# Scientia

## FROM NEUROTRANSMISSION TO COMPLEX COMPUTATIONS

**EXCLUSIVES:**

- The American Academy of Neurology
- The Alliance for Aging Research

**HIGHLIGHTS:**

- The Genetics of Epilepsy
- The Mitochondrion: The Powerhouse Behind Neurotransmission
- Growing Old Gracefully: The Science Behind Aging
- Improving our Healthcare System with Queueing Theory

Issue: #107

# WELCOME...

This edition of Scientia celebrates the diversity of scientific endeavour by showcasing research across three different themes. Each project has a healthcare or biological focus, but this edition is notable for its inclusion of a wide variety of research disciplines from neuroscience through aging to computational biology. We hope this variety will make for an interesting and enlightening read, regardless of your chosen profession.

To kick things off we feature some recent breakthroughs in neuroscience research. To introduce the section, we talk to the American Academy of Neurology about their activities in promoting neuroscience and neurology in the US and further afield. We then showcase no less than nine exciting projects, ranging from helping patients with brain trauma to hunting for new drug targets to treat Alzheimer's disease. Here, we detail the work of Dr David Greenberg and his team at the Research Institute at Nationwide Children's Hospital, who study the genetic determinants of inherited epilepsy. We also introduce Professor Elizabeth Jonas and her team at Yale university, who investigate the mitochondrion's role in neurotransmission.

The next section deals with improving the universal experience of aging. With increasing life spans and an aging population, this research is more important than ever before. Here, we have had the pleasure of speaking with Susan Peschin, the president of the Alliance for Aging Research – a non-profit organisation dedicated to accelerating scientific discoveries that vastly improve our aging experience. From here, we highlight the work of five researchers, each dedicated to improving our quality of life as we age, from investigating the effects of aging on gene expression, to improving palliative care for the dying.

Our final section deals with how mathematical approaches can be applied to bioscience and healthcare. Among three other exciting projects, we feature the work of Dr David Stanford of the University of Western Ontario. Dr Stanford uses Queueing Theory mathematics to provide solutions to real-world healthcare problems, such as emergency department wait times and organ donor priority lists, making wait times shorter and fairer for those in need.


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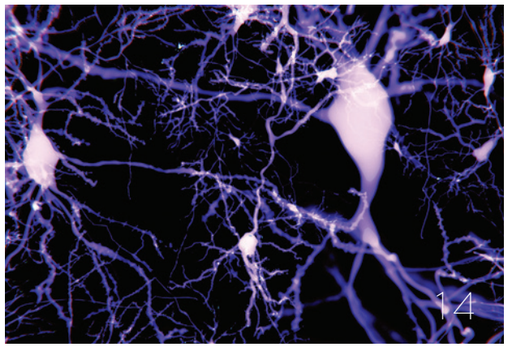


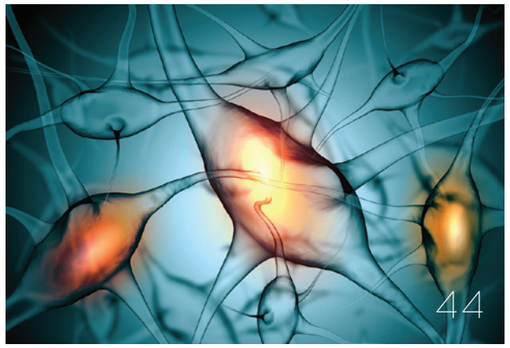



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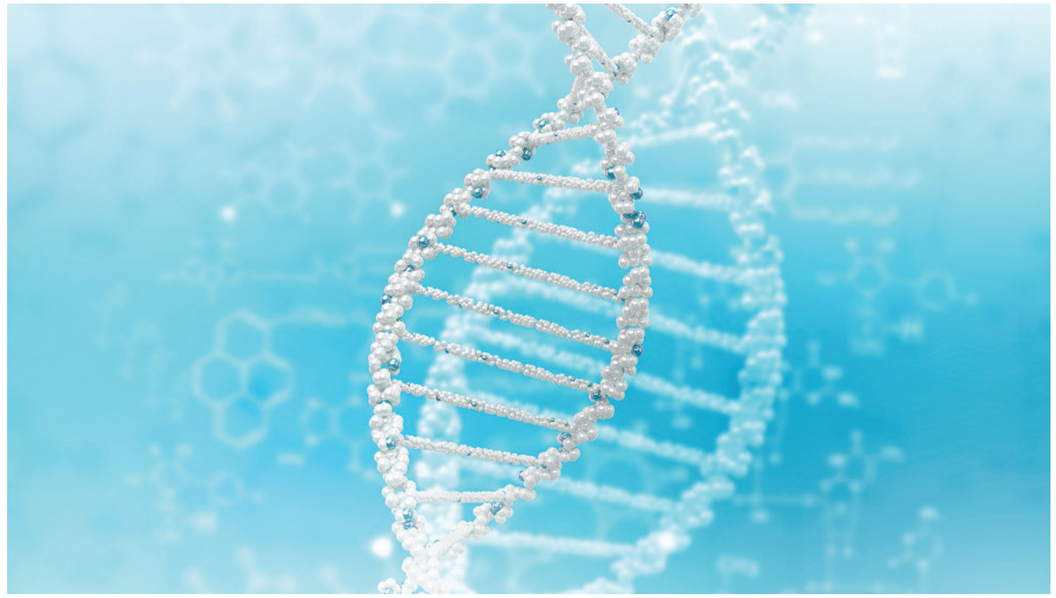


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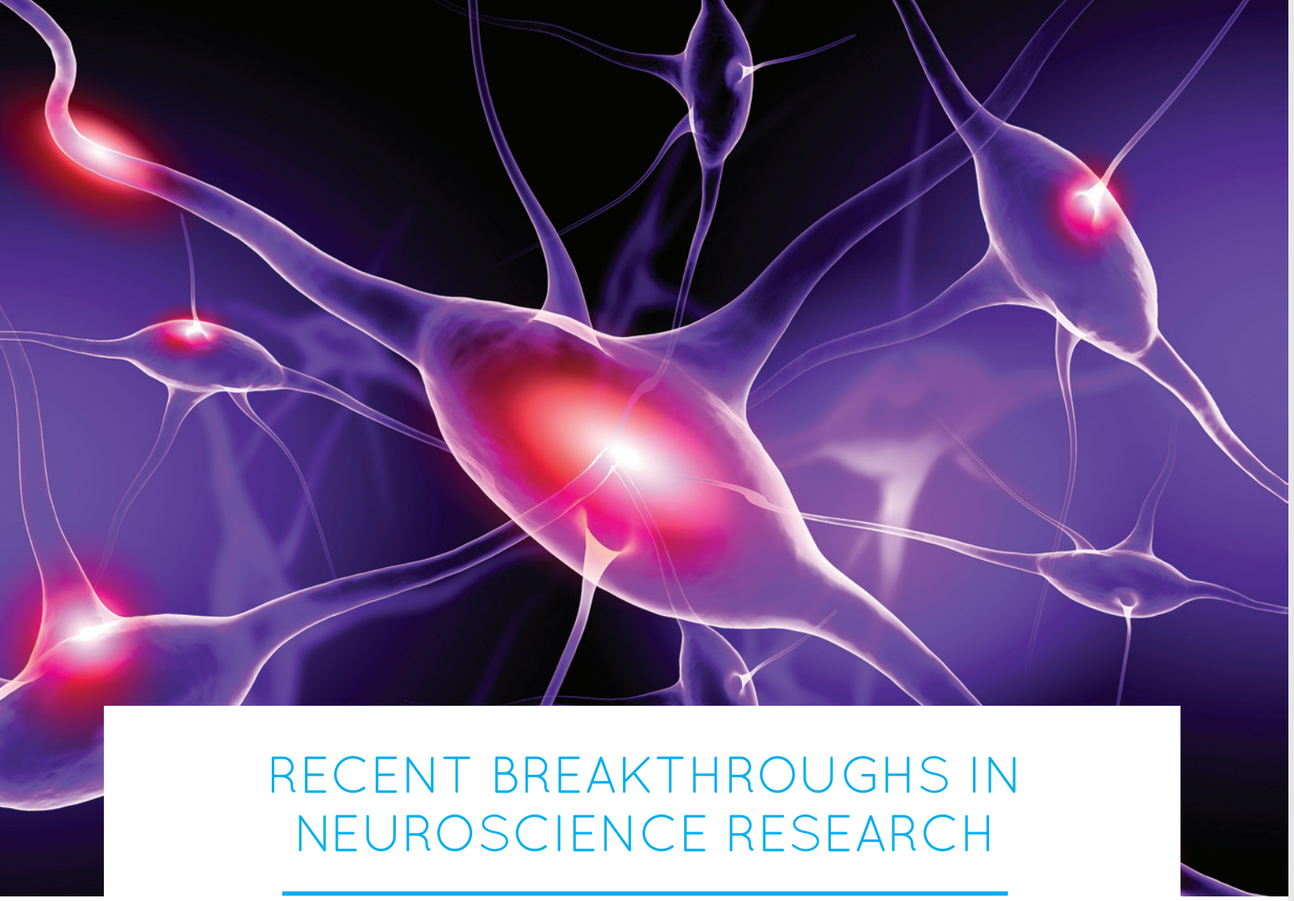


# RECENT BREAKTHROUGHS IN NEUROSCIENCE RESEARCH

In this section of the magazine, we explore the latest breakthroughs in neuroscience, highlighting research across the entire spectrum of this fascinating field – from unveiling the genetics behind epilepsy to investigating the role of the mitochondrion in neurotransmission.

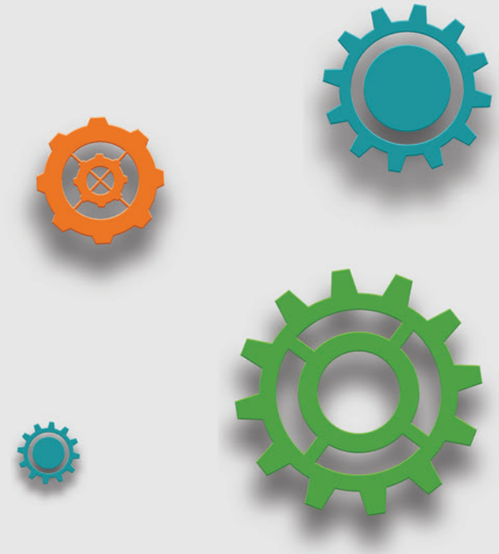
To introduce this exciting discipline, we have had the opportunity to talk to Dr Terrence L. Cascino, the President of the American Academy of Neurology (AAN), who tells us all about the AAN's activities in promoting neuroscience and neurology in the US and further afield. From here, we introduce Professor Jing-fei Dong of Bloodworks Research Institute and the University of Washington, who is working hard to help people suffering from devastating traumatic brain injuries (TBI). Many patients with TBI present a condition known as coagulopathy, where they cannot form blood clots and thus bleed uncontrollably. Professor Dong aims to unveil the mechanisms behind coagulopathy, with the eventual goal to design new therapies for this often fatal condition.

Our tour of the nervous system then takes us to the prefrontal cortex – the region of the brain associated with complex cognition, decision making, expression of personality and social behaviour. Here we showcase the work of two researchers, the first of whom is Professor Amy Arnsten at Yale University. Professor Arnsten and her team have discovered new insights into how our prefrontal neurons operate at a molecular level, and how they work together to influence our ability to remember, pay attention, and control our thoughts and actions. This fascinating research has led to the development of new pharmaceutical treatments for both ADHD and PTSD. Working in a similar field is Professor Chiye Aoki, who explores the neurobiological processes that underlie anxiety disorders such as anorexia nervosa. Professor Aoki and her team perform this research by looking into how environmental factors can influence how the hippocampus and prefrontal cortex develop in juvenile and adolescent brains.

On the topic of how environmental factors can affect the developing brain, we introduce Professor Irva Hertz-Picciotto at UC Davis, who has gained new insights into how poor nutrition and exposure to environmental pollutants during pregnancy can lead to the development of autism spectrum disorders in the unborn child. Professor Irva Hertz-Picciotto's concern for children has motivated her to launch two large-scale projects called MARBLES and CHARGE, that aim to revolutionise the way we view environmental risks and early markers of autism spectrum disorders. Next, we explore how diet modification can even be used as an effective therapy for autism. By first investigating the mechanisms that make the ketogenic diet so effective for treating epilepsy, Professor Susan Masino and her team at Trinity College, Connecticut, are using what they've learned to apply this diet as a treatment for other neurological conditions, such as autism spectrum disorder.

From here we are plunged into the bewildering world of genetics, where we first look at the genes responsible for epilepsy. Here we introduce Dr David Greenberg and his team at the Research Institute at Nationwide Children's Hospital, who studies the genetic determinants of inherited epilepsy. In particular, his group discovered the gene BRD2, found on human chromosome 6, which is associated with Juvenile Myoclonic Epilepsy – one of the most common forms of epilepsy. In their continued investigations into this gene and its interactions, the root cause of the disease will become clearer, leading to the development of new treatments. Also working at the interface between neuroscience and genetics is Professor Naoko Tanese at New York University School of Medicine, who investigates transcriptional and post-transcriptional gene regulatory pathways. Specifically, Professor Tanese and her team examine the function of the Huntington's disease protein 'huntingtin' and look at how it differs between healthy individuals and those with the disease. This research may reveal new pathways for drug targeting, resulting in new approaches to treat Huntington's disease.

Next, we further explore the nuts and bolts behind neurodegenerative disease, first by introducing a project led by Dr Tamàs Fülöp at the University of Sherbrooke. His team are investigating neglected aspects of Alzheimer's disease in order to find new drug targets, by looking at the role of viral and other infectious agents in the pathogenesis of the disease. Finally, we introduce the pioneering work of Professor Elizabeth Jonas at Yale university, who investigates the mitochondrion's role in neurotransmission. Professor Jonas and her team characterise how channels in the mitochondrial membrane affect neuronal function during processes such as memory formation and learning. Because these processes can go very wrong in neurodegenerative diseases like Alzheimer's or Parkinson's, this research is unveiling new insights into the molecular basis of neurodegeneration.



# THE AMERICAN ACADEMY OF NEUROLOGY

Founded in 1948, and now representing more than 30,000 individuals worldwide, the American Academy of Neurology (AAN) is the world's largest association of neurologists and neuroscientists. From funding and disseminating the latest research, all the way through to encouraging young people to pursue careers in neurology, the AAN's mission is to promote the highest quality patient-centred neurologic care and enhance the career satisfaction of its members. For this edition of Scientia, we had had the pleasure of speaking to **Dr Terrence L. Cascino,** MD, the President of the American Academy of Neurology, who tells us all about the AAN's activities in education, research dissemination, funding, policy making and more.



**Please give our readers a brief introduction to the AAN, and tell us a little but about your mission.**

The AAN was founded in Minneapolis, MN, in 1948 by A.B. Baker, MD, FAAN, chair of the University of Minnesota neurology department. Starting with fewer than 400 members, the AAN now represents more than 30,000 neurologists, with approximately 24,000 in the US and 6,000 international members. We are the world's largest professional association of neurologists, and the leading online resource for neurologists across the globe. The AAN's vision is to be indispensable to our members. Our mission is to promote the highest quality patient-centred neurologic care and enhance member career satisfaction.

**Who are your members, and what are the benefits of being a member of the AAN?**

Our members include medical students, residents and fellows, neurologists in private practice and in academic institutions, and neuroscientists. Some memberships are tiered and customised to the particular needs of those in the profession, such as nurse practitioners, physician assistants, and business administrators. We also have senior members who have retired or are moving toward retirement yet want to stay on top of the latest developments in neurology.

Most member benefits are available to all members, such as significant discounts on conferences, services, and products. Members receive free issues of our suite of *Neurology®* journals, our patient education magazine *Neurology Now®* that they can share with patients, and the monthly member magazine *AANnews®*. We offer a wealth of resources, including free continuing medical education for maintenance of certification, evidence-based practice guidelines and advisories. Our NeuroTracker™ tool provides members with a convenient one-stop shop for tracking both AAN and non-AAN activities, such as CME credits.

We also offer a variety of leadership development programs to nurture talents across a diverse range of members. Our advocacy program provides opportunities to travel to Washington, DC, to take our messages to Congress, and intensive advocacy training to members who want to create change in their practices, institutions, and communities.

**Please tell us about the many ways that the AAN supports neuroscience research. What grants are available for researchers, and what areas of research do you consider the most important?**

For more than 20 years, AAN research awards have been a crucial resource for young, promising researchers and a springboard to future grant support from the National Institutes of Health and other organisations. We recently made a significant expansion of our investment in research, bringing the total amount to $2.8 million for 2016 – and we plan an even larger increase next year. Our research support is made possible by funds from the AAN; the American Brain Foundation; association partners such as the Parkinson's Disease Foundation, the ALS Association, the Tourette Association of America, and the National Multiple Sclerosis Society; partners in the pharmaceutical industry; and from our generous members. This has enabled us to create two new awards for career development and neurology research training, in addition to our scholarships and awards for clinical research training, clinician-scientist development, practice research training, and mentored clinical and population research. We don't rank any as 'most important' as they all serve vital needs in finding cures and treatments for people with neurologic disease and improving delivery of care. Promoting research is a mainstay of our Annual Meeting, where we share as many as 2,700 abstracts with more than 12,000 attendees from the neuroscience community. Our Breakthroughs in Neurology and Sports Concussion conferences also feature important cutting-edge research. We also share this knowledge through our Neurology publications. And we recognise leading researchers – from medical students to legends of the labs – with highly coveted awards at our Annual Meeting.

**Does the AAN facilitate international collaboration? Tell us about**

**how the AAN supports research that may not be entirely US-based.**

The AAN offers a number of significant opportunities to assist and promote international collaboration. Our flagship journal *Neurology* is a prime example: In 2015, about 48% of our papers were from international authors, and the journal publishes international translated editions for Argentinian, Chinese, and Korean audiences. There also is an Indian edition in which the articles are English.

*Neurology: Without Borders* is a regular journal feature and an accompanying web blog that examines neurologic medical education, research endeavors and clinical care in global, resource-limited settings with a forum for timely and relevant communications about the most prescient issues, activities, and developments pertinent to the advancement of neurology in these regions of the world.

The Academy supports a vibrant Global Health Section comprised of interested neurologists, and integrated sessions focusing upon global health issues have become standard at the AAN's Annual Meeting, which attracts thousands of international neurologists. And we recognise the accomplishments of international researchers with fellowships and prestigious scientific awards.

**Describe some of the ways that the AAN supports clinical trials, and how do you help researchers achieve more meaningful outcomes for patients from these trials?**

Clinical trials are featured extensively in Annual Meeting programs, and the AAN promotes peer-reviewed results from clinical trials through the journal *Neurology* and press releases to media. The AAN has advocated for legislation to protect individuals from losing private insurance health coverage for routine medical care when participating in clinical trials for neurologic disease. The Academy also regularly informs readers of *Neurology Now* about opportunities and advances learned in clinical trials; the June/July 2015 issue provided an extensive primer on such trials, explaining what they are and how readers can get involved and make a significant contribution to science.

**Please describe one or two major achievements in neuroscience research that the AAN has been involved with in the last few years.**

The AAN is committed to making a profound difference in the lives of researchers, and in turn making a difference in the lives of patients with brain disease.

The new 2017 AAN Research Program exemplifies our dedication to promoting neurology and neuroscience research and training, marking our pledge to support all types of research across all career levels and discovery stages.

Scientific and medical communities have called the shortage of investigators a crisis that will impact far more than the 50 million Americans currently affected by a neurologic disease. Recognising this critical need for neurology research, the AAN has dedicated its grant-making efforts to fulfilling this need.

**What journals and magazines do the AAN publish, and what is the AAN's position on knowledge dissemination and education?**

One of the main reasons the AAN was founded was to provide continuing education to neurologists to help them stay current on the latest developments in the profession. Published since 1950, *Neurology®* is the premier peer-reviewed journal for clinical neurologists. In recent years, it has become the 'hub' for several 'spoke' journals that serve particular interests of our members, such as *Neurology® Clinical Practice, Neurology® Genetics and Neurology® Neuroimmunology & Neuroinflammation.*

*Continuum: Lifelong Learning in Neurology®* is the official continuing medical education journal of the AAN. It is an in-depth, clinically oriented review journal for the practicing neurologist, residents and fellows, and medical students. Written by authors who are experts in their field, each bimonthly issue contains up-to-date knowledge around a single topic area in neurology geared toward the practicing neurologist.

*Neurology Today®* reports on breaking news, issues, and trends in the practice and science of neurology, reaching more than 20,000 professionals. This twice-a-month magazine delivers credible, up-to-the-minute, balanced, cutting-edge reporting and commentary for today's busy neurology professionals. *AANnews®,* the AAN's monthly member magazine, promotes the Academy's conferences and Annual Meeting; free education opportunities for maintenance of certification; public and regulatory policy updates and grassroots advocacy opportunities; research fellowships and scientific awards; and other news, products, and services designed to enhance the work of our members.

Finally, the AAN also publishes the bimonthly patient education magazine *Neurology Now®,* which provides patients and their caregivers with credible, up-to-the-minute, balanced coverage of the latest advances in neurology research and treatment, all written in concise and easy-to-understand language.

**Please tell us about the 'Neuroscience is…' campaign, and the AAN's position on public outreach.**

Neuroscience Is…™ is the AAN's new four-pronged campaign to build public awareness and demonstrate the importance of neuroscience research in care of neurology patients and the development of cures for brain diseases. The campaign seeks to provide children and young adults with access to education on the brain and nervous system, and inspire a love of neuroscience from an early age, and to encourage a desire in college and medical students to seek careers in neurology, and support residents and fellows pursuing research as a career. Furthermore, it aims to make the connection in the public and in practicing physicians that neuroscience and neurology research are the reasons that cures, therapies, and treatment options are available. Finally, the campaign ensures that the criticality of brain research is so 'in the muscle' of our legislative process that funding for neuroscience is a given each year, and not something which must be fought for and justified each year.

The Neuroscience Is… campaign has four components:

Neuroscience Is…™ Cool is geared toward K-12 students. It incorporates unusual and fun facts about the brain and nervous system to attract the attention of young minds and help shape a lifelong interest in and commitment to the neuroscience field.

Neuroscience Is…™ Rewarding will inspire medical students to select a career in neurology by increasing their awareness of the impact that neuroscience has on the prevention, diagnosis, and treatment of as many as 600 known types of brain diseases.

Neuroscience Is…™ Essential speaks to practicing neurologists and other neuroscience professionals by using compelling facts to demonstrate how research is critical to patient care and day-to-day practice.

Neuroscience Is…™ Critical underscores the simple fact that without support from government agencies, industry, and the general public, there is not enough funding to sustain neuroscience research. Without research, there are no treatments. And without treatments, there are no cures. This initiative raises awareness of the AAN's support of researchers and access to practitioners to conduct trials and trial recruitment.

The AAN hosts a daylong family-friendly Brain Health Fair for the communities where we hold our Annual Meetings, sharing the latest developments in neuroscience with thousands of people living with brain disorders as well as their caregivers and families. We have expert speakers on hand and special activities to encourage young people to better appreciate and care for their brains—and perhaps even become neurologists as well. We also have an annual Neuro Film Festival that solicits and shares on YouTube short, personal homemade videos about the effects of brain disorders on people and the need for more research.

Our outreach continues with our patient magazine *Neurology Now* and the series of disease-specific *Neurology Now* Books that help educate the public – particularly patients and caregivers – about a range neurologic disorders.

**How does the AAN engage with policy makers to ensure patients receive the benefits of ongoing neuroscience research? Tell us about one or two of your achievements in this regard to date.**

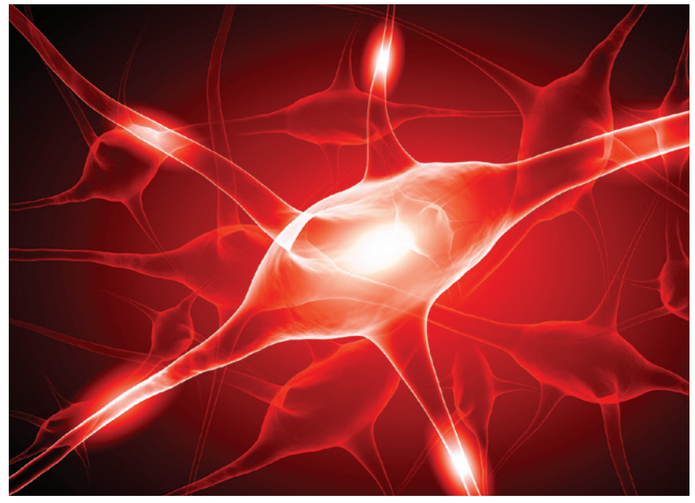
The AAN has a robust US-focused advocacy program that represents neurologists and their patients on local, state, and national levels. We have a strong grassroots involvement of our members, and have had a staffed office in Washington, DC, since 2005. Some of our most significant advocacy 'wins' have focused on lobbying Congress for increased research spending. Just last March, 185 AAN member advocates went to Capitol Hill to ask Congress for increased funding for the BRAIN Initiative at the National Institutes of Health (NIH). In June, the Senate Appropriations Committee approved a $100 million increase for the BRAIN Initiative ($250 million total) for fiscal year 2017. The Senate package also included significant increases in funding for the overall NIH budget by $2 billion to a total of $34 billion, which included a $400 million increase for Alzheimer's disease research ($1.39 billion total). We also have been in the forefront of successfully advocating for greater research and improved treatment for traumatic brain injury and sports concussion.

**Finally, what do you consider to be the biggest challenges currently facing neurology and neuroscience today? How is the AAN working to overcome these challenges?**

Every day, our staff of 160 professionals and hundreds of volunteer members are working to demonstrate the value, quality, and safety of neurology and neuroscience. Research is of paramount importance if we are to have more effective treatments and cures and we are continually advocating for increased funding from outside agencies to support new discoveries and breakthroughs.

We are steadfastly committed to ensuring the viability of various practice types, from solo and small practices to large clinics and hospitals. As our recently completed study of job burnout among neurologists has verified, practicing neurologists are overwhelmed by the constant regulatory hassles that take time away from their patients and rob them of the enjoyment of their careers. This can drive away young, promising medical students and residents who might become neurologists at a time when we are facing significant shortages to treat our aging population. So we have created a new Live Well campaign to help our members with tools and resources to identify, mitigate, and prevent burnout. And we continue to fight for them against unnecessary and overly complicated regulations so they can spend more time and energy with the patients they serve.

**'One of the main reasons the AAN was founded was to provide continuing education to neurologists to help them stay current on the latest developments in the profession'**



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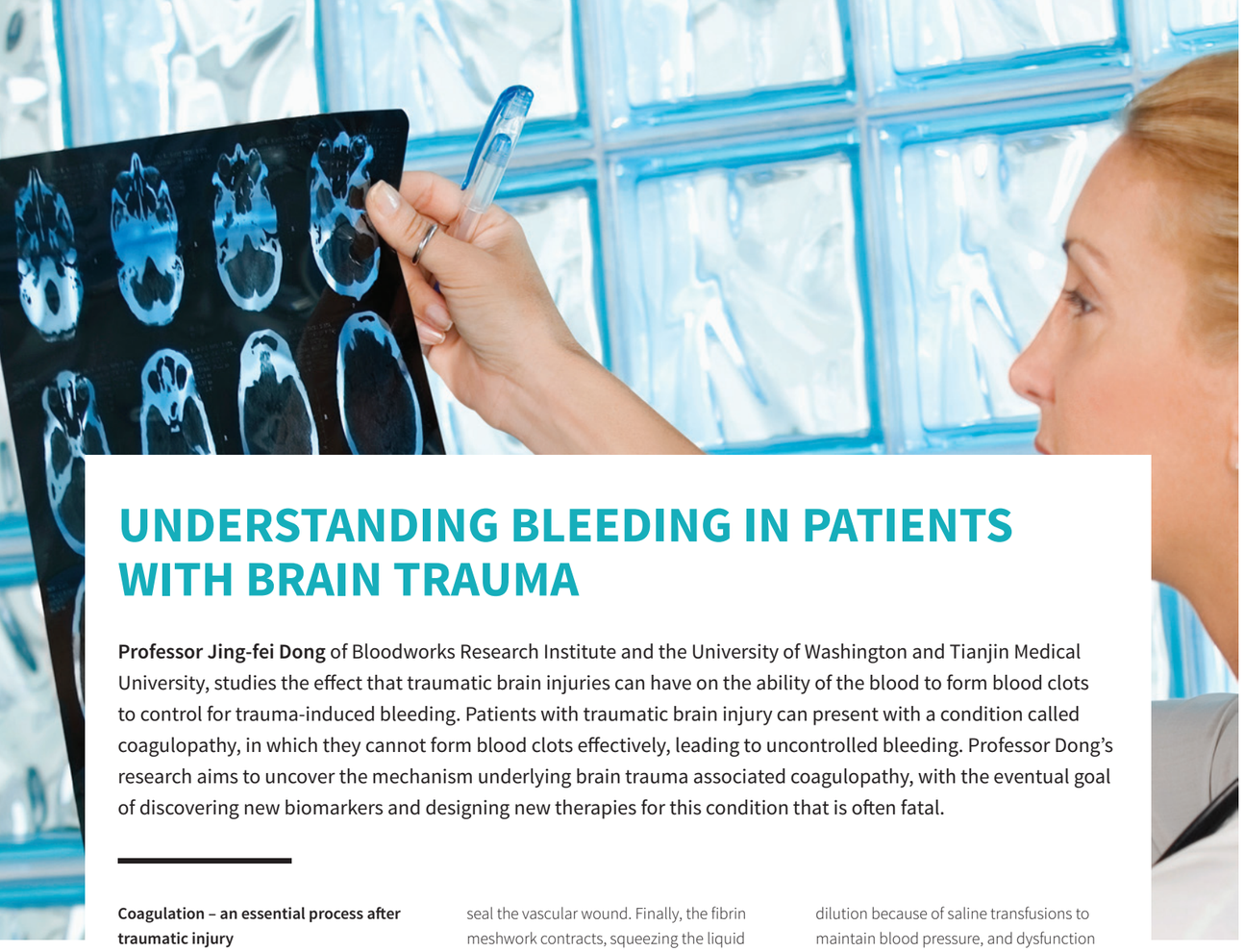


# UNDERSTANDING BLEEDING IN PATIENTS WITH BRAIN TRAUMA

**Professor Jing-fei Dong** of Bloodworks Research Institute and the University of Washington and Tianjin Medical University, studies the effect that traumatic brain injuries can have on the ability of the blood to form blood clots to control for trauma-induced bleeding. Patients with traumatic brain injury can present with a condition called coagulopathy, in which they cannot form blood clots effectively, leading to uncontrolled bleeding. Professor Dong's research aims to uncover the mechanism underlying brain trauma associated coagulopathy, with the eventual goal of discovering new biomarkers and designing new therapies for this condition that is often fatal.

**Coagulation – an essential process after traumatic injury**

Blood clotting, also known as coagulation, is the process by which liquid blood forms a semi-solid or gel-like bolus. This clot functions to stop liquid blood loss from damaged blood vessels, which is a mechanism known as hemostasis. Coagulation is a multifactorial process and is initiated when blood is exposed to tissue underlying the lining of blood vessels. This can occur following blood vessel damage due to trauma, for example. One of the first steps in coagulation is the binding of very small cells known as platelets in blood to the site of injury. The platelets form an initial plug at the site of injury. A complex biochemical cascade subsequently occurs that involves the activation of the platelets, which go on to release a cocktail of substances that are pro-coagulatory. A host of factors sequentially participate in the coagulation reaction. The final outcome is the conversion of fibrinogen, a soluble protein present in blood plasma, to fibrin, which forms at the injury site as sticky threads. The fibrin threads form a sticky mesh, which traps platelets, other blood cells and plasma proteins to make a clot to seal the vascular wound. Finally, the fibrin meshwork contracts, squeezing the liquid in the clot out and helping to make the clot more solid.

Coagulation is particularly important in cases of traumatic injury. Extensive damage to blood vessels during trauma can result in rapid blood loss, through both internal or external bleeding, which can be life threatening. In fact, uncontrolled bleeding causes 30–40% of trauma fatalities. Consequently, it is important that effective coagulatory processes can operate to slow blood loss as rapidly as possible. Trauma is a major cause of death among all adults and is the leading cause of death among younger people (45 years old and younger). Coagulopathy is a major reason underlying uncontrolled bleeding in trauma injuries.

**Coagulopathy – when blood clotting fails**

Coagulopathy is a condition in which the blood is not able to form clots effectively, leading to uncontrolled bleeding. Coagulopathy can occur for a variety of reasons. In trauma patients, coagulopathy can occur as a result of blood loss, blood dilution because of saline transfusions to maintain blood pressure, and dysfunction of components of the coagulatory system itself. Coagulopathy also contributes to, and is enhanced by, low body temperature, and blood becoming metabolically acidic (acidosis). In fact, seriously injured trauma patients can experience what is known as the 'death triad' of coagulopathy, hypothermia and blood acidosis, where a vicious cycle of these processes is self-perpetuating and difficult to treat, ultimately resulting in death. Professor Dong tells Scientia about the current clinical situation and lack of new therapies: 'Doctors are left with very limited treatment options, especially compared with the recent rapid advances in the diagnosis and treatment of cancer, heart attack and stroke.' Therefore, it is imperative to find effective ways to rapidly diagnose and treat coagulopathy.

**Coagulopathy as a result of brain trauma**

The brain is a particularly serious location to obtain a traumatic injury. Traumatic brain injuries are a significant source of disability and death. Professor Dong explains the scale of the problem and the major sources of traumatic brain injury: 'While it has often been associated with wars in a historical context, traumatic brain injury has increasingly been linked to modern society as it is often caused by road traffic accidents, construction accidents, sport injuries and violence. In 2013 alone, about 2.5 million emergency department visits, hospitalizations, and deaths in the United States were associated with traumatic brain injury, either alone or as a part of polytrauma that also involves the trunk and limbs. More importantly, patients with traumatic brain injury have very high rates of mortality and morbidity. However, the pathogenesis of traumatic brain injury and its complications remain poorly understood.'

Patients who suffer traumatic injuries of the brain also commonly suffer coagulopathy, which can complicate their recovery and increase their chances of death. Incidences of coagulopathy are reported to vary from 10–97% in such patients. However, interestingly, the major risk factors for coagulopathy in traumas of the trunk and limbs, namely, significant blood loss and blood dilution as a result of saline transfusions, are not typically present in patients with isolated brain trauma. Injuries of the brain produce significantly less blood loss, and fluid transfusions are often restricted to prevent brain swelling. However, brain trauma patients can go on to develop coagulopathy nevertheless, suggesting that the pathogenesis of brain injury-derived coagulopathy and that caused by injuries of the limbs and trunk are different. The mechanism of traumatic brain injury in producing coagulopathy remains incompletely understood and this is what drives the research of Professor Dong. 'Our research could provide new insights into the pathogenesis of coagulation and other complications secondary to traumatic brain injury and lead to new and targeted therapeutics that can improve the survival of patients and their quality of life,' he explains. The lack of mechanistic insight into this traumatic brain injury-derived coagulopathy has prevented early detection, prevention and effective treatment to date.

**The basis for brain trauma-induced coagulopathy**

Professor Dong's work has focused on the biochemical factors involved in coagulation and how they relate to brain trauma. Several of these molecules are highly expressed in cells of the brain. These include platelet-activating factor, tissue factor and anionic phospholipids. When brain tissue is injured through trauma, the blood-brain barrier (a specialized form of tissue lining the blood vessels of the brain) is compromised and brain tissue components can be released into systemic circulation. These pro-coagulatory molecules can then initiate diffuse coagulation elsewhere in the body. As a result, platelets and coagulation factors are used up, leading to a deficit in the areas they are needed and potentially resulting in localised defects in hemostasis at the areas of vascular injury. This is known as consumptive coagulopathy. In particular, Professor Dong is interested in how these factors are released from the cerebral tissue, how they travel about the body and how they cause coagulation by interacting with other biochemical factors in the blood.

**'Our research could provide new insights into the pathogenesis of coagulation and other complications secondary to traumatic brain injury and lead to new and targeted therapeutics that can improve the survival of patients and their quality of life'**

**Cellular microparticles**

Cellular microparticles are small (under 1

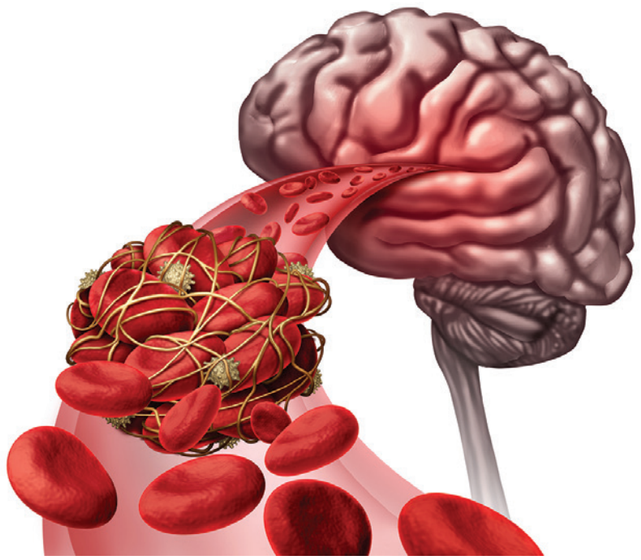
μm in size) cell fragments, composed of cell membrane and cellular organelles, that are shed and released by cells undergoing programmed cell death (this process is called apoptosis) or cells undergoing a process called microvesiculation. Such cellular microparticles are rich in signalling molecules that can affect coagulation. Professor Dong and his team hypothesised that brain-derived cellular microparticles may be a key mediator of systemic clotting abnormalities under conditions of brain trauma. The team thought, that through their release into systemic circulation and interaction with coagulation factors in plasma, brain-derived microparticles could produce consumptive coagulopathy.

**Key results to date**

In 2015, the team showed that producing traumatic brain injuries in mice resulted in the systemic release of brain-derived cellular membrane microparticles that expressed pro-coagulatory molecules such as tissue factor on their surfaces. The microparticles were responsible for coagulation abnormalities present after brain trauma. This finding comprised a novel mechanism for brain trauma-induced coagulopathy.

In addition to membrane-derived microparticles, more recently the team have discovered organelle-derived cerebral microparticles that are released into the systemic circulation of mice following brain trauma. Specifically, they found mitochondrial microparticles in the systemic circulation of such mice. The mitochondrion is a cellular organelle primarily involved in energy production in intact cells. The team found that a phospholipid called cardiolipin on the surface of the mitochondrial microparticles was responsible for producing systemic coagulation in the mice. In addition, they found that the mitochondria present in such microparticles produce reactive oxygen species, which cause the activation of platelets and also act as a source of oxidative stress, causing inflammation.

With the exciting discovery of this mechanism of brain trauma derived coagulopathy, the team hope that the microparticles and signalling molecules they have identified could be used as biomarkers and potential drug targets in the treatment of coagulopathy. In the short term, they plan to see if the removal of cellular microparticles from systemic circulation can ameliorate the colagulopathy associated with brain trauma.



**Motivation and future outlook**

Professor Dong shared with us what initially interested him in this type of research: 'My medical training is in neurosurgery and I had practiced it for 10 years, with strong clinical and research interests in traumatic brain injury. As a neurosurgeon, I have encountered numerous cases where coagulation abnormalities, not traumatic brain injury, claimed patients' lives so I am fully aware of this severe complication of traumatic brain injury and its impact on patients' recovery. This bleeding problem in part contributed to my desire to study platelets and adhesion molecules for thrombotic and bleeding disorders at the Gladestone Research Institute of Cardiovascular Disease, University of California at San Francisco.' Professor Dong also explains what makes this research interdisciplinary and unique: 'While studying hemostasis was intended to be a short-term training experience and a side project, it somehow evolved into a full-time research career in hemostasis and thrombosis. I am, therefore, in a unique position to study traumatic brain injury-associated bleeding abnormalities because of my training in two seemingly very different medical specialties: neurosurgery and hemostasis.'

**'In 2013 alone, about 2.5 million emergency department visits, hospitalizations, and deaths in the United States were associated with traumatic brain injury'**

The team hope to apply what they have learnt so far to discover even more about brain trauma-induced coagulopathy with the goal of developing therapies and finding new biomarkers. 'Using knowledge that we have gained so far, we are actively investigating the contributions of other brain-derived molecules to the pathogenesis of traumatic brain injury-associated clotting deficiency.' Professor Dong summarises. 'We are also conducting several state-of-art experiments to identify new markers to predict the development of the clotting abnormalities found in patients with traumatic brain injury. The biomarker development will also help us develop new and targeted therapeutics that can significantly improve the outcomes of patients who suffer from traumatic injury and associated coagulopathy.'



# Meet the researcher

**Professor Jing-fei Dong**
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Professor Jing-fei Dong is a Professor of Medicine in the Division of Hematology in the Department of Medicine at the University of Washington and is an Adjunct Professor at Tianjin Medical University and at the Section of Cardiovascular Sciences in the Department of Medicine at the Baylor College of Medicine. He has expertise in neurosurgery and hemostasis, with a particular focus on clotting disorders under conditions of traumatic brain injury. He is involved in the search for new biomarkers and therapies for brain trauma-induced coagulopathy. He is the recipient of an award for Excellence in Research, from Baylor College of Medicine and an Established Investigator Award from the American Heart Association This project has received funding from the National Institute of Health in the U.S. and the Nature Science Foundation in China.

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UNIVERSITY of WASHINGTON

# STAYING IN CONTROL: HOW THE PREFRONTAL CORTEX HELPS US BE HUMAN

**Professor Amy Arnsten** and her team at Yale University have pioneered new insights into the unique ways that the prefrontal cortex is regulated at the molecular level, altering our ability to remember, pay attention, and control our thoughts and actions when we feel stressed and out of control. This research has led to two medications now in human use: guanfacine for the treatment of ADHD and other prefrontal cortical disorders, and prazosin for the treatment of Post-Traumatic Stress Disorder.

Professor Amy Arnsten found inspiration early for what would later become her life's work studying the complex role of the brain in human cognition. 'As a teenager back in the 1970s, I had the opportunity to volunteer in Greystone Park State Psychiatric Hospital in New Jersey,' she recalls, 'I was able to see that mental health care was still in the middle ages compared to fields like cardiology, and realised that there was an urgent need for scientific understanding of the brain circuits underlying mental disorders in order to develop more rational strategies for treatment. Moreover, I was able to work closely with patients, and learn about their symptoms and their needs.'

'One particularly inspiring moment occurred when I was speaking with a gentleman with schizophrenia, who had been an astrophysicist. When we spoke about astronomy, he was completely coherent and at ease. But when I simply mentioned the name of a cruel doctor, he instantly disintegrated into "word salad", the loose associations of thought disorder. In that moment I knew that understanding how chemical actions during stress alter higher brain circuits would be a major clue in understanding the symptoms of schizophrenia. I have been studying how molecular events during stress alter brain function ever since.'

A professor of neuroscience at Yale University and a leading researcher in the molecular and neurobiological components of human cognition, Professor Arnsten has authored over one hundred fifty research papers and book chapters, and is a recipient of a prestigious Pioneer Award from the Director of the National Institutes of Health, and of the 2015 Goldman-Rakic Prize for Outstanding Achievement in Cognitive Neuroscience. Her research had yielded ground-breaking new insights into how the arousal systems rapidly change the strength of neural connections in the prefrontal cortex to alter our higher cognitive abilities, and how genetic or environmental insults to this process contribute to cognitive deficits in mental illness and in age-related disorders.

**The prefrontal cortex**

To understand the higher cognitive functions of the brain, researchers such as Professor Arnsten have turned their attention to the prefrontal cortex (PFC), that region of the brain located just behind our forehead. Neuroscientists such as Professor Patricia Goldman-Rakic identified the key role that the prefrontal cortex plays in working memory, i.e., the ability to keep in mind an event that has just occurred and also to retrieve information from long-term memory to control how we think, feel and believe. Also referred to as our mental 'scratch pad', the prefrontal cortex allows us to carry out a wide range of complex short-term memory tasks. When you are attempting to hold a telephone number in your head for long enough to make a call, that's your working memory in action.



Working memory depends on networks of neurons in the prefrontal cortex that excite each other to generate mental representations that keep information "in mind" in the absence of sensory stimulation. These mental representations are the fundamental building blocks of abstract thought and reasoning, contributing to executive functions such as planning and organisation, and metacognitive processes such as insight about oneself and others. Even language production requires the prefrontal cortex, in a specialised region called Broca's area. However, these higher cognitive abilities are impaired when the connections between prefrontal neurons are weakened, for example due to chemical changes during uncontrollable stress.

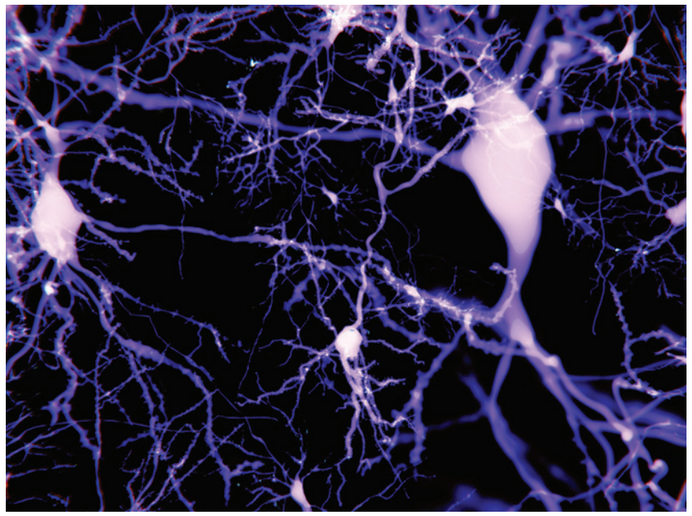
**How our state of arousal dramatically alters our higher cognitive abilities**

Our mental state changes dramatically during different arousal states, for example, we are unconscious during deep sleep, and aware and responsive when awake. Research has shown that the arousal systems change the brain's chemistry to help coordinate events in our environment with our brain's mental state. Arnsten's lab has shown that these changes in arousal chemicals can produce rapid changes in the strength of prefrontal cortical neuronal connections that dramatically alter our cognitive abilities.

For example, during deep sleep, many of our arousal systems are turned off, and prefrontal networks are unable to connect. One of the main arousal systems releases a chemical called acetylcholine, and the Arnsten lab has shown that acetylcholine stimulation of nicotinic alpha-7 receptors is required for prefrontal neurons to excite each other. The absence of acetylcholine during deep sleep and the subsequent loss of prefrontal neuronal firing may explain why we are unconscious during this stage of sleep.

The arousal systems turn on when we are awake, and they release chemicals that strengthen prefrontal networks connections and cognitive function. Some of the arousal systems, such as the cells that make acetylcholine, norepinephrine or dopamine, are known to give a small burst of chemical release when there is an interesting event in the environment. For example, modest levels of norepinephrine release can stimulate alpha-2A receptors which strengthen prefrontal connections and enhance prefrontal network firing. This leads to improved working memory, attention and impulse control. Similarly, acetylcholine is released in response to rewarding events, and as described above, strengthens prefrontal connections. Interestingly, caffeine enhances acetylcholine release, and nicotine in cigarettes stimulates nicotinic alpha-7 receptors, suggesting that these common agents may help us to work better by strengthening prefrontal cortical functions.

**'By understanding the needs of prefrontal neurons, we can design strategies that protect and strengthen these circuits'**



In contrast to arousal states when we feel safe and in control, during uncontrollable stress there are high levels of dopamine and norepinephrine release in brain, chemicals in the catecholamine family that are similar to epinephrine (adrenaline). These high levels of catecholamine release weaken prefrontal connections and impair cognitive abilities, but also strengthen many of the more primitive brain areas, such as the circuits that process sensory information, mediate motor habits or evoke emotional responses. For example, high levels of norepinephrine release engage alpha-1 receptors that cause prefrontal cells to stop firing, but strengthen the functions of the amygdala, an older brain region that evokes unconscious emotional reactions. Thus, the release of high levels of catecholamines during uncontrollable stress rapidly switches the brain from a thoughtful, reflective state to a more unconscious, reflexive state. This may save our lives when we are in danger, but it is often unhelpful when we need top-down control to properly guide our actions and decisions. Interestingly, even an acute mild stressor can cause these brain changes if the stressor makes you feel out of control.

**Relevance to cognitive disorders**

A large number of cognitive disorders involve dysfunction of the prefrontal cortex. For example, the impaired attentional and impulse control of Attention Deficit Hyperactivity Disorder (ADHD) has been associated with slower growth of the parts of the prefrontal cortex that help us inhibit inappropriate responses. These same prefrontal areas are weaker in bipolar disorder during the manic phase of the illness, when patients lose insight and impulse control. Many disorders, including bipolar disorder and schizophrenia, are aggravated by stress, and others such as Post-Traumatic Stress Disorder (PTSD) can be caused by exposure to a traumatic stressor.

Professor Arnsten's research suggests that chemical changes in the prefrontal cortex during stress may contribute to a number of these cognitive disorders, particularly when there are genetic or environmental insults that erode the brain's ability to stop the stress response. For example, many of the genetic insults linked to the increased risk of schizophrenia target molecules that normally rein in the stress response in prefrontal cortex, and stress is known to increase symptoms of thought disorder and to precipitate descent into illness. The aging process also afflicts molecules that normally stop stress signals in prefrontal cortex, and this may contribute to phosphorylation of tau, one of the pathological events in Alzheimer's Disease. Thus, learning about how arousal chemicals influence these higher brain circuits is providing clues about what may cause cognitive disorders. And research in the Arnsten lab has already identified two extremely promising new treatments that are currently in human use.

**Promising new treatments**

The first of these, prazosin, is an alpha-1 adrenergic receptor blocker that was originally developed to treat hypertension. As described above, high levels of norepinephrine release during stress stimulate alpha-1 receptors that impair prefrontal function and strengthen amygdala. Prazosin can block these effects of stress, and is now in widespread use to treat PTSD in veterans, active military personnel and in civilians such as victims of rape.

A second treatment developed by Professor Arnsten's group involves the use of guanfacine for a variety of disorders that involve dysfunction of the prefrontal cortex. Guanfacine is a selective alpha-2A-receptor agonist that was approved by the Food and Drug Administration under the brand name Intuniv™ for the treatment of ADHD in children aged 6 to 17. By acting on post-synaptic alpha-2A-adrenergic receptors in prefrontal cortex, guanfacine helps to strengthen prefrontal network connections and boost working memory, reduce distractibility and regulate impulse control. Animal studies show that guanfacine also protects the prefrontal cortex from emotional or physiological stress (e.g. hypoxia), protecting prefrontal cortical gray matter. These actions may explain why guanfacine is useful in treating children who have been traumatised or abused.

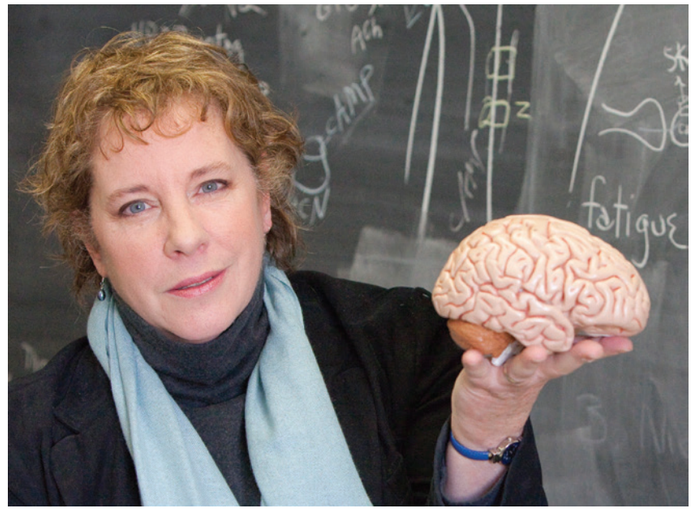
**'We are now trying to understand how dysregulation of stress signaling pathways leads to loss of cortical gray matter and to the phosphorylation of tau, a key step in neurodegeneration in Alzheimer's Disease'**

**Future research**

When asked about what lay in the future for her research, Professor Arnsten seems as determined as ever to continue finding better forms of mental health care. 'We are now trying to understand how dysregulation of stress signaling pathways leads to loss of cortical gray matter and to the phosphorylation of tau, a key step in neurodegeneration in Alzheimer's Disease,' she explains, 'we are trying to understand why certain highly interconnected neurons are so vulnerable to degeneration, for example in schizophrenia and Alzheimer's Disease, while other neurons (such as in the primary visual cortex) are quite resistant to degeneration. We are finding that there are striking differences in their molecular regulation that may provide important clues to what makes a neuron vulnerable vs. resilient in the face of stress and aging.'

As Professor Arnsten pointed out in a recent paper, the impact of stress on the networks of the prefrontal cortex is an increasing challenge in the 'information age' when we need our higher cognitive functions to thrive. This is particularly a challenge for an aging society, when higher cortical circuits are at particular risk. More than ever before, we need our brains to function at peak efficiency in order to think and behave effectively. Having a better understanding of how the prefrontal cortex responds to molecular events may give us the tools to protect these invaluable brain circuits.






# Meet the researcher

**Professor Amy Arnsten**
Professor of Neuroscience and Psychology
Yale School of Medicine
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Professor Amy Arnsten is a full professor of neuroscience and psychology at Yale University as well as a member of Yale's Kavli Institute of Neuroscience with cross-appointments to Yale's Child Study Center and the Albert J. Solnit Integrated Training Program. A native of Maplewood, New Jersey, she received her undergraduate degree in Neuroscience from Brown University in 1976, and went on to complete her doctorate in Neuroscience from the University of California at San Diego in 1981. After completing postgraduate work at Cambridge and Yale under the supervision of the legendary research Patricia Goldman-Rakic, she became part of Yale's vibrant neuroscience faculty. A leading researcher in the neural basis of higher cognition, she has authored more than 130 papers exploring stress-related and age-related neurological disorders as well as the molecular basis of memory and emotion. Research conducted at her laboratory has yielded critical insights into prefrontal cortical functioning as well as improved treatments using prazosin and guanfacine for ADHD, PTSD and dementia.

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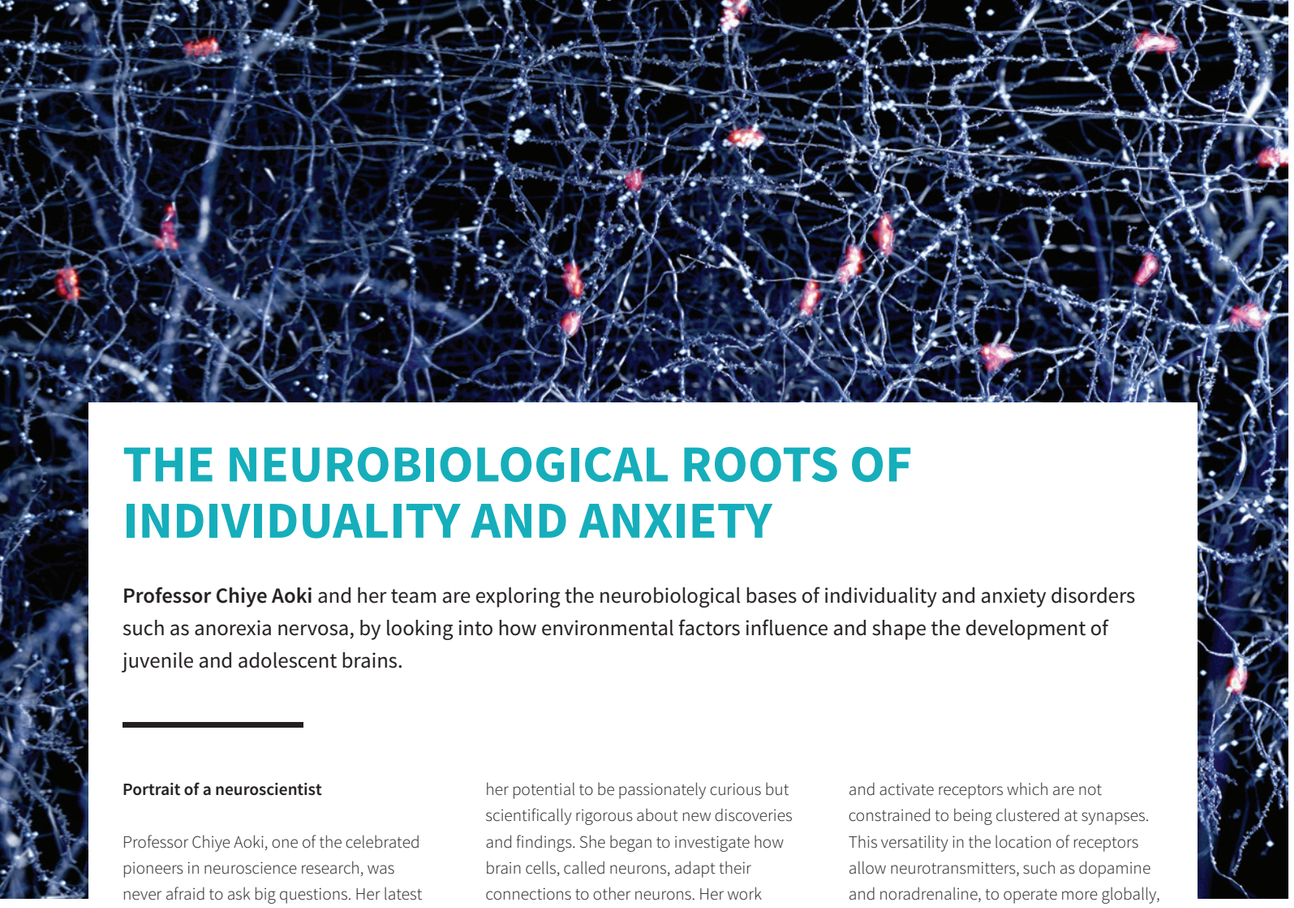
Yale University



# THE NEUROBIOLOGICAL ROOTS OF INDIVIDUALITY AND ANXIETY

**Professor Chiye Aoki** and her team are exploring the neurobiological bases of individuality and anxiety disorders such as anorexia nervosa, by looking into how environmental factors influence and shape the development of juvenile and adolescent brains.

**Portrait of a neuroscientist**

Professor Chiye Aoki, one of the celebrated pioneers in neuroscience research, was never afraid to ask big questions. Her latest research focuses on how changes at the synapse relate to resilience or vulnerability to anorexia. However, her interest in neuroscience was spiked by an interesting observation she made regarding her childhood years. She first learned to speak in the United States but she later moved to Japan for a few years. Much to her surprise, she had to relearn English upon her return to the US. However, this adaptation was much easier for her, still a child, than it is for adults. To her it became clear that changes within the maturing brain must be the cause of such differences. This observation placed her onto the path of asking deeper questions, regarding how human individuality – the self – is shaped by experiences and what might be the neurobiological basis of this process. By asking such questions, she seeks to understand the physical changes in the brain and the mechanisms that shape the character of the sense of self.

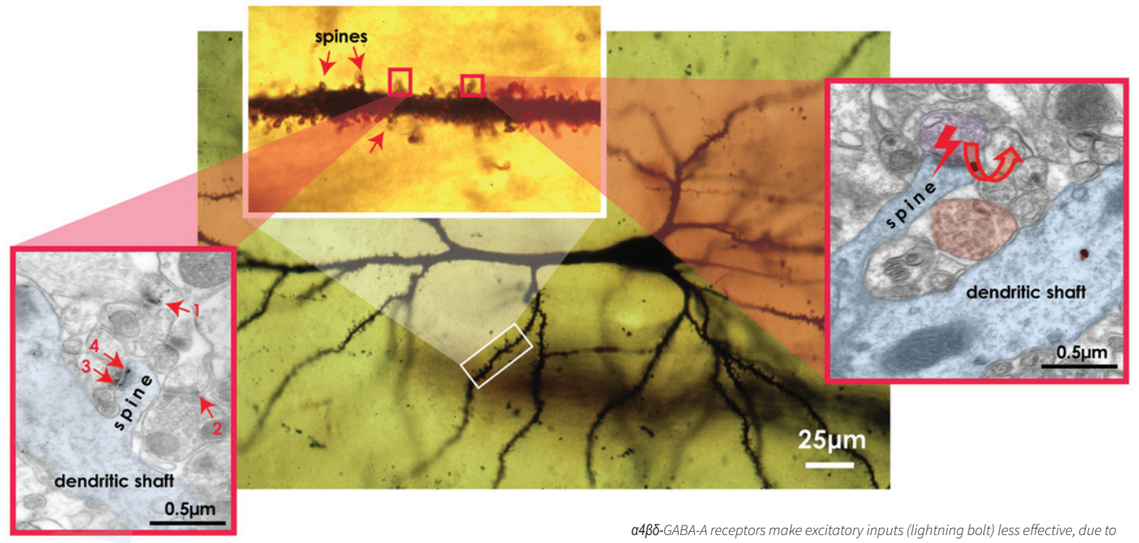
During her undergraduate years, Professor Aoki thrived in a climate where neuroscience was a still developing science. She enjoyed being able to challenge the existing knowledge and make new discoveries about neuroscience. This approach shaped her view of the field and helped her understand her potential to be passionately curious but scientifically rigorous about new discoveries and findings. She began to investigate how brain cells, called neurons, adapt their connections to other neurons. Her work led to finding a correlation between how malleable the shape of a neuron is and how adaptable a neuron's connectivity to other neurons is – a term called neuronal plasticity. This functional link is particularly interesting because it acts during a period critical for the development of the visual cortex and language acquisition regions, thus disruptions in neural activity in these areas have life-long consequences. For her discovery, she received her PhD degree in 1985 from The Rockefeller University.

During her postdoctoral fellowship with the Cornell University Weill Medical College in the laboratory of Dr Virginia Pickel, Professor Aoki learned how to view molecular interactions at synapses, which are structures specialised for neuron-to-neuron connections. Synapses, although so vital for brain function, are extremely small, requiring an electron microscope to magnify the structure at least 20,000 fold to be viewable. The techniques involving the use of electron microscopes enabled her to observe the behaviour of new or established synapses and helped her make some interesting discoveries. One of her findings was that neurotransmitters such as dopamine and noradrenaline propagate over the gaps between neurons and activate receptors which are not constrained to being clustered at synapses. This versatility in the location of receptors allow neurotransmitters, such as dopamine and noradrenaline, to operate more globally, rather than through pathways specified by point-to-point synaptic connections between neurons. Such global actions of neurotransmitters may be the key to their role in affecting mood or vigilance, rather than relaying specific events or features that an individual also experiences through synaptic activity.

Another discovery she made involved a separate group of brain cells called glia, which enshroud, protect, and feed neurons. She found that these cells participate in the conversion of glutamate, an excitatory neurotransmitter, into its non-toxic form, glutamine, which plays a role in the synthesis of proteins and production of cellular energy. Later on, she researched nitric oxide, a very important molecular compound with a role in cardiovascular signalling and an extremely short lifetime, which makes it difficult to study. In fact, nitric oxide was so difficult to find that its discoverer was awarded the 1998 Nobel Prize in Physiology. Professor Aoki found that nitric oxide can be generated by neurons with glutamate receptors – called NMDA receptors. Following this line of research, she found that glutamate receptors can be found on both ends of a synapse and that they aggregate in clusters on the main



*Spines, seen by light microscopy at a magnification of 1,000x. Both light micrographic images were created by Professor Dominick P. Purpura, Professor Emeritus of Neuroscience at Albert Einstein Medical College and past president of the Society for Neuroscience*




*The presence of NMDA receptors at excitatory synapses is revealed by first using antibodies that specifically recognize only those proteins that form these receptors. The binding of antibodies to the NMDA receptors at excitatory synapses are then detected by electron microscopy at a magnification of 40,000x, which enables detection of colloidal gold particles that have been linked to these antibodies (tiny dark spots 1, 2, 3 and 4).*

*α4βδ-GABA-A receptors make excitatory inputs (lightning bolt) less effective, due to shunting inhibition (arrow, indicating leakage of the excitatory synaptic current). The presence of α4βδ-GABA-A receptors at excitatory synapses is revealed by using a similar antibody-colloidal gold method for electron microscopy, but this time, using antibodies that specifically recognize only those proteins that form α4βδ-GABA-A receptors (dark spot under the arrow).*

**Professor Aoki's research paves the way for new treatments for anorexia nervosa, and contributes to a better understanding of the neurobiological bases of plasticity.**

trunks of neurons, called dendritic shafts, during early life, clearly before they specialise into forming a synapse.

To the present day, her research uses electron microscopy to gaze into the processes going on between transmitters and receptors – processes having potential roles in synaptic plasticity. The phenomenon of synaptic plasticity is a response to the level of activity of neurons. In one of the many types of plasticity, synapses can strengthen when they are used and weaken when the activity level decreases. This variation of the synapse strength with activity level is what makes cognition a 'use it or lose it' type of process and forms the basis of the importance of education and repetition in improving intellectual ability. Through observations made using electron microscopy, Professor Aoki was able to confirm that neurotransmitter receptors are brought to the neural membranes on both ends of a synapse in a way that depends on activity. Moreover, neurons show a measurable change in response to activity in less than ten minutes in adult brains.

More recently, Professor Aoki and her team have been exploring the hypothesis that the proteins that form filaments to maintain the shape of cells have a role in the circulation of synaptic molecules within dendritic spines, the small protrusions of a dendrite specialised to receive input from other neurons. For this reason, the team is researching what happens when two molecules are present or absent from specific neural sites. These molecules, drebrin and neurexin, help recruit synaptic molecules and enroll them into forming new synapses. Neurexin is a protein mostly found in the cell membrane of the sending axon terminal – the presynaptic membrane – while drebrin is hypothesised to play a role in plasticity on the receiving, dendritic side of synapses.

Professor Aoki's research is also focused on the molecular and cellular mechanisms involved in acetylcholine and gamma-aminobutyric acid (GABA) regulation. Acetylcholine is a neurotransmitter present in both the central and peripheral nervous systems, which activates muscles and plays a key role in the regulation of attention and memory within the brain. GABA, on the other hand, functions as an inhibitor of neuronal excitability in the brain, which helps to refine brain function by counter-balancing excitation and also prevents epilepsy and

uncontrolled anxiety. Professor Aoki's interest is to understand why there are so many types of GABA and acetylcholine receptors in the brain. Moreover, she wants to understand how these receptors regulate anxiety through modulation of neuronal pathways in three key regions of the brain - the hippocampus, prefrontal cortex and amygdala. One possibility she has considered is that GABA and acetylcholine receptors are distributed along neuronal pathways formed by excitatory neurons. The neuronal pathways formed by excitatory neurons enable specific processes in each region of the brain, such as for the motor cortex to generate very precise visually guided behaviour, such as a baseball player to hit a homerun. If so, variations in GABA and acetylcholine receptor distribution along an excitatory pathway would allow for modulation (boosting or dampening) of that pathway, thereby providing much more flexibility and individuality to our thought processes, perception, behaviour, and decision-making.

To test her working hypothesis, Professor Aoki and her team are feeding neuronal sites with neurotransmitters that modulate neuron activity. To analyse their findings, the scientists are statistically comparing the measured response in neural activity with the map showing the distribution of receptors across neurons belonging to the activated pathway.

**Measuring the impact of stress on the young brain**

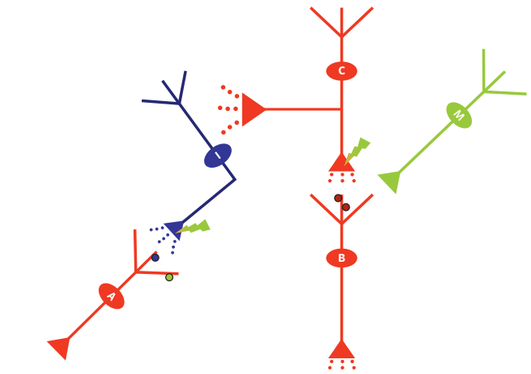
As Professor Aoki explains, plasticity is a term widely used in neuroscience to describe many types of phenomena. For example, plasticity is used to describe the reorganisation of neuron-to-neuron connections in large areas of the cortex, or axons' regeneration, the refresh rate of neurotransmitter receptors, creation of new neurons, regulation of neurotransmitter secretion, and so on. This wide applicability of the term is due to the realisation that the brain actively adapts to the environment and its own activities, or acts such that it can compensate for damage or prevent overdrive of the nervous system.

The phenomenon of plasticity is extremely useful in learning, because it allows for the creation of new pathways and strengthens existing ones – for example when learning a new language, which is an example particularly interesting for Professor Aoki. Yet the plasticity of the brain can be hijacked by harmful processes, such as addiction or stress, and can be vulnerable to the action of hormones. When a behavioural pattern becomes fixed in the brain, it can lead to stress reactions even in the absence of a stressful stimulus, based only on previous experience. Traumatic stress, for example, can determine individuals to overreact to otherwise innocuous environments and triggers.

Brain plasticity is more prominent in young individuals. Moreover, it plays an important role in the formation of the future adult brain. For this reason, Professor Aoki used a clever experimental setup to study the effects of the vicious circle of stress and anorexia nervosa. She published her results in a paper called 'Synaptic changes in the hippocampus of adolescent female rodents associated with resilience to anxiety and suppression of food restriction-evoked hyperactivity in an animal model for anorexia nervosa'. Her working hypothesis was that a GABA-dependent mechanism within the hippocampus controls the regulation of anxiety in individuals – anxiety which has a strong influence on the vulnerability of the respective individual to activity-based anorexia (ABA).

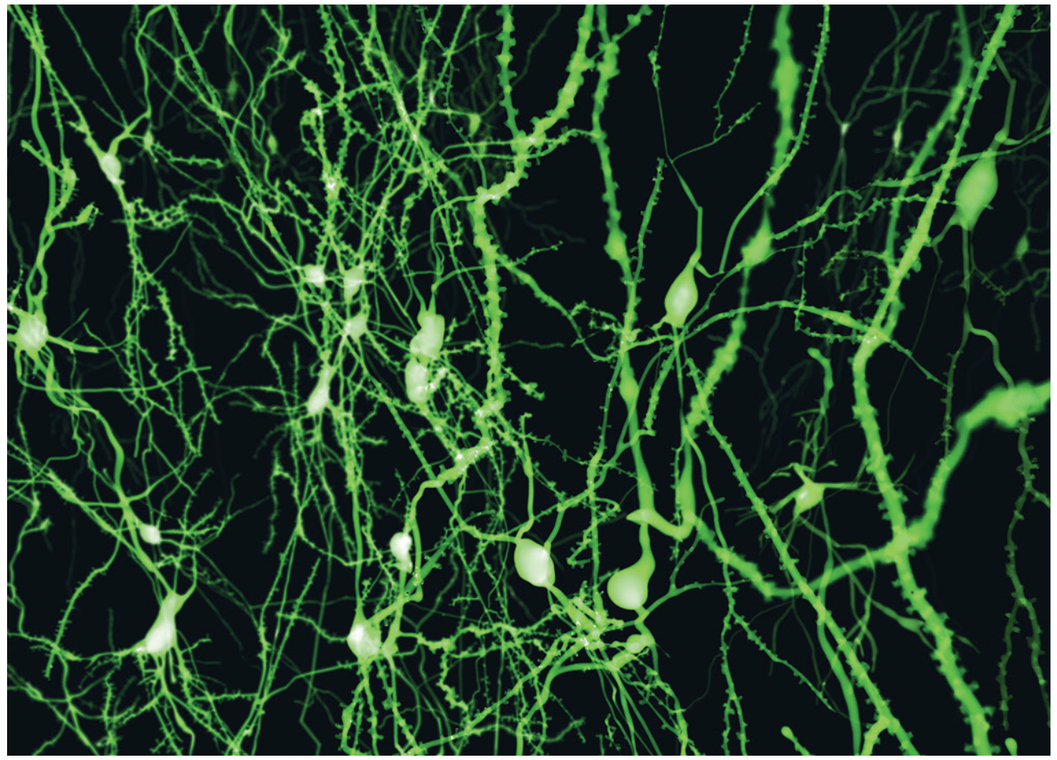
Anorexia nervosa is a mental illness affecting mostly adolescents, with a mortality rate 200 times larger than that of suicide. The disease is known to affect females nine times more often than it affects men, suggesting that sex hormones may play a role in this condition. The current research points to a paramount importance of the prefrontal cortex, amygdala, striatum, and hypothalamus regions of the brain, and anomalies in the function of neurotransmitters such as dopamine and serotonin as most prominent causes of the disease. Professor Aoki's contribution has been to hypothesise GABA action in the hippocampus and prefrontal cortex as additional important factors in the risk of developing anorexia nervosa.

Anorexia nervosa manifests through heightened anxiety, extreme abstaining from food despite hunger, intense fear of gaining weight, and a preference for excessive exercise. Since there is no pharmacological cure for this condition and the relapse rate is larger than 25%, Professor Aoki decided to use an animal model of the illness so that she was able to study brain changes in her subjects, which were adolescent female rodents. The ingenuity of her setup was that it exposed rodents to two bouts of activity-based anorexia separated by a period of recovery, thus mimicking the natural course of the illness. In the first stage, the rodents were introduced to a running wheel. After that, their access to food was restricted to only 1-2 hours per day. The researchers made two important observations. Firstly, the rodents became extremely anxious and began preferring to run excessively on the running wheel even when they had access to food, instead of eating. Secondly, many, but, importantly, not all of the rodents became excessive runners: those that did were the more anxious types that lost the greatest amount of body weight.




**A response** **An Alternative response**

*Red neurons are excitatory, providing a neuronal pathway through synaptic connections that generate behavior. The blue neuron is inhibitory. The green neuron is modulatory. In this schematic, Excitatory Neuron A generates a simple response, whenever excited. However, this response can be suppressed through a higher command neuron, C, that activates an inhibitory neuron, I, that is set in place to inhibit Neuron A. The command neuron also excites an alternative excitatory neuron B that generates an alternative response. The neuromodulator can activate one type of receptor (green lightning bolt) to boost neurotransmitter release, thereby favoring the elicitation of the alternative response. The same neuromodulator can active another type of receptor (green ball on neuron A) to suppress excitability of Neuron A. Receptors for neuromodulators are not constrained to reside at synapses. This allows for neuromodulators to operate more widely and in a varied fashion, sculpting overall behavior of an individual.*



For a better understanding of the results, we should recall that GABA inhibits neural excitability, whereas the hippocampus plays a central role of enabling memory to form for long periods. Besides that, the amygdala, together with the hippocampus, processes emotional reactions while prefrontal cortex is involved in decision-making processes. Professor Aoki found that one type of GABA receptor, called α4βδ-GABA-A receptor, is particularly well-correlated with the rodents' propensity to give up running on the wheel, so as to feed more efficiently during the limited hours of food availability. It is a type of GABA receptor that is not receptive to benzodiazepines, like Valium. This may explain why benzodiazapines have not been so effective in treating anorexia nervosa, even though they are usually effective in reducing anxiety. Her work also suggests that drugs favouring the recruitment of the special type of GABA receptors found in brains of the more efficient feeders may help treat the more vulnerable individuals.

Although the setup cannot capture the complex social causes leading humans to anorexia nervosa, such as peer pressure to be thin, it is extremely valuable because the neurobiological changes in the rodents' brains should capture changes that are similar in human brains triggered by self-imposed food restriction and excessive exercise. Even though previous studies creating animal models of anorexia are available, the novelty of this research is that it specifically considers the role of anxiety, plasticity, and chemical regulation during the development of the young hippocampus and prefrontal cortex.

Another finding was that the number of NMDA-type glutamate receptors associated with excitatory neurons in the hippocampus varies proportionally with the weight loss. Therefore, Professor Aoki and her team were able to propose that, at least in some cases, the excitability of glutamate receptors associated with anorexia can be dampened with the help of GABA.

Many have asked Professor Aoki why rodents choose to run, especially when they are hungry. This seems paradoxical. Why don't animals quiet down, so as to conserve energy? Professor Aoki speculates that these hungry rodents might be exhibiting a form of foraging behaviour. Foraging is an adaptive innate behaviour, enhancing the chance for an individual to locate a new source of food, rather than perishing in a food-scarce environment. Recall that some, but not all, rodents become hyperactive when hungry. Could it be that a select number of animals and people are born to become foragers, to ensure that its gene pool survives famine? If so, Professor Aoki continues to surmise, then the individuals that run less on a wheel are doing so, because they have learned to suppress a powerful innate behaviour, achieved through active inhibition of key brain areas, such as the prefrontal cortex and the hippocampus. Professor Aoki's research paves the way for new treatments for anorexia nervosa in particular, and contributes to a better understanding of the neurobiological bases of plasticity. In the following years, the resourcefulness and ingenuity shown by Professor Aoki's research will help her uncover additional mechanisms that shape the developing brain.

# Meet the researcher

**Professor Chiye Aoki**
New York University Center for Neural Science
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Professor Chiye Aoki is currently leading a lab with the New York University Center for Neural Science. She and her team explore neurotransmitter expression and stress modulation in the brain, as well as neuronal plasticity. She received her PhD in 1985 from The Rockefeller University and continued with a postdoctoral fellowship at Cornell. In 1999, she was appointed as Director of the CNS Summer Undergraduate Research Experience Program and her work has been funded since 1985 through a variety of sources, including the NIH FIRST Award, the NSF Presidential Faculty Fellowship Award, The International Human Frontiers Science Program and the Klarman Family Foundation Grants Program in Eating Disorders Research. Throughout her career, she has written more than 100 papers and received almost 7,500 citations. She has been an invited speaker at over 50 conferences.

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# THE CONCEPTION OF AUTISM SPECTRUM DISORDERS

**Professor Irva Hertz-Picciotto** has been fascinated by early development since childhood. Her interest brought on a life long journey, studying the dangers lurking around the corner for the not yet born – now focusing on risks for autism spectrum disorders.

**Among your multiple other epidemiological interests, the focus on environmental effects on pregnancy outcomes has followed you from the very start of your career. What spurred this interest in the first place?**

When I was 11, my mom announced that she was pregnant. I was incredibly excited and curious about what was happening inside her! I went to the library to find books but what I found did not provide enough to satisfy me. My brother was born, and I loved being the big sister of a tiny baby. Shortly thereafter, the newspapers and television were showing pictures of deformed babies, who had been exposed to thalidomide – a medication given to pregnant women to treat nausea. It was shocking, and drove home for me how vulnerable the foetus is, and that some are luckier than others.

**What inspires you in your work?**

A few things energise me. One is my deep concern for children, who need so much from the rest of us – being fully dependent on, and intensely affected by the people who take care of them. A second motivator has been the sense of urgency felt by many families dealing with a child who has an autism spectrum disorder. Parents are justifiably frustrated at the lack of answers about what caused the disorder, and what they can do to make their child's life the best possible. Third is the energy and creative thinking of the students and post-docs that I have been privileged to mentor and work with. Fourth is the sheer pleasure of delving into the process of scientific discovery and challenging my brain to tackle big problems that concern real people by searching for a new perspective.

**You have shown that there is an increased risk of autism spectrum disorders following exposure to substances commonly used in our everyday lives. Are law and policymakers regulating use of these substances in a timely manner?**

Overall, there is a bias in our society that favours rapidly moving new products onto the market. Once on the market, it is a completely different story when questions arise regarding adverse effects on health from industrial or consumer products: action is usually very slow. There is a high bar for eliminating or restricting use or production of the chemical agents under scrutiny. In fact, we have a dual problem. First, there are thousands of chemicals that were grandfathered in when regulation began to address potential health effects by requiring testing, but mainly for new products. Second, the testing that is required is inadequate. Under certain laws in the U.S., testing of new chemicals covers cancer, gross malformations, and acute toxic effects, but does not examine long-term adverse developmental effects stemming from prenatal or early life exposures. Additionally, the problems of multiple exposures and their interactions remain a major gap. We have little or no information about the impact of a complex environment in which we are exposed, simultaneously, to a multitude of compounds, and whether the combined effects exceed what one might expect from simply adding up the individual influences on health or development. Thus, tens of thousands of chemicals are on the market for which we cannot say that they are safe for foetuses and small children, either individually or in combination. Moreover, our regulatory apparatus is vulnerable. It is subject to serious pressures from stakeholders representing financial and political pressures who often succeed in delaying action to restrict or eliminate chemicals for years or even decades – even when abundant evidence of toxicity has accrued. In short, regulation of toxins that influence brain development has been slow, some say glacial, leaving many children unprotected.

**Your studies also focus on gene – environment interactions. You have shown that in children with a variant COMT gene, associated with reduced efficiency for the repair of our DNA and the neurotransmitters necessary for brain function, folic acid supplementation reduced autism risk by 82 per cent. How can this data be used to target at risk children?**

Yes, there appear to be several genetically

defined subsets of the population in which we found folic acid supplementation to have quite a huge protective effect against the development of autism spectrum disorder, or ASD. I would hasten to add the caveat that this particular genetic variant of the COMT gene that you mention was fairly rare in the children. The other gene with a pronounced protective effect in combination with folic acid supplementation was found in the mother: her MTHFR677a. For public health prevention, we might do better targeting the mom before she has even conceived her child, since the benefits of supplementation with prenatal vitamins are primarily for the three months before and the first month of pregnancy. Before embarking on such a targeted direction, however, replication of the roles of those genes is needed. At this stage, the first step would be to replicate these results. I have not yet seen an attempted replication: either some researchers have tried but decided not to publish their results, or no one has yet examined these gene-by-environment interactions.

If another team does succeed in replicating the gene-by-environment interaction, then your question about how to move from scientific evidence to a real intervention on those at elevated risk would become a high priority. A possible recommendation to consider is for all girls, when they reach reproductive age, to be tested for these genetic variants. Those with the high risk genetic profile could be put on prenatal vitamins for their entire reproductive years. Alternatively, this could be suggested only for those planning a pregnancy, but about half of pregnancies are not planned.

**You have received the Goldsmith Lifetime Achievement Award from the International Society for Environmental Epidemiology. What did this mean to you privately and professionally?**

I am a firm believer that the legacy of someone in my position lies at least as much in those we train and mentor, as it does in the specific scientific discoveries that we might make. After all, in 20 years, our papers may hardly be noticed, being supplanted by newer and better research methods. Our students, postdocs, and other faculty that we may influence are the ones who carry the torch, including the passion for doing rigorous science, and hopefully they pass the inspiration to the next generation.

# ENVIRONMENTAL-METABOLIC-GENE INTERACTIONS IN AUTISM

Professor Irva Hertz-Picciotto's concern for children has led her to launching the MARBLES and CHARGE studies, two large-scale projects that may trigger a paradigm shift in the way scientists view environmental risks and early markers of autism spectrum disorders.

**Our current understanding of what causes autism**

In 2013, it was estimated that almost 22 million people live with autism, a neurodevelopmental disorder characterised by a set of core symptoms that include impairments in communication ability and in social interaction, and presenting in the first three years of life. However, individuals with this disorder manifest varying degrees of severity, comorbidities and idiosyncratic behaviours. Despite this variation, however, all autism types are encompassed by the umbrella term: autism spectrum disorders (ASD). Autism rates have increased during recent decades, partly due to greater awareness and improved diagnosis and reporting of ASD. However, Professor Hertz-Picciotto found that a substantial proportion – potentially more than half – of the increase cannot be attributed to younger age at diagnosis, changes in clinical practice and/or broadened diagnostic criteria.

After 15 years of research and over a billion dollars invested in studies of genetic risk factors for ASD, the scientific community has identified a large number of rare and very rare variants, adding substantially to the body of knowledge about etiology of this condition. Using databases documenting the functions of genes and linking them to molecular mechanisms, the field has moved from specific genes to biologic pathways. For instance, in the case of ASD, scientists are confident that aberrant synaptogenesis – the formation of junctions where one neuron transmits its message to the next one – plays a role in the brains of children with ASD. Yet heritability studies have increasingly shown that, as compared with genes, environmental factors play an equal or greater causal role in ASD. Based on the complexity of ASD and decades of research on other complex conditions such as heart disease and cancer that repeatedly established links with both genetic and environmental factors, Professor Hertz-Picciotto reasoned that early life exposures likely contributed to ASD. Moreover, she realised that a search for modifiable environmental factors was necessary in order to lay the foundation for intervention that could ultimately halt the steady rise in autism and potentially decrease severity of impairments in those affected. From this line of thinking emerged the complementary CHARGE and MARBLES studies, seeking to explore the impact of early life exposures, both maternal and postnatal, on the emergence of autism. To date, these studies have focused on several areas such as maternal nutrition, metabolism, and exposure to certain substances during pregnancy.

**A possibly disturbed metabolic pathway**

The CHARGE Study takes a retrospective approach. Specifically, children with either ASD or typical development are identified at the same ages, and then compared, with data collected to learn about differences in their early life exposures. The MARBLES study follows children from long before birth to their third birthday, an age when a firm diagnosis can usually be made. MARBLES recruits pregnant mothers who already have had an autistic child and therefore have an increased risk of a second affected child. The study also aims to identify markers of autism in infants, because, first, an early diagnosis facilitates severity-decreasing interventions that can improve the skills and autonomy of the children, and second, a connection of such markers to environmental exposures may hold the key to understanding both environmental causes and their mechanisms. The MARBLES study team therefore collects blood, urine, hair, placental tissue, and breast milk to both measure the exposures and explore biomarkers on potential pathways through which they may influence the development of the foetal and infant brain.

The data in the CHARGE study demonstrated that mothers of children with ASD had a different metabolic profile during pregnancy than those of typically developing children, even after adjusting for a wide array of potential confounders, encompassing maternal demographic and socioeconomic characteristics, as well as child's sex and age. The metabolic conditions included type 2 diabetes, gestational diabetes, and obesity, and the CHARGE team also validated these from prenatal medical records. Four independent groups corroborated the findings of CHARGE, including several very large studies, providing a robust body of evidence and thus reinforcing the idea that a disrupted metabolism can influence infant brain development through direct or indirect pathways.

Inflammatory processes produced by several types of immune cells, fat cells, and other types of tissue produce inflammatory proteins. One of Professor Hertz-Picciotto's working hypotheses was that, besides dysregulation of insulin and resulting impacts on cell energy metabolism, the maternal production of inflammatory proteins can alter immune signalling in the child. Because immune cells are abundant and in tight communication with neurons in the brain while it is undergoing exceedingly rapid growth, neuro-inflammation is a potential and plausible pathway linking metabolic conditions to ASD. Further research by Professor Hertz-Picciotto's team and others detected antibodies against the foetal brain that are found in blood of over 15% of mothers of ASD affected children but less that 2% of those with typical children. Despite mounting evidence regarding the link between maternal immune activation and autism, scientists are not yet able to draw a definitive conclusion as to whether the maternal

immune alterations found in humans are causal of ASD, or her immune disturbance is a consequence of the disorder, or the child's ASD and the maternal immune changes have no direct connection and are simply connected through a common upstream factor.

To further pursue this question, Professor Hertz-Picciotto obtained new-born blood from CHARGE children and analysed cytokine levels – proteins that act to either promote or dampen inflammatory responses. Her team was able to confirm the association between elevated levels of the pro-inflammatory cytokines IL-1ß and IL-4 (interleukins) at birth and subsequent increased risk of ASD, nailing down one link in the chain she hypothesised. Additionally, maternal metabolic conditions clearly precede both the presence in the new-borns of these proteins, and the development of autism, thus hinting at the possibility to demonstrate causation in future work.

**Maternal nutrition may reduce risk**

A second set of studies by Professor Hertz-Picciotto's team has focused on maternal nutritional status. Mothers in CHARGE reported on their use of vitamin supplements and their intake of grains and cereals that are rich in the B-vitamins. Interestingly, those mothers who took the recommended prenatal supplements before or during the first month after conception had a 40% lower likelihood of having a child with ASD! These prenatal vitamin supplements are formulated with folic acid, and the recommendations to take them before and early in pregnancy, are aimed at reducing the occurrence of neural tube defects, a type of birth defect in which the brain does not form properly in the embryonic period. Although retrospective maternal report can sometimes be unreliable, these CHARGE study results have now been replicated in a larger study that collected information about use of prenatal supplements prospectively, i.e., before the mother knew about her child's diagnosis. Not only did women from another population experience lower risk if they took the prenatal supplements, but the window of protection was consistent with what was observed in CHARGE.

However, the CHARGE study went further: mothers and children were genotyped for targeted genes involved in metabolism of folic acid to

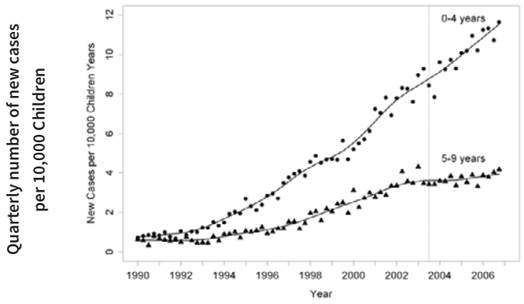
folate and in the role of folate as a donor of methyl groups. Methyl groups, consisting of a carbon linked to three hydrogen atoms, are epigenetic marks that sit outside the DNA code and regulate gene expression. They are also essential for production of neurotransmitters, chemicals such as dopamine and serotonin, that carry signals across synapses. In the CHARGE Study, gene variants that confer a less efficient conversion of folic acid to folate (e.g., MTHFR677A), and other gene variants that impart a less efficient transfer of methyl groups to DNA (e.g., COMT), were associated with the greatest reduction in risk from folic acid intake. This finding is significant for several reasons. First, it represents the first gene-by-environment interaction to be discovered in the field of ASD etiology. Second, it identifies a genetically vulnerable subset of the population that may gain virtually all of the benefit from an environmental – in this case nutritional – intervention. Third, it is actionable and extends the positive impacts of nutritional supplements that are already strongly recommended. Fourth, it identifies a critical window around the time of conception for which nutritional supplementation may reduce risks for autism. Fifth, it supports site-specific epigenetic mechanisms (i.e., DNA methylation for particular genes) in ASD etiology.

**Environmental hazards in CHARGE and MARBLES**

Humans are exposed to environmental chemicals in air, water, food and soil, as well as from a wide variety of household and personal care products. For example, pesticides are present as residues in foods, including fruit, vegetables, dairy, meat and fish. Other sources are commercial applications in agriculture, golf courses and around highways that result in drift at substantial distances (e.g., 5-10 km); indoor applications at malls, and household uses. Pesticides are notably different from most other environmental pollutants such as metals, plasticizers, and flame retardants, in that their primary purpose is to kill animals of other species, usually by acting on nerve cells to disrupt ion channels that regulate transmission of messages across the synapse. This type of action is also relevant to human beings and hence it should not be surprising that most pesticides are neurotoxic. However, until recently, most information on pesticide neurotoxicity pertained to its short-term acute effects, rather than long-term impact from prenatal exposures, a period when half a million cells in the foetal brain are dividing every minute.

To address this developmental window, Professor Hertz-Picciotto used the CHARGE Study to examine the impact of living close to agricultural applications by mapping the residences before and during pregnancy. These addresses were then linked with a database of recorded use on crops by commercial applicators. Professor Hertz-Picciotto then showed that the children in families living near applications of certain pesticide classes during gestation were more likely to develop autism than children from families living further away. These classes included organophosphates, which in the U.S., are now banned for residential uses, and pyrethroids, a group of synthetic mimics of the naturally occurring pyrethrins; the pyrethroids are far more potent than the pyrethrins.

With these results, along with suggestive reports from other populations, Professor Hertz-Picciotto is now turning to the MARBLES Study to measure biomarkers of exposure to the pregnant mother. Multiple urine specimens have been collected in each trimester for determining both organophosphate and pyrethroid metabolites. Because these compounds have short half-lives before being excreted,




a single urine sample measurement can be misleading as to the level of exposures throughout the gestational period.

While some people might argue that short-lived compounds pose little or no threat to health, Professor Hertz-Picciotto points to the role of these substances during critical development periods: 'Biologic activity does not require a long-term continuous exposure. For embryonic and foetal development, or even early childhood, a short-term exposure can have a profound effect if a critical process is taking place during that time window', she notes. 'Moreover, many short-lived compounds are used repeatedly. This is especially true of certain household and personal care products, for example, phthalates, which are present in vinyl flooring and in most scented products such as air fresheners, hand and body lotions, and shampoos.'

**Why it matters**

Taking a step back from her large program of research on pre- and post-natal neurodevelopment, Professor Hertz-Picciotto comments that 'many of the chemicals of concern appear to affect multiple organ systems. In other words, the same chemicals can interfere with early child brain development, influence metabolism to increase risk for obesity or diabetes, disrupt thyroid or sex hormones, and affect immune responses in the lungs. This multiplicity of effect underscores the need for a more pro-active approach to regulation of environmental chemicals.' Unlike drug safety, pre-market testing for effects on the foetal brain has been minimal at best, and non-existent for the vast majority of the thousands of chemicals in commerce. Meanwhile, studies have repeatedly shown that new-borns already carry detectable levels of dozens of chemicals considered to have adverse health or developmental consequences.

By understanding the consequences of environmental exposure, finding the exact pathways by which these factors impact brain development, and detecting early signs of autism through the MARBLES study, Professor Hertz-Picciotto's work aims to identify risk factors amenable to reduction, protective substances that can be promoted, and the broad field of co-factors that can be modulated. As she explains: 'I am confident that we can substantially reduce the incidence of this disorder, and decrease the severity of symptoms in those who are genetically predisposed to develop autism spectrum disorders.' Tackling the environmental exposures, from nutrition to chemical pollutants, and the epigenetic, immune, and metabolic mechanisms that can alter brain development, she hopes to one day see the rates of ASD finally decline to their pre-1990 levels.



# Meet the researcher

**Professor Irva Hertz-Picciotto**
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Starting out as a high school math teacher, Professor Irva Hertz-Picciotto found her true passion in Environmental Epidemiology later in life. She now directs the Program in Environmental Epidemiology of Autism, at UC Davis MIND Institute. She is the founder and director of CHARGE, the first large, population-based study to identify risk factors, amenable to intervention, for autism spectrum disorders, and MARBLES, a study searching for early biomarkers of autism. She has over 300 publications investigating effects of environmental factors on pregnancy outcomes and early child development. In 2011, she received the Goldsmith Lifetime Achievement Award from the International Society for Environmental Epidemiology.

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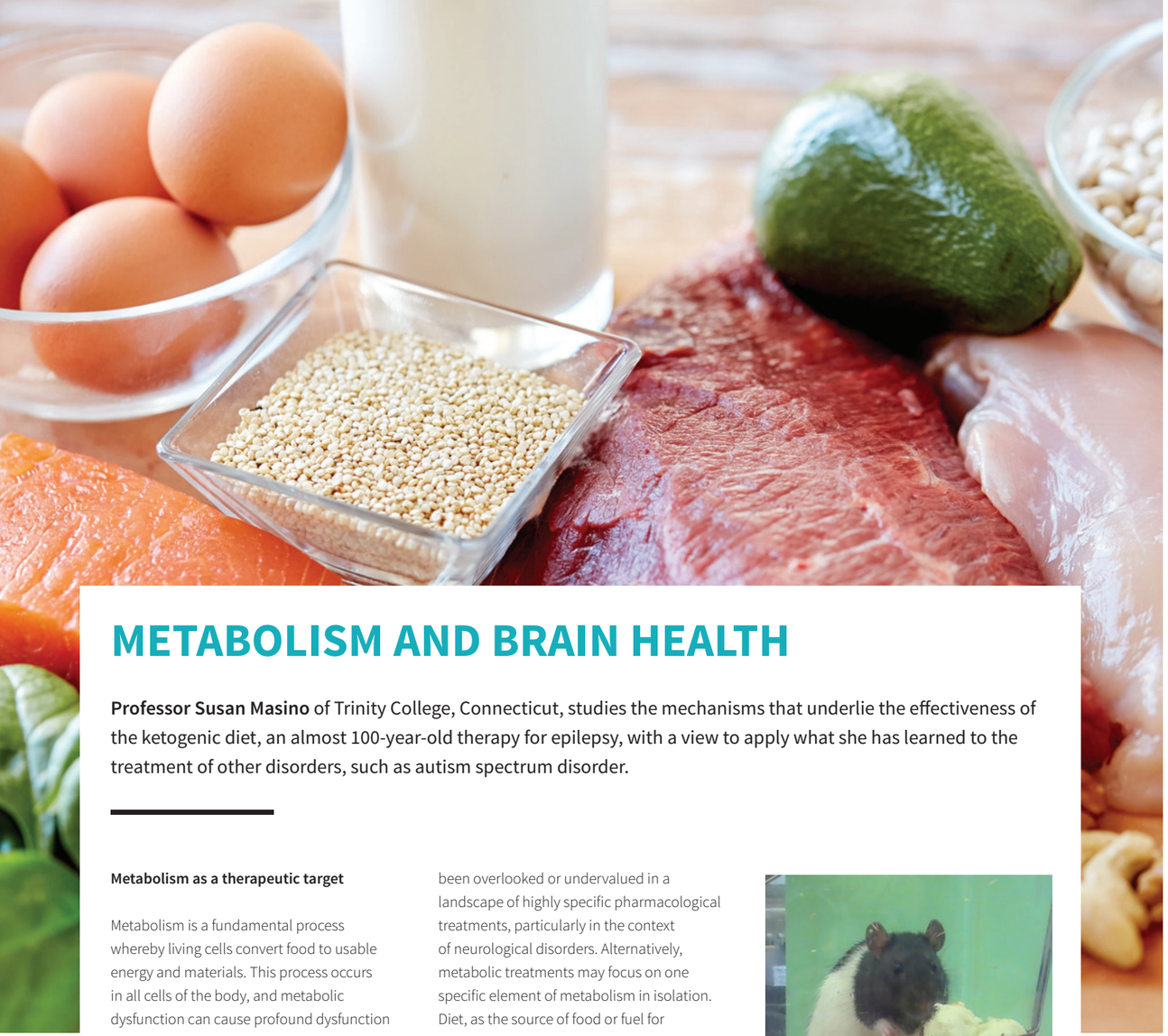


# METABOLISM AND BRAIN HEALTH

**Professor Susan Masino** of Trinity College, Connecticut, studies the mechanisms that underlie the effectiveness of the ketogenic diet, an almost 100-year-old therapy for epilepsy, with a view to apply what she has learned to the treatment of other disorders, such as autism spectrum disorder.

### Metabolism as a therapeutic target

Metabolism is a fundamental process whereby living cells convert food to usable energy and materials. This process occurs in all cells of the body, and metabolic dysfunction can cause profound dysfunction in physiological systems. Not surprisingly, metabolic disturbance may play a major role in numerous diseases, including neurological diseases, even those it is not typically a therapeutic target. Classical pharmacological approaches aim to identify and modulate highly specific targets that contribute to the symptoms of disease - or its underlying cause, if known. However, the goal of specificity notwithstanding, many pharmacological treatments cause off-target side effects or produce unforeseen consequences by modulating their targets in ways that would not occur under normal physiological conditions. Furthermore, pharmacological effects are often not long lasting, requiring chronic repeated treatment – and are often masking their target symptoms rather than promoting or restoring health.

As metabolism is ubiquitous it has perhaps been overlooked or undervalued in a landscape of highly specific pharmacological treatments, particularly in the context of neurological disorders. Alternatively, metabolic treatments may focus on one specific element of metabolism in isolation. Diet, as the source of food or fuel for metabolic processes, has enormous potential in treating metabolic disorders and in overall health and disease prevention. Changing our diet can have multifaceted and lasting effects on our physiology, providing a simple but highly effective means to ameliorate diseases by maintaining healthy metabolism. Moreover, the disease-modifying changes brought about by changing diet can help us to learn more about our physiology and the root of the disease processes that afflict it, and could contribute to the development of more effective pharmaceutics that can target the underlying cause of the disease, rather than palliative treatment of symptoms.

While diet-modification and metabolic approaches do not fit with the highly specific targeting of traditional pharmacology, producing change in multiple physiological systems at once may be what is required to effect significant therapeutic benefit, and particularly in patients for which traditional pharmacological therapies have failed. Furthermore, due to its high metabolic demand, brain function may be particularly sensitive to metabolic problems. This requires a paradigm shift, from the treatment of specific symptoms of a specific disease, to the modulation of metabolism throughout the body to support and enhance overall health and optimise metabolic processes to negate existing dysfunction. This approach has gained significant support recently.



*Rat tucking in to a ketogenic meal.*
*Credit: David Ruskin*



However, this is somewhat of a renaissance, as this concept is not new. One such example of a therapeutic diet for the treatment of epilepsy is the ketogenic diet, which has been in existence for nearly 100 years.

### The ketogenic diet and epilepsy

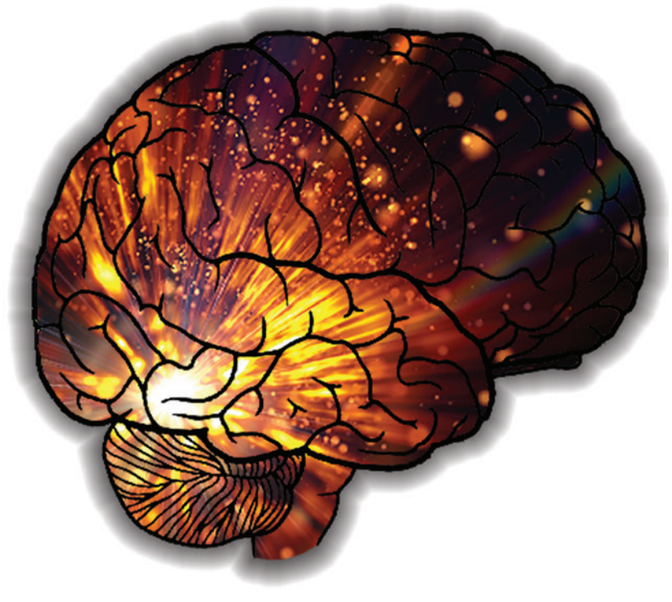
The ketogenic diet has been in use for the treatment of epilepsy since 1921. It had long been noticed that fasting reduced the incidence of epileptic seizures and the ketogenic diet was developed to circumvent the obvious limitations of fasting as a long-term treatment. Patients on the ketogenic diet limit strictly their intake of carbohydrates, and eat sufficient but not excessive protein, meaning that the majority of their energy is derived from dietary fats which are converted into ketone bodies. These ketone bodies can pass into the brain and are used instead of carbohydrate-derived glucose as a source of energy. The diet is often prescribed for patients for whom conventional pharmacological anti-convulsant therapies have failed – but is arguably underutilised. Professor Masino tells Scientia about the advantages of the ketogenic diet for particular epileptic patients: 'Today there are many drugs available to control epileptic seizures, yet this metabolic therapy can stop seizures even when all medications fail: for some patients a ketogenic diet is superior to all known drug treatments.' The mechanisms underlying the therapeutic effects of the ketogenic diet in epilepsy are unclear. However, Professor Masino has posited a theory based on a molecule with inherent involvement in the metabolic process: adenosine.

### The ketogenic diet and the adenosine hypothesis

Adenosine is a nucleoside neuromodulator that is involved in cell energy transfer; it is the core of adenosine triphosphate (ATP), the main cell energy molecule. Adenosine is regulated in response to cell stress and metabolic demand and it has been shown to have neuroprotective, anti-seizure and disease modifying properties. Professor Masino tells Scientia about the importance of adenosine and how her early work with it has shaped her current hypothesis: 'Adenosine immediately links cell energy (ATP is successively dephosphorylated into adenosine) and neural activity (adenosine is a neuromodulator acting at G-protein coupled receptors) and exerts lasting epigenetic changes as a product of DNA methylation. Initially, adenosine was hailed as an endogenous neuroprotective molecule that increased during events like hypoxia or injury. My initial electrophysiological research in the laboratory of Tom Dunwiddie helped to reveal the dynamic regulation of adenosine by diverse ongoing physiological changes at the cellular level. This led to my current hypothesis on the regulation of adenosine by ketogenic diet.' She explains the therapeutic potential and limitations of adenosine as a standalone therapy and how she believes it links with the ketogenic diet: 'Adenosine has long been a highly coveted therapeutic target as an anticonvulsant and a neuroprotectant, but peripheral side effects have made drug development challenging. Both a ketogenic diet and adenosine can stop seizures that are refractory to all available medications, and both link metabolism to neuronal activity. Therefore, if a ketogenic diet increases adenosine signalling it sheds light on two long standing mysteries: 1) how does a ketogenic diet work, and 2) how can we regulate adenosine.' The team has investigated the ketogenic diet in rodents, to determine if adenosine signalling is increased.

## 'Ultimately my real passion is brain health – I believe it should be part of all of our health visits'

*Credit: McCarron Art*



### Key findings to date

The team has discovered some compelling evidence for adenosine's involvement in the ketogenic diet's effects. They observed changes in adenosine signalling in the brains of rats that had been fed a ketogenic diet. They found that the effectiveness of the ketogenic diet in preventing seizures is eliminated in animals that do not possess adenosine receptors or in those that are administered a drug that inhibits the action of these receptors, suggesting that adenosine's action on its receptors is a key mediator of the ketogenic anti-seizure effect in epilepsy. Recent work suggests that a ketogenic diet may have long-term epigenetic effects via adenosine signalling independent of adenosine receptors, providing a potential explanation for the long-term therapeutic effects experienced by some epilepsy patients on the ketogenic diet even after cessation of the diet. The team has some preliminary evidence that these effects are mediated through changes in DNA methylation. However, Professor Masino is determined to see if the findings the team had made concerning the ketogenic

diet and adenosine are relevant to other neurological conditions, in keeping with the concept that metabolic therapies could be applicable to maintenance and enhancement of overall health: 'It turns out that adenosine is neuroprotective, but not just during pathological events. I believe that it promotes a dynamic homeostasis, which is essential for a healthy brain. In fact, we are beginning to appreciate that both adenosine and the ketogenic diet regulate brain function in a homeostatic way and may have disease-modifying properties. This could yield a new approach where rather than treating a specific disease we focus on restoring health.'

**Beyond epilepsy – autism spectrum disorder**

Based on a parallel hypothesis about autism spectrum disorder and adenosine the team broadened the scope of their research and began to study the effect of the ketogenic diet in a mouse model of autism spectrum disorder. While dietary treatments, such as the gluten-free diet, have been considered for autism previously, none were shown to be broadly effective in clinical trials. One small clinical study had previously shown that the ketogenic diet might have potential in the treatment of autism, but it was not a standard protocol and there has been little follow up. Professor Masino's team investigated the effect of the ketogenic diet in a mouse model of autism and was able to show a reduction in autistic behaviours compared with mice fed a normal diet. This included a reduction in repetitive behaviours such as grooming alongside increased sociability and awareness of social cues. The team was curious if the anti-seizure effects of the ketogenic diet were involved in its ability to reduce autistic behaviours, given that patients with autism spectrum disorder often experience seizures. However, the mice demonstrated no electrical or behavioural evidence of seizures, and no evidence of overall changes in brain activity when fed a ketogenic diet – suggesting that even though the ketogenic diet does stop seizures, the improved behaviours were not due to this effect. The results are exciting – a subset of patients with autism spectrum disorder have co-morbid epilepsy, and severely affected patients who also have seizures tend to have very poor outcomes. An established therapy such as the ketogenic diet could be particularly helpful in treating patients with autism spectrum disorder and seizures and may already be eligible for ketogenic diet therapy.

**Looking to the future**

Professor Masino explains her hopes for metabolic therapy, including complementary or synergistic co-therapies: 'New research has provided evidence that alternatives which can substitute for or complement the ketogenic diet – and potentially augment its efficacy – may be close at hand. Evidence is also mounting that for some people ketogenic diets can reverse chronic health conditions and provide general health benefits beyond treating any particular disease. Understanding key mechanisms underlying the success of metabolic therapy is of the highest biomedical significance: it is anticipated these mechanisms will apply to provide breakthroughs for multiple common, chronic, and poorly-treated disorders.' She explains that, for her, the multifaceted nature of dietary therapies is their key strength in treating complex and multifaceted disease states: 'While the ketogenic diet in particular is challenging conceptually for those who want to identify very specific mechanisms, I see this as a strength whereby a different set of key mechanisms may be needed address any disorder. Traditionally the ketogenic diet has been used to treat epilepsy, and my hypothesis about the ketogenic diet and adenosine predicted additional conditions that would benefit from this metabolic approach, such as

**'Evidence is mounting that for some people ketogenic diets can reverse chronic health conditions and provide general health benefits beyond treating any particular disease.'**

pain and autism spectrum disorder. These are challenging conditions with diverse underlying causes, and we are actively pursuing the short and long term efficacy and mechanisms of metabolic therapy in these and other conditions.' While the research group is interested in identifying the mechanisms underlying the efficacy of metabolic treatments, such as the ketogenic diet, Professor Masino would like to see healthcare providers and legislators adopt a more pre-emptive treatment approach to brain health, where disease modification and improvement in overall health are adopted in conjunction with treating specific symptoms and targets: 'Ultimately my real passion is brain health – I believe it should be part of all of our health visits – and it should be a top priority for public policy decisions and funding. A brain-health focused approach could prevent or delay disease, and complement any disease-specific treatment. I believe a key place to start is with our food system. We subsidise production of processed foods and refined carbohydrates and then pay the medical bills for the chronic diseases they precipitate. People who eat a higher proportion of subsidised food – often refined carbohydrates – have worse cardiometabolic health. I am sure that the conclusion would be the same for brain health, and the economic and societal costs of neurological disorders are staggering.'



# Meet the researcher

**Professor Susan Masino**
Vernon Roosa Professor of Applied Science
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Susan Masino obtained her PhD from the University of California, Irvine in 1996, following which she pursued postdoctoral research at the University of California, Irvine and the University of Colorado. She is currently the Vernon Roosa Professor of Applied Science and appointed jointly in the Psychology Department and Neuroscience Program at Trinity College, Connecticut. She is a member of the Society for Neuroscience, American Epilepsy Society and American Physiological Society, among others. She studies the regulation of adenosine and mechanisms underlying the ketogenic diet with a focus on brain health and neurological conditions such as epilepsy and autism.

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# THE GENETICS OF EPILEPSY

Searching for disease-causing genes is no simple task. **Dr David Greenberg** has been studying the genetic determinants of inherited epilepsy for 30 years and explains how the search can be hampered by deeply ingrained, but incorrect, assumptions within the field

Epilepsy is thought to affect around 1% of the worldwide population. It is defined as a neurological disease, or group of diseases, causing epileptic seizures. The seizures can have a broad range of symptoms, severity, and duration, depending on the specific type of epilepsy. Seizures can be very brief and practically undetectable but in more severe cases can cause prolonged and vigorous episodes of shaking and/or loss of awareness. Seizures are a manifestation of a wave of unregulated electrical activity in the brain.

One of the most useful tools on the route to developing treatments for any disease is a good knowledge of its causes. We have known for a long time that many types of epilepsy are heritable diseases. Some very rare forms of epilepsy can be seen in one generation after the other in a family, but such generation-to-generation manifestation of epilepsy is not the common pattern. The most common forms of the epilepsies with a strong genetic effect are the idiopathic generalised epilepsies (IGEs) (also called Genetic Generalized Epilepsies (GGE)), which constitute around a third of all cases of epilepsy and include various sub-types, such as juvenile myoclonic epilepsy (JME) and juvenile absence epilepsy (JAE), among other forms. The familial nature of these syndromes makes it likely that the basic causes are genetic, that is, they are caused by mutations to genes and are not caused by anything environmental. However, not only have the genes involved proven difficult to identify, there is virtually no knowledge of what has gone wrong in brain wiring or function that leads to these epilepsies. Therefore, if the mutated gene, or genes, can be identified, we will be well on the way to understanding the causes of the IGEs, allowing researchers to have a target for developing better treatments and even cures. Unfortunately, the reality of this research is not quite so simple. Dr David Greenberg has been studying the genetic causes of epilepsy for over 30 years and in addition to his own research on the subject, has written articles describing some of the core issues in the way geneticists search for disease causing genes.

**Improving the search with careful diagnosis**

Modern scientific technology allows researchers looking for the genetic causes of disease to generate large quantities of data more quickly than ever before. When Dr Greenberg began his research into this area, sequencing a gene could take years. Today this task can be performed in just a few hours, and entire genomes are sequenced with relative ease. Generating the data is no longer the hurdle it used to be. However, the technology does not help in what is perhaps the most important part of studying the genetic origins of epilepsy, namely, finding and choosing the human subjects for study, for the utmost care must be exercised in selection of subjects. One of Dr Greenberg's main criticisms with the way research is carried out in this area concerns this selection, specifically at the very beginning of the process: the diagnosis of epilepsy. As previously mentioned, 'epilepsy' is really a group of diseases, producing a wide spectrum of symptoms consisting of different types of seizures, different timings, different seizure triggers, different ages at which the seizures start, and different treatments. There is little evidence to suggest that just because these symptoms are all called 'seizures', that they are all produced by perturbing the function of any particular gene, or group of genes. In order to find the genes associated with a particular type of IGE, a detailed system of disease classification is first required.

Psychiatry as a field is often criticised for making an ever expanding list of diseases with more and more specific symptoms. Where once a person may have been diagnosed with just 'anxiety', there is now a vast range of specific disorders that contain 'anxiety' as a symptom. After receiving criticism for dividing so many disorders into various subcategories, a trend developed in psychiatry to try to simplify the field by lumping many of these disorders back into single categories. One example of this is the recent decision to do away with the specific diagnoses of Asperger's syndrome, pervasive developmental delay (PDD) and autism, and, instead, to replace these definitions with the blanket term of 'autism spectrum disorder'. Though there may be some organisational benefits to this type of approach, it causes problems when trying to find specific genetic factors that determine the expression of diseases such as epilepsy because 'epilepsy' is a symptom (recurrent seizures) not a single disease. What we call epilepsy, even IGE, is a heterogeneous group of diseases that are difficult to differentiate without a carefully considered diagnosis.

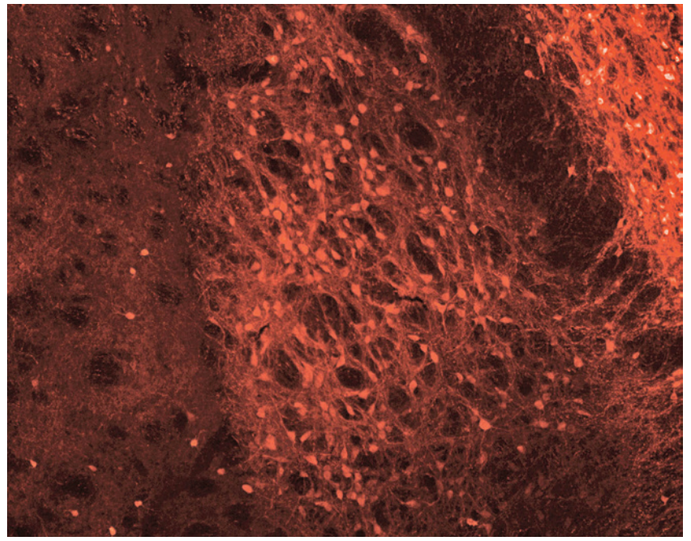
That careful diagnosis, which is based on accurate descriptions of known types of epilepsy, is critical because, without it, genetic analysis becomes incredibly difficult. Sufferers who display similar, but not identical, symptoms may be categorised as having the same disease, but these disorders may be due to mutations in completely different genes or different combinations of genes. Using genetic data from hundreds of people to look for mutations associated with a particular disease becomes an uphill battle if the patients have a wide range of different diseases, and therefore different causes. This is the problem of genetic heterogeneity, perhaps the biggest problem in trying to understand epilepsy as well as other common diseases.

**The accepted dogma of the causes of epilepsy**

Dr Greenberg's critique of much of the research into the genetic determinants of epilepsy extends to some of the finer points of statistics and genetic analysis, but often what he takes issue with is the actual logic of how experiments are designed and the assumptions that can dictate how data are interpreted. A pervasive idea is that genetic diseases are often 'caused' by a single particular gene. This idea oversimplifies the reality of disease genetics. Although life would be much easier for researchers if diseases were usually caused by a single gene, the truth appears to be that often, multiple mutations (or variations) are required for a particular disease to rear its head (this is also known as gene-gene interaction). Rather than looking for rare versions of genes which are quite uncommon in epilepsy sufferers, Dr Greenberg suggests that we should also be looking at the pairs (or more) of common genes which only appear together in IGE sufferers. Mathematically, this makes sense, but means that when looking for a gene which causes a disease affecting only 1% of people, you might now be looking for a gene mutation (or variant) that can be found in as much as 10% or even 25% of the population but that 10% or 25% do not have the other gene (or genes) necessary to have the disease. Despite the high prevalence of these genes in the population, the disease will only manifest itself when both genes are inherited at the same time in the same person, in agreement with the observed frequency of epilepsy sufferers.

Dr Greenberg also warns his fellow scientists against susceptibility to 'blinders' in their study of epilepsy genetics. For many years, the field's most widely accepted idea was that epilepsy is a 'channelopathy', in that it is caused by problems in the proteins that channel the flow of ions across cell membranes. That flow of ions is the basis of electrical signals transmitted through the nervous system. This idea fits with observations of what happens to the brain during an epileptic seizure (a dysregulation of that ion flow), but the idea that most epilepsy is due to such 'broken' proteins is not supported by actual evidence. Dr Greenberg argues that this dogma caused researchers to be blinkered and adopt a biased interpretation of their data. During a genetic screen to look for mutations associated with epilepsy, where genetic data has been collected from a large number of people with and without epilepsy, the screen will first narrow down the search to a particular region of the genome. This region might contain a large number of genes, but researchers often became fixated only on those regions that happened to contain channel genes, which they assumed were the gene causing the epilepsy, focussing on these at the expense of any proper investigation of any of the other genes. Harmless variations exist in all our genes (those variations are what make us different from one another). If a family being studied has one of the benign variations in a gene pre-accepted as a cause

**'We still understand very, very little about how the genome works. We have the letters of the book, but having the letters does not mean we understand the words'**



*The glowing neurons have been stained for the parvalbumin molecule. These special neurons, which express the neurotransmitter GABA (gamma-aminobutyric acid), are critical to controlling electrical activity in the brain*

epilepsy (e.g., a channel gene), then we can end up with the kind of circular reasoning which leads to the 'channelopathy' hypothesis supporting itself without a leg to stand on. The presence of a mutation within the channel gene (or any other), also might not tell us much without the knowledge of how often mutations (or variations) are acquired by that gene in the general population over the course of time, which is a question often left unaddressed.

**Our current understanding**

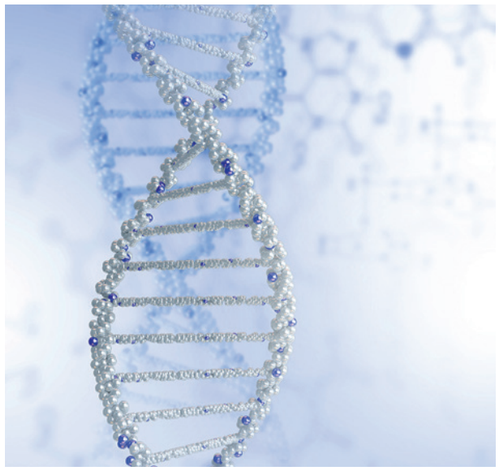
Some genes have been identified thus far in the study of epilepsy genetics but most of the proven genes are for the rarest, most devestating forms of epilepsy. The first gene for an IGE (in fact, the first epilepsy-related gene discovered), was BRD2, and it was reported by Dr Greenberg's research group in 1988. BRD2, found on human chromosome 6, is associated specifically with Juvenile Myoclonic Epilepsy (JME), one of the most common forms of IGE. 'By first doggedly pursing and then proving our original finding of a gene on chromosome 6, we could then develop a mouse model that we have shown has symptoms very close to what we see in human JME', Dr Greenberg tells *Scientia*. 'This gives us a way to study what the gene is actually doing'.

What became clear is that BRD2 is not a channel gene, and instead belongs to a class of genes known as transcription factors. These genes exert control over the way other genes are expressed , or 'read' (i.e., copied onto RNA), and ultimately translated into proteins, depending on the type of cell and the requirements of that particular cell. When the function of the gene is knocked out entirely, the mice fail to develop a functional nervous system during gestation and do not survive, showing that the gene is required for normal brain development. However, when its function is reduced by half, as in the genetically modified mice, they have less of a particular type of neuron in parts of the brain, and they develop sensitivity to seizures on the same 'schedule' (i.e., after puberty) just as is seen in humans.

The specific location of the BRD2 mutation associated with JME is located within a 'non-coding' part of the gene, the part that does not directly determine the sequence of amino-acids making up the BRD2 protein. In the early days of gathering sequence data, geneticists believed non-coding DNA, which makes up 98% of the human genome, to be 'junk'. Sequencing of the coding regions only makes the task of sequencing much quicker and easier, but as the case of BRD2 shows us, it is not safe to assume that all disease-related mutations will be located in the part of the gene that becomes protein, or that parts of the genome without a clear function are 'junk'.

Another gene identified in a similar manner, and with some confidence as having a part to play in susceptibility to an epilepsy, is ELP4, which is associated with a common form of epilepsy called rolandic epilepsy (RE). Again, ELP4 is not a channel gene, and the presumptive causative mutation was not located within the coding region of the gene. Similar to BRD2, ELP4 may have a role in the development of the nervous system. ELP4 has a role in the construction of cellular structures called microtubules, part of the cell's 'cytoskeleton'. Microtubules are critical for the correct structural organisation of cells and the tissues which they are part of.

Both of these groups of studies employed a type of genetic analysis called 'linkage analysis'. Unlike an association analysis, where genetic markers (or signposts) in a large group of people with a particular disease are compared to the markers in people who do not have the



disease, a linkage analysis uses data collected from entire families. The benefit of this analysis is that it provides information about inheritance, which association analysis does not. One can see more clearly which genes cause disease when, for example, an affected parent carries a particular mutation and has several children, but only the ones who inherited the mutation also have from the disease. A disadvantage to this approach is that it is less sensitive for identifying genes with a more minor effect on the expression of the disease.

Another way of strengthening this analysis for identifying IGE genes, is, in addition to counting as 'affected', those with an actual epilepsy diagnosis, also counting those with epilepsy-associated brain activity by performing electroencephalograms (EEG) on the family members, even if those subjects do not have seizures. IGE patients usually have abnormal EEG findings, even when they are not having a seizure. Family members in families identified through and IGE patient have a higher frequency of these same EEG traits, even though they may never have had a seizure. In a particular family with a known history of epilepsy, after careful analysis of their genetics, we might see that the same genes are linked to the EEG trait as well as the epilepsy. When combined with the EEG data, the causative genes can be more readily identifiable by looking at who really displayed the neurological characteristics of the IGE, rather than just counting who suffers from epileptic seizures. This technique was used effectively in the identification of both ELP4 and BRD2.

The conclusion appears to be that rather than mutation within the channel genes being the root of the problem, many epilepsies may be caused instead by subtle anomalies in brain structure, which can cause or predispose a person to epileptic seizures.

**The next steps**

Dr Greenberg's research group plans to continue researching BRD2 to determine the mechanism by which it contributes to epilepsy susceptibility. They also aim to investigate what other genes BRD2 might interact with in people with epilepsy. As the root causes of the disease become more clear, so too will potential treatments. On the subject of increased knowledge of the causes of epilepsies and affected types of brain cells, Dr Greenberg says optimistically, 'not only can this lead to better treatments, but also even a cure for epilepsy'.



# Meet the researcher

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Dr David Greenberg began his career in theoretical chemistry before changing fields to neurobiology and then on to human genetics. Working at the Harbor Hospital in Los Angeles, he researched the genetic causes of celiac disease and type 1 diabetes. Following this work, he moved on to the Comprehensive Epilepsy Centre at UCLA, to begin his research on the genetics of epilepsy. He now works at the Battelle Centre for Mathematical Medicine at the Nationwide Children's Hospital, Columbus, Ohio. Dr Greenberg has also developed computer software for conducting genetic analysis simulations. He has published extensively on these and developed a suite of programs used to teach linkage analysis and association analysis to researchers and students.

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# HUNTINGTIN: ITS ROLE IN GENE EXPRESSION

**Professor Naoko Tanese** and her research team at New York University School of Medicine investigate transcriptional and post-transcriptional gene regulatory pathways. Specifically, Professor Tanese is interested in identifying the post-transcriptional functions of the Huntington's disease protein huntingtin and how they differ in the disease state.

**Huntington's Disease**

Huntington's disease is a rare, but devastating hereditary disease that causes the breakdown of nerve cells in the brain over time. The average age of onset is between 30 and 50 years; however, the disease can occur earlier or later. The result is a progressive decline in cognitive, psychiatric and motor abilities. Most literature reports motor impairments occurring early in the disease followed by cognitive/psychiatric dysfunction. Cognitive impairments include difficulty in prioritising and focusing, lack of impulse control and self-awareness, and difficulty learning new information. Memory can also be severely compromised. This decline is accompanied by the development of psychiatric symptoms, typically depression and anxiety, but other symptoms such as obsession, compulsion and irritability can also occur. Another hallmark of Huntington's disease is the development of involuntary and unwanted movements in early stages of the disease. Clinical diagnosis occurs by the onset of motor symptoms. These movements start out as small, muscle twitches in the face and extremities that eventually progress to the rest of the muscles in the body, causing movements to become more rigid and difficult to initiate. Particularly aggressive forms of the disease display symptoms early in life. When Huntington's disease develops before the age of 21, it is known as Juvenile Huntington's disease or JHD. While very similar to the adult form, JHD differs from Huntington's disease in that specific motor symptoms develop at different times during the progression of the disease and seizures can also occur. Some studies also suggest that JHD progresses more rapidly than the adult onset disease. While there are treatments available to manage the symptoms, there are currently no cures that prevent the decline associated with the progression of the disease.

Huntington's disease is caused by an inherited mutation in the Huntingtin gene, *HTT,* that results in over 40 repeats of the amino acid glutamine in the encoded huntingtin protein (Htt). A typical huntingtin protein has between seven and 35 repeats. Huntington's disease is an autosomal dominant genetic disorder, meaning that just one of the two genes that are inherited from one's parents needs to be mutated for the disease to arise. *HTT* is widely expressed throughout the body and is essential for normal development. It has also been implicated in many cellular functions. In her latest project, Professor Naoko Tanese and her research team have discovered novel functions of Htt in gene expression. Understanding these new Htt functional roles and how they differ in the case of mutant Htt may reveal novel pathways and targets for future therapeutic interventions.

**Post-transcriptional Regulation of Gene Expression**

Gene expression is the process by which information encoded by a gene is used to synthesise a gene product, typically a protein. Protein synthesis occurs in three main steps: transcription, post-transcriptional processing, and translation. During transcription, an enzyme called RNA polymerase reads DNA of the gene and creates a copy called messenger ribonucleic acid (mRNA) or a transcript. The transcript then undergoes post-transcriptional processing to improve recognition of the mRNA by the translational proteins and remove any portions of the sequence that should not be translated. During translation, the transcript is decoded by a multi-protein structure called the ribosome to generate a string of amino acids known as a peptide. The peptide then folds into secondary and tertiary structures to form the protein.

Gene expression can be regulated by several factors at different steps in the protein synthesis process. Gene expression is regulated post-transcriptionally by RNA binding proteins or RBPs. Some RBPs interact with one end of the transcript called the 3' untranslated region (UTR) to affect its stability or distribution in the cell in a number of ways. RBPs can influence gene expression by sequestering mRNA in processing bodies. Processing bodies (P-bodies) are small granules or aggregates of mRNA-degrading proteins in the cytoplasm. Through degradation or storing of mRNA, the P-bodies reduce the amount of protein that can be made and thus downregulate gene expression. Gene expression can also be regulated post-transcriptionally through mRNA localization, or transportation of the transcript to specific locations in the cell.

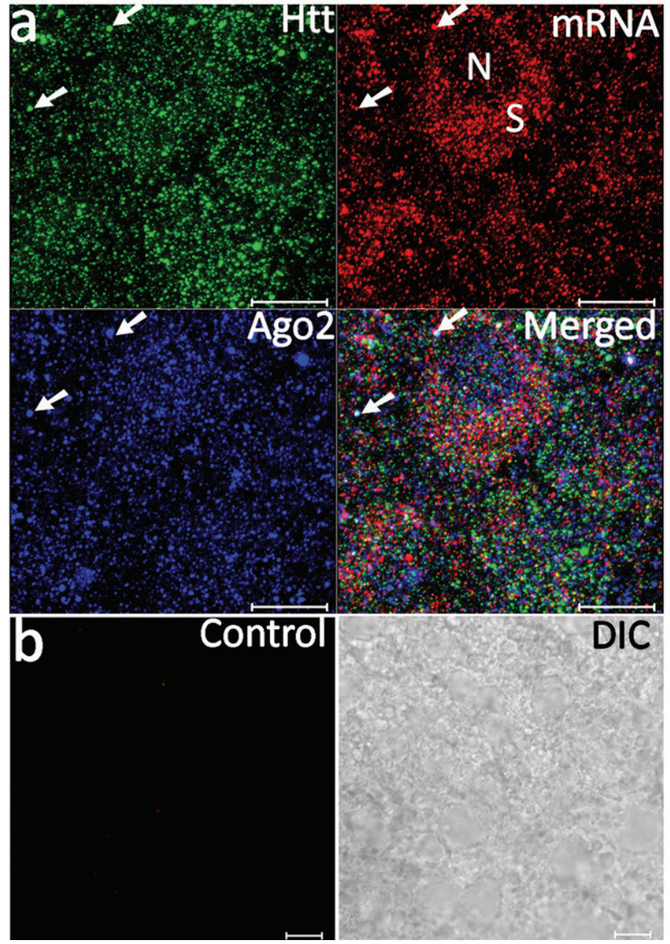
Localisation of mRNA is particularly important in nerve cells, or neurons. Neurons send signals to other neurons through a gap between the two cells called the synapse. This interaction is strengthened or weakened based on how much activity the synapse experiences over time. This change in strength is known as synaptic plasticity. Synaptic plasticity is vital for learning and forming new memories. Mounting evidence suggests that the transport and translation of mRNAs to the synapse contributes to synaptic plasticity by rapidly replenishing proteins to branches of the neuron that are far away from the cell body, especially following synaptic transmission.

**Htt in RNA Transport and Translation**

Professor Tanese and her research team have extensive experience in transcriptional and post-transcriptional gene regulation. More recently, they have turned their attention to the function of Htt. Because the normal function of Htt and the mechanism by which its mutant counterpart contributes to Huntington's disease is still largely unknown, they became increasingly interested in the role of Htt in post-transcriptional gene regulation as a novel approach to understanding the function of Htt normally and in the disease state.

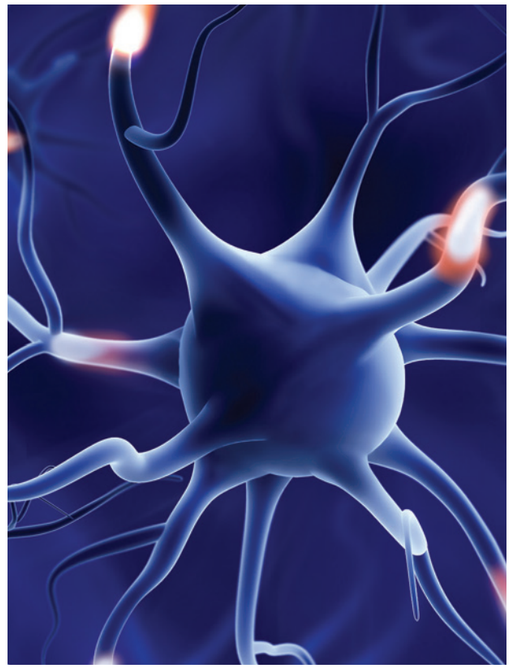
The team began their investigation by determining where the Htt protein was located in neurons. Using advanced imaging and microscopy techniques, the research team discovered that Htt could be found near neuronal RNA granules and P-bodies. RNA granules are large RNA-protein complexes responsible for transporting mRNA to specific areas in the cell. To determine whether Htt influences mRNA localisation, the research team reduced the level of Htt in neurons grown in culture and examined its effect on transcript transportation. They found that the reduction of Htt in the cells disrupted mRNA localisation, suggesting that Htt may contribute to the structure of RNA granules during RNA transport.

**'Understanding the mechanism of disease is critical to identifying targets for therapeutic intervention and treatment especially for HD for which there is no cure'**




*Visualisation of BDNF mRNA with huntingtin and argonaute proteins in the cortex of rat brain. Ma B, Culver BP, Baj G, Tongiorgi E, Chao MV and Tanese N, Mol Neurodegener., 2010, 27, 22.*

To obtain a better picture of the processes that Htt is involved in and how they may differ for mutant Htt, Professor Tanese and her team identified proteins that interact with each Htt form. By identifying the primary functions of the proteins that interact with Htt, the team could gain a better understanding of the processes that Htt may influence. Analysis of Htt protein binding partners revealed that both normal and mutant Htt interact with proteins related to RNA metabolism and protein synthesis, including complexes involved in translation. 'It is difficult to tease out various functions of Htt in this context,' Professor Tanese explains, 'but we have evidence supporting its role in RNA metabolism.' The mutant Htt associated with RBPs and translation factors more than the normal Htt, suggesting that dysregulation of post-transcriptional gene regulation and translation may contribute to the development of the diseased state in Huntington's disease.



Now that the research team knew that Htt interacted with translational proteins, they wanted to determine the function of the association between Htt and these proteins. To determine its function, the team examined translation in cells with normal and high concentrations of Htt. They discovered that translation was inhibited in cells with a higher concentration of Htt. Taken together with additional experiments the data support a role for Htt in mRNA transport and translation.

**Htt Self-Regulation**

During their investigation, Professor Tanese and her team noticed that, in addition to proteins, Htt also associates with mRNA for ß-Actin – a protein essential for cell structure and rearrangement. This led them to ask whether Htt post-transcriptionally regulates gene expression through interaction with mRNAs and if so, what mRNAs did Htt regulate. To answer these questions, the research team strove to identify all other mRNAs that the protein associates with using a technique called RNA immunoprecipitation followed by RNA-Sequencing. To their surprise, they found that both the normal and mutant Htt protein strongly associated with their own *HTT* mRNA. The function of this association was investigated further by determining whether Htt regulates its own expression by inhibiting its translation through interactions with the *HTT 3'* UTR. This is not uncommon, as many other proteins, such as TDP-43 (a protein whose mutation leads to the development of ALS, another neurodegenerative disease), regulate their own mRNA through interactions with the 3'UTR sequence. Professor Tanese found that when she linked another gene to the *HTT 3'* UTR, there was a decrease in the linked protein's expression level. Increasing the level of Htt protein further decreased the expression of the linked protein while reducing the Htt concentration had the opposite effect and increased the linked protein's synthesis. This series of experiments provided strong evidence that Htt does in fact regulate translation of the *HTT* mRNA via the 3' UTR sequence.

**'I am passionate about basic science research in which we strive to define molecular pathways and players that sustain cell homeostasis'**

**Mutant Htt and Mis-spliced mRNA**

Examination of the huntingtin protein's association with its own mRNA also revealed that mutant Htt associates with a mis-spliced *HTT* mRNA. Splicing is a common post-transcriptional modification that mRNA transcripts undergo to remove unnecessary strings of sequence. During the process, RBPs recruit proteins to remove the extraneous sections of RNA (introns) and splice together the remaining sections (exons). The RNA can be spliced in several different ways to produce multiple transcripts from one gene. The mis-spliced *HTT* mRNA has been incorrectly spliced resulting in a truncated protein encoded by exon 1. The fact that this association only occurs in the presence of mutant Htt and not normal Htt brings up the possibility that it may play a role in development of Huntington's disease. Understanding the function of this truncated mRNA and its relationship to the mutant Htt could reveal a new target pathway for future Huntington's disease treatments.

**Dissecting Htt's Post-transcriptional Functions**

Professor Tanese continues to tease out the role of Htt in the regulation, transport and translation of its own normal and mis-spliced mRNA. Currently, her lab is investigating the relationship between the mutant Htt and the mis-spliced mRNA further by identifying the mRNA sequence that allows them to associate, identifying any other proteins that may be involved in the interaction, and determining whether the 3' UTR sequence of the mis-spliced mRNA has a function similar to its function in the normal *HTT* mRNA by regulating its translation.

In addition to its own mRNA, the research team confirmed that Htt associates with other mRNA. This led them to hypothesise that Htt may regulate the transport and translation of mRNA that are vital to neuron survival. Upcoming studies by this group will focus on identifying the other mRNAs that associate with Htt and mutant Htt during translation, the function of Htt in these relationships and how it differs between the normal and mutant protein. 'In addition to investigating the nature of Htt protein – Htt mRNA interaction, we would like to identify other mRNAs selectively targeted by normal and mutant Htt protein.' Professor Tanese explains. 'We want to test the hypothesis that they have a role in the survival of neurons in the striatum, a brain region most susceptible to mutant Htt toxicity. We shall see what new direction the next discovery will take us.'

The team has uncovered novel roles for the normal and mutant Huntington's disease protein huntingtin in post-transcriptional gene regulation and translation in neurons. These novel functions have several implications for the development of Huntington's disease. Through understanding how Htt supports neurons with these novel functions, she and her team could reveal more accurate and effective pathways to target for Huntington's disease treatment development.



# Meet the researcher

**Professor Naoko Tanese**
Professor of Microbiology
Associate Dean for Biomedical Sciences
Director, Sackler Institute of Graduate Biomedical Sciences
New York University School of Medicine
New York, USA

Professor Naoko Tanese is a Professor of Microbiology, Associate Dean for Biomedical Sciences and Director of the Sackler Institute of Graduate Biomedical Sciences at New York University School of Medicine. She received her undergraduate degree in Chemistry at the University of Chicago in 1981. She went on to obtain her masters and PhD in biochemistry at Columbia University. She continued her postdoctoral training in biochemistry at the University of California, Berkley. She then made the transition to quite a different field of research, and now focuses on transcriptional and post-transcriptional gene regulatory pathways with an emphasis on the role of the huntingtin protein in these pathways and its influence on Huntington's disease pathogenesis. In addition to her research, Professor Tanese is highly dedicated to student mentorship. In 2014, she was appointed Dean of the Sackler Institute of Graduate Biomedical Sciences. Her responsibilities include recruiting prospective students to the graduate program and providing oversight in the PhD training process.

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# A NEW PERSPECTIVE FOR ALZHEIMER'S DISEASE TREATMENT

Nearly all of the clinical trials for Alzheimer's treatments have failed, leading many to wonder whether pharmaceutical companies have been chasing the wrong targets. **Dr Tamàs Fülöp** and his colleagues at the University of Sherbrooke are leading the charge to investigate neglected aspects of the disease in order to find new targets.



**Alzheimer's disease– what we know**

Alzheimer's disease causes progressive damage in the brain, leading to irreversible problems with memory, cognition and social engagement, eventually leaving the patients unable to care for themselves. It is the most common form of dementia in the world and the risk of being affected increases with age. Current projections predict that the number of cases will continue to rise dramatically in the future as our life expectancy increases, placing a heavy burden on the health care system and care givers.

The hallmarks of the disease are numerous. Diagnostic criteria include neuronal loss and synaptic dysfunction which together amplify the breakdown in communication between neurons, ultimately leading to their death. A protein called amyloid precursor protein (APP) normally found in healthy neurons, is chopped into peptides of varying lengths according to two different pathways. One is called the non-amyloidogenic pathway and is not associated with pathology. In contrast, the other pathway called the amyloidogenic pathway generates fragments that include a 42 amino acid-long amyloid beta fragment that may become toxic to the brain. Under pathological conditions such as in Alzheimer's disease, amyloid beta accumulates and aggregates on the outside of neuronal cells, forming plaques in the brain. Another hallmark of Alzheimer's involves the Tau protein. This protein is normally found in abundance in neural tissue, but becomes irreversibly modified to form fibrous tangles that aggregate and deposit inside neuronal cells. This causes these proteins to lose their important role in axonal transport, which is to help molecules shuttle from the cell body, where the nucleus is housed, to its synapse – a specialised structure where information is exchanged between adjacent neurons.

The question remains whether these two hallmarks are the cause of Alzheimer's disease or merely the consequence of another upstream triggering event. Amyloid beta aggregation, for example, can be seen before the symptoms of Alzheimer's appear, placing it as an early event but without evidence of a direct connection with onset of the disease. The question thus arises: what are the events that initiate Alzheimer onset and progression?

**A renegade immune system**

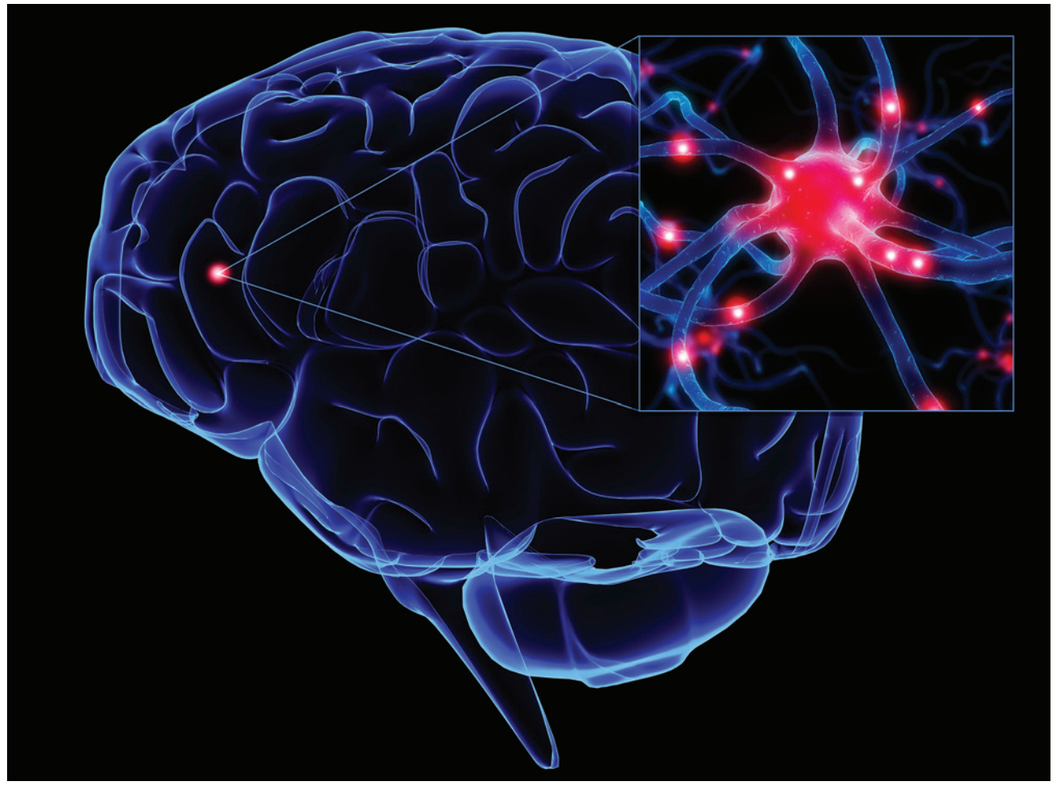
Whereas the exact mechanism of Alzheimer's disease remains elusive, some links have been made to a host of factors including gene susceptibility, metabolic disorders and brain inflammation. In addition, vascular risk factors such as hypertension and hypercholesterolemia have been suggested as additional factors that may contribute to the development of Alzheimer's disease. One indisputable characteristic of the disease is chronic inflammation of the brain and the periphery. The system that comprises the brain and spinal cord in humans, termed the central nervous system, is considered an immunologically privileged site, which means that cells outside this system cannot enter due to a protective 'wall' – the blood-brain barrier. This wall, however, is not without its cracks. It can be compromised following insult, or infections leading to a permissive communication between mediators of inflammation in the periphery (cells, inflammatory proteins) and the brain.

The response of the brain is propagated by members of the innate immune system such as microglial cells, which guard the brain and its occupants. Normally in a resting state, they become activated by plaque formation due to increasing numbers of amyloid beta peptides. They produce other mediators such as pro-inflammatory cytokines, neurotrophic factors and proteases to remove agglutinated amyloid beta, and to combat infection. All of these mediators combine to eliminate the aggression through engulfing and clearing debris, such as amyloid beta peptides, thus protecting the cells against damage. At the end of the aggression, they contribute to the resolution of the inflammation. However, under circumstances of sustained aggression this situation becomes chronic. Consequently, increased levels of pro-inflammatory cytokines are found in the brain and its surrounding fluid in Alzheimer's patients compared to controls, corroborating this mechanism.

The response to amyloid beta aggregates is triggered by the expression of specific receptors on the brain reactive cells such neurons, astocytes and microglia. The greater the amount of amyloid beta peptides, the more microglial cells that will be activated in response, subsequently releasing more cytokines, and creating a positive feedback



## One indisputable characteristic of Alzheimer's disease is chronic inflammation of the brain and the peripheral nervous system.



loop. Therefore, this normally regulated process can become aberrant, or renegade, leading to a sustained inflammatory response. This is also exacerbated by 'CNS immune senescence', a process whereby age-related structural changes in microglial cells occur, subsequently causing deterioration of their ability to provide neuroprotection.

Outside the central nervous system, a peripheral immune surveillance team is also hard at work patrolling for unwanted guests. Evidence also shows that members of this team can also be summoned into action during the course of the disease. This includes members of the adaptative immune system, a much more sophisticated system than that of the innate response. Unlike the innate immune response, the adaptative response is very specific to the particular insults that induced them, and can provide long lasting protection. However, they can also exacerbate the response by releasing more pro-inflammatory cytokines and further contributing to inflammation.

**Leaving no stone unturned**

Part of Dr Fülöp's work concerns natural killer cells, a rarely-studied component of the innate immune system as it relates to Alzheimer's disease. His team performed the first analysis of these cell types in patients suffering from amnesic mild cognitive impairment, believed to be an early stage of the disease associated with prominent memory decrease with no functional changes. In most cases, this affliction will become more severe and progress to Alzheimer's disease within a time as short as three years. Natural killer cells survey the central nervous system for signs of infection or stress. True to their name, they kill target cells that have become useless or are detrimental to the host. They are classified on the basis of the proteins they express on their cell surface which directly relates to their specific function. If, for example, one subpopulation of these killer cells decreases in number with age, they may be unable to effectively destroy an upstream mediator of amyloid beta plaque formation and this may serve as a clue for the early detection of immune alterations in the progression of Alzheimer's disease.

What the team found highlighted the complexity of this disorder. Differences in natural killer cell populations did not correlate with a pattern of progression

from healthy to mild cognitive impairment to full-blown Alzheimer's. Rather, it appeared to be a signature unique to the individuals with mild cognitive impairment, which then disappeared upon disease progression. Dr Fülöp theorised that natural killer cells may therefore be involved in an active attempt by the immune system to respond to an as-yet-unidentified pathology during this early stage of memory impairment. When attempts to stop this pathological insult fail, progression to Alzheimer's disease occurs. This situation is similar to that which is seen in rheumatoid arthritis, for example. It nonetheless proves that no immune component should be left unscrutinised when searching for potential drug candidates.

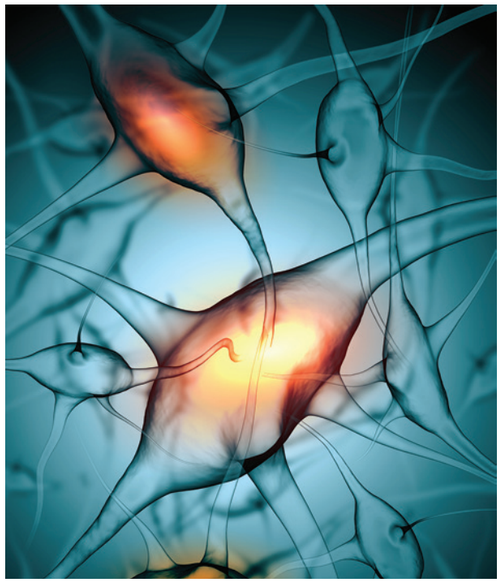
**Viruses – unlikely suspects**

Most new Alzheimer's drugs try to eliminate the characteristic plaques within the brain caused by amyloid beta deposits and quell the immune response. These drugs have, up to now, led to severe side effects and have sadly shown that clearing these plaques alone does not result in any cognitive improvement.

Once again breaking from tradition, Dr Fülöp is among a team of scientists who believe that microbes could be the instigators of this cascade of events, and by focusing more research efforts on their etiology, one may be able to slow or even arrest Alzheimer's disease progression. These infectious agents, including herpes simplex virus type 1 (HSV1), *Chlamydophila pneumoniae* and spirochetes can enter the central nervous system and remain there in a dormant state. The protective effect of the immune system declines with age allowing these pathogens to undergo reactivation in the brain. As a consequence, they cause neuronal damage by direct microbial activity or by triggering inflammation. This can happen repeatedly, leading to or contributing to hallmarks of the disease.

There are many lines of evidence to support an infectious component of Alzheimer's disease. Viruses and other microbes are found within the brain of most elderly people. In the brains of immunosuppressed patients, HSV1 DNA has been found to be expressed in greater quantities. Moreover, in the brains of Alzheimer's patients, HSV1 DNA was expressed within amyloid beta plaques. The inflammatory cascade of the disease is similar to that which occurs during infection, and features of the disease are transmissible by inoculation of the Alzheimer's disease brain to primates or mice. Finally, genome wide association studies, which investigate correlations between genetic alterations and disease, found that variations in a gene which modulates immune function and susceptibility to infectious disease, apolipoprotein E, and especially apoE4, also carries a risk of Alzheimer's disease.

Evidence further supports that the infectious agents are not just innocent bystanders in this saga. Brain infection is linked with Alzheimer's disease-like pathology in humans, while in mice and cultured cells amyloid beta accumulation was observed following infection with HSV1. Antiviral drugs, such as acyclovir, were also able to block HSV1-induced pathology in cultured cells. The last clue arises from an early symptom of Alzheimer's disease – olfactory dysfunction. The olfactory nerve leads directly to an area of the brain where the characteristic Alzheimer's pathology is routinely seen and eventually disseminates. This nerve is also a likely entry point of HSV1 and other viruses into the brain, thus implicating viruses at this initial site of damage.



**Dr Fülöp theorised that natural killer cells may be involved in an active attempt by the immune system to respond to an as-yet-unidentified pathology during early stages of memory impairment.**

Previously unsuspected, amyloid beta induction may initially have a protective role against pathogenic agents. Amyloid beta is normally produced by neurons at a rate required to fulfill their everyday function. This includes maintaining synaptic plasticity, the ability of the synapse to strengthen or weaken over time in response to a change in their activity, memory and antimicrobial protection. Dr Fülöp's group was one of two groups to present evidence that amyloid beta possesses antiviral activity against HSV-1 as well as other viruses. They hypothesise that production of amyloid beta may represent an initial self-defence attempt by the brain to curtail viral, and possibly other, aggressions. This is done through amyloid beta phagocytosis by microglial cells and the subsequent release of pro-inflammatory factors that sustain microglia activation. The situation turns awry when viral reactivation becomes more frequent, particularly with aging when microglial cells become less efficient at eliminating viruses and amyloid beta. Amyloid beta is then overproduced and accumulates, interfering with its normal, physiological function and triggering a critical inflammatory state leading to neuronal loss and eventually, the development of Alzheimer's disease. In short, a vicious cycle is set into motion where damage is amplified over time and becomes irreversible.

**The future of Alzheimer's treatment**

The failure of 413 trials of Alzheimer's therapy carried out over the past fifteen years is a clear indication that the research community is ready for some new ideas. Dr Fülöp is among the voices proposing a deeper look at alternative treatment routes to overcome this frustrating impasse. The role of viral and other infectious agents in the pathogenesis of Alzheimer's disease may be a promising start.



# Meet the researcher

**Dr Tamàs Fülöp**
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Dr Tamàs Fülöp is Assistant Director of the Research Center on Aging and Full Professor at the Faculty of Medicine and Health Sciences of the Université de Sherbrooke in Quebec, Canada. He obtained his PhD degree in immunology and gerontology from the University of Debrecen in Hungary, and he then carried out postdoctoral research training in the field of biochemistry of connective tissues at Paris, University Val de Marne' , in France. He also holds a MD Internal Medicine/Geriatrics degree, and is currently the head of the Immune Inflammation Laboratory. His current research interests include immunity in relation to aging.

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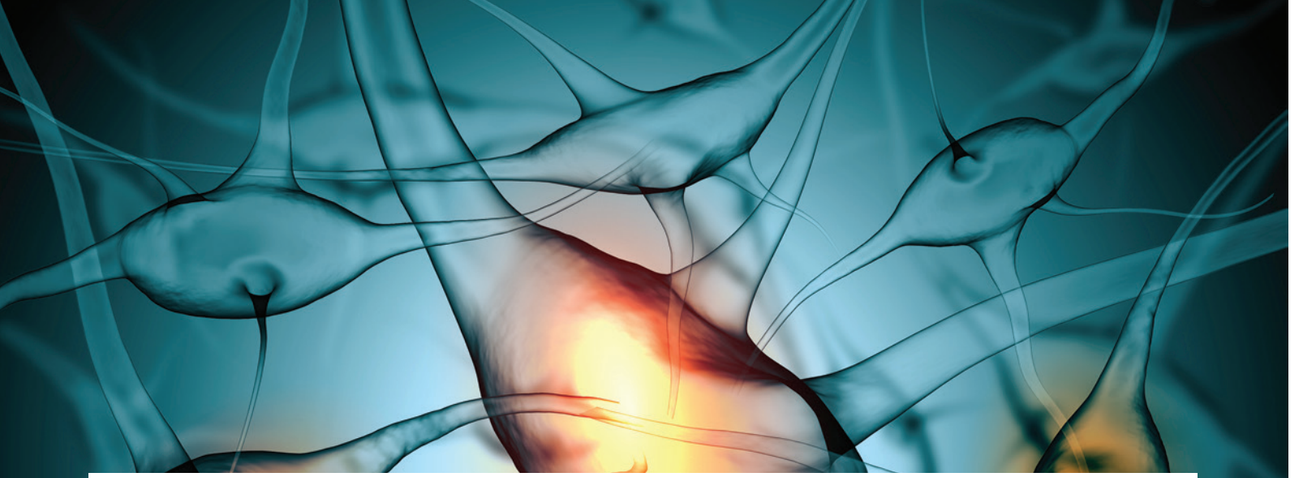


# THE MITOCHONDRION: THE POWERHOUSE BEHIND NEUROTRANSMISSION

**Professor Elizabeth Jonas** and her colleagues at Yale University study the function of cell components called mitochondria and their role in neurotransmission. In particular, Professor Jonas is interested in characterising how channels in the mitochondrial membrane affect neuronal function during processes like memory formation and learning, and how they enhance or reduce neuronal viability during disease.

**Neurotransmission – firing on all cylinders.**

Neurons form an incredibly complex network throughout our bodies, and play a fundamental physiological role. Neurons form the link between brain and body by transmitting information to and from the brain and spinal cord to peripheral tissues such as our muscles. The brain can encode information as patterns of neural impulses that pass from neuron to neuron. Neurons also comprise the brain and spinal tissue itself and normal neuronal function forms the basis for a diverse set of processes including thought, memory formation and movement. Neurotransmission is the procedure by which neuronal cells communicate with each other. Typically, neurons do not directly touch each other, but rather exchange information at specialised structures surrounding a very thin gap between neurons called a synapse. At a synapse, an activated neuron, through which a neural impulse has travelled, will release molecules called neurotransmitters which can travel across the synaptic cleft and bind to receptors in a second neuron. In this manner, the neural impulse can propagate along the second neuron and continue on its way. As you might have guessed, this process requires energy, and alterations in the mechanism by which this energy is provided can have profound effects on neuronal function and viability, and implications in disease.

**The nuts and bolts of neurotransmission: the mitochondrial system.**

Much of the energy demands of neurotransmission are met by a small organelle present in neurons called the mitochondrion. Mitochondria are membrane-bound structures which produce a substance called adenosine triphosphate (ATP), which is used by the neuron as a source of energy during neurotransmission. Mitochondrial efficiency refers to how much ATP is produced per molecule of glucose or oxygen taken up by a cell or organism, and when this process is inefficient less ATP is produced. Neurotransmission also involves the uptake of calcium through small channels or pores in the neuronal membrane. Calcium facilitates the release of neurotransmitters from the neuron. However, once neurotransmission is complete, the calcium that entered the neuron needs to be cleared, to allow the system to reset. Mitochondria play a role in helping to mop up this calcium through an intricate system of channels in their membranes. They also re-release the sequestered calcium and so not only do they help to reset calcium levels in the neuron, but they participate in carefully regulating calcium levels during neurotransmission. This process also has important effects on ATP production by the mitochondria and can affect how excitable the neuron is. If the calcium-regulating or ATP-producing processes become altered this can change the excitability of a neuron over time, or can even cause the death of the neuron. In fact, channels in mitochondria are important factors in cell death and may contribute substantially to the permanent alteration in neuronal function in the brain that underlies learning and memory formation and other forms of neural plasticity. These processes may go very wrong in neurodegenerative diseases like Alzheimer's or Parkinson's disease; therefore, mitochondrial dysfunction is heavily implicated in these diseases.

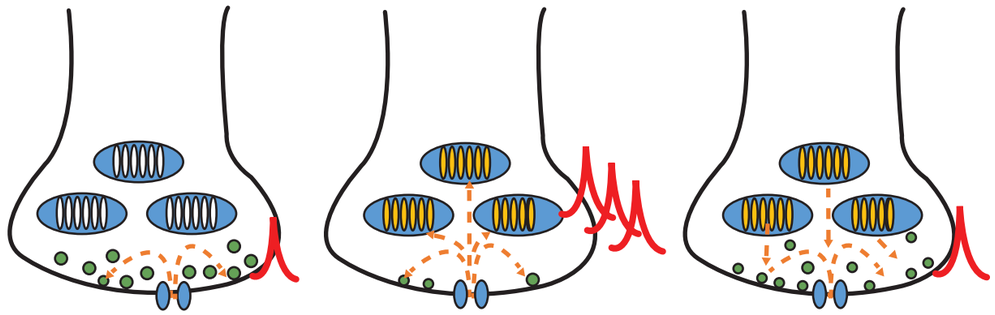
**How is this process regulated?**

Professor Elizabeth Jonas and her team are interested in the regulation of ion transport across the mitochondrial membrane, with a view to understanding the role of the mitochondrion in normal activities such as learning and memory, as well as its role in disease processes which result in abnormal neuronal activity or neuronal cell death. The team focuses on identifying the most important molecular players in the energy and calcium dynamics of neurotransmission and on studying how these interact, like filling in pieces of a jigsaw puzzle. In fact, Professor Jonas is credited with developing a completely new technique to study ion channels inside living cells, using two electrodes, whereby one electrode is sheathed inside another and is used to probe for ion channels in the membranes of organelles, like mitochondria. Interestingly, her initial goal when applying this technique was to find ion channels on the membranes of large secretory granules in invertebrate neurons. She was surprised when instead, she discovered calcium release channels in mitochondria, a discovery which sparked her intense interest in mitochondrial channels and the role they play in disease. Understanding what substances pass in and out of the mitochondrion, when they move in and out, and what molecules permit or block their movement and why, are key elements of Professor Jonas' research. For example, the team discovered that a segment of an enzyme called ATP synthase, which is involved in the production of ATP in the mitochondrion, functions as a channel in the inner mitochondrial membrane, where it is embedded. Professor Jonas believes that in this discovery, the team has uncovered the identity of a very important channel, called the mitochondrial permeability transition pore. The channel can open and close, allowing mitochondrial contents to travel through it. Exposure to high concentrations of calcium results in permeability transition pore opening which may act physiologically in the neuron to help re-release sequestered calcium, but, if open for a longer time, may also disrupt mitochondrial function. These events ultimately lead to loss of normal neurotransmission and neuronal death. In addition, the team is interested in other molecules that can interact with, and affect the behaviour of mitochondrial pores or otherwise modulate mitochondrial activity and neurotransmission. The Bcl-2 family of proteins, originally identified in cancer cells, is involved in the regulation of mitochondrial membrane permeability among other processes. Several members of the family tend to promote cell death, while others prevent cell death. This comprises a particularly important function for cancer cells, which rely on anti-death Bcl-2 family members to promote their cancer-inducing behaviour. In neurons the anti-death Bcl-2 family members prevent death due to many forms of injury or aging, and this function in neurons is termed neuroprotection. One member of the family is called Bcl-xL. Bcl-xL spans the mitochondrial membrane and its classical role is to prevent other members of the Bcl-2 family from initiating a cell death program known as apoptosis. However, Professor Jonas' team has found that Bcl-xL can also improve the efficiency of mitochondrial function during neurotransmission by interacting with the ATP synthase enzyme and the mitochondrial permeability transition pore. Jonas' team finds that Bcl-xL essentially contributes to prevention of leaking of H+ ions through the mitochondrial permeability transition pore, which improves the ability of the mitochondria to increase ATP production, while reducing the metabolic demands on mitochondria. These processes may partially underlie the potential of Bcl-xL to protect neurons from degeneration and may also play a role in memory formation in the brain.

**Synaptic plasticity**

So how do these complex phenomena on a small scale in neurons relate to appreciable effects in human beings? One mechanism these factors can affect is synaptic plasticity, which is the tendency of a synapse to become stronger or weaker over time, as a result of changes in its activity. It is hypothesised that this process could underlie our ability to create memories and

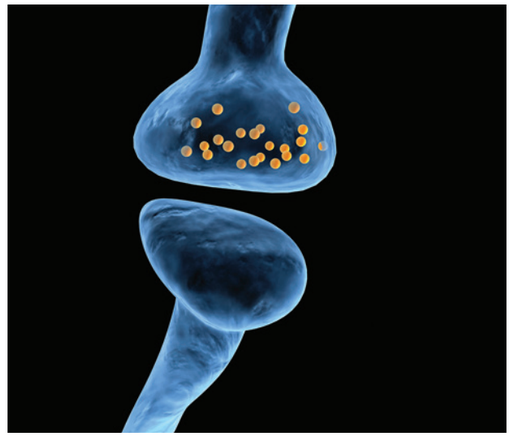
## 'We think we have found a key molecule that forms a major cell death-inducing mitochondrial ion channel'




*1. An action potential invades the terminal. Some vesicles fuse, releasing neurotransmitter.*

*2. Repeated action potentials (tetanus) invade terminal. Many vesicles fuse. Calcium is taken up into mitochondria.*

*3. Calcium is released from mitochondria. When another action potential invades the terminal, the increased calcium levels from mitochondrial release plus plasma membrane Ca2+ influx increase vesicle fusion, potentiating neurotransmission.*



retain learned information, in addition to potential involvement in pathologies like addiction, depression, autism, Alzheimer's Disease and in movement disorders such as Parkinson's Disease. Mitochondrial ion channels play a role in synaptic plasticity, through changes in the uptake or re-release of calcium into the neuron, which can enhance or suppress neurotransmitter release into the synapse, but also perhaps through long lasting changes in the efficiency of energy production over time (mitochondrial plasticity). In these ways, increased levels of Bcl-xL can enhance the potential of a neuron to release neurotransmitter over the long-term. Therefore, changes in the activity of mitochondrial ion channels could potentially play a significant role in brain development and in preventing brain aging.

**Cell death**

In addition to changing levels of neuronal activity, mitochondrial ion channels also control cell death. In diseases such as stroke, the blood supply to an area of the brain is cut off by the blockage of a blood vessel feeding the brain. The neurons in the affected area are deprived of nutrients and oxygen and die, potentially leading to impairments in cognition and mobility, including paralysis. Some of the affected cells undergo a process called apoptosis, during which a complex biochemical chain of events causes them to be destroyed. This process involves mitochondrial ion channel activity and Bcl-2 proteins. Professor Jonas' team investigated the role of Bcl-xL in neurons which had been starved of nutrients and oxygen during the process known as neuronal ischemia. They discovered that Bcl-xL was a key component of the apoptotic cascade in this type of cell, and that during stroke or ischemic brain injury, neurons tended to form a slightly different form of Bcl-xL, that induced, rather than protected from, apoptosis. Surprisingly, fighting this pro-death form of Bcl-xL was easy. The group used a drug that was already known to block the anti-apoptotic full length form of Bcl-xL in cancer cells. In the Jonas team hands, the drug, ABT-737, also appeared to effectively interact with the pro-death form of Bcl-xL, significantly reducing the amount of cell death caused by the brain ischemia. The team concluded that Bcl-xL might represent an important drug target in the treatment of diseases like stroke. If a drug could inhibit the pro-apoptotic form of Bcl-xL in stroke patients, then perhaps levels of cell death and functional impairment could be dramatically reduced.

**Professor Jonas and her team have hypothesised that neuroprotective molecules like Bcl-xL may prevent the onset of neurodegenerative disease through their interactions with mitochondrial ion channels.**

While pro-apoptotic Bcl-xL appears to contribute to neuronal cell death processes in diseases like stroke, full length Bcl-xL may promote neuronal survival under non-ischemic conditions. In fact, Professor Jonas and her team have hypothesised that neuroprotective molecules like Bcl-xL may prevent the onset of neurodegenerative processes and consequently neurodegenerative disease. Conversely, the loss of neuroprotective molecules like Bcl-xL, could permit neurodegenerative processes to occur. Neurodegeneration results in the loss of synaptic function, or in neuronal death, and underlies a host of diseases including Alzheimer's disease, Parkinson's disease and Huntington's disease among others. These conditions are a significant source of mortality and suffering. Consequently, research that could provide answers about the molecular basis for neurodegeneration, such as that undertaken by Professor Jonas, could be very valuable to society.

**The next steps for Professor Jonas' team.**

'We now want to find out exactly how Bcl-xL assists the mitochondria to alter their efficiency during formation of memories in the hippocampus and how Bcl-xL works in other neurons in the brain' Professor Jonas tells Scientia. The team is also interested in finding out more about how this interaction may go awry in neurodegenerative conditions. These results could aid in finding new drug targets to help in the treatment of such conditions. Future research will look at other proteins that interact with ATP synthase to improve the efficiency of mitochondrial function. One example is DJ1. This protein is highly expressed in the brain and in cancer cells, and is mutated in a familial form of Parkinson's disease, suggesting a strong link with the pathogenesis of Parkinson's disease.

In addition to looking at neurodegenerative disease, Professor Jonas also plans to study the role of mitochondrial efficiency in neurodevelopmental disorders. Fragile X syndrome is one such disorder in the category of autism spectrum disease, resulting in intellectual disability. Fragile X disorder is caused by an abnormality in the FMR1 gene which encodes the FMRP protein. 'Preliminarily, we have found that Bcl-xL and FMRP work together to protect neurons and particularly synapses during brain development' says Professor Jonas. The FMRP and Bcl-xL combination may work to regulate the efficiency of mitochondrial function and this in turn seems to regulate protein synthesis efficiency during synaptic plasticity. The team suspects that the abnormal FMRP protein may disrupt mitochondrial function and thereby prevent the normal regulation of protein synthesis in Fragile X patients' brains. These findings could underlie in part the pathogenesis of the disorder.

The implications of the results of this neurological research are applicable to a variety of diverse disease states. The team with Dr Kambiz Alavian at Imperial College, London, have also recently started to determine the role of the ATP synthase channel they have identified as the mitochondrial permeability transition pore, in the growth of cancer. In other studies at Yale, they have begun to characterise the structure of the channel in detail using a technique called cryo-electron microscopy, in order to better understand how it functions.



# Meet the researcher

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Professor Elizabeth Jonas is an Associate Professor in the Department of Internal Medicine (Endocrinology) and the Department of Neuroscience at Yale University. After completing a BA at Yale University, Prof. Jonas completed her M.D. at New York University. Subsequently, she completed residencies in Neurology and Internal Medicine at the Yale University School of Medicine and a Postdoctoral Fellowship in Pharmacology at the same location. She began an Assistant Professorship in the Department of Internal Medicine at Yale University before becoming an Associate Professor in the Department of Internal Medicine (Endocrinology) and the Department of Neuroscience at Yale University.

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# IMPROVING THE UNIVERSAL EXPERIENCE OF AGING

Our life expectancy continues to soar year after year – people born in 2016 are estimated to live a whopping 15 years longer than those born 50 years ago. This is a direct consequence of advances in health science, including vaccines, which have led to the massive decline and even eradication of contagious diseases such as mumps and smallpox, and anti-biotics – that can quickly treat otherwise fatal bacterial infections. However, accompanying our longer life-spans is a new breed of ailment – age-related disease – which includes everything from cancer to Alzheimer's. In order to ensure that we can each enjoy a healthier, happier old age, it's now more important than ever to focus our research efforts on improving the aging experience. In this section of the edition we explore the field of aging research, showcasing projects ranging from improving palliative care to investigating the effect of dietary restriction on cellular aging.

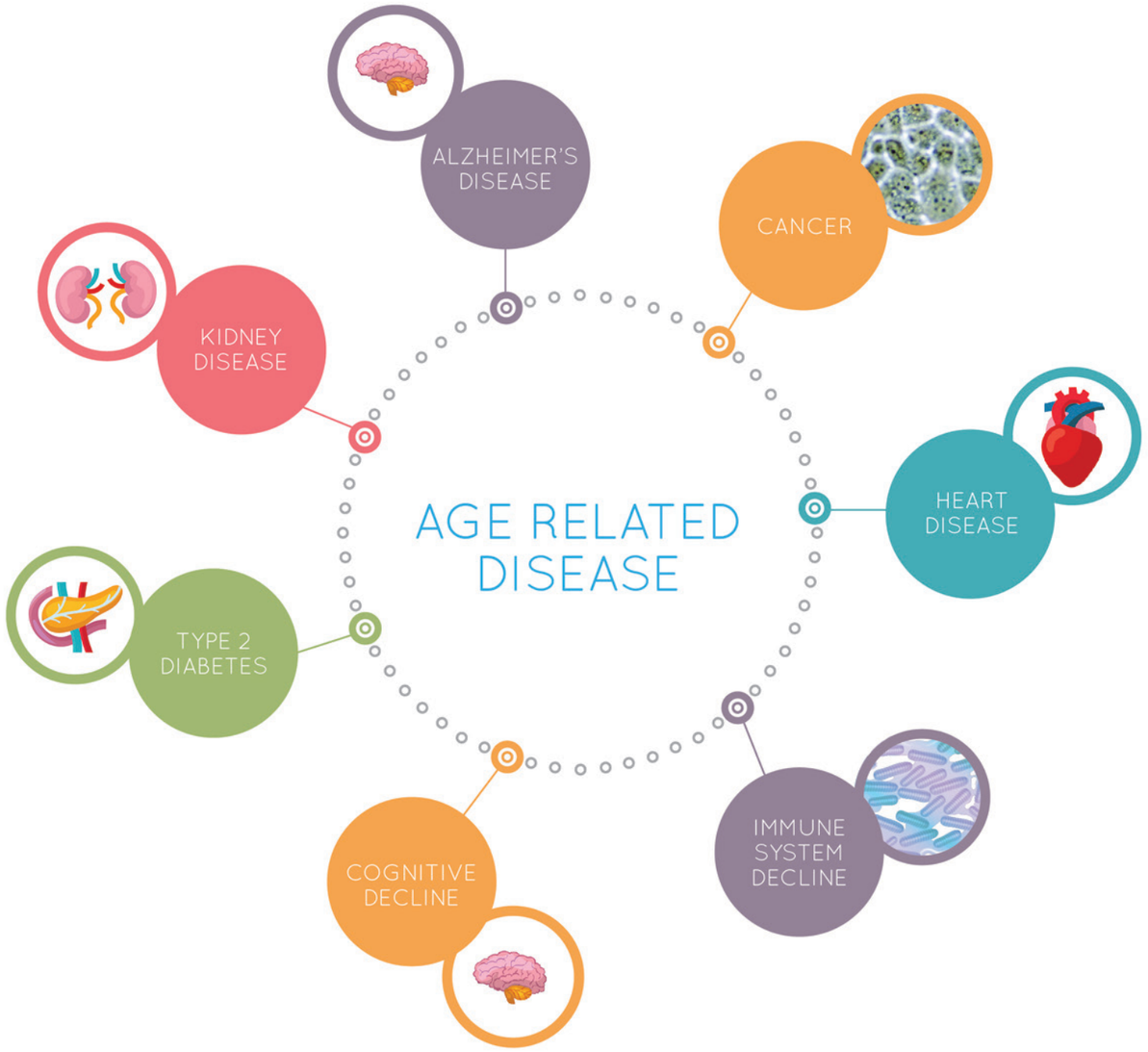
To introduce the field of age-related research, we have had the pleasure of speaking with Susan Peschin, MHS, the president and CEO at the Alliance for Aging Research. This US non-profit organisation is dedicated to accelerating the pace of scientific discoveries and their application in order to vastly improve the universal human experience of aging. Sue discusses the Alliance's activities in advancing aging research, promoting education, influencing science policy and more.

From here, we feature four ambitious research projects, each dedicated to enhancing our experience of aging. Our first researcher in this section, Dr Hsien Seow at McMaster University, is on a mission to change how people are cared for at the end of their lives. By demonstrating the benefits of homecare, developing palliative homecare teams, and informing healthcare policy, he aims to revolutionise how palliative care is provided in the home for Canada's most vulnerable patients. Also helping Canada to become a world-leader in aging research is Dr Janet McElhaney at the Northern Ontario School of Medicine, who, alongside her colleague Dr George Kuchel at the University of Connecticut, is on a mission to reduce the impact of influenza in older adults. By examining the relationship between aging, vaccine type and immune response, the pair is working to enhance the effectiveness of influenza vaccines in the elderly.

Next, we introduce Professor Arlan Richardson and his team at the University of Oklahoma Health Sciences Center, who investigate the biological mechanisms behind aging. In particular, Professor Richardson looks at the effects of aging and dietary restriction on gene expression, and tests the oxidative stress theory of aging in mice. Most recently, he has been exploring the effect of the drug rapamycin on aging and age-related diseases, in a study that has shown promising results thus far. We then move on to discuss the latest research into Hutchinson-Gilford Progeria Syndrome, a rare genetic disorder where children show symptoms of aging at a very young age, and can expect to live only into their late teens or early twenties. Professor Karima Djabali and her colleagues at the Technical University Munich have been on a mission to find a cure for this devastating disorder, while at the same time revealing new insights into normal aging.



## AGE-RELATED DISEASE



# THE ALLIANCE FOR AGING RESEARCH

The Alliance for Aging Research is a national non-profit organisation dedicated to accelerating the pace of scientific discoveries and their application in order to vastly improve the universal human experience of aging and health. Founded 30 years ago in 1986, the Washington D.C. based organisation works to advance innovation that supports the health care needs of older Americans and their caregivers, through targeted federal advocacy and education campaigns. Over the next few pages we speak with **Susan Peschin,** MHS, the president and CEO at the Alliance for Aging Research, who tells us all about the organisation's activities in advancing aging research, promoting education, influencing science policy, and more.



**To start, please describe some of the many ways that the Alliance works to advance science in the field of aging research.**

Since its founding, the Alliance has advocated for increases in federal funding at the National Institutes of Health (NIH). The Alliance was one of the lead organisations to successfully push for the doubling of the NIH's budget in 1999, and more recently helped direct the charge for the 200 percent increase in Alzheimer's research dollars since 2010.

The Alliance has also played a primary role in raising the profile of the rapidly evolving field of geroscience and its promise to extend our heathy years of life, or 'healthspan'. We launched a nationwide 'Healthspan Campaign' in 2012 on Capitol Hill, produced an award-winning film, *The Healthspan Imperative,* narrated by Martha Stewart, supported published research on the potential financial value of healthspan extension and created an ever-growing library of interviews with key aging biology researchers.

We have also developed an unparalleled expertise on FDA regulatory issues. The Alliance chairs coalitions focused on Alzheimer's disease and sarcopenia that bring together patient advocacy organisations, industry, academics, and government agencies to engage with the FDA review divisions on broad issues in clinical development. Our Aging in Motion (AIM) coalition, which focuses on sarcopenia, led the effort to secure an ICD-10-CM code for the condition.

**Does the Alliance directly work with funding bodies such as NIA, or the other NIH Institutes? If so, in what way?**

Yes, we do. We coordinate frequently with the Director of the National Institute on Aging (NIA), Dr Richard Hodes, and his colleagues on educational events to help raise the profile of the institute's research. In 2013, we partnered with the Foundation for the NIH (FNIH) to help host the first-ever NIH geroscience summit, and NIA experts are also featured in our Healthspan Imperative film. We have worked with several other NIH institutes and centers on the geroscience effort, on geriatric cardiac issues, cancer, nutrition and aging, vision loss, and on public-private partnership initiatives in Alzheimer's disease and related dementias. It is an honour and a pleasure for us to advocate for increased NIH funding overall, and for NIA funding specifically.

**Explain how you work directly with US policymakers to establish legislation that will advance medical breakthroughs. Tell us about a few of your success stories in this regard.**

The Alliance has worked on several, high-profile, successful legislative initiatives since its founding. We led a broad coalition opposing Congressional efforts to limit research involving human embryonic stem cells. Thanks in large part to the work of this coalition, these limitations were lifted by Presidential Executive Order in 2009 and replaced with a framework for funding ethical stem cell research with full protections against abuse.

The Alliance was instrumental in advocating for the inclusion of the Patient-Centered Outcomes Research Institute, which funds comparative effectiveness research, into the Affordable Care Act. We have also actively participated in several rounds of the Prescription Drug User Fee Authorization (PDUFA) process, as well as the similar process for medical devices (MDUFA), successfully advocating for provisions that increase the U.S. Food and Drug Administration's (FDA) focus on patient preferences, institute needed changes in FDA hiring practices, and provide industry support to fund such initiatives.

More recently, we devoted significant efforts to advocating for successful passage of the U.S. House of Representatives' 21st Century Cures Act, landmark legislation that aims to transform how biomedical research is conducted and how treatments are approved in the U.S. A version of the bill is currently being considered in the Senate.

**In addition to promoting aging research and influencing US policy, in what other ways does the Alliance work to enhance the lives of the elderly?**

The Alliance serves as a source for reliable information on the health and well-being of older adults. Our Silver Book® program has curated a digital library of informative health education resources on a variety of age-related diseases and general health topics for policymakers, health care professionals, press, and the public. Our 'pocket films' are short in length, animated, and explain complex medical topics and conditions in understandable language. They include topics such as: Alzheimer's disease, atrial fibrillation, heart valve disease, and sepsis, as well as nutrition and aging and medication safety.

The Alliance also publishes white papers and public opinion surveys, and develops community leader kits so that local organisations can share our information directly with older adults. For more of our education resources, please visit our health information page.

**Tell us about one or two promising treatments currently being developed to increase the healthspan of human beings. Describe the role of the Alliance in the development of these treatments?**

The Alliance is not directly involved in funding or designing medical research studies, but we regularly collaborate with top experts at the NIH, FDA, and academic centres to raise awareness about the latest advances.

There are a few interventions that have shown promise in animal studies for the increase of healthspan. Researchers fused the circulatory systems of young and old mice to create a shared blood supply, a process called parabiosis. In the old mice, the young blood triggered new muscle and more neural connections, and follow-up studies revealed that their memory formation improved. The researchers discovered that a gene called Creb prompts the rejuvenation. Block the protein produced by Creb, and the young blood loses its anti-aging magic. Another team discovered that a factor called GDF11 increased the number of neural stem cells and stimulated the growth of new blood vessels in the brains of older animals. These intriguing experiments have led to clinical trials that are exploring the impact of plasma from young people on middle aged and elderly Alzheimer's patients. If the results are positive, this could mark a turning point for the study of diseases of aging.

**'We remind those in power that there is nothing more consequential they can do than to support the health and well-being of those they serve'**

Another potentially promising treatment to slow the aging process that has garnered much attention over the last year is the diabetes drug metformin. Experts at Albert Einstein College of Medicine have initiated the Targeting/Taming Aging with Metformin (TAME) study to test this potential in humans.

**As scientists continue to unravel the biological mechanisms behind aging processes, and develop new ways to increase peoples' healthspans, do you believe that someday aging may no longer be considered a burden?**

Interesting framing. I would say first that burden is a part of life, no matter what age, and that degree of burden is subjective. There is a tremendous amount that we gain from an aging society, but I think that we are not culturally mature enough in the United States to realise that. The perception of burden comes from viewing older adults as non-productive economically, and from increased utilisation of entitlement programs and health care.

There is no question that the science of aging biology has the potential to not only improve overall healthspan, but to reduce health care costs in the long run. An October 2013 *Health Affairs* piece, which the Alliance partially funded, found that a successful intervention in delayed aging could increase life expectancy by an additional 2.2 years, most of which would be spent in good health and with an economic value estimated to be $7.1 trillion over fifty years. The authors point out that, should this come to pass, reasonable adjustments would need to be made to entitlement programs.

However, in order for us to realise the potential of geroscience, there needs to be significant public and private investment in this area of research, such as there has been for disease-specific research. To this end, the Alliance is pursuing a cross-government geroscience coordinating committee to improve funding prospects and partnerships across research agencies—much like what has been done for diabetes, autism, and breast cancer.

In the meantime, we will continue to push institutions, researchers, health care providers, and decision-makers to put themselves in the shoes of senior patients and their family caregivers. We do this in order to bridge the gap between what health care providers say and what patients actually hear; to improve development of, and access to, much-needed treatments for age-related diseases; and to remind those in power that there is nothing more consequential they can do than to support the health and well-being of those they serve.

Alliance FOR AGING RESEARCH

**www.agingresearch.org**



# CHANGING HOW PEOPLE ARE CARED FOR AT THE END OF LIFE

By demonstrating the benefits of homecare, investigating palliative homecare teams, and informing healthcare policy, **Dr Hsien Seow** is aiming to revolutionise how palliative care is provided in the home for Canada's most vulnerable patients.

**Failing the dying when they need the health system the most**

Although we live in an age where medicine is ever advancing and life expectancy is on the rise, all of us will inevitably die. For most, the journey of dying will be filled with serious chronic disease, old age, and disability. Unfortunately, the current health system is still too focused on curing disease that it neglects how to live well, and eventually die well, with serious chronic illness. Too often this leaves those who face serious illness feeling unprepared, fearful, in pain, and unaware of the options available. This affects not just the individual's quality of life but their loved ones and carers too. This is why the provision of palliative care is so important. The ethos of palliative care can be summed up by Dame Cicely Saunders, the founder of the hospice movement: 'You matter because you are you, and you matter to the end of your life. We will do all we can not only to help you die peacefully, but also to live until you die.' The palliative approach aims to improve quality of life and relief from suffering through the holistic care of both the dying person and their family, encompassing the psychological, social and spiritual, as well as the physical. In spite of the benefits for patients and loved ones, it is estimated that only 15 to 30% of Canadians receive palliative care. Underutilisation of palliative care also has economic disadvantages. It is estimated that 10-25% of the government's healthcare budget is spent on those in their final year of life, and the economic value lost by unpaid carers comes in at $25 billion annually in Canada. As the elderly population continues to grow and illnesses become more complex, inadequate access to end-of-life care will result in more people dying with pain, fear and poor quality of life.

Dr Hsien Seow, Associate Professor at McMaster University and the Canada Research Chair in Palliative Care and Health System Innovation, is seeking to change this. From early in his career, Dr Seow realised the importance of supporting those at the end of life, noting that 'the experience of dying, or caring for the dying, is often still filled with uncertainty, unpredictability and confusion'. As he further explains: 'We have designed a health system that support us well during pregnancy and birth, but abandons us during the journey of dying'. What would a better system look like? Dr Seow and his team are proposing a shift from overcrowded hospitals to home and community care. 'We need to provide more and better palliative care in the home and community because it is what most patients want, and it can be high-quality, and lower cost.'

Although 80% of Canadians state that they would prefer to die at home, 75% are hospitalised in their final year of life and 65% die in acute settings. By developing multidisciplinary palliative care teams in the community and providing more homecare nursing, patients can be better empowered to make decisions about their care and can have better quality of life. Teams also provide greater supports for caregivers.

The economic benefits for both family and state are notable. Inpatient costs can make up 50 to 70% of total costs in an individual's final year of life. Comparing the $1000 daily cost of hospitalisation to the cost of homecare nursing – around $160 a day – emphasises the health system savings if unnecessary hospital admissions are reduced.

In spite of all the advantages, there are major knowledge gaps associated with providing increased access to palliative homecare nursing services. Dr Seow aims to fill this gap through his research on palliative care by demonstrating the impact of palliative homecare and exploring how to integrate homecare nursing into larger multidisciplinary palliative care teams in the community.

**First steps: homecare reduces late-life hospital stays**

In a 2010 study, Dr Seow and his colleagues investigated the link between homecare services and hospital admissions at the end of life. Taking a large sample from the population of Ontario, Canada, the team measured the odds of admission to an emergency department or inpatient services in the final two weeks of life. The study revealed several interesting associations. Firstly, the team found that individuals admitted to homecare earlier than six months before death were 35% less likely to be hospitalised or die in an acute setting

## ‘We have designed a health system that support us well during pregnancy and birth, but abandons us during the journey of dying ’

than those admitted three to four weeks before death. Secondly, they found that patients who received seven or more hours of nursing care per week were 50% less likely to be hospitalised that those receiving one hour of care a week. It should also be noted that these results followed a dose response. This means that the earlier a patient was admitted to homecare and the more nursing hours they received, the less likely they were to be hospitalised. Overall, these results suggest that early and increased homecare services could alleviate the demand on acute healthcare services for palliative resources.

But this was just the beginning. Questions remained about whether the impact of homecare could be generalised to other regions and whether it led to overall cost savings to the health system. Further research was needed.

**Next steps: the impact of homecare across Canada**

Dr Seow and colleagues carried out another study in 2015 across Canada, namely in Ontario, Nova Scotia and British Columbia, in order to assess whether there was an association between the provision of homecare nursing with reduced hospitalisation across multiple provinces in the last six months of life. The team based their analysis on whether nursing hours in one week reduced hospital admission in the subsequent week. Even though each of the provinces have different systems to organize and deliver homecare, the results consistently showed that palliative nursing care reduced incidents of hospitalisation by 17 to 34% across the final six months of life. The study also revealed the efficacy of standard nursing care in the final month of life – 5 or more hours per week lowered hospital admissions by 15 to 23%. We see from these results the importance of nursing in end-of-life care, not due to provincial homecare system or the individual expertise of the nurse, but as an effect in itself. Therefore, it may be beneficial to train more nurses with the skills needed to manage palliative care needs.

Further study in the same year examined the financial implications of palliative homecare on healthcare system costs in the final six months of life. The researchers explored the association of increased homecare nursing costs on subsequent relative hospital costs and also on total costs to the public healthcare system. Dr Seow and colleagues found that particularly in the final month of life, increased nursing costs were correlated with lower total system costs, though no dose response pattern emerged. Again, these results were consistent across Ontario, British Columbia and Nova Scotia. Dr Seow explains: ‘This means that the patients across Canada receiving more nursing hours in their final month of life had overall lower health care costs because of avoided or shortened hospitalizations, even when we added in the costs of the extra home nursing hours.’ Although the numbers may seem small – ranging from several hundred dollars to approximately $1000 per person – these individual savings magnified to the population definitely add up.

**The future: building homecare teams in all communities**

The benefit of palliative homecare nursing is evident. However, since end of life homecare is not just the domain of nurses, Dr Seow and his colleagues have broadened their scope to include multidisciplinary palliative care teams, which include palliative care specialists, nurses, general practitioners and other health professionals, working in the community.

To investigate the effectiveness of palliative care teams, Dr Seow and colleagues in a 2014 study examined 11 multidisciplinary palliative care teams working to deliver palliative care in individuals’ homes in diverse communities across Ontario. Although these teams varied in terms of size, location and composition, they all had the same goal – to manage symptoms, provide education and guidance, coordinate services and be available to their patients at any time, day or night. Because of the diversity of the teams, they predicted that only some of the teams would be effective and those would be the ones to replicate and scale. But the results were astonishing. Compared with usual homecare alone, individuals who were treated by any of the teams in their homes were significantly less likely to be admitted to hospital or the emergency department. This patient group was also half as likely to die



in hospital than the control group. The consistent results across many diverse teams in real world settings strongly support the evidence for their effectiveness. ‘Our results showed there was not one best model as we had predicted. Instead, they all were effective in their own local context’, Dr Seow summarizes.

Why did the teams reduce hospital and emergency department use? In usual care, patients and families often encounter a system that is fragmented, inconsistent, inaccessible, or of variable expertise in palliative care. These lead to failure to cope and inadequate symptom control, which are the main reasons for inpatient admission. These palliative care teams can provide patients and their loved ones with essential integrated support. As well as the individual and system level benefits, healthcare providers themselves report the advantages of working as part of a multidisciplinary community team, such as the professional and emotional support from other team members, a reduction in provider grief, and improved job satisfaction. By generating further knowledge on how to develop palliative care teams, providers can work in more effective models of care and individuals will have greater access to holistic care at the end of life.

## ‘There is no one-size-fits-all community model. The core principles of the model have to be adapted to the local culture and context. That is how we achieve standardised care without a cookie-cutter approach ’

Dr Seow reveals a key finding in his research so far: ‘What we’ve learned is that every community and institution is a mini-ecosystem, with its own complex network of providers and resources. Therefore, to deliver palliative care successfully in patients’ homes, every community has to have its own unique model of care that needs to build on those local resources and partnerships. Thus, there is no one-size-fits-all community model. The core principles of the model have to be adapted to the local culture and context. That is how we achieve standardised care without a cookie-cutter approach.’

**What’s next?**

While much has been achieved, there is still more to be done. Dr Seow’s focus is now on developing frameworks to show how such teams evolve and developing tools and materials to help other communities build palliative care capacity for teams that are tailored to the local community. The community teams together can create a system which provides effective, sustainable and accessible end of life care for all patients who want to die at home. ‘We are at a point where we have lots of evidence that palliative care in the home is beneficial’, Dr Seow explains. ‘The future of my research is working on how to build the palliative care capacity in community providers and implement the optimal model of care for their local context. This ensures we have a system where all patients and families can receive palliative in their homes. It’s not enough to know that palliative care in the home is a good thing. We need to know how to deliver on that promise to patients and families, which means the hard work of building local capacity for multidisciplinary palliative care teams.’

# Meet the researcher

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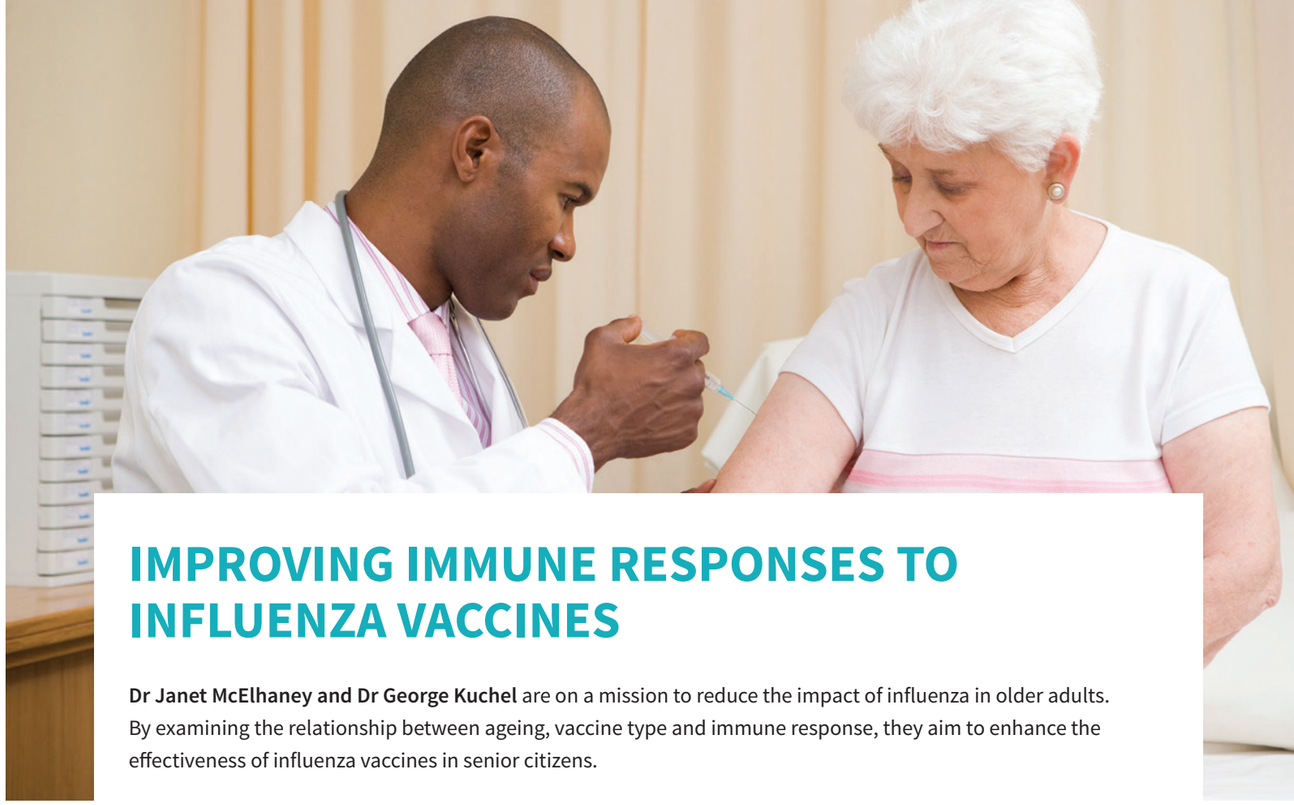

# IMPROVING IMMUNE RESPONSES TO INFLUENZA VACCINES

**Dr Janet McElhaney and Dr George Kuchel** are on a mission to reduce the impact of influenza in older adults. By examining the relationship between ageing, vaccine type and immune response, they aim to enhance the effectiveness of influenza vaccines in senior citizens.

### Preventing Flu Related Health Complications

Every year during winter, flu season comes upon us and we are often advised by healthcare professionals to be vaccinated with a flu shot. This is especially true for the older population – influenza causes 200,000 hospitalisations and 36,000 deaths per year in the United States, 90 per cent of which occur in those aged 65 or over. Influenza related morbidity and mortality increases when combined with factors such as age, chronic disease, dementia and history of pneumonia. Flu is also the most common cause of viral pneumonia in the elderly and has been linked to the development of heart disease, strokes and other illnesses which reduce quality of life. Therefore the goal of vaccinating senior citizens is to provide clinical protection and prevent future disability.

So how do vaccines work? Immunity is usually established by injecting a weak or inactive sample of a virus into the body. The body then produces strain-specific antibodies against the glycoproteins that are found on the surface of the pathogen. The immune system can do this in two ways: naïve T-cells and B-cells generate a response to the newly introduced pathogen, or memory T-cells (which have come in contact with the pathogen before) are boosted. With influenza, the latter is often what takes place as the majority of people have previously come in contact with the virus. However, this system doesn't always work, particularly amongst the elderly. Firstly, there may be a mismatch between the specific viral strains that are used in the vaccine and the influenza strains which are circulating in a particular flu season. This leads to a reduction in vaccine efficacy for all age groups. One randomised control trial estimated that influenza vaccines had only 50 per cent efficacy in a healthy cohort of older people, and this could be as low as 30 to 40 per cent for some members of the population. Secondly, older people do not always have the same level of immune functioning as their younger counterparts.

### About the Ageing Immune System

Immunosenescence can be defined as the gradual deterioration of the immune system as a consequence of ageing. This affects both the body's response to infections and the development of immune memory after illness or vaccination. Immunosenescence therefore increases susceptibility to influenza while also reducing the effectiveness of the vaccine. The degree of immunosenescence experienced by an individual is also influenced by any underlying chronic illnesses they may have, their functional dependence and their level of frailty. All of these factors then contribute to the risk of serious outcomes from influenza. Frailty was of particular interest to the research team, who utilised the Frailty Index in their studies. The Frailty Index is a 40 point clinical tool based on clinical and laboratory markers of health variables such as medical conditions, cognitive and emotional health, and functional status amongst others.

Inflammaging is a chronic elevation of inflammatory cytokines in the blood due to the ageing process and is also associated with increased frailty. Such inflammation and other age related changes in tissues, can increase susceptibility to infection. As we age, changes occur in our mucosal barrier functions (which act as a protective barrier against pathogens), making them less effective. The loss of mucosal barrier function in the lungs makes older people particularly susceptible to influenza and other respiratory infections so any vaccine-induced protection also has to overcome this hurdle.

Finally, age-related changes in immune cells known as T-cells are involved in reduced responses to vaccines. As we age, the thymus (where T-cells are made) shrinks and the

**‘The findings of this research will lead to a new way of testing vaccines much earlier in the process. Ultimately it will bring new vaccines for an aging population to market quickly and more cost-effectively.’**

output of naïve T-cells declines leading to poorer immune responses and poorer vaccine efficacy. There is also a proportional increase of memory T-cells, specifically a subset known as CD8+ T-cells, and to a lesser extent, CD4+ T cells. These are the dominant effectors against the influenza virus. CD4+ (helper) T cells produce cytokines that stimulate B cells to produce antibodies in response to the surface glycoproteins, which are strain-specific. CD4+ T cells also stimulate CD8+ (cytotoxic) T cells that recognize the internal proteins of the virus and kill virus-infected cells and so are able to protect against different subtypes of influenza A. However, age related changes in these cells contribute to poor vaccine response and an increased risk of complications.

Cytomegalovirus (CMV) infection is common in older adults with around 90 per cent of those aged 80 and above being seropositive for CMV infection. Although CMV is generally an asymptomatic infection in older adults, the immune response that contains CMV replication leads to an expansion of CMV-specific T cells, and has been associated with a general decline in immune responsiveness, and also linked to functional decline.

Therefore, it is evident that T-cell mediated clearance of the influenza virus is an important pathway to explore in providing clinical protection against infection.

**Difficulties in Development**

It is clear that there is a need to develop more effective vaccines, so why is this so difficult to achieve? For one, results can be confounded by a number of factors, such as functional status, frailty, chronic disease burden, vaccination history and previous exposure to the virus. The interaction of immunosenescence, inflammaging and reduced immune responses also lead to major challenges in effective vaccine development.

Even establishing a suitable sample population can prove troublesome as researchers must enrol adequate numbers of subjects and retain them over a number of years due to the high variability in influenza attack rates. Influenza infection can also present itself atypically in older adults, therefore surveillance and documentation must be rigorous. This may involve making weekly phone calls to participants, taking note of any and all acute respiratory symptoms and analysing nose and throat swabs to confirm infection in those suspected of being ill.

Earlier research has shown that the serum antibody titres which are used to test the efficacy of vaccines do not distinguish between older adults who develop influenza from those who do not. Dr McElhaney explains: ‘The tests we have now to evaluate a vaccine’s effectiveness do not work very well with older adults. The findings of this research will lead to a new way of testing vaccines much earlier in the process. Ultimately it will bring new vaccines for an aging population to market quickly and more cost-effectively.’ Therefore, they determined that it was essential to include other immunologic measures when assessing vaccine efficacy in the older population.

Drs McElhaney and Kuchel had several long term goals for this innovative five-year research project. The first was to identify T-cell responses which could act as biomarkers (indicators of biological processes) for serious complications of influenza in older adults. The second aim was to develop a clinical tool and set of biomarkers which could be used at the point of care to predict how the patient would respond to the influenza vaccine. They also sought to develop more useful tests to gauge the effectiveness of new vaccines.

A number of novel methods were involved in the investigation. This research project will be the first to use transcriptome sequencing to evaluate and analyse T-cell responses to identify a set of biomarkers that predict vaccine failure. This investigation into the correlates of effective T-cell response when compared to ineffective responses can help researchers understand at a population level how and for whom new vaccines should be developed.

The research team also investigated granzyme B activity as a biomarker for vaccine response. Granzyme B is an enzyme found in granules along with perforin, which when released by immune cells, enters the virus-infected cells through perforin and kills the cell to clear the pathogens. They found that increased granzyme B activity induced by influenza virus could predict a positive response to the vaccine which in turn prevented infection.

Other biomarkers proved more complicated



to pin down. One cytokine, IL-6, has both pro- and anti-inflammatory properties. On one hand, it has been linked to inflammaging, frailty and poorer clinical outcomes. On the other hand, IL-6 plays a key role in mounting an immune response to the influenza vaccine and infection. While it is a valid predictor of clinical outcomes when measured from peripheral blood samples, it can be a more difficult determinant within specific tissues and organs where levels tend to be more tightly regulated. Therefore, the ways in which the dysregulation of such biomarkers contribute to vaccine responses require further investigation.

**Predicting Vaccine Efficacy**

Taking the above into account, Drs McElhaney and Kuchel sought to establish Frailty Index scores and positive CMV status as correlates of disease severity and vaccine efficacy by analysing cytokine and granzyme B responses to the influenza vaccine in 150 older adults and 20 young adults. They postulate that CMV seropositivity and high levels of frailty predict an increase in the damaging effects of extracellular granzyme B which in turn affected the body’s overall response to the vaccine. This may be due to the abnormally high baseline levels of GrzB in the resting T-cells of CMV positive individuals, which do not express perforin in response to influenza and are thus associated with a dysfunctional memory response to an influenza challenge. Previous results have shown that low GrzB activity in influenza-stimulated cells was correlated with developing influenza infection and increased disease severity. Current work is further defining how inflammation or anti-inflammatory responses, levels of frailty and CMV status could predict vaccine efficacy and risk for functional decline during influenza illness in older adults.

With the same sample of 150 older adults, the team explored whether a high dose vaccine induced a protective response in more subjects than a standard dose. Within each group, levels of frailty, inflammatory cytokines and GrzB activity were compared between those who developed influenza symptoms and those who did not. It was found that the increased dose of the vaccine boosted the immune response more effectively in older adults.

The project is still in its early days, but the results so far are impressive. The team have succeeded in developing and validating highly sensitive assays of T-cell responses to the influenza vaccine and identifying biomarkers of protection against disease. The development and manufacture of a new vaccine could cost up to 1 billion US dollars, therefore the industry requires robust means of confirming the effectiveness of new vaccines in as short a period as possible.

The project thus far has made it clear that vaccines that stimulate enhanced T-cell responses will improve primary prevention of influenza in older adults. The research suggests that the age related decline in immune function is reversible and it would be possible to improve protection through vaccination strategies which improve presentation of the internal proteins of the virus to the defence system.

**Future Project Goals**

The next steps of the project involve taking a closer look at the source of IL-10 production, a cytokine which impedes immune response when in excess. There will also be greater emphasis on the effects of adjuvants to the influenza vaccines and there potential to enhance protection.

They also hope to focus on developing a point of care test to vaccine responsiveness so as to develop clinician’s knowledge of how to assess and modify disease risk. With such knowledge, healthcare providers can advise appropriate prevention strategies to their patients in addition to vaccination so as to reduce influenza related disability. With such high morbidity and mortality related to influenza infection in adults over 65, Dr McElhaney and Dr Kuchel’s research is a major step in protecting the years and quality of life of our older population.

# Meet the researchers


**Dr Janet McElhaney**
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Dr Janet McElhaney is the VP Research and Scientific Director of the Health Sciences North Research Institute and Professor in Clinical Sciences Division at the Northern Ontario School of Medicine. She is also Health Sciences North Volunteer Association Chair in Healthy Aging. After completing her medical degree in the University of Alberta, she completed her fellowship in geriatric medicine. Her research interests include the impact of immunosenescence on immune responses to vaccination, immunological biomarkers of protection mediated by vaccination and how vaccination plays a role in preventing disability in older adults. Her 13-year collaboration with Dr Kuchel has synergised the expertise in inflammation and frailty, and its impact on immune function and functional decline related to infectious diseases.

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Dr George Kuchel is Professor of Medicine in the University of Connecticut. After completing his medical degree in McGill University, Kuchel specialised in Geriatric Medicine at Harvard Medical School. He currently serves as director of the UConn Centre on Ageing, Division Chief of Geriatric Medicine and Citicorp Chair in Geriatrics and Gerontology. His research interests include pathophysiology of common geriatric syndromes, the role of inflammatory molecules in mediating muscle loss and sarcopenia in older adults, and the design of targeted interventions capable of altering the natural history of common geriatric syndromes.

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# GROWING OLD GRACEFULLY: THE SCIENCE BEHIND AGING

**Professor Arlan Richardson** and his colleagues at the University of Oklahoma Health Sciences Center investigate the biological mechanisms behind aging. Professor Richardson is specifically interested in the effects of aging and dietary restriction on gene expression, testing the oxidative stress theory of aging in mice and most recently, studying the effect of the drug rapamycin on aging and age-related diseases.

### The biology of ageing

Worldwide, scientists are curious to understand how and why we age. These questions are complex, and the answers remain unclear. Research focusing on certain cellular components, such as DNA and the mitochondria, has aimed to understand the aging process. In addition, researchers have explored the food we eat and even the air we breathe to find answers to these questions.

Philosophers and scientists have proposed theories of how and why we age since ancient times. In the 1990s, it was estimated that there was about 300 theories surrounding this topic. Scientists continue to narrow down these theories and categorise them, in order to better understand the mechanisms behind aging. This knowledge goes much deeper than simply trying to find the 'fountain of youth'; understanding the mechanisms that lead to the decline of the human body may lead scientists to interventions for age-related diseases, such as cancer, Alzheimer's disease and kidney failure. Ultimately, answering these questions might mean longer, fuller and healthier lives for a vast number of people. Professor Richardson has devoted his career to aging research for the past 40 years, and thus, he is committed to advancing our understanding of the biology of aging.

### Reducing calories to increase lifespan

The aging process increases certain pathologies and decreases most physiological functions. Dietary restriction (or caloric restriction) increases lifespan by reducing or delaying these aspects of aging in numerous genotypes of laboratory rodents. In fact, dietary restriction increases the lifespan of a wide variety of organisms ranging from invertebrates to various strains of rats and mice, certain breeds of dogs and rhesus monkeys. These data led scientists to believe that the effect of dietary restriction on longevity and aging is universal, but recent contradictory data using over 40 different lines of mice with different genotypes have put this hypothesis under question because dietary restriction did not always increase lifespan and many of the genotypes showed a decrease in lifespan. One reason for these contractor data is that there might be a certain level of dietary restriction that is required to increase lifespan depending on the genetic background of the animal and higher levels of dietary restriction are negative.

In support for this explanation for the contradictory data, Professor Richardson and his team recently showed that 10% dietary restriction surprisingly increased the lifespan of rats similar to that observed in the rats on a 40% dietary restriction. Currently, his laboratory is studying the effect of 10, 20, and 40% restriction in 8 different lines (genotypes) of male and female mice.

Based on other similar studies involving rodents, it had been assumed that for dietary restrictions to have a similar effect in humans, a 30–40% reduction in food consumption is necessary. For most people, such a dramatic reduction in calories is very difficult and for some even impossible. The data from Professor Richardson's study have important translational implications because

## ‘Dietary restriction is the first intervention shown to delay aging and increase lifespan in rodents, which has been shown in a variety of animal models’

it suggests that even a 10% caloric restriction might achieve most of the benefits of dietary restriction for humans.

**Understanding the oxidative stress theory of ageing**

In the 1950s, the ‘free radical theory of aging’ was proposed. This theory postulates that that through normal metabolism the body generates oxygen free radicals that play a role in the aging process. This is because the free radicals cause oxidative damage to large molecules in cells, which accumulate with age. This theory has since been modified and is now called the ‘oxidative stress theory of aging’ because oxygen species, such as peroxides and aldehydes, which are not actually free radicals, also induce oxidative damage to cells.

Over the years, data from studies involving invertebrates and rodents show a correlation between increased lifespan and resistance to oxidative stress. However, Professor Richardson’s group addressed the fact that there is limited direct evidence showing that altered oxidative damage/stress plays a role in aging. In order to better examine this theory, Professor Richardson and his colleagues collected all of their lifespan data, from 8 years of research, on transgenic/knockout mice with alterations in a wide variety of genes involved in the antioxidant defence system. Surprisingly, their data demonstrated that almost all of the alterations in the antioxidant system of mice had no effect on lifespan, and according to Professor Richardson’s team, this suggested that oxidative stress/damage does not plays a major role in the molecular mechanism of aging in mice.

After conducting these studies, Professor Richardson realised that one aspect surrounding the outcome needed further clarification. That is, when analysing the data, the endpoint used to measure aging was lifespan, which is the gold standard in determining whether a particular mutation or treatment affects aging, and there is little direct evidence to support the oxidative stress theory of aging when lifespan is used as the measurement. However, if the expression of antioxidant genes is studied in the context of age-related disease models, an altered antioxidant defence system significantly impacts disease progression or severity, which is what is predicted by the oxidative stress theory of aging.

Professor Richardson has proposed two possible reasons for these contradictory observations on the effects of manipulating antioxidant genes on lifespan and age-related pathologies. The first explanation is that oxidative stress does not affect aging but affects the progression of age-related pathologies, which means that oxidative stress/damage plays a role in health span or healthy aging. The second explanation for these conflicting findings is that the effect of oxidative stress on aging depends on the environment. With these potential explanations, Professor Richardson has proposed that in order to clarify the role of oxidative stress in regulating lifespan, then both health and lifespan under various environmental conditions must be clarified. Under optimal conditions for the rodents, lifespan is relatively unaffected by oxidative stress, but healthy aging (measured by health span) is altered by oxidative stress. Thus, Professor Richardson suggests that if both the health and lifespan of antioxidant mouse models under optimal, healthy conditions as well as under chronic stress or disease states are examined then we might have a better understanding of the larger role that oxidative stress plays in healthy aging.

**The Tithonus effect**

Rapamycin is a drug that is approved by the FDA for cancer patient and those undergoing organ transplantation. Mice treated with rapamycin demonstrate an increased mean and maximum lifespan and show a delay in all competing causes of mortality (i.e., age-related diseases), which suggests that rapamycin slows aging. These data imply that rapamycin might be an anti-aging drug, but Professor Richardson cautions that before this conclusion can be made, rapamycin must be assessed to determine if it produces a ‘Tithonus effect’. By this, he means that the increased lifespan might come with more disability and disease and a greater loss of physiological function, reducing the quality of life, which was vividly depicted in the Greek myth about Tithonus, a prince of Troy. The goddess Eos kidnapped Tithonus and she asked Zeus to make him immortal. Alas, Eos failed to ask for eternal youth—hence, the ‘Tithous effect’.

Professor Richardson examined the available data to determine whether mice that are fed rapamycin demonstrate a Tithonus phenotype. In other words, does rapamycin increase lifespan without improving the quality of life? After examining the data, he first found that there was not a general increase in the pathology at death. Actually, some pathologies were reduced. Secondly, he observed that certain key physiological functions were enhanced by rapamycin. Lastly, he found that, in addition to cancer, several diseases are delayed by rapamycin in mice models of human diseases, including atherosclerosis, Alzheimer’s disease, Hutchinson-Gilfords progeria syndrome and Huntington disease. Rapamycin appears to broadly affect a large number of age-related diseases that are important in human health.

In addition, after examining all of the data, Professor Richardson found that the mice that are given rapamycin live three to four months longer, which is the equivalent of about a decade in human years, and show a similar health status/quality of life at the time of death as mice that do not receive rapamycin. Actually, the mice on rapamycin seemed to show an improved function in certain physiological parameters as well as a reduced incidence/severity of some age-related diseases. Altogether, this means that they do not exhibit the Tithonus phenotype.

Professor Richardson believes that these findings are important because the data support the possibility of clinical trials to study the efficacy of rapamycin in treating diseases that affect the elderly, especially those that are debilitating and have no known treatment, such as Alzheimer’s disease and other neurodegenerative diseases.

**Combining rapamycin with dietary restriction to extend longevity**

Separately, rapamycin and dietary restriction have been shown to increase lifespan (as discussed above). Rapamycin targets a protein in cells called mTOR (mammalian target of rapamycin). Part of the mTOR signal transduction pathway involves the major nutrient sensing pathway in mammals, and because of this, it was initially thought that the mechanism of how rapamycin increases lifespan was similar to dietary restriction. Several research groups have conducted studies

to test the hypothesis that dietary restriction and rapamycin affect lifespan through similar pathways, and the results show that the two treatments share similar mechanisms. However, even though similarities were demonstrated, differences were also revealed. Therefore, whether they actually share similar molecular mechanisms remained unclear.

Professor Richard’s team aimed to further clarify the combinatorial effect of dietary restriction and rapamycin on extending longevity. They compared the expression of genes (the transriptome) and the metabolic markers (the metabolome) in the liver of mice that were given a regular diet, rapamycin, a dietary restriction or a combination of rapamycin and a dietary restriction. The data analysis showed that rapamycin and dietary restriction are distinct groups. When the mice were treated with rapamycin or a dietary restriction, more than 2500 genes were significantly changed compared with the mice fed a regular diet, and more than 80% of the genes were unique to either dietary restriction or rapamycin.

When the metabolic markers were analysed, there was an even greater difference between rapamycin and dietary restriction. No metabolic markers were significantly different between the rapamycin-treated mice compared with the mice on a regular diet. In contrast, 173 metabolic markers were different in the dietary restriction mice. Interestingly, when the mice were treated with a combination of rapamycin and a dietary restriction, the number of genes significantly changed was twice as large as the number of genes significantly altered by the treatments alone. Thus, the overall effects of dietary restriction or rapamycin on the liver are very different, and when rapamycin and a dietary restriction are combined, the results show alterations in a large number of genes and metabolic markers that are not significantly changed by with the treatments or by themselves. These results suggest that the combinatorial treatment would be more effective at extending longevity than either treatment alone.

# Meet the researcher


**Dr Arlan Richardson**
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Professor Arlan Richardson is a professor of Geriatric Medicine and the Donald W. Reynolds Endowed Chair of Aging Research at OUHSC and is a Senior VA Career Scientist at the Oklahoma City VA Medical Center. He has received the Nathan Shock Award from the Gerontology Research Center at the National Institute on Aging for his pioneering research on the effect of dietary restriction on gene expression, the Robert W. Kleemeier Award for outstanding research in the field of gerontology from the Gerontological Society of America, the Harman Research Award for research contributions in the field of aging and dietary restriction from the American Aging Association, and the he Lord Cohen Medal for Services to Gerontology from the British Society for Research on Ageing.

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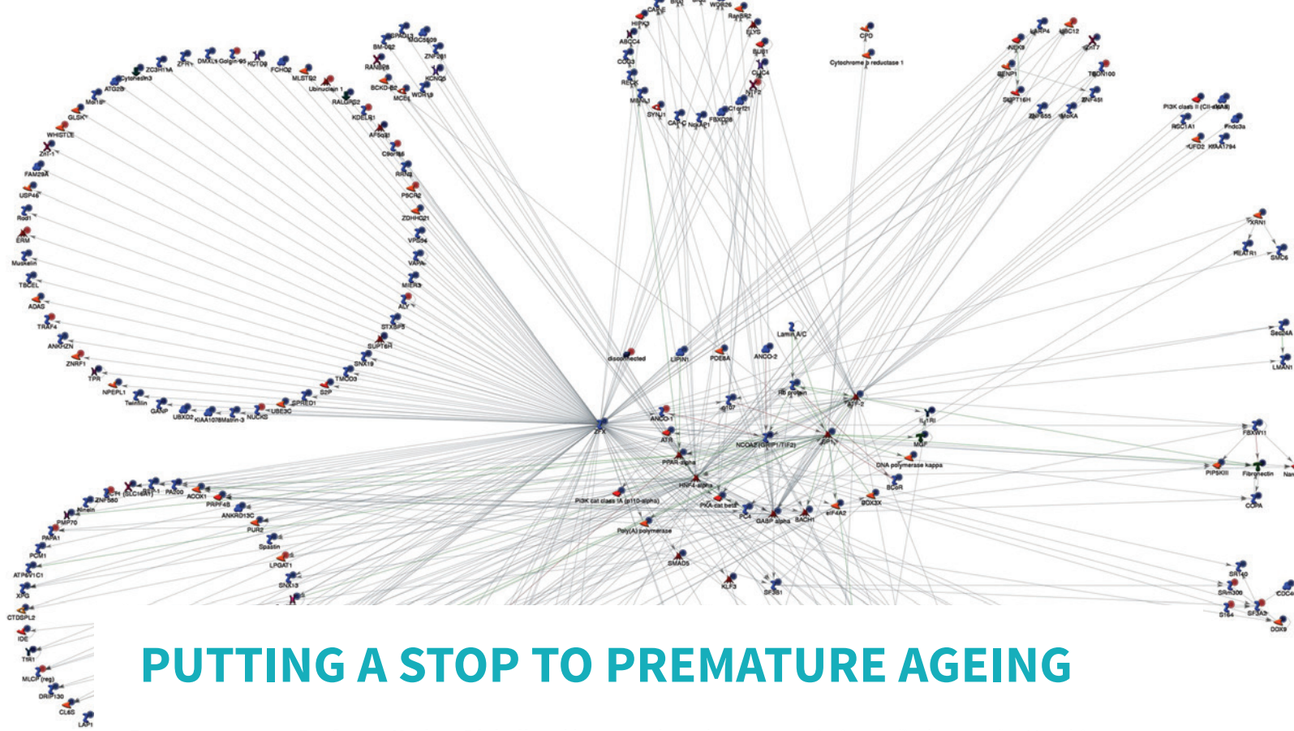
The University of Oklahoma Health Sciences Center





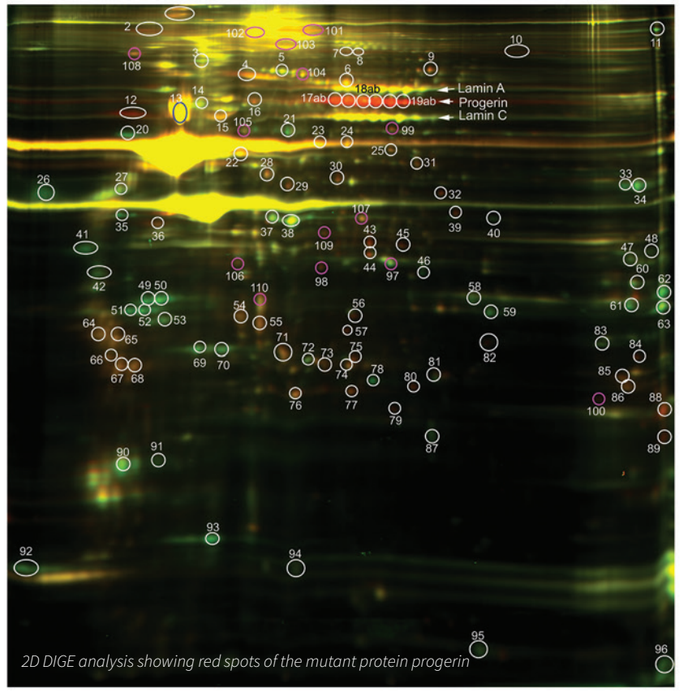


# PUTTING A STOP TO PREMATURE AGEING

For over ten years, **Professor Karima Djabali** and her team have been on a mission to find a cure for Hutchinson-Gilford Progeria Syndrome. This cutting edge research can inform both interventions for this fatal disorder and reveal further insights into normal ageing.

**What is premature ageing?**

Hutchinson-Gilford Progeria Syndrome (HGPS) is a harrowing diagnosis to receive. This rare genetic disorder can be characterised by premature ageing, particularly of the vascular system, leading to severe atherosclerosis (narrowing of the arteries) and consequently, stroke or heart attack. Other symptoms of the disorder include growth delay, loss of body fat and osteoporosis. Young people diagnosed with HGPS have a life expectancy of just seven to 20 years. So what is being done to combat this tragic disease? Professor Djabali and her team are working to define the molecular and cellular processes that lead to the development of HGPS and to find effective therapeutic interventions for children affected by the condition.




*2D DIGE analysis showing red spots of the mutant protein progerin*

**Discovering the Mechanisms of Disease**

There have been significant gains in knowledge surrounding HGPS pathophysiology since 2003. Researchers associated with the Progeria Research Foundation research focus now know that HGPS is caused by mutations on the LMNA gene, the role of which is to provide

## Hutchinson-Gilford Progeria Syndrome is caused by mutations on the LMNA gene which encodes lamin A and C proteins that are critical components of the nuclear envelope that protects the genome integrity.

*Normal aging*

instructions for the production of lamin A and lamin C. Lamins are proteins which line the inside of the nuclear envelope and play an important role in the organisation and the scaffolds of the nucleus of the cell. There are two categories of these proteins: A-type lamins and B-type lamins. In almost 90% of HGPS cases, mutation occurs on a part of the LMNA gene which leads to the deletion of 50 amino acids in the lamin A protein. The deletion of these amino acids means that the cells can no longer produce mature lamin A and instead a mutant form of lamin A (known as progerin) is formed. Progerin then accumulates in the nucleus and causes severe deformations in the nuclear membrane and major reorganisation of the chromatin. This affects the cell cycle and cell migration and promotes premature senescence (the deterioration of cellular function).

The cardiovascular system is the primary target of HGPS. Progerin accumulates predominantly in the nuclei of the vascular cells with the consequence of dramatically accelerated cardiovascular disease in young patients with HGPS. The disease process of HGPS is similar to that of the vascular pathology of ageing – they both feature hardening and narrowing of the arteries and small blood vessels, as well as prominent adventitial fibrosis (the scarring and thickening of the outer layer of the arteries.)

Most recently, Professor Djabali and her team reported on how the build-up of progerin in the nucleus impairs chromosome maintenance during mitosis. Progerin accumulation disrupts cell division by displacing centromere protein F, a protein which attaches the chromosome to the spindle fibre in the middle phase of mitosis. The end result is delayed reformation of the nuclear envelope, genomic instability and cells with two nuclei rather than one. The cumulative effect of these defects with each round of mitosis then predisposes cells to premature dysfunction.

**Journey to a cure**

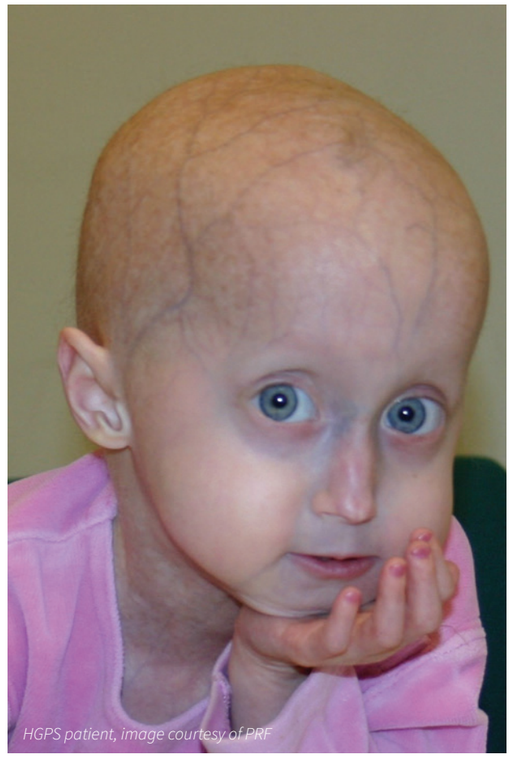
With each study, Professor Djabali and her colleagues get one step closer to a cure. Their 2010 paper explains the influence of protein farnesylation on the pathology of HGPS and how this could be inhibited. Protein farnesylation in this case refers to the retention of a farnesyl group (a carbon-based organic compound) to prelamin A, the precursor to lamin A. As stated above, the LMNA mutation associated with HGPS leads to the deletion of amino acids, including the amino acid which usually signals the cleavage site for the farnesyl group from prelamin A in order to form mature lamin A. Instead, the farnesyl group remains attached making progerin toxic to the nucleus. The team's research suggests that this impacts the lamin A-retinoblastoma protein (pRB) signalling network, causing pRB to interact differently with its downstream transcription factors and regulators, thus implicating this network as a key factor in HGPS pathogenesis. Now that the mechanism starts to unravel, the team treated HGPS fibroblasts with a protein farnesyltransferase inhibitor (FTI) and found that this reversed abnormal gene expression in 288 of the 352 genes found in fibroblasts from HGPS patients. This outcome suggests that modulation of the lamin A-pRB signalling network could be a key factor in slowing down premature ageing.

A 2012 study by Professor Djabali and her colleagues provided the first in vivo evidence (evidence from within a living organism) that progerin is produced in adult stem cells (visualised on skin sections). Through the development of a new method of isolating naïve multipotent skin derived precursor cells from human fibroblast cultures, the team were able to observe a number of cell properties. These precursor cells had similar stem cell properties to cells isolated from skin biopsies. The cells could self-renew and differentiate in smooth muscle cells and fibroblasts. They were also found to express nestin (a marker of neuronal stem cells). A subset of these cells exhibited progerin positive signals which could reiterate the same molecular process which leads to progerin accumulation in adult stem cells. This study provides a viable alternative to skin biopsy samples from HGPS patients, which are not readily available for research, and opens new avenues to study the pathogenesis of other rare diseases.

In a 2014 study, Professor Djabali and her team investigated the use of sulforaphane (SFN) as a treatment for HGPS. SFN is an organic compound which can be found in cruciferous vegetables, such as young broccoli and cauliflower sprouts. The team aimed to test the ability of SFN to induce progerin clearance and improve disease phenotype in HGPS cell cultures using two-dimensional differential in a gel electrophoresis (2D-DIGE) approach. Progerin accumulation results in alterations to several components of protein degradation pathways. It leads to the impairment of autophagy (the cell's ability to degrade and recycle cellular components) and proteasome activity (the breakdown of damaged or unneeded proteins). As a potent inducer of antioxidant and detoxification enzymes, SFN also enhances the protein degradation systems and promotes progerin clearance and proteostasis (the process by which cells control the quantity and folding of proteins). SFN does this through modulation of the nuclear factor erythroid 2-related factor (Nrf2) signalling pathway.

The Nrf2 signalling pathway can be looked upon as a 'master regulator' of antioxidant defence within the cells. Nrf2 targets genes which promote resistance to cancer and other diseases through the transcription of detoxifying and anti-inflammatory proteins. It may also play a role in preventing premature ageing. So how does SFN fit in? It's all down to the interaction between SFN and a sensor known as Keap1. Keap1 is part of the body's stress response system and inhibits Nrf2 signalling. SFN disrupts this process and allows Nrf2 to accumulate in the nucleus and activate the transcription of its target genes some of which were identified as downregulated in HGPS cells in the 2D-DIGE analysis.

In the study, cells treated with SFN exhibited significant increases in growth rate, proteasome activity and autophagy. SFN treatment also led to more efficient DNA damage repair in the cell, increased ATP levels and normalisation of the nuclear envelope. Significantly, the increase in proteostasis and progerin clearance remained high over 12 weeks, supporting its efficacy as a long term therapeutic strategy in cell based system and show potential for children with HGPS. Therefore, Professor Djabali's team are conducting further studies to determine the extent to which modulation of the Nrf2 pathway with SFN could be a candidate target for future clinical trials.



*HGPS patient, image courtesy of PRF*

Additionally, stimulating the Nrf2 pathway may be beneficial to normal cells, progerin and prelamin A also accumulate in some cells of the skin and vascular system in unaffected individuals. Because the number of cells expressing this abnormal protein – progerin – seems to increase with age, further studies are underway to test the potential of manipulating this pathway in the context of physiological ageing. Remarkably, these studies show once again that important insight into the ageing process can be gained by studying HGPS. HGPS may not only pinpoint therapeutic avenues for fighting this rare genetic disorder, but might also teach us more about normal ageing and provide clues for how to slowdown ageing processes in general.

**The Latest Cutting Edge Research**

More recently, the findings of a 2016 study by Professor Djabali and her colleagues further explain the underlying mechanisms of genomic instability in HGPS cells. By investigating the effects of progerin on the dynamics of mitosis, the team uncovered evidence which appears to directly link progerin action to defects in chromosome segregation, nuclear envelope reformation and other cell defects. The first part of the study analysed the distribution of proteins in relation to the components of the nuclear lamina and envelope during mitosis. The findings showed that progerin began to cause defects in chromosome segregation as early as the metaphase (shortly after the breakdown of the nuclear envelope). This led to delays in nuclear envelope reformation as well as trapped cell components and proteins in the endoplasmic reticulum during cell division.

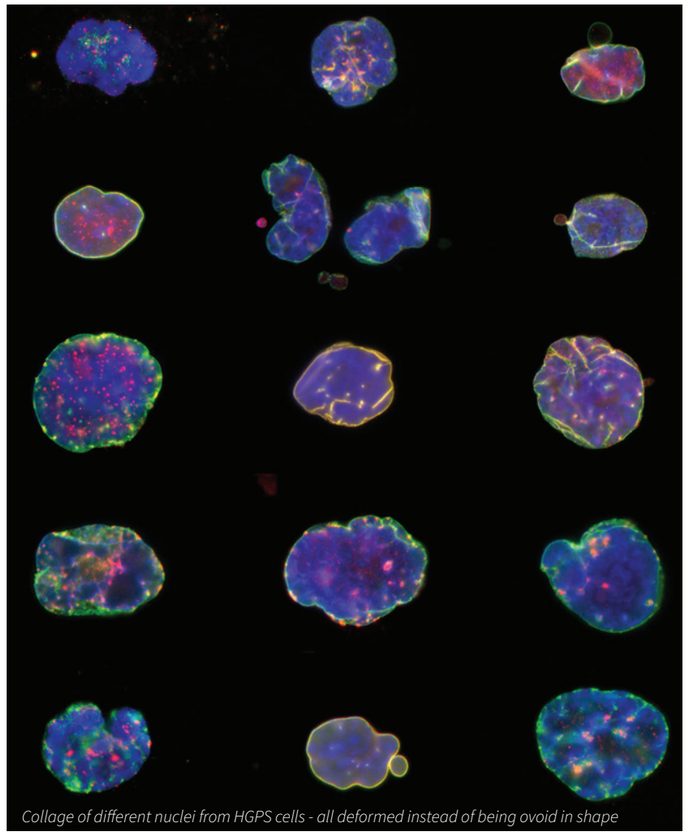
The second part of the investigation looked at the effect of progerin on the localisation of two proteins, CENP-E and CENP-F, on kinetochores.

Kinetochores are protein structures which allow chromatids to attach to spindle fibres and are required for proper segregation of chromosomes. While distribution of CENP-E was similar to that in normal cells, CENP-F was displaced from kinetochores during the metaphase. This suggests that CENP-F could be a target of progerin during mitosis. The team also found evidence of increased chromatin lagging and genomic instability which increased proportionally with progerin levels. These progerin dependent defects accumulate with each round of mitosis, suggesting that high levels of progerin predispose cells to undergo premature senescence.

**Delaying Age Related Disease**

There is also a lot to learn from this research regarding physiological ageing outside of disease processes. By examining the processes underlying premature ageing syndromes, Professor Djabali and her colleagues can determine whether or not similar mechanisms underlie normal ageing. It may also lead to insights in the pathology underlying other age related diseases, particularly vascular and neurological disorders affecting the elderly population. Therefore, the team aim to define isolate and characterise skin stem cells in order to determine how ageing might impact stem cell renewal and differentiation. This unique approach to the study of ageing makes the laboratory the only one in Germany to examine the characteristics of ageing from this angle.

So far, studies into HGPS have led to some interesting discoveries in the processes of ageing. An examination of skin biopsies from individuals across the life span showed a comparable frequency of progerin at a low level in all age groups, but increased levels and deeper distribution of the protein were found in the older population. This suggests that progerin expression could be used as a biomarker for normal cellular ageing. Likewise, in a histological comparison of vascular pathology in HGPS and vascular pathology of ageing, the team and collaborators detected progerin in both samples, supporting the theory that progerin has a role in cardiovascular ageing amongst the general population. Similar results were found in a later study examining the link between atherosclerosis in ancient humans, premature ageing syndromes and normal ageing. After imaging studies of ancient mummies revealed vascular calcification, researchers became interested in the possibility of a genetic predisposition to atherosclerosis. In their 2014 study, the team found that progerin can be detected in vascular cells. The resulting loss in cells from the vascular walls and replacement by fibrous tissues promotes hardening and calcification of the blood vessels. Although vascular progerin is found in low levels in the general population, these levels increase with age, suggesting a potential role for progerin in age-related atherosclerotic processes.



*Collage of different nuclei from HGPS cells - all deformed instead of being ovoid in shape*

**Next steps in ageing research**

So what's next for Professor Djabali and her team? As well as continuing her search for a cure for HGPS, the knowledge gained from the research on progeria could drive future research in Alzheimer's disease (AD). By dissecting the underlying mechanisms of ageing cells in patients with AD, it may be possible to better understand which processes are inducing neuronal degeneration.

Research into treatments for HGPS may also be useful in identifying new targets for cancer therapy. In simple terms, cancer is the uncontrolled division and growth of cells. One of the major risk factors for cancer is ageing. What is remarkably intriguing is that cellular ageing leads to cell cycle exit via down regulating signalling pathways controlling the proliferation of cells. In cancer some of the overlapping signalling triggers the opposite outcome. How does the balance between these signals dictate such opposite cellular fate? Can HGPS teach us which signalling or gene targets could lead to anti-cancer treatments? Professor Djabali is searching for answers.

Finally, a greater understanding of how adult stem cells work can inform work in regenerative medicine and drug therapies. Studying the mechanisms by which protein breakdown regulates the processes of ageing could be the key to identifying therapeutic interventions which promote healthy cellular function and ameliorate age-related pathologies.



# Meet the researcher

**Professor Karima Djabali**
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Professor Karima Djabali is a faculty Professor at the school of Medicine, Technical University Munich. She was previously Assitant Professor in the Department of Dermatology in Columbia University, New York, and scientist at the CNRS, France. Since receiving her PhD in Biology from the University of Paris 7, Professor Djabali has undertaken a number of prestigious postdoctoral fellowships and research positions and has received grants from the National Institute of Health, the Alexander von Humboldt Foundation, the Progeria Research Foundation, and the DFG. She has also received a number of awards for her research, including the Irving Clinical Research Career Award and Christine Kühne Center for Allergy Research and Education (CK-CARE) Award. For more than a decade, Professor Djabali and her team have focused their research on cellular ageing under normal and disease conditions, specifically in Hutchinson-Gilford progeria syndrome a very rare segmental premature aging syndrome. Her lab also aims to define, isolate and characterise adult stem cells for basic science, disease modeling and therapy. Together with outstanding international collaborators, she hopes that one day they can defeat HGPS disease and possibly slow down other age-related conditions.

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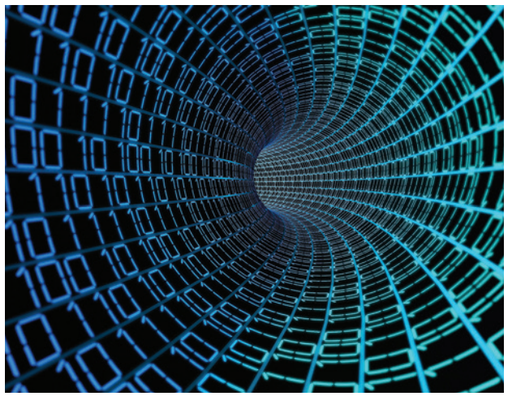
# MATHEMATICAL APPROACHES TO BIOSCIENCE



The use of mathematical modelling has proven to be a valuable tool for bioscience applications. A variety of modern bioscience disciplines rely heavily on the use of computers and mathematical approaches to perform complex virtual modelling or enormous quantities of calculations, too tedious or numerous to even contemplate performing manually. From the humble pocket calculator to the largest supercomputers, the way we conduct bioscience research has been changed irrevocably by the digital revolution. Numerous biological processes are inherently complex, and are difficult to characterise and examine in a traditional wetlab context.

The investigation of such processes is well served by the processing power of modern computing technology. For example, researchers involved in drug discovery now routinely use virtual modelling to screen millions of 'virtual' compounds for potential activity at a specific biological target. In this manner, they avoid the nearly impossible task of synthesising and screening those same compounds in a traditional wetlab setting. This means that expensive synthesis and screening only needs to happen for those compounds that show the most promise in the virtual models.



In this section we explore the work of several researchers who utilise computer modelling in all or part of their work to derive cutting edge results, that would otherwise be difficult or impossible to obtain. In the first article of this section we look at the work of Dr Mathias Currat of the University of Geneva. Dr Currat is a population geneticist and computer scientist who studies human evolution. Here, we discuss his latest project which uses computer modelling and genetic data to investigate the demographic and migratory events that helped to form the human European gene pool. In the second article of this series we talk to Dr Heiko Reutter of the University of Bonn who also studies human genetics. Dr Reutter studies the genetic basis for birth defects of the uro-rectal area, oesophagus and trachea using genetic sequencing. Genetic sequencing involves enormous datasets which require computer based bioinformatics approaches for analysis.

From here, we introduce the research of Dr Jing He of Old Dominion University in Virginia. Dr He's research team use advanced computational techniques to analyse 3-dimensional electron microscopic images of frozen proteins, with the ultimate goal of determining their 3-dimensional structures. They also employ powerful machine learning methods to determine secondary protein structures. Last but not least we discuss the work of Dr David Stanford of the University of Western Ontario. Dr Stanford uses Queueing Theory mathematics to provide solutions to real-world healthcare problems. The solutions devised by Dr Stanford can be applied to emergency department wait times and organ donor priority lists, making wait times shorter and fairer for those in need.

# SIMULATING GENETIC PATTERNS IN EUROPEAN HUMAN EVOLUTION

**Dr Mathias Currat** is a population geneticist and computer scientist interested in human evolution. Here he describes a new project combining computer simulations and molecular genetic data to understand the role played by various demographic and migratory events that established the human European gene pool.

**For starters, could you tell us a little more about your background and what attracted you to the field of human evolution?**

Having studied biology at the University of Geneva, I started to investigate more specifically human evolution when I joined the department of anthropology for my Master degree. I was attracted by the multidisciplinary frame of mind of the research carried out in that field, as well as by the new computational methods developed by my host lab, which is at the cutting edge in this domain. I was fascinated by the crossover between various disciplines such as biology, computer science and history, which fulfilled my hunger for learning. I consider myself fortunate to learn new things on a daily basis through my research and teaching in subjects as diverse.

**You hold degrees in both biology and computer science. Do you consider population genetics an 'ideal cross-over' between the two?**

Nowadays, computer skills are a primary tool in most areas of biology and more generally of science. This is particularly true in the field of population genetics which is mainly based on theoretical models, mathematical and statistical developments which can be implemented in computer programs. Moreover, the accumulation of genetic and genomic data over the last decades made their analysis more complex and it is now almost impossible to avoid computational methods to store and analyse these large datasets. In that context, computer simulations have become an indispensable tool because they allow studying complex models. For instance, they allow me to incorporate in the models both the effects of population demography and the molecular evolution of DNA, which is essential when analysing genetic and genomic data.

**SERIAL SPLATCHE is a new simulation modelling technique you developed to tackle your main research questions. In what way is it different from existing simulation techniques?**

SERIAL SPLATCHE is derived from SPLATCHE, a program developed initially with my colleagues Prof. L. Excoffier (University of Bern) and Dr N. Ray (University of Geneva), which allows making spatially-explicit simulations. It simulates the movements of people and hence of their genes through time within a given geographic area. There are few programs of that kind available, and SPLATCHE is the most versatile of them, allowing the simulation of realistic scenarios, such as the effects of migration, demographic growth, competition for the resources, mixing between species, mutation, and many other factors. Moreover, SERIAL SPLATCHE is the only spatially explicit program capable of simulating ancient DNA data retrieved from fossil bones, all other approaches including only DNA from modern populations.

**You attempt to combine ancient and modern DNA data. A problem with using ancient DNA is that the sample size is usually limited. How can your approach overcome this problem?**

Because of the scarcity of ancient DNA, methods specifically designed to handle it, such as SERIAL SPLATCHE are needed to extract maximum information from it on the evolution of our species. Our approach tries to overcome the limited sample size of ancient DNA by combining into the analyses the large databases of modern DNA in a joint fashion. In that way, we hope to increase the power of our estimations by adding direct picture of the past diversity through ancient DNA to the detailed knowledge of genetic diversity of today's humans.

**You mention that to understand how and when genes diffused in Europe can have important implications in biomedical studies. Can this help in the understanding and possibly the treatment of certain present-day genetic defects in humans?**

Our simulation approach aims at better understanding their evolution and why their occurrence may be so different among populations. Genetic diseases such as cystic fibrosis or iron overload are more frequent in Western Europe than elsewhere. Their negative effects could have been overcome by demographic processes, thus explaining their higher frequency in some populations. Large demographic growths as the arrival of the first Homo sapiens 40,000 years ago and the Neolithic transition 10,000 years ago could possibly have spread some disease-associated variants in European populations. Spatially-explicit computer simulation has precisely described this purely demographic process affecting genetic data. Studying the behaviour of diversity during complex demographic events may serve to understand basic patterns for the spread of genetic diseases in certain populations.



# COMBINING MODERN AND ANCIENT DNA ON A SPATIALLY EXPLICIT SCALE

The genetic diversity of human population is shaped by various processes, such as natural selection and demographic events. The research of Dr Mathias Currat of the University of Geneva is centred on the use of simulation models in reconstructing human evolution on the European continent and determining the importance of demographic events, thereby using a combination of ancient and modern DNA data in a spatially defined context.

**A modelling approach in human evolution**

The composition and genetic diversity of the contemporary human population is shaped by a myriad of processes. Natural selection is clearly one of the major structuring force. New mutations arise in time; unfavourable ones might become extinct whereas others have more chance to be passed on. Past demography and large population migrations also leave their imprint on the gene pool. By statistically analysing these data we can attempt to unravel the evolution of populations and determine the main processes that formed them.
Different approaches exist to tackle these questions. One common approach is comparing the genetic material present amongst existing populations. Populations are defined on the basis of geographic or cultural criteria. Another approach is the use of models. By modelling evolutionary scenarios, we can obtain a theoretically expected genetic diversity that can then be compared to real data. We can use this method to confront evolutionary scenarios with observed data, and try to explain which scenarios are more likely to have shaped present genetic diversity.

A challenging aspect of human population genetics is that there are so many factors acting simultaneously on the gene pool. Considering all these factors together leads to immensely complex models. Fortunately, sophisticated computer programs exist that allow for simulating quite complex models. Thereby we can generate an expected result of genetic diversity under various evolutionary scenarios, combining demographic and genetic parameters and accounting for archaeological and environmental information. The goal of these models is not to reproduce the actual past (which is impossible), but to understand the main processes that may have shaped the genetic diversity we observe today.

**Incorporation of ancient DNA data**

Thus far, mainly contemporary genetic material has been used to study human genetic diversity. Recent advances in DNA extraction, recuperation (sequencing) and analysis techniques have given a spectacular boost to the field, enabling the analysis of DNA from ancient fossils. This ancient DNA or aDNA in short, may be broadly defined as the retrieval of DNA sequences from museum specimens, archaeological finds or fossil remains. This has opened up whole new avenues of paleogenetic research.
By recovering genetic data from different periods in the past (heterochronous genetic data), we can follow changes in genetic diversity over time. aDNA samples have strongly enlarged our knowledge of our own evolution, and new insights include the identification of the Neanderthal portion of our genome and the discovery of a new relative of ours, the Denisovan.

Climatological conditions in Europe, especially the northern and central areas, have promoted the preservation of prehistoric fossils. Therefore, the majority of aDNA data so far has been recovered from this continent, thereby providing a great potential for reconstructing the processes that lead to its genetic diversity. 'Despite several decades of multidisciplinary research on the settlement history of Europe, there are still many open questions and hot debates about the genetic impact of the main demographic and migratory events which shaped the diversity of the populations living today on this continent', says Dr Currat.

For instance, it remains unknown what the genetic legacy is of the first anatomically modern humans (AMHs – Homo sapiens) that entered Europe around 45,000 ago and rapidly colonised the continent and what is of the subsequent settlement episodes. Another pressing question considers the extent of the interaction with the Neanderthal population that preceded AMH. Then there are debates regarding the roles played in shaping genetic diversity of population migrations during the Last Glacial Maximum (between 26,500 and 19,000 years ago) and the Neolithic transition (between 10,000 and 5,000 years ago).
The results of ancient mitochondrial DNA (mtDNA) are particularly challenging to interpret, as they suggest a general pattern of

genetic discontinuity between Neolithic and contemporary populations of the same area. This is in conflict with the view that our present diversity has been largely formed by demographic events during the Neolithic or even before, as analysing DNA from contemporary populations seems to indicate. These are the questions Dr Currat and his collaborators hope to answer in a project funded by the Swiss National Science Foundation.

**SERIAL SPLATCHE, a powerful simulation tool**

This project, entitled 'Reconstructing Europeans' genetic evolution through computer simulations and heterochronous molecular data' aims to synthesise and integrate data from various sources to gain a better understanding of European evolution. The first problem is the lack of tools capable of simultaneously considering aDNA analyses and demography and population migrations. As Dr Currat explains: 'Our work tries to fill this lacuna by developing a new, highly flexible, spatially-explicit approach which is specifically designed to study aDNA at the population level, including the incorporation of spatial elements (geographic locations, movement of populations)'.

This approach has taken shape in the form of SERIAL SPLATCHE, a newly developed simulation method developed by Dr Currat and collaborators. SERIAL SPLATCHE has several unique advantages over existing models. First of all, it is capable of accounting for the geographic characteristics of the study area and exactly positioning populations, samples and migrations. Secondly, it is at present the only spatially-explicit model that can integrate data from both modern and ancient DNA. Thirdly, SERIAL SPLATCHE enables the simulation of complex interactions between populations, such as competition for resources and the mixing of populations.

The project consists of three main research lines. The first line, carried out by PhD student Nuno Silva, focuses on the discrepancies that arose from the mtDNA results. The second line, for which PhD student Jérémy Rio is responsible, aims to optimise the amount of information that can be extracted from aDNA sequences. In the third line, Masters student Nicolas Broccard studies the extent to which local historic processes may have affected genetic diversity without having erased the signature of more ancient demographic events.

**Prospects and other applications**

The simulation approach is already rendering surprising results, which could not have been obtained in other ways. Simulation results indicate a much larger contribution of pre-Neolithic hunter-gatherers than assumed thus far and underline the potential impact of ancient demographic events on the present genetic diversity. 'We believe that our work holds the promise of solving current interrogations and long-standing debates on Europeans' genetic evolution', says Dr Currat.

Although the principal aim of this project is to improve our understanding of European human evolution and the processes that lead to today's genetic diversity, there are also applications in other fields. SERIAL SPLATCHE is a powerful and flexible simulation method, which can in principle be applied to any area around in world and at any scale. Dr Currat explains that it can also be applied to other species: 'as long as their mode of reproduction and dispersion is relatively similar to that of humans'.

The results of Dr Currat's studies also have important implications for biomedical studies. Certain genetic diseases or traits, such as iron overload, cystic fibrosis and lactase persistence occur more in Europe than elsewhere in the world. By understanding how and when genes dispersed in Europe we can obtain an understanding of the spreading and evolution of these diseases or traits, and why their occurrence is so varied amongst different populations.



# Meet the researcher

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Dr Mathis Currat is Senior Lecturer of the Anthropology Unit, Department of Genetics & Evolution of the University of Geneva. He has studied biology and computer science. In 2004, he obtained his PhD in science from the University of Geneva. He also used to work as a freelance web developer and expert. His research specialises in the ancient history of human settlement and evolution in Europe.

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UNIVERSITÉ DE GENÈVE

# STUDYING CONGENITAL HUMAN MALFORMATIONS CAUSED BY TOO MANY GENES

Genetics researcher **Dr Heiko Reutter** and his colleagues at the Institute of Human Genetics of the University of Bonn, Germany, think that some congenital malformations of the uro-rectal area, oesophagus and trachea are caused by too many copies of genes that govern the structure of those organs.

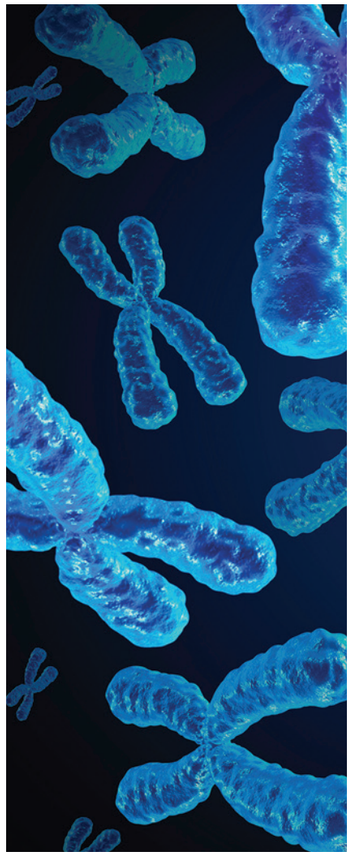
### Genes Make the Man

As every school child learns, we human beings are who we are in large part because of the 46 chromosomes that carry our genetic information. We get 23 chromosomes from our mothers, 23 chromosomes from our fathers and we start our lives with a 46-page blueprint for how we are to look, grow and function. Because of the way chromosomes are passed from parents to child, we expect tall parents to have tall children, brown-eyed parents to have brown-eyed children and so on. Those chromosomes contain roughly 20,000–25,000 human protein-coding genes – the individual basic biological units of heredity – that individually code for various structural proteins, enzymes and the myriad of molecules needed for our bodies to function. It's a complicated system that more often than not runs smoothly. Sometimes, however, defects or malfunctions in those genes can cause structural or functional problems – disease. Paediatric genetics researcher Dr Heiko Reutter has dedicated his professional life to investigating the role of gene abnormalities as a cause of some malformations found in babies at the start of their lives.

### When are too many copies of a gene too much?

The topic of birth defects is a complex one. Some birth defects are caused by obvious external forces. For example, the latest worry in the news is the Zika virus, a virus that can cause microcephaly – an abnormally small head – in babies affected during pregnancy. Another defect doctors address is foetal alcohol syndrome in babies whose mothers drink during pregnancy. Perhaps the majority of birth defects, however, appear to be caused by abnormalities in the baby's DNA that cause structural or functional abnormalities. Some of these defects seem to arise spontaneously, but many are passed down from parent to child. If a parent has an abnormal gene, it can be passed on to the baby as a genetic disease.

Usually, when one thinks of human genetic disease, you think of single mutations. After all, a change in one gene is a simplistic way to think of changes in DNA – mutations. One nucleic acid change due to, say, radiation damage, changes one gene and causes a problem. Humans were thought to have 46 chromosomes, those chromosomes





**As more is known about copy number variations (CNV), more diseases are found to be associated with abnormally large areas of CNV in one or more genes. Dr Reutter and his co-workers have found that CNV may, in fact, be associated with a variety of birth defects**

*The team, from left to right: (upper line) Catharina Luise von Lowtzow, Alina Hilger, Johanna Magdalena Schmidt, Rong Zhang; (lower line) Greta Große, Charlotte Schramm, Heiko Reutter, Valerie Weitensteiner, Franziska Kause, Florian Marsch; (missing) Gabriel Dworschak, Thomas Bogs*

had about 20,000–25,000 human protein-coding genes, and each gene was almost always present in two copies. If there is a malformation or mutation of one of those genes, disease may result. Unfortunately, things are not that simple. Scientists have now discovered that a large number of genes, perhaps 5–10% of the human genome, have a number of copies. These so-called *copy number variations* (CNVs) can have one, three, or even more copies. Sometimes the genes are missing altogether – *a deletion.* But often there is extensive copying that can cause be a cause of genetic variation.

If the CNV results in a large number of copies of the gene or parts of the gene, there may be an imbalance of the product or structure produced by that gene that causes disease. This phenomenon is of great interest to Dr Heiko Reutter, a genetics researcher at the Institute of Human Genetics of the University of Bonn. Some cases of Alzheimer's disease and schizophrenia are thought to be associated with CNVs. As more is known about CNV, more diseases are found to be associated with abnormally large areas of CNV in one or more genes. Dr Reutter and his co-workers have found that CNV may, in fact, be associated with a variety of birth defects.

### On the Trail of the Causes of Birth Defects

Birth defects are perhaps one of the saddest things in the practice of medicine. We normally think of people as being overjoyed with the birth of the fine, healthy 'bouncing baby'. But what if that baby is born with some congenital anomaly that portends a life of suffering, or at least significant difficulty? Dr Reutter has personally seen babies born with, for example, genitourinary deformities like exstrophy of the bladder. The birth defect called bladder exstrophy is the protrusion of the urinary bladder through a defect in the abdominal wall, often associated with abnormalities of the pelvic bones, pelvic muscles, and sex organs. While bladder exstrophy is thankfully rare, when it occurs it can be a cause of great disability for babies, often condemning them to multiple surgeries and perhaps some permanent dysfunction. Even if babies with bladder exstrophy are successfully treated and ultimately have children of their own, the risk of similar malformations in their own babies is increased about 400-fold. Plus, there are higher concordance rates among monozygotic compared to dizygotic twin pairs. Clearly this is a genetic disease.

Early on in his career, Dr Reutter took an interest in the genetics of bladder exstrophy

and related defects. He connected with the German and Austrian Bladder Exstrophy Support Group and with the Association for the Bladder Exstrophy Community in U.S. and Canada, and reported six new families with bladder birth defects. He reviewed these families' histories and the literature, and determined that there was a significant genetic predisposition for susceptibility to this defect. He published this research in the American Journal of Medical Genetics in 2003. While continuing to deal with this defect from a clinical standpoint – he's a paediatrician, after all – Dr Reutter continued to search for a genetic cause. In 2008 Dr Reutter reported in the Journal of Urology an epidemiologic of 238 European families with affected babies. His analysis showed that the problem must be genetic, since such things as paternal age, reproductive history and other exogenic factors seemed to be unrelated to the risk of exstrophy.

For example, in 2011, Dr Reutter and some colleagues published a report in the Journal of Pediatrics of several hundred U.S. and European patients with bladder exstrophy and related abnormalities that correlated various maternal characteristics and the severity of the defect in the baby. It turned out that taking folic acid during pregnancy was related to less severe disease. While exstrophy might be genetic in origin, it looks like there is still something that can be done to positively influence it or prevent it.

He and his co-workers published a number of other clinical papers on families affected with exstrophy, but Dr Reutter decided to expand his study beyond epidemiologic and clinical investigation. He decided to apply complex laboratory genetic techniques to the question and actually look for abnormal genes and gene sequences in the patients themselves. In a breakthrough analysis of an Iranian family with two children with exstrophy, Dr Reutter found that regions of chromosome 4 and chromosome 19 were possibly related to at least one form of exstrophy. A further study that Dr Reutter published in PLoS Genetics just last year included a genome-wide association study of 110 exstrophy patients and 1,177 controls of European origin. Here, an association was found with a region on chromosome 5.

To make a long story short, the cumulated results of current research recently allowed Dr Reutter to review and summarise the scientific evidence that exstrophy is indeed a genetic disease. He observed that recent identification of CNVs associated with exstrophy and similar defects, identification of susceptibility regions and genes through the systematic application of DNA array based analysis, and results of candidate gene and genome-wide association studies provide strong evidence that exstrophy and related syndromes are genetically determined. Like many genetic illnesses, there may be different versions of the disease related to different genes or chromosomes, but the search for truth goes on. In the meantime, exstrophy isn't the only thing Dr Reutter is interested in.

**Looking for Causes of Other Defects… and Finding CNVs**

Like bladder exstrophy and its associated conditions, oesophageal atresia with or without tracheoesophageal fistula are anatomical congenital malformations believed to be caused by multiple genetic and environmental factors. These congenital anomalies are rare, but potentially serious. As Dr Reutter investigated exstrophy, he became interested in oesophageal atresia. Sometimes it occurred in patients with exstrophy. Many of the same techniques, both laboratory and clinical, that he uses for exstrophy can be applied to oesophageal atresia. In fact, he and his colleagues recently reported on 375 patients in a combined Dutch, American and German cohort who were investigated with DNA microarray studies. Researchers compared the CNV profiles of the affected individuals with their unaffected parents and published controls and identified 167 rare CNVs containing genes. One CNV had been previously associated with oesophageal disease. There was an association with chromosomes 15, 16 and 22. They published this study this year in the European Journal of Human Genetics, concluding that CNVs could indeed be a cause or contributor to these types of defects. But there is more.

In a report submitted to the Journal of Neurodevelopmental Disorders for publication, Dr Reutter and his group employed molecular karyotyping and genetic analysis on 35 terminated foetuses with isolated central nervous system (CNS) malformations. They detected five disease-causing CNVs in four foetuses involving regions of chromosome 6, 16 and the X chromosome. They also detected a probably disease-causing CNV involving a region of chromosome 3 in one foetus. The conclusion? CNVs are related to CNS malformations, too, adding to data they published last year showing similar findings in patients with CNS abnormalities as well as anorectal malformations.

**So Close Yet So Far**

Dr Reutter and his colleagues have made a lot of headway in the last decade unravelling the question of the genetics in a number of birth defects. A promising candidate for some of their questions are CNVs. But there's more work to be done and more puzzles to be solved. What is certain is, genetics is more than just a 23 chromosomes from each parent. That's just the start. Be assured, though, that Dr Reutter's dedication to this important field will lead to more answers as babies continue to be born.



# Meet the researcher

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Dr Heiko Reutter was awarded his M.D. from the University of Tübingen in Tübingen, Germany, in 2000. After graduation, Dr Reutter did his residency in Paediatrics and Human Genetics and Fellowship in Neonatology at the University of Bonn in Bonn, Germany. In 2013 he joined the faculty of the University of Bonn in the Department of Neonatology and Paediatric Intensive Care, as well as becoming a Senior Researcher at the Institute of Human Genetics there.

Dr Reutter's research interests initially included the genetic and non-genetic causes of the exstrophy-epispadias complex. From 2004 until 2009 his attention turned to research on the genetic causes of orofacial clefts. Since 2008 he has expanded his research to anorectal malformations. From 2009 until 2012 he coordinated the German Network for Congenital Uro-Rectal Malformations. Since 2011 he is the spokesman of the research centre for rare uro-rectal malformations at the centre for rare disease of the University of Bonn. In 1998, he co-founded the German support group for those affected with the exstrophy-epispadias complex.

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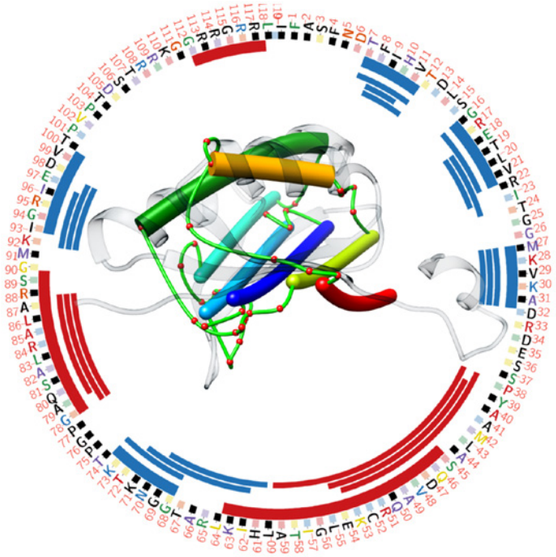
# USING ADVANCED COMPUTATIONAL TECHNIQUES TO DERIVE PROTEIN STRUCTURES FROM 3D CRYO-ELECTRON MICROSCOPIC IMAGES WITH INSUFFICIENT RESOLUTION

Scientist **Dr Jing He** and her colleagues at Old Dominion University in Virginia use advanced computational techniques to interpret 3-dimensional electron microscopic images of frozen proteins to determine their 3-dimensional structures.

**The World of Molecular Structures**

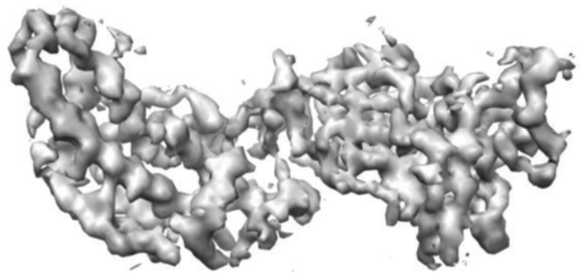
Proteins contribute to all biological functions in cells. An average protein is about a few nanometers in size. What do they look like and how do they work? Our understanding of molecular structures has been improved tremendously over the last half a century. The first molecular structure was proposed by American biologist James Watson and the English biophysicist Francis Crick for the DNA molecule in 1953. The first high-resolution protein structure was determined for myoglobin by John Kendrew and Max Perutz in 1958. Now, there are over 120,000 molecular structures determined and archived in the Protein Data Bank (PDB) that is publicly accessible worldwide. These molecules were extracted from many different organisms and each has an important role in a biological process. In order to understand the mechanism of a biological process, 3-dimensional structures



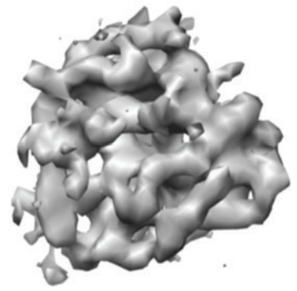
*Matching secondary structures (alpha helices (thicker sticks) and beta-strands (thinner sticks)) detected from cryo-EM density map (extracted from EMDB-1780) and those (red and blue bars) predicted from protein sequence.*



**‘My work is to develop advanced computational methods and tools to interpret 3-dimensional cryo-EM images with insufficient resolutions’**



**3.8Å resolution image (extracted from EMDB-5199)**



**8.6Å resolution image (extracted from EMDB-5223)**

of molecules are absolutely necessary, because the 3-dimensional structure provides a snapshot of all the details about the molecule. A molecular structure lets you see the details about the molecule. You get to see the locations of all major atoms and how they are related. With modern sciences, it is often possible to figure out what molecules are involved in a biological function. However, it is very hard to figure out how it happens. Scientists want to figure out how molecules interact with each other so that they can design tricks to alter or to control the interaction in curing various diseases.

**The Challenge Of Large Molecular Complexes And Cryo-Electron Microscopy**

Rapid determination of molecular structures lies in the development of two important techniques: X-ray crystallography and Nuclear Magnetic Resonance. In order to take pictures of small molecules you cannot use visible light – you need to use radiation of small wavelengths, such as X-rays. For X-ray crystallography to be useful, proteins need to be lined up in an ordered array, or a crystal, which can occur under right conditions in the solution. Once good quality crystals are formed, the positions of atoms can be calculated from the X-ray diffraction patterns. Although these two techniques are mainly used in the structure determination of molecules, large molecular complexes and membrane-bound proteins are particularly challenging for these techniques. Large complexes such as ribosomes and various viruses may have tens to thousands of molecules bound together. In general, it is hard to grow crystals for large complexes and membrane-bound complexes. Structural knowledge of large molecular complexes is important, since the structure of large complexes reveals detailed relationship among multiple molecules. Each biological function is performed by multiple molecules. The inter-relationship between them provides important information regarding how the biological function is performed. Cryo-electron microscopy (cryo-EM) is a powerful technique for determining the structures of large molecular complexes. This technique is not limited by the crystallisation of proteins, for which the size of the protein complex matters a lot. The idea behind this technique is to produce many 2-dimensional images of the molecular complex using an electron microscope. As our experience of taking a picture using a camera tells us, the object needs to be still to get a good picture. Thus, in this technique, molecules are fixed in ice by plunging them in a liquid nitrogen bath. But how are two-dimensional pictures converted into a 3-dimensional image? Suppose that we want to know the 3-dimensional volumetric image of a car in great details using a camera that can only produce 2-dimensional images of the car. We can randomly lay many copies of the same car in a parking lot and take pictures of all cars. Each 2-dimensional image of the car contains information about the car from a particular angle. Given sufficient such images, it is possible to computationally merge them into a 3-dimensional volume of the car that agrees, in principle, with all 2-dimensional pictures. Cryo-EM techniques have improved dramatically over the last twenty years and it is only recently that the determination of atomic-resolution structures is possible for many molecular complexes. As of August 2016, there have been 1134 atomic structures of molecular complexes resolved using cryo-EM techniques and they are all deposited in PDB.

**Figuring Out the Structure from 3D Images with Insufficient Resolutions**

In order to derive atomic structures using cryo-EM, a 3-dimensional volumetric image, or so-called density map, must be interpreted. If the density map is produced at a high-resolution of around 3Å, then their atomic structures can be resolved, since sufficient details about atoms can be figured out from such images. When most components of a car are distinguishable from an image, it is easier to tell the entire structure of the car. Similarly, the location of most major atoms can be figured out directly from such a high-resolution density map.

The lower the resolution of the image, the harder it is to figure out the structure of the protein. If your specimen only allows you to obtain data at medium resolution, about 5–10Å say, then less information is

obtained from such images than from the high-resolution images. This is similar to the problem of drawing the original car based on the car in the junk yard. Molecules are delicate, and it is challenging to obtain high-resolution images for many molecules. A method to derive atomic structures from medium resolution images will allow us to push the limits of structure determination for those molecules that are more dynamic and only have density maps with insufficient resolution. Dr He and her colleagues' specialty is using advanced computational techniques to combine both the volumetric data and protein sequence data to figure out the structure of the protein from a 'cryo-EM density map with 5-10Å resolution.

**Attacking Two Critical Steps in Interpreting Cryo-EM 3D Images**

To make sense of a sub-standard image of a car, two critical steps are needed: to distinguish the individual components as much as possible, and to make connections between the components in order to set up the framework of the car. The individual components seen may have errors, and therefore a framework needs to be created with the consideration of such uncertainty. Dr He looks at this problem as three different objectives directed at the same general goal, in describing the structure of large molecules. First, she wants to improve the accuracy in secondary structure detection from cryo-EM density maps at medium resolutions. 'The most characteristic components in the medium-resolution density maps are secondary structures such as ọ-helices and ß-strands,' she explains. The accuracy of secondary structure detection plays an important role in the determination of the tertiary structure of the protein. The ọ-helix is a compact helical structure formed by a polypeptide chain. They have the right sequence of amino acids to allow a close, stable curling of the coils. A ß-strand is a segment of chain that is 'stretched', and when multiple ß-strands come together, they form a sheet-like shape called a ß-sheet. You might imagine a helix as a piece of traditional telephone wire, and a ß-sheet as multiple ladders laid out and tied together with soft strings, where each half of a ladder is a ß-strand. It is amazing how the molecular world is not actually that chaotic at all. The same insight used to stabilise objects in the real world is also used to stabilise protein structures.

Dr He also wants to develop computational methods that derive the topologies – a mathematical term used to describe the framework of the car – of secondary structures when inaccurate data is used in the first place. Finally, she wants to turn the complex computational methods that she develops into user friendly tools that anyone can use, even someone that is not a trained mathematician or computer scientist.

**Computation Makes You See that You Do Not See**

Addressing her first objective, Dr He and her co-workers and students have developed computational methods to detect ọ-helices and ß-sheets using their characteristic shape properties. Computer programs are written to search the 3D image of a protein for cylindrical shape regions as helices and plane-like regions asß-sheets. This process is called pattern recognition and is a powerful technique to distinguish objects as long as they have good characteristics and the image is of sufficient quality. Generally, if your eyes can distinguish an object from the background, computer programs can be designed to find it automatically. However, what happens when your eyes cannot see the object? Would it still be possible to detect it computationally? This question puzzled Dr He for a long time until she and her student found a way to push pattern recognition beyond its capability by introducing modelling into the problem. Since the spacing between two neighbouring ß-strands is about 4.5–5Å, they can't be resolved in a 3D image of 5–10Å resolution. When we can't see ß-strands, is it still possible to detect them? Dr He and her student Dong Si discovered that the arrangement of ß-strands is linked to the twist of the ß-sheet. It had been discovered in the 70s that all ß-sheets exhibit right-handed twisting, which means that they are not flat. They utilised the asymmetric nature of the ß-sheets to model ß-strands. This work demonstrated that it is possible to extract the location of ß-strands from medium resolution cryo-EM images. The location of ß-strands is one of the two treasures existing in medium-resolution images. This further justifies the value of images at medium resolutions. 'What we learned is that advanced computational methods are powerful in detecting objects that are not resolved in certain cases.' Dr He explains. This work is included in StrandTwister, a method to predict the trace of ß-strand from ß-sheet image at medium resolutions.

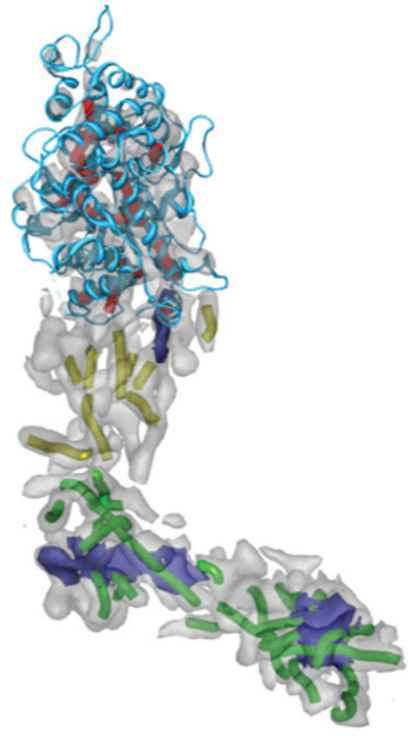
**Machine Learning to Utilise Large Amounts of Data**

As more 3D cryo-EM images and their corresponding atomic structures are deposited in the public database Electron Microscopy Data Bank (EMDB) and PDB, it is possible to involve machine learning techniques in pattern recognition of secondary structures. Machine learning techniques uses existing data to train a computer program so that it remembers the patterns and will be able to recognise similar patterns automatically when they appear in a new set of data. Dr Jing He, Dr Dong Si (previously a student of Dr He) and their collaborator Dr Shuiwang Ji developed a support-vector-machine (SVM)-based learning method to detect both ọ-helices and ß-sheets from cryo-EM maps. Recently they developed a deep learning approach using a convolutional neural network (CNN) for the same problem. Advanced computational techniques such as modelling and machine learning have enhanced the capability of pattern recognition because they allow us to explore patterns beyond the current image. Those weak patterns in the current image may become more evident when other alternative patterns are modelled or when other images are brought into the analysis.

All of this work dramatically improves Dr He's ability to determine secondary protein structure in cases of medium resolution cryo-EM data. But that was only the first of her three objectives.

**Good Algorithms Mean Less Effort Dealing with Errors in Data**

Deriving structures from 3D images with insufficient resolutions requires effort in modelling uncertainty or possible errors in the data. Using the analogy of figuring out the framework of the car, we need to figure out how the protein chain threads through the 3D image. You can imagine a protein as a chain of beads (or a necklace), where each bead resembles an amino acid. A helix/ß-strand is in fact a segment of the chain often consisting of 3 to 30 consecutive amino acids. The secondary structures on the protein sequence show their identity but not their location in 3-dimensional space. The secondary structures detected from the cryo-EM 3D image provide their location but not identity. The idea is to combine the 3D image and the protein sequence to get both the identity and spatial location of secondary structures. Since secondary structures are major components of a structure, once they are figured out, the entire framework of the structure can be derived. A naïve way of combining the protein sequence with the 3D image involves trying out all possible ways to thread the sequence through the image. This means huge computation since the computational time becomes exponential as the number of the alternatives and the number of components increase. Dr He's team, including her collaborators Dr Desh Ranjan, Dr M. Zubair, and Dr Abhishek Biswas (previously a student of Dr Ranjan and Dr Zubair), developed a smart way to try out alternatives with significantly reduced computation using what is called a dynamic programming algorithm. To give an example how much a good algorithm can cut down computational effort, using a naïve way to try out all possible alternatives takes 493718.75 seconds, but only 22.35 seconds using the dynamic programming method developed for the same task. A good method needs to be implemented in a user-friendly tool before it is actually useful. Dr He and co-workers are trying to provide researchers with secondary structure detection and analysis tools that are easy to use.



**Herpes Virus VP5 protein**

**Riding the Wave into the Future**

Dr He explains that she has always been curious about biology: 'It is a field with so many unanswered questions, and computation has become such an important component of biology.' And now, modern biology has become quite dependent on Dr He's specialty of mathematical computation and computer science. 'Cryo-EM has evolved dramatically over the last twenty years. Very few people used to believe in it, yet now many people want to use it, since it is becoming a mature and powerful technique for structural determination of large biological complexes.' And now, Dr He is riding the wave of progress into the future. When asked what the future holds for her research, Dr He says: 'advanced computational methods have been shown to cut down the computational time and to enhance the accuracy in interpreting medium-resolution cryo-EM maps. I believe that integrating advanced computational methods deeply in biological problems is the future approach for complex biological problems.'

# Meet the researcher

**Dr Jing He**
Associate Professor
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Dr Jing He received her BSc in Applied Mathematics in 1990 from Jilin University in China. She received an MSc in Applied Mathematics in 1994 from New Mexico State University, and was then awarded her PhD in Structural & Computational Biology & Molecular Biophysics from Baylor College of Medicine, in Houston, Texas, in 2001. She joined Department of Computer Science at New Mexico State University in 2002 and then Old Dominion University in 2009.

Dr He's research interests include developing computational approaches to derive the structure of proteins from data obtained by cryo-electron microscopy. At a medium resolution such as 5–10Å, it is extremely difficult to determine protein structure directly from the volumetric data, so she tries to develop computational methods and tools in order to automate the determination of protein structures from the cryo-electron microscopy density maps. Dr He has authored and co-authored more than 50 papers in peer-reviewed journals and scientific conferences dealing with cryo-electron microscopy imaging and bioinformatics, as well as several book chapters.

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OLD DOMINION UNIVERSITY



# IMPROVING OUR HEALTHCARE SYSTEM WITH QUEUEING THEORY

**Dr David Stanford's** research at the University of Western Ontario involves applying the mathematics of Queueing Theory to real-world problems in healthcare, such as emergency department wait times and organ donor priority lists.




**To begin with, what motivated you to build a career in Queueing Theory?**

My motivation for being interested in waiting times dates back to my time growing up in Montreal. During my secondary education, I commuted some 25 km to a Jesuit high school, crossing the St. Lawrence River each day. The bridges were always congested, and I kept thinking 'What a waste of time!'

Later in university, there was a specific incident I'll always remember: I queued for about an hour one day just to cash my monthly assignment-marking paycheque, which made me late for the next class. I remember thinking 'There has to be a better way to do this'. When I arrived late to class, it was the introductory lecture of Queueing Theory! I was hooked then and there.

**Your queueing methods utilise priority gain over time to balance wait time and importance. Could you explain, for us non-mathematicians, how your methods are able to determine the best rate of priority gain?**

The field of Key Performance Indicators (KPIs) provides the key. The idea behind KPIs is that patients should commence treatment by a time point specific to their level of urgency or acuity so as to avoid adverse outcomes. Occasionally, due to randomness in how patients arrive (which usually cannot be controlled), a fraction of patients will miss their targets. It is the occurrence of these situations we seek to minimise, both in terms of their frequency of occurrence and the amount by which they miss the specified delay. An accumulating priority mechanism implicitly keeps track of how long such patients have waited, so that by adjusting the rates of priority accumulation, we can find the right fit so that we succeed in maximising compliance with the specified waiting time targets for each acuity level.

Of course, if a facility is understaffed, an accumulating priority queue cannot produce a miraculous solution. The healthcare field is full of people saying things like 'Think outside of the box' as if creativity alone were capable of making all the queues disappear. Rather, a sound grounding in waiting time principles is needed to discern when a creative solution can make a difference, and when it amounts to 'rearranging the deck chairs' on the proverbial Titanic.

**Why do you think that methods such as these, which are fairly well established in telecommunications, are only now being examined for use in healthcare?**

That's an excellent question. In Canada, and I think in most countries still, no training is given to doctors and other healthcare professionals on waiting times and queues, and their mathematical foundations. That is a critical mistake: in my view, all such professionals should have some idea as to the common physical phenomena that apply to all situations where people arrive at random for a capacitated service. Instead, we have all these smart individuals who have no training on something most are likely to

have to deal with on a daily basis! As a result, people tend to think 'It's a queue; how hard could that be?' when in fact understanding the consequences of workload, variability in arrival and treatment times, in the pooling of resources and in prioritisation is key to achieving a suitable solution. In short, we need to see a formal exposure of all healthcare professionals for whom access is a responsibility to queueing principles during their training, if we are to hope to see the types of benefits in the healthcare field that have been applied in telecom for the past hundred years.

**You have mentioned that queue surges can be reduced by 'pooling' resources. How easily can this be applied to fields such as healthcare, where hospital locations have relatively 'local' catchment areas?**

This is a matter of balance that requires a team-based approach to resolve. I am not a specialist in the agglomeration of regional healthcare facilities, but there is no need for me or any other queueing theorist to play that role. Healthcare professionals equipped with the right skill set, or even better, a team-based approach that includes a quantitative specialist (such as an industrial engineer, an operations researcher, a statistician or similar analytics professional) is key to determining that balance.

**Where would you like to take your research from here? Have you a 'dream' subject, as it were?**

It may sound silly, but I feel I have really found a niche in the area of KPIs and the use of the Accumulating Priority Queue (APQ) method to resolve them. There remains a wealth of aspects of the APQ still to be explored, relating to such things as the 'affine' case in which patients start with a base level of priority credit that depends upon their acuity, which then accumulates further from that point.

I'm also particularly interested in access as it applies to Emergency Medicine, whether you can it A&E, ED, or ER, and in transplantation wait times. I may not be able to heal patients as my father did, and as my brother and niece continue to do. But I hope I can continue to work with teams of medical professionals interested in applying queueing theory principles to heal healthcare systems. THAT is my dream!

# TAKING NUMBERS AND GETTING THEM IN LINE

Medical treatment is overused and under-budgeted, leading to long wait times for those in need. How do we make these queues shorter and fairer? With the help of Queueing Theory, naturally.

It's early morning; you're on the way to work and want to pick up some coffee. Walk into the store, stand in line, and wait… and wait… The day has barely started and already you are frustrated and annoyed, all because of a simple queue on the way to get a coffee. Now think how much worse it would be if you were stuck in an interminable line and waiting for something *really* important – somewhere such as the emergency room of the local hospital.

Waiting times at a hospital are naturally more important than those at a café, but they both come down to some general rules which occur whenever people are lining up and queueing for something. The most basic of these is the underlying disconnect between how often people join the queue, and how often they leave. People arrive in a chaotic manner, with quiet lulls broken up by groups of customers – think of a train-station café with bursts of activity as a carriage of caffeine-starved bankers arrive. But they will leave the queue at a defined, regular rate – the barista from said café can only make two coffees per minute. The mixture of chaotic arrivals versus regulated departures almost guarantees that queues will occur.

**Chaos is come again**

The queueing problem becomes even more difficult when we move from cafes to hospitals. Emergency rooms need to prioritise patient treatments based on the urgency of their symptoms, a problem not shared by café owners (a heart attack will



**'I hope I can continue to work with teams of medical professionals interested in applying queueing theory principles to heal healthcare systems. THAT is my dream!'**

be treated before a broken finger, but the cappuccino does not need priority over the latte). To do this, emergency rooms will use a triage system, splitting patients into priority classes based on their need – patients in these groups must then be treated within a set period of time. In Canada, for example, patients are categorised as Resuscitation (treat them right now), Emergency (treat within 15min), Urgent (30min), Less Urgent (1h) and Not Urgent (2h).

Having ranked and categorised incoming patients, the challenge then is to determine the order in which they should be treated. Many hospitals use a simple ranking system: if a patient with higher priority comes in, they are treated first. This is excellent for critical cases but can be difficult for a patient with a 'less urgent' problem, who can be kept waiting for hours as new arrivals take their place in the line. In a sufficiently busy hospital, a less-urgent case can end up waiting for half a day before there is enough of a lull to let them reach the head of the queue.

So how can we solve this problem? One approach is to mix waiting time and importance. In this situation, every patient comes in and begins accumulating a figurative 'priority score'. This score goes up with time, at a rate which is dependent on the classification of the patient. Broken arm? Your priority score will rise at a faster rate than the patient next to you with a strange rash. Every time a doctor is free, the waiting patient with the highest priority score will be admitted for treatment. By mixing time spent and importance, this hybrid system makes overall waiting times fairer for every type of patient.

This type of approach has been long used in the telecommunications industry, allowing the transmission of large amounts of data in an orderly manner. It and other techniques stem from a vast field of research known as Queueing Theory (appropriately enough), which delves into the mathematics behind queues in cafes and telephone lines. Some of these insights have also been adapted for the field of medicine through the work of mathematicians such as Dr David Stanford, of the Department of Statistical and Actuarial Sciences at Western University, Canada. His work includes the development of hospital queueing systems, as in our previous example, but also touches on a number of other healthcare fields.

**Blood hath bought blood**

For example, he has worked on the relatively knotty problem of organ transplants. As with hospitals, organ transplants involve patients waiting in a queue for a limited supply of medical help, in this case organ donors. Such queues are often growing in length, thanks in part to a mismatch between the numbers of donors and transplant patients. However, the system is more complicated than this, and can in fact be considered as a set of interlinked queues – each comprising a different blood type.

Blood types are caused by differences in the molecular sugars coating our red blood cells, and play an essential role in both blood transfusions and organ transplants. Combinations of genes give rise to several major groupings: Type O patients can donate to any recipient, but can themselves only receive Type O blood. Type A or Type B can receive their own blood types or Type O, but not AB. AB Patients can receive donations from any blood type, but can themselves only donate to other AB patients. Some combinations of organ donor and patient are thus simply impossible, and therein lies the problem.

How do you decide who gets a donor organ? Imagine a Type O liver, which can be transplanted into any patient. Should it be provided to the next patient on the list, regardless of their blood type? This is known as ABO-Compatible donation, in which patients are eligible for any donation which they can physically receive. Great for the A's and B's, but Type O patients now find that the only organ type they can actually receive is being used to keep other blood types alive – the consequence of which is that Type O patients now spend much longer waiting for a compatible donation than all other blood groups.

Alright then, what if we say that patients can only receive donations from those who have the same blood type as themselves? This is known as ABO-identical donation, and it solves the problems of O-Type patients waiting significantly longer than others. But of course, nothing is that easy – these systems thus bring in their own type of delays, this time those with less common blood types such as AB and B, who end up waiting for much longer thanks to their small pool of potential donors.

How do we resolve these two methods into a form that is fair to everyone concerned? Here the Queueing Theory mathematics utilised by Dr Stanford can come into play, and indeed the application of these techniques to organ donor queues is one of his research interests. Mathematical modelling and a solid dose of theory have allowed the development of a hybrid system, in which blood types are partially 'pooled' or joined together so that, for example, Type O can donate to Type B (but only occasionally), while Type A can only donate to Type AB (likewise, only occasionally), but no other cross-donations can occur. This links the less-common blood types to more common ones while keeping the groups relatively separate, thus allowing for fairer distribution of organs to needy patients than either of the previous methods.

**Queues and Q's**

Both of these innovations utilise the power of Queueing Theory mathematics to develop more efficient ways of dealing with limited supplies – be they donor organs or doctor's time. In a time characterised by aging populations and ever more expensive medical costs, these increases in efficiency are sorely needed to help keep healthcare budgets under control. Dr Stanford's work is already being examined for implementation by Canadian healthcare authorities, and may eventually end up in hospitals around the country.

If hospitals give Queueing Theory its due, then perhaps one day the worst hospital queue will be the one by the coffee stand.

# Meet the researcher

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Professor Stanford obtained his PhD at Carleton University in 1981, and he now holds the Undergraduate Chair position at the Department of Statistical and Actuarial Sciences at the University of Western Ontario, Canada. His research there involves applying the mathematics of Queueing Theory to real-world problems in healthcare, such as emergency department wait times and organ donor priority lists.

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## REFERENCES

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DA Stanford, JM Lee, N Chandok, V McAlister, A queuing model to address waiting time inconsistency in solid-organ transplantation, Operations Research for Health Care, 2014, 3, 40–45.

Click HERE to view the research poster on 'Addressing Waiting Time Inconsistencies due to ABO Status in Transplantation'

See below for Professor Stanford's short tutorial 'How Queueing Theory Can Improve Wait Times':
https://www.youtube.com/watch?v=SRqI_Ntrcnc
(also available in French at https://www.youtube.com/watch?v=KNLxLWV_IYM)

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