none have shown superiority. Other barriers to providing effective interventions in correctional settings include the limited funding and availability of resources. As such, treatment is often only offered to prisoners with the most severe diagnoses. Nonetheless, the benefits of mental health interventions are likely to have a positive influence on prisoners who show only a few symptoms but lack the skills to function adequately in society or in prison.

Dr. Robert Trestman at the Carilion Clinic and Virginia Tech Carilion School of Medicine identified the need for a more tailored psychiatric treatment in correctional environments. With colleagues, he has developed the START NOW program which embraces a strengths-based approach and focuses on learning coping skills.

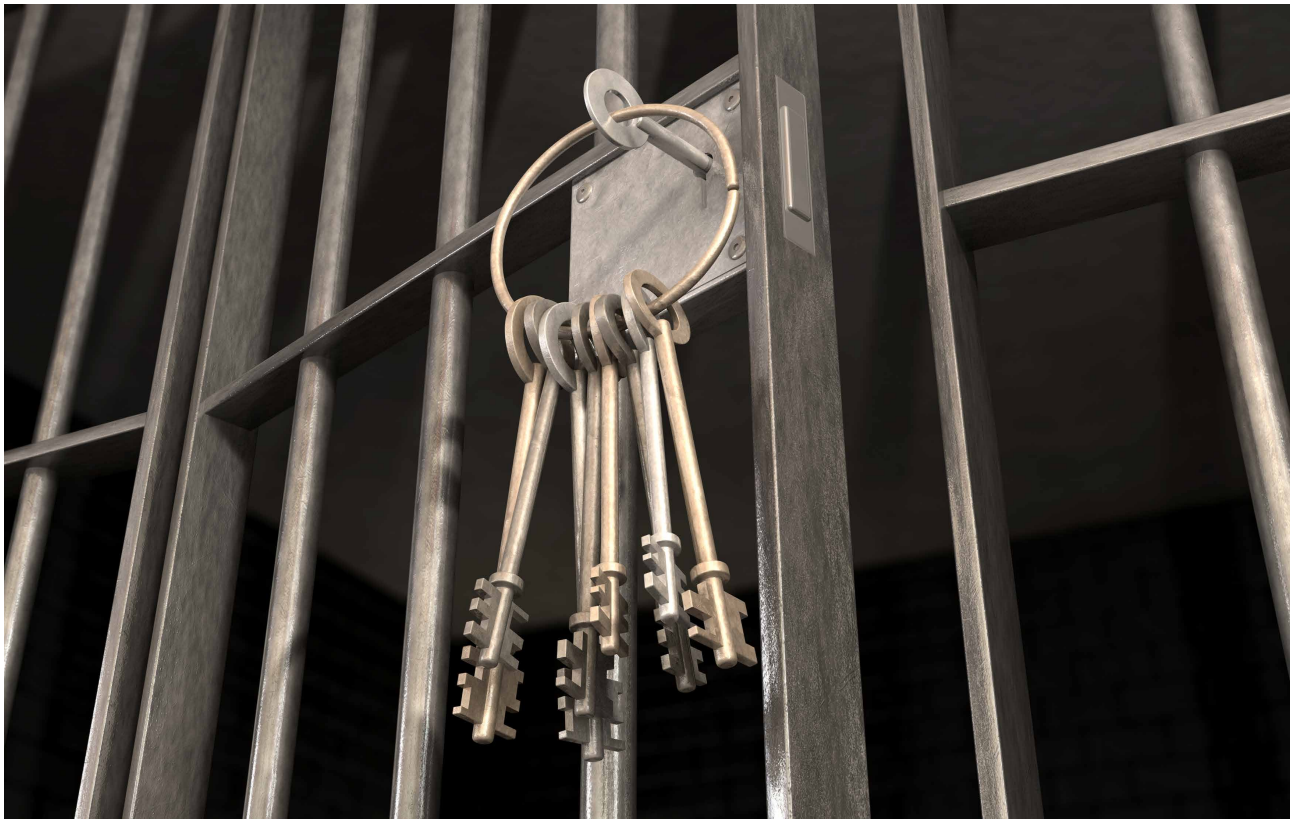
START NOW Program: Who, What and Why?

START NOW is for any individual with impairments relating to impulse control, emotion regulation, and the management of interpersonal relationships. The program places emphasis on individuals being responsible for their own learning and development, and encourages skill building in a non-judgemental



environment. Using group sessions is cost-effective but also allows for feedback and support amongst participants.

An easy to read and jargon-free manual with instructions for each session is provided. A typical program consists of 32 sessions broken down into four different units. The first involves developing self-control, dealing with stressors, and becoming ready for behavior change. The second focuses on understanding and coping with feelings and emotions. The third is about building relationships and the final unit covers setting and reaching personal goals. Based on CBT, the START NOW intervention helps participants understand how their interpretation



of events can affect their mood and behavior. Greater understanding of these cognitive processes can increase awareness of behavioral triggers and allow the modification of thoughts and attitudes.

Another approach used in START NOW is Functional analysis, and this is used to help break down behavior patterns by examining the causes and consequences of behavior. New skills are practiced within and between sessions using role-play, exercises, and group discussions. Motivational interviewing is also incorporated to help improve participants' drive to engage, learn and change their behaviors. Although individuals in correctional institutions may be reluctant, the START NOW intervention acknowledges and accepts that ambivalence to change is normal. Stimulating exercises and the reinforcement of positive behaviors are used to help overcome hesitancies.

A high rate of traumatic brain injury is found in correctional institutions, and this can affect verbal processing and concentration. In addition to providing instruction manuals and sessions designed for individuals of all cognitive abilities, START NOW includes trauma-sensitive care. This incorporates some elements of dialectical behavior therapy including mindfulness, a type of meditation focusing on being present in the moment.

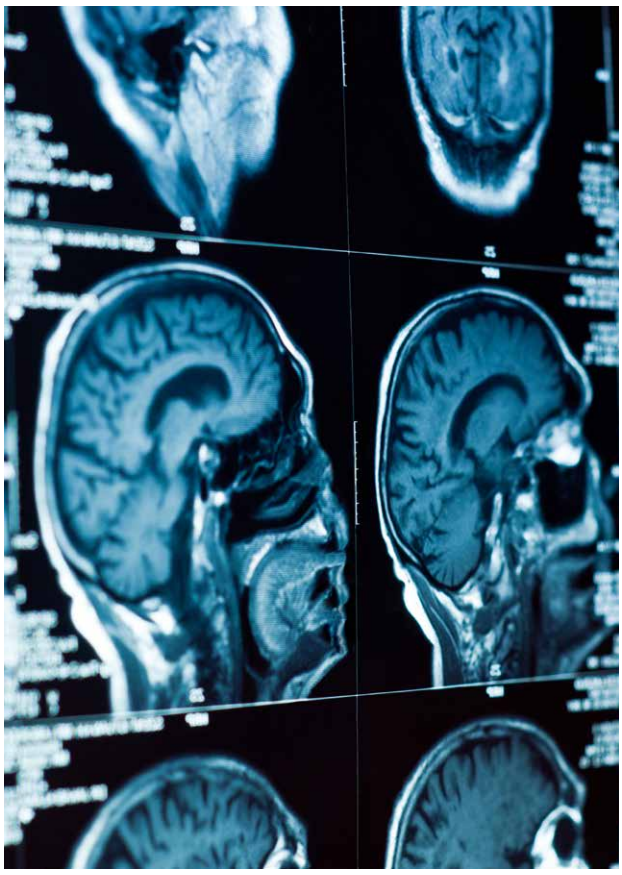
Evidence for Success

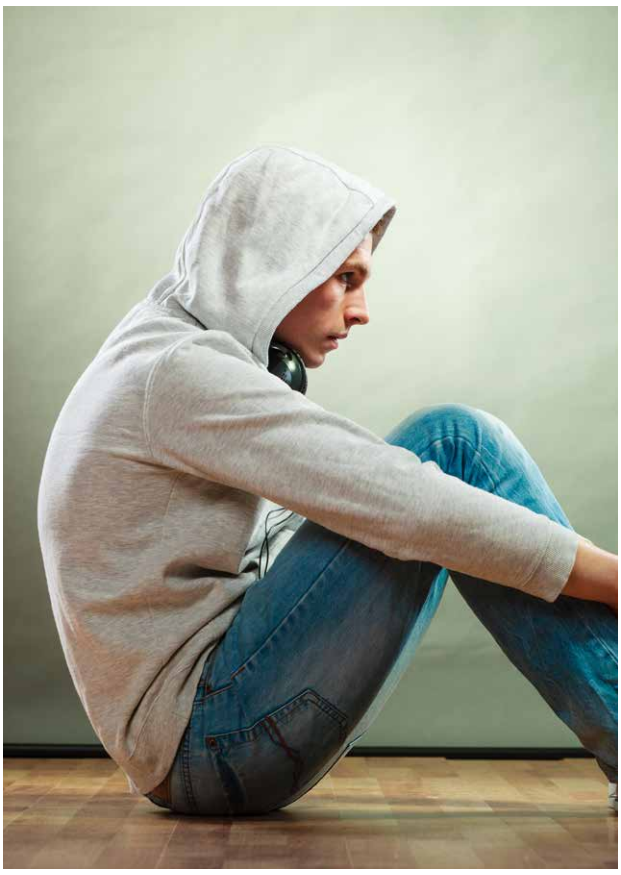
START NOW is already used within correctional, forensic, and community settings across 20 states in the USA and five different countries. Dr. Trestman and his colleagues have undertaken research to ascertain its effectiveness within correctional environments. A recent example is the evaluation of START NOW in 15 Illinois prisons. Of the 344 male prisoners who initially attended the program, 58% completed it. While this rate may seem low, it is consistent with – or even better than –completion rates found for other interventions in this population. To further determine the effectiveness of START NOW, Dr. Trestman and colleagues examined its impact on disciplinary violations. Impressively, the number of violations dropped by 57% during engagement with the program, and after it was completed, the level was still 27% below the average level.

There are several additional indicators of success. The percentage of prisoners placed in segregated or restrictive housing has been found to reduce during the program and even further afterward. Self-reported aggression is also reduced and participants report increases in their levels of satisfaction. These highly positive findings are consistent with research conducted across the Connecticut Department of Corrections. These positive results demonstrate the benefits of using START NOW across correctional systems in the USA and beyond.

Adaptation for Adolescents

Following its success within the prison system, START NOW has already been adapted for use in other populations, including adolescents. Across Europe, it has been observed that youths are displaying increased violence, aggression, and related mental health problems. Children and adolescents diagnosed with conduct disorder (CD) and oppositional defiant disorder (ODD)





often display impulsive, argumentative behavior leading to quarrels and possibly acts of cruelty. This is a highly vulnerable group who are at risk themselves and also perhaps to others. Although typically considered a male disorder, research has suggested a rising prevalence rate amongst females.

It was recognized that the START NOW program could offer skills-based training that could help adolescents with CD and ODD to enhance their emotional regulation capabilities. Prof. Christina Stadler and her colleagues undertook a study to evaluate the use of START NOW amongst female adolescents living in youth welfare centers. All 128 participants fulfilled the diagnostic criteria for CD and/or ODD and were randomly assigned to a test or control group. Modifications included cartoons and film clips to make the program more age-appropriate. Impressively, a significant reduction in symptoms as assessed by an interview, pre-, and post-treatment were found. Furthermore, participants reported being satisfied with the program and did not feel stigmatized by taking part. Overall, START NOW was considered to have a positive impact in this environment, empowering both adolescents and social workers.

Impact on Opioid Use Disorder

Dr. Trestman has also demonstrated success in using the START NOW intervention to treat people with opioid use disorder (OUD). In 2019, around 3.7% of the USA population had misused opioids in the past year. However, access to addiction treatment is often limited with only 18% of those with opioid addiction issues typically receiving medication assisted treatment (MAT). In 2000, the Drug Addiction Treatment Act required physicians to combine MAT with psychotherapy.

Recently, Dr. Trestman and his colleagues investigated the effectiveness of combining the START NOW program with MAT in treating OUD. The study assessed the views of 44 people with OUD through satisfaction surveys and focus groups. Constructive feedback pointed to the benefits of greater inclusion of strategies to target impulsivity and future planning and overall, results were favorable. With some modifications START NOW looks to become an effective program for people with OUD.

The next stage of Dr. Trestman's research was to conduct a clinical trial assessing the effectiveness of START NOW in treating OUD. Preliminary assessments of behaviors (impulsivity, aggression, and interpersonal problems), retention in treatment, and drug screen results indicate non-inferiority of the START NOW arm as compared to Treatment-As-Usual. Both patient and clinician satisfaction were positive for the START NOW program.

With robust evidence supporting its success and lots of exciting research on the horizon, Dr. Trestman is keen to raise awareness of START NOW, particularly around the possibilities of adapting the program for use with different populations.

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HOW DO CHILDCARE ARRANGEMENTS IMPACT EDUCATION OUTCOMES?

A stimulating and nurturing early childhood experience is critical to achieving better educational outcomes in later life. But what are the best childcare arrangements? Is it better to be looked after by family members or a nanny at home, or would care provided by qualified carers in a more structured environment bring additional benefits? A large study by **Dr Gabrielle Garon-Carrier**, from the Université de Sherbrooke, Quebec, Canada, sought to find answers to these burning questions.

The Childcare Conundrum

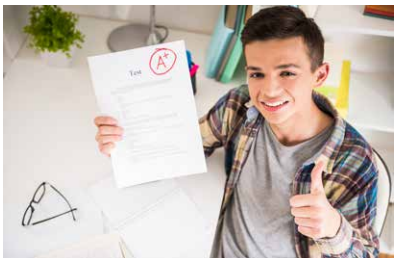
Parents all over the world face difficult decisions about childcare arrangements. Should their child be looked after by a family member, friend, or neighbour until school age or would hiring a nanny with relevant experience and references be better? Would the child benefit from spending time in a professionally organised and run childcare facility? Financial implications, the potentially hurt feelings of willing relatives, and considerations about the location and opening hours of organised childcare facilities make these decisions particularly hard to reach. The stakes are high: A positive early experience is likely to influence later educational attainment and will have a profound influence on the future of the child. Sporadic news articles confuse the issue further, with some suggesting a benefit from participating in organised care, others questioning it.

So, what do scientists studying early child development think about this thorny issue? Canadian child psychologist and education expert, Dr Garon-Carrier from the Université de Sherbrooke, points out that this problem is more complex and controversial than many of us might think.

A Confusing Picture

Formal childcare can take two forms, in which children either attend an organised centre or can be looked after by a trained, officially accredited carer in the home environment. The common feature of these arrangements is the professional training the caregivers receive. Informal care, in contrast, is provided by a willing family member, neighbour, or friend. For decades, scientists have been exploring how the outcomes of these arrangements compare, but unfortunately, the picture that emerges is both complex and confusing.

Dr Garon-Carrier explains that previous studies have often been limited in scope. She further notes that often children have been observed over only a short period of time and researchers have omitted crucial details from their evaluations. While difficult to record, follow and interpret, features such as



the education of the carers, the financial arrangements and social status of the family, and other circumstances will have a significant effect on outcomes.

For example, informal care provided by a well-educated grandparent in an affluent home is likely to be very different from what a child experiences when the carer is less educated, financially disadvantaged, or unemployed. Similarly, drawing conclusions on the effects of formal care is complicated by a range of factors, such as the number of hours of participation in organised care – the 'dose effect'. Even the sex of the children seems to have an influence – perhaps surprisingly, boys seem to benefit more from formal childcare than girls. Obtaining a comprehensive picture is made even more difficult by

the reportedly different benefits of attending formal childcare arising from studies conducted in the USA, Canada and the UK.

Searching for Clarification

This lack of definitive data prompted Dr Garon-Carrier and her co-workers to embark on a large-scale longitudinal study on how childcare arrangements influence educational aspirations later in life. The researchers took a long-term view and examined the likelihood of the children embarking on higher education. They obtained access to a large database of children held by Statistics Canada, a state-run national information database.

From this database, families with at least one child aged 24 to 36 months in 1994/5 were selected. The families were interviewed every two years between 1994 and 2008, and information was gathered about childcare arrangements, child behavioural problems such as depression and separation anxiety, family income, and the working hours of parents. The working patterns of parents were ascertained and critically, information about formal and informal childcare. Establishing attendance at higher education was achieved through Canadian tax arrangements.

Of the participating children, 51% had never attended childcare and were exclusively looked after by a parent (usually the mother). The majority of these families were from lower socioeconomic classes. To analyse the remaining families, the researchers used complex statistics to compensate for the large number of factors that could have potentially influenced the educational achievements later in the life of a given child. Briefly, this approach established pairs of children where the parental income, work arrangements and other parameters were comparable, and the pair differed only in having received childcare in either formal or informal settings.

Some Unexpected Findings

On average, 83% of the initially studied children completed at least one year in higher education. However, this number was lower (79%) amongst individuals attending formal childcare and higher (89%) in those who were looked after informally in their childhood. As might be expected, Dr Garon-Carrier and her co-workers also found that girls and children of well-educated parents usually achieved better. However, there were two unexpected trends. First, children attending informal childcare were more likely to pursue higher education. Second, children in middle- or higher-income families attending formal childcare were less likely to participate in higher education. In contrast, children from poorer, often out-of-work households were helped by attending formal arrangements.

Dr Garon-Carrier and her team did not expect to see such a difference between the outcomes for children from lower vs higher-income families attending formal childcare arrangements. She was even more surprised that children attending informal childcare were doing better in terms of enrolment in higher education, regardless of the financial state of the families.

To further understand these unexpected findings, the researchers undertook more in-depth analyses in which they compared two types of formal arrangements: childcare provided in organised central facilities or provided at home by a trained and accredited carer.

Explaining the Benefits of Informal Arrangements

Despite further investigation, no differences in the probability of pursuing higher education were found between children attending formal childcare arrangements in a central facility or at home, which further underlined that informal arrangements resulted in better long-term educational prospects.

Dr Garon-Carrier herself believes that selecting 24- to 36-month-old children for the study may have been a reason to explain these findings. She points out that at this age, interpersonal interactions with an emotionally involved carer may be more important than developing social skills by interacting with a group of other children. At a later age, the importance of these factors may change. Thus, selecting an older age group at the start of the study may have resulted in different conclusions. These findings also imply that similar children from underprivileged backgrounds do not always benefit from the same level of emotional support in informal care arrangements. Consequently, this vulnerable group seems to benefit more from the presence of a qualified carer from a younger age.

Dr Garon-Carrier also believes that the practicalities of investigating the influence of early childcare on the likelihood of attending higher education also affected outcomes. To allow such long-term follow-up, children who were aged 24 to 36 months had to be selected from 1994/95. It is important to mention that the quality of organised formal childcare in Canada has changed significantly since the mid-90s. Providers are better trained, the facilities are more strictly regulated, and are increasingly being inspected by local government agencies to ensure education quality. Thus, repeating this study in a decade could bring about different outcomes.

Important Conclusions

The importance of childcare provision does not only affect individuals and their families. Improving educational outcomes for vulnerable groups is the best way to improve their life chances, benefitting both the individual and society. Unfortunately, recent studies have shown that Canada lags behind comparable countries in providing childcare for working families. Recognising the importance of these early interventions, the Canadian government has announced the provision of a $30 billion investment over the next 5 years for the development of a nationwide childcare network.

The work of Dr Garron-Carrier and her co-workers demonstrates the long-term effects of attending childcare arrangements at the age of 24 to 36 months, with measurable consequences in educational attainment 20 years later, in early adulthood. In particular, demonstrating the beneficial effects of early organised childcare attendance on the futures of vulnerable children will be critical in shaping educational policies for years to come.

Meet the researcher

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Dr Garon-Carrier completed her undergraduate studies at the Université Laval and then her PhD at the same university in 2016. This was followed by postdoctoral research at Goldsmiths, University of London. After working for the Canadian Government, she became an assistant professor in the Department of Education at Université de Sherbrooke. Her work is focused on the early-life factors affecting school readiness and educational achievements in later life. She has published extensively on the development of early numeracy and its role in scholarly achievements, the factors influencing motivation during school attendance, and the development of separation anxiety during pre-school childcare. The importance and impact of this work have led Dr Garon-Carrier to hold a Tier 2 Canada Research Chair on school readiness, the inclusion of vulnerable populations and social adjustment, and to see her work feature regularly in the media.

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FUNDING

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UdS Université de Sherbrooke

CONFRONTING COMPLEX SOCIETAL ISSUES THROUGH RESEARCH

Dr Teresa Silva at Mid Sweden University has turned her focus from investigating individual risk factors towards understanding how society is contributing to behavioural problems and mental health issues. She is currently undertaking research in a number of critical areas including child protection and domestic abuse against males. This work is providing vital evidence to support the development of interventions and policies that are more effective.

Family Relationships: Risks and Interventions

A range of factors affect our behaviour and mental health, and often we look inward to try to uncover what has caused these issues. Dr Teresa Silva, based at Mid Sweden University, has reversed this focus to examine how societal factors, often beyond our control, can negatively impact our mental health. A key area of her research is family relationships and institutional support to families, particularly those that are dysfunctional. At a young age, children rely on their parents for security and comfort. When this does not exist, long-term emotional issues may ensue. Similarly, in adulthood, our mental health can be affected when our closest relationships become harmful or abusive.

Dr Silva emphasises how important it is that services are in place to provide support if these relationships become dangerous or harmful, ensuring no further damage is done to our mental health. These are usually institutions such as social services or the family/criminal justice system. Much of Dr Silva's research focuses on exposing how these important institutions often do not take appropriate responsibility for identifying risks and providing effective interventions. This can lead to the most vulnerable members of our society becoming harmed by the organisations that should be there to protect them.

Childhood Emotional Abuse: Parental Alienation

Dr Silva's research underscores the critical influence of parental behaviour on child mental health. In certain situations, particularly when relationships break down between parents, children can become stuck in the middle.

Her recent research focuses on parental alienation (PA for short), which is a form of childhood emotional abuse that occurs when a parent instrumentally uses their child to inflict psychological harm on the other parent. This act of revenge is usually associated with high conflict separation or divorce. Although the main aim of the 'alienating parent' is to inflict sabotage on the relationship between the child and the so-called 'target parent', this behaviour can indirectly cause harm to the child itself.

The key tactics used by alienating parents include undermining the target parent and making it appear as if they have rejected the child. In extreme cases, the alienating parent will fabricate instances of abuse, which sadly, are often successful in ensuring there is a legal separation from their child. Other actions include stopping contact, in person and/or through phone calls and messages.

These manipulative behaviours from the alienating parent often result in the child being resistant to having contact with the target parent. They may even engage in a pattern of verbal abuse and aggressive behaviour towards the target parent. In severe cases, the bond between the target parent and the child is broken, something that is very difficult to mend even with family therapy.

PA can cause the child to experience feelings of abandonment, loss and fear, and Dr Silva notes it can have a tremendously negative impact on their psychological and behavioural development. In the medium to long term, they may have issues with low self-esteem, anxiety and depression as well as displaying violent behaviour, alcohol and substance misuse. Research has also shown that it can also cause problems with relationships later in life including difficulties in forming and maintaining attachments with others, breakdowns and even alienation from their own children.

As PA is very under-researched, we are unable to know the true extent of the negative impact it has on society. There has been much discussion around whether it should be categorised as a psychiatric condition, which Dr Silva believes may have overshadowed work to find an appropriate solution. Currently, there is no consistent monitoring of its prevalence and there are no specialised services in place to address issues relating to PA.

This lack of support can leave target parents feeling hopeless, especially if they have been through numerous attempts to resolve the situation through the family justice system. For some, it can lead to doubts about whether they should continue their fight, whereas for others it can cause desperation, even leading them to undertake extreme actions such as abducting the child or committing violence towards the alienating parent.

Dr Silva believes that prevention and intervention should be prioritised in cases of PA, and that all the professionals involved should take a shared responsibility to find a solution. Her research emphasises that protecting the child must be the main priority, whereas to date, often the focus of family courts has been on finding a legal resolution. She believes it is the responsibility of the family justice system to monitor individuals and ensure that the parties involved are cooperating with mental health services and attending family therapy as required. Dr Silva has called for more research in relation to PA, particularly around its impact upon children's long-term psychological and behavioural development. She emphasises that only by broadening our understanding will we be able to design and implement effective solutions.

Dysfunctional Families and Social Services

Even when parental relationships have not broken down, dysfunctional family life may negatively impact a child's psychological state. Dr Silva is currently undertaking a piece of research to help identify the risk factors amongst disadvantaged families, which may lead to developmental problems within children. Her latest research is driven by a notable rise in the number of families within a rural region in Sweden, that have had children placed into foster care due to severe dysfunctional family situations.

These children are being placed into care at a younger age than ever before, and policymakers are very concerned about the wellbeing of children in the region. Dr Silva's research will help to provide answers about why these alarming trends have occurred, as well as identify the risk factors involved. The policymakers will then use this information to improve current measures and to inform the development of more effective preventative interventions.

The research will focus on children who have family risk factors that are likely to increase the development of psychological and behavioural issues such as anxiety, depression, self-harm and drug use and delinquent behaviour at a very young age. Dr Silva will utilise data that has been collected by social services, relating to children in very disadvantaged situations that need foster care due to the inadequate conditions in their own families. These data will be compared with data on a school sample of children of the same age and gender. During her analysis, Dr Silva will evaluate parental competence

and use family systems theory to understand the development of pathological relationship dynamics between all the family members. When such dynamics are established, parental bonds and the attachment between the child and the primary caregivers can be heavily affected. As a result, the child might lose trust in adults and find him/herself with no emotional support at a stage of his/her life when it is most needed. Dr Silva's analysis is currently ongoing, with results due in autumn 2022. She hopes the outcomes will help social services to identify risk factors and intervene at an earlier stage in the situations in which families are not able to provide adequate care for their children.

Intimate Partner Violence and the Justice System

Beyond childhood, our most significant relationships are often with our spouse or partners. Dr Silva has researched the impact it can have upon our mental health when these relationships become abusive or violent. This is referred to as intimate partner violence, which is defined as abuse conducted by a former or current partner that can be physical, verbal, emotional, economic or sexual.

Much of Dr Silva's research has been focused on male victims, revealing that law enforcement, the criminal justice system and even family members are less likely to believe testimonies when they are from men. These male victims have often been falsely accused of being the violent spouse and feel discriminated against by police authorities and also in relation to accessing support.

Dr Silva believes another failure of the justice system is the lack of proper assessment of alleged victim credibility, especially when the alleged victim is a female. This is a particularly poignant topic, as it can impact child custody rights. Dr Silva noted that false accusations of abuse are something that can be used as a mechanism within parental alienation to ensure that the target parent does not win custody rights within separation or divorce proceedings.

Whether or not child custody is involved, Dr Silva argues that structured assessments of witness credibility should be undertaken as standard within the justice system. She believes that often statistical evidence is being ignored and emphasises that there are serious consequences of not believing real victims or falsely convicting someone who is innocent. Such consequences affect not just the victim and perpetrator but their families and loved ones as well.

Dr Silva has analysed a real-life case report and demonstrated that undertaking a more structured approach to assessing the credibility of the alleged victim can lead to different judgement outcomes. The case she analysed was a libel claim relating to the actor Johnny Depp who was accused of several accounts of assault by his former partner Amber Heard. The claim was filed against a UK tabloid paper that published allegations in 2018 that labelled Mr Depp as a 'wife-beater'.

Dr Silva used documents that were published publicly to undertake two different structured assessments to verify the credibility of the alleged victim. The first was the Six-Factor Test, which evaluated the plausibility of these allegations whilst assessing with psychological characteristics of the victim and perpetrator. The second was the Brief Spousal Assault Form for the Evaluation of Risk (B-SAFER) Test, which focuses on the analysis of risk relating to the perpetrator's history of violence and psychological functioning.

During the trial, the judge found the alleged victim to be very credible. However, Dr Silva's analysis revealed her testimony to be of low credibility, meaning it is likely she lied about the violence. Dr Silva believes these findings demonstrate the advantage of using structured tools and provide evidence for their consistent usage and implementation across the justice system.

As we have seen, Dr Silva does not shy away from tackling even the most difficult societal issues. By embracing multifaceted approaches to understanding the complexity of issues such as child protection and domestic abuse against males, her research is providing the necessary understanding and evidence-base to improve interventions and make critical changes at the policy level.

Meet the researcher

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In 2016, Dr Teresa Silva was appointed Associate Professor of Criminology at Mid Sweden University. Throughout her career, Dr Silva's research has focused upon public health, psychology, psychiatry and criminology. Having previously worked in forensic psychology, she completed her PhD in 2009 at the University of Valencia, researching psychopathy within the Spanish juvenile justice system. Currently, Dr Silva is undertaking two active projects. The first is funded by Mid Sweden University and Örnskoldsvik Kommun, in which she is exploring the individual, social and situational factors that may be causing an increase in dysfunctional behaviour among children. The second project is exploring the victimisation experiences of men who have suffered abuse and violence from an intimate partner, and who struggle against the authorities, institutions and society that fail to believe them.

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Mid Sweden University
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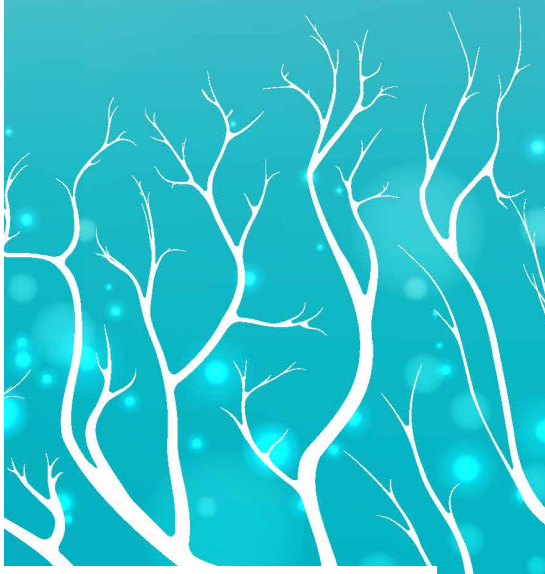
BUILDING RESILIENCE IN PUBLIC SAFETY PERSONNEL

While it is impossible to imagine a stress-free working environment, border services personnel, correctional workers, firefighters, operational and intelligence personnel, paramedics, police, public safety communicators, and search and rescue personnel are regularly exposed to dramatic, potentially psychologically traumatic experiences. Unsurprisingly, people working in these professions suffer from mental health challenges more often than the general population. The research of **Professors Anderson** and **Carleton** focuses on improving the well-being of these key workers in Canada.



The Emotional Toll of Work

Had a bad day in the office or a disagreement with a work colleague? All working lives have downsides but compared to the challenges faced routinely by public safety personnel – individuals working in the emergency services – many work-related stresses can appear trivial.

It is widely appreciated that firefighters, paramedics (including emergency medical technicians) and police officers can be exposed to very challenging, potentially psychologically traumatic experiences. Other public safety personnel, such as correctional workers and public safety communicators (such as call handlers and dispatchers at emergency services) fulfil less visible roles that are equally as important and often similarly challenging. The list of stressful situations that these individuals can find themselves in daily is limited only by our imagination, and few civilians will have a true comprehension of the extreme stressors that may be confronted.

We can also instinctively see that many people working in these roles will suffer psychological injuries as a result of their daily work, leading to various mental health challenges, including symptoms of post-traumatic stress disorder, major depressive disorder, generalised anxiety disorder, and even suicidal thoughts. Perhaps fewer people realise that the consequences of these mental health challenges are not limited only to these individuals and their families. When affected people leave their highly skilled jobs, it stretches existing resources, and the recruitment and training of those replacing them require a considerable financial commitment.

Despite the importance of these problems, the science of objectively investigating the experiences of public safety personnel, and the exploration of potential coping mechanisms that may help these individuals, is in its relative infancy. Professors Anderson and Carleton are pioneers in examining stress mitigation and the provision of treatment for post-traumatic stress disorder in these populations. They have spent decades investigating the challenges facing public safety personnel and trying to develop innovative solutions to support the mental well-being of these key workers.

The Scope of the Problem

In any scientific discipline, defining a problem usually starts with establishing the number of affected individuals. However, gaining accurate numbers indicating how widespread mental health challenges are amongst public safety personnel is difficult. The definition of these disorders is continually evolving, and the methods used for gathering information show considerable variability between studies.

Without getting into the nuances of these considerations, let's look at some findings. In a relatively recent Canadian study, Professor Carleton and

colleagues found that out of 5,813 public safety personnel more than 15% had symptoms of at least one mental health disorder, while more than 28% reported signs during the three-month study period that were compatible with the presence of two or more mental health disorders. Similar studies in paramedics indicated that 49% of the questioned population showed some signs of one or more mental health disorders, while in nurses the number is around 45%. A bleak picture indeed.

However, as Professors Anderson and Carleton point out, the situation is even worse for a subset of these workers. Ex-military service personnel are often employed by the police or other public safety-related organisations. A large study found that individuals with previous military experience were 1.5 times more likely to suffer from post-traumatic stress disorder, or problematic symptoms related to anxiety, depression, or stress, than those with no army experience.

The team also point to a particularly worrying statistic: mental health disorder symptoms appear correlated with suicidal thoughts and suicide plans. However, somewhat surprisingly, the professors and their colleagues found that most actual suicide attempts were made by civilian members of the police force – specifically, individuals in administrative and support roles.

Organisational Issues

Of course, it is not possible to eliminate potentially psychologically traumatic events from the lives of public safety personnel, as dealing with these events is an integral part of the job. Data from Canada indicate that the average emergency worker will experience hundreds or even thousands of events involving threatened or actual physical assault, sexual violence, fires, explosions, violent or sudden deaths, and catastrophic injuries during their active working life.

However, it is increasingly being recognised that these potentially psychologically traumatic events represent only a fraction of stressors affecting public safety personnel. As the work of Professors Anderson and Carleton has demonstrated, so-called organisational and operational issues, such as shift work, limited resources, lack of equipment or training, actual or perceived lack of support from colleagues and family, and related issues can have a pronounced cumulative negative impact on the mental health of public safety personnel, above and beyond the potentially psychologically traumatic events. In fact, the most prominently reported causes of stress were the feeling that workers constantly needed to prove themselves, chronic fatigue, social stigma associated with the job, and the poor reaction of colleagues after somebody took time off due to sickness or injury.

Some Lesser-investigated Consequences of Stress

Apart from its effects on mental health, both acute and chronic stressors can impact the functioning of individuals in challenging environments. While most work in this field has investigated how stressors impact mental tasks such as decision-making and problem-solving, Professor Anderson decided to focus his attention on the previously ignored topic of how stressors impact movements – so-called motor skills.

There is objective evidence that in situations of imminent danger, for example when facing an armed opponent, the precision of movements deteriorates. In the context of policing, this translates to reduced accuracy while performing critical shooting skills. This deterioration of precise skilled movements is combined with increased blinking – resulting in less visual contact with a potential target – and will inevitably affect the performance of officers when facing dangerous scenarios. Other movement-based tasks, such as actions during self-defence or arrest situations also deteriorate significantly in threatening situations.

Based on a review of the literature and their own research, Professor Anderson and his team created recommendations for the police force on how to train officers to reduce the stress-related deterioration of such motor skills.

Help at Hand

Despite expensive research on the consequences of mental health challenges among public health personnel, past attempts to reduce the impact of these problems invariably focused on interventions after challenges had already developed. In contrast, in view of the inevitability of public safety personnel being exposed to potentially psychologically traumatic events, and the organisational and operational stressors identified during their earlier work, Professor Anderson and his colleagues started to wonder whether it was possible to implement proactive strategies designed to build resilience, the ability to 'bounce back' after negative events.

As a first step towards this goal, they designed and created a 6-hour online programme designed to promote resilience by explaining psychological concepts and demonstrating potential coping strategies. The effectiveness of this intervention was initially tested in paramedic and nursing students. Participants in these early trials took a baseline test of their coping strategies, completed the training programme, and were retested for their resilience after the completion of the online training, and 3, 6 and 9 months later. The results demonstrated the effectiveness of the training material, although initial improvements started to slowly erode by 6 months and decreased even further at 9 months.

Professors Anderson and Carleton and their co-workers also analysed the literature for the effectiveness of existing interventions in proactively reducing post-traumatic stress disorder among public safety personnel. They found that most of the 36 previously trialled interventions provided some benefit, with multimodal strategies performing better.

To aid the development of better resilience training, the group conducted one of the largest studies of self-reported coping strategies of experienced public safety personnel. Professor Anderson views resilience as an 'eco-system', a combination of three mechanisms that help the individual cope with traumatic events. The first one of these is based on the personal coping strategies of individual public safety personnel. Participating in training programmes, admitting the existence of problems, and reaching out for help when it was needed helped individuals to develop successful coping strategies.

The second pillar of promoting resilience is the family environment. Previous work has shown that while families are generally proud of public safety personnel, unsocial working hours, alternating night and day shifts, and psychological factors can have a negative impact on family relationships. Nonetheless, this work also identified positive habits underpinning well-functioning supportive families in which emergency workers and their partners and children could flourish.

The third pillar of resilience is a well-organised workplace. As mentioned earlier, the team already provided scientific evidence-based recommendations for the training of police officers, and their previous work contained valuable data that could be utilised in the training of managers, allowing them to develop more supportive working practices and environments.

The Road Ahead

Given the irreplaceable contribution of public safety personnel to society, it is essential to create environments where individuals exposed to potentially psychologically traumatic events can work without sacrificing their own mental well-being. Achieving this goal would not only help the individuals providing these vital services but could minimise absenteeism, stress-related illnesses and injuries, and help to develop a more reliable, less stretched and happier workforce, benefiting society most broadly. The dedicated work of Professors Carelton and Anderson and the team undoubtedly represents the first steps towards solutions that will eventually transform the lives and working conditions of these valued professionals.

Meet the researchers

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Professor Gregory Anderson is the Dean of the Faculty of Science at Thompson Rivers University. He studied exercise physiology at the University of British Columbia and later gained his PhD in applied physiology at Simon Fraser University. He worked as the Dean of the Office of Applied Research & Graduate Studies at the Justice Institute of British Columbia between 2011 and 2020 during which time he volunteered as the Associate Director – Police Sector at the Canadian Institute for Public Safety Research and Treatment between 2016 and 2019. He took up his current post at Thompson Rivers University in 2020. His research interests include occupational health and wellness and the physiology of physically demanding occupations. He has developed and assessed an online learning tool for first responders to improve their personal resilience prior to deployment and published extensively on topics covering occupational and organisational stress and proactive mental health support programmes (https://publicsafetyresilience.trubox.ca/). He is passionate about improving the well-being of Canadian public safety personnel, their colleagues, organisations, and their families through world-class research and the promotion of evidence-based practices, policies and programmes for all public safety personnel.

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Nicholas Carleton, PhD is a Professor of Clinical Psychology, a registered clinical psychologist in Saskatchewan, and is currently serving as the Scientific Director for the Canadian Institute for Public Safety Research and Treatment. He has published more than 200 peer-reviewed articles and book chapters exploring the fundamental bases of anxiety and related disorders. He has completed more than 400 national and international conference presentations. He also serves as an active member of several national and international professional associations. As a principal or co-principal investigator, he has been awarded more than $60M in competitive external funding. He has received several prestigious awards and recognitions, including recent induction as a Member of the Royal Society of Canada's College of New Scholars, Artists and Scientists, and as a Fellow of the Canadian Academy of Health Sciences, and was awarded the 2020 Royal-Mach-Gaensslen Prize for Mental Health Research.

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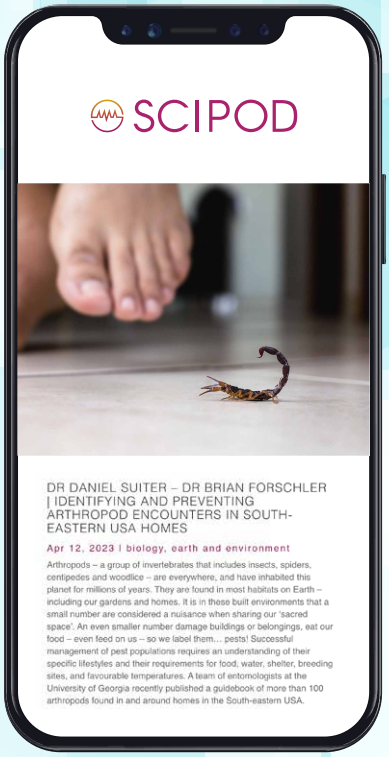

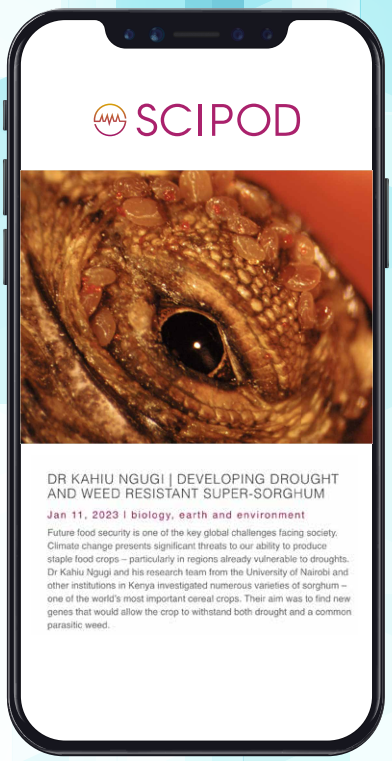

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