EXCLUSIVES:
• The National Institute of General Medical Sciences
• The American Society for Cell Biology
• The Natural Sciences and Engineering Research Council of Canada
• Centre for the Aids Programme of Research in South Africa

HIGHLIGHTS:
• Mechanobiology – Exploring the Mechanics of Cell Behaviour
• Unravelling the Mystery of Chronic Asthma
• The Social Gradient in Musculoskeletal Health
• Child’s Play: Revealing Human Nature Through Early Competition
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WELCOME...

It is my absolute pleasure to introduce this diverse edition of Scientia, where we explore the multitude of ways that scientific research enhances our health and happiness, from cell biology to social science.

We kick-start the edition by investigating human health at the most fundamental level – the molecular processes that occur in our cells. Here, we showcase the work of several research teams, each dedicated to unravelling these intricate chemical mechanisms, and the ways in which they can go wrong. As we’ll see, this type of basic research lays the foundations for elucidating the biochemical origins of many diseases, such as cancer and Alzheimer’s disease, and thus helps scientists in developing targeted treatments.

Next, we highlight a collection of research projects, where scientists are applying this fundamental knowledge in the hunt for new pharmacological treatments. In this diverse section, we meet researchers who are finding innovative new ways to treat a huge array of non-communicable diseases, including cancer, diabetes, cardiovascular disease, asthma, eczema and haemophilia. Infectious diseases are also a huge source of suffering and mortality worldwide, so the latter part of this section showcases novel approaches to combatting HIV, influenza and infection-driven preterm birth.

Although pharmaceutical intervention is behind much of our improved healthcare in the twenty-first century, greater focus is being placed on alternative solutions that can provide huge benefits without the need for costly drug development. In the next section of the edition, we showcase the work of several scientists who are applying the latest technology to develop innovative non-pharmaceutical person-centred approaches to treating a variety of conditions.

After showcasing the latest innovations in healthcare, both pharmaceutical and non-pharmaceutical, we then conclude the edition with another paradigm shift, towards the human as a whole, and our complex interactions with one another in society. In this final section, we explore the ways in which these social structures and interactions influence our health, happiness and even how our species developed.

Meet The Team...

DIRECTOR
Nick Bagnall
nick@sciencediffusion.com

EDITOR-IN-CHIEF
Dr Nelly Berg
nelly@sciencediffusion.com

EDITOR
Dr Catriona Houston
catrina@sciencediffusion.com

DESIGN MANAGER
Mimi Jones

PUBLICATION MANAGERS
Brett Langenberg
brett@sciencediffusion.com

Nick Powers
npowers@sciencediffusion.com

Marie Serrano
marie@sciencediffusion.com

Tom Render
tom@sciencediffusion.com

CONTRIBUTING WRITERS
Margaret Unkefer, MSc
Conn Hastings, PhD
Sherwin Barretto, PhD
Jenny Chik, PhD
Meghan Maslen Kelly, MRes
Sherwin Barretto, PhD
Ingrid Fadelli, BSc, MA
Miriam Grace, PhD
Kate Stewart, BSc
Joseph Pastorek, MD, JD
Alma Ionescu, BSc
Fiona Williams, BSc
Chris Harrison, PhD
Matthew Aitkenhead, PhD
Kevin Pollock, PhD
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All plant and animal life on our planet is formed from the same fundamental units. Since the term ‘cell’ was first described by Robert Hooke and published in Micrographia (1665) there has been a fascination with and a need to understand these most basic elements of life. We begin this edition of Scientia by exploring the molecules and cells that form the basic building blocks of all life on Earth, from unravelling the language of DNA, to investigating how cells respond in outer space. Here, we will explore some of the latest approaches to basic research in the cellular sciences, and how this is enhancing our understanding of the fundamental processes of life, helping to pinpoint how diseases progress and allowing long-term human spaceflight to become a reality.

Research into the molecular mechanisms of cellular biology has led, over the last 50 years, to incredible breakthroughs in the treatment of disease, as well as wider ranging implications for the development of other medical technologies. Since the work of Francis Crick, James Watson, Rosalind Franklin and Maurice Wilkins in uncoiling the structure of DNA, there has been an explosion in our understanding of genetics and in cellular and molecular biology. This has provided the foundation for the significant advances in healthcare that have taken place since the 1950s. Basic research in the cellular sciences is still essential for finding new ways to treat debilitating life-threatening conditions such as heart disease, cancer, Alzheimer’s and Parkinson’s disease.

We begin this section of the edition with an exclusive interview with Dr Erika Shugart, Executive Director of the American Society for Cell Biology (ASCB). Since it was founded in the ‘60s due to growing interest in the inner workings of the cell, the ASCB has worked to support scientists and research in molecular and cellular biology. As cell biology has expanded to include advancements in biochemistry, genetics and microscopy, the ASCB’s membership has also grown and has included 32 Nobel prize winners since 1960. Currently, cell biology as a field is expanding further to embrace new technologies in high resolution imaging, genomic sequencing and massive data handling.

Next, we introduce Dr Julio Collado-Vides who is applying new techniques in data analysis using computational mathematical models at the Center for Genomic Sciences at the National Autonomous University of Mexico, to unravel the language of DNA and to understand the ‘grammar’ of transcription factors and gene regulation. His team are also using their bioinformatic skills to look at research itself, towards creating a database that allows for the integration of vast amounts of published research from across the biomedical sciences.

As techniques in molecular biology and biochemistry have developed, the amount of information available to biologists has become increasingly complex and difficult to integrate and understand. This has given rise to a generation of mathematicians who are applying their skills in the fields of molecular and cellular biology. One such researcher is Dr Wenxuan Zhong who runs the Big Data Analytics Lab at the University of Georgia together with her colleague Dr Ping Ma. In this section, we showcase her team’s work in developing new statistical approaches to unravelling huge datasets, enabling researchers to understand the mysteries of DNA methylation that regulates gene expression. This work will help researchers to investigate the progression of diseases such as heart disease, diabetes and cancer, with the ultimate aim of inspiring novel treatments.
Part of the explosion of biological big data in recent years has come from the advancement of structural imaging techniques such as NMR spectroscopy, X-ray crystallography and, more recently, cryo-electron microscopy. The acquisition of detailed information on the three-dimensional structure of large biologically important molecules is useless unless researchers can visualise these molecules and their interactions in motion. This empowers researchers to investigate the function of different proteins and how mutations can lead to disease, allowing the development of novel drugs and agents that can interact with them. Solving this problem is Dr William Ray at The Ohio State University and the Research Institute at Nationwide Children’s Hospital in Columbus in the US. Dr Ray has turned his expertise in computer science and computer graphics to develop new ways of visualising proteins and molecules in motion, allowing scientists to more accurately probe their roles in disease.

One organisation making this type of fundamental biomedical research possible is the National Institute of General Medical Sciences (NIGMS) – one of the US’s National Institutes of Health. Here, we feature an exclusive interview with Dr Jon Lorsch, Director of NIGMS, who talks to us about the Institute’s role in supporting basic biomedical research, from advancing cryo-electron microscopy to finding new ways to analyse, share and integrate huge amounts of complex biological data in this new era of big data biology. Recognising that at the most basic level, all life is virtually identical, Dr Lorsch also emphasises the huge importance of using model organisms to help shed light on human biology. He highlights several breakthroughs in human health that have been achieved thanks to model organisms, such as mice, zebrafish, fruit flies, and even the humble roundworm.

Indeed, the roundworm (*Caenorhabditis elegans*) is the model organism of choice for Dr Robert Fairman at Haverford College. Next, we highlight his team’s latest developments in the technique of analytical ultracentrifugation (the spinning of proteins at incredibly high speeds) to investigate how proteins stick together into fibres and bundles. The
aggregation of these protein bundles is behind the degeneration and death of neurons in Alzheimer’s and Parkinson’s diseases, which are currently untreatable. Using these techniques to unravel how these bundles of protein stick together is vitally important for developing new strategies to combat these debilitating diseases.

Meanwhile at the University of Illinois in the US and the Max Planck Institute for Medical Research in Germany, Dr Taher Saif and Dr Andrew Holle have been working to understand how cells interact with their outside environment. In the emerging new field of mechanobiology, this team of scientists is pioneering the investigation of how mechanical stress impacts on the proteins of a cell to produce a response. These processes play an important role in the progression of cancer, as cancer cells interact with their environment physically to invade surrounding healthy tissue. By working to understand how cancer cells squeeze themselves into tiny gaps and push their way through their local environment, the team hope to find ways to stop them and halt the progression of tumours.

Understanding the impact of the environment on cells is also of utmost importance to a team of dedicated young researchers at the NASA Ames Research Center, who are working to understand the biological impact of long-term space flight on the blood, bone and reproduction systems of astronauts. Just like Dr Fairman, the team use model organisms such as fruit flies to probe how microgravity might impact human cells. Their research is not only making long-term space missions a reality, but is also providing valuable insights into the progression of diseases here on Earth, such as osteoporosis.

By studying the basic mechanisms and functions of the smallest units of life, all of the scientists featured in this section of Scientia are paving the way for significant improvements to human health, as well as contributing to our exploration of the solar system, and beyond.
Founded over half a century ago, the American Society for Cell Biology (ASCB) is an inclusive, international community of biologists studying the cell – the fundamental unit of all life on Earth. The Society aims to advance scientific discovery, advocate sound research policies, improve education, promote professional development, and increase diversity in the scientific workforce. In this exclusive interview, we have had the pleasure of speaking with Dr Erika Shugart, Executive Director of ASCB. Here we discuss the ways in which ASCB supports biological research, advocates for sound research policies, boosts diversity in the scientific workforce, and much more.
To start, please tell us a bit about the history of ASCB. How was the organisation founded?

The American Society for Cell Biology was first organised at an ad hoc meeting in the office of Keith R. Porter at Rockefeller University on May 28, 1960. In the 1940s, Porter was one of the first in the world to use the revolutionary technique of electron microscopy (EM) to reveal the internal structure of cells. The other early leaders of the ASCB – George Palade, Don Fawcett, Hewson Swift, Arthur Solomon, and Hans Ris – were also EM pioneers. All were concerned that existing scientific societies and existing biology journals were not receptive to this emerging field that studied the cell as the fundamental unit of all life. The ASCB was legally incorporated in New York State on July 31, 1961. The first ASCB Annual Meeting was held November 2–4, 1961, in Chicago where 844 attendees gathered for three days.

The ASCB did not remain an EM society. New technologies and new discoveries in molecular biology, genetics, biochemistry, and light microscopy quickly widened the field. Cell biology has continued to expand ever since, extending its impact on clinical medicine and pharmacology while drawing on new technologies in bioengineering, high-resolution imaging, massive data handling, and genomic sequencing. ASCB membership has grown to 9,000 worldwide (with 25% of ASCB members working outside the United States). Since 1960, 32 past or current ASCB members have won Nobel Prizes in medicine or in chemistry.

Mention the many ways through which ASCB supports cell biology research in the US and further afield.

ASCB supports cell biology research in the US and internationally through the work of its various committees. Domestically, we support our researchers through the work of our Membership Committee, Women in Cell Biology Committee, Committee for Postdocs and Students (COMPASS), and Minorities Affairs Committee, each of which offer programs, training, career development, fellowships, grants, mentorships, and networking opportunities for diverse populations of cell biologists working in academia, industry, and the government. Likewise, our International Affairs Committee (IAC) works to enhance the engagement of Society members residing outside the United States, as well as foreign nationals living in America. Recently, our IAC has established several memoranda of understandings (MOUs) with comparable scientific societies around the world. The goal of these MOUs is to promote international scientific exchange, to contribute to building capacity in cell biology worldwide, to transcend the complex political issues facing the world today, and to set an example of how to cooperate productively, sharing ideas, and moving forward creatively.

ASCB’s Annual Meeting is the preeminent event for cell biologists to learn about the latest discoveries in their field. In 2017, the meeting is being held jointly with the major European life sciences research organisation EMBO. Additionally, during the meeting the Society hosts events that foster collaboration between domestic and international researchers, such as roundtable discussions and a research and training exchange fair. Science has always been an international enterprise. The international dimension has been strengthened by the 21st century globalisation, which now allows for easier collaboration across countries and cultures. The objective of our partnerships is to take advantage of global connections to enable societies to expand their reach internationally.

ASCB also publishes two journals – Molecular Biology of the Cell, which is our science-focused journal, and CBE Life Sciences Education, which presents education research in cell biology and in the life sciences more broadly.
Tell us a bit about how ASCB works to advocate sound research policies in the US, and mention one or two of your success stories to date.

ASCb has focused on public policy since 1988. Our current Public Policy Director, Kevin Wilson, has worked tirelessly on Capitol Hill to advocate for sound research policies. In addition, our Public Policy Committee members are dedicated to the task of advocating for evidence-based scientific policy and for legislation that supports the public funding of research. Kevin keeps our Public Policy Committee members informed about critical issues affecting science. In turn, they regularly make trips to Capitol Hill, send messages about critical legislative action to local legislators, write letters to the editor and op-ed columns, and act as conduits through which local leaders and neighbours find out what is happening in their own backyard.

In terms of success stories, there was a media and political firestorm in 2011 and 2012 about revelations that certain federal agencies had spent large amounts of money for employees to travel to and participate in what were portrayed as frivolous team-building activities. In an effort to quell the media storm, the Obama administration issued a directive placing limits on the use of federal funds for employee travel and conference participation. Congress took it a step further, and included the administration’s directives in several bills, making the limitations the law of the land. There was only one problem. Both the administration and Congress failed to carefully define the word ‘conference’. The problem was so big that the Department of Health and Human Services had to issue a document entitled ‘Typical Meetings and Events That Are Not Conferences’.

As the ASCB Program Committee worked to finalise speakers for the 2013 Annual Meeting, many of its National Institutes of Health (NIH) speakers began to cancel their plans to participate. We learned that in an effort to enforce the travel restrictions, NIH was limiting intramural researchers to only one scientific conference each year. To resolve the dilemma, ASCB, joined by other professional societies, worked with Congress to correct the mistake. In 2014, Congress attempted a temporary solution, acknowledging that the fix was ‘to ameliorate an unintended consequence, which has been reducing the attendance of US scientists at international scientific meetings’.

One of the numerous provisions in the 21st Century Cures Act recently signed into law by President Obama is Section 3074, ‘Scientific Engagement’. The section says, in part, that ‘scientific meetings that are attended by scientific or medical personnel, or other professionals… for whom attendance at such meeting is directly related to their professional duties… shall not be considered conferences’ for either compliance with travel reporting requirements or travel restrictions.

While the mistake and the fix impacted only a small number of scientists, it was important to the entire scientific community. Science is collaborative and depends heavily on interaction and the exchange of ideas and information that can take place face-to-face only at a scientific meeting. A reduction in the number of federal scientists at scientific meetings would adversely affect research done by those who collaborate with federal scientists and benefit from and depend on the advances made at federal laboratories.

Along with leading an effort to fix an error in federal travel policy, the ASCB issues White Papers and Position Papers on various topics of concern to the scientific community. For example, in 2014, the ASCB issued a White Paper that examined key opportunities in the field of stem cell research. The paper made a number of important recommendations on how to move the field forward. That report has

‘Our Women in Cell Biology Committee promotes gender equality and diversity by providing career development programs, mentoring, speaker referral lists, achievement awards, child care grants and web-based resources for women working in the field of cell biology’
served as the foundation for policy decisions by both the National Center for Advancing Translational Sciences at the NIH and at the Allen Institute for Cell Science.

Describe how the organisation achieves its goal of increasing diversity in the scientific workforce, in the field of cell biology.

ASCB has always been at the vanguard of groups working on increasing diversity in the biomedical sciences. In 2004, ASCB was awarded a US Presidential Award for Excellence in Science, Mathematics and Engineering Mentoring for the work of its Minorities Affairs Committee (MAC). We are pleased to offer programs that support the goal of increasing diversity in the scientific workforce for both women and underrepresented minority groups.

The goal of the MAC is to significantly increase the involvement of underrepresented minority scientists in all aspects of the Society. A long-range goal of the committee is to contribute to the nation’s effort to increase the number of underrepresented minority scientists. To that end, ASCB offers travel awards for members of these populations to attend our annual meeting. At the meeting, we offer mentoring and professional development programs aimed at trainees. Our Visiting Professorship Program supports professional development of faculty at primarily undergraduate teaching institutions that serve minority students and scientists by funding them to spend a summer working alongside an ASCB member at a research-intensive institution.

The Faculty Research and Education Development (FRED) Program is a year-long program offered by the ASCB to promote grant funding success of junior faculty at institutions with a strong commitment to recruiting students from backgrounds underrepresented in STEM to the field of cell biology. We also offer an intensive summer workshop for postdocs and junior faculty to help them be successful in their careers.

Our Women in Cell Biology (WICB) Committee promotes gender equality and diversity by providing career development programs, mentoring, speaker referral lists, achievement awards, child care grants and web-based resources for women working in the field of cell biology. During the annual meeting, WICB offers career discussion and mentoring roundtables, as well as a ‘mentoring theatre’, where workplace scenarios are performed and discussed. And ASCB has an extensive network of women in cell biology who support one another through mentoring and outreach as well as a career advice column in the ASCB Newsletter.

How do ASCB help students and postdoctoral researchers achieve success early in their careers?

In addition to the professional development programs mentioned above, ASCB provides a number of professional development and support resources for early career scientists. Our early career meeting grants, which are available to both domestic and international researchers, encourage graduate students and postdocs to team up to host regional scientific meetings. Other resources include ASCB’s online CV review, career development help on our website, our Dear Labby advice column and WICB columns in the ASCB newsletter, and an impressive collection of career workshops presented at the yearly meeting. Workshops at the annual meeting cover topics from how to apply for grant funding, science writing and science policy, advocacy communication, teaching and administration skills, oral presentation skills, mentoring, and research opportunities abroad, just to name a few.

In the summer, we offer our very popular and highly competitive biotechnology
management course, presented in conjunction with the Keck Graduate Institute in California. This week-long residency course provides PhD and postdocs with an introduction to and an overview of the skills needed to launch a non-academic scientific career. Past students rave about the networking connections they have made and the course content, which includes a team project.

Finally, please describe your work in the areas of education and public outreach. How does the ASCB help to inspire school children, college students and the general public to develop an interest in cell biology?

Public outreach is a critical part of ASCB’s mission. In order for government funding of science to continue, scientists must help people understand the importance of our work. In 2016, ASCB’s Committee for Postdocs and Students (COMPASS) Outreach Grant program made 11 programs possible to bring fun and interactive science programs to children of all ages. The projects, which were proposed by ASCB members, funded projects in places as far flung as Brisbane, Australia, and inner city Philadelphia. These grants, of up to $1,000, help ASCB members engage with local schools, science fairs, and society.

In the summer of 2014, ‘Life: Magnified’, an exhibit of 46 eye-popping colour images of life on the cellular level, opened in the airport’s Gateway Gallery in Concourse C. ‘Life: Magnified’ was a collaborative project of ASCB, the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH), and the Metropolitan Washington Airports Authority (Airports Authority) with support from ZEISS.

ASCB’s Public Information Committee (PIC) is the outreach arm of the Society, promoting public awareness of the latest advances in cell biology and the crucial importance of basic research for human health. PIC’s major activities are member lab-generated commissioned videos, and a One-Minute Selfie Elevator Speech Contest. Year round, PIC supports science communication training and outreach media projects to open the eyes and ears of the world to cell biology.

ASCB’s Education Committee (EdComm) also has been at the forefront of professional scientific societies to respond to the call for undergraduate life science education reform and offers two programs that allow participants to develop the student-centred teaching skills necessary for promoting maximum student success through long-term one-on-one mentorship. The Promoting Active Learning and Teaching (PALM) Network is a joint venture among ASCB and several other life science professional societies that focuses on developing active learning strategies for reform of the traditional lecture classroom. The Mentoring in Active Learning and Teaching (MALT) program focuses on mentorships that extend participant research (or other research projects) into course-based undergraduate research experiences (CUREs) that will lead students to experience authentic scientific research. While both PALM and MALT are dedicated to promoting sustainable reform to undergraduate life science teaching practices, their specific foci in attaining this reform are distinct.

And although the primary audience for our website is our membership base, anyone is free to read the articles we publish to the ASCB Post or that are archived in our ASCB Newsletter.

www.ascb.org
Whether it be letters or ideograms, standardised street signs or smiley faces in instant messages, communication is one of the most important tools which humans have. The ability to share information, perhaps better known as language, has taken us from our first co-ordinated hunts on the savannah all the way through to present-day gossip over the Royal family.

Language is a way of conveying information, but the simple use of words by themselves is not sufficient. Simply saying ‘bird’ does not tell your partner whether the bird is watching them, hiding behind the bushes, cooked and ready to eat, or indeed behind them and flying away with their sandwich. Thus, words need to be linked together to provide context, using a series of rules which together make up our grammar. Despite our suffering in school, grammar is normally so well-understood for native speakers that even errors in nonsensical sentences can be identified (the classical example from Chomsky is ‘colourless green ideas sleep furiously’ – a sentence that makes no sense but is recognisable as acceptable grammar).

Common rules and context play a vital part in communicating information. This is not only limited to the world of human speech, but occurs in a multitude of other processes which rely on information exchange. The most fundamental of these, underlying all life itself, is that of DNA. DNA strands encode all of the information necessary for life, and yet are themselves composed of a four-letter alphabet (C,G,T,A), which codes for roughly twenty syllables (the amino acids and STOP codons) – yet this limited selection allows combination into all of the words (proteins) required to sustain life.

Yet, as we saw before, words are useless if you do not know the context. The scientists who finally sequenced the entire human genome were surprised to find that there were far fewer genes than they had expected. Instead the difference between, say, a skin cell and a neuron, came down to differences in context – some genes were activated in some circumstances, some in others, some were cut up and rearranged during protein production to make newly spliced variants. All of these changes were brought about as a result of genetic instructions present on the DNA strand, each of which provided part of the context required for correct understanding of the genetic ‘word’. The question then is, given that DNA has words and context, can we treat it as a language?

PUTTING LIFE IN CONTEXT

How do you determine important scientific links when you are flooded by new publications each day? Professor Julio Collado-Vides and his team at the National University of Mexico appear to have the answer.

Speaking DNA

The attempt to answer this question has long been a goal of Professor Julio Collado-Vides, of the National University of Mexico. His original studies revolved around the role of transcription factors in genetic control. Transcription factors control the first step in the production of proteins, the transcription of the DNA gene into a short-lived RNA copy. By binding to the DNA in or around the vicinity of the gene, they can control if and how well the RNA transcription machinery can interact with the gene. Although incredibly complex in practice, most transcription factors can be classified as repressors (which reduce the chance of a gene being transcribed) or as activators (which increase the chance).

Professor Collado-Vides’ early work also involved modelling the organisation of transcription factors and gene regulation as a genetic grammar. By leveraging the significant amount of research done on language grammar, he was able to develop a model of ‘transcription factor grammar’ – a series of rules which allowed new transcription factor sites to be identified with far higher specificity than possible before.
This was the starting point for over twenty years’ research in the growing field of bioinformatics – the use of computers and mathematical models to understand biological systems. This syntactic approach helped in predicting and labelling transcription factor sites in the early days of genomics – with the sequencing of the entire *E. coli* bacterial genome. Professor Collado-Vides is still highly active in the bioinformatics area, and lately he has focused on extending the initial genetic grammar to a more complex set of data – the GENSOR Units.

**Mapping GENSORs**

Transcription factors are a well-studied part of the genetic molecular machinery, dating back to the discovery of the bacterial LacI transcription factor in 1959. LacI acts as a repressor – binding to a strand of DNA and preventing the nearby genes from being transcribed. The repressor will detach from the DNA strand only when the correct environmental conditions are achieved – it thus acts as a link between the environment and the genetic response.

In the case of LacI, an environmental signal (lactose) is transduced into a signal that can directly affect the genetic regulator (here through the transformation of lactose into allolactose), which is then responsible for a switch in the genetic environment (the expression of lactose metabolising genes), and then leads to a response (the cell can now effectively metabolise lactose).

This combination of elements – signal, signal transduction, genetic switch and response – can be linked together by researchers, and considered as a single higher-level unit. This unit is referred to by Professor Collado-Vides and his group as a Genetic Sensory Response Unit, abbreviated to the catchier name of GENSOR Unit. Using GENSOR Units helps humans to quickly capture the incredible complexity of cell signalling, which can involve tens or hundreds of individual components, and allows comparatively simple networks of information flow to be constructed. ‘I see GENSOR Units as either a higher level of integration after concepts like operon and regulon, as a tool for understanding when analysing global genomic experiments, and as a first step to systematically describe fluxes of regulated information,’ says Professor Collado-Vides.

He and his collaborators attempted to construct a comprehensive GENSOR map of the bacteria *Escherichia coli* – known around the world to be the trusty workhorse of the microbiology lab. Their previous efforts had netted a collection of 189 transcription factors, each with differing targets, responding to different signals, and leading to different effects. The challenge, now, is to put these transcription factors together into a clear and usable network.

The group used an approach that leveraged a number of different databases, based on their experience in bioinformatics approaches. The transcription factor proteins were used as a starting point, and steadily widening automated database searches were used to find interacting proteins, known genes that were controlled by the process, and the cellular effect of those genes. The resulting pile of data was then sieved to identify the most important links. In total, 189 GENSOR elements were constructed. Given our current knowledge, only 89 are a complete genetic sensor and response systems, while for others, this approach helps in predicting some of the missing components.

The majority of these GENSOR Units were found to be controlled by some form of feedback loop – the activity of the GENSOR
is affected by its own output. These loops were predominantly simple, though some transcription factors were shown to be controlled through multi-step metabolic changes. Alongside these feedback loops were a number of different metabolic factors that either directly controlled or were directly controlled by the GENSOR Unit. In other words, a single GENSOR Unit may control the production of several different molecules, but is itself only affected by one of these.

The true advantage of GENSORS, however, lies in the ability to merge several individual units into a larger network. For example, *E. coli* have a set preference that they will follow when choosing which carbohydrate to use as a food source. Glucose is used first, then lactose, and only later come molecules such as arabinose and xylose. A number of transcription factors, and thus GENSOR Units, are involved in carbohydrate metabolism, and the interactions of these can be used as to model just how the bacteria will behave when faced with any combination of deliciously edible sugars.

Before genomics, microbiologists were devoted to studying defined capabilities of cells, like carbon degradation, nitrogen assimilation, cell division, or responses to different environmental stresses. Transcription factors were named according to those cellular capabilities. With the advent of genomics, we now know that there is a lot of cellular integration. ‘There is no elementary sensing’, as Dr Collado puts it, because most molecules have multiple consequences in the cell. The GENSOR Unit is a concept adequate to describe the interconnections supporting this integrative physiology in explicit diagrams in databases.

The Artificial Librarian

What works for genetic grammar can also work for normal language, and so it is perhaps natural that Professor Collado-Vides’ team would apply their knowledge to scientific communication as well. The group has long been involved in the manual collection and curation of papers covering bacterial gene regulation, and their work underlies several open databases (see for instance: [http://regulondb.ccg.unam.mx/](http://regulondb.ccg.unam.mx/)) that are regularly used by scientists to determine the current state of knowledge. However, manual curation has a number of limitations: reading papers is time consuming, the efforts of experts are required to adequately capture the findings, and the information that can be searched is limited by the database format.

Bioinformaticians, of course, are experts in drawing information out of large, complex data sources. The group asked themselves if they could use machine learning and computer-based curation in order to create a new and heavily cross-linked database of gene regulation. ‘The dream,’ says Professor Collado-Vides, ‘is to generate methods that will impact the whole domain of people devoted to gather, organise, integrate and enable navigation of large corpora of data, information and knowledge in the biomedical sciences.’

The first step towards this dream is the integration of data mining and text analysis into several of the databases that the team is participating in. Several tools have been developed that specialise in extracting information from life science publications – they use a knowledge of how English sentences are structured to extract an overview of the results and what links have been demonstrated. These were built upon using similarity algorithms that determine ‘similar’ words – a search does not need to bring up an exact match to be relevant, rather a close match will also be correct. These were then integrated into an interface system that allows users to see and decide the correct piece of knowledge based on what the computer is proposing – it will highlight words and sentences it believes are involved in gene regulation and display how they link to one another.

The end result of all this work is an intelligent database that curators can use. As they read through a paper, the system automatically provides links to other publications dealing with the same subject, links determined entirely by the artificial intelligence behind the database. This means that curators no longer have to spend their valuable time hunting down correlating publications or references. Although the final process is still manual, the research team has determined that the system can increase curation speed, but most important, it will enhance the traceability of pieces of knowledge linking them to their original publication, and will enrich databases in novel ways.

Science Communication Outside the University

In yet another application of these curation ideas, Dr Collado-Vides has embarked in a non-profit adventure of what is expected to become an interactive encyclopaedia, where knowledge is organised both within an ontology (similar to the organisation of knowledge within the British Encyclopaedia), but also ordered by different levels of understanding, linking texts for laymen with more advanced texts. Conogasi ([http://conogasi.org/](http://conogasi.org/)) will initiate its activities the fall of 2017.

The Context of the Flood

In the modern genomic era, high-throughput sequencing and the expansion of research to laboratories across the world provide us with a flood of genetic knowledge as never before. However, the sheer pressure of information prevents us from understanding or even reading it all, human minds are simply not up to the task. Instead, machine learning systems allow us to simplify the flow and pull out the most useful links, be it from scientific publications or gene regulation databases. Both of these require that we understand the context of the information, the details, genetic or written, which allow us to make sense of what we see.

It is in solving this problem that the work of Professor Collado-Vides and his group truly shines. By helping to automate the recognition of context, they provide a means for us to tame and understand the flood of information. This, in turn, means that other scientists can work effectively and spend time on their true calling, the extension of human knowledge.
Meet the researcher

Professor Julio Collado-Vides
Center for Genomic Sciences
National Autonomous University of Mexico
Cuernavaca
Mexico

Professor Julio Collado-Vides received his MSc in Physical Chemistry in 1985 and went on to achieve a PhD in Biomedical Research in 1989 from the world-renowned National Autonomous University of Mexico. After his PhD, he went on to do three years of postdoctoral research at MIT. He is currently a Professor of Computational Genomics in the Center for Genomic Sciences at the National Autonomous University of Mexico. With a research career in bioinformatics and genetics spanning over two decades, he has published over 100 papers, and has been cited over 21,000 times – leading him to be recognised as one of the most highly-cited scientists in the world. He has supervised almost 20 students, sits on numerous boards, and has been awarded a number of honours – a number which can only increase. His research has been a team effort of current and past members of his laboratory (http://www.ccg.unam.mx/en/ComputationalGenomics).

CONTACT

E: collado@ccg.unam.mx
T: (+52) 777 313 9877

KEY COLLABORATORS

Daniela Ledezma-Tejeida (PhD student working on the GENSONs)
David Rosenblueth at IIMAS, UNAM (computer scientist who implemented the programs of the grammatical model http://turing.imas.unam.mx/~drosenbl/)
Jacques van Helden, Université d’Aix-Marseille, France (http://jacques.van-helden.perso.luminy.univ-amu.fr/)
Fabio Rinaldi, Swiss Institute of Bioinformatics (http://www.sib.swiss/rinaldi-fabio/rinaldi-fabio-sub)

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The process of scientific discovery has always opened up exciting new insights into the natural world. As technology has developed, the amount of information scientists can collect has increased dramatically. This data offers unprecedented opportunities to understand the ways in which complicated biological and physical systems behave, to collect observations on how they change, and even to make predictions. These huge datasets record information on many scales: from telescopes monitoring the movements of stars, to the genetic sequence unique to every individual. The vast volumes of biochemical information now available to scientists have the potential to greatly improve healthcare. Identifying the specific data signatures associated with disease could allow the rapid detection of harmful substances, and unlocking the mechanisms of disease to opening up routes towards new treatments.

Smell-Seeing with the ‘Optical Electronic Nose’

A striking invention whose powers of data detection have a variety of health-related applications is a little device sometimes known as the ‘optical electronic nose’.

More technically called the colorimetric sensor array (CSA), it looks like a square with 36 coloured circles printed on it. But when the array is exposed to a mixture of chemicals, the dots change colour in a way that depends on the exact chemical profile of the mixture. Like the human nose and its ability to identify a smell based on the pattern of electrical signals it triggers in sensory receptors, the CSA produces a specific set of colour changes for the chemicals it detects, hence its nickname. CSAs can be small, cheap and accurate. Their many uses include detecting markers of disease in breath, including lung cancer and infections; identifying pathogenic bacteria based on the volatile substances they produce; and sensing volatile toxicants as part of security systems.

Noses to Numbers

The set of all the changes undergone by each dot produces a sort of fingerprint for the chemical signal, and can be represented numerically, as the change in each of the dot’s red, green and blue colour components. This is written as a table with red, green and blue entries for each dot, also called a matrix. This representation allows scientists to compare several colour changes. In turn, knowing which colour changes correspond to which chemical mixture makes it possible to identify the chemicals in an unknown mixture.

However, classifying the chemicals accurately depends on statistical methods that can perform this classification well.

One common way of telling different groups apart from each other is a technique called linear discriminant analysis (LDA). LDA works by finding combinations of the properties, or predictors, of the group members, in such a way that when the groups are compared by these combinations of predictors, the differences between groups are larger than the differences between group members. However, this method is not ideal for CSA data, because the CSA data has a matrix structure.

To be suitable for LDA, the CSA data must be converted from its two-dimensional matrix...
structure to a simple, one-dimensional form. This can lead to a substantial loss of the structural information we had when each dot's colour change in, say, red, was recorded separately. Another problem that comes up with this transformation is that each colour change for each dot is recorded as a separate property, or variable. This means that if we have 36 colour changes in each colour component, we end up with 108 separate variables. Thus, the parameters to be estimated are tripled. One of the main principles of statistical methods is that if we have a small set of experiments, but a large number of variables that we observe, it is very difficult to say anything meaningful about the differences between the variables we observe through our experiments. This is called the ‘curse of dimensionality’. So, methods that are designed for the sort of data that CSA produces would be a great step forward in improving the accuracy of the data’s interpretation, which is important in situations such as breath analysis to identify diseases.

New Methods for Making Sense of CSA Data

One scientist at the forefront of this type of statistical research is Professor Wenxuan Zhong. Professor Zhong runs the Big Data Analytics Lab at the University of Georgia, where she develops new statistical approaches to tackle the search for patterns in large, difficult datasets. She and Kenneth Suslick at the University of Illinois, who invented the original CSA technique, have created a new classification method tailored to CSA data. Presented in her 2014 publication in the journal Technometrics, Professor Zhong’s ‘matrix discriminant analysis’ (MDA) approach takes the CSA matrix of colour changes and transforms it into two components, one for the rows, based on dyes, and one for the columns, based on colour changes. This preserves the information contained in the matrix structure, and allows us to escape the curse of dimensionality we’d get with 108 variables.

Professor Zhong has refined her MDA approach to work around another problem of CSA analysis, which is that not all dots produce a meaningful colour change for every chemical. So, incorporating the information from these misleading changes can lead to classification mistakes. The ‘penalised MDA’ approach reduces these errors, improving the accuracy of identifying chemicals. Professor Zhong tested the performance of her PMDA method in a real-life scenario – the detection of low levels of volatile toxic chemicals. Even in small quantities, long-term exposure to such chemicals can be dangerous. Professor Zhong’s approach consistently outperformed the simpler LDA method, with lower classification errors.

The MDA approach to classifying CSA-like data can be taken a step further. Professor Zhong has developed an algorithm she calls ‘Sequential Iterative Dimension Reduction’ – SIDRA for short, which she describes in her 2015 publication in Wiley Interdisciplinary Reviews: Computer Statistics. Just like in the MDA method, SIDRA compresses the information from CSA-like data, more technically defined as a tensor, into lower dimension, which makes it easier to analyse. Importantly, the method ensures that no information about the data structure is lost in the process.

Understanding the Genetic Causes of Disease

Now Professor Zhong hopes to apply her statistical expertise to tackling some other tricky problems in biological data. She’s still interested in improving health outcomes, but this time she’s focussing on understanding
the causes of complex diseases, at the most basic level – DNA. As humans, our cells contain the genetic code for our species, but our bodies also host diverse microbial communities with their own DNA, such as the intestinal microflora. We now know that the exact microbial species we host, and their different abundances, can play an important part in regulating our health. This is thought to be a factor in conditions such as inflammatory bowel disease, type 2 diabetes, and obesity. Scientists can now take a sample of human gut flora and use next-generation sequencing techniques in a metagenomics approach to quickly build up a picture of the patient’s complete microbial profile, without all the pitfalls of trying to cultivate microbes in the lab. However, there are still significant challenges to accurately identifying the species in samples like this.

Professor Zhong and her team have invented a powerful new method which sidesteps the need to use a reference genome, which can lead to biases in differentiating closely related species and in estimating their abundances. Metagenomic data consists of short DNA sequences from all the species in the sample. Unlike other methods, her MetaGen algorithm assumes that each species will have a unique relative abundance of a contig across the multiple samples – a sample profile. The sample profile of a contig should be the same as that of the species genome, and contigs with similar sample profiles are likely to be derived from the same genome. MetaGen uses this information to group the contigs into different species bins, and a method from information theory, the Bayesian information criterion, to determine the number of species. Professor Zhong hopes that MetaGen’s fast and accurate approach to identifying microbial species and associated abundances will make it easier to associate microbial species profiles with specific diseases, and bring us a step closer to finding cures. Nick Nystrom, the Senior Director of Research and the Principal Investigator for the Bridges Supercomputer at Pittsburgh Supercomputing Center, commented that ‘this was a really aggressive simulation or calculation where they were looking at 378 billion base pairs.’

**Beyond Genes – Epigenetics**

However, some diseases are thought to be shaped by DNA in ways that go beyond the genetic information it encodes. These ‘epigenetic’ mechanisms lead to potentially longer-term changes in gene transcription – the process in which RNA is produced from DNA, before being translated into proteins – without affecting the DNA sequence. Although some epigenetic mechanisms are a normal part of development, certain changes can lead to disease. Epigenetic processes can include the modification of histones, the proteins around which DNA is wrapped in the cell, and DNA methylation, in which a methyl molecular group is added to DNA.

DNA methylation is a relatively recent discovery, but has already been found to have an important role in essential processes such as the differential regulation of specific genes in tissues. While DNA methylation may occur in stable patterns that are inherited, it can also be altered in development and ageing, and by environmental factors. Faulty regulation of DNA methylation appears to be one of the causes of heart disease, diabetes and cancer. Next-generation genome sequencing techniques, first developed in the 1990s, have been further refined to allow the detailed mapping of DNA methylation across genomes.

Understanding how different DNA methylation patterns are associated with specific diseases could open up new routes to treatment, but presents a particular statistical challenge due to the high volume of data, and the interference of other signals, or ‘noise’. Current methods of comparing methylation levels between cell types focus on aggregating the methylation levels at different genome sites into a single statistic, and comparing the statistics obtained between cell types.

However, this method overlooks the pattern of methylation at different sites, and this information is known to play an important part in regulating gene expression. The relationship between levels of DNA methylation and expression also appears to be complex, and classical correlation approaches do not accurately describe the way in which gene expression varies with methylation.

Professor Zhong thinks she may have invented a method that could accurately classify these challenging DNA methylation patterns, and allow us to identify how individual patterns could be characteristic of particular diseases. Their idea is based on building a mathematical model which predicts the methylation level of a gene, and uses information about its cell type and methylation levels at particular sites. They have already conducted a preliminary test to see whether the new method can distinguish between the different DNA methylation patterns in the genomes of patients with two variants of leukaemia. Comparing the graphs of DNA methylation levels that the model predicts from methylation sites and cell types, there are striking differences between the two sets of patients, proving that the model has the potential to perform well.

Professor Zhong now plans to upscale the model, identifying differentially methylated regions across the human genome and linking these to cell types. With some additional statistical ideas drawing on her earlier work on dimension reduction, this will allow her team to predict changes in gene expression linked to differential methylation patterns. Finally, she hopes to make the model widely available by designing a user-friendly piece of software that researchers can use, giving us another exciting new tool that could revolutionise prospects for treating a suite of diseases.
Meet the researchers

Professor Wenxuan Zhong
Department of Statistics
University of Georgia
Athens, USA

Professor Wenxuan Zhong completed a PhD in Statistics at Purdue University in 2005, and went on to carry out postdoctoral research in the Department of Statistics and FAS Center for Systems Biology at Harvard University until 2007. She then became Assistant Professor at the Department of Statistics of the University of Illinois at Urbana-Champaign. Professor Zhong is currently an Associate Professor at the Department of Statistics of the University of Georgia, where she leads research on developing statistical theory and methodology to address the challenges of analysing large volumes of genetic data. She is the founding director of the Big Data Analytics Lab, and its Principal Investigator together with Professor Ping Ma.

CONTACT
E: wenxuan@uga.edu
T: (+1) 706 542 0120
W: http://www.stat.uga.edu/people/wenxuan-zhong

FUNDING
National Institutes of Health
National Science Foundation

REFERENCES


Professor Ping Ma
Department of Statistics
University of Georgia
Athens, USA

Professor Ping Ma completed a PhD in Statistics at Purdue University in 2003, before carrying out a postdoc from 2003–2005 at Harvard University in Bioinformatics and Computational Biology. In 2005 he became Assistant Professor at the Department of Statistics of the University of Illinois, and was Associate Professor from 2011 to 2013. In 2014 he moved to the University of Georgia to take up the role of Associate Professor at the Department of Statistics, and since 2015 he has been Professor at the Department. Together with Professor Wenxuan Zhong, he is Principal Investigator of the Big Data Analytics Lab.

CONTACT
E: pingma@uga.edu
T: (+1) 706 542 0714
W: http://www.stat.uga.edu/people/ping-ma


Visualising the Moving Parts of Molecules

We have all seen models and graphics of complex molecular structures, like proteins or DNA sequences. Some look like coloured balls connected by lines – the balls representing molecular nuclei or even whole amino acid groups, the lines representing molecular bonds. Other representations may be a bit more elaborate, with more curves and rounded areas. While this might suffice for teaching that child about proteins and enzymes and such, it certainly doesn’t suffice for the serious scientist. There simply aren’t enough balls and sticks in the world to do this, for one thing. For another, you can’t tell how a protein’s function will change with a change in chemical composition simply by switching out a coloured ball in the model for a different coloured ball.

Since the description of the structure of DNA in the mid-twentieth century, there has been an explosion in our knowledge of DNA structure – that makes up genes and chromosomes – and of proteins – those important macromolecules that are coded for by DNA. Whole databases have come online with massive volumes of data, such as the internationally renowned Human Genome Project (HGP) and the Protein Data Bank (PDB). The HGP aims to identify and map all the genes of the human genome. The PDB maintains a database of the three-dimensional structures of large biological molecules, such as DNA, RNA and proteins, that result from imaging proteins with technology such as NMR spectroscopy, X-ray crystallography or cryo-electron microscopy. Data from researchers around the world is collected in such large databases and made available to scientists everywhere. But simply knowing the sequence or static structure of these molecules doesn’t tell us about their function. It also doesn’t give us an easy way to predict how small changes in the molecules – mutations – will affect their function. There are just too many pieces and too many connections. Sometimes more data is too much data. This is what some call ‘Big Data’ – data collections that are large and complex and sometimes simply become information overload. This is where Dr William Ray and his colleagues in Ohio are hard at work. They want to graphically represent this type of data in a way that is easy to ‘see’ and use, to allow scientists to understand the moving parts of molecules.

Wearing Two Hats – A Jack of Two Trades

Dr Ray is a scientist trained in Computer Science, specialising in both computer graphics and human-computer-interfaces. He is also a Biophysicist, focused on the molecular biology of ancient bacterial transcription through the lens of microbiology. His computer graphics background superimposed upon his microbiological background has made him particularly interested in visual representations of data, most specifically protein and nucleic-acid sequence data.

Dr Ray’s experience and work in this area and networking with that scientific community lead him to believe that the de-facto use of sequences – which coloured balls go in what order in a molecular model – as a way of communicating about proteins and nucleic acid chains fundamentally colours the way that researchers think about these molecules and how they work. ‘Traditional representations of the data as literal character sequences have historically impeded full understanding of actual molecular products,’ he tells us. This static representation itself produces significant inherent biases in tools – such as computer algorithms and modelling programs – that...
Dr Ray has developed alternative ‘sequence’ encodings and visualisations that avoid the analytical and communication limitations of pure sequence-based representations for proteins and nucleic acids. He and his group have demonstrated that their improved encodings enable significantly more insight into the functional consequences of changes to ‘sequence’. In other words, if you change an amino acid or nucleic acid in your molecule, what happens to the ultimate function of that molecule or the molecules that result from it? Dr Ray and his colleagues have developed computer-based tools that have contributed fundamental new insights into the basic molecular determinants of function and disease. In fact, some of the visualisations produced by them have been featured in art exhibits around the world. The group has received funding from the NSF to continue improving their encodings and visualisation tools, and NIH funding to apply these tools to several different biological questions. ‘We are simultaneously working towards applying the visualisation and analysis insights they have gleaned from molecular biology to other life-sciences domains, such as clinical outcome measures and genetic cancer research,’ Dr Ray explains.

The IEEE – originally founded in 1884 with the rise of the electrical industry and telegraph – is the Institute of Electrical and Electronics Engineers. But since everything runs on electricity now, membership today includes computer scientists, software developers, information technology professionals, physicists, medical doctors and many others. In 2010, the IEEE became interested in the science of biological data visualisation. It’s as if the world had just beat a path to Dr Ray’s door. The IEEE added a conference track to their other standard topic tracks and called it BioVis. They invited Dr Ray, along with several others with his interests, to participate in the party.
other researchers working at this nascent interface of biology and data visualisation. They had the honour of being invited to develop and lead an international BioVis symposium. Since Dr Ray (under his biophysics hat) happened to be the most senior biological expert in this group, he developed a biological data analysis contest for the symposium. This contest introduced grand-challenge biological data visualisation and analysis problems from the bio/life-sciences to computational experts around the world. The expertise and capabilities of a 395,000-member organisation was now focused on Dr Ray’s crusade, visualising complex biomolecular data.

For five years Dr Ray was responsible both for the day-to-day operation of the contest, as well as for providing the biological expertise to train the computationalists so that they could better address the actual end-user needs. After all, any software program that a biologist can use in his laboratory should be easy enough for a biologist to actually use. ‘One of the teams I mentored developed a novel approach to visualising different protein stabilisation choices made by different branches of a phylogeny, which was so appealing that it was displayed at the New York Museum of Modern Art, and so useful that it has been added to the standard collection of web analysis tools provided by EMBL (Europe’s version of the NIH),’ Dr Ray recalls. Under his leadership, the data analysis contest portion of BioVis has become so successful that it was adopted by the SAGE BioNetworks DREAM challenge team for management and incorporation into future international DREAM challenge projects. SAGE BioNetworks is a non-profit research organisation that works to develop predictors of disease and encourage research into health by facilitating open access to the scientific data and encouraging patient engagement in the research process.

And as far as networking, Dr Ray has collaborated with essentially all of the other senior practitioners in the growing BioVis domain. He continues to work with the BioVis community, this year in the capacity of co-chair of the Posters track, and will be leading other aspects of their symposiums and workshops in the future, spreading the gospel of graphics. But even with all of this activity, he’s still hard at work back in Ohio.

Meanwhile, Back at the Lab

Over the years, Dr Ray has had his fingers in a few scientific pies. For example, he has collaborated with a number of experts in viral vector therapy to use a structure-based design to genetically modify an adenovirus for use as a viral therapy for neurologic disease. He has also collaborated with experts regarding the structure of the F-protein in RSV. Current collaboration topics also include pre-eclampsia and biofilms.

Dr Ray’s lab has developed and continues to enhance a visual analytics tool they call ’StickWRLD’, that creates an interactive 3D representation of biological data. The tool was originally developed for visualising DNA sequences, and later expanded include protein sequences. Recently, the lab has adapted StickWRLD to visualise SNP data and canceromics data. The StickWRLD user can dynamically change the statistical thresholds of their analysis and pan and zoom the graphics generated by the program. Using this program on protein data, they found that they can identify positions on a protein that are functionally required versus those that aren’t necessary for function. This type of information is important if you want to engineer a protein for a certain function. Do you need this part or that part for the protein to function, or can you substitute something else here and not adversely affect the function?

Dr Ray also worked with his OSU colleagues on a system to produce high-quality renderings of molecular motion. They used the pathlines of atoms in a molecule (the route an atom moves through time within the molecule) versus the usual timeline rendering – the shape a molecule is overall at different times. They call their web-based system for generating time-lapse molecular-flow images and motion structures ‘MoFlow’. It can take protein structure files from the PDB and create an interactive web-based visualisation of molecular motion that can even be printed on a 3D printer. They found that pathlines are more easily understood than timeline representations. As well, pathlines also represent motion directly, rather than representing structure with inferred motion. And MoFlow is probably an apt title for Dr Ray himself. Between work at the OSU and the Battelle Center, and his work with IEEE’s BioVis and the NIH and NSF, he is definitely a moving and flowing researcher.
Meet the researcher

Dr William Clarence Ray
The Ohio State University
Columbus
USA

Dr William Ray received his Ph.D. in Biophysics in 2000 from The Ohio State University. Thereafter he did a postdoctoral fellowship at the Columbus Children’s Research Institute in Columbus, and then took a research scientist position there for a number of years before becoming a Research Assistant Professor of Paediatrics at The Ohio State University in 2008. In 2011 he was named an Assistant Professor of Paediatrics at OSU, where he teaches graduate courses, as well as supervising the thesis research of PhD students and mentoring undergraduate and high school students. He is also Principal Investigator in the Battelle Centre for Mathematical Medicine at The Research Institute at Nationwide Children’s Hospital, where he was recruited as a faculty member in 2002 to build the infrastructure necessary to support research computing at the institute, and he has served as director of the research computing core, as well as serving on the institutional research-computing advisory committee, and the research-computing executive committee.

CONTACT
E: ray.29@osu.edu
T: (+1) 614 355 5645
W: http://www.stickwrld.org

KEY COLLABORATORS
Dr Christopher W. Bartlett, The Research Institute at Nationwide Children’s Hospital
Dr Irina Buhimschi, The Research Institute at Nationwide Children’s Hospital
Dr Mark Peeples, The Research Institute at Nationwide Children’s Hospital
Dr Thomas Magliery, The Ohio State University

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THE NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES

As one of the 27 Centers, Institutes and Offices within the National Institutes of Health (NIH), the National Institute of General Medical Sciences (NIGMS) supports basic research that increases our understanding of biological processes, laying the foundation for finding new ways to predict, prevent, diagnose and treat disease. Often referred to as NIH’s ‘training Institute’, NIGMS’s second aim is to foster the best trained, most innovative and productive biomedical research workforce possible.

With a budget of $2.5 billion USD per year, NIGMS funds scientists at universities, medical schools, hospitals and research institutions throughout the US. Scientists funded by NIGMS investigate how living systems work at a range of levels, from molecules and cells to tissues, whole organisms and populations. Key areas of NIGMS investment include cell biology, biophysics, genetics, developmental biology, biochemistry, organic chemistry, biomedical technology, bioinformatics and computational biology. The Institute also supports research in certain clinical areas, primarily those that affect multiple organ systems, such as pharmacology, anaesthesiology, wound healing, physical trauma, burn injury and sepsis.

In this exclusive interview, we have had the pleasure of speaking with Jon R. Lorsch, Director of NIGMS, who describes the Institute’s unique and varied approaches to supporting basic biomedical research. He also elaborates on several cutting-edge techniques that are advancing our understanding of human health and disease, and shares his hopes for the future of NIGMS and fundamental biomedical research.

NIGMS supports biomedical research that focuses at many levels, including individual molecules, cells and whole organisms. CREDITS: V Schramm, Albert Einstein College of Medicine of Yeshiva University; D Bushnell, K Westover & R Kornberg, Stanford University; National Center for Microscopy and Imaging Research, University of San Diego; ibid; O Ruz & G Eisenhoffer, University of Texas.
You have had quite a successful and varied academic career – what motivated you to join NIGMS in 2013 as Director?

While at Johns Hopkins I steadily took on more leadership and managerial roles. I noticed that by serving in these roles, one can have a very big (and hopefully, positive) impact on a large number of people. In fact, it became clear that I could have a bigger impact on biomedical research – and potentially on society – through scientific leadership, management and administration, than I could by just focusing on my own lab research. I decided that helping to steer the scientific ship is the most important way I can spend my time.

NIGMS focuses on supporting investigator-initiated research, whereby the individual scientists identify the most important questions and develop the strategies needed to answer them. How successful is this approach?

History indicates that letting scientists ‘follow their noses’ is the most productive path to medical and technological breakthroughs. Targeted projects focused on a particular application can be vitally important, for instance, the development of a vaccine for an emerging viral disease. But investigator-initiated research, which involves a combination of curiosity, expertise, creativity and serendipity, has repeatedly proven to yield discoveries that are the most broadly applicable and transformative. By emphasising investigator-initiated fundamental research, NIGMS is unleashing the creativity and energy of scientists across the country.

Biomedical research is a dynamic enterprise that creates opportunities to explore new ideas. We are committed to giving investigators the freedom and flexibility to pursue emerging research questions that can lead to novel scientific insights.

We also encourage researchers to collaborate with each other, especially across disciplines, to form research teams. Only by combining the approaches and expertise of multiple scientific fields can we fully understand the complexities of living systems.

‘No matter how counter-intuitive it may seem, basic research has proven over and over to be the lifeline of practical advances in medicine’

– Arthur Kornberg, 1959 Nobel Laureate in Physiology or Medicine

Researchers captured this detailed view of cellular scaffolding using a ground-breaking technique called stochastic optical reconstruction microscopy (STORM). The 2014 Nobel Prize in Chemistry honoured STORM and related microscopy techniques that are revolutionizing cellular imaging. CREDIT: Xiaowei Zhuang, Howard Hughes Medical Institute, Harvard University, and Nature Publishing Group.
To continue making discoveries, scientists need cutting-edge tools. To that end, NIGMS supports the development of, and widespread access to, high-quality technologies, such as laboratory instruments and techniques, computational technologies, chemical reagents, biological repositories and database resources. Such new technologies enable and expedite scientific advances that, in turn, drive the development of additional technologies.

Even more significant, novel technologies open unexplored fields of knowledge by allowing new types of questions to be asked. Think about what the discovery of microscopes allowed us to do – see life at the cellular level, diagnose diseases, and now, even manipulate single molecules and cells. Or another example – the ability to obtain complete genome sequences made it possible to compare genomes across individuals, revealing the genetic basis of a wide variety of human diseases. None of these advances would have occurred without the enabling technologies that made them possible.

‘Basic technological innovation can open new realms of scientific possibility’

– Francis Collins, Director, National Institutes of Health

We recently launched two initiatives to kick-start the development of new research tools and technologies. The initiatives are called Exploratory Research for Technology Development and Focused Technology Research and Development. Unlike the way technology development has historically been supported by NIH, these initiatives focus entirely on developing tools, not on applying them. Although every funded project must be motivated by an unmet need or new opportunity in biomedical research, the researchers are free to focus completely on the technical aspects of designing innovative new tools, rather than optimising existing tools to answer specific biomedical questions or explore particular organisms, systems or diseases.

NIGMS funds many scientists who develop and apply cryo-electron microscopy in their research. Tell us a bit about this powerful technique and its potential to increase our understanding of life at the molecular level.

Figuring out a molecule’s detailed, three-dimensional structure – the position and orientation of every atom inside it – is one of the ways researchers can learn about the molecule’s role in the body. This knowledge can then help pave the way for a wealth of medical advances, including the prevention and treatment of diseases.

In past decades, the most popular technique for studying the structure of molecules was X-ray crystallography, a technique unsurpassed in its ability to reveal intricate, atomic-level details, even for large molecular complexes. But this technique also has serious drawbacks – most notably, that it requires scientists to grow crystals of the molecules they are studying.

Growing crystals of most biological molecules is difficult, and occasionally impossible. Coaxing crystals to form requires putting the molecules in an unnatural environment and, at times, lopping off parts of them. Even then, the crystals may never form, or if they do, the data collected after taking these extreme measures might not accurately represent the structure of the molecule in its native environment.

Cryo-electron microscopy (cryo-EM) is emerging as complementary – and, in some cases, a promising alternative – to X-ray crystallography. In the past, cryo-EM was only able to reveal the fuzzy outline of a molecule, while crystallography provided exquisite internal details. Now, the visualisation power of cryo-EM is beginning to rival that of X-ray crystallography, thanks to recent advances in the cameras used to collect cryo-EM data and the computational methods used to process the data. Because it doesn’t require sample crystallisation, cryo-EM has the added advantage of enabling scientists to view specimens frozen from their natural or near-natural state. The power of cryo-EM improved so quickly and dramatically that the journal Nature Methods named it the Method of the Year in 2015.

Cryo-EM works on samples that are cryogenically frozen at liquid nitrogen temperatures. The flash-freeze is so quick that water molecules do not have time to form ice crystals, which could disrupt the normal arrangement of molecules. Samples prepared this way are very stable, and many images can be collected from them, allowing researchers to repeatedly refine their data, removing artefacts and creating even sharper images than ever before.

Some scientists are using cryo-EM to create three-dimensional models, or maps, of molecular complexes. To make these models, scientists use sophisticated software...
programs to stitch together thousands of two-dimensional images taken at slightly different angles. The resulting high-resolution three-dimensional models are already yielding new information about the molecular underpinnings of health and disease. For example, recent advances towards a possible HIV vaccine depended on information from cryo-EM studies about how antibodies recognise the virus.

The Institute also focuses on data-intensive and data-driven biomedical research. Describe the importance of improving researchers’ ability to take full advantage of the ever-growing volume of complex biological data.

Over the past decade, the life sciences have been deluged with data, much of it stemming from genome-based technologies. We now have vast quantities of information about molecular networks, cellular processes and diseases. We have collections of databases, knowledge bases and repositories. The challenge is to figure out how data from multiple sources can be shared, analysed, integrated and interpreted in a seamless and synergistic way.

To get a feel for the scale of the problem, take genomic repositories as an example. There are more than a dozen of them, each focusing on a particular research organism – mouse, fruit fly, E. coli, etc. These repositories were created by and for the relevant community of scientists and evolved organically over many years. As an unfortunate result, the various resources are organised differently, maintained separately, and not integrated with one another.

There are also databases about biomolecules: protein structures go in one place, chemical structures in another. Other resources catalogue the DNA, RNA, proteins and small molecules involved in metabolism, signal transduction, immune system function, disease progression and many other processes. But there is no free and easy flow of information between these resources.

Scientists and organisations worldwide are working to get all these biomedically relevant resources to ‘talk’ to each other. They even came up with an acronym for the goal – FAIR, for Findable, Accessible, Interoperable, Reusable. The idea behind the FAIR Principles is to not only benefit human researchers doing data-driven projects but also enable machines – computers – to find and analyse data from multiple resources in an automated way.

For our part, NIGMS fosters a stable, robust and FAIR data ecosystem by supporting new technologies in biomedical computing, informatics and data sciences. NIGMS-funded researchers are forging new applications that will help bridge the gap between the current model of data production and a newer model of data-derived knowledge.

NIGMS also supports a variety of projects that use research organisms. Give a few examples of how this type of research has advanced the understanding of human health and disease.

Much of what we know about biology was learned through research on organisms such as bacteria, fruit flies and mice. These studies taught us how cells grow and divide, how inheritance works, how organisms store and use energy, how certain parts of the brain function, how diseases develop, and what drives behaviours such as eating and sleeping. These lessons have led to new methods for addressing health and disease in humans.

At the most basic level, all life is virtually identical. All organisms rely on the same
systems, structures and substances to live. That’s why research on non-human organisms can shed light on human biology. Regardless of where on the tree of life a creature resides, it uses similar genes, proteins, hormones and other molecules to sense and respond to the environment; digest, store and utilise food; build, transport and dispose of materials; and grow, develop, reproduce, age and die. As a result, a well-designed experiment in a tiny roundworm could shed light on a biological process in people.

In general, research organisms are small, relatively easy and inexpensive to work with, and widely available to scientists. They might also have characteristics that are desirable for certain research projects. For example, zebrafish grow quickly and have see-through embryos, making them ideal for studying how organs develop. Mice that have specific diseases or genetic defects are available by mail order and have been used to investigate cancer, diabetes, infectious diseases, neurodegenerative diseases, autoimmune disorders, birth defects, drug addiction and hundreds of other disorders.

Studies in fruit flies and tiny worms revealed key aspects of how fertilised eggs develop into complex organisms. This research also led to the unanticipated discovery that genetically controlled cell death plays a critical role in cancer and other diseases. As another example, scientists studied yeast to sort out the orderly sequence of events that cells follow to duplicate their contents and divide into two, a process called the cell cycle. This information impacts millions of people, as many anti-cancer drugs interfere with the cell cycle. Furthermore, research in nematode worms has yielded important information about ageing. This research is very difficult to do in people and other organisms that have long life spans.

Studies in research organisms have also produced many powerful scientific tools, including CRISPR, RNA interference, DNA chips and genome-wide scanning methods. These tools are now being used in human health studies worldwide.

Researchers are constantly seeking and discovering new research organisms. A relatively new example is the bobtail squid – a strawberry-sized creature that lives in knee-deep waters around Hawaii. These miniature squid have a symbiotic relationship with bioluminescent bacteria known as Vibrio fischeri. The bacteria inhabit a special light organ on the squid’s underside. There they are fed a nutrient-rich solution in exchange for providing light that a bobtail squid can tune to match its surroundings. Cloaked in ambient light, the squid’s silhouette and shadow essentially disappear, making it invisible to predators. Juvenile bobtail squid actively encourage V. fischeri bacteria to colonise their light organs. If no colonisation occurs, the squid cannot reach sexual maturity.

Humans also have symbiotic relationships with bacteria and other microorganisms. These creatures live everywhere in and on us: our guts, skin, noses, mouths, lungs. They are essential for proper nutrition and may be involved in normal immune system development, neurological function and more. They may also play roles in the development of diabetes and other diseases.

Scientists have begun studying the symbiosis between bobtail squid and V. fischeri as a model to better understand the complex relationship between humans and our resident microorganisms and how this dynamic partnership influences our health and development.

Finally, what do you see as the biggest challenges facing basic biomedical research over the next decade? Tell us about the Institute’s plans to tackle these challenges.

Over the past decades, basic biomedical research has flourished, leading to a deeper, more sophisticated understanding of living systems. As we build on these advances, we aim to deploy our resources in the most efficient ways possible. We also need to include a wide variety of scientists so we can benefit from the full spectrum of perspectives on biomedical questions. To accomplish these goals, we need to re-optimise the scientific enterprise.

Specifically, I’d like to see NIGMS:
stable, flexible and efficient research environment. We hope it will falls within the NIGMS mission. MIRA is designed to produce a more mechanism called the Maximizing Investigators’ Research Award (MIRA). Rather than requiring scientists to focus on specific projects, this award provides support for any research in their laboratories that must apply for additional grants. Any time spent writing and launching new projects must be new, five-year funding mechanism called the Maximizing Investigators’ Research Award (MIRA). Rather than requiring scientists to focus on specific projects, this award provides support for any research in their laboratories that falls within the NIGMS mission. MIRA is designed to produce a more stable, flexible and efficient research environment. We hope it will allow scientists to be more productive and more innovative – to take scientific risks and to pursue important, new scientific questions that arise during the course of their research. We have a companion MIRA program for early-stage investigators that aims to help young scientists overcome hurdles in becoming independent scientists.

Modernising graduate school education: Training the next generation of biomedical researchers is fundamental to the NIGMS mission. Toward that goal, NIGMS is actively supporting efforts to catalyse the modernisation of biomedical graduate education. We have undertaken several initiatives to stimulate this process, such as hosting a symposium to showcase innovations in biomedical graduate education, career development and skills development. Future training issues we want to address include: enabling institutions to innovate to find optimal models for training scientists in the 21st century, identifying best practices for educators and mentors as they design and implement new education and training models, and tracking student outcomes and evaluating program results.

Rigor and reproducibility: To accurately reveal the complexities of living systems, biomedical research must be rigorous. Experiments should be robust and unbiased, and they should produce results that are high quality, reliable and reproducible. In recent years, a number of publications have suggested that the rigor and reproducibility of biomedical experiments are eroding. NIH is leading the effort to address this concern and has been joined by the research community, scientific publishers, universities, industry and professional organisations.

NIGMS is addressing these issues through several initiatives, including co-hosting a trans-NIH workshop on reproducibility in cell culture studies, providing extra funds to more than a dozen training grants to teach graduate students how to design high-quality experiments; and supporting, along with nine other NIH components, grants for training modules to enhance data reproducibility, then publishing the products of these grants in an online clearinghouse.

Diversity and inclusion: Achieving diversity in the workforce is also a key priority for the Institute. The biomedical workforce has not kept pace with changes in the nation’s demographics. As a result, the field is not benefiting from the creativity, energy, skill sets and viewpoints of many within the US populace. To better leverage the rich diversity of thinking and experiences within our country, NIGMS offers several programs designed to develop talented people from underrepresented populations.

In addition, NIGMS aims to broaden the geographic distribution of NIH funding. Through the Institutional Development Award (IDeA) program, NIGMS boosts research capacity through faculty and student development and building research infrastructure in states that historically have not received high levels of NIH funding.

The overarching goal for all of NIH is to advance medical science and improve human health. My hope is that, through our support of investigator-initiated research, effective training programs and new technologies, NIGMS will play an important role in reaching that goal.

Alfred Atanda, Jr., is a paediatric orthopaedic surgeon and researcher at Nemours/Alfred I. duPont Hospital for Children in Wilmington, Delaware. He receives grant support through an Institutional Development Award (IDeA) that provides mentoring and professional development opportunities to investigators in the early stages of their careers. CREDIT: Cynthia Brodaway, Nemours/Alfred I. duPont Hospital for Children.

• Create more efficient and sustainable funding mechanisms for investigators at different stages of their careers.
• Modernise graduate school education to take advantage of the latest educational methods and equip our future scientists with 21st-century skills.
• Ensure rigor and reproducibility in research.
• Increase diversity in the workforce and in the institutions, geographic regions, scientific topics and approaches we support.

We have covered all these topics in the NIGMS Feedback Loop blog, and will continue to do so, but here are a number of highlights:

**Funding mechanisms:** Historically, most NIGMS research grants funded individual projects that had specific goals defined at the beginning of a four- to five-year project period. Sometimes, during a project, observations and insights reveal exciting new research questions or unexplored areas. Under the current system, it can be difficult for investigators to pursue new directions using funds that were allocated for a specific purpose. In addition, scientists who want to launch new projects must apply for additional grants. Any time spent writing and reviewing grant applications means less time for conducting research.

To address these issues, NIGMS initiated a new, five-year funding mechanism called the Maximizing Investigators’ Research Award (MIRA). Rather than requiring scientists to focus on specific projects, this award provides support for any research in their laboratories that falls within the NIGMS mission. MIRA is designed to produce a more stable, flexible and efficient research environment. We hope it will allow scientists to be more productive and more innovative – to take
Research using model organisms has allowed scientists to reveal the basic processes of life and develop an understanding of human disease.

**Escherichia Coli**

*E. Coli* is the bacterial workhorse of molecular biology. Matthew Meselson and Franklin Stahl used these bacteria to establish the semi-conservative nature of DNA replication in 1958, confirming that each old strand acts as a template for a new one.

**Drosophila Melanogaster**

Because of their size and quick growth, they are easy to maintain in a small space—including outer space! Edward Lewis, Christiane Nusslein-Volhard and Eric Wieschaus used these flies to determine how genes control embryonic development in multicellular organisms.

**Caenorhabditis Elegans**

This tiny transparent worm contains just 302 neurons, whose connections have been mapped to form a complete ‘connectome’. This helps scientists to unlock how neuronal networks control simple behaviour.

**Mus Musculus**

The humble mouse is to thank for huge advances in medical sciences, including the development of the Polio vaccine. Today, the mouse is the most widely-used and important model of human disease including cancer and HIV/AIDS.

**Danio Rerio**

The translucent zebrafish develops rapidly and can be easily genetically modified. Jane Marion Oppenheimer used zebrafish to illuminate vertebrate body development in the 30s, and George Streisinger went on to develop the zebrafish as an important model of vertebrate genetics.

**Metabolomics, proteomics, connectomics and the new era of Big Data biology. Will computer models start to replace model organisms?**
Proteins are the building blocks of life. The genetic code within each cell of our bodies controls the sequence of molecules called amino acids, which assemble together to form each protein. But this is only the first step. Amino acids are small charged molecules that interact with one another as they join together to form strings of peptides (polypeptides), which spontaneously develop into different structures known as secondary structures. This can involve the formation of sheets or helical spirals following certain rules determined by the order of the amino acids. These secondary structures then interact with one another to form larger tertiary structures of proteins that control and mediate every aspect of cell function.

‘Ever since I was an undergraduate student, I’ve always been fascinated with a chemical view of biology,’ Professor Fairman tells Scientia. He first became fascinated with proteins and their structure during a research internship at Brookhaven National Laboratories, where he worked on the assembly of a virus that infects bacteria called a bacteriophage. During his graduate studies, he became particularly interested in a quantitative approach to protein folding and protein design, and says that, ‘much of my work over my career has taken advantage of protein design approaches to study interesting problems in protein assembly.’

Gumming Up the Works

Many genes encode long sequences of the amino acid glutamine. Alterations in the DNA encoding these regions can lead to repetitions and an increase in the length of these sequences. When these chains of glutamine become too long, protein aggregates can start to form. These aggregates are formed from structures known as β-sheets, which assemble into tiny fibres (fibrils) that cannot be dissolved. This leads to the death of particularly vulnerable cells such as neurones – the elaborate and sensitive cells in our brain. This is the basic mechanism that causes the debilitating and currently untreatable neurological disorder Huntington’s disease, which is characterised by a disruption of normal movements and a decline in mental ability, resulting in dementia.

‘The relationship of this aggregation to disease is poorly understood, and differences in protein structure may underlie differences in toxicity,’ Professor Fairman admits. ‘Many neurodegenerative diseases (Alzheimer’s, Huntington’s, Parkinson’s) have a similar underlying molecular mechanism of protein aggregation, involving the formation of an extended polypeptide chain that can aggregate through β-sheet formation.’

As such, it is vital to understand how these protein aggregates are formed and the intermediary steps that lead to the development of small fibres and larger structures that somehow disturb normal cell chemistry and function in neurones.

Professor Fairman’s team use the latest developments in a technique called analytical ultracentrifugation, which involves spinning solutions of peptides at very high speeds to separate them according to their sizes under the influence of elevated G-forces (gravity). More recently, they have combined this technique with detecting specific proteins, which have been tagged with fluorescent markers. ‘Our work takes advantage of a key biophysical method that allows for the study of protein aggregation in solution, and more specifically, with the use of fluorescence detection, allows such characterisation in complex mixtures that better represent cellular conditions,’ says Professor Fairman.
This tool satisfies my innate interest in quantitative approaches to biology research.

Using these techniques, Professor Fairman’s team looked at how regions in the protein known to cause Huntington’s disease could interact and mediate the formation of aggregates.

One region with a helical structure in the huntingtin protein, which is adjacent to the glutamine rich β-sheets, is known to be important for the formation of aggregates.

In order to investigate the role this structure plays in the process, this 17 amino acid long helical region was fused to a glutamine sequence. This same glutamine sequence was fused to another previously well studied coiled-coil structure consisting of two helices or coils twisted together like a rope. Comparing the two allowed the team to test different models of how these regions of the protein interact with one another. The presence of the coiled-coil acted to reduce aggregation and supported the model that interaction between regions is an important factor in the formation of aggregates from β-sheets.

They went on to examine the appearance and structure of the fibres produced by these synthesised peptides in order to reveal the mechanisms that produce much larger structures, from layers of β-sheets, like those found in neurones. They observed that the presence of the coiled-coil helical structure did not prevent β-sheet structure from forming, but it reduced the bundling of fibres that produces these larger structures. Understanding these mechanisms behind the assembly of larger structures can facilitate new approaches for designing inhibitors of protein aggregation. In addition, it allows researchers to design peptides that favour particular shapes, in order to investigate how these different structures lead to cell death in both cells and animal models.

Glowing Worms

Fibres formed from β-sheets can become tangled together and can drag in other molecules within the cell to produce much larger structures called inclusion bodies.

In more recent work published in the last year, the team have developed the use of...
analytical ultracentrifugation combined with a fluorescence detection system to look at the intermediate steps in protein aggregation and inclusion body formation in an animal. This is of fundamental importance to understanding which of these intermediate steps is toxic or protective and contributes to cell death and disease.

Advances in genetics now make it possible to label particular proteins of interest with fluorescent markers. A commonly used system involves genetically manipulating the worm *Caenorhabditis elegans* to allow visualisation of such proteins within the living animal. Professor Fairman’s team have shown that crude extracts from these worms can be analysed using ultracentrifugation methods to detect a wide range of intermediate aggregates of protein from the smallest fibres of β-sheets to the larger inclusion bodies characteristic of neuronal damage in humans. This is proof of the concept that this method can be used in a whole animal model of disease using complex mixtures formed within a multi-cellular animal. The technique also allows the different types of intermediate aggregates of protein to be studied under the microscope and compared to those seen in the living animal. This has opened up the possibility of exploring the effects of specific genetic mutations in the proteins involved and how different factors such as ageing and the environment can influence aggregation.

**From Worm to Fly and Beyond**

Professor Fairman says his team are, ‘hoping to continue our work in the use of the analytical ultracentrifuge with fluorescence detection to study other neurodegenerative diseases, such as Parkinson’s disease.’ These techniques have demonstrated their great potential in the team’s proof of principle experiments. Professor Fairman and his collaborators have applied for a grant to purchase new state of the art equipment, which will help them to further unravel the mechanisms of protein aggregation in neurodegenerative diseases. The new equipment, alongside the team’s specialist expertise in this novel technique, will act as a hub for a wide range of projects. ‘Haverford will become a site for training in the use of this technology, open to the global community,’ Professor Fairman states. ‘It is exciting to be involved at the cutting edge of this technology, particularly bringing this approach to my teaching as well.’ Students have been actively involved in work that has been published this year after working on bacterial enzymes that synthesise biologically useful peptides like anti-bacterial agents. This work could aid the redesign of such synthases for use in bioengineering projects. The teaching labs also provide students with a unique experience of the scientific process, as well as rigorous quantitative training in protein structure.

Professor Fairman and his team plan to continue investigating the early steps in the aggregation of proteins with long glutamine sequences, and the influence of flanking regions in animal models such as *Caenorhabditis elegans* and the fly *Drosophila melanogaster*. Both of these model species can be genetically modified to label proteins of interest with fluorescent markers, making them ideal candidates for the application of this technique.

The same approach can then be applied to the study of other protein aggregation systems such as the protein α-synuclein, which forms the aggregates characteristic of Parkinson’s disease. In *Caenorhabditis elegans*, the team plan to introduce mutations to α-synuclein and manipulate other genes and proteins that may influence aggregate formation. Using flies as a model system, the team also plan to look at a common mutation that is known to cause motor neurone disease (amyotrophic lateral sclerosis, or ALS) which encodes a set of five repeating amino acids that also have a tendency to form aggregates leading to neuronal damage.

Using these methods, Professor Fairman’s team will unlock the mechanisms that cause neurodegenerative disease, allowing the discovery of future treatments. The advancement of these techniques also aids the development of novel biomaterials for use in engineering and biotechnology, and offers the next generation of young scientists access to the world of research.
Meet the researcher
Professor Robert Fairman
Department of Biology
Haverford College
Pennsylvania
USA

Professor Robert Fairman developed a fascination in biochemistry as an undergraduate studying at Long Island University and during a research internship at the Brookhaven National Laboratories. He went on to obtain a PhD at Stanford University where he became interested in protein design, folding and assembly. He followed this with a postdoctoral fellowship at DuPont Merck Pharmaceutical Co. and as a research investigator at Bristol-Myers Squibb. He was appointed to teach biochemistry in 1997 in the Department of Molecular and Cell Biology at Haverford College and is currently Professor of Biology where he is actively involved in teaching advanced topics in protein science. Amongst other courses, he teaches a junior-level hypothesis driven laboratory course (the ‘superlab’) and has developed cross department laboratory courses in biochemistry. The superlab course is one example of how his scholarship and teaching are highly intertwined. His group carries out research, using new techniques in analytical ultracentrifugation, into the development of novel biomaterials and the process of protein aggregation in neurodegenerative disease.

CONTACT
E: rfairman@haverford.edu
T: (+1) 610 896 4205
W: www.haverford.edu/biology/fairman/fairman.html

FUNDING
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NSF

REFERENCES

KEY COLLABORATORS
Bashkim Kokona, Department of Biology, Haverford College
Tom Laue, University of New Hampshire
Chris Link, University of Colorado Boulder
Nancy Bonini, University of Pennsylvania
Lou Charkoudian, Department of Chemistry, Haverford College
The students who drive the research
MECHANOBIOLOGY – EXPLORING THE MECHANICS OF CELL BEHAVIOUR

Understanding how cells interact with the physical world around them is at the core of mechanobiology, a growing subfield connecting the arenas of cell biology and bioengineering. Two leading researchers, Professor Taher Saif and Dr Andrew Holle, with the support of an international body of scientists, are spearheading work in this field that aims to uncover how cells are impacted by their mechanical microenvironment in a physiologically relevant context.

A Union of Biology and Engineering

Like the humans in whom they reside, cells within our bodies are constantly seeking to make sense of the physical world around them. In the same way that we are compelled to feel and touch our surroundings in order to better understand them, cells also need to explore and interact with their environment in order to know how and when to function. Exactly how this happens is currently under investigation by researchers working in the emerging field of mechanobiology – a union of biology and engineering that focuses primarily on how physical forces and changes in the mechanical properties of cells and tissues contribute to development, physiology, and disease. Two innovators in this exciting arena are Professor Taher Saif from the Department of Mechanical Science and Engineering at the University of Illinois, USA, and Dr Andrew Holle from the Department of Cellular Biophysics at the Max Planck Institute for Medical Research in Heidelberg, Germany. Together, they are aiming to bring mechanobiology to the forefront of cell biology. ‘This is a growing field, as almost every type of cell behaviour worth studying can be affected by the physical characteristics of the material around them,’ says Dr Holle.

There is an increasing body of evidence that suggests that mechanical forces, both extracellular and intracellular, have a profound influence on a variety of cell functions, including cell division, cell death (apoptosis), proliferation, and differentiation. Understanding how cells interact with the physical world around them is core to determining their behaviour and function says Professor Saif: ‘The focus of our research for the last ten years is to understand the effect of mechanical micro-environments on cell functionality. The ultimate goal is to explore the underlying mechanism of cellular mechanotransduction in a physiologically relevant context, particularly in human diseases.’

Mechanotransduction, a novel term being used with increasing frequency, refers to the various mechanisms by which cells sense their physical three-dimensional environment. This form of sensory transduction converts mechanical stimulus (or force) – exerted by properties of the extracellular matrix (ECM; a non-cellular scaffold-like component that is present within all tissues and organs, and capable of biochemical support), neighbouring cells and physical stress – into biochemical signals. In turn, these signals result in the adjustment of cellular and extracellular structures. This mechanosensitive feedback modulates a myriad of cellular functions and is vital for organ development and homeostasis.

The Mechanobiology of Cancer Cells

The accomplishments of Professor Saif and Dr Holle have brought together a number of expert researchers, namely Professor Joachim Spatz and Dr Jennifer Young at the Max Planck Institute for Medical Research and the University of Heidelberg in Germany, Professor Ralf Kemkemer at the Max Planck Institute for Intelligent Systems and Reutlingen University in Germany, Professor Yu Suk Choi at the University of Western Australia, and a group of US-based researchers comprising Professor Adam Engler at the University of California, San Diego, Professor Mark Kuhlenschmidt at the University of Illinois at Urbana-Champaign, Professor Paul Janmey at the University of Pennsylvania, and Post-doctoral Associate Xing Tang at Harvard University. This international collaboration, whose expertise spans the fields of stem cell biology, biophysics, bioengineering, and mechanical science, is currently focusing on the mechanobiologics of cancer cells, with the aim of identifying the mechanisms by which cancer cells navigate different types of extracellular environments.

Death from cancer is predominantly caused
by the development of secondary growths (metastases) and not by the parent tumour. During metastasis, malignant cells break away from the parent tumour and spread through the blood or lymph system to invade new tissues and organs. Consequently, one of the first steps in the spread of cancer is the invasion by malignant cells of normal tissue surrounding the tumour. This invasion is initiated and controlled by various mechanical interactions in the tissue, particularly through the ECM. These mechanical interactions can take two forms: the first involving remodelling of the ECM by nearby contractile cells on the tumour surface, and the second involving the alignment of fibres in the ECM and adjacent cytoskeleton – an active, complex and multifunctional network of interlinking tubules and actin filaments that extend throughout the cytoplasm in all cells. These actions enhance the tendency of cancer cells to follow alignment; thus, enhancing their invasion of neighbouring tissue via contact guidance.

Although great strides have been made in unearthing the underlying mechanics of cancer cell invasion, the physical-chemical mechanisms and parameters within the cellular microenvironment that initiate the onset of metastasis are, as yet, not fully understood. ‘For cancer cells to escape from their primary tumour and metastasise, they have to poke around and feel their environment, then make changes to their cell programming,’ explains Professor Saif. ‘If we can figure out exactly how these cancer cells get this information, then we can prevent them from learning about their environment and hopefully stop them from metastasising.’

From Stem Cells to Cancer Cells

As a basis for his exploration into the mechanobiology of cancer cells, Dr Holle, who currently holds the post of American Association for Cancer Research Basic Research Fellow, as well as a Max Planck Institute Postdoctoral Fellowship, has conducted considerable research into mechanotransduction pathways in stem cells – unspecialised cells that can differentiate into many different cell types. It is well known that stem cells receive a myriad of chemical and mechanical cues from their microenvironment that must be translated into signals that dictate cell behaviour. More recently, however, light has been shed on the underlying mechanical forces that shape organismal development and direct disease response. In particular, Dr Holle’s graduate research focused on the interaction between stem cells and the ECM.

Through building a platform that combines the application of analytical computational techniques (bioinformatics) with a small (or short) interfering RNA (siRNA) screen capable of identifying novel proteins and high content cell imaging and analysis, Dr Holle was able to study the influence of focal adhesion proteins – multi-protein structures that form mechanical links between the intracellular and extracellular environment – on stem cell differentiation. In doing so, they have identified new candidate focal adhesion proteins that play a significant role in cell differentiation. It is hoped that the development of interdisciplinary tools such as this can be applied to cancer mechanobiology, with target proteins and resultant cell behaviour assayed on a high content/throughput scale.

Dr Holle went on to investigate the interplay between cancer cell-ECM interactions and the reorganisation of intermediate filaments comprising the cytoskeleton. He found that by freeing the mechanically-active actin component of the cytoskeleton from the more ‘dampening’ intermediate filament cytoskeleton, cancer cells were able to
display enhanced contact guidance. As cancer cells are generally softer, faster, and more sensitive to nanoscale topography than normal cells, this vein of research presents an interesting possibility for future work in slowing cancer cells down by enhancing their intermediate filament cytoskeleton.

Dr Holle was also part of an international team that developed a new method for fabricating linear gradient stiffness in hydrogels – macromolecular gels constructed of a network of water-laden, cross-linked polymers. Using hydrogels of varying stiffness gradients spanning the entire in vivo physiological and pathological mechanical landscape, he and his fellow researchers were able to monitor different stiffness-dependent processes in stem cells and, thus, find optimal stiffness values for inducing desired cell behaviour, such as cell migration. These findings have significant relevance, in that some cancer cell lines are inversely sensitive to substrate stiffness and, consequently, show markedly different migration phenotypes. ‘Simple tools like these, which are straightforward to build, use, and analyse, will help make investigations into cancer cell mechanobiology more accessible and widespread,’ says Dr Holle.

Understanding Cancer Cell Mechanics

Similarly, Professor Saif, who has a background in mechanical science and engineering, is keen to understand the effect of a cell’s mechanical microenvironment on its functionality. Towards this goal, he and his team have developed a number of modalities for measuring cell forces, including new imaging methods that allow the visualisation of cells and their intracellular dynamics while they are under mechanical stress, and biophysical modelling of cellular microenvironment. To assist his research, Professor Saif utilises a variety of nanoscale quantitative tools, including very-high-resolution microscopes (atomic force microscopy and traction force microscopy), and computational and theoretical modelling. Notably, his lab at the University of Illinois (Saif Lab) has pioneered a number of methods and concepts, including microfluidics, nanofabrication, micro and nanotechnology, and novel stages for mechanical manipulation.

Professor Saif’s work involves both in vitro and in vivo models, and he has been conducting research into the behaviour and mechanics of cancer cells since 2007. Initially, he and his team investigated human cancer cell lines, discovering that tumour mechanical microenvironment is a critical player in cancer cell metastatic transition. More recently, Professor Saif has been focused on the mechanobiology of primary human colon tumour cells and tissues. Findings from his studies have shown that human colon cancer cells with low metastatic potential exhibit a metastatic-like phenotype when cultured on appropriately soft substrates, whereby they dissociate from each other and become migratory. This behaviour led to the upregulation of several oncogenes playing important roles in cancer cell migration, invasion, proliferation, and the suppression of apoptotic genes – resulting in the cancer cells becoming much more tumorigenic compared to the parent tumour.

Currently, Professor Saif and his research group, in collaboration with Carle Foundation Hospital and Presence Hospital in Illinois, and the Mayo Clinic, are working to address the following questions: (i) what is the role of intra-cellular forces in transforming the parent HCT-8 cells to metastatic ones? (ii) Can cell forces be used as a marker for drug screening? And, (iii) is there a mechanical signature in metastatic colon cancer cells from human patients and can such signatures be used for cancer prognosis?

The Future of Mechanobiology

The next step in Professor Saif’s and Dr Holle’s research is to take what they have learned and attempt to alter it to facilitate their understanding of cancer cell mechanobiology. ‘If we know how cancer cells can squeeze through very tight passages, our goal is to treat those cells with a chemical or protein that stops them from doing so,’ they tell us. Their combined body of work provides fundamental insight into the critical role the tumour mechanical microenvironment may play in the metastatic transition of cancer cells, and hence cancer progression.
Meet the researchers

Professor Taher Saif
Department of Mechanical Science and Engineering
University of Illinois, Urbana-Champaign, Illinois, USA

Professor Taher Saif began his training in Civil Engineering at Bangladesh University of Engineering and Technology before moving to continue his studies at Washington State University, USA. He achieved his PhD in Theoretical and Applied Mechanics at Cornell University in 1993, going on to become a Research Associate in Cornell’s National Nanofabrication Facility in 1996. He joined the faculty at the University of Illinois in 1997, where he is currently Gutgsell Professor in the Department of Mechanical Science and Engineering. He is a member of the Board of Directors for the Society of Engineering Science and a Fellow of the American Society of Mechanical Engineers. He is Associate Editor of the Journal of Applied Mechanics and on the editorial board of the International Journal of Applied Mechanics.

CONTACT
E: saif@illinois.edu
T: (+1) 217 333 8552
W: http://saif.mechse.illinois.edu/

Dr Andrew W. Holle
Cellular Biophysics Department
Max Planck Institute for Medical Research
Heidelberg, Germany

Dr Andrew W. Holle received his BSE in Biomedical Engineering from Arizona State University, USA and went on to achieve his PhD at the University of California, San Diego with a thesis entitled ‘Focal adhesion proteins are mechanosensitive and regulate stem cell differentiation’. From 2014 to 2017, he performed postdoctoral research on cancer invasion in synthetic microchannels in the Department of New Materials and Biosystems at the Max Planck Institute for Intelligent Systems in Stuttgart, Germany. He is currently continuing this research in the Department of Cellular Biophysics at the Max Planck Institute for Medical Research. He was awarded a Basic Cancer Research Fellowship from the American Association for Cancer Research from 2016 to 2017.

CONTACT
E: holle@is.mpg.de
T: (+49) 711 689 3625

KEY COLLABORATORS
Professor Joachim Spatz, Max Planck Institute for Medical Research and University of Heidelberg
Professor Ralf Kemkemer, Reutlingen University and Max Planck Institute for Intelligent Systems
Dr Jennifer Young, Max Planck Institute for Medical Research
Professor Yu Suk Choi, University of Western Australia
Professor Adam Engler, University of California, San Diego
Professor Mark Kuhlenschmidt, University of Illinois at Urbana-Champaign
Professor Paul Janmey, University of Pennsylvania
Dr Xing Tang, Harvard University

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The human body has evolved over millions of years within an Earth-bound environment. The biomechanics of our cellular tissues are constrained by the physical laws of life on Earth. It is only within a tiny fraction of evolutionary time that we have developed the ability to leave our planet’s protective atmosphere and experience the weightlessness of space. As we are developing our new-found potential for extra-terrestrial exploration, the consequences of placing human bodies into space long-term have to be carefully considered. At NASA Ames Research Center in California’s Silicon Valley, a team of dedicated biologists is working to understand the effects of space flight on the human body, and is developing countermeasures to protect astronauts and colonists of the future as they embark on missions to other planets in our solar system.

NASA needs new scientists to be trained to continue this work into the future of space flight, and support the innovations in medical science that must accompany explorations of this kind. Dr Sanjoy Som, director of the Blue Marble Space Institute of Science, is a researcher at NASA Ames in the field of astrobiology – the study of the origin, distribution, and future of life on Earth and beyond. Alongside a team of other dedicated scientists, he has developed a unique training programme for early career scientists preparing to pursue postgraduate training. The programme gives students access to the cutting edge research environment, resources and expertise at NASA to develop projects aimed at supporting human life long-term in space. The programme also supports training in ethics and a consideration for the wider implications of their research projects. Science communication is also a big part of the training programme, helping future scientists and NASA to engage the public with current projects and ongoing research.

Living Bone

Bone is a dynamic living tissue that constantly adapts to the environment. The mechanical load on bone tissue acts to stimulate repair and regeneration to maintain a constant level of mineral content and mass. This process of remodelling occurs through bone resorption by specialised cells called osteoclasts, and the formation of new mineralised bone tissue by osteoblasts. In space, under reduced gravity (microgravity), resorption of bone is stimulated, leading to a loss of bone mass similar to that seen in osteoporosis. Scientists at the NASA Ames Research Center are currently working to unlock the mechanisms behind this process, in order to find ways to prevent it from happening. Back here on Earth, this research has major implications for understanding the degeneration of bone that occurs in disease, or when mechanical stimulation is lost due to prolonged bed rest in hospital.

Meg Cheng-Campbell, working with Dr Elizabeth Blaber and Dr Eduardo Almeida within the Bone and Signaling Laboratory, enjoys the challenge and satisfaction of fundamental biology research. The team have previously found that mice, exposed to microgravity for 15 days on the space shuttle Discovery or 30 days on the Bion M1 mission, experienced a loss of bone mass. Because the cells that make up bone are continuously replaced by new cells, the team hypothesised that these losses may be caused by a decreased capability of the bone to renew its own cells. This suggests that being exposed to gravity stimulates the production of new bone forming cells. In space, this loss of cell-renewal ability is combined with an increase in bone loss, leading to a reduction in bone mass. The team’s research has implications for other parts of the body that rely on a constant supply of new cells to repair and regenerate.
Ryan T. Scott is working with Dr Joshua Alwood to investigate the molecular mechanisms that lead to bone loss during weightlessness and how space radiation affects bone strength. Ryan further investigates the effects of weightlessness on the female reproductive system. He has a special interest in the study of exercise science and physiology. He is working with Dr Alwood to discover how increasing activity in the limbs, such as during exercise, can counteract the effects of weightlessness and radiation when undertaken together with drug treatments. Indeed, in the future, it may be possible to take such drug treatments to prevent the loss of bone mass, and so the team are also investigating the possible side-effects these treatments might have if taken over a long time.

Samantha Torres, also in the Bone and Signaling Laboratory, currently works with Dr Ruth Globus on a project to investigate the physiological, cellular and molecular responses of bone to simulated galactic radiation and weightlessness. This work has implications for ground-based health problems such as prolonged bedrest and radiotherapy. The team showed that exposure to similar total doses and types of ionising radiation as those predicted during long duration space exploration, results in the activation of molecular mechanisms related to inflammation, stimulates bone-resorbing osteoclasts, and promotes bone resorption. The researchers are finding ways to protect against ionising radiation, such as consumption of dried plums, which have anti-oxidant and anti-inflammatory properties.

It's in the Blood

In the Vascular Analysis Laboratory, Matthew Murray is working with Dr Patricia Parsons-Wingerter on the vessel generation analysis (VESGEN) project. Every cell in the body requires a constant supply of energy for metabolism and immune support, through highly branched blood vessels. Each cell is no more than a fifth of a millimetre away from the smallest blood vessels (capillaries), while these tiny vessels are at least forty branch points away from the heart. The vascular fingerprint for each person or animal is unique, and constantly adapts to changes in the environment by adding and removing capillaries. The growth of new blood vessels is important in embryonic development and also the development of pathological tissue in cancer. This same process also occurs during an inflammatory response to injury, and is required for proper wound healing.

The Vascular Analysis Laboratory has developed a software system for rapid and accurate analysis of the vascular fingerprint of animals or humans, to allow a detailed investigation of how these blood vessel patterns can change during space flight. This will enable the team to observe how these changes might affect health or disease progression during long-term missions. This vascular signature gives an accessible and quickly measured read-out of the complex molecular and cellular signalling pathways that are initiated to promote remodelling and growth of new vessels in response to stress or injury. The team has already used similar techniques to look at the changes in blood vessels that occur in the retina during diabetes, which can lead to sight loss.

Matthew and the team are currently interested in how changes in blood vessels in the retina change during space flight, in order to understand a condition known as Vision Impairment and Intracranial Pressure syndrome, which can cause vision loss after long exposure to reduced gravity. The team hope to measure the remodelling of blood vessels in the retina by comparing images before and after astronauts take a trip to the International Space Station. This will aid development of effective countermeasures for astronauts, but also potential future treatments for patients on Earth with similar medical conditions.

Space Sex

The number of women becoming astronauts was much less than the number of men in the first few decades of space flight, but this is rapidly changing. In fact, the NASA astronaut class of 2013 had equal numbers of men and women. Eric Moyer is working with Dr April Ronca to explore how space flight might affect the physiology of males and females differently, with the aim of improving the health of all astronauts travelling beyond our planet. They are investigating how to protect reproductive health, so that communities in space might adapt to conception, pregnancy, birth and development. They are also investigating the longer-term implications of how child development might be affected by exposure to the harsh environment and stresses of space flight.

Recent research has shown that mice who have spent weeks in space experience a disruption to their reproductive health and a loss of fertility. But this is only part of the problem. Technology for mouse studies in space has been developed at the Ames Research Center, allowing Eric and Dr Ronca to now analyse the effects of space flight on mouse behaviour, which can inform all future studies involving mammals in space. Remarkably most behaviour such as eating, drinking and grooming was very similar to the mice on the ground, but mice in space quickly learned to propel themselves around the habitat, with their activity levels actually increasing over time in flight. The development of these mouse habitats in space allows the team to research the effects of space flight on mammalian behaviour –
which needs to be addressed before more complex intergenerational experiments can be attempted.

This is important because the team’s future experiments will investigate how radiation and reduced gravity conditions unique to space flight might affect the reproductive cycles of adults and also the development of offspring. They will be looking to expand past research investigating the effects of altered gravity, such as that seen during a space flight launch, on foetal development via patterns of gene expression and the maturity into adulthood, which has demonstrated a strong influence on health and disease throughout life. The goal will be to identify which environmental factors lead to changes in gene expression across the entire lifespan and how these factors may be transmitted to subsequent generations. For men and women to live beyond our planet and start forming communities, there is a lot to be understood about how life in space might affect the conception and development of offspring.

Ready to Launch

Meanwhile in the Biomodel Performance Laboratory led by Dr Sharmila Bhattacharya, students Iman Hamid and Christina Cheung are busy preparing their payload for an upcoming launch. Dr Bhattacharya’s team are using fruit flies to explore how life in space alters the immune response to a microbial infection. They are also using software and video tracking to observe changes in fly behaviour. By comparing results from flies that have been to the International Space Station with flies back on Earth, they plan to look at a number of physiological processes important for future space travel, such as the roles that different genes play in protecting against reactive oxygen species and radiation.

The new rodent and fruit fly facilities on the International Space Station, combined with the biological research on the ground, allows this group of young scientists to gain unique hands-on research experience and to develop new strategies for long range exploration and colonisation of our solar system. The opportunity to take part in these projects is preparing these future leaders to accomplish dizzying heights.
Meet the researchers

Meg Cheng-Campbell
Margareth ‘Meg’ Cheng-Campbell is currently a Master’s student at Santa Clara University studying Bioengineering. Meg was introduced to NASA Ames Research Center as a Research Associate through the Space Life Sciences Training Program, where she worked with the Human Performance Centrifuge. Since then, Meg has worked with Dr Eduardo Almeida and Dr Elizabeth Blaber in the Bone and Signaling Laboratory. Her current work focuses on understanding the complex mechanisms underlying bone tissue maintenance and stem cell regeneration.

E: margareth.a.cheng-campbell@nasa.gov

Ryan T. Scott
Ryan T. Scott is a Research Associate at NASA Ames Research Center within the Bone and Signaling Laboratory working with Dr Joshua Alwood. His research seeks to investigate the molecular, cellular, biomechanical, and physiological effects of spaceflight, to understand how the musculoskeletal system, reproduction, endocrinology and nervous system are all impacted together. His future work is focused on development and testing of countermeasures to enable long-term spaceflight missions.

E: ryan.t.scott@nasa.gov

Samantha Torres
Samantha Torres is a graduate student at San Francisco State University. She is currently studying for a Master’s degree in Public Health with an emphasis on Epidemiology. She is also a Research Associate in the lab of Dr Ruth Globus at NASA Ames Research Center, where she seeks to understand the effects of radiation and microgravity on the skeletal system. Her research is focused on human biology in space and the effects of comparable conditions such as radiotherapy and musculoskeletal disuse here on Earth.

E: samantha.m.torres@nasa.gov

Matthew Murray
Matthew Murray graduated from Santa Clara University with a Bachelor’s degree in Bioengineering. He is currently working with Dr Patricia Parsons-Wingerter in the Vascular Analysis Laboratory at the NASA Ames Research Center on diabetic retinopathy, bed rest, and crew member studies. This forms part of the vessel generation analysis (VESGEN) project, which aids in determining the cause of Vision Impairment and Intracranial Pressure syndrome – a condition causing visual impairments often experienced after long-term exposure to microgravity.

E: matthew.c.murray@nasa.gov

Eric Moyer
Eric Moyer is a biology researcher and lab manager for Dr April Ronca at the Reproduction and Development Laboratory at the NASA Ames Research Center. His research is focused on the molecular biology of rodent gene expression and the serum proteins produced within stress and metabolism regulation pathways following ground-based models of spaceflight exposure. He contributes to the study of animal behaviour on the International Space Station conducted using NASA’s Rodent Research Facility.

E: eric.l.moyer@nasa.gov

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In this next section of Scientia, we are delving into how basic research is applied in the pursuit of new treatments for some of the world’s most dangerous and debilitating diseases.

From the development of vaccines that stopped the polio epidemics in the early twentieth century, to the discovery of the role of bacterium *Helicobacter pylori* in gastritis and peptic ulcer disease, to the breakthroughs that led to the identification of the virus that causes cervical cancer, ground-breaking research in cellular and molecular biology is constantly laying the foundations for the development of radical new ways to prevent, diagnose and treat diseases.

To introduce this section, we have had the pleasure of speaking with Dr. B. Mario Pinto, President of the Natural Sciences and Engineering Research Council of Canada (NSERC). In this exclusive interview, Dr. Pinto talks to us about the outcomes of the recent 2017 Global Research Council meeting, hosted by NSERC, where the heads of research funding agencies across the globe meet to discuss international collaboration between funding bodies. He speaks about diversity and equality in science, and the importance of viewing basic and applied research as part of the same integrative process to promote innovation.

Next, we introduce several researchers who are breaking down the frontiers between basic and applied research, starting with those dedicated to finding novel ways to treat cancer. Our first researcher featured in this section is Dr. Varda Shoshan-Barmatz at the Ben-Gurion University of the Negev in Israel, who is unpicking the inner workings of cancer cells to find a way to make them self-destruct. She is applying her knowledge of mitochondria, the powerhouses of the cell, to find proteins that can control the fate of cancer cells. Her team’s work has led to the development of novel compounds for the treatment of skin cancer.

Cancer cells are hungry for energy, and determining how mitochondrial function drives their division, and growth of a tumour, is an important strategy for the development of novel therapies. At the University of Pennsylvania, Dr. Ian Blair and his team are leading the way in the new field of metabolomics, using biological biochemical markers to investigate cancer cell metabolism. This approach will allow the team to identify ‘finger prints’ of metabolites, produced during the process of metabolism, which can be used to identify and diagnose different disease states. This will provide knowledge of how cancer cells use energy, in order to target them for destruction.

Every cell in our bodies relies on the complex interconnecting network of vessels that forms the circulatory system, which carries the nutrients and chemicals required for metabolism. At Cornell University, Dr. Annarita Di Lorenzo and her team are investigating the function of the cells that line our blood vessels – endothelial cells – in order to better understand the causes of high blood pressure and cardiovascular disease, leading causes of death worldwide. Their ground-breaking research into special fats called sphingolipids is providing valuable insight into the biochemical processes that regulate the function of endothelial cells and how they might contribute to increased blood pressure and heart damage.

Meanwhile, Dr. Ashley Cowart at the Medical University of South Carolina is also working to understand the role of sphingolipids in health and disease. Here, we feature her team’s work exploring the function of sphingolipids in the formation of insulin resistance in diabetes, potentially opening up new treatment targets for people suffering from metabolic disease.

Our next scientists, Drs. Petra Kienesberger and Thomas Puliniikunnil are exploring the link between these two most deadly diseases – heart disease and diabetes – by working to unravel the processes of metabolism in the
cells of the heart. Their team at Dalhousie University have identified how the metabolism of cardiac cells changes in obese and diabetic patients, and how cells start to build up waste products that can cause tissue damage. Many diabetic and obese patients suffer from heart damage, and so understanding how metabolism and heart function are linked is of fundamental importance to treating this form of heart disease.

Disruptions to blood flow can be caused by the restriction of major blood vessels, due to the build-up of fatty plaques in a process known as atherosclerosis. This is particularly dangerous when the blood supply to the heart or brain is compromised, leading to irreversible damage and potentially a heart attack or stroke. Dr Gábor Csányi and his group at Augusta University are working to understand how the cells that line our blood vessels absorb fats such as cholesterol, leading to the formation of plaques. Identifying the key biomolecular players in this process is fundamentally important to identify new targets for the prevention and treatment of this devastating disease.

The function of the circulatory system is intricately linked with that of the lungs, which act to enrich blood with the oxygen needed for cell metabolism. The intricate branching pathways of our lungs can also become constricted, causing considerable distress as observed in those suffering from asthma. Dr Dale Tang and his team at Albany Medical College are investigating the mechanisms of disease progression in asthma, and are working to understand how the smooth muscle cells that line the airways increase in number and contract, causing airflow restriction. Unlocking these cellular mechanisms will contribute to the understanding of this distressing condition and allow novel treatments to be developed for the millions of chronic asthma sufferers across the globe.
The influenza pandemics of the early twentieth century before the widespread adoption of vaccination programs led to the deaths of millions of people worldwide. Influenza continues to be the most widespread respiratory disease in humans and it also increases mortality related to other conditions in vulnerable groups such as the elderly. Vaccines work by exposing people to one small portion of a virus so that the real thing can be recognised and destroyed by the immune system. However, the influenza virus is a slippery character that randomly mutates and changes at a rapid rate, meaning that vaccines often only remain effective for a short period before the virus changes so much that the immune system no longer recognises it. At the Icahn School of Medicine at Mount Sinai, Dr Peter Palese is taking a novel approach to developing a more effective influenza vaccine. Here, we describe how his team's universal flu vaccine will overcome many of the limitations faced by available vaccination approaches and, ultimately, provide long-lasting protection against seasonal and pandemic influenza.

Next, we move on to an even deadlier virus – HIV. Here, we have had the pleasure of speaking with Dr Salim Abdool Karim of the Centre for the AIDS Programme of Research in South Africa (CAPRISA), who tells us about the centre's ground-breaking work in preventing and treating HIV and AIDS. South Africa is currently in the midst of a devastating HIV epidemic, and there are an estimated 7 million people living with the disease in South Africa alone. Dr Karim discusses the cutting-edge research being carried out at the centre on developing a vaccine for HIV and other prevention technologies that could halt the spread of this life-threatening disease.

The ability of a virus to hijack the machinery of human cells is actually something we can use to our advantage. This is the goal of Dr Arun Srivastava and his team at the University of Florida, who are working to develop new gene therapy techniques to revolutionise the treatment of blood disorders, by harnessing the power of viruses – nature's own genetic modifiers. The team is adapting a relatively harmless human parvovirus, so that upon hijacking a human cell, it can produce proteins that can replace defective ones in human blood cells. In addition to working towards new treatments for blood disorders, the team’s approach is also opening up therapeutic opportunities to treat many other diseases, immunodeficiencies and even AIDS.

Moving from viruses to bacteria, our next researcher is working to prevent preterm births caused by bacterial infection and inflammation. Although it is the leading cause of death and disability in children under 5 years old, the rates of preterm birth in have remained static recent years and are particularly high in developing areas such as sub-Saharan Africa. Here, we introduce Dr Jeff Keelan and his colleagues at the Western Australian Preterm Birth Prevention Initiative, who are carrying out research to better understand how preterm birth is triggered, identify women at risk and prevent it from happening.

The development of antibiotics is one of the greatest advances in healthcare in the twentieth century and is something that we have come to take for granted. However, untreatable bacterial infections still account for millions of deaths each year and the antibiotic-resistance in many strains of bacteria is fast becoming a major cause for concern across the globe. At the University of Melbourne, Dr Roy Robins-Browne and his team are developing a novel strategy to combat these infections by unlocking the genetic and molecular mechanisms that allow bacteria to colonise a host. They hope to develop novel compounds that prevent bacteria from becoming harmful without actually killing them, making it much less likely for them to become resistant. This has the effect of rendering dangerous bacteria harmless, while also preventing the loss of the beneficial bacteria in our bodies.

Indeed, beneficial bacteria have recently taken centre stage in many research projects across the globe. This is because we exist within a symbiotic relationship with billions of bacteria that are vital for our good health. Next, we introduce Azim Munivar and Travis M Whitfill at Aztra Inc., who are using novel genetic techniques to work with a naturally occurring bacteria that safely inhabits human skin. Their aim is to manipulate these bacteria, considered beneficial to the immune system, to produce proteins that could provide safe and stable long-term treatments for skin conditions such as eczema. The team’s new technologies are currently on the way to clinical trials.
Comprising the heads of research funding agencies worldwide, the Global Research Council (GRC) is an organisation dedicated to fostering multilateral research and collaboration across continents to benefit both developing and developed nations. The GRC holds annual meetings to promote the sharing of data and best practices, and to facilitate strong collaboration between funding bodies. The 2017 meeting, held in May, was co-hosted and organised by the Natural Sciences and Engineering Research Council of Canada (NSERC), together with the Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica of Peru (CONCYTEC), in partnership with the International Development Research Centre (IDRC).

In this exclusive interview, we had the pleasure of speaking with Dr B. Mario Pinto, President of NSERC, who highlighted the key outcomes of the meeting. In particular, he discussed how to achieve gender equity and diversity in science, ways to assess research impact, and the importance of viewing basic and applied research as one dynamic ecosystem.
To start, please give us a brief introduction to NSERC, and tell us about your aims, vision and the types of research that you support.

NSERC is a federally funded organisation. It's one of the three major granting councils in Canada, and its mandate is to fund research in the natural sciences and engineering. At NSERC, we have a budget of $1.2 billion a year, and we fund about 11,500 professors, as well as 30,500 students and postdoctoral fellows, and we also work with about 3,700 industrial partners, who partner with our academics.

Our vision is very simple – to make Canada a country of discoverers and innovators, and that really is the aim of everything we do. We strive for research excellence, both on the discovery side and on the innovation side – and we don’t make a distinction between the two, because we do both of these activities as part of one ecosystem, and we have feedback loops between different sectors to optimise our research inventions if they should arise.

In terms of the types of research, NSERC covers everything in the natural sciences and engineering – including, engineering of different types, e.g., biomedical engineering, biomedical sciences involving cell biology, chemical biology, molecular biology, physiology, and physics, chemistry, biology, mathematical and statistical sciences, geosciences and computational sciences, etc. – so, it really is probably the broadest mandate of the granting councils.

The GRC selected NSERC, in partnership with the National Council of Science, Technology and Technological Innovation of Peru, to co-host the annual meeting in Ottawa. Please tell us about the purpose of this meeting and how it ties in with NSERC’s aims.

The GRC was founded in 2012 and hosts annual meetings. There are bids each year to host the next meeting. NSERC won the bid for this year, together with our co-host, Peru. The objective of all of these meetings is to bring the heads of research councils together to share best practices, develop policies that will guide us into the future, while admitting that we all have different strengths, and also different problems. It is critical that we learn from each other so that we don’t repeat mistakes of the past as we go forward together. In addition, this forum allows us to set up bilateral and multilateral collaborations that address some of the critical issues in research and innovation, and it’s very much a collaborative venture now.

If you want to accomplish something in a reasonable timeframe, generally speaking, collaboration is the way. Even on something very fundamental – as fundamental as quantum physics, for example – because there are bright minds all over the world, tapping into complementarities is really the productive way forward. So, the purpose of the GRC meeting is to bring together the heads of the research agencies. Together, we manage about 80% of the public research funds in the world. We have 70 partner countries on board, and it’s an attempt to provide some direction to our respective clienteles in different countries.

‘One should not obsess about the difference between basic and applied research. One should focus instead on doing excellent research.’
The meeting’s first session focused on the dynamic interplay between research and innovation. Please tell us what was discussed on this topic, and what creative ideas arose for boosting the translation of fundamental research findings to innovative new technologies.

This has been a big debate – some have been quick to put into boxes the fundamental research, on the one hand, and what people call ‘applied research’ or ‘innovation’, on the other hand. We feel that this particular categorisation has been restrictive and limiting.

So, what we discussed in great detail was this artificial distinction between fundamental research and applied research, and we pretty much agreed with each other. We also had a keynote lecture from an eminent scientist at Harvard, who supported this view that one should not obsess about the difference between basic and applied research. One should focus instead on doing excellent research and in fact, that’s how things come to pass. If there’s a potential for application, by all means, make it happen.

The picture I paint is based on a concept – R&D&D – popularised by Robert C. Dynes. It stands for research, development, and the second D stands for delivery – of impact, either societal impact, economic impact, or policy decision-making impact. We view R&D&D as an ecosystem in which there’s a two-way flow between all of those sectors, and, in fact, that’s how research takes place. One doesn’t worry about where one enters that ecosystem because we recognise that one goes back and forth through a continuous optimisation process. It’s not a pipeline, in which researchers send off a fundamental research idea to industry. That’s not how it works – it may have worked like that 50 years ago, but it certainly does not work like that now.

In the discovery space, we believe in training students in the art of critical and creative thinking, and invention comes out of discovery every once in a while. It can come about by conception, misconception or accident, as articulated by the late Derek Barton, a Nobel Prize winner. It is recognising the invention under each of those three scenarios that’s very important.

Commercialising inventions is extremely difficult, because you need knowledge of markets, you need knowledge of global value chains, supply chains, and you need capital to invest very carefully. In addition, you have to have a different set of standards (than research standards), and you have to be able to kill projects quickly, because you have to have a very different lens if you’re going to do innovation. You have to be hard-nosed, and you have to ask critical questions in the beginning and not at the end. That’s not necessarily in the academic nature. When doing innovation, you have to be focused on delivering on that second D (delivery), which is evaluated based on a different set of criteria. So, you have to bring in different players than just those in academia.

This is why NSERC forges partnerships with industry, so that at the outset university researchers can partner with people in industry who have knowledge of business practices, global markets, etc. R&D&D is the ecosystem that NSERC is trying to help develop, in which there’s continuous optimisation.

Do you think that the other funding bodies present at the meeting will derive some inspiration from NSERC’s approach to merging basic and applied research?

We came to that conclusion through the statement of principles, because it turns out that R&D&D is also a useful framework for making the case to governments. Should one invest in fundamental research? Should one invest in applied research? If other granting bodies can provide a construct like we have, in terms of R&D&D, which demonstrates that, naturally, all of the three endeavours are essential, but one focuses on impact, then the researchers also have a responsibility. They have to think about where their fundamental research may have some value. I was in Malaysia recently, and their government officials embraced this concept because, like many other governments, they were wrestling with this issue.

R&D&D was embraced by granting councils from other countries in the last two days, and I believe they will find it useful to convince their governments that they need to support fundamental research, but as part of an entire ecosystem. Because governments have the interests of the country in mind, they’re interested in economic and societal impact, and they’re interested in jobs, jobs, jobs, as we are here.

The second topic in the meeting focused on capacity-building and connectivity among granting agencies worldwide. Would you like to explain some of the ideas that arose during this meeting towards achieving these aims?

Yes, one of our priorities at NSERC is going global, and that’s why the format of the Global Research Council was of great interest to us, because it helps us to go global. But going global comes with responsibilities – to work with the developing world, to share best practices and to deal with global challenges.

This meeting has been very helpful, because we could share best practices in building capacity. For example, how does one manage effective peer review? It may be obvious to some, but others may be struggling with it. How does one build capacity to move towards gender equity in the long term? We discussed the issues of achieving gender equity and improving the status of women in the research enterprise in different countries. At the meeting, Ireland, a leader in the area, shared its practices that...
ensure gender equity with respect to faculty appointments. So, how can Global Research Council participants learn from these and other practices shared by other countries, to build capacity and adopt best practices to empower individuals in different countries to take charge of their own destiny?

So, what came out in terms of concrete actions here? I was very impressed that we focused the discussion on research impact, and it comes back to R&D&D. One of the things we discussed was, How does one assess research impact? Here we shared practices from some countries that are ahead of others in measuring research impact. Once again, Ireland is a leader in this area. They set up a two-level evaluation system for grant applications. The first evaluates the proposal and the candidate, as we all do. The second level consists of a separate panel who evaluates the impact; it is a rigorous system, but it involves a great deal of ‘person power’, and that’s their way of asking the question: What is the impact? In this system, evaluators are also realistic about the timeframe for different types of endeavours.

For example, quantum technology is going to take a long time, maybe 20 years, for application. Drug development also takes a long time, whereas digital technology development is much faster. This is an extremely useful practice to share, because I think talking to different stakeholders about realistic timeframes is where we’ve missed the boat.

Therefore, it is very useful for different countries to hear the value of educating all stakeholders about the different ‘runways’, as I put it, for the different fields of endeavour so that one manages expectations.

The other focus was based on one of the UN’s sustainable development goals: ‘No one left behind’. This is very important for embracing diversity, and for asking the question: How can we help each other in building international efforts of the future, and in bringing everyone together? Our representation this year from the sub-Saharan African countries was outstanding, as was that of the Latin American countries. It was just phenomenal. As we build the global research family, everyone gains familiarity, relaxes, engages in more informal talks, and feels included, and that is one of the goals of the Global Research Council.

Finally, on capacity-building, we also took away some valuable lessons in how we share data. We discussed open data. How do we address that on a global scale? How do we move forward on communication? How do we move forward on collaboration? How do we move forward on developing content? These were all part of the discussions about capacity-building.

Finally, what are the main outcomes of this meeting that you hope to witness in the coming year?

We’ll be moving forward on several important issues.

On gender equity, we’ve been very involved with developing practices for equity with respect to placement of women and under-represented groups in the academic workforce. So, we will be moving forward to develop more concrete proposals on how we can address those deficits.

In fact, we’ve already started. For example, a number of the GRC member granting councils, representing 22 countries of mainly European Union countries and the developing world, have begun a collaborative effort to do research, development and delivery (R&D&D) on best practices for water purification, security, distribution, policy-making, etc. We wish to pursue those types of linkages using very large existing programs. None of us has lots of money to spare, so we have to be circumspect and critical about what we invest in, but we’ve decided to tackle some very pressing global challenges.

Visit www.globalresearchcouncil.org for more information.
The Problem with Glioblastoma

For the majority of patients, it starts with frequent headaches that, as time progresses, are accompanied by bouts of nausea, vomiting and changes in vision. Many individuals go on to experience seizures, problems with speech, and shifts in mood and personality. Investigation by magnetic resonance imaging (MRI) then reveals a lesion or mass in the brain, leading to the fear-inducing diagnosis of a malignant tumour.

Glioblastoma is the most common form of brain cancer. It is highly aggressive and particularly difficult to treat, which is reflected in its survival rate. Approximately 88% of patients die within 14–36 months of diagnosis despite undergoing surgery, chemotherapy, targeted therapy and radiotherapy. This high mortality rate results largely from the tumour’s ability to resist treatment.

Heterogeneous in nature, a glioblastoma comprises a number of different cellular population subsets, one of which consists of glioma stem cells. These are self-renewing cells that have the potential to become cancerous. Typically, glioma stem cells remain dormant until activated and, as such, they are unaffected by conventional chemotherapies that target dividing cells. Like sleeper agents, they wait patiently until the onslaught is over, only then do they reawaken to re-establish the tumour. This covert action results largely from the tumour’s ability to resist treatment.

The Hallmarks of Cancer Development

A cell undergoes changes and acquires several traits that mediate its transformation from normal to malignant. These acquired traits, often referred to as the hallmarks of cancer, enable the cell to thrive when under normal circumstances it would die. Briefly, the cell gains the ability to produce its own growth factors and reprogram its metabolism to support persistent development. In addition, it adopts strategies that enable self-replication and evasion of signals instructing it to die. This uncontrolled growth leads to the formation of a mass. Once the mass reaches a certain size (approximately 2 mm) it induces the creation of blood vessels, which provide it with the nutrients and oxygen supply required to expand and invade into surrounding tissues. Given that these adaptations are central to cancer development, it is essential to understand the mechanisms involved in order to create potential strategies for treatment. In her research, Professor Shoshan-Barmatz ‘hijacks’ two important hallmarks of cancer development and survival, turning them against cancer cells.

The Mitochondrion – The Cellular Power Plant

Metabolic reprogramming and avoidance of cell death are two of several acquired traits of cancer cells that rely on the rewiring of an organelle called the mitochondrion. Mitochondria are housed in the cytoplasm, or in association with other organelles (for example, the endoplasmic reticulum), where they communicate with other cellular compartments via multiple activities. They play a fundamental role in metabolising different substrates to produce chemical energy, in the form of the energy-carrying molecule adenosine triphosphate (ATP), which the cell requires to carry out normal processes. Mitochondria are also involved in a number of other functions, including the synthesis of many compounds, such as cholesterol, regulation of the cell redox state, calcium homeostasis, cell signalling events, inter-organelle communication, cell proliferation, cellular ageing, and disease development. As cancer cells possess reprogrammed metabolism including that of the mitochondria, Professor...
Shoshan-Barmatz explored a new code for reprogramming cancer cell metabolism, thus reversing tumour oncogenic properties.

In addition to serving as the cell’s powerhouse, mitochondria also play a pivotal role in regulating programmed cell death, commonly referred to as apoptosis. This is an evolutionarily conserved and genetically regulated process that begins during embryonic development, which maintains homeostasis within body tissues throughout adulthood. Apoptosis allows the efficient removal of unnecessary or menacing cells. Consequently, deficiencies in the regulation of apoptosis are linked to numerous diseases, including neuronal degenerative diseases, tumorigenesis, autoimmune disorders, and viral infections. In cancer, resistance to apoptosis contributes not only to tumour development, but also to resistance to conventional anticancer therapies, such as radiation and chemotherapy. In her research, Professor Shoshan-Barmatz discovered a new mechanism for activating apoptosis and developed novel molecules to activate this mechanism as a strategy for treating cancer.

VDAC1 – A Novel Molecular Target

The search for new treatments to tackle the cancer heavyweights such as glioblastoma has prompted the research of Professor Shoshan-Barmatz, at Ben-Gurion University of the Negev in Israel, noted for her work on the mitochondrial protein voltage-dependent anion channel (VDAC) 1, as a definitive therapeutic target in cancers such as glioblastoma, liver cancer and others.

VDAC1, a protein that resides within the mitochondrial outer membrane, is a beta barrel protein containing a large pore that enables the movement of metabolites and ions into the mitochondrion from the cytoplasm and vice versa. As the gatekeeper of the mitochondrion, VDAC1 assumes a crucial position in the cell, serving as the main interface between mitochondrial and cellular metabolisms, and controlling cross-talk between mitochondria and the rest of the cell. Consequently, VDAC1 is responsible for regulating mitochondrial energy production and maintaining metabolism. VDAC1 is over-expressed in cancer cells. Professor Shoshan-Barmatz has shown that it is required for their development and survival. VDAC1 has also been recognised as a key protein in mitochondria-mediated apoptosis by assisting in the release of inter-membranal apoptotic proteins from the mitochondrion into the cytosol and due to its association with pro- and anti-apoptotic proteins. The expression levels of VDAC1 are increased in many cancers, including breast, lung and glioblastoma. Thus, VDAC1 is emerging as a promising target for controlling apoptosis.

Importantly, VDAC1 serves as a hub protein, interacting with diverse sets of cytosolic, endoplasmic reticulum, mitochondrial and other proteins that together regulate cell survival and cellular death pathways. The most notable of these proteins belong to the family of hexokinases; important enzymes that catalyse the first step of glycolysis, the process of metabolising glucose. One of the signature characteristics of highly malignant, poorly differentiated tumours is their high rate of glycolysis, a property that is frequently dependent on the marked over-expression of VDAC1-bound hexokinase in cancer cells. Subsequently, the glucose consumption of cancer cells is greater than that of non-malignant cells, a characteristic that is the basis for the PET scan. Recently, however, there has been a shift in the understanding of the role that interaction between hexokinases and VDAC1 plays in mitochondrial function. Two hexokinases, hexokinase-1 (HK-1) and hexokinase-2 (HK-2) are overexpressed in cancers and by interacting with VDAC1 they gain direct access to the mitochondrion generated ATP, thus enhancing glucose metabolism. Interestingly, HK-1 and HK-2 interaction with VDAC1 also impedes apoptosis and it is this relationship that may be instrumental in mediating not only how cancer cells reprogram metabolism to gain the energy they require to grow, but also how they acquire the ability to evade cell death. It is suggested that the increases in VDAC1 and HK-1 and HK-2 expression levels in cancer contribute to the acquisition of two of the hallmarks of cancer: reprogrammed metabolism and evasion of apoptosis. Professor Shoshan-Barmatz identified the
Novel Strategies for Cancer Therapies

Further research by Professor Shoshan-Barmatz and her team has led to emergence of several potential strategies for cancer therapy. The first, involves the screening, identification and development of several novel, patent protected small molecules that can activate the pro-apoptotic activities of the protein. By targeting VDAC1-bound HK-1 and HK-2, and VDAC1-mediated apoptosis, these small molecules are able to bring about cell death. These molecules led to the establishment of a company, ViDAC Pharma, Ltd., that is currently carrying out a phase II clinical study on non-melanoma skin cancers, and treatments for other cancers are under development.

A second strategy involves VDAC1-based peptides, a chain of amino acids derived from VDAC1 sequence serving as the binding sites for HK-1, HK-2 and other anti-apoptotic proteins, which impair energy homeostasis and minimise the anti-apoptosis self-defence mechanisms of cancer cells. To date, over 40 versions of cell penetrating VDAC1-based peptides have been designed and screened, and the three shortest, most stable, and effective at inducing cell death in cancer cell lines but not in non-cancerous cells have been identified. The peptides were tested in mouse models of cancer and were found to prevent energy production in the tumour, inhibit cell proliferation and invasion, induce cell death, including of cancer stem cells from which the cancer re-develops. This multi-pronged attack on tumours was obtained regardless of the cancer type and mutation status, with perceived specificity towards only cancerous cells.

Investigations with these peptides have been conducted in animal models of lung, breast, and liver tumours, all showing equal success in inhibiting both tumour growth and the metastasis of melanoma to the lung or the brain. Favourable results, including dramatic cell death induced by the peptides in B-cell chronic lymphocytic leukaemia (CLL), and selective killing of lymphocytes obtained from CLL patients, have been obtained, while sparing those obtained from healthy donors. This work was prompted by a prestigious three-year award from the American Leukemia & Lymphoma Society.

Professor Shoshan-Barmatz and her team have more recently demonstrated the ability of the engineered VDAC1-based peptide, Tf-D-LP4, to effectively induce cancer cell death in a panel of genetically characterised glioblastoma and glioblastoma-derived stem cell lines and in animal models of human glioblastoma. Ultimately, the Tf-D-LP4 peptide was able to cross the blood-brain barrier and launch a multi-pronged attack, disrupting glioblastoma cell metabolism, dramatically reducing intra-cranial tumour growth, invasiveness, proliferation and, importantly, increasing mouse survival by over 5 months (equivalent to several years in humans) even after treatment had ended. Thus, the Tf-D-LP4 peptide offers an innovative therapeutic strategy that is capable of preventing the replication of not only cancer cells, but also glioma stem cells in the tumour, thereby preventing tumour regrowth and the relapse of the patient.

A third strategy is based on the overexpression of VDAC1 in cancers and involves the silencing of VDAC1 expression using molecules called small interfering ribonucleic acids (siRNAs). Rather than using molecules to interfere with cellular metabolism and activate cell death pathways at the protein level, Professor Shoshan-Barmatz’s team have shifted their attention to disabling it at the source. Deoxyribonucleic acid (DNA) is the genetic blueprint from which all proteins are constructed. In a series of well-orchestrated steps, genes are transcribed, or copied, into messenger RNA (mRNA), which is subsequently translated into proteins. Therefore, if this process is interrupted at any point, the protein will not be produced and all cellular functions associated with that protein would be affected. Specific siRNAs (modified for stability) have been developed that can interrupt the translation of the VDAC1 protein from its mRNA template. Research has shown that this results in the depletion of VDAC1 within the cell, thereby inhibiting growth, disrupting energy production, and disabling the abnormal metabolic behaviour of these cancer cells. Research using a cervical cancer, lung cancer, and a glioblastoma mouse human xenograft model, showed that siRNA-VDAC1 inhibited tumour growth and caused regression of established tumours.

Using glioblastoma as a platform for ‘proof of concept’, the potentiality of siRNAs was tested in several glioblastoma cell lines and patient-derived cells, and subcutaneous or intracranial-orthotopic glioblastoma xenograft mouse models. Silencing of VDAC1 expression resulted in significant inhibition of tumour development. When encapsulated within a nanoparticle, siRNA was able to reach the brain, where it reversed cancer-induced metabolic reprogramming, preventing the proliferation of cancer cells, and transformed glioma stem cells, the perpetrators of disease recurrence, into normal, non-proliferating neuronal-like brain cells.

Implications of VDAC1-Based Therapeutics

Professor Shoshan-Barmatz’s work has demonstrated that VDAC1 plays a fundamental role in mediating how cancer cells acquire the metabolic and apoptotic adaptations necessary for development and progression. These findings represent a major breakthrough in the development of anti-cancer strategies that are capable of simultaneously targeting numerous hallmarks of cancer development. The results implicate VDAC1 as a significant control point in oncology and thus, an emerging cancer drug target with enormous potential. Professor Shoshan-Barmatz’s new anti-cancer agents have broad therapeutic impact and are expected to result in huge clinical benefits, not only for glioblastoma, but for other cancers as well.
Meet the researcher

Professor Varda Shoshan-Barmatz
Department of Life Sciences
Ben-Gurion University
Beer-Sheva, Israel

Varda Shoshan-Barmatz is a full Professor in the Department of Life Sciences, Ben-Gurion University (BGU), and the Hyman-Kreitman Chair in Bioenergetics. She received her PhD in Biochemistry from the Weizmann Institute of Science in Israel. She went on to carry out post-doctoral research at the University of Wisconsin-Madison in the USA and Toronto University in Canada. Since 1982, Professor Shoshan-Barmatz has been a faculty member of the Department of Life Sciences at Ben-Gurion University in Israel, where she was promoted to full Professor in 1995 and served as Chair (2000–2004). Professor Shoshan-Barmatz was founder of the National Institute for Biotechnology in the Negev (NIBN), served as Deputy Director (2004–2006) and Director (2006–2015). She is the Principal Investigator on 40 research grants and has authored over 140 articles and reviews. Her work on VDAC1 formed the basis for the establishment of a company, ViDAC Pharma, and she holds 13 patents on novel strategies for human cancer therapies and biomarkers. Over the course of her career, Professor Shoshan-Barmatz has mentored over 60 graduate students and 14 post-doctoral fellows. In addition, she has served on a number of granting committees and has been an ad-hoc reviewer for 16 international scientific journals. Professor Shoshan-Barmatz has earned prestigious awards for her contributions to science, including the Hestrin Prize for Excellence in Biochemical Research, a Teva Research Award for Young Scientists (1993) and Teva Founders Award in recognition of pioneering scientific achievements in cancer treatment research (2016). She was selected by the Lady Globes Journal as one of the 50 most influential women in Israel (2009) and the five women who made breakthroughs in science in Israel (2016). She has been nominated twice by BGU and the city of Beer-Sheva for the Excellence in Teaching Award and Excellent Scientist Award, respectively.

CONTACT

E: vardasb@bgu.ac.il
T: (+972) 864 61336
W: http://in.bgu.ac.il/en/natural_science/LifeSciences/Pages/staff/Varda_Shoshan-Barmatz.aspx

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Over the past decade, there has been much progress in the development of metabolomics, one of the key ‘omics’ sciences (along with genomics, proteomics, transcriptomics and others), that are revolutionising biology and medicine. Metabolomics uses cutting-edge analytical chemistry techniques to characterise repertoires of small molecules present in cells and tissues, known as metabolites. Metabolism – the vast, complex network of chemical reactions that occurs within living systems – involves the conversion of thousands of metabolites, each reaction being catalysed by specific enzymes. This vital web of metabolic pathways is what keeps us alive, although occasionally things go wrong, and the promotion or inhibition of certain pathways can result in disease. Elucidating the metabolic basis of disease states has become a lively field of study over the past few decades. In particular, the ‘metabolic reprogramming’ that occurs in cancer has been implicated in disease pathogenesis and progression.

While metabolomics may be considered a posterchild of 21st century science, its roots are historic. Over a century ago, Sir Archibald Garrod (1857–1936), a British physician, was the first to link metabolism with disease, and coined the term ‘inborn errors of metabolism’, based on his studies on alkaptonuria (black urine disease). Since then, a plethora of analytical methods and computational tools have emerged to characterise the vast array of metabolites in biological tissues. The three major workhorses of modern metabolomics that have developed in the last 15 years are nuclear magnetic resonance (NMR) spectroscopy, gas chromatography mass spectrometry (GC-MS) and liquid chromatography MS (LC-MS). NMR discriminates between metabolites’ proton resonance spectra, whereas mass spectrometry differentiates qualitatively based on metabolites’ size and structure. Appropriate validation and quality control samples are needed to provide adequate rigor. Other technologies commonly used include magnetic resonance spectroscopy (MRS), positron emission tomography (PET), matrix-assisted laser desorption/ionisation (MALDI)-MS, secondary ion MS (SIMS) and desorption electrospray ionisation MS (DESI-MS) techniques.

Professor Ian Blair of the University of Pennsylvania is an eminent authority within the emerging field of metabolomics. His research group focuses on the development of metabolomics methodologies, for biomarker analysis and validation, probing the metabolic basis of a number of diseases, particularly cancer, and investigating the impact of drug candidates on target pathways.

Biomarker Analysis and Validation

A major component of metabolomics research is the analysis of low molecular weight biomarkers. Biomarkers are chemical, physical or physiological indicators of a disease that can be detected and quantified for disease diagnosis and prognosis and to inform treatment. Robust biomarkers are needed for effective screening, surveillance and primary prevention of disease states. Biomarkers can be molecular such as proteins, carbohydrates, small molecule metabolites, or genetic, such as gene variants (alleles) or mutations that indicate the presence and severity of a disease.

The use of protein biomarkers has been developed in recent years, though these are sometimes inadequate. For instance, serum prostate antigen (PSA) – the standard biomarker for monitoring prostate cancer treatment – is notorious for having low specificity and sensitivity. In fact, its use in prostate cancer screening is controversial and not recommended. Likewise, no clinically useful biomarkers exist for colorectal cancer,
with colonoscopies being standard for diagnosis. Similarly, for pancreatic cancer, the most useful biomarker, CA19–5 antigen found in sera, is also elevated in non-malignant jaundice and liver cirrhosis. While protein-based biomarkers are commonly used for cancer screening and surveillance, the presence of these proteins in non-diseased states and other conditions compromises sensitivity and specificity. In contrast, metabolite spectral ‘fingerprints’ tend to be unique to a disease state. There is increasing interest in the use of metabolomics-based biomarkers for disease states. Professor Blair’s group is developing and validating methods for analysis of oestrogens in the sera of postmenopausal women using liquid chromatography-tandem mass spectrometry (LC-MS/MS), as an early-stage biomarker of breast cancer risk.

A major part of Professor Blair’s focus is the development of metabolic biomarkers for diseases. One such disease that his team are studying is Friedreich’s ataxia, a recessive inherited disease that causes progressive damage to the nervous system. Friedreich’s ataxia is caused by a mutation in frataxin, a small protein required for iron-sulphur protein assembly in the mitochondria. Unfortunately, the inability to access the highly affected neuronal and cardiac tissues has hampered metabolic evaluation and biomarker development. As a metabolic biomarker, the team developed a metabolic assay based on incorporating 13C labelled glucose into acetyl-CoA and succinyl-CoA, metabolites used in the TCA cycle, in patient-derived platelets. Successful incorporation is indicative of functional aconitase, an iron-sulphur cluster-based enzyme, which requires functional frataxin to work.

The selection of in vivo cells or tissues for metabolomics analysis is important. A wide variety of biological specimens have been used including urine, faeces, blood, saliva, cerebrospinal fluid and exhaled breath condensate. Biological samples are fairly messy and often need to be ‘cleaned’ prior to MS or NMR.

Mitochondrial Dysfunction in Diseases and Therapeutic Approaches

As well as using metabolomics for disease diagnosis, Professor Blair’s research group also targets metabolism for disease therapy, especially for cancer. Tumour cells are known to have altered metabolism compared to healthy cells. Respiration, the metabolic ‘burning’ of food molecules to release vital energy is an essential feature of all living cells, including diseased and tumour cells. A particular focus of Professor Blair’s team is targeting respiration in abnormal cells, either for disease therapy or to gain mechanistic insights into the respiratory aberrations that are often part of disease pathogenesis, particularly in cancer and neuronal defects. Moreover, fast-growing tumour cells usually have high respiratory activities and lactate accumulation due to high levels of inefficient anaerobic respiration based on glycolysis (the so-called ‘Warburg Effect’).

A brief explanation of respiration is necessary here. Sugars and fats are progressively oxidised to release energy, in the form of ATP (the ‘energy molecule’). Respiration begins with glycolysis in the cytoplasm, and continues in the mitochondria. In glycolysis, glucose, a sugar molecule with 6 carbon atoms (6C) is split into two pyruvate molecules (3C). Pyruvate then diffuses into the mitochondria and is further oxidised and decarboxylated (a carbon atom is removed, forming CO2) to make acetyl-CoA. In the mitochondria, pyruvate enters the tricarboxylic acid (TCA) cycle (also known as the citric acid cycle). In this cycle, acetyl-CoA (2C) combines with oxaloacetate (4C) to form citrate (6C). Citrate undergoes a sequence of reactions, involving two decarboxylations.
Ultimately oxaloacetate is reformed and the cycle continues. During both glycolysis and the TCA cycle, NAD+ is reduced to NADH, which means that it gains electrons. The electron gained by NADH is then shuttled into the electron transport chain (ETC). During this, the electron races through three mitochondrial membrane complexes before being accepted by oxygen to form water. The ETC process generates a H+ (proton) gradient, which drives the formation of ATP. ATP is also formed during glycolysis and the TCA cycle. This high-energy molecule then goes on to deliver vital energy to the cell.

Professor Blair and his team have investigated a number of compounds that are found to de-rail respiration in abnormal cells, either as causative agents of disease or as potential therapies. One particular compound of interest is rotenone – a broad-spectrum pesticide – which has been implicated in Parkinson’s disease in humans and animals. Farmers routinely exposed to rotenone were found to have a 2.5-fold greater risk of developing Parkinson’s disease. While the associations of pesticides and human health is nothing new (think of the DDT scandal), rotenone has largely slipped the public radar. Indeed, rotenone is often used in ‘organic’ gardening to kill beetle pests (due to its natural occurrence) and by US government agencies to kill fish in lakes and rivers, though its use in conventional farming is being phased out.

Professor Blair has conducted a number of studies to elucidate the potential mechanisms of rotenone in mitochondrial dysfunction, which is thought to play an important role in Parkinson’s disease. Rotenone is known to inhibit mitochondrial complex 1 of the ETC, blocking electron flow. The group’s previous research found that even at low levels, rotenone reduces levels of succinyl-CoA, indicating that rotenone inhibits glucose-derived glycolysis. In the absence of carbohydrate-derived sugars, cells often carry out beta-oxidation of fatty acids to synthesise acetyl-CoA to feed the TCA cycle. Recent research by the team found that with rotenone’s inhibition of glucose-mediated glycolysis, cells are more susceptible to carry out beta-oxidation of fatty acids as a compensatory pathway. This finding is significant as disruptions to these compensatory pathways in neurons may contribute to cell death in Parkinson’s disease.

The inhibition of glycolysis and mitochondrial metabolism have been proposed as novel approaches to anti-cancer treatments. Lonidamine (LND), an anti-tumour drug, has been used in combination with other therapeutic agents to improve the efficacy and response to cancer treatment. LND is known to block respiratory pathways in cancer cells, and one of Professor Blair’s priorities is to elucidate the mechanisms behind this. Recently, his group determined that LND targets mitochondrial complex II of the ETC, and also induces formation of toxic reactive oxygen species (ROS) through complex II, inducing cell death in DB1 human melanoma cell lines. Moreover, recent research by the group suggests that LND also inhibits pyruvate uptake of the mitochondria and lactate efflux from cells. As lactate accumulation is toxic to cells, this may offer another mechanism for tumour cell killing.

Metabolomics – The Way Forward

Metabolomics is an emerging field, and there are a number of challenges that it must face. The development of robust sample collection and preparation procedures, with the elimination of sample variability and bias, is of utmost importance, as is the validation and quantification of biomarkers. As a key ‘omics’ science, metabolomics should not be used in isolation, but as part of a suite of multi-omics, including genomics, proteomics and lipidomics. Indeed, BluePen Biomarkers, a comprehensive multi-omics biomarker discovery and validation company co-founded by Professor Blair does just that. It is hoped that this synergistic approach will lead to the discovery of clinically-relevant biomarkers against a range of diseases.

Finally, metabolomics, as a discipline, should become both more mechanistically- and therapeutically-oriented. Changes in metabolite spectra can speak volumes about disease pathogenesis as well as inform treatment modality. Biomarker discovery, disease pathogenesis and therapeutic modalities that specifically target aberrant metabolism should not be considered in isolation, but should be addressed together. And the Blair Lab provides a rare and exemplary illustration of this approach.
Meet the researcher

Professor Ian A. Blair
Center for Cancer Pharmacology
University of Pennsylvania
Philadelphia, USA

Professor Ian Blair completed his BSc in Chemistry at the University of London in 1968, and went on to gain a PhD in Organic Chemistry in 1971, under the tutelage of Nobel Laureate Sir Derek HR Barton. He has since had an illustrious career as a lecturer and researcher in a number of institutions, including Makerere University, Uganda, Australian National University, Adelaide University, Australia, Vanderbilt University, Nashville, Tennessee, and University of Pennsylvania – his current institution. He is currently the vice-chair of the Department of Systems Pharmacology and Translational Therapeutics, and Director of the Translational Biomarker Core, Center of Excellence in Environmental Toxicology, at the University of Pennsylvania. His research is focused on the metabolic characteristics of disease states, and his pet research interests include the use of MS for ‘omics’ and DNA analysis, oxidative stress, lipidomics and biomarker discovery and validation.

CONTACT
E: ianblair@upenn.edu
T: (+1) 215 573 9885
W: https://www.med.upenn.edu/blairlab/faculty_blair.shtml

KEY COLLABORATORS
Garret A. FitzGerald, MD
Trevor M Penning, PhD
Clementina Mesaros, PhD
David R. Lynch, MD, PhD
Jerry D. Glickson, PhD
Anil Vachani, MD
Tilo Grosser, MD
Nathaniel W. Snyder, PhD
Wei-Ting Hwang, PhD

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**SPHINGOLIPIDS: FATS THAT PROTECT THE HEART**

Despite current therapies, cardiovascular disease is a leading cause of death worldwide, suggesting that alternative drug targets are urgently needed to preserve cardiovascular health. Sphingolipids – a class of biologically active molecules and components of cell membranes – are a potential target, but how their synthesis is regulated in cardiac disease remains an elusive mystery. Dr Annarita Di Lorenzo and her group at Weill Cornell Medicine have begun to elucidate the molecular mechanisms regulating this pathway, which may lead to novel therapeutic treatment of cardiovascular diseases.

**The Need for Alternative Targets in Cardiovascular Disease**

Cardiovascular diseases represent a huge global disease burden, and are a leading cause of death worldwide – approximately 63% of global deaths are due to non-transmissible diseases, with half of those attributed to cardiovascular diseases (30%). One of the major risks factors for cardiovascular disease is high blood pressure or hypertension. Hypertension causes approximately 7.5 million global deaths annually and nearly 1 billion people are estimated to be affected. In addition, it has been linked to a higher risk of coronary heart disease and stroke. Despite the use of drugs to control hypertension, it still remains one of the most common causes of heart failure, kidney impairment and stroke, suggesting alternative drug targets are needed for therapeutic treatment.

Physiologically, the dysfunction of the endothelium, which is made up of endothelial cells that line the inner surface of blood and lymphatic vessels, has been linked to hypertension. In healthy individuals, the endothelium is a complex system that is crucial for the maintenance of vascular homeostasis, functioning through cell signalling in response to various physical, biological and chemical stimuli. Moreover, endothelial cell dysfunction is an early event contributing to the disruption of vascular tone, or the partial contraction of the arteries to maintain blood flow, and thus, regulate blood pressure.

*Sphingolipids – Bioactive Molecules with a Role in Cardiovascular Dysfunction*

In terms of biological stimuli, endothelium-released nitric oxide is known to be crucial for the regulation of blood pressure. However, sphingolipids are also emerging as biologically active molecules with cardiovascular effects, including blood pressure control. Sphingolipids are a class of lipids with a characteristic 18-carbon amino-alcohol backbone referred to as the sphingoid base or sphingosine. First discovered from brain extracts in the 1870s, they were named after the mythological Sphinx because of their enigmatic nature. Their complexity arises from the various chemical groups that can be attached to sphingosines. The most well-characterised sphingolipid is sphingosine-1-phosphate, created by adding a phosphate chemical group to sphingosine.

Functionally, sphingolipids are essential structural components of the cell membrane, as well as being bioactive signalling molecules. They are involved in many cellular processes such as cell stress responses, cell survival, differentiation, migration and inflammation. They are synthesised through the sphingolipid de novo (from scratch) biosynthesis pathway in the endoplasmic reticulum – the largest organelle in eukaryotic cells. Although the synthetic pathway is well-defined, little is known about how this pathway is regulated.
during physiological or hypertensive conditions. Hence, Dr Annarita Di Lorenzo and her research group sought to uncover this mystery.

Dr Di Lorenzo’s research on endothelial cell dysfunction first began during her PhD at the University of Naples, Italy. Building on her PhD training, she joined the lab of Dr William Sessa at Yale Medical School for her postdoctoral training in 2006, where she continued to examine the mechanisms of endothelial cell dysfunction. One line of research led her to study Nogo-B – a membrane protein highly expressed in the walls of blood vessels. This study unveiled an important role of Nogo-B in blood vessels in the development of inflammation. However, how Nogo-B influenced the onset of inflammation was still unknown.

The research in her laboratory at Weill Cornell eventually led to the major discovery that Nogo-B is a negative regulator of sphingolipid biosynthesis, which has implications for cardiovascular functions and treatments.

**Nogo-B as a Therapeutic Target to Control Hypertension**

Through a series of experiments *in vitro* and *in vivo*, Dr Di Lorenzo and her research team at Weill Cornell Medicine demonstrated that Nogo-B negatively regulates sphingolipid de novo synthesis by inhibiting serine palmitoyltransferase (SPT) – the first and rate limiting enzyme of the sphingolipid synthesis pathway. The Nogo-B protein has two functionally similar variants, called Nogo-A and Nogo-C, which are abundantly expressed in the central nervous system, and Nogo-C is also found in skeletal muscle. On the other hand, Nogo-B is highly (but not exclusively) expressed in endothelial cells. Specifically, Dr Di Lorenzo and her colleagues found that mice without Nogo-A and Nogo-B variants (also known as Nogo-A/B-deficient mice), had markedly lower blood pressure compared to control (or wild-type) mice. Since nitric oxide lowers blood pressure, not surprisingly, Dr Di Lorenzo also found that nitric oxide production was notably increased in the blood and in mesenteric arteries (arteries that drain from the aorta) of Nogo-A/B-deficient mice compared to controls. Therefore, Nogo-B may be responsible for activating endothelial nitric oxide synthase (NOS) – the enzyme that catalyses the formation of nitric oxide – to increase nitric oxide production and alter blood pressure.

Since the locations of Nogo-B and endothelial NOS in the cells were different, Dr Di Lorenzo and her group hypothesised that these two proteins did not interact directly. Rather, they believed that Nogo-B was likely to regulate sphingolipid synthesis, as one of the sphingolipids, S1P, is known to be vastly important in controlling blood vessel function. Indeed, Nogo-B was found to interact with SPTLC1 – a subunit of the SPT enzyme – in cells from mouse lungs and lung lysates (disintegrated lung cells), suggesting an interaction between these proteins. In addition, sphingosine-1-phosphate (S1P) was suppressed in both Nogo-A/B-deficient and control cells isolated from mouse lung when SPT – the rate-limiting enzyme of sphingosine synthesis – was inhibited by the compound myriocin. These findings indicate that Nogo-B also exerts its effects through inhibiting SPT. Thus, in the absence of Nogo-B, the inhibition of SPT is relieved, and sphingolipid and nitric oxide production in the cell is increased, thereby lowering blood pressure.

Most importantly, Dr Di Lorenzo and her group found that Nogo-B mediated vascular dysfunction in a mammalian model of hypertension. When Nogo-A/B-deficient mice were continuously infused with the peptide hormone angiotensin II, wild-type mice showed sustained levels of hypertension. In contrast, it only induced a mild increase in blood pressure in Nogo-A/B-deficient mice. Not only do these findings indicate that Nogo-B inhibits SPT, leading to higher blood pressure, it also demonstrates that Nogo-B, SPT and the sphingolipid synthesis pathway can be used as novel pharmacological targets to regulate blood pressure.

**Nogo-B-mediated inhibition of sphingolipid biosynthesis plays an important role in the pathogenesis of hypertension, heart failure and inflammation.**
Can Inhibiting Nogo-B Protect the Heart Against Cardiac Dysfunction?

Apart from hypertension, cardiac hypertrophy – an increase in the mass of the heart due to pathological stimuli – can also lead to cardiac dysfunction. Cardiac hypertrophy can be induced by pressure overload, but not all hypertrophy is considered detrimental. The mechanism behind adaptive and pathological hypertrophy remains poorly understood. To this end, Dr Di Lorenzo and her group found that Nogo-B regulated sphingolipid synthesis within the endothelium to protect the heart in a mouse model of cardiac failure. The team established this mouse model by surgically tightening the aortas in the mice – a procedure called transverse aortic constriction (TAC). In sham-operated mice, who underwent ‘placebo surgery’ so they could be used as an accurate control, nylon string was only tied loosely around their aortas. Following TAC, the control mice developed cardiac hypertrophy after 2 weeks, with a 2-fold increase in heart size reached at 3 months. In contrast, the heart size of Nogo-A/B-deficient mice was reduced compared to sham-operated controls and in mice at 2 weeks and 3 months post-TAC. The absence of Nogo-A/B did not greatly alter the magnitude of hypertrophy. However, the cross-sectional areas of cardiac muscle cells in Nogo-A/B-deficient mice were significantly smaller than those of the control TAC-operated mice. Moreover, ultrasound results revealed that Nogo-A/B deficiency protected the heart from failure 3 months post-TAC.

Cardiac inflammation is intimately linked to hypertension and scarring, which can compromise the heart’s pumping capacity and oxygen diffusion to hypertrophied cardiac tissue. Notably, Dr Di Lorenzo’s team found that the Nogo-A/B-deficient mice were resistant to pressure-induced inflammation and scarring at 2 weeks and 3 months following TAC. Mice lacking Nogo-B specifically in endothelial cells were also similarly resistant to pressure-induced vascular permeability and inflammation compared to control mice. Cardiac function was also preserved in these mice 3 months after TAC. These findings suggest that the loss of Nogo-A/B may sustain cardiac muscle function in response to long-term pressure overload by maintaining cardiac cell function and reducing vascular permeability and inflammation.

Next, Dr Di Lorenzo and her group investigated whether SPT activity was altered following TAC. As expected, in the absence of Nogo-A/B, SPT activity was notably increased in sham-operated hearts and in hearts 3 days post-TAC. Critically, when SPT activity was inhibited with myriocin 1 day before, on the day of and 2 days following TAC, vascular permeability and inflammation from pressure overload in Nogo-A/B-deficient mice were elevated and returned to control levels compared to wild-type mice. Further ultrasound tests revealed that the heart in these Nogo-A/B-deficient mice were no longer protected – in fact, cardiac dysfunction had been re-established to wild-type levels. Taken together, the inhibition of SPT mediated by Nogo-B plays a crucial role in pressure overload-induced cardiac dysfunction. Therefore, Nogo-B and the sphingolipid synthetic pathway may also be targeted to protect the heart from long-term cardiac dysfunction.

Future Perspectives

Although the sphingolipid synthesis pathway had previously been well-defined, limited was known about its effects on blood pressure and the mechanisms underlying its regulation in physiological and hypertensive conditions. In view of this, the innovative work of Dr Di Lorenzo and her team has shed light on the crucial regulatory role of Nogo-B-mediated sphingolipid synthesis in hypertension and pressure overload-induced cardiac hypertrophy. Specifically, her team found that Nogo-B is a negative regulator of SPT and subsequent decrease in sphingolipid synthesis leads to an increase in blood pressure. Nogo-B also regulates cardiac cell function and its absence protects the heart from dysfunction in response to long-term pressure overload. These observations also suggest that sphingolipid synthesis may play a causative role in development of cardiovascular diseases.

Nevertheless, some open questions about the regulation of sphingolipid synthesis remain. For example, our understanding of the molecular mechanisms relieving the inhibitory actions of Nogo-B on SPT and the structure of SPT is currently incomplete. And what about the role of SPT and sphingolipid synthesis in other diseases? The research of Dr Di Lorenzo and her group forms a solid framework to investigate the role of sphingolipid synthesis in other pathological conditions associated with lack of Nogo-B, such as asthma, liver scarring and inflammation. The team’s seminal findings also provide compelling evidence that Nogo-B and the sphingolipid synthesis pathway are promising therapeutic targets for the treatment of hypertension and cardiac hypertrophy. Given the involvement of sphingolipids in numerous cellular processes, it would not be surprising for Dr Di Lorenzo’s team to discover that modulating this pathway may impact therapeutic treatment of many human diseases in the near future.
Meet the researcher

Dr Annarita Di Lorenzo
Department of Pathology and Laboratory Medicine
Weill Cornell Medicine
Cornell University
New York, NY
USA

Dr Annarita Di Lorenzo received her PhD in pharmacology and vascular biology in 2004 under the supervision of Drs Maria Rosaria Bucci and Giuseppe Cirino at the University of Naples ‘Federico II’ Italy. She went on to complete her postdoctoral training at Yale Medical School in the United States between 2006 and 2011. Subsequently, she established her own laboratory in the Center of Vascular Biology directed by Dr Timothy Hla, in Department of Pathology and Laboratory Medicine at Weill Cornell Medical College, where she studies the role of sphingolipid signalling in cardiovascular function and disease. Her research has been published in high profile journals including Science Signalling, PNAS, Hypertension and Nature Medicine. She is also a member of the Editorial Advisory Board of the Journal of Pharmacology and Experimental Therapeutics.

CONTACT
E: and2039@med.cornell.edu
T: (+1) 212 746 6476
W: http://vivo.med.cornell.edu/display/cwid-and2039

KEY COLLABORATORS
Dr Timothy Hla, Harvard Medical School, Boston, USA
Dr William Holland, UT Southwestern Medical Center, Dallas, USA
Dr Bodo Levkau, University Hospital Essen, Germany
Dr Giuseppe Faraco, Weill Cornell Medicine, New York, USA
Dr Teresa Sanchez, Weill Cornell Medicine, New York, USA
Dr Costantino Iadecola, Weill Cornell Medicine, New York, USA
Dr Maria Rosaria Bucci, University of Naples ‘Federico II’, Italy

Dr Luigi Gnudi, King’s College London, London, UK
Dr Carl Blobel, HSS Research Institute, New York, USA
Dr Xian-Cheng Jiang, SUNY Downstate Medical Center, Brooklyn, NY, USA
Dr Mario Gaudino, Weill Cornell Medicine, New York, USA
Dr Alessio Accardi, Weill Cornell Medicine, New York, USA
Dr Matthew E. Fink, Weill Cornell Medicine, New York, USA
Dr Richard L. Proia, NIDDK, Bethesda, MD USA

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SMALL SCALE ROOTS OF METABOLIC DISEASE

Although metabolic diseases plague Western countries, we still don’t fully understand the molecular mechanisms that trigger these diseases. Therefore, biochemists such as Dr Ashley Cowart at the Medical University of South Carolina strive to uncover the cellular pathways behind such diseases, in the hope of finding new treatments.

According to the World Health Organisation, a body mass index of over 25 means that a person is overweight, whereas one over 30 indicates obesity. Both of these categories are major risk factors in cardiovascular and metabolic diseases. Moreover, the International Diabetes Federation reports that in 2012, almost 400 million people worldwide were living with diabetes – a number that is expected to climb to more than half a billion people by 2030. A whopping 80% of these patients are located in developed countries, and the incidence of type 2 diabetes continues to rise in both Europe and the United States. This type of diabetes is strongly linked to abdominal obesity and can be confirmed based on hyperglycaemia in blood plasma. Although metabolic ailments such as obesity and diabetes manifest on a large scale, scientists have found that they start small – on a cellular level. In order to understand and treat these diseases, researchers such as Dr Ashley Cowart and her colleagues are exploring the interactions and behaviours of a group of molecules called ‘sphingolipids’ inside living cells.

Sphingolipids are a class of lipid that contain an 18-carbon amino-alcohol, called a ‘sphingoid base’. This basic structure has several variations, each giving rise to a unique member of the sphingolipid family such as sphingosine, sphingosine-1-phosphate, ceramide, sphingomyelin and glycosphingolipids. ‘This class of compounds was discovered by Johann Thudichum who described them in his book, A Treatise on the Chemical Constitution of the Brain, published in 1884,’ Dr Cowart tells us. ‘At that time, sphingolipids were thought of simply as insulation for the nervous system, similar to the rubber coating on electrical wiring. It was not until the late 1900s that scientists found that these lipids were dynamically generated or broken down in response to stress, and that they bound to protein targets in the cell to elicit cell stress responses.’ In Thudichum’s experiments, he noted that these lipids must somehow be chemically distinct from other lipids. Because at that time he did not understand the composition and structures of these odd lipids, he named them after the mythological Sphinx.

Solving a Lipidic Riddle

Sphingolipids possess special signalling and structural properties, and these compounds have been found to play major roles in the behaviour of tissues affected by diabetes. In the past few decades, research has shown that sphingolipids are more than just simple structural components of cell membranes – they also help to control numerous crucial processes, such as growth, differentiation, chemical signalling and programmed cell death or apoptosis. In addition, these lipids trigger insulin resistance – an initial step in diabetes. They have also been found to promote inflammation associated with diabetes and are now thought to mediate outcomes of diabetes including non-alcoholic fatty liver disease and cardiovascular disease.

When sphingolipids become abnormally regulated, they can trigger cell death and even dysfunction of vital organs such as the heart and the pancreas, or in the aetiology of cancer and multiple sclerosis. Moreover, researchers have found clues suggesting that the levels of sphingolipids in blood plasma may act as a warning sign that the process of gaining insulin resistance has begun. However, while many new discoveries have emerged in the field of sphingolipid research in the past few decades, their precise roles remain obscure. Thus, there is an urgent need for the development of novel experimental approaches to uncover
the exact molecular mechanisms that lead to changes in sphingolipid function, so that new treatments can be developed.

Motivated by the immense impact of these lipids on human health and life expectancy, Dr Cowart and her colleagues investigate the ways that dietary and plasma fats affect the metabolism of sphingolipids present in tissues. This is important because the concentration of lipids in plasma increases in obesity and diabetes, and this increase can change the sphingolipid content in many tissues, such as those in the heart, liver, adipose tissue and skeletal muscles. The team wondered if these lipids may contribute to tissue illness such as insulin resistance, inflammation, abnormal growth of cardiac muscle, and fatty liver disease.

To test their hypothesis, Dr Cowart and her colleagues created experimental lab setups using rodent and tissue culture models. Through the course of these experiments, the team found that rodents placed on high saturated fat diets had dramatic changes in specific sphingolipids in their tissues. These changes resulting from high-fat diets can trigger major problems in gene regulation and chemical signalling pathways. By creating a novel mouse model based on high saturated fat-feeding, Dr Cowart and colleagues have been able to pinpoint several facts.

‘This class of compounds was discovered by Johann Thudichum who described them in his book published in 1884. At that time, sphingolipids were thought of simply as insulation for the nervous system, similar to the rubber coating on electrical wiring. It was not until the late 1900s that scientists found that these lipids were dynamically generated or broken down in response to stress, and that they bound to protein targets in the cell to elicit cell stress responses.’
‘Essentially, this line of work began with our findings published in 2009 that excess saturated fat does not drive sphingolipid biosynthesis in a non-specific way, as was the going thinking at the time, but that sphingolipid metabolic pathways reconfigure in response to saturated fats, which suggests that sphingolipid synthesis may in part mediate stress responses to dyslipidemia,’ Dr Cowart explains. ‘Our studies in the liver, muscle, and heart have mechanistically linked this “reconfiguration” to cell processes including inflammation, maladaptive autophagy, endoplasmic reticulum stress and apoptosis.

Most of the earlier studies were in skeletal muscle or skeletal muscle model systems, then we moved into cardiac muscle, and we have some more recent work in liver.’

**Research Directions in Lipidomics**

In a series of papers published between 2008 and 2016 in some of the most reputable scientific journals, Dr Cowart’s team reported many new insights into the roles and normal and abnormal functions of sphingolipids in tissues. Some of the team’s findings were related to the behaviour of cardiac tissue in diabetes, the pathway of myocardial sphingolipids, the phenomenon of hepatic inflammation caused by diets rich in saturated fats and mediated by sphingolipids, and the role of saturated fats in the stimulation of genes responsible for promoting steatosis, or fatty liver disease.

Type 2 diabetes can trigger changes to the heart tissue of otherwise healthy individuals. Although they display none of the factors of traditional heart disease, these people can suffer from diabetic cardiomyopathy – an abnormal enlargement of the cardiac muscle that vastly increases the risk of heart failure. In mouse models of diabetic cardiomyopathy, heart dysfunction and enlargement happen due to a lipid overload caused by dietary saturated fatty acids. At the same time, this phenomenon occurs in the presence of the synthesis of new sphingolipids. However, it was not clear whether the effects are caused by saturated fatty acids and sphingolipids in general, or by certain species of lipid.

Dr Cowart’s team showed that diets rich in saturated fatty acids caused cardiac muscle enlargement, functional systolic and diastolic problems of the left ventricle, and intracellular degradation leading to cell death. Furthermore, they discovered a specific metabolic pathway involving dietary saturated fatty acids and sphingolipids, which together cause lipotoxicity and ultimately heart disease. More precisely, the fatty acid myristate was found to induce cell degradation and hypertrophy by its metabolism to sphingolipids, while palmitate did not. Finally, Dr Cowart’s group was able to determine that these effects occurred through specifically the (dihydro)ceramide synthase 5 form.

The sphingolipid base backbone formation is catalysed by an enzyme called serine palmitoyltransferase (SPT), which, if inhibited, reduces cardiac disease appearance in those with lipid overload. However, the metabolites involved in these phenomena were not previously known, along with their relevance for living tissue. Dr Cowart and her colleagues sought to determine whether the so-called d16 sphingolipids derived from myristate occurred in the myocardial tissue and if their metabolism was different from that of sphingolipids derived from palmitate. The study found that the bases of d16 lipids are present in more than a third of the sphingolipids in the myocardium. The team also found that dietary saturated fats promoted their production. Moreover, only d16 sphingolipids and not d18 contributed to cell death and chemical changes in tissue. This was the second specific sphingolipid pathway that Dr Cowart and colleagues identified, and they now propose that either of these pathways may serve in the future as therapeutic targets for diabetic heart pathology.

Fatty hepatitis – or steatohepatitis – is present in about 20% of patients suffering from fatty liver disease and results in cirrhosis, fibrosis, and risk of hepatocellular carcinoma. Having already demonstrated that lipid overload stimulates the production of sphingosine kinase 1 (SphK1), an enzyme responsible for generating sphingosine-1-phosphate (S1P), Dr Cowart and her team sought to test whether S1P promotes the inflammation of the liver in obesity. The team discovered a twofold increase of the enzyme in human livers affected by non-alcoholic fatty liver disease, a finding which was confirmed in mouse models with high-fat diets. All mice livers also showed inflammatory factors and infiltration of immune cells – all except for the mice without SphK1. Additionally, the team found that SphK1 is promoted in muscle tissue by palmitate, and that the levels of interleukin-6, an immune modulator, were significantly lower in obese mice lacking SphK1.

The team’s investigations have paved the way to a better understanding of the action of sphingosine kinase 1 in dietary lipid overload, and offer future therapeutic targets for treating individuals suffering from metabolic disease.
Meet the researcher

Dr L. Ashley Cowart
Department of Biochemistry and Molecular Biology
Medical University of South Carolina
Charleston, South Carolina
USA

Dr L. Ashley Cowart obtained her PhD in biochemistry in 2001 from the Vanderbilt University in Nashville, Tennessee. She is now an Associate Professor at the Department of Biochemistry and Molecular Biology at the Medical University of South Carolina and also a research health scientist at the Ralph H. Johnson VA Medical Centre. Her team studies how plasma lipids affect tissue sphingolipid metabolism, and their roles in mediating diabetes-associated disease. Dr Cowart, who also serves as the co-Director of the Lipidomics Core Facility at the Medical University of South Carolina, is a member of the American Society for Biochemistry and Molecular Biology, serves on multiple committees in both the Ralph H. Johnson VA Medical Center and the Medical University of South Carolina, and has published over 40 papers in peer-reviewed journals.

CONTACT
E: cowartl@musc.edu
T: (+1) 843 876 2787
W: http://academicdepartments.musc.edu/biochemistry/faculty/cowartl.htm

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Heart disease has been the leading cause of death worldwide for over 15 years, killing millions of people each year. Diabetes recently rose to the sixth leading cause of death worldwide, and is projected to move further up the list in the future. These two diseases are often associated and share risk factors, as well as distinct metabolic and molecular signatures. While scientists have a grasp on many factors that are associated with heart disease and diabetes, often the underlying mechanisms are not well understood, presenting a barrier to the development of novel treatments for these conditions. The cornerstone of Dr Kienesberger and Dr Pulinilkunnil’s research program is illuminating the role of cellular energy metabolism in governing outcomes of metabolic heart disease.

**Autophagy, Obesity, and the Heart**

Diabetes and obesity are often linked with heart disease. In particular, many diabetic and obese patients suffer from a group of conditions known as cardiomyopathy – weakening of the cardiac muscle. The progression of cardiomyopathy often leads to heart failure, thus an understanding of the factors that contribute to and predict cardiomyopathy may help doctors to develop new treatments to reduce and prevent heart disease related deaths in the future. Drs Kienesberger and Pulinilkunnil noted that cardiomyopathy in obese and diabetic patients is often associated with changes in cardiac metabolism that lead to glucolipotoxicity, an accumulation of toxic levels of glucose and fatty acids and their metabolites in the bloodstream and in the cardiac cells. When this occurs, cardiac cells start to struggle with energy regulation and stop disposing and recycling unneeded and damaged proteins, which can ultimately lead to cell death. The Pulinilkunnil laboratory hypothesised that glucolipotoxicity interferes with the cells’ natural waste disposal system and sought to identify and characterise the underlying mechanisms.

Cells get rid of waste, such as used proteins, via intracellular organelles called lysosomes. These tiny vessels engulf unwanted organic materials and digest them through a process known as autophagy. Through autophagy, the cell is able to break down and recycle cellular waste to provide energy for the cell and create fresh building blocks for new proteins and cellular products. When lysosomes are unable to perform autophagy, the cell begins to build up unwanted proteins and waste, which can damage the health of the cell. Lysosome autophagy is controlled by a suite of specialised signalling proteins called transcription factors. One of the most important of these proteins is master regulator transcription factor EB, or TFEB for short. TFEB signals for cells to produce new lysosomes, and coordinates the activity of active lysosomes to keep autophagy running smoothly in the cell. The team speculated that TFEB activity and lysosomal function were disrupted in glucolipotoxic conditions.

To test this, they started with observing the heart tissue of obese and diabetic mice. They found that TFEB was present in lower levels in diseased animals than in healthy controls. Next, they grew rodent cardiac cells in a petri dish, and exposed them to differing levels of glucose and fatty acids, allowing them to observe the response of the cells in detail. The isolated cells exposed to glucolipotoxic conditions expressed lower levels of TFEB and showed impaired lysosomal autophagy. Notably, a particular subset of fatty acids seemed to be most toxic, indicating that this mechanism could be targeted in obesity and diabetes to ameliorate cardiomyopathy. To test whether the rodent results held up in humans, small samples of heart tissue were collected from human patients undergoing heart surgery for various cardiac disorders. Analysis of this tissue revealed that indeed, morbidly obese patients with heart disease had lower levels of TFEB than non-obese patients. These results highlighted the connections between conditions and disease, and revealed impoverished TFEB as a potentially critical player in obesity and diabetes related heart disease.
The Complex Role of Fats in Heart Health and Disease

An interest in the consequences of cardiac cell metabolism and glucolipotoxicity led Dr. Kienesberger and colleagues to review the role of lipids in cardiac disease, in an effort to tease out the most damaging fats. Cardiac lipotoxicity, the over-accumulation of specific lipids in heart tissues, often leads to damaged cardiac cells, insulin resistance, metabolic dysfunction, and ultimately cell death. However, there are many types of lipids, and how they interact with cardiac tissue is not well understood, leading to the classifications of ‘good’ and ‘bad’ fats. However, much of the research in this area has focused on a single lipid type, and may be neglecting the bigger picture as to how fat molecules and cardiac cells interact. Dr. Kienesberger performed a comprehensive survey of the available research on lipotoxicity and found trends that may indicate a complex role for how lipids interact with cardiac tissue in the development of heart disease.

Heart tissue often prefers to consume fatty acids for energy, but healthy cardiac cells have a more flexible metabolism, consuming both fatty acids and sugars present in the blood stream to keep the heart pumping. Diseased hearts often start to show less metabolic flexibility, relying on fewer energy sources and making cells more vulnerable to impairment. Disease states are often associated with the build-up of fatty plaques in the heart tissue, a situation that occurs when there are more fatty acids available than the heart can metabolise. In studies focused on single types of fats, certain lipids appear to be more toxic than others when they build up in these plaques, while others have been labelled as harmless or ‘good’. Dr. Kienesberger’s analysis indicates that the reality is much more complex. Some lipids that appear harmless on their own may have components that are toxic or interact with other molecule to cause damage. Dr. Kienesberger argues that more comprehensive research on the networks of these molecular interactions is necessary to form a full picture of the processes that underlie lipotoxicity. However, a consistent finding across all studies is that limiting fatty acid uptake prevents the fatty over-accumulations that contribute to heart disease. Therapeutics that aim to limit fatty acid uptake may help prevent heart damage, regardless of lipid type.

A New Understanding of Chemotherapy Induced Heart Disease

The Pulinilkunnil laboratory further expanded this research to other models of heart failure commonly observed in drug induced cardiomyopathy – specifically that induced by Doxorubicin, a drug used for cancer treatment. Doxorubicin, or DOX, is an effective chemotherapy medication commonly used to treat many different kinds of cancer and notably breast cancer. Chemotherapies are often known for their unpleasant side effects such as hair loss and nausea, but DOX is also known to increase susceptibility to cardiomyopathy specifically in women, through mechanisms that are not fully understood. Prolonged treatment with DOX often leads to heart failure, requiring physicians to balance cancer treatment with heart disease risk. DOX is known to suppress mitochondrial metabolism, alter calcium levels, and interfere with protein breakdown in many kinds of cells, but its effects on cardiac cell waste processing has only recently been uncovered.

Prior work established that there is a perturbation of lysosomal autophagy activity in the cardiac muscle of DOX treated patients, but it was unclear how DOX might be
influencing these processes. The Pulinilkunnil laboratory demonstrated that DOX repressed the expression of TFEB and reduced the activity of cathepsin B – a proteolytic enzyme essential for lysosomal autophagy. Overall, they found that the loss of TFEB reduced the expression of many proteins involved in autophagy, disrupted the normal flux of autophagy activities in the cells, reduced the availability and activity of cathepsin B, and increased the activation of cell death programs.

While DOX appears to disrupt mitochondrial activity, restoration of mitochondrial function has largely failed to prevent the drug’s negative effect on heart health. Dr Pulinilkunnil argues that a component of DOX’s apparent effect on mitochondrial activity is related to its influence on autophagy processes; cardiac cells possess a particularly high number of mitochondria, and when unable to recycle, defective mitochondria quickly become overloaded and unable to function. The Pulinilkunnil laboratory’s careful investigation of the DOX-influence pathways associated with lysosomes and autophagy reveal that the drug interferes with autophagic processes at multiple levels, and the disruption of these pathways is what ultimately results in cardiac damage.

In cardiac tissue, DOX interferes with autophagy by altering lysosome structure and impairing transcription factors that regulate lysosomal activity, including TFEB. This results in a build-up of waste in cardiac cells, creating a toxic environment inside the cell and leading to cell death, causing widespread cardiac tissue damage and eventually resulting in heart failure. Taken together, these effects can lead to widespread cardiac tissue damage, increasing the chances of heart complications following cancer treatment with DOX. Despite this grim result, the team found a silver lining: when TFEB was restored to the cells, many of these effects were reduced and cardiac cells were less likely to undergo programmed cell death. This highlights the potential for TFEB as a research target for preventative cardiac health treatment.

In this regard, the Pulinilkunnil laboratory recently published an in-depth review on this topic, detailing what is known about the molecular relationships between these components of health and illuminating novel pathways through which DOX influences heart health. Dr Pulinilkunnil has grown particularly fascinated with the connections between lysosomal autophagy and cardiomyopathy. Studying the effects of metabolites, drugs, peptides, novel compounds, dietary substances, nutrients on lysosomes and TFEB allows him to reveal the pathways involved in autophagy processes and better appreciate how these entities influence functional outcomes in healthy individuals, with the hope that this increased understanding will lead to advances in our understanding of metabolic heart disease and help discover new therapeutics to boost cardiac health down the line.

**Tying Obesity to Disease**

For both heart disease and diabetes, a high level of body fat is a risk factor, and excess adipose tissue plays a role in disease development. While these effects are routinely attributed to alterations in metabolism associated with obesity, the hormonal and molecular pathways that contribute to disease progression are not fully known. Autotaxin, or ATX, is a protein produced by adipose cells that is released into the blood stream. Once secreted, ATX produces a fat molecule that triggers important signalling cascades in many cell types of the body. ATX has been implicated in cardiovascular disease and, more recently, obesity and diabetes, but it is currently unclear whether it aggravates or mediates progression of obesity and diabetes. The Kienesberger laboratory sought to better understand the behaviour of this protein and its relationship with blood sugar regulation and metabolism.

The team began by examining ATX blood serum levels in mice that had been raised on either regular chow diet or a diet containing high fat and high sugar (HFHS), and had blood collected either closely following a meal or after a 16 hour fast. Mice on the HFHS diet were overweight and had higher levels of ATX. Mice that had fasted prior to blood collection had lower levels of ATX regardless of body condition. These results confirmed that ATX levels are related to overall fat accumulation, but may fluctuate with meals.

Next, the researchers grew adipose cells in petri dishes to more closely analyse ATX secretion in response to varying doses of glucose and insulin, which controls many of the body’s metabolic processes. They found that fat cells with induced insulin resistance due to their inability to properly respond to insulin showed increased ATX secretion, and this effect could be prevented by adding an insulin sensitiser to the mix. Glucose appeared to have a dose-dependent effect on ATX secretion overall – predictably increasing ATX levels as glucose levels went up. Insulin had a more nuanced effect – a short spike of insulin increased ATX, but chronic exposure to insulin decreased ATX levels. ATX levels appeared to be influenced by both short- and long-term changes in nutritional states, blood sugar levels and insulin.

**Making Strides in Human Health**

The team’s future research directions will continue to pursue a deeper understanding of human health. With the help of a grant from the Heart and Stroke Foundation of Canada – New Brunswick and the New Brunswick Health Research Foundation, Dr Kienesberger intends to delve deeper into the mysteries of ATX, further teasing apart the relationship between this elusive protein, diabetes, and heart health. Dr Pulinilkunnil recently received a grant from the Canadian Diabetes Association to study the role of TFEB and autophagy in mediating heart disease, with the hopes of developing novel treatments for cardiomyopathy.
Meet the researchers

Dr Petra C. Kienesberger
Department of Biochemistry and Molecular Biology
Dalhousie University
Saint John
Canada

Dr Petra C. Kienesberger received her PhD in Biology with specialisation in Biochemistry and Molecular Biology at the Karl-Franzens University of Graz, Austria in 2009, and went on to complete a postdoctoral fellowship in Cardiovascular Pathophysiology at the University of Alberta from 2009 to 2013. She joined the faculty of medicine at Dalhousie University in 2013 as an Assistant Professor in the Department of Biochemistry and Molecular Biology, where she currently runs a laboratory group researching the role of fat metabolism and signalling in energy homeostasis, specifically during obesity, diabetes, and heart disease progression.

CONTACT
E: pkienesb@dal.ca; pkienesb@gmail.com
T: (+1) 506 636 6971
W: http://www.biochem.dal.ca/faculty-staff/faculty/kienesberger.php

Dr Thomas C. Pulinilkunnil
Department of Biochemistry and Molecular Biology
Dalhousie University
Saint John
Canada

Dr Thomas C. Pulinilkunnil received his PhD in Cardiovascular Metabolism from the University of British Columbia in 2005. From 2005 to 2008 he completed postdoctoral work in Endocrinology, Diabetes and Metabolism at Harvard Medical School, and from 2008 to 2012 he engaged in a postdoctoral fellowship studying Energy Metabolism at the University of Alberta. In 2012 he accepted a position in the faculty of medicine at Dalhousie University as an Assistant Professor in the Department of Biochemistry and Molecular Biology, where his laboratory investigates the role of autophagy signalling and lysosomal function in metabolic perturbations underlying obesity, diabetes, breast cancer and congenital disorders.

CONTACT
E: tpulinil@dal.ca; tpulinil@unb.ca
T: (+1) 506 636 6973
W: http://www.biochem.dal.ca/faculty-staff/faculty/pulinilkunnil.php

KEY COLLABORATORS
Dr Monte Willis, UNC, Chapel Hill USA
Dr Abhinav Diwan, Washington University, Missouri
Dr Andrea Ballabio, TIGEM, Telethon, Italy
Dr Daniel Kane, STFX University, Canada
Dr Bryan Crawford, UNB, Canada
Dr Erin Kershaw, University of Pittsburgh
Dr Vassilis Aidinis, Alexander Fleming
Dr Mohamed Touaibia, Université de Moncton

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DALHOUISIE UNIVERSITY
HDL, LDL and Atherosclerosis: The Good, the Bad and the Ugly

Atherosclerosis is a chronic inflammatory disease in which lipid rich plaques build up in the walls of major arteries, restricting the flow of oxygen-rich blood to different tissues and organs. Atherosclerotic plaques result from the accumulation of fatty deposits along the vessel wall. Growing plaques can protrude into the arterial lumen, eventually causing a partial or complete obstruction of blood flow. Angina pectoris is a medical condition caused by a gradually growing stable plaque and subsequent decrease in coronary blood flow. Unfortunately, atherosclerotic plaques can become unstable and rupture, leading to thrombus formation, rapid decrease in blood flow and low tissue oxygen levels. Atherosclerosis can lead to serious problems, including heart attack, stroke and peripheral artery disease.

The major player in atherosclerosis is cholesterol. As a fat, or lipid, cholesterol cannot be dissolved and float freely in the blood. To overcome this, the body has specific proteins, called lipoproteins, which bind to it and carry it where it needs to go. Two types of cholesterol carriers are low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs). Clinically, they are known as ‘bad’ and ‘good’ cholesterol, respectively.

Atherosclerotic lesions typically develop where arteries branch. In response to disturbed blood flow that naturally occurs in these forked regions, blood vessels thicken. When this happens, the endothelial cells lining the interior vascular surface become activated and exhibit increased permeability, making it easier for LDL to enter into the intima – the innermost layer of the vessel wall. Individuals with high cholesterol have abnormally elevated levels of LDL in the blood, which increases the number of LDLs accessing and colonising the vascular wall. A major problem here is that when LDLs are exposed to the new environment, they undergo oxidative modifications that transform them into pro-inflammatory molecules, which release factors from the endothelium that initiate and maintain inflammation in the vessel wall.

Several cells of the immune system respond to these pro-inflammatory signals. One particular type, called monocytes, migrate from the blood to these inflammatory sites in the vessel wall and transform into larger, mature macrophages. Macrophages are armed with the necessary machinery to recognise and remove oxidised LDL from the vascular space. Proteins located along the macrophage membrane, called scavenger receptors, recognise oxidised LDL and bring it inside to be used or eliminated by the cell. However, accumulated LDL in these diseased areas increases the internalisation rate, which transforms these highly mobile removal workers into fat-laden and less mobile ‘foam’ cells. The lipids engulfed by the macrophage give it a ‘foamy’ appearance (red colour on the right). Paralysed, these foam cells die and become part of the expanding fatty plaque they were previously trying to prevent. Over time this plaque expands, narrowing the vessel lumen and reducing the volume of blood that can pass through. If and when this plaque ruptures, a blood clot will emerge and obstruct the vessel entirely, starving the organ it serves of its oxygen supply. If it serves the heart, it can cause a heart attack, while if it occurs in the brain, it is called stroke.

MACROPINOCYTOSIS: THE MACROPHAGE DRINKING PROBLEM BEHIND ATHEROSCLEROSIS

Dr Gábor Csányi and his team at the Vascular Biology Center of Augusta University explore the molecular mechanisms involved in an alternative pathway of macrophage LDL cholesterol uptake, with hopes of finding new targets for the treatment of atherosclerosis.
Pinocytosis: An Unexplored Target in Atherosclerosis

Dr Gábor Csányi and his group have contributed to a better understanding of how macrophages ingest bad cholesterol and transform into lipid-laden foam cells. They have shown that LDLs do not actually need to be oxidised to be internalised, and also, that another mechanism of uptake is involved in macrophage foam cell formation. Essentially, in addition to relying on the selective uptake of modified LDLs by scavenger receptors, macrophages use pinocytosis to ingest unmodified LDLs. Pinocytosis, often referred to as ‘cell drinking’, is the engulfing of extracellular fluid – liquid that surrounds the cell. This process is nonspecific, because the cell swallows everything in the fluid, including random dissolved particles. The rate of pinocytosis and pericellular solute concentration are the primary factors that determine the extent of solute internalisation.

So why do cells risk allowing entry to everything? Immune cells, like macrophages, use pinocytosis to survey the surrounding environment for antigens. They bring samples inside and evaluate them to establish whether there are foreign invaders that might be potentially harmful and need to be eliminated. Additionally, the cells use pinocytosis to internalise extracellular nutrients to support their growth and metabolic needs. Macrophages use two forms of pinocytosis to internalise fluid and associated solutes – macropinocytosis and micropinocytosis.

As their names suggest, they ingest large and small volumes of extracellular fluid, respectively. While both types of pinocytosis may participate in the ingestion of unmodified LDLs, Dr Csányi and his group are specifically interested in the role that macropinocytosis plays in this process.

Macropinocytosis starts when extracellular signalling molecules interact with specific protein receptors housed on the cell membrane. When the cell receives the initiating signal, membrane ‘ruffles’ form, creating folds! and protrusions that extend into the surrounding environment. These protrusions move back towards the plasma membrane, much like a swimmer doing the breast stroke, capturing fluid and solutes in their wake. Membrane protrusions can also transition into curved ruffles and form ice cone like macropinocytotic cups, which close on the top, capturing extracellular fluid and solutes. Following this, the newly formed fluid sacks pinch off from the plasma membrane to form irregular shaped and sized vesicles called macropinosomes, which travel into the cytoplasm – the cell’s interior.

Dr Csányi’s research group has delved deeper into the mechanisms by which macropinocytosis of lipids are regulated. ‘My research focuses on a better understanding of the mechanisms leading to atherosclerosis and applying this understanding to develop and implement new strategies for its prevention and treatment,’ he explains. ‘Utilising a broad range of innovative technologies and strategies, we investigate the mechanisms by which cardiovascular risk factors, inflammatory mediators, matrix proteins, and genes promote the transition from a healthy “normal” vessel wall to a “diseased” vasculature.’

The role of Nox2 in Macropinocytosis

In their research, Dr Csányi and his team demonstrated that Nox2 participates in macropinocytosis. Belonging to the NADPH oxidase (Nox) family of protein oxidases, Nox2 functions in generating reactive oxygen species (ROS) and mediating intracellular redox reactions. Briefly, this enzyme complex is responsible for transferring electrons to molecular oxygen to produce superoxide anions – the precursor radicals of ROS. Low levels of ROS regulate signalling pathways that are important for maintaining healthy cellular physiology, while increased ROS generation leads to oxidative stress and contributes to vascular and other diseases.

Dr Csányi chose Nox2 not only because of its importance in ROS...
production in macrophages, but also because it exists downstream in a signalling chain of molecules, namely Protein Kinase C (PKC) and Ras-1, known to function in macropinocytosis. Therefore, he proposed that these other proteins may send signals to Nox2 to help them initiate macropinocytosis. Dr Csányi and his team conducted their initial experiments using macrophages in the lab. They used 4ß-PMA, a molecule known to induce macropinocytosis in macrophages, to test whether Nox2 participates. A clever way to explore whether a particular molecule is important in a biological process is to disrupt its function. Firstly, they deactivated the Nox2 protein and found that 4ß-PMA-induced macropinocytosis and the amount of lipid ingested was significantly reduced. To clarify these findings, they silenced the Nox2 gene before it was able to be fully translated into functional protein. Again, macropinocytosis was inhibited.

While evaluating the biological processes in cells on their own was informative, it was important to test whether similar results translated to animal models of atherosclerosis. To test this, the team extracted macrophages from wild-type mice, whose genetics have not been manipulated, and Nox2 knockout mice, where Nox2 expression is genetically removed. These macrophages were treated with 4ß-PMA to induce macropinocytosis and subsequently injected into the peritoneal cavities of ApoE—/— mice – bred to develop high cholesterol to induce macrophages to manipulate, and Nox2 knockout mice, where Nox2 expression is genetically removed. These macrophages were treated with 4ß-PMA to induce macropinocytosis and subsequently injected into the peritoneal cavities of ApoE—/— mice – bred to develop high cholesterol similar to that seen in atherosclerotic patients. A day later, Dr Csányi and his colleagues extracted and analysed the macrophages and found that macropinocytosis and lipid uptake was significantly diminished in those taken from the Nox2 knockout mice. Cumulatively, these experiments highlighted Nox2 as a principal player in the induction of macropinocytosis. Next, Dr Csányi’s laboratory sought to identify the signalling mechanisms downstream of Nox2 involved in macropinocytosis.

In further investigations, Dr Csányi and his team found that intracellular Nox2 signalling activates cofilin (an actin-binding protein) to trigger membrane ruffling – the first stage of macropinocytosis. It does this by stimulating a secondary signalling pathway, called the phosphoinositide 3 kinase/Akt pathway (PI3K/Akt). For the first time, Dr Csányi and his colleagues demonstrated that Nox2-mediated intracellular redox signalling plays a pivotal role in a mechanism that initiates macrophage LDL macropinocytosis.

**CD47 and Nox1: Partners in Crime**

Dr Csányi, Dr Pagano and their colleagues defined a physiologically relevant pathway that mediates macrophage macropinocytosis of unmodified LDL. The extracellular matrix surrounding a cell’s exterior provides structural and biochemical stability. It is composed of different molecules, including a group of matricellular proteins, which help to regulate pathways that are important for normal cell maintenance. One type of matricellular protein, called thrombospondin-1 (TSP1), is elevated above healthy levels in the intimal layer of the vessel wall in individuals with atherosclerosis. Therefore, Dr Csányi proposed that TSP1 may act as a physiological stimulator of LDL macropinocytosis in macrophages.

The research team found that incubating macrophages with physiologically relevant concentrations of TSP1 leads to extensive plasma membrane ruffling, macropinosome formation and increased uptake of unmodified LDL compared to untreated controls. Subsequently, they demonstrated that TSP1 binds to a specific protein receptor, CD47, to carry out this process.

While these findings were new and exciting, the team sought to uncover the remaining parts of the chain involved in transmitting the instructions to initiate macropinocytosis. Dr Csányi and colleagues genetically blocked the activity of different molecules in the pathway to see whether they are involved in TSP1-CD47-induced membrane ruffling and macropinocytosis. It has previously been shown that TSP1 signals through Nox1, another isoform of the NADPH oxidase family, to induce superoxide anion production in the vascular smooth muscle. Based on these findings, Dr Csányi proposed that TSP1 may use the same mechanism in macrophages to stimulate macropinocytosis. Indeed, they measured increased levels of superoxide anions inside macrophages upon TSP1 treatment. Additionally, macrophages exhibited reduced membrane ruffling, macropinocytosis activity and lipid uptake in response to TSP1 when Nox1 was inhibited. The in vivo importance of this pathway was recapitulated in a mouse model of atherosclerosis.

However, the final piece of this signalling puzzle was still missing. How does the TSP1/CD47/Nox1 axis trigger membrane ruffling and macropinocytosis? Similar to the previous study, the link was cofilin. TSP1 was found to induce cofilin activation when CD47 and Nox1 were functioning in wild-type macrophages but failed to do so when CD47 and Nox1 had been genetically blocked. The study by Dr Csányi and his team demonstrated for the first time that a signalling pathway involving CD47, Nox1 and cofilin contributes to TSP1-induced macropinocytic uptake of unmodified LDL by macrophages.

**What Does It All Mean?**

Dr Csányi’s work has given us important new insights into the specific mechanisms involved in macrophage uptake of LDL. In addition, it has clarified that macrophages also use macropinocytosis to internalise unmodified forms of bad cholesterol, contributing to the formation of foam cells that become part of atherosclerotic lesions. Dr Csányi states that, ‘these findings support a new paradigm in which redox signaling-mediated lipid macropinocytosis independent of extracellular lipid oxidation propagates initiation and development of vascular inflammatory disease.’ Finally, and perhaps most importantly, this research has highlighted specific mechanistic components that could be targeted for atherosclerosis prevention and treatment.
Meet the researcher

Dr Gábor Csányi
Vascular Biology Center
Department of Pharmacology & Toxicology
Augusta University
Georgia, USA

Dr Gábor Csányi graduated from the University of Szeged in Hungary with a Masters of Pharmacology in 2003. He completed his PhD in Medicine at the University of Szeged and Jagiellonian University in Kraków, Poland in 2008. During his PhD, much of his work was focused on characterising functional alterations of endothelium-derived vasodilators that occur in several cardiovascular pathologies, including atherosclerosis, ischemic heart failure, dilated cardiomyopathy and diabetes mellitus. Following his PhD, Dr Csányi moved to the USA to carry out his postdoctoral training in the laboratory of Dr Patrick Pagano at the University of Pittsburgh in Pennsylvania. In his second year, Dr Csányi received an American Heart Association (AHA) Postdoctoral Fellowship. At present, he is an Assistant Professor in the Vascular Biology Center at Augusta University in Georgia, USA and his research is funded by the NIH Pathway to Independence Award (K99/R00).

CONTACT
E: gcsanyi@augusta.edu
T: (+1) 706 721 1437
W: http://www.augusta.edu/centers/vbc/csanyi.php

KEY COLLABORATORS
Patrick Pagano, PhD, Vascular Medicine Institute, University of Pittsburgh, USA
Stefan Chlopicki, PhD, Jagiellonian Centre for Experimental Therapeutics, Krakow, Poland
David Fulton, PhD, Vascular Biology Center, Augusta University, USA

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LABORATORY
Gabor Csanyi, PhD
Pushpankur Ghoshal, PhD
Bhupesh Singla, PhD
Huiping Lin
Madison Carpenter

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UNRAVELLING THE MYSTERY OF CHRONIC ASTHMA

Understanding the underlying processes that contribute to the progression of asthma is essential to improve our limited knowledge of the disease, and to assist in the generation of novel therapies. Professor Dale Tang and his team in the Department of Molecular and Cellular Physiology at the Albany Medical College in New York are pioneers in a new arena of research investigating the potential role of smooth muscle proteins in the development of airway hyperresponsiveness and airway remodelling – prominent characteristics of chronic asthma.

A Commonplace Enigma

The seriousness of asthma is widely appreciated – with nearly 300 million people affected globally. It is one of the most common diseases of the respiratory airways and although its onset is predominantly in childhood, it occurs in individuals of all ages and ethnicities regardless of socioeconomic status. However, despite its significant impact on the population, chronic asthma is still considered an unmet need, with many sufferers struggling to successfully manage their symptoms.

Professor Dale Tang, a renowned researcher with a broad background in physiology, molecular biology, cell biology, and biochemistry, was initially drawn to the field of asthma research when he was a medical student at Tongji Medical University in Wuhan, China. ‘I saw an asthmatic patient who was coughing and wheezing,’ he tells us. ‘We gave him medications, but he did not respond very well. My attending physician told me that our understanding of asthma is limited and we do not have many medications to treat this disease – this is one of the major reasons that attracted me to this field.’

Asthma is a dynamic disease that changes over time. Knowledge of the cellular processes that underlie its development – or pathogenesis – is surprisingly limited. Indeed, scientists are still striving to accumulate fundamental data that can be used to enhance our understanding of the mechanics that drive disease progression.

The Role of Smooth Muscle

Much of the musculature of internal organs and the digestive system is lined with smooth muscle, a non-striated muscle that lacks voluntary control. Together with the airways and the lungs, smooth muscle makes up a major part of the respiratory system. Moreover, the contraction of smooth muscle lining the respiratory airways plays an essential role in regulating the functions of the respiratory system. During breathing, the mechanical process of oscillation works to overcome the resistive and elastic properties of the lungs and chest wall. This mechanical stretch of the airways results in the contraction of smooth muscle by inducing the tensing and relaxing of smooth muscle cells (myocytes).

Recent evidence has shown that the action of smooth muscle contraction is compromised in asthma. ‘We know that abnormal airway smooth muscle contraction causes breathing difficulty in asthmatic patients, but the mechanisms that control airway smooth muscle contraction are not fully understood,’ says Professor Tang.

It is well known that asthma is characterised by airway hyperresponsiveness, airway remodelling, mucus hypersecretion, and airway inflammation – all of which lead to impaired respiratory airflow. Considerable information exists about the development of mucus hypersecretion and airway inflammation, however less is known about the mechanisms of airway hyperresponsiveness and airway remodelling. Both of these processes are largely attributed to increased airway smooth
muscle cell contractility and proliferation. Funded by a grant from the National Institute of Health, it is the aim of Professor Tang and his team, Research Associate Guoning Liao, PhD student Brennan Gerlach, Senior Research Technicians Olivia Gannon and Alyssa Rezey, Laboratory Manager Ruping Wang and Postdoctoral Fellow Jiaoyue Long, at the Albany Medical College in New York to investigate the mechanisms that control smooth muscle cell proliferation and migration, and determine their role in the possible pathogenesis of airway remodelling in asthma.

Properties of the Actin Cytoskeleton

Within the body’s cells exists a complex network of interlinking tubules and filaments known as the cytoskeleton – a structure that has a myriad of functions including giving the cell shape and providing mechanical resistance to prevent deformation of the cell. The cytoskeleton also participates in the regulation of contraction, thereby distorting the cell and its environment, and allowing the cell to migrate. The filaments that comprise a chief component of the cytoskeleton are constructed from a globular protein called actin. Along with myosin – a molecule capable of generating force – the actin cytoskeleton occupies a substantial proportion of the cytoplasm within smooth muscle cells and forms the apparatus that allows the entire smooth muscle to contract. Professor Tang and his team were the first researchers to propose that smooth muscle contraction is similar to the moving of a car, with myosin functioning as an ‘engine’ for smooth muscle contraction and the actin cytoskeleton serving as a ‘transmission system’ in smooth muscle. A number of protein kinases – enzymes capable of chemically modifying other proteins – have been reported to regulate actin cytoskeleton activity in smooth muscle, including Abelson (c-Abl, Abl) tyrosine kinase, p21-activated kinase (PAK), focal adhesion kinase (FAK), and integrin-linked kinase (ILK). The long-term goal of Professor Tang’s research is to unveil the mechanism of these actin-associated proteins in airway smooth muscle contraction and determine their role in the pathogenesis of asthma.

‘We are the first to discover that c-Abl tyrosine kinase regulates smooth muscle contraction by controlling structural changes in the cell’

c-Abl Tyrosine Kinase – a Key Player in Asthma Development

Initial investigations conducted by Professor Tang and his team have focused on c-Abl, a non-receptor tyrosine kinase that is widely expressed in cell cytoplasm and necessary for smooth muscle force development. Results from pilot studies in human airway smooth muscle cell cultures show that the silencing of c-Abl in airway smooth muscle significantly inhibits actin cytoskeleton dynamics, diminishing contractile response and inhibiting smooth muscle cell proliferation. These findings, explains Professor Tang, are the first evidence that c-Abl is a crucial component of the cellular processes that regulate activity of the actin cytoskeleton and a key player in regulating airway smooth muscle contraction and cell proliferation. In order to identify the role of c-Abl in the development of asthma, Professor Tang and his fellow researchers went on to conduct studies in airway smooth muscle cells taken from patients with severe asthma and animal models mimicking allergen-induced asthma in humans. Interestingly, results from these studies revealed that the level of c-Abl was significantly higher in asthmatic cells than in normal cells. This discovery is of noteworthy relevance when considering airway hyperresponsiveness – increased sensitivity that stems primarily from hyperreactivity of airway smooth muscle. Conditional knockout of c-Abl – a technique involving the elimination of the gene encoding c-Abl in smooth muscle – was found to reduce airway smooth muscle hyperreactivity in vitro and lessen airway resistance in mice sensitised and challenged by an allergen. Further
studies in animal models of chronic asthma encouragingly supported this finding, with substantially lower airway resistance and lower contractile response being observed compared to control models.

Increased expression of c-Abl in smooth muscle was also found to contribute to the development of airway remodelling in chronic asthma. The pathogenesis of airway remodelling involves a number of factors: the formation of excess fibrous connective tissue (fibrosis), enhanced accumulation of extracellular matrix protein, epithelial injury, airway smooth muscle growth (hypertrophy), and proliferation of airway smooth muscle cells.

The team also found that conditional knockout of c-Abl led to a reduction in smooth muscle mass and diminished cell proliferation markers, while treatment with pharmacological c-Abl inhibitors was found to have similar effects. Thus, Professor Tang and his team have confirmed that c-Abl is required for smooth muscle cell proliferation and increased expression of c-Abl in smooth muscle may contribute to the development of airway remodelling in chronic asthma. ‘We are the first to discover that c-Abl tyrosine kinase regulates smooth muscle contraction by controlling structural changes in the cell,’ says Professor Tang. ‘Disruption of this kinase inhibits asthmatic symptoms in animal studies. Thus, this kinase may be a new biotarget for the development of new therapy to treat asthma.’

The Importance of Cortactin and Profilin-1

Two other proteins with potential involvement in smooth muscle contraction have also been the focus of research for Professor Tang and his team. The first, cortactin, is an actin-regulatory protein capable of regulating actin filament assembly. In human airway smooth muscle cells and tissues, cortactin is able to control actin activity and the development of smooth muscle force without affecting myosin activation. The second protein, profilin-1, is an actin-binding protein that promotes actin functionality and has a role in modulating smooth muscle contraction.

Using airway smooth muscle cells prepared from human bronchi and tracheas, bronchial rings taken from human lungs, and allergen-sensitised and allergen-challenged animal models, Professor Tang and his group of researchers have successfully unveiled a novel cellular mechanism by which the interaction between cortactin and profilin-1 results in smooth muscle contraction.

Disruption of this interaction was found to inhibit smooth muscle hypercontractility in cell cultures and airway hyperresponsiveness in asthmatic animal models. Importantly, c-Abl appears to have a probable role in regulating the coupling of cortactin with profilin-1.

Implications for the Future

Professor Tang is one of the few investigators in the world to have studied both the myosin and cytoskeletal pathways in smooth muscle. His identification of several protein molecules that are critical for the regulation of actin dynamics and smooth muscle contraction has substantially changed people’s views on how smooth muscle contraction is regulated. With the support of his team, he plans to continue his research into the multifaceted role of c-Abl in the development of asthma. ‘The expression of this kinase is upregulated in asthmatic human airway smooth muscle cells;’ he tells us. ‘Currently, we plan to understand how this happens.’

Professor Tang believes that activation of c-Abl by contractile stimulation mediates cortactin activity and promotes actin functionality and smooth muscle contraction. To test this hypothesis, he and his team plan to explore the role of c-Abl in mediating cortactin activity, and the association of cortactin with other actin-associated proteins implicated in airway smooth muscle contraction. They also plan to investigate the possible roles of c-Abl and actin activity in regulating the mitogen-activated protein kinase (MAPK) pathway – a chain of protein kinases that allow the communication of signals from the cell surface to deoxyribonucleic acid (DNA) inside the cell nucleus.

Finally, Professor Tang hopes to confirm that allergen exposure may be responsible for the increased expression of c-Abl in asthmatic human airway smooth muscle cells and that this may be the pivotal contributor to allergen-induced airway hyperresponsiveness, airway remodelling, and airway inflammation. These anticipated findings will have a markedly positive impact on the development of efficacious asthma therapies and will undoubtedly provide long-needed hope for the millions of individuals whose lives are affected by chronic asthma.
Meet the researcher

Professor Dale D. Tang
Department of Molecular and Cellular Physiology
Albany Medical College
Albany, New York
USA

Dr. Dale Tang is a professor at the Albany Medical College in New York, USA, where he also currently heads the Cytoskeletal Signalling and Asthma Research Program at the Department of Molecular and Cellular Physiology. He completed his MD at Tongji Medical University in Wuhan, China, in 1983, before going on to complete a PhD in biochemistry and physiology in 1990. He went on to conduct postdoctoral research in these subjects at the University of Texas Southwestern Medical Center in Dallas, USA and the University of Calgary in Alberta, Canada. He worked in the Department of Physiology at the Indiana University School of Medicine for several years before taking up the post of Associate Professor, and then Professor, at the Albany Medical College in New York, USA. He sits on the Editorial Boards of several key medical journals and he has been the recipient of a number of research grants. His research focuses on the function of the actin cytoskeleton in smooth muscle contraction and its potential role in the pathogenesis of asthma.

CONTACT
E: TangD@mail.amc.edu
T: (+1) 518 262 6416
W: http://www.amc.edu/Profiles/tangd.cfm

KEY COLLABORATORS
Dr. Guoning Liao, Albany Medical College, USA
Brennan Gerlach, Albany Medical College, USA
Olive Gannon, Albany Medical College, USA
Alyssa Rezey, Albany Medical College, USA
Ruping Wang, Albany Medical College, USA
Jiaoyue Long, Albany Medical College, USA
Yinna Wang, Albany Medical College, USA

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REFERENCES


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The Need for a Universal Influenza Virus Vaccine

Catching “the flu” is something we all dread and go to great lengths to avoid. However, influenza viruses are tricky characters, prone to fast evolutionary modification and a high mutation rate. This changeable nature allows their evasion of our body’s humoral (antibody-mediated) immune response. Causing a wide range of symptoms, influenza viruses are considered significant human respiratory pathogens. They are one of the most common causes of acute respiratory disease and can result in high mortality and morbidity, particularly in high-risk populations such as infants, the elderly, and those with chronic illnesses. Surveillance data show that influenza viruses result in 2–5 million cases of severe illness and up to 250,000–500,000 deaths per year worldwide.

Furthermore, influenza viruses are responsible for seasonal, endemic infections and can emerge periodically to cause unpredictable and devastating pandemics. Vaccination is currently the best, and most cost-effective, countermeasure against influenza virus infections in humans. Although available vaccines offer effective prophylactic protection, particularly in healthy individuals and children, they are limited in scope and are effective against only the circulating strains that are closely related to the vaccine strain. Through a mechanism called “antigenic drift”, the genetic variation of influenza viruses allows them to escape immune responses quickly. Therefore, revaccination with reformulated vaccines is required annually to ensure continued protection – an inconvenient, impractical and costly undertaking. In addition, because vaccine strain selection is based on surveillance and prediction, mismatches between vaccine strains and circulating viruses frequently occur, resulting in reduced vaccine efficacy.

Four distinct types of influenza viruses are currently circulating in the human population: two influenza A viruses (the 2009 H1N1 pandemic strain and H3N2) and two divergent lineages of the influenza B virus. Consequently, effective vaccine formulations need to contain at least the two influenza A virus strains and one influenza B virus strain, which further complicates the manufacturing process.

Thus, despite existing vaccination programs, seasonal influenza virus epidemics represent a significant public health burden. To meet this challenge, Dr. Peter Palese, an eminent researcher in the fields of infectious diseases and virology and Chair of the Department of Microbiology at the Icahn School of Medicine at Mount Sinai in New York, believes that to overcome the shortcomings of seasonal influenza virus vaccines and to enhance our pandemic preparedness, game-changing influenza virus vaccines that confer broad, ideally universal, and long-lasting protection are needed. “We all have heard that influenza virus vaccines are not perfect. They have to be administered every year so that we are protected against the seasonal appearance of new strains. Every year, old vaccine components have to be changed. This can be
“Every year, old vaccine components have to be changed. This can be cumbersome and expensive. Our efforts are to develop a universal influenza virus vaccine that does not have to be administered every year, but will last for 20 years or even hopefully for a lifetime.”

To achieve this goal, Dr. Palese, with the assistance of numerous experts, is embarking on an exciting influenza vaccine project – substantially advancing research in the field of virology. His ultimate aim is to develop a human universal influenza vaccine that will abolish the need for annual revaccination.

Hemagglutinin – a Viable Vaccine Target

The majority of the humoral (antibody-mediated) immune response against influenza viruses is directed against hemagglutinin (HA) – one of the major surface glycoproteins of the influenza virus. There are eighteen subtypes of HA (H1 through H18), of which only H3, H2 and H1 are found in human influenza viruses. Importantly, H5, H6, H7 and H9, which are found in highly pathogenic avian influenza viruses, can occasionally affect humans.

at a low rate. The HAs are responsible for binding the influenza virus to sialic acid – a widely distributed monosaccharide (sugar) that is present on the surface of host cells. This allows the influenza virus to replicate and spread infection throughout the host’s tissues. Attack from antibodies directed against HA drives antigenic drift, as the head region of the HA molecule exhibits high plasticity (phenotypic adaptability). This plasticity, however, is constrained by the two functions of HA – mediation of the binding of the virus to host cell-surface receptors (through the receptor binding site) and the fusion between viral and endosomal membranes (through the stalk domain). To stay viable, the virus needs to retain the structure of both the binding site and the stalk domain. These regions are therefore conserved in the influenza virus HA.

Recognizing the importance of HA in modulating the spread of influenza virus infection, Dr. Palese and his collaborators in this universal influenza vaccine project – including Dr. Florian Krammer, also based in the Department of Microbiology at the Icahn School of Medicine at Mount Sinai, and Dr. Adolfo García-Sastre, Director of the School’s Global Health and Emerging Pathogens Institute – recently developed multiple, broadly-neutralizing, monoclonal, therapeutic vaccine antibodies capable of targeting the conserved regions of HA. Two main classes of these antibodies have been proposed – those directed against the conserved, membrane proximal stalk (or stem) domain of the HA, and those directed against the membrane distal receptor binding site (the head).

Promising Vaccine Candidates Against Influenza

Dr. Palese believes that it will be possible to create a universal influenza virus vaccine by reducing the immunodominance of the influenza virus HA head domain, thereby increasing the immunogenicity of the HA stalk domain. His approach is based on the observation that sequential exposure to influenza viruses with divergent head domain but conserved stalk domain of HA refocuses the immune response towards the conserved stalk domain. He and his team have developed broadly protective vaccine candidates based on chimeric HAs – combinations of globular head domains from an HA of one influenza virus subtype or strain and the conserved stalk domain from an HA of another subtype/strain. “We are looking at
pre-existing immunity, as everyone above 2 or 3 years of age is likely to have antibodies,” says Dr. Palese. “We are hoping to give a vaccine that is a chimeric construct, which, in people who have been infected at one point, will cause the immune system memory to recognize these stalk regions and amplify antibody titre against the conserved regions of the virus.”

Initially, Dr. Palese and his team designed two vaccine constructs, each comprising a chimeric combination of globular head domains from exotic influenza A viruses and stalk domains from an H1 influenza virus. Both chimeric vaccine constructs were sequentially administered to mice that had been exposed (primed) to an H1 stalk antigen. The animals were then challenged four weeks after the last vaccination with a lethal dose of diverse influenza A viruses. Control groups included prime-only animals that received irrelevant protein vaccinations, vaccination-naive animals, and standard of care (SOC) positive-control animals that received two doses of a human seasonal trivalent vaccine matched to the challenge strain – a regimen similar to the two-dose regimens recommended for the vaccination of children.

The study resulted in a significant difference in survival between groups vaccinated with the chimeric vaccine constructs and naive animals, with all naive animals and a large proportion of prime-only animals succumbing to infection. Notably, both the chimeric construct and SOC vaccination methods provided complete protection from mortality. These encouraging findings led Dr. Palese and his team to further test vaccination with the chimeric construct against challenges with antigenically distinct influenza virus strains. This allowed the comparison of the effectiveness of the novel vaccination regimen with that of the current SOC against antigenically mismatched viruses. Again, the results were promising, with the universal vaccination regimen providing complete protection from morbidity and mortality.

Broadly Protective Antibodies

To determine if sequential vaccination with chimeric vaccine constructs resulted in the production of broadly reactive antibodies, the team went on to analyze the response of the vaccines to purified influenza virions (entire virus particles, consisting of an outer protein shell and an inner core of RNA). A broad range of influenza virus strains were tested, representing strains that had circulated among the human population from 1940 to 2013. In all cases, the novel vaccination approach induced relatively constant antibody responses. This finding has significant value, in that it demonstrates that vaccination with these chimeric constructs promotes the amplification of cross-reactive, neutralizing antibodies against a wide range of diverse influenza viruses and, importantly, these antibodies could protect from future virus challenges.

This is an exciting discovery for Dr. Palese and his colleagues, and they are continuing their research in order to further understand exactly how these antibodies work. “We are now trying to understand the mechanism by which these broadly protective, stalk-specific antibodies mediate antiviral activity and how they differ from regular antibodies that just recognize the head and prevent attachment,” says Dr. Palese.

Neutralizing antibodies to influenza virus HA are thought to act predominantly by inhibiting virus attachment to sialic acids on the surface of host cells. Traditionally, these antibodies were thought to interact exclusively through the antibody’s variable region – the fragment antigen-binding or Fab region. However, Dr. Palese, in collaboration with Dr. Jeffrey Ravetch from The Rockefeller University, has put forward compelling evidence to suggest that to confer protection, broadly neutralizing HA stalk-specific antibodies require interactions between their tail ends (Fc region) and Fc immunoglobulin gamma receptors (FcγRs) situated on the surface of effector cells that constitute the immune system, such as B lymphocytes, macrophages, neutrophils and natural killer cells. In short, the activated effector cells destroy the viruses or the infected cells by antibody-mediated phagocytosis or antibody-dependent cell-mediated cytotoxicity.

A Vaccine for the Future

Although considerable progress has been made towards demonstrating the potential usability of headless (stem-only) and chimeric HA-based antigens, further research is needed to determine how best to direct the antibody response. Dr. Palese’s universal influenza vaccine project continues in its efforts to provide a better understanding of the mechanisms underlying the immune system’s response to influenza viruses, infection, and vaccination that will enhance efforts to improve vaccine design.

Development of improved vaccines, including a potentially universal vaccine, will undoubtedly offer broader protection from drifted influenza virus strains, induce long-lived immunity against seasonal strains, and allow for the rational design of vaccines that can be stockpiled for use in an arising pandemic. The use of these vaccines may also prevent, or limit, the emergence of drug resistant strains of influenza virus in human populations – redefining and reducing the negative impact of influenza.
Meet the researcher

Peter Palese, PhD
Professor and Chair
Department of Microbiology
Icahn School of Medicine at Mount Sinai
New York, USA

Dr. Peter Palese is currently Professor and Chair of the Department of Microbiology at the Icahn School of Medicine at Mount Sinai in New York, USA. His research is in the area of RNA-containing viruses with an emphasis on influenza viruses. Specifically, he established the first genetic maps for influenza A, B and C viruses, identified the function of several viral genes, and defined the mechanism of neuraminidase inhibitors (which are now FDA-approved antivirals). He pioneered the field of reverse genetics for negative strand RNA viruses, which allows the genetic manipulation of these viruses. This technique is crucial for the study of the structure and function relationships of viral genes, for investigation of viral pathogenicity, and for development and manufacturing of novel viral vaccines. At present, Dr. Palese’s group works with Drs. Adolfo García-Sastre and Florian Krammer on the development of a universal influenza virus vaccine. Dr. Palese is a Member of the National Academy of Sciences, a Member of the National Academy of Medicine (formerly IOM) and a Fellow of the American Academy of Arts and Sciences.

CONTACT
E: peter.palese@mssm.edu
T: (+1) 212 241 7318
W: http://labs.icahn.mssm.edu/paleselab/

KEY COLLABORATORS
Dr. Adolfo García-Sastre, Icahn School of Medicine at Mount Sinai, New York
Dr. Florian Krammer, Icahn School of Medicine at Mount Sinai, New York

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The Centre for the AIDS Programme of Research in South Africa (CAPRISA) was established in 2002 with a National Institutes of Health (NIH) grant award. The organisation was created as a partnership between five institutions: University of KwaZulu-Natal, University of Cape Town, University of Western Cape, South African National Institute for Communicable Diseases, and Columbia University in New York. Now a well-established and highly regarded AIDS Research Centre, CAPRISA conducts innovative research into HIV pathogenesis, TB-HIV treatment and HIV prevention. In this exclusive interview, we have had the privilege of speaking with Professor Salim Abdool Karim, Director of CAPRISA. Here, Professor Abdool Karim tells us about CAPRISA’s cutting-edge research to treat and prevent HIV infection in South Africa and across the globe.
Describe CAPRISA’s mission, and the ways by which CAPRISA conducts and supports HIV/AIDS research in South Africa.

CAPRISA’s vision is an AIDS-free world. The main goals of CAPRISA are to conduct locally responsive and globally relevant research on HIV/AIDS and tuberculosis (TB), while building the research infrastructure and providing research training opportunities for the next generation of scientists.

CAPRISA concentrates its research on developing new interventions to reduce new cases of HIV infection and HIV-related deaths in Africa. Hence, CAPRISA’s research focuses on co-infection with HIV and TB, which is the most common cause of deaths in South Africa, and on new prevention technologies for young women, who have the highest HIV infection rates in southern Africa. The aim of its studies is to prevent HIV infection in young women and reduce HIV-TB deaths in South Africa.

How prevalent is HIV/AIDS in South Africa? Is the disease a leading cause of death?

South Africa bears a disproportionate burden of HIV infection. The country has about 19% of the global HIV burden despite being home to less than 1% of the global population. The South African epidemic is described as a generalised, hyper-endemic epidemic where, despite active HIV prevention and treatment programmes, there continues to be high rates of new HIV infections. South Africa has the highest number of people living with HIV in the world. According to UNAIDS, there are an estimated 7 million people living with HIV in South Africa and each day there continues to be about 1,000 new infections. About one fifth of all the people living with HIV in the world are in South Africa.

In South Africa, HIV and TB are individually among the leading causes of death. However, it should be noted that many individuals with TB are also co-infected with HIV. People with both HIV and TB infections at the same time have a much higher risk of dying, as the two infections have a higher death rate when they occur together than separately. The risk of developing symptoms of TB, including cough, fever and night sweats, is highest soon after a person becomes HIV positive and becomes pronounced again when the person develops advancing HIV related immune suppression. The risk of rapid progression to TB disease is much greater among individuals with HIV infection, because HIV impairs the body’s ability to contain TB infection.

Please describe CAPRISA’s work in combatting the high death rate caused by HIV – Tuberculosis co-infections.

People with HIV have the highest risk of dying due to TB. However, with scientific advancement, policy change and programmatic implementation, the number of people dying from HIV-associated TB worldwide has fallen by 32% since 2004. Currently, deaths from HIV-TB...
account for 25% of all TB deaths and one third of the estimated 1.2 million deaths from HIV.

The decline in deaths in people with both HIV and TB infection is a consequence of 3 things: (1) expansion of AIDS treatment globally, (2) improvements in HIV-TB case finding through implementation of HIV screening in TB programs and improved TB diagnostics leading to earlier TB diagnosis, and (3) integration of antiretroviral therapy (ART) with the medications taken for TB.

A radical decline in antiretroviral drug prices together with investments from governments and donor agencies such as the Global Fund to fight AIDS, TB and Malaria and the President’s Emergency Plan for AIDS Relief made AIDS treatment a reality for those most in need. By the end of 2016, an estimated 18.2 million people were accessing ART, an impressive scale-up from 7.5 million in 2010. In one rural South African community, for example, scale up of ART therapy between 2003 and 2011 has reduced the risk of acquiring HIV by 38%, while increasing life expectancy by 11.3 years.

CAPRISA's scientific research over the last decade has led to new interventions, policies and guideline changes, all aimed at improving the clinical management of HIV-TB co-infection. Results from the CAPRISA 003 HIV-TB treatment study undertaken in Durban at the TB clinic in Warwick Avenue are widely used in guiding the way people with this dual infection are treated throughout the world. This study demonstrated that the key to saving lives of people with the dual disease is to integrate the care they receive for HIV with their TB treatment. The results from the CAPRISA 003 study provided pivotal data contributing to the revision of the WHO, US-DHHS and South African guidelines on the treatment of TB-HIV co-infection. CAPRISA's findings on reducing deaths from HIV-TB have already been implemented in several countries and it is estimated that the implementation of the integrated approach to TB-HIV treatment could prevent about 10,000 deaths each year in South Africa alone. By 2014, the percentage of identified HIV-positive TB patients who started or continued on ART reached 77%, demonstrating a giant leap forward in the effective management of dually infected patients.

HIV disproportionately affects young women in South Africa. What are the reasons behind this, and what work is CAPRISA involved with to specifically target this problem?

While global HIV trends indicate a decline in the number of new cases of HIV infection (from 3.4 million in 2001 to 2.1 million in 2015), these trends mask the continued spread of HIV in certain regions, populations, and age groups. Of the 10 countries that contribute two-thirds of all HIV infections globally, seven are in eastern and southern Africa. In these regions, women account for 59% of all people living with HIV, and adolescent girls and young women aged 15 to 24 years are particularly vulnerable. Of the estimated 3.9 million young people aged 15 to 24 years living with HIV in 2014, 2.3 million (58%) were young women. Throughout sub-Saharan Africa, HIV prevalence among adolescent girls and young women exceeds that of their male peers, with HIV prevalence up to 6 times higher in young women in South Africa.

Why are young women so vulnerable?

While the cause of this vulnerability has not been fully elucidated, it is compounded by a complex interplay of biology, gender-power disparities, social, political and economic factors. In sub-Saharan Africa, adolescent girls and young women tend to acquire HIV infection at a much earlier age than their male peers. This age–sex disparity in infection rates is a consequence of young girls partnering with men who are about 8–10 years older than them, and who may have recently acquired HIV or who are already living with HIV but are not on treatment with antiretroviral medicines. Adolescent girls and young women engage in sexual relationships with older men for multiple reasons. While some relationships are based on love or sexual curiosity, in some instances, particularly for those from impoverished backgrounds, young women may engage in transactional sex and form relationships with older men for financial and social security.

Young people are often inexperienced in sexual risk-taking and many are not able to negotiate condom use with older partners. Understanding the drivers of this partnering pattern and learning more about these male partners is critical for addressing the prevention needs of adolescent girls and young women.

In addition to unknowingly choosing a sexual partner who may be already infected with HIV, early sexual debut, teen pregnancies, early school drop-out, and sexual violence also increase the vulnerability of adolescent girls and young women to acquiring HIV infection and maintain them in vicious cycles of poverty and dependency.

The high HIV incidence rates observed among adolescent girls and young women in sub-Saharan Africa suggest that factors beyond behaviour may be contributing to the heightened vulnerability in this group. Women are biologically more vulnerable to HIV and are, on average, twice as likely as men to become infected after a single sexual encounter. The biological mechanisms that make women more vulnerable than men in acquiring HIV are related to the high levels of immune cell activation (which is the
viral target for infection) in the female genital tract and the increased expression of HIV co-receptors in cervical cells (compared to foreskin cells) may explain why women have a higher per-act risk of HIV acquisition than men. Genital trauma, experienced as a result of forced or unwanted sexual intercourse, can also facilitate HIV transmission.

Inflammation in the female genital tract may also be an important risk factor. Analysis of female genital tract samples from the CAPRISA 004 tenofovir gel trial showed that genital inflammation, defined by combinations of elevated pro-inflammatory cytokines, was associated with a 3-fold increase in the risk of HIV acquisition. The causes of this inflammation are still unclear, but CAPRISA’s research suggests that one of the causes is a vaginal bacterium known as Prevotella Bivia. A better understanding of the immunological basis of HIV transmission in young women could yield useful clues to future HIV prevention technologies and strategies.

Given the scale of the HIV epidemic in young women and the dire need for solutions to reducing risk of HIV in this group, CAPRISA conducts research on the epidemiology, pathogenesis and prevention of HIV infection, with a special emphasis on HIV prevention in women. This research is also part of CAPRISA’s DST-NRF Centre of Excellence (CoE) in HIV Prevention. The CoE was awarded by the NRF in 2015 to CAPRISA as the host organisation and the University of KwaZulu-Natal as co-host with the University of Cape Town, University of the Western-Cape and the National Institute of Communicable Diseases as partners. The CoE’s research programme has been planned to generate new knowledge that contributes to the development of new HIV prevention approaches.

The main goal of the CoE in HIV Prevention is to undertake research, training, information sharing and policy support aimed at understanding and ameliorating the high risk of HIV in women, especially young women, in South Africa. The CoE’s research agenda will advance technologies such as pre-exposure prophylaxis, microbicides and vaccines to impact on HIV transmission in one of the most difficult sub-populations in Africa, where traditional HIV prevention has had little, if any, impact.

The pivotal study in this research programme is the CAPRISA 002 Acute Infection study on HIV immunology and pathogenesis, which conducts basic science studies to generate new knowledge on the transmitted virus, the mucosal milieu for viral entry, the earliest innate immune responses, the T-cell and humoral adaptive immune responses, and the host genetic factors associated with HIV in order to inform the future development and testing of immune-based prevention and treatment strategies.

How likely is the transmission of HIV from a mother to her child during birth or breastfeeding? Please tell us a bit about CAPRISA’s work in preventing this spread.

At CAPRISA research on the prevention of mother-to-child transmission (pMTCT) is conducted in partnership with the Women’s Health Unit at the University of KwaZulu-Natal. CAPRISA hosts a NIH-funded Clinical Trials Unit (CTU) and studies in the IMPAACT (International Maternal Pediatric Adolescent AIDS Clinical Trials) Network are conducted as part of the CAPRISA CTU.

HIV can be transmitted in utero (pre-partum) during the process of childbirth (intra-partum) and post-partum through breastfeeding. In the absence of any intervention, the mother-to-child transmission rate is between 15% and 45%. The risk of perinatal HIV transmission is influenced by the severity of HIV disease in the mother (high RNA viral load and low CD4+ count), the route of delivery (caesarean section versus vaginal delivery), and the type of breastfeeding practices (exclusive breastfeeding or mixed feeding) and duration of breastfeeding. Notable advances have been made in reducing mother-to-child transmission of HIV to very low levels through the use of antiretroviral drugs, obstetric practices including caesarean delivery, and management of breastfeeding. Several countries have been successful in eliminating mother-to-child transmission.

Although thousands of children are newly infected with HIV in South Africa each year, major gains have been achieved in scaling-up of interventions for the prevention of mother-to-child transmission in South Africa. Within 10 years of initiating the country’s programme on preventing HIV in newborns, 95% of all health facilities were providing this service. By 2015, South Africa was one of six countries that met the Global Plan target of reducing mother-to-child transmission by 90%. More than 98% of women receive an HIV test during pregnancy and 95% of HIV-positive mothers are receiving antiretroviral treatment during childbirth (intra-partum) and post-partum through breastfeeding. Intra-partum transmission rate is 1.5% and just over 4% at 18 months’ follow-up.

As availability of ART to reduce mother-to-child transmission during childbirth increases, breastfeeding is assuming a proportionately greater role as a source of HIV spread to newborn babies in settings where formula-feeding is not an affordable option. Breastfeeding, particularly in poor countries, can account for one-third to one-half of all mother-to-child transmissions. This risk is reduced substantially if the mother exclusively breastfeeding her baby since mixed feeding (breastmilk plus formula milk or any other feeds, including water) increases the risk of HIV transmission to the baby. Duration of breastfeeding also affects the rate of transmission.

What are broadly neutralising antibodies? Please tell our readers the ground-breaking work carried out by CAPRISA on this topic.

A neutralising antibody (NAb) is an antibody that defends a cell from an...
antigen or infectious body by neutralising any effect it has biologically. Broadly neutralising antibodies (bNAbs) are antibodies (Abs) that can kill multiple strains of a particular virus.

Although Abs against HIV develop in all infected individuals, they are ineffective at controlling viral replication because these Abs can only kill a single strain of HIV. Since these strain-specific Abs target variable regions of the HIV envelope, the virus readily escapes these Abs. Abs targeting more conserved regions of envelope arise in some individuals but these bNAbs fail to fully control replication because they appear too late in infection and are also ultimately evaded. Nevertheless, isolation of bNAbs from HIV-infected individuals has enabled us to identify vulnerable sites on the HIV envelope and provided us with important reagents to demonstrate that these types of antibodies are protective in animals.

A large number of highly potent and bNAbs have been isolated from several HIV-infected donors over the past 5 years. Collectively, these monoclonal Abs target 5–6 conserved neutralisation-sensitive epitopes on the outer envelope covering of the virus including sites on gp120, the CD4 binding site (CD4bs), glycans on the V1, V2 and V3 loops, the membrane proximal external region (MPER) of gp41 and epitopes that span the gp120 and gp41. Those that target V3 and V1V2 glycans show the highest potency (IC50 of 0.01 - 1 µg/ml) while those targeting the MPER and CD4bs are generally broader (neutralise >90% of global isolates).

The CAPRISA team has isolated a number of monoclonal antibodies (mAbs), which target the V2 region of the HIV-1 envelope glycoprotein and are N160 glycan independent, from a South African woman participating in the CAPRISA 002 Acute Infection study. These mAbs preferentially target clade C viruses making them suitable for use in countries such as South Africa. One particular antibody from patient CAPRISA 256, referred to as CAP256-VRC26.25, is among the most potent monoclonal antibodies currently available, and neutralises 73% of clade C viruses.

Importantly, unlike any other V1V2-directed antibodies, the most potent members of the CAP256-VRC26.25 lineage are not dependent on the N160 glycan (glycan dependence can result in incomplete neutralisation). The exceptional potency and N160 glycan independence of the CAP256-VRC26.25 makes it an attractive candidate for further testing for HIV prevention and even as a potential therapeutic approach.

The CAPRISA consortium developing the CAP256-VRC26.25 Ab, which includes the National Institute for Communicable Diseases humoral immunology team, have partnered with the Ragon Institute of MGH, MIT & Harvard and the US NIH National Institute of Allergy and Infectious Disease’s Vaccine Research Centre to develop this antibody and test it in monkeys. While the 6 monkeys that received the sham injections became infected when challenged, all 12 monkeys that received the CAP256-VRC26.25 antibody were protected when challenged with SHIV. Following these promising results in monkeys, the CAP256-VRC26.25 Ab is being manufactured by the NIH’s Vaccine Research Centre for human trials. It is anticipated that the first human studies of this Ab will begin early in 2018, initially on its own and subsequently in combination with other Abs.

If this Ab works in humans to prevent HIV infection, the next step will be to use it as prophylaxis where young women could receive an injection of the Abs every 4-6 months to protect them from acquiring HIV infection.

Finally, how close to you believe we are to developing an effective vaccine for HIV? Once developed, do you think it will be challenging to distribute the vaccine to people throughout the most affected countries?

Although there have been major advances in understanding HIV pathogenesis and the human immune system over the past three decades that continue to contribute to HIV vaccine development, several unique challenges remain before an effective vaccine is developed. Firstly, HIV attacks CD4+ T cells, the very cells that orchestrate the immune system to combat intruding viruses. Secondly, this retrovirus continuously mutates and recombines resulting in an extensive diversity of viral strains. For a vaccine to be effective at a global level, it would have to protect against a large number of evolving and diverse strains of HIV. Thirdly, there is not a single known case of an HIV positive person naturally clearing the infection, which would enable scientists to study potential correlates of protection. The RV144 trial that demonstrated partial efficacy, has provided some new clues on what immune responses may be required and once better defined, will inform new immunogen designs that could accelerate the path to an effective vaccine.

While a safe and effective vaccine is still several years away, CAPRISA is contributing to the global effort to develop new prevention technologies. Specifically, CAPRISA is working with several partners to develop new antiretroviral prophylaxis approaches, broadly neutralising Abs, and other technologies that could empower women to protect themselves from HIV. Without this, there is little prospect of reaching a world without AIDS.
THE AAV CLUB: APPLYING AAV VECTORS TO GENE THERAPY

The sci-fi vision of gene therapy for curing genetic diseases is fast becoming a reality as more therapies are entering clinical and commercial development. Dr Arun Srivastava and his team at the University of Florida are investigating the use of the next generation of AAV vectors for gene therapy.

The Scope and Limitations of Gene Therapy

The notion of ‘gene therapy’ has intrigued scientists, biotechnologists, clinicians, futurists and the public for decades. The ‘biopunk’ subgenre of science fiction has long exploited society’s fascination with this paradigm, and the prospect of medical enhancement by means of genetic engineering has provided a rich theme in both utopian and dystopian visions of the future. However, gene therapy is no longer science fiction, but is fast becoming ‘science fact’.

Many diseases are driven by defective genes, leading to expression of dysfunctional proteins. Gene therapy aims to deliver fully-functioning genes to specific tissues, replacing or counteracting malfunctioning genes, thus driving sufficient expression of the functional protein. Gene therapy has its roots in the work of pioneering molecular biologist, Joshua Lederberg, who discovered that genetic material is transferred between bacteria by ‘conjugation’ – a primitive version of bacterial sex. Later, Lederberg with Norton Zinder discovered another mechanism of bacterial genetic transfer. They found that bacteriophages – small viruses that infect bacteria – also transfer genetic material between bacteria, known as ‘transduction’. In transduction, DNA or RNA become incorporated in the bacterial genome.

The first proof-of-concept model for human gene therapy was developed by husband-and-wife team, Waclaw and Elizabeth Szybalski, who demonstrated viral transduction in cultured human bone marrow cell lines. This showed the unthinkable – that viral transduction is also possible in eukaryotic cells (complex cells with a nucleus and membrane-bound compartments)! Transduction into the host cell’s nuclear genome, using viruses as delivery vectors, became the preferred method for gene therapy. While the concept is simple, the practicalities of delivering functional genes to targeted tissues, while evading immune responses, and maintaining a long-term effect, are challenging.

Currently, over 1800 approved clinical trials involving gene therapy have been conducted or are underway globally.

Investigating AAV and B19 Parvoviruses for Gene Delivery

For almost four decades, Dr Srivastava and his team at the University of Florida College of Medicine have investigated the biology of human parvoviruses as potential vectors for gene therapy. Parvoviruses are a group of some of the smallest viruses that infect animals, with a virion (virus particle) diameter of only 25 nanometres – tiny even by viral standards! Dr Srivastava and his lab have focused their research efforts on two human parvoviruses – the adeno-associated virus (AAV) and the parvovirus B19 (also called primate erythroparvovirus 1). Both AAV and B19 are prevalent in the human population and relatively harmless, with AAV regarded as non-pathogenic, and the mildly-pathogenic B19 being responsible for common childhood rash. The small size, genomic simplicity and relative non-pathogenicity of the human parvoviruses make them ideal vectors for human gene therapy. Dr Srivastava’s group is interested in the application of human parvovirus transduction of blood cells for the gene therapy of haematological diseases, and they are working hard to make this a reality! Their major focus is treatment of haemoglobin deficiencies with AAV6 vectors. They are also focussing on the application of AAV3 vectors for treating haemophilia (clotting factor disorders), and hepatocellular carcinoma and hepatoblastoma (liver cancers) as an alternative to chemotherapy.

Dr Srivastava’s interest in parvoviruses began early, and rather serendipitously. As a first-year graduate student, he ‘became hooked’ when he stumbled upon a 1974 Nature paper on the mechanism of single-stranded DNA (ssDNA)
replication, by the famous prokaryote taxonomist, Dr Thomas Cavalier-Smith. ‘Later, I learned that a non-pathogenic human parvovirus, the adeno-associated virus (AAV) contains ssDNA,’ he explains.

Without these formative inspirations, Dr Srivastava may never have pioneered the application of parvoviruses as vectors for gene therapy. ‘I started working on AAV in 1980, and have continued ever since.’

The wild-type adeno-associated virus 2 (AAV2 serotype) was discovered in 1965. About 90% of humans have anti-AAV2 antibodies, implying that most of us have been exposed to AAV2, yet there is no conclusive evidence that infection leads to any known disease. Pioneering studies undertaken by Dr Srivastava in the 1980s elucidated the remarkable simplicity of the AAV genome and proteome. The AAV genome is a small linear ssDNA molecule containing 4680 nucleotides (for the AAV2 serotype), which encodes four replication proteins, three capsid proteins, and an assembly activating protein.

For development of viral gene therapy vectors capable of effectively entering cells, discovery of viral-specific receptor protein molecules on the surface membrane of target cells is important. Receptors are like ‘doorways’ into cells, allowing viruses to enter. In 1996, Dr Srivastava and colleagues demonstrated that AAV infection of cells is receptor-mediated, having identified a cell line (human megakaryocytic leukaemia cells, MB-02) resistant to AAV infection as it lacks specific receptors. This finding sparked a quest for the discovery of cell surface receptors indicative of transduction success, which became a major endeavour for the team. In 1999, they identified a cell receptor associated with AAV2 infection – human fibroblast growth factor receptor 1 (FGFR1) as a cellular co-receptor for AAV. In 2003, they discovered a co-receptor – α5β1 integrin – as a cellular co-receptor for parvovirus B19, and in 2010, identified human hepatocyte growth factor receptor (HGFR) as a cellular co-receptor for AAV3. As well as AAV2, the group is also investigating transduction efficiencies and tissue-specificities of other virus serotypes. While AAV2 is ideal for liver- and retina-directed gene therapy, Dr Srivastava and other groups have found that AAV3 and AAV6 are more effective for human liver and blood cells, respectively.

Blood clotting (coagulation) after an injury prevents bleeding and promotes wound healing – vital to prevent blood loss and haemorrhaging. Therefore, conditions affecting clotting are particularly dangerous. Haemophilia is a debilitating inherited disease that impairs the blood’s ability to clot, and sufferers are susceptible to prolonged post-injury bleeding, easy bruising and increased risk of internal bleeding in the joints and brain – potentially causing severe, permanent damage. Blood clotting is a complex pathway involving several specialised blood proteins. These include blood clotting factor proteins produced in liver hepatocyte cells that facilitate the clotting process. There are two types of haemophilia – haemophilia A and haemophilia B – that result in the deficiencies or absence of clotting factors VIII and IX, respectively. A number of clinical trials are currently underway investigating the clinical efficacy of clotting factor replacement by gene therapy. These involve the simple procedure of infusion of the therapeutic AAV viruses into the hepatic artery of patients (delivers blood to the liver) for delivery of corrective genes that encode deficient clotting factors to the hepatocytes. Dr Srivastava’s lab focusses on clotting factor IX replacement for haemophilia B treatment in hepatocytes, using ‘humanised’ mouse and non-human primate models.

Engineering AAV Vectors for Gene Therapy

To overcome the limitations of the wild-type AAV, recombinant strategies, involving genetic modification of the virus, have been deployed by Dr Srivastava’s group to improve the delivery, transduction and expression of corrective genes to target tissues. Ironically, while high dosages of AAV virus are typically necessary for efficient gene therapy, high dosages can also activate T-cell mediated immune responses that attack the viral vector. Therefore, a major component of Dr Srivastava’s work is to improve transduction efficiency, so that curative outcomes can be achieved at lower viral dosages.
A major aim of the lab is to elucidate cellular mechanisms that attack and destroy the viral vector. Inside cells, unwanted proteins destined for destruction are tagged with ubiquitin – a regulatory protein that serves as a signal for protease enzymes to break down unwanted proteins. Dr Srivastava’s group discovered that this is responsible for around 80% of AAV2 vectors failing to enter the nucleus, and are trying to circumvent this destructive pathway to improve gene therapy efficiency.

The group found that the presence of tyrosine, serine or threonine residues (amino acids prone to phosphorylation) on the AAV capsid surface destines the virus to ubiquitination-mediated destruction. Hence, they have genetically modified wild-type AAV viruses, by mutating these surface amino acids to phenylalanine or valine (both amino acids), which do not undergo phosphorylation, and thus bypass the cell’s ubiquitination-based defences. This strategy proved highly effective for improving transduction rates. Tyrosine-to-phenylalanine mutation in AAV2 led to 10-times lower viral doses needed for adequate transduction in mice. While mouse-based models are standard in Dr Srivastava’s lab, the group are shifting the focus of their modified AAV studies from mouse models to human models, as the next step to feasible human gene therapy. This poses a challenge, as AAV transduction efficiencies have typically been much lower in human cells than in mouse cells. The AAV3 serotype, once rejected as a potential vector because of poor in vivo transduction in mouse livers, has turned out to be effective in transducing human hepatocytes. The group has therefore focussed on genetically engineering the AAV3 virus to further increase transduction rates in human hepatocytes. In 2016, the group demonstrated that just two mutations, of a serine and threonine to valine, in AAV3 gave 8 times and 80 times greater transduction rates, respectively, than AAV8 and AAV5 (two favoured gene therapy vectors for clinical development) in human hepatocytes in a ‘humanised’ mouse model.

Even if transduction is successful, and the virally-delivered gene-of-interest has been delivered to the target cell, there is no guarantee that the corresponding corrective protein will be sufficiently produced (‘expressed’) to cure the target disease. While the ssDNA is a defining feature of AAV as a naturally occurring virus, Dr Srivastava, along with others in the AAV community, speculate that engineering the wild-type single-stranded AAV (ssAAV) to have a double-stranded ‘self-complimentary’ genome can improve corrective gene expression through improved transcription (transfer of genetic information from DNA to RNA).

Single-stranded DNA is transcriptionally-inactive, and the viral synthesis of a second strand is a rate-limiting step in cell culture studies. Unfortunately, second strand DNA synthesis is typically suppressed by host cells, often leading to only 5% of mouse hepatocytes expressing the protein after transduction. Dr Srivastava’s group has identified a cellular chaperone protein (involved in folding newly-expressed proteins), called FKBP52, phosphorylated forms of which inhibit the second-strand synthesis by binding to ssAAV ssDNA. Indeed, the vast majority of AAV gene therapy clinical trials have been carried out using ssAAV. Several research groups, including Dr Srivastava’s, are working on developing next-generation AAV gene therapy vectors.

In Dr Srivastava’s group, hepatocytes are not the only targets for AAV gene therapy for clotting disorders. The group is also interested in AAV transduction of hematopoietic stem/progenitor cells (HSPCs) as agents of gene and cell therapy. HSPCs are stem cells produced in bone marrow that differentiate to form all other types of blood cells (multipotency). They therefore have greater therapeutic scope, with potential to treat beta-globin deficiencies, leukodystrophies, immunodeficiencies and even AIDS. HSPCs are a promising choice for autologous stem cell/gene therapy. This would involve extracting HSPCs from patients, in vitro culturing the extracted HSPCs, and viral transduction (gene delivery, gene editing) to introduce the corrective gene into the cultured HSPCs. Next, the modified HSPCs are inserted back into the same patient so they differentiate into the functional cells of interest. Alternatively, the modified HSPCs may be differentiated in culture by adding certain chemicals before infusion into patients. For the development of feasible stem cell/gene therapies, efficient transduction procedures must be established. Unfortunately, poor transduction and corrective protein expression in HSPCs are hampering their applicability to cell therapy. In 2016, the group demonstrated a novel highly efficient approach to viral transduction of HSPCs using AAV6 vectors. These insights are highly promising, and could be the basis of feasible high-efficiency transduction and gene editing approaches.

**AAV Gene Therapy – the Way Forward**

Dr Srivastava’s group has pioneered the application of AAV gene therapy in pre-clinical models, identifying AAV-specific cellular co-receptors and in vivo mechanisms that may hamper gene delivery, and engineering wild-type vectors for improved robustness. The group hopes to continue further optimisation of the AAV genome and capsid proteins, and improve the tissue-specificity of different vectors for more targeted therapy. There is overwhelming evidence for the safety and efficacy of recombinant AAV gene therapy with 162 clinical trials conducted to date.
Meet the researcher

Professor Arun Srivastava
Cancer & Genetics Research Complex
Departments of Pediatrics
University of Florida
Gainesville, USA

Professor Arun Srivastava is Chief of the Division of Cellular & Molecular Therapy in the Departments of Pediatrics, and the Powell Gene Therapy Center, at the University of Florida. He received his PhD from the Indian Institute of Science in Bangalore, India. After completing his postdoctoral training at the Memorial Sloan-Kettering Cancer Center in New York, he worked as a Research Associate at the University of Florida, before a two-decade sojourn at Indiana University School of Medicine, Indianapolis, where he rose to the rank of Professor. He then returned to the University of Florida in 2004. He has served on various NIH Study Sections, and is also on the editorial boards of several esteemed gene therapy-related journals. He was appointed as Honorary Professor and Advisor, Shenzhen Institute of Xiangya Biomedicine, Central South University, Shenzhen, PR China, and University of Florida Research Foundation Professor, and Children’s Miracle Network Scholar. For nearly four decades, Dr Srivastava’s research has focused on the virology and pathophysiology of two human paroviruses, the adeno-associated virus (AAV), and the parvovirus B19, and the application of these as vectors for gene therapy. His laboratory has shed light on many fascinating insights into the field of paroviruses, including molecular mechanisms of viral entry into cells, intracellular viral trafficking and regulation of AAV DNA replication and encapsidation, and parvovirus-induced pathogenicity in transgenic and knockout mouse models.

CONTACT
E: aruns@peds.ufl.edu
T: (+1) 352 273 8259
W: http://research.pediatrics.med.ufl.edu/researchers/research-faculty/arun-srivastava/

KEY COLLABORATORS
Dr Chen Ling, University of Florida
Dr Mavis Agbandje-McKenna, University of Florida
Dr Roland Herzog, University of Florida
Dr Mervin Yoder, Indiana University
Dr Emmanuel Payen, University of Paris
Dr Philippe Leboulch, University of Paris
Dr Alok Srivastava, Christian Medical College
Dr Barry Byrne, University of Florida

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PREVENTING PRETERM BIRTH AND IMPROVING INFANT OUTCOMES

Professor Jeff Keelan and his colleagues at the Western Australian Preterm Birth Prevention Initiative are on a mission to lower rates of child morbidity and mortality through research into the prediction, treatment and prevention of premature labour and birth.

**Landmark Initiative in Lowering Prematurity**

The leading cause of death and disability in children under the age of five isn’t a contagious disease or genetic condition—it’s complications associated with preterm birth (PTB). The rate of PTB, defined as the birth of a baby at less than 37 weeks’ gestation, is around 5–8% in developed regions such as Europe and Australasia, but is much higher (between 16 and 18%) in developing areas such as sub-Saharan Africa or South-East Asia. According to the World Health Organisation, nearly one million infants’ lives are lost annually due to PTB. Unfortunately, despite intensive research, the rates of PTB have remained static for decades—and have even risen in some populations.

Much of Professor Jeff Keelan’s research is affiliated with the Western Australian Preterm Birth Prevention Initiative which was launched in 2014 under the leadership of leading Maternal-Fetal Medicine specialist, Professor John Newnham. Made up of a team of clinicians, researchers, public health professionals and patient advocate groups, the aim of the Initiative was to safely reduce the rate of PTB in the state by a third over a five-year period. This comprehensive programme encompasses new clinical guidelines, a cervical length screening program, an outreach programme for healthcare practitioners, a public health scheme for women and families and a new dedicated PTB prevention clinic for referral of high risk pregnancies. The initiative, supported by the Women & Infants Research Foundation, also incorporates ongoing research into the prediction, treatment and prevention of prematurity and PTB. A population study assessing the effects of the Initiative, which was just published in the American Journal of Obstetrics & Gynecology, showed an 8% reduction in the rates of PTB after just 18 months of operation—the lowest rate in the state in six years and a major achievement.

Despite the achievements of the Initiative to date, current strategies do not target key aetiologies such as infection and inflammation, and available pharmacological interventions have had little impact on the preterm birth rate to date. In order to develop effective therapies, Professor Keelan and his team are focussing on two challenges: firstly, they must determine how best to identify the women who are at risk of PTB, and secondly, they must develop effective and safe ways to treat amniotic and fetal infection and inflammation.

**Infection and Preterm Birth**

So what triggers PTB? Well, there are many different causes, but in about 10–20% of cases, the cause is intrauterine infection (IUI). IUI is caused by microbial invasion of the amniotic cavity (the fluid-filled sac which protects the fetus during pregnancy). This occurs when the bacteria residing in the vaginal mucosa breach the cervical barrier and colonise the fetal membranes and amniotic fluid. This then triggers an inflammatory response from mother and fetus, which can lead to chorioamnionitis (inflammation of the fetal membranes), preterm contractions, rupture of membranes, fetal inflammatory reaction and a range of serious neonatal complications including cerebral palsy. Importantly, the risk of IUI increases markedly as gestational age at delivery decreases. The majority of extremely preterm babies (those born more than 12 weeks premature) have evidence of an infectious aetiology—these babies typically have the greatest risk of death or life-long disability.

But which pathogens are responsible? A variety of bacterial culprits that commonly cause infection-driven preterm birth have been identified—some are relatively innocuous bacteria commonly found in the reproductive tract of pregnant women, some are more typically associated with the oral microbiota, while others are only found in women with abnormal vaginal flora, explains Professor Keelan. ‘However, the bacterial species most commonly isolated from the amniotic cavity with preterm birth are *Ureaplasma*, tiny microorganisms present in around 50% of pregnant women. It remains a mystery why, in some women, vaginal *Ureaplasma* colonisation—which is usually harmless and undetected—transforms into intraamniotic infection. However, it is known that the ascension process does not occur until the second half of pregnancy, which means that we have the first 20 weeks to give any antibiotic therapy and prevent the complications of IUI-associated PTB.’

**Identifying Women at Risk**

Before one can treat the infections leading to PTB, one has to know who to target for treatment. Most women with *Ureaplasma*, for example, will not deliver early, while the ‘classic’ vaginal microbial abnormalities are poorly predictive of preterm delivery, and only present in a small percentage of women. Antibiotics need to be administered conservatively in pregnancy in order to prevent antibiotic resistance and also to preserve the ‘healthy’ bacterial environment that is required for normal fetal development. Professor Keelan and his colleagues, based
at King Edward Memorial Hospital in Perth, Western Australia, have been developing a test to identify the presence of high risk vaginal microbiota in mid-pregnancy based on molecular profiling of bacterial DNA at 18–20 weeks’ gestation. Through this microbial profiling, they believed it should be possible to identify and treat women who screen positive with appropriate antimicrobial drugs or probiotics to prevent PTB and its consequences. This novel test employs polymerase chain reaction (PCR) to detect and quantify a specific set of bacterial DNA sequences in self-collected vaginal swabs. Excitingly, their research has identified a specific genotype of *Ureaplasma* that is associated with increased risk – but only when present in combination with two other bacterial species. Based on these observations, the team has developed a new microbial DNA test that is far more sensitive and specific than standard microbiological methods for identifying women destined to deliver preterm. Moreover, the test allows the identification and treatment of women positive for *Ureaplasma* at high risk of PTB, for the first time. The team plans to commence trials to prevent IUI-driven PTB based on screening of women using the bacterial DNA test in the near future.

**Novel Antibiotic Therapy Against PTB**

As it stands currently, trials that have given antibiotics to women to prevent PTB have had limited success. There are several reasons why antibiotics have been ineffective: many antibiotics do not cross the placenta at sufficient levels to kill particular bacteria; antibiotics were administered too late in the pregnancy to prevent infection; antibiotics were ineffective against the specific organisms associated with IUI; and finally, there has been little attempt to mitigate inflammation in the fetus or amniotic cavity.

The team has identified a new antibiotic, called solithromycin, which has great potential for preventing and treating perinatal infections. The drug was created by a small USA-based biotech company, Cempra Inc., by adding three molecular groups to a well-known macrolide antibiotic – clarithromycin – in order to change the uptake of the drug and its ability to overcome resistant bacteria. Professor Keelan’s team discovered that solithromycin is unique in that it can cross the placenta much more efficiently than other macrolide antibiotics. Using a pregnant sheep model, they found that solithromycin was capable of reaching effective concentrations in the fetal circulation and amniotic fluid within four hours of a single maternal dose. These findings were subsequently confirmed in a human placental model. Solithromycin is active against all the major microorganisms associated with PTB, including *Ureaplasma*, making it an effective treatment for IUI that can be administered orally. It is also highly effective against a wide variety of antibiotic resistant bacteria, and has the additional benefit of being an effective anti-inflammatory agent.

Professor Keelan explains the potential of the new drug: ‘We’ve done some measurements..."
in sheep and human placentas and estimate that the crossover is about 50% compared to just 2–4% for the older antibiotics. It’s also 10 to 100 times stronger, and works on antibiotic resistant bugs. It really does open the door for the effective treatment of a range of intrauterine infections and offers significant advantages over existing antibiotics.

Their next step is to carry out preliminary studies in pregnancy to assess the degree of fetal exposure and also solothymycin’s effects of vaginal bacteria. In an unexpected turn of events, work on solothymycin was recently halted after the approval of the drug for the treatment of community-acquired pneumonia was blocked by the FDA on safety grounds. Professor Keelan and his team are waiting to hear from the manufacturer on their strategy going forward before progressing their studies.

Exploring Cytokine Suppressive Anti-inflammatory Drugs

While intrauterine infection is undeniably an important and preventable cause of PTB, inflammation without signs of infection is almost twice as common in preterm deliveries. Inflammation is a normal part of labour and delivery, but in some pregnancies, it is excessive and causes early birth. The triggers of so-called ‘sterile inflammation’ may include oxidative stress, placental breakdown and senescence, uterine stretch, and hormones produced by maturing fetal organs. The answer to effectively treating excessive inflammation may lie in a group of drugs known as cytokine supressive anti-inflammatory drugs (CSAIDs). Cytokines are a broad group of proteins that act as signalling molecules to regulate immune responses and inflammation. Cytokines and bacterial products activate common signalling pathways inside cells that turn on inflammation-related genes and activate cellular inflammation. One of the key pathways that coordinates this response is called the NF-κB pathway – activation of two enzyme complexes (IKK2 and TAK1) is critical in the regulation of this pathway.

Professor Keelan and his colleagues have examined the potential use of CSAIDs to target pathways that lead to intraamniotic inflammation and PTB, using a variety of in vitro and ex vivo models. After screening many different compounds and performing concentration-response studies, they identified two drugs that appear to be particularly effective in blocking activation of inflammation-associated genes and which are likely to be safe and effective in pregnancy. The first is an IKK inhibitor called TPCA-1. This drug, which has been investigated in several animal models of inflammation, inhibited the expression of multiple inflammation-associated genes in human placental cells stimulated with bacteria. Another drug, (S2)-7-Oxozeaenol (OxZ, a TAK1 inhibitor), was found to be even more effective than TPCA-1 in blocking placental inflammation. Importantly, neither drug caused placental cell death, which can be a side-effect of some anti-inflammatory drugs.

In a recently published preclinical evaluation, Professor Keelan and his team found that TPCA-1 and OxZ were both effective in reducing inflammation in human extraplacental membranes delivered following spontaneous preterm labour. Production of prostaglandins – lipid-derived factors produced in response to inflammation which cause the uterus and trigger contractions – was significantly inhibited by the two drugs. TPCA-1 and OxZ were equally effective in membranes delivered with or without evidence of IUI, suggesting that they could be used to block the effects of both sterile and infection-driven inflammation. ‘This is important,’ says Professor Keelan, ‘because clinically it is difficult to diagnose an IUI until after delivery, when placental histology and microbiology can be performed and the diagnosis made’.

The effects of two CSAIDs were explored in a sheep model of endotoxin-driven chorioamnionitis. Both drugs (administered via intraamniotic injection) prevented signs of inflammation in the fetal lung and fetal membranes, lowering cytokine concentrations in both the fetus and the mother and levels of prostaglandins and cytokines in amniotic fluid. Studies are now underway in rodent models to confirm that the drugs prevent PTB and improve fetal outcomes at safe doses. Additional studies are required to assess fetal toxicity at high and/or chronic doses, and determine the extent of passage across the placenta. These drugs, if they are introduced clinically, would be used in conjunction with antibiotics to ensure that an underlying infection is not allowed to flourish once the inflammation has been suppressed.

Next Steps in PTB Research

The team are also working on the efficacy and safety of a novel peptide drug called ryvela as a therapeutic strategy for protecting the fetus from IUI. Ryvela is an allosteric antagonist that partially blocks the activity of the IL-1 receptor. IL-1 is a key cytokine responsible for amplifying inflammation in the pregnant uterus and neonate. Ryvela suppresses IL-1 driven inflammation in diseases such as inflammatory bowel disease and ischemic retinopathies, and in mouse models has shown remarkable results in stopping PTB and preventing inflammation-driven damage in the fetus. The team must now determine a number of factors: whether or not ryvela can cross the placenta, how efficacious it is in humans, the dose and mode of administration that best reduce inflammation with minimal side effects, and the short and long term benefits of the drug on fetal organ development and physiological function. The team, which involves collaborators across Australia and Canada, will use a number of animal and human tissue models to answer these questions.

The potential advantages to ryvela are numerous. Pilot studies have demonstrated that the drug has greater specificity, pharmacological stability, potency and efficacy than its counterparts that are currently sold and marketed around the world. Its selectivity reduces the risk of adverse effects and its increased bioavailability means it can be administered through minimally invasive routes. It is also likely to be less costly than similar available drugs. The data from this study will inform the design of clinical trials of antenatal ryvela administration. The team hopes that this will result in another anti-inflammatory option to complement antibiotic therapy directed at fighting IUI and PTB.

While the above drugs are being investigated for the treatment of preterm labour and protection of the neonate from the harmful effects of perinatal inflammation, Professor Keelan also has plans to explore alternative therapies for chronic administration throughout the second half of pregnancy. For example, the team is currently considering several plant-based dietary supplements (nutraceuticals) which have proven anti-inflammatory effects, to see if these effects can be replicated in the human placenta at feasible doses. This approach could be a safe and patient-friendly way of suppressing inflammation during pregnancy in women at high risk. The results of the preliminary studies will be known by the end of 2017.
Meet the researcher

Professor Jeff Keelan

School of Women’s and Infants’ Health
University of Western Australia
Perth, Australia

Professor Jeff Keelan is Professor of Obstetrics and Head of Laboratories at the School of Women’s and Infants’ Health at King Edward Memorial Hospital (KEMH), University of Western Australia. He is also Director of the Women and Newborn Health Research Network at KEMH, Deputy Director of the Women and Infants Research Foundation, Scientific Director of the Western Australia Preterm Birth Prevention Initiative and Co-Chair of the WA Microbiome Consortium.

After finishing his undergraduate degree in Applied Biology in the UK, Professor Keelan went on to complete a PhD in Obstetrics and Gynaecology at the University of Auckland. Throughout his career, he has received numerous distinctions. He was an invited member of the Faculty of 1000 Biology in 2007, became a fellow of the Society of Reproductive Biology in 2011 and was named on the NHMRC External Assessor ‘Outstanding Contribution’ Honour Roll in 2014. He teaches undergraduate and postgraduate courses in the areas of human reproductive biology, pharmacology and obstetrics. His current research is centred on the pharmacological treatment of intraamniotic infection and inflammation, nanoparticle-based drug delivery in pregnancy and the intrauterine microbial and endocrine environment.

CONTACT

E: jeff.keelan@uwa.edu.au
T: (+61) 8 6458 1880

KEY COLLABORATORS

Professor John Newnham, University of Western Australia
Dr Matthew Payne, University of Western Australia
Associate Professor Matthew Kemp, University of Western Australia
Dr Demelza Ireland U University of Western Australia WA
Dr Peter Mark, University of Western Australia
Professor Brendan Waddell, University of Western Australia
Professor David Olson, University of Alberta
Professor Sarah Robertson, Robinson Institute, University of Adelaide

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With antibiotic resistance rapidly emerging among many important bacterial pathogens, it is imperative that new classes of antimicrobials are developed. Professor Roy Robins-Browne and his team at The University of Melbourne are taking a novel approach to antimicrobial therapy in developing a strategy to combat antibiotic-resistant bacteria.

According to Sally Davies, the Chief Medical Officer for England, the ‘antibiotic apocalypse’ may already be upon us. It has been estimated that every year, 50,000 people in Europe and the United States die from infections that antibiotics have lost the power to treat. Novel classes of antimicrobials are urgently required, and studying bacterial virulence factors could lead to the development of entirely new classes of antimicrobials.

Professor Roy Robins-Browne and his research team at The University of Melbourne in Australia are working to understand how virulence factors are switched on and what can be done to limit this functionality. Their findings are important in helping to identify virulence gene expression and potentially to generate novel antibacterial molecules that inhibit the activation of virulence.

Untreatable Infections in a Post-Antibiotic Era

Dame Sally Davies has assumed a global leadership role in tackling antimicrobial resistance (AMR) and has described the threatened loss of antibiotics as being on a par with terrorism and climate change. To give a startling example, there are an estimated 480,000 cases a year of multi-drug resistant tuberculosis, 40% of whom will succumb to their disease. Moreover, a UK Government Review has estimated that by 2050, deaths from untreatable infections will exceed 10 million per year at a cumulative cost of more than $100 trillion.

As well as offering a therapeutic cure for infections, antibiotics are also essential in preventing certain infections. Without these important drugs, surgery would once again become life-threatening. Furthermore, organ transplant recipients rely on antibiotics for their survival because their immune system cannot fend off infections while it is suppressed by the drugs they take to prevent organ rejection. Even childbirth could become much more dangerous, where it was historically common for women to succumb to postpartum sepsis.

Seven decades on from the introduction of penicillin, the prevalence of multi-drug resistant micro-organisms has skyrocketed. This may lead to a scenario where general practitioners and secondary care clinicians are unable to treat minor wound infections. Even simple urinary tract infections caused by *E. coli* or *Klebsiella pneumoniae* may result in fatal outcomes if the bacteria are resistant to ‘last-resort’ antibiotics, such as carbapenems.

As a startling example, in 2016, a woman returned to the USA from a trip to India, harbouring a new resistant pathogen called New Delhi Metallo-Beta-Lactamase-Producing *Klebsiella pneumoniae*. Antimicrobial susceptibility testing indicated that the isolate was resistant to more than 25 different antibiotics, including all aminoglycosides and polymyxins, and even showed intermediate resistance to tigecycline (a drug developed in response to emerging antibiotic resistance). The patient died from septic shock, simply because there were no antibiotics that could stop her infection from progressing. This scenario is likely to be a taste of things to come.

The Ability of Many Bacteria to Cause Disease is Genetically Regulated

‘Many bacteria that cause disease only produce the factors they need for this when they are at the site of infection in their hosts,’ explains Professor Robins-Browne. Bacteria do this by sensing where they are in the body and then activating the genes they require to colonise the site of infection and produce the factors they use to cause disease. This process of specific gene activation often involves bacterial proteins – called transcriptional regulators – that bind to DNA in order to activate relevant genes. If this process can be stopped (Professor Robins-Browne describes this as ‘disarming virulence’), the bacteria will remain harmless.

‘Advantages of this type of approach to treatment compared with traditional antibiotic
therapy are that (1) unlike all current antibiotics, this process does not interfere with bacterial growth, and therefore is far less likely to lead to resistance, and (2) this type of treatment does not affect the harmless, commensal, “good” bacteria in our bodies, and therefore should have few side effects,” says Professor Robins-Browne.

*Citrobacter rodentium* as a Model for *E. coli* Virulence

Established members of the attaching and effacing (A/E) family of pathogens responsible for human disease include enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC). EPEC commonly causes infections in infants, resulting in life-threatening diarrhoea, while EHEC is less common, but can cause serious foodborne outbreaks of bloody diarrhoea and the potentially fatal, haemolytic uraemic syndrome.

In order to evoke A/E lesions, bacteria need a particular suite of genes termed the locus of enterocyte effacement (LEE). This virulence factor allows bacteria to bind avidly to intestinal epithelial cells. Professor Robins-Browne and his colleagues used a mouse model of infection where a bacterial species, *Citrobacter rodentium* (*C. rodentium*), infects mouse gut epithelial cells in a fashion similar to that of EPEC/EHEC infection of humans. The research team aimed to identify novel colonisation factors of *C. rodentium* using a mutant library of *C. rodentium* in mice.

Intriguingly, they discovered a particular gene that they called regA, which is very similar to perA of human EPEC and other genes encoding members of the AraC family of transcriptional regulators. These regulatory proteins facilitate the production of specific messenger RNA from a DNA template within cells – a process known as transcription. The transcription of DNA to produce messenger RNA is an essential step in the synthesis of all proteins, including enzymes and structural proteins that are essential for life.

Professor Robins-Browne and his team performed a series of experiments to elucidate the properties of regA. When the team deleted this gene in *C. rodentium*, they observed that the pathogen’s ability to colonise mouse intestines was greatly reduced. They also showed that the RegA protein strongly stimulates the transcription of several genes involved in virulence. Thus, the scientists demonstrated that RegA is an important virulence regulator in *C. rodentium*, and is similar to AraC-like virulence regulators in important human intestinal pathogens, such as EPEC, *Salmonella*, *Shigella* and cholera bacillus. Having identified this key virulence regulator, further studies were required to determine if its action could be inhibited by drug-like chemicals.

**Regacin – a Prototypical Inhibitor of Virulence**

Because the expression of virulence by many intestinal pathogens is tightly regulated at the transcriptional level, Professor Robins-Browne and his colleagues decided to focus on a strategy to target pathogens with drugs that interfere with virulence activation. Many Gram-negative intestinal pathogens including *E. coli*, *Salmonella*, *Shigella*, *Yersinia* and *Vibrio cholerae*, require virulence activators, equivalent to RegA, to cause disease.

Professor Robins-Browne and his team investigated the *C. rodentium* virulence...
regulator, RegA – the regulatory protein encoded by the regA gene – to see if it could be a potential drug target. By searching through a library of small molecules and using chemical optimisation, they were able to identify two small molecules that specifically prevented RegA from activating its target genes in C. rodentium, consequently reducing the expression of several genes required for virulence. These include a gene for another transcriptional regulator that is required to activate genes of the LEE.

In addition to this ground-breaking work, the team performed biophysical, biochemical, genetic and computational analyses, which showed that the inhibitors of RegA act by binding directly to RegA in a way that prevents it from binding to its DNA targets. They named the more potent of these compounds: ‘regacin’. When mice were fed regacin, either 15 minutes before or up to 12 hours after orally infecting them with C. rodentium, the team noticed a dramatic reduction in the numbers of this bacterium in the intestines of the mice. These findings demonstrated that chemically inhibiting RegA’s ability to bind to DNA is a viable strategy towards the development of drugs that inhibit virulence regulation.

Discovering a CfaD Inhibitor in E. coli

A challenge for Professor Robins-Browne and his team was to translate their work with C. rodentium to relevant pathogens of humans. Enterotoxigenic E. coli (ETEC) is one of the most common causes of infectious diarrhoea in children who live in developing countries, and is also notable for its ability to cause traveller’s diarrhoea. Traveller’s diarrhoea is a common problem, affecting millions of tourists each year, thus creating a large economic burden.

To cause disease, ETEC must first colonise the human gut epithelium. Once it does this, it delivers one or more toxins to the cells. CfaD is an AraC-like transcriptional regulator of ETEC, which plays an essential role in virulence gene expression in these bacteria. Might this mechanism be similar to that observed in earlier studies by Professor Robins-Browne and colleagues in their mouse model of infection?

The team analysed an ETEC strain that was grown under different conditions and found a set of genes that were regulated by CfaD. The team characterised a number of these genes, and were able to figure out how CfaD mediates their activation. They found that the expression of a cluster of known virulence genes, called the etpBAC operon, was activated by CfaD. This highlighted the importance of CfaD in inducing the production of a variety of surface molecules (called adhesins) that help bacteria bind to cells, thus allowing ETEC to colonise its host, and avoid being washed away by intestinal fluids and gut motility.

Finally, the team searched through a commercial small molecule library and found a molecule, they called CH-1, that specifically disrupts the regulatory function of CfaD. They then went on to discover a second compound, called CH-2, which had greater potency. The critical importance of CfaD in the control of ETEC virulence suggests that this has great potential as an authentic target for new types of drugs to combat ETEC infections.

A New Class of Antibiotics?

Many scientists now fear that mankind is standing on the precipice of an antibiotic doomsday scenario. However, Professor Robins-Browne’s team’s research has identified mechanisms through which bacterial virulence can be switched on, providing hope that these key molecular pathways may be targeted by future drugs.

The translational nature of the research activities of Professor Robins-Browne and colleagues is exemplified by their development of Travelan®, a commercial product that uses antibodies from cattle to prevent traveller’s diarrhoea. Now, by employing an ingenious molecular strategy, his team has shown that it is possible to subvert previously successful pathogenic mechanisms that result in significant morbidity.
Meet the researcher

Professor Roy Michael Robins-Browne
Department of Microbiology and Immunology
The University of Melbourne
Victoria
Australia

Professor Roy Robins-Browne was awarded his medical degree and PhD by the University of the Witwatersrand in South Africa. In 1982, he joined the Department of Microbiology at the University of Melbourne, Australia, as an Associate Professor. He went on to become a full Professor in 1996, and between 1998 and 2013, was Head or Deputy Head of the Department. In addition to his post at the University of Melbourne, he is also currently the co-leader of the Infectious Diseases and Microbiology Group at the Murdoch Childrens Research Institute at the Royal Children’s Hospital in Melbourne. The main focus of Professor Robins-Browne’s research is the pathogenesis of bacterial infections of the gastrointestinal tract, with a particular focus on \textit{E. coli}. He has authored or co-authored more than 300 peer-reviewed papers on this and other topics concerning bacterial pathogens. He has also developed a commercial product for the prevention of traveller’s diarrhoea. Apart from his research, Professor Robins-Browne has played a central role in coordinating microbiology teaching for medical, biomedical and science students at University of Melbourne.

CONTACT

E: r.browne@unimelb.edu.au
T: (+61) 3 8344 8275

KEY COLLABORATORS

Dr Ji Yang, Peter Doherty Institute, The University of Melbourne
Dr Marija Tauschek, Peter Doherty Institute, The University of Melbourne
Professor Michael Parker, St. Vincent’s Institute of Medical Research, and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne
Dr Jessica Holien, St. Vincent’s Institute of Medical Research

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Azitra Inc. is a company with one purpose – to deliver innovative, affordable and sustainable treatments for skin disease. How are they doing this? Through utilising the potential of the microbiome. The microbiome is the combined genetic makeup of the microorganisms present in a particular environment, in this case, the skin. The microbiome comprises billions of bacteria, which play a critical role in human health, particularly in inflammation and immune regulation. By manipulating the microbiome, Munivar and Whitfill have engineered a novel bacterial vehicle capable of secreting therapeutic treatments for dermatological conditions. As Whitfill explains: ‘The microbiome is an explosive field, and we’re using cutting-edge genetic tools to enhance natural and safe bacteria as a platform for novel therapeutics.’

Microbiome start-ups are increasingly being recognised as the way forward by pharmacological companies and funding organisations alike. So far, Azitra has received $3.75 million in funding, including a $2.9 million venture round led by Bios Partners. They also make up part of Peter Thiel’s Breakout Labs programme – a funding and support mechanism for up and coming companies in scientific fields.

This funding allows the team at Azitra to push forward with developing therapies for a wide range of skin conditions, from dry skin and eczema to MRSA and rare genetic conditions. Skin diseases constitute a high level of disease burden and will affect 30 to 70% of individuals at some point in their lives. In spite of this, current treatments for skin diseases are frequently limited to antibiotics, anti-inflammatories or steroids, due to the wide variability of cause and presentation amongst skin disorders. These are often ineffective and short lasting, with potentially negative side effects. Whitfill explains why: ‘Many patients with skin disease are suffering from symptoms that are not fully addressed with current treatment options, and we hope to provide effective therapeutics that treat the root cause of eczema and eventually other skin diseases with our platform.’ The team aim to treat the underlying causes of conditions by introducing therapeutic bacteria into the affected zone, allowing them to colonise the area and begin to correct the microbial imbalance.

Foundations of Novel Drug Delivery

Azitra applies sophisticated microbiological, molecular and genetic techniques to develop patient friendly treatment options for skin conditions. ‘We have created enhanced probiotics for treating skin disease,’ says Whitfill. ‘These probiotics use safe, naturally occurring bacteria from skin, and we have enhanced them to make natural proteins that can have added benefit to bacteria alone.’

The probiotic in question is a reengineered version of the bacterium Staphylococcus epidermis (SE). SE is a bacterium that naturally lives on human skin and is usually non-pathogenic. Certain strains of SE have also shown beneficial immune effects in skin. Although this species of bacteria has historically been very difficult to modify, the Azitra team (in partnership with Jackson Laboratory and Yale University) has optimised methods of manipulating SE to develop a version that can make and secrete therapeutic proteins as it colonises the skin. In this way, it is possible to treat skin diseases that are caused by a deficient protein.

Azitra’s current phase of research and development aims to demonstrate the feasibility and potential of genetically engineered SE as a vehicle for drug delivery.
One of the advantages of this approach is that SE can establish residence on the patient’s skin and continuously deliver therapeutic proteins, removing the inconvenience of frequent reapplications. Another benefit is the modularisation of the delivery system, which will allow genetic targets to be controllably swapped. This will in turn allow the team to apply the technology to a wide range of skin diseases.

Finally, it is a safe and stable treatment option. ‘Our treatment is very safe. Everyone has bacteria on their skin, and in many times their microbiome is imbalanced (i.e. dysbiosis),’ says Munivar. ‘There is evidence that by applying healthy bacteria to the skin, it can restore the dysbiosis and help restore healthy skin. Additionally, these bacteria have been shown to bolster the skin’s immunity and have conferred protective effects to the skin against pathogens and antigens. We have considerable data to show that this approach is safe, but we’ve also engineered our bacteria with additional safety features.’

**Tackling Genetic Skin Disorders**

The main goal of the team is to demonstrate the ability of SE to secrete a range of functional proteins with real world clinical potential in two different skin diseases: atopic dermatitis and Netherton’s syndrome. This approach will demonstrate the flexibility of the system across a range of cellular properties and functions.

Atopic dermatitis, or eczema, is a chronic skin disease which affects 10 to 15% of children under the age of 16. Symptoms include areas of dry skin, itching, redness and, in severe cases, skin thickening, cracking and bleeding. It is associated with high costs and disease burden and there is no known cure. Mutations in the gene encoding filaggrin (a structural protein) cause the skin barrier to become disrupted, leading to inflammation and dysbiosis in the skin. ‘Studies have repeatedly shown that these patients have an imbalanced microbiome, and in the case of eczema, they often have an overgrowth of *Staph aureus*,’ says Whitfill. ‘There is also evidence that our strain of bacteria can kill some strains of *Staph aureus*, which is one of the reasons we chose it as a chassis.’

As topical filaggrin has been shown to restore the skin barrier in mice with eczema, the Azitra team have engineered SE to supplement filaggrin in the skin. Early trials confirm the potential of SE as a treatment for eczema, with patients showing increased
skin water and fat content, lower skin pH and a dramatic reduction in *Staphylococcus aureus* (a bacterium responsible for skin infections). The treatment also colonises the skin over time, a feature that would remove the need for frequent reapplication of emulsifiers and antibiotics.

Netherton’s syndrome is a genetic disorder caused by a mutation in the gene encoding LEKTI – a protein involved in the regulation of desquamation (skin peeling). The condition presents at birth with red skin covered by fine scales, eczema and elevated systemic levels of Immunoglobulin E (an antibody associated with allergic reactions). There are currently very few treatment options for patients suffering from this condition. The Azitra team is engineering SE to secrete LEKTI onto the skin to provide an effective and convenient therapy for individuals with Netherton’s syndrome.

Key Features of the Delivery System

However, the concept of ‘bugs as drugs’ isn’t without its challenges. In order to generate a robust and flexible drug delivery system, the Azitra team must engineer some key characteristics into their design. As well as overcoming the difficulties of creating a stable chassis, there is also the issue of penetrating the stratum corneum (the outermost layer of the epidermis). Challenges that arise include susceptibility to enzymatic digestion, obstacles in solubility and diffusion due to the hydrophobic (water repellent) surface of the skin, and the layers of tightly linked cells that comprise the stratum corneum. The team have optimised human skin importability (how well the protein can permeate the layers of the skin) by engineering cell penetrating peptides (CPP) into SE. The have also maximised bacterial cell exportability, which refers to the ability of the system to export therapeutic proteins to the skin.

As part of Azitra’s ongoing research, the team are characterising the effectiveness of engineered SE at colonising human skin. This involves investigating the penetration depth and length of residence of SE on the skin, and evaluating different doses and application times on human explants (tissues cultured outside the body). The team’s preliminary studies in mice suggest that bacteria will colonise the surface and deep grooves of the stratum corneum and maintain a constant presence for up to a week at a time and have been engineered to rapidly die off for safety and commercial purposes. These data are essential in translating these in vitro studies to human clinical trials, and give valuable information on potential dosing strategies.

Towards the Future

So, what else is in store for Azitra? Moving to a commercial product may be a long journey, but the team has high aims for the remainder of 2017. They are establishing a well-controlled and defined cell bank and are working on developing a final strain that incorporates advanced genetic and functional features. The team is also focusing on the practicalities of developing a real product, a process which involves establishing a stable formulation at room temperature and setting up a manufacturing system that ensures products are consistent and of high quality. In the final quarter of 2017, Azitra is planning an investigator-led safety and proof-of-concept human clinical study in collaboration with their partners at Jackson Laboratory.

The coming year will see the beginning of clinical trials of SE for cosmetic indications, such as rashes, dryness and itching. Alongside this, Munivar and Whitfill will be meeting with the FDA to seek permission to carry out clinical trials of SE for eczema and other clinical indications. From there, the team can begin to carry out the studies needed to get to the clinical trial stage, which is envisioned to begin in late 2018 or 2019.

“We are aiming to go two routes using our platform: a cosmetic application and a pharmaceutical application,” says Munivar. “We are concurrently talking with the FDA for the pharmaceutical approach for treating atopic dermatitis while also heading towards a study as a cosmetic (e.g. dry or itchy skin). In the future, we hope to develop other iterations of this platform for treating additional skin diseases. We are hoping to partner with or out-license parts of this platform to pharmaceutical and consumer health companies.”
Meet the researchers

Travis M Whitfill
Azitra Inc.
400 Farmington Ave
Farmington, CT
USA

Azim Munivar
Azitra Inc.
400 Farmington Ave
Farmington, CT
USA

Travis M Whitfill is co-founder and Chief Scientific Officer of Azitra Inc. After training in biochemistry and microbiology at the MD Anderson Cancer Center and Duke University, Whitfill completed a Master’s of Public Health in molecular epidemiology at Yale University. He is the principal investigator on numerous federally funded grants and partnership discussions, and has raised $3.8 million in funding to date. Whitfill is also a research scientist at Yale and senior analyst and partner at Bios Research, a venture capital focused on therapeutics and medical technology. He has been presented with several awards including the Thiel Foundation Grant and SCCM Top Paper Award and the IMSH/SAEM Top Ten Papers in 2015 and 2016.

Azim Munivar is co-founder and Chief Executive Officer of Azitra Inc. He holds a BS in both Engineering (focusing on Chemical Biomolecular Engineering) and Economics (specialising in Finance and Healthcare Management) from the University of Pennsylvania. He also completed the Jerome Fisher Program in Management and Technology and has recently completed his medical degree at Yale University.

CONTACT
E: travis@azitrainc.com
T: (+1) 203 599 0700
W: https://www.azitrainc.com/

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KEY COLLABORATORS
Julia Oh PhD, Jackson Laboratory for Genomic Medicine
Leonard Milstone MD, Yale University
Jeff Bose PhD, University of Kansas Medical Center
Although pharmacological treatments still form the vanguard of our improved healthcare in the twenty-first century, more focus is being placed on alternative solutions that can provide huge benefits without the need for costly drug development. Moving towards a more person-centred approach to treatment, which considers all aspects of a patient’s health and well-being rather than a single set of symptoms, is starting to be viewed as an essential next step in the development of modern health care. In this section of the edition, we showcase the work of several scientists who are working towards this goal.

First, we meet the HealthMeasures team, a group of NIH scientists dedicated to using modern communication technologies to develop new ways to assess and record our health and wellbeing. Using a set of four novel measurement tools, they hope to allow researchers and clinicians access to a wealth of data from different people with many different health concerns. The HealthMeasures tools enable researchers to view a cross-section of health outcomes including data on physical, mental and social health throughout a person’s life, using advanced and flexible computer tests that choose questions based on previous responses to give an accurate score in less time, reducing the burden for the patient. This clever application will allow clinicians and researchers to monitor a patient’s progress over time and assess the wider effectiveness of various treatment programmes. This revolution in data-driven smart healthcare could potentially have huge benefits for patients and will allow clinicians to make better use of the healthcare strategies we already have in place.

Next, we feature the Washington University Neurofibromatosis (NF) Center, where Dr David Gutmann and his team are taking many different approaches to treat this devastating genetic condition that affects one in 3000 people. In addition to conducting ground-breaking research and offering the latest pharmaceutical treatments, the NF Center also provides complementary care programs to boost their patients’ health and overall wellbeing. These programs include Beat NF, which harnesses the power of jazz to help pre-school children develop their social and motor skills, and Teen NF, which helps young people to face the challenges of being a teenager with NF.

The smart use of technology and taking a patient-centred view of how people engage with treatment in rural communities has allowed our next featured researcher to develop a radical new strategy to help stroke survivors recover the use of their limbs. By harnessing the power of video games, Dr Mary Galea at the University of Melbourne and her team have developed a rehabilitation system to help stroke survivors recover the use of their limbs. The addictive power of the video game motivates the patients to participate and engage in various movements that are important for recovery but can be physically difficult to maintain. The team further developed their technology so that it could be used at home with on-line remote support, enabling patients to engage in recovery from the comfort of their living rooms.

Also utilising gaming technology to help stroke survivors recover their motor function is Dr Fei Hu and his team at the University of Alabama. His team integrates virtual reality, robot and motion capture technology, to develop a low-cost solution that is both highly effective and more accessible than current rehabilitation methods. The research of both Dr Galea and Dr Hu will enable greater access to treatment in rural communities that are far from hospitals, drastically improving outcomes for survivors of stroke.
**HealthMeasures: A National Person-Centered Assessment Resource**

HealthMeasures is a novel set of four measurement systems that assess physical, mental, and social health, symptoms and wellbeing, along with physical function, social function, sensory function, and cognitive function across the human lifespan. These tools allow researchers and clinicians to compare individuals and groups across diseases and conditions.

**Introduction to HealthMeasures**

HealthMeasures consists of four comprehensive health outcome measurement systems including PROMIS® (Patient-Reported Outcomes Measurement Information System®), Neuro-QoL (Quality of Life in Neurological Disorders), ASCQ-MeSM (Adult Sickle Cell Quality of Life Measurement Information SystemSM), and NIH Toolbox® (NIH Toolbox for Assessment of Neurological and Behavioral Function®) that assess wellbeing and function. HealthMeasures aims to assess domains that are meaningful to people in their daily lives in both clinical and research settings. The assessment tools are brief and easy to understand, psychometrically sound and interpretable, and scores can be expressed on a common scale. They cover individuals from early childhood to older adulthood and can be used across various diseases, languages, literacy levels, and ethnic groups. HealthMeasures can provide valid, clinically relevant data for use in clinical care, research, or health policy development.

**Flexible Administration**

The methodology underpinning the development of HealthMeasures is Item Response Theory (IRT). IRT enables the creation of item banks (large groups of questions measuring a single domain, such as fatigue). This approach allows for assessment using brief, fixed length forms or more flexible computer adaptive tests (CATs). CATs allow for a tailored, computer-assisted assessment in which questions are chosen intelligently, based on responses already given. CATs produce brief and accurate scores with little burden to the person being tested.

**A Common Metric**

A major benefit conferred with the use of HealthMeasures is the reporting of results on a common metric, or common scale. Because different fixed length short forms and CATs from the same item bank produce scores on the same scale, HealthMeasures enables comparing your results to those of others. In addition, measures developed outside of the HealthMeasures family can often be linked to a standardized HealthMeasures metric with “PROsetta Stone®” ([www.ProsettaStone.org](http://www.ProsettaStone.org)). This enables one to ‘cross talk’ between measures by placing them on the same metric.

**Applications of HealthMeasures**

The applications of HealthMeasures are wide ranging. Healthcare practitioners can use these systems to monitor the symptoms and functioning of their patients over time, as well as evaluate the quality of the care they provide. These measurement tools can be used in clinical, comparative effectiveness, epidemiological, or health services research. Within an educational setting, HealthMeasures may also be used for screening, determining eligibility for services such as special education, or monitoring progress.

To find more information, download copies of the measures, and see demonstrations of the NIH Toolbox, go to [http://www.HealthMeasures.net/search-view-measures](http://www.HealthMeasures.net/search-view-measures).
PROMIS®: PATIENT-REPORTED OUTCOMES MEASUREMENT INFORMATION SYSTEM®

PROMIS is a unique measurement system developed by researchers at multiple academic institutions and the National Institutes of Health to assess health and wellbeing in adults and children from the general population as well as those living with chronic conditions.

Person-Centered Measurement Systems

PROMIS (Patient-Reported Outcomes Measurement Information System) is a comprehensive, accurate, flexible, and accessible set of tools to measure self-reported physical, mental, and social health, including symptoms, functioning, and general perceptions of health and wellbeing, in people ages 5–90. PROMIS is a result of great efforts to advance the science of measuring patient-reported health status and has been developed and validated using state of the art methods to be psychometrically sound and relevant across diverse conditions. PROMIS measures have greater precision (with the administration of fewer items) and a larger range of measurement than the majority of conventional measures. This decreases respondent burden, lowers floor and ceiling effects, and increases power in studies without increasing sample size. Featuring more than 200 measures for adults and almost 100 measures for pediatric populations, PROMIS can be used to assess symptoms and functions across lifespan, disease state, and demographic groups.

PROMIS Measures

PROMIS measures are available in short forms (made up of a fixed set of two to ten questions per domain), profiles (fixed collections of short forms from multiple domains), and as computer adaptive tests (CATs; in which questions are dynamically selected from the item bank based on the respondent’s previous answers). Measures include physical health (e.g., physical function, pain, fatigue, sleep, sexual function, symptoms), mental health (e.g., depression, anxiety, anger, positive affect, cognitive function, substance use, self-efficacy for management of chronic conditions, stress experiences), and social health (e.g., ability to participate in social roles and activities, satisfaction with participation in social roles and activities, social support, isolation, family relationships). Learn more about available PROMIS measures at www.HealthMeasures.net/PROMIS.

Development and Validation of Comprehensive Measures

PROMIS was constructed and validated using rigorous methods with more than 50 research protocols and over 60,000 people contributing data. Measurement development methods included a comprehensive literature review of existing measures, focus groups testing existing and de novo questions with diverse patient groups, cognitive interviews, and measurement expert review. Psychometric testing confirmed the factor structure of each health domain and allowed for analysis at the item and bank level. Substantial qualitative and quantitative evidence supports the validity of PROMIS measures.

Interpretable Scores and a Common Metric

One of the major benefits of PROMIS is the interpretability of scores and the ability to translate other health outcome measures to the PROMIS metric so comparisons can be made (www.ProsettaStone.org). PROMIS uses standardized scores known as T-scores which can be evaluated against a reference population (which is usually the U.S. general population). Using this metric, a score of 50 is the average of the reference population with a standard deviation of 10. Therefore, a score of 60 means the individual is one standard deviation above the reference population. Depending on the domain, this could be desirable or undesirable as high scores indicate more of the concept being measured. For example, a high fatigue score indicates high levels of fatigue whereas a high physical function score indicates better physical function.

Interpreting PROMIS® T-Scores

*Please see web link for details on interpreting PROMIS® T-scores. Within a given condition or PROMIS domain, thresholds may differ.
NEURO-QoL: QUALITY OF LIFE IN NEUROLOGICAL DISORDERS

Sponsored by the National Institute for Neurological Disorders and Stroke, Neuro-QoL is a measurement system designed to evaluate and monitor the physical, mental, and social effects experienced by adults and children living with a range of neurological conditions.

Evaluating Function and Wellbeing Across Conditions

Neuro-QoL (Quality of Life in Neurological Disorders) is a psychometrically sound measurement system developed and validated for neurological disorders using state of the science methods by researchers at Northwestern University and other academic institutions. Neuro-QoL is a set of measures, many of which are linked to PROMIS (Patient-Reported Outcomes Measurement Information System), that assess and report on symptoms, function, and wellbeing across a range of common neurological conditions, including multiple sclerosis, stroke, Parkinson’s disease, epilepsy, amyotrophic lateral sclerosis (ALS), and muscular dystrophies in people ages 8 to 90. Neuro-QoL measures can be administered via paper, web, and app-based versions and are designed to minimize the burden on the patient.

Adult and pediatric versions are available and can be completed by a proxy responder when necessary. The measures can be used alongside PROMIS or NIH Toolbox instruments that assess other aspects of health and function. Learn more about Neuro-QoL at www.HealthMeasures.net/Neuro-QoL.

Holistic Person-Centered Assessment

Because living with a neurological disorder can affect numerous facets of an individual’s life, Neuro-QoL evaluates aspects of physical, mental, and social health.

Physical health measures include lower extremity function and mobility, upper extremity function and fine motor skills, fatigue, and sleep disturbance. Mental health measures assess anxiety, depression, positive affect and wellbeing, emotional and behavioral dyscontrol, cognitive function, communication, and stigma. Social measures include the ability to participate in social roles and activities and satisfaction with social roles and activities. This includes one’s degree of involvement and fulfillment in usual roles, responsibilities, and activities within society.

Development and Validation of a Standardized Measurement System

The Neuro-QoL development process was a collaborative multi-site research initiative. After an extensive literature review, researchers undertook two phases of in-depth expert interviews, as well as patient and caregiver focus groups and interviews. Through an iterative process, 17 content areas for adults and 11 content areas for children were selected. Measures were calibrated against the U.S. general population and clinical samples using Item Response Theory and validated in adult and pediatric clinical samples.

Interpretable Scores

Neuro-QoL uses standardized scores known as T-scores that can be evaluated against a reference population. A score of 50 is the average of the reference population with a standard deviation of 10. Therefore, a score of 60 means the individual is one standard deviation above the reference population. High scores indicate more of the concept being measured which can be desirable (e.g., ability to participate in social roles and activities) or undesirable (e.g., anxiety).
ASCQ-MeSM: ADULT SICKLE CELL QUALITY OF LIFE MEASUREMENT INFORMATION SYSTEMSM

ASCQ-Me is a patient-reported outcome measurement system developed to evaluate the physical, mental, and social wellbeing of adults with sickle cell disease. Sponsored by the National Heart, Lung, and Blood Institute, ASCQ-Me can be used by physicians and researchers to support care for affected patients.

Monitoring Effects of Sickle Cell Disease

According to the Centers for Disease Control and Prevention, sickle cell disease (SCD) affects approximately 100,000 individuals in the U.S. and 300,000 individuals worldwide. The Adult Sickle Cell Quality of Life Measurement Information System (ASCQ-Me) is a psychometrically sound and content valid measurement system for monitoring symptoms and function in those affected by the disease. ASCQ-Me measures are appropriate for individuals ages 18 to 90 living with SCD.

As well as documenting the effects of SCD on adult functioning and wellbeing, ASCQ-Me can support treatment planning, clinical and health services research, evaluate the effectiveness of therapies, and inform programs to improve delivery of healthcare. It provides a comprehensive view of health and function when used with PROMIS or the NIH Toolbox.

Flexible Administration

ASCQ-Me measures include short forms (fixed set of five items per domain), checklists (fixed sets of descriptive items) and computer adaptive tests (CATs, in which items are dynamically selected based on the respondent’s previous answers). The measures are freely available and can be delivered on paper or by computer.

Holistic Person-Centered Assessment

As well as assessing the severity of pain, ASCQ-Me measures the impact of the severity and unpredictability of pain on the person’s functioning and the number and frequency of pain episodes. It also evaluates the effect of SCD on getting to sleep and staying asleep, and the severity of stiffness a person experiences. A SCD Medical History Checklist is available to record symptoms, treatments, organ and bone damage, and the quality of healthcare the individual has received. Mental and social health are taken into account through measures of the effects of SCD on social functioning and the emotional impact of SCD in terms of worry, loneliness, and depression.

Development and Validation of ASCQ-Me

ASCQ-Me was developed and validated by scientists with state of the art methods to measure wellbeing and to be complementary with physiological measures of disease severity. Development included a comprehensive literature review to identify important aspects for assessment, focus groups to create a suitable conceptual framework, and field testing of the items through geographically diverse U.S. clinics. Psychometric testing confirmed the factor structure and allowed for analysis at the item and bank level.

The validity of ASCQ-Me is based on substantial qualitative and quantitative evidence. Content validity is supported by structured interviews with patients and experts and is grounded in the life experiences documented in participant interviews.

Learn more about ASCQ-Me at www.HealthMeasures.net/ASCQ-Me.
NIH Toolbox is a diverse set of measures which can be used to assess cognitive, sensory, motor, and emotional function across various study designs, settings, and conditions. It is available as an iOS app for an iPad.

Comprehensive Measures Straight to your iPad

The NIH Toolbox is a multidimensional set of brief measures that assess health and function across four domains: cognition, emotion, motor, and sensation. Each domain can be assessed using a 30-minute battery or any of the NIH Toolbox’s over 100 measures can be used alone. Because it is non-disease specific and normed for ages 3 to 85, the NIH Toolbox can be used within numerous study designs with a particular emphasis on measuring outcomes in longitudinal epidemiologic studies and prevention or intervention trials. This suite of measures is cutting edge, psychometrically sound, cost efficient, and can be administered with minimal training. Measures can be utilized in a variety of settings, among individuals in the general population, and those living with chronic conditions.

Learn more at www.HealthMeasures.net/NIHToolbox.

Wide Array of Assessment

Through the utilization of Item Response Theory and computer adaptive testing, measures can be brief while remaining precise and valid. The NIH Toolbox contains two types of measures: performance based tests of function and self- or proxy-report measures.

The cognition battery assesses the mental processes involved in gaining knowledge, comprehending, communicating, problem solving and planning, and executing complex behaviors. Individual measures include attention, episodic memory, working memory, language, executive function, and processing speed. The cognition battery also includes summary scores, namely a cognitive function composite score, a fluid cognition composite score, a crystallized cognition composite score, and an early childhood composite score.

The emotion battery evaluates four domains: psychological wellbeing, stress and self-efficacy, social relationships, and negative affect. The self-report battery is recommended for ages 8 and over and there is a parent proxy version for parents of children between the ages of 3 and 12. The constructs assessed by the emotion battery include anger, fear, sadness, positive affect, general life satisfaction, meaning and purpose, perceived stress, self-efficacy, social support, companionship, and social distress. The parent proxy battery includes measures of positive affect, general life satisfaction, positive peer interaction, social withdrawal, peer rejection, empathic behaviors, self-efficacy, perceived stress, sadness, anger, and fear.

The motor battery measures an individual’s ability to use and control muscles and movements. The battery appraises dexterity, strength, balance, endurance, and locomotion. The early childhood battery assesses dexterity, grip strength, standing balance, and endurance.

Finally, the sensation battery assesses sensory input and interpretation. It measures audition, vision, olfaction, pain, and taste. The early childhood sensation battery measures visual acuity and olfaction.

Development and Validation of NIH Toolbox

NIH Toolbox was developed by more than 250 contributing scientists at 80 institutions whose aim was to enhance data collection in large cohort studies and advance neurobehavioral research. More than 16,000 participants were involved in field testing, calibration, and validation. Studies were conducted across age ranges and were statistically compared to gold standard measures. Thanks to a large national standardization study, normative scores are available for targeted, accurate comparison throughout life stages.


**ABOUT HEALTHMEASURES**

**About the Team**

Many researchers, experts, and participants (including patients) contributed to the development of each of the four measurement systems included in HealthMeasures. From 2014–2018, a multi-institutional HealthMeasures team is supported by a cooperative agreement with Northwestern University (# U2C CA186878). This trans-NIH collaboration includes scientific and program support and input from 12 NIH Institutes, Centers, and Offices:

- National Heart, Lung, and Blood Institute (NHLBI)
- National Institute on Aging (NIA)
- National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
- National Institute on Deafness and Other Communication Disorders (NIDCD)
- National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
- National Institute on Drug Abuse (NIDA)
- National Institute of Mental Health (NIMH)
- National Institute of Neurological Disorders and Stroke (NINDS)
- National Institute of Nursing Research (NINR)
- National Center for Complementary and Integrative Health (NCCIH)
- Office of Behavioral and Social Sciences Research (OBSSR)
- Office of Research on Women’s Health (ORWH)

The HealthMeasures team is made up of psychometricians and measurement science experts from Northwestern University, University of California Los Angeles, University of California San Diego, American Institutes for Research, University of Pittsburgh, University of North Carolina, and the National Institutes of Health. After 2018, HealthMeasures will operate as a non-profit, self-sustaining enterprise.

**Measurement Expertise**

The team’s expertise lies in measure development, validation, translation, and expansion as well as the application of measures across a range of settings, diseases, and conditions. Members of the HealthMeasures team are available as consultants and collaborators through contracts and as co-investigators on grants conducting psychometric analyses, consulting on research design, constructing linking tables using PROsetta Stone methodologies, conducting cross-cultural validation and translation of measures, evaluating and interpreting the effects of interventions, applying HealthMeasures in clinical practice, and developing custom assessment technology in clinical, educational, and research settings. HealthMeasures experts routinely provide training and workshops. HealthMeasures assessment platforms are developed and supported by individuals with expertise in informatics, software development, quality assurance testing, and business analysis. This expertise was integral to the development and support of assessment technologies for research and clinical applications including the NIH Toolbox iPad app, the PROMIS iPad app, Assessment Center™, and the Assessment Center Application Programming Interface (API).

To learn more about collaborating with a member of the HealthMeasures team, contact help@HealthMeasures.net.
What is Neurofibromatosis?

Neurofibromatosis (NF) encompasses a set of complex genetic disorders that affects nearly every organ system, causing tumors to grow in the brain and throughout the body. There are three distinct types of NF:

• Neurofibromatosis type 1 (NF1)
• Neurofibromatosis type 2 (NF2)
• Schwannomatosis

While half of people with NF inherit the disorder from an affected parent, new cases can also arise spontaneously through mutations in the NF and related genes. Diagnosed most often in children and young adults, NF occurs in all races, ethnic groups, and both sexes.

The most common of these conditions is NF1, affecting over 1 in 3,000 people worldwide. Children with NF1 typically come to medical attention when they are noted to have birthmarks (café-au-lait macules) on their skin. Individuals with NF1 are prone to develop a wide variety of medical complications, including, but not limited to, developmental delays, autism, bone defects, sarcomas, nerve tumors (neurofibromas) and brain cancers (gliomas). Importantly, every individual with NF1 is unique, such that the clinical problems and their severity vary from person to person. This variability limits our ability to predict what types of complications will arise or to provide personalized care.

Much less common than NF1, individuals with NF2 can develop cataracts and many different types of brain and nerve tumors, causing problems with vision, hearing and balance. Similarly, individuals with Schwannomatosis are prone to spine and peripheral nerve tumors, resulting in chronic pain and disability.

Current treatments for NF are aimed at controlling symptoms, including surgery for painful and disfiguring tumors. As such, there is an urgent need to identify and evaluate new molecularly-based treatments for NF1, NF2 and Schwannomatosis. Progress in this area will require the seamless integration of advanced laboratory investigation with clinical research and care.

THE NF CENTER: EXCEPTIONAL CARE THROUGH GROUNDBREAKING RESEARCH

An international leader in research and treatment of neurofibromatosis (NF), the Washington University NF Center unites outstanding basic laboratory and clinical investigators with expert clinicians to provide comprehensive treatment options for children and adults with these complex genetic conditions.
The mission of the Washington University NF Center is to translate groundbreaking research into exceptional patient care. Using an integrated approach, we combine innovative laboratory science with multidisciplinary clinical care and novel complementary care programs. The collective focus of these researchers and clinicians is directed toward developing and optimizing treatments for children and adults with NF.

Cutting Edge Research: The Treatments of Tomorrow

Through research at the Washington University NF Center, we aim to achieve three major goals critical to improving the lives of people living with NF:

1. Discover the factors that contribute to the clinical variability seen in individuals with NF
2. Determine which medical problems are likely to occur and require therapy in each person with NF
3. Identify new therapies that specifically treat NF-related medical problems

Research performed in the Washington University NF Center is multifaceted, and involves the collective talents of over twenty researchers representing different clinical specialties and research disciplines. To this regard, we are focused on:

- Discovering new treatments for brain and nerve tumors in NF1 and NF2
- Exploring the cellular and molecular basis for NF1 optic glioma development and vision loss
- Identifying prognostic and therapeutic strategies for neurofibromas and malignant sarcomas in NF1
- Understanding the factors that contribute to autism, ADHD, and learning deficits in children with NF1
- Defining risk factors for the development of cancer in NF
- Developing personalized therapeutic approaches to tailor treatments to the specific problems arising in any given individual with NF (precision medicine)

Center investigators employ a wide variety of technologies and approaches to unravel the mysteries of NF, ranging from genomic sequencing and mathematic analyses to behavioral studies and cellular engineering strategies. By leveraging the expertise of a diverse group of laboratory and clinical researchers, we are able to accelerate progress in the field.

Laboratory researchers in the Center employ a diverse number of genetic, molecular, and cellular methods to understand how the NF genes function in health, and how mutations in these genes lead to the development of nervous system abnormalities, such as brain tumors. Coupling these approaches with a unique collection of human biospecimens and small-animal models, ongoing studies are designed to discover the causes for the various medical problems that arise in people with NF, which are used, in turn, to identify new treatments.

In addition to these basic and translational research efforts, there are a large number of clinical studies underway. These investigations involve novel NF patient databases and registries, genomic and biochemical analyses, patient-focused studies and clinical treatment trials in which promising new drugs are evaluated as part of national consortium efforts.

The Center boasts a long list of significant research achievements to date, which include the first whole genome sequencing of NF1 brain tumors, the identification of novel brain and nerve tumor therapies, the largest international study characterizing autism in NF1, and the discovery of causes for vision loss from NF1 optic gliomas. Furthermore, scientists at the center have developed numerous unique small-animal models of NF1 and NF2, which continue to be used to design personalized therapies, some of which have been successfully translated into novel clinical trials for affected individuals.

More information about ongoing research can be found on the Washington University NF Center website (nfcenter.wustl.edu) or the Gutmann Laboratory website (gutmannlab.wustl.edu).
Patient Care: A Multidisciplinary Approach

The Washington University NF Clinical Program at St. Louis Children’s Hospital and Barnes-Jewish Hospital is an internationally-renowned program specifically designed to provide multidisciplinary care for children and adults with NF, bringing together dedicated specialists with extensive experience in NF. With experts in fields as diverse as dermatology, neurology, neurosurgery, orthopedics, oncology, neuropsychology, and endocrinology, these healthcare professionals are able to provide cutting-edge treatments for every facet of NF.

Children and young adults with NF1 and NF2 are seen at St. Louis Children’s Hospital by a core NF care team composed of two neurologists, a pediatric nurse, a pediatric physical therapist, and a clinical research coordinator. During the clinic visit, a thorough evaluation is performed, which includes assessment for developmental delays, behavior and learning issues, and NF-related neurological problems. Additionally, families are given a personalized health organization binder and relevant educational brochures. Clinical appointments with other subspecialists are typically coordinated for the same day.

Families are approached to participate in NF Center clinical research studies to increase our understanding of NF and to improve risk assessment and find targeted therapies. When appropriate, children and adults are enrolled in clinical trials using promising new agents targeted to the molecular and cellular abnormalities seen in tumors arising in people with NF1 and NF2.

Complementary Care Programs: Extending Patient Care Beyond the Clinic Walls

The three complementary care programs offered by the NF Center are designed to address the myriad of developmental and behavioral issues that affect children with NF1.

Beat NF is a jazz music motor therapy program which integrates jazz music and physical therapy approaches to promote socialization and gross motor development in preschool-aged children with NF1.

Utilizing a number of organized play-based group events, Club NF offers structured physical and occupational therapy to target motor, behavioral and social impairments seen in school-aged children with NF1. Club NF engages children in a wide variety of activities, ranging from chess and gardening to ice skating and acting classes.

Designed and directed by St. Louis Children’s Hospital clinical neuropsychologists, Teen NF focuses exclusively on improving the social and behavioral skills of teens. The program includes parallel discussions for parents, helping them to deal with the challenges specific to raising a teenager with NF1.

These complementary care programs are offered at no charge to families due to the generosity of the Doris and Donald Schnuck Fund for Children in Need and the St. Louis Children’s Hospital Foundation.

“To improve the lives of people with NF, research should be focused on improving our ability to predict what medical problems might arise in a particular individual, and then treating patients with therapies tailored to their specific condition.”
Dr. David Gutmann is one of the world’s leading laboratory scientists and clinical experts in neurofibromatosis (NF). He currently is the Donald O. Schnuck Family Professor and Director of the Washington University NF Center, while also serving as Donald O. Schnuck Family Professor in the Department of Neurology. Dr. Gutmann obtained his Ph.D. in Microbiology and Immunology in 1984, followed by his M.D. with distinction in 1986 from the University of Michigan. After completing his Neurology residency at the University of Pennsylvania, Dr. Gutmann joined the laboratory of Francis S. Collins, M.D., Ph.D., a world-renowned physician-geneticist. During his postdoctoral research fellowship with Dr. Collins, Dr. Gutmann identified the protein encoded by the Neurofibromatosis type 1 (NF1) gene and defined its function as a tumor suppressor.

In 1993, Dr. Gutmann was recruited as faculty to the Washington University School of Medicine, where he established the NF Clinical Program at St. Louis Children’s Hospital. Recognizing the need to accelerate the pace of scientific discovery and its application to the care of individuals with NF, he founded the Washington University NF Center in 2004.

Dr. Gutmann’s research laboratory has been highly productive, generating many small-animal models of NF, which have provided critical insights into the pathogenesis of brain and nerve tumors, as well as normal brain development. Importantly, these studies have led to the discovery and evaluation of several new treatments for NF-related tumors and medical problems, some of which are currently being studied in human clinical trials.

Dr. Gutmann has published over 400 peer-reviewed manuscripts, and served on many national and international advisory boards, including the National Institute of Neurological Disorders and Stroke Advisory Council. He has been recognized for his achievements with numerous prestigious honors, such as the 2012 Frederich von Recklinghausen Award, 2013 Washington University Distinguished Faculty Research Award, 2016 Research Program Award from the National Institute of Neurological Disorders and Stroke, and 2017 Alexander von Humboldt Research Award.

Leading the Charge

Over the last decade, The Washington University NF Center has grown to become one of the largest and most comprehensive NF centers in the world. From research excellence to outstanding multidisciplinary patient care to integrative complementary care approaches, the NF Center is pushing the boundaries of what is possible for people affected with NF.

Today, the Washington University NF Center is internationally recognized as one of the premier clinical and research programs focused entirely on NF.

Partner with the Washington University NF Center

The Washington University NF Center receives funding from many sources, including the National Institute of Health; however, the NF Center also relies heavily on private funding which is essential for advancing research goals and providing resources and complementary care programs to families affected by NF.

There are many ways to support the Washington University NF Center in their mission to provide exceptional care through groundbreaking research.

To learn how you can get involved, please visit nfcenter.wustl.edu/give/

CONTACT

Email: NF@neuro.wustl.edu

For more information regarding the Washington University NF Center, please visit nfcenter.wustl.edu

To learn more about the groundbreaking research being conducted in the Gutmann Laboratory, please visit gutmannlab.wustl.edu

Follow the Washington University NF Center on Facebook: facebook.com/WashingtonUniversityNFCenter
Freedom and control of movement forms a fundamental part of our daily lives. Loss of voluntary control of muscle movement can occur as a result of damage to the brain or the neurones that project from the brain, through the spinal cord, to the muscles controlling the arms and hands. In stroke this is caused by disruption of the blood supply to the brain, leading to damage and death of neurones in the affected area. Often this occurs in regions of the brain that initiate and control voluntary movement. Although brain tissue and nerves cannot regrow, the brain is a dynamic organ with the capacity to reorganise itself to allow motor function to recover following injury.

Stroke is a major cause of disability worldwide, affecting 5 million survivors who are left permanently disabled. The persistent loss of voluntary movement that occurs after stroke has a significant effect on quality of life. It affects people's work and social life and has an impact on their psychological well-being, as well as placing an additional strain on carers and support agencies. During rehabilitation, much emphasis is placed on the function of lower limbs and finding ways to enable patients to walk again. However, a large number of day to day tasks important in our lives, require accurate control of hands and arms: from eating, to dressing yourself, to opening the front door with a key. Perhaps as a result of this, patients report that the loss of movement in their arms, is as important to them as loss of function in their lower limbs.

Professor Mary Galea of the University of Melbourne, Royal Melbourne Hospital, trained first as a clinical physiotherapist and has worked to rehabilitate survivors of stroke, traumatic brain injury, spinal cord injury, and other neurological conditions. She then went on to study neuroscience, and during her PhD she focused on the factors that promote recovery of the nervous system after injury. However, she reflects that she went on to develop ‘a special interest in the recovery of arm and hand function.’

Rebuilding the Brain

‘Despite the increase in knowledge about recovery after stroke, very little time is devoted to upper limb rehabilitation because it is not a priority in getting a patient ready for discharge from rehabilitation,’ Professor Galea explains. She believes this is because ‘there are low expectations regarding the potential for recovery.’

A large proportion of stroke survivors (around 30 to 60%) fail to recover the use of their arm and hand. Research in animals suggests that after a brain injury occurs, repeated use of paralysed limbs stimulates reorganisation of the undamaged cortical areas of the brain. Repetitive exercises specifically targeted to improve a particular task stimulate the neurones in the cortex to produce new connections in a process called synaptogenesis. This process underlies the recovery of motor function and the ability to make voluntary movements after a stroke. In humans, clinical evidence suggests that high intensity task-specific training leads to the best outcome in terms of recovery, compared to simple repetitive exercises.

‘Activity of the affected arm and hand, whether volitional or induced through electrical stimulation or robotic means, helps to preserve the muscles and nerves and to drive reorganisation of the nervous system to promote recovery,’ Professor Galea states. She also believes that despite advances in our understanding of what is required to induce recovery, the current provision of physiotherapy in Australia is not sufficient to drive the cortical rearrangement required for recovery of upper limb movement. Due to the lack of successful rehabilitation, fewer resources are being devoted to the upper limbs, and there is currently little opportunity after discharge to continue with rehabilitation, especially in remote communities. ‘I would like to change the low expectations of health professionals regarding recovery of upper limb function’ says Professor Galea. Indeed, her team have shown that with appropriate exercise opportunities significant recovery of arm and hand function is possible.

Addressing the Evidence–Practice Gap

In order to address this gap between clinical practice and our understanding of what’s needed to drive recovery, Professor Galea’s team have developed an upper-limb rehabilitation programme using new inexpensive computer based technology and tested its effectiveness in a real-world setting.
‘My whole career has been focused on recovery of function after brain injury. I have made this a priority in my research because I believe we can do better if we think laterally, and think of ways to provide patients with the opportunity to help themselves.’

Patients with a range of different symptoms at the busy, resource limited, rehabilitation centre at the Royal Melbourne Hospital were given access to a range of devices to create additional exercise opportunities to promote the recovery of arm and hand movement in a ‘Hand Hub’.

In this study, 92 patients, most of whom had suffered a stroke (and some with multiple sclerosis and loss of arm function due to tumours) underwent a six-week programme at the hospital where they visited the Hand Hub for an hour of arm and hand training at least three times a week for six weeks. The Hand Hub consists of three devices that patients can use, each getting progressively more difficult as movement improves or depending on the range of movement a patient has after injury. The first – the Able-M – is a table-top device for patients who struggle with very limited arm movements, which leads onto the Able-X, a light-weight handle-bar. Finally, the most challenging device is the ReJoyce station, which consists of a spring-loaded arm and involves practising tasks with daily household objects such as a doorknob, a key and two coin simulators.

Playing the Game

This equipment is run by game software that provides instant visual feedback on a patient’s progress, and allows them to move through different levels of difficulty. It also provides constant monitoring and information that the physiotherapist can use to assess progress. Up to five patients can use the equipment at once under the supervision of one physiotherapist and one health care assistant, increasing the number of people who can receive treatment quickly and cutting waiting times.

The games are interesting, challenging and fun, and therefore sustain the participants’ focus and motivation. The games have been designed so that after a while, patients stop thinking about the arm movement and focus on obtaining the goal of the games. This is thought to be a critical aspect of motor learning and encourages people to continue with the intensive exercise programme. After the six-week programme the participants showed significant improvements in their upper limb strength. This had a direct impact on their quality of life, improving their psychosocial well-being and cognitive abilities. Importantly, patients reported an increased ability to use their affected limb in everyday life, reducing the burden on carers.

This preliminary study revealed the potential of this novel approach. Now, Professor Galea’s team are now developing a tele-rehabilitation programme based on the positive results of the Hand Hub trial, to allow patient treatment to continue after discharge from hospital, in the comfort of their own homes.

Into the Future: Tele-rehabilitation

Access to rehabilitation services in the
community is limited. During early rehabilitation, the number of arm movements made by patients to aid recovery is low compared to the hundreds of repetitions that have been shown to promote changes in cortical structure in animals. Tele-rehabilitation uses advances in robotics, sensors and game technology to dramatically increase the amount of treatment a patient receives. Patients in the preliminary Hand Hub study had to travel up to 550 km to reach the hospital for treatment. The fact that patients were willing to travel such a large distance was perhaps a reflection of the lack of resources in their local area, and how strongly people felt about recovering their hand function.

In order to help people in rural communities, the team have developed a system based on the same technology as that used in the hospital, which can be used at home and remotely monitored by a physiotherapist. Professor Galea’s aim is ‘to show that tele-rehabilitation is effective and to see it rolled out nationally, so that patients who currently cannot access rehabilitation for their affected upper limb, whether because of distance from rehabilitation services or lack of them, can benefit.’

A trial is currently underway with an increased number of patients in a rigorous randomised control trial to test the effectiveness of tele-rehabilitation with the Hand Hub and its cost effectiveness. Each participant in the trial has access to the Able-M and Able-X equipment at home, which are controlled by a set-top box through Wi-Fi, and can be connected up to any available TV or digital screen. Software is accessed through a cloud server, and therapists can remotely access performance and training records and guide people through on-line communication as their training progresses. The participants use the Hand Hub at home for an hour, every five days over eight weeks, and this is compared to a control group who have access to a web based game designed to enhance memory and attention. The control group is required in order to determine the effect of the Hand Hub exercises separate from the cognitive enhancement associated with playing the game.

Patients will be tested after eight weeks, six months and twelve months to observe long term changes. Evidence suggests that cortical rearrangement stimulated by such high intensity training can produce improvements that last for years. As confidence develops in using the affected limb, patients start to spontaneously use the affected arm or hand, leading to further improvements.

Access for Everyone

If the trial is successful it can be applied nationally in Australia and could be adopted in other countries across the world to enhance arm function recovery following stroke. The Hand Hub is already being adopted elsewhere in other rehabilitation units and in Australian communities by local stroke support groups.

This technology is adaptable and can be used to allow more cost and resource effective access to appropriate physiotherapy after different types of brain injury. Allowing everyone access to an enjoyable, motivating game that can improve quality of life, reducing the impact of loss of arm and hand function on the lives of the people affected and their carers. Professor Galea and her team would like to show how effective tele-rehabilitation can be, and to see it rolled out nationally and even internationally, so that stroke survivors everywhere can benefit, regardless of their location.
Meet the researcher

Professor Mary Galea
Department of Medicine
Royal Melbourne Hospital
University of Melbourne
Melbourne, Australia

Professor Mary Galea trained as a physiotherapist at the Lincoln Institute of Health Sciences and obtained a PhD in neuroscience at the University of Melbourne in 1992. She was then awarded a postdoctoral fellowship University of Melbourne, which led on to a senior lectureship and an appointment as Foundation Professor of Clinical Physiotherapy from 2001–2012. She was also the director of the Rehabilitation Sciences Research Centre at the University of Melbourne and Austin Health from 2004–2011. Currently, Professor Galea is a Professorial Fellow at the University of Melbourne in the Department of Medicine at the Royal Melbourne Hospital, where she carries out basic and clinical research into nervous system recovery after injury, with a focus on recovery of arm function. She has won numerous awards for her work including the Sir Winston Churchill Memorial Fellowship in 2007 and was inducted into the Victorian Honour Roll of Women in 2014.

CONTACT
E: m.galea@unimelb.edu.au
T: (+61) 3 8387 2017
W: http://bit.ly/MaryGalea

KEY COLLABORATORS
Professor Fary Khan, Director of Rehabilitation, Royal Melbourne Hospital
Dr Bhasker Amatya, Research Fellow, Royal Melbourne Hospital
Dr Eduardo Cofré Lizama, Research Fellow, Department of Medicine (Royal Melbourne Hospital), The University of Melbourne
Dr Andisheh Bastani, Research Fellow, Department of Medicine (Royal Melbourne Hospital), The University of Melbourne
Ms Marlena Klaic, Senior Occupational Therapist, Royal Melbourne Hospital
Dr Imogen Windle, Rehabilitation Registrar and PhD student, Royal Melbourne Hospital
Dr Arthur Hsueh, Health Economist, The University of Melbourne
Professor Leonid Churilov, Head of the Division of Statistics and Decision Analysis, Florey Neuroscience Institutes

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Department of Medicine, University of Melbourne

REFERENCES
An estimated 15 million people a year suffer strokes, and stroke survivors are often afflicted with motor disabilities, due to damage in areas of the brain. Current therapies for helping patients restore their motor functions are expensive and labour-intensive, and the estimated cost of post-stroke treatment in the USA alone is $28 billion per year. With current treatments, patients can only expect to partially recover their movement capabilities, resulting in a severely reduced quality of life.

Recent advances in robotics have improved patient access to automated stroke rehabilitation systems. These systems can aid patients in recovering their lost motor skills, but are often bulky and expensive, and so cannot be used in the patient’s home. Alternative equipment that is both cheaper and portable would greatly increase access, leading to greater rehabilitation success and reduced costs. One of the most promising areas of development involves the use of virtual reality, robots, and smart sensors.

Motion sensing technology in computer games has been available for several years. These systems operate in a number of ways, but rely on the ability to identify the user’s movements and translate them into in-game actions. If motion sensing could be applied to detecting the movement and posture of a stroke survivor, it could potentially be used for rehabilitation, helping them to recover their lost movement. Several types of motion or gesture sensing systems exist, including video or thermal cameras, accelerometers, gyroscopes and Microsoft Kinects, etc. Some of these systems are small enough to be built into smartphones, enabling the movement and orientation of a patient’s phone to be measured.

Bringing Technologies Together

In computer games, the movements sensed are usually delicate and only small changes may be made as time goes on. This requires sensitive motion pattern detection. Also, the systems used by computer games rely on the recognition of specific actions and are relatively blunt in how they measure the user’s activities. The sensors used today typically rely on accelerometers, which can detect movement, but cannot obtain accurate information about the actual position or posture of the user’s limbs or body. One way of solving this problem is to use a thermal camera to detect the position and posture of the user. Dr Fei Hu and his colleagues at the University of Alabama have developed a system to achieve just this.

Proving feedback to the user on how well they have carried out an action would help the user to learn and improve, but the technology would need to be highly sensitive and sophisticated to achieve this. If this technology could be realised, it would provide a mechanism by which people with motor difficulties could re-learn a wide number of actions, and regain their motor skills in the process. Dr Fei Hu and his team have taken this concept further, integrating a number of systems (including virtual reality,
video games, sensors, robot, etc.) together into a cyber-physical system (CPS) that can be used for physical rehabilitation. This technology is highly versatile, and has the potential to help patients suffering from a wide range of conditions.

The team’s CPS combines motion sensing with a virtual reality headset that shows the user their own motions in a virtual environment. This innovative low-cost system can accurately recognise patient movements and gestures, and provide feedback, and thus is ideal for automated rehabilitation applications.

Recent improvements by Dr Hu and his team have made the sensors on this system much more sensitive to the user’s movement, while at the same time reducing the amount of power they require. They have also redesigned the software that recognises movements, such as grabbing a cup or kicking a ball. Distinguishing between small, relatively delicate actions such as picking up a cup or grasping a door handle is vital if patients are to be able to recover their ability to perform these everyday tasks.

However, detecting movement is not the only important thing. Sensing disorders in the patient’s movements allows for diagnosis as well as training, and would enable the system to identify the type of rehabilitation required. The patient’s performance can also be recorded, allowing progress to be monitored remotely from a hospital, while the patient is at home.

Virtual Learning for the Real World

So, what does the team’s virtual environment look like? The virtual environment does not need to be totally immersive, nor does the patient need to be totally encased in a network of sensors. Using a wearable data glove, the system can detect both the position of the patient’s arm and their hand movements.

Through changing the shapes and colours of different objects, the environment provides feedback to the patient. This ‘biofeedback’ information can be displayed on a standard computer screen, and this will provide the patient with enough information. However, using virtual reality headsets, which are becoming much cheaper over time, can enhance the patient’s visualisation of the virtual environment and allows biofeedback to be overlaid on the real world.

When physical disabilities are more severe, more sophisticated systems are needed, such as a platform capable of dealing with sensors and feedback mechanisms for multiple parts of the body. Dr Hu and his team are currently developing such a system, with the support from the US National Science Foundation (NSF). ‘This platform can be used to perform post-stroke rehabilitation, pilot training, gesture correction, and other body training applications,’ says Dr Hu. The platform includes a virtual reality system with a suite of medical sensors to monitor patient condition and movement, and a software system to integrate the hardware units and signals from multiple sensors.

‘In the future, we aim to upgrade our virtual reality system to augmented mixed reality, add new sensors to the data gloves to measure more delicate hand motions, program the robot in a more flexible style to emulate complex climbing actions, and create more exciting animation videos for virtual rehabilitation purposes’
Currently, the team’s multi-interface platform combines control and virtual reality systems with a reprogrammable treadmill, a data glove, an EMG (electromyography, used to assess muscle activity) and an ECG (electrocardiograph, used to measure heart activity). The platform’s virtual reality system contains multiple simulations and scenarios, to allow for different patient training arrangements. However, the platform’s architecture is designed to be flexible and is capable of being combined with other types of sensors and equipment.

One of the most important aspects of this platform is how it integrates readings from multiple sensors to give information about the patient’s condition. Because each type of sensor produces data with different characteristics, Dr Hu and the team needed to develop a different data processing method for each sub-system in the platform. To do so, the team utilised their exceptional programming expertise, creating multiple ‘dll’ files to help the different components of the platform communicate with each other. These dll (dynamic link library) files are a common aspect of modern-day computing, and enable the sub-systems of most of our current technologies to work together.

However, none of this would work without the ability to properly identify patient movements, and a lot of work has also been carried out by Dr Hu and his team to develop different data processing methods for each sub-system in the platform. To do so, the team utilised their exceptional programming expertise, creating multiple ‘dll’ files to help the different components of the platform communicate with each other. These dll (dynamic link library) files are a common aspect of modern-day computing, and enable the sub-systems of most of our current technologies to work together.

Future Work

Dr Hu’s goal is to develop a system that integrates highly sensitive motion sensors with a suite of other patient monitoring systems. He also aims to include RFID (radio-frequency identification) sensors that can provide information about the patient’s environment (for example, to identify medicines in specific containers, or objects in the room as they move through it).

Another important goal that Dr Hu aims to achieve is the development of a sophisticated virtual/mixed ‘game’ environment that can be used for measurable patient rehabilitation. This environment would be able to automatically adjust itself based on the patient’s training progress, and the patient’s own condition. As the patient progressed or their condition improved, the virtual environment would change to maximise the effectiveness of the training program.

As mentioned above, properly fusing all the signals together from multiple sensors is vital, to allow a system like this to operate intelligently. Additionally, the correlations among all signals must be analysed in real time, to ensure that the simultaneous movements in different parts of the body are captured accurately as meaningful, specific motions. Dr Hu’s team is working on a system that brings sensor signals together and simplifies their integration to recognise complex features, objects and specific actions.

‘We aim to upgrade our virtual reality system to augmented mixed reality, add new sensors to the data gloves to measure more delicate hand motions, program the robot in a more flexible style to emulate complex climbing actions, and create more exciting animation videos for virtual rehabilitation purposes,’ says Dr Hu.

In this future work, Dr Hu wants to integrate EEG signals from the patient’s brain with their own movements, to provide deeper insights into how the training and rehabilitation process changes brain structure. His team also intends to work on machine learning approaches that will enable more complex patterns of the movement to be recognised from heterogeneous sensors.
Meet the researcher

Dr Fei Hu
Electrical and Computer Engineering
University of Alabama
Tuscaloosa, Alabama, USA

Dr Hu is currently a Professor at The University of Alabama in the US. After obtaining a BS at the School of Computer Science & Engineering of Shanghai Tiedao University in China in 1993, he graduated from the same university in 1996 with a Master's degree. In 1999, he achieved a PhD in Communication & Signal Processing at Tongji University in China, and a second PhD from Clarkson University, New York, USA in 2002. From 1999 to 2002, he acted as a Teaching Assistant then a Research Assistant at Clarkson University, and from 2002 to 2008 was an Assistant Professor at Kate Gleason College of Engineering at RIT in New York. After achieving an Associate Professorship in 2008, Dr Hu moved to the University of Alabama, where he became a Professor at the Department of Electrical and Computer Engineering in 2015. Dr Hu’s research focuses on wireless networks, machine learning, Big Data and cybersecurity. He teaches a number of Electrical and Computer Engineering courses, and was voted the Best Teacher Award in 2007 at RIT. He has been an Associate Editor for a number of journals in his field, including Communications and Computer Security, International Journal of Communications, International Journal of Telemedicine & Applications and the IEEE Journal of Communication Surveys & Tutorials. He has over 200 publications in international journals and at conferences. His research has been supported by US NSF, DoD, and industry.

CONTACT
T: (+1) 205 348 1436
E: fei@eng.ua.edu
W: http://feihu.eng.ua.edu/

KEY COLLABORATORS
Dr Yogendra Patil, former PhD student
Dr Ting Zhang, former PhD student

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We started this edition of Scientia by looking at the inner workings of the human cell. The edition then progressed to showcase some of the latest advancements in medical treatments, both pharmaceutical and non-pharmaceutical. We finish this issue with another paradigm shift, towards the human as a whole, and our complex and fascinating interactions with one another, and how these interactions influence our health, healthcare, social amenities and even how our species has developed.

We start the section by showcasing the work of Dr Sharon Lee Brennan-Olsen and her team at the University of Melbourne and the Australian Institute for Musculoskeletal Science. Dr Lee Brennan-Olsen and her colleagues investigate the effect of socioeconomic status on how people suffer and recover from musculoskeletal disorders. The team have identified a social gradient in people who suffer from these conditions, where people from disadvantaged communities tend have much poorer health outcomes compared with their privileged counterparts. They are currently focused on identifying why the gradient exists and how they can make targeted interventions to improve the care and health of those who are most at risk of developing these conditions.

Our next featured researchers are also concerned with the welfare of the most vulnerable, in this instance elderly people in rural communities. Dr James Mjelde and Dr Rebekka Dudensing of Texas A&M University are economists working to illuminate the issues surrounding rural public transportation for the elderly. As our population ages, there is an increasing number of elderly people living in rural areas, where amenities and access to basic services such as healthcare are severely lacking. Adequate public transport is often in short supply, and the team are currently advising state agencies on the best way to finance and facilitate transportation for these vulnerable citizens.

Human movement is also one focus of our next researcher, but in this case the movement consists of human migrations throughout history and how this has influenced the language we speak to each other. Dr Marian Klamer of Leiden University specialises in the study of languages, with the aim of opening a window onto areas of the world that have very few historical records. In particular, she has devoted her career to the complex task of researching Austronesian and Papuan languages.

While language is one very obvious way we communicate, much of how we understand ourselves and each other is unspoken, and still largely unknown. In our final article in this edition of Scientia, we introduce Dr Beate Priewasser and Dr Josef Perner at the University of Salzburg, who play games with young children to reveal the nuances of social development. Getting the children to play competitive board games tells the researchers how we represent and evaluate our own motives and perspectives, and recognise alternative motives and perspectives in other people.
The Social Determinants of Musculoskeletal Disorders

Health and disease are shaped not only by biology but also by several social, demographic, economic, policy and behavioural factors – the ‘social determinants of health’. Numerous analyses attest to the associations between socioeconomic indicators and health outcomes. It is well-documented that socially disadvantaged populations have worse health outcomes than their privileged counterparts – a phenomenon termed the ‘social gradient of health’. Indeed, many physical diseases and poorer health outcomes are observed to disproportionately affect disadvantaged and marginalised people, including – as we shall see – musculoskeletal disorders. While this may often be attributed to ‘poverty’, the reality is far more complex, and the social gradient is indicative of deep social and economic inequalities. As well as having low economic capital, poorer individuals may have lower social capital, less extensive social networks with fewer support mechanisms, fewer choices, and often limited access to, or understanding about, health care services – factors that negatively influence health outcomes.

The skeleton, together with muscle, cartilage, tendons, ligaments, joints and other connective tissues, constitutes the musculoskeletal system. This forms a robust internal framework that provides support, stability and movement to the body – although there is plenty of scope for damage or ‘insult’ to the musculoskeletal system. Musculoskeletal disorders (MSDs), such as arthritis, osteoporosis, sarcopenia, osteosarcopenia, musculoskeletal trauma and injury can be extremely debilitating. These disorders can all result in a reduced quality of life and increased dependence on others. As such, they can severely affect an individual’s life and make everyday activities that most of us take for granted – walking the dog, grocery shopping, making the bed, lifting objects – extremely difficult. In addition, the prevalence of MSDs can impose significant personal burden, and dramatically increase the economic burden on healthcare systems. MSDs can also result in earlier mortality. Unsurprisingly, the social gradient in health is strongly represented in musculoskeletal disease.

Dr Sharon Lee Brennan-Olsen of the University of Melbourne and the Australian Institute for Musculoskeletal Science (AIMSS) is interested in understanding the social determinants of MSDs and related health service utilisation. ‘My program of research focuses on understanding why the social gradient exists and identifying potential points for intervention, including underlying biological factors such as inflammation,’ she says.

The Social Gradient of Arthritis

Arthritis is an umbrella term for more than 100 chronic, painful, debilitating diseases that most often affect joints. The most common forms are osteoarthritis and rheumatoid arthritis. Osteoarthritis is characterised by progressive degeneration of joint cartilage and swollen joints, mainly affecting the fingers, knees, and hips, amongst other joints. Rheumatoid arthritis is an autoimmune disorder that results in systemic inflammation of the joints, and subsequently influences many of the body’s systems.

Worldwide, arthritis affects one in four adults: 43% of those will have activity limitations due to their arthritis. Arthritis is not contained only to those living in higher-income
countries. Rather, it presents a particularly complex problem for residents of lower- and middle-income countries – countries that have 90% of the global burden of arthritis, but only 12% of global health spending. For those with arthritis in lower- and middle-income countries, the disease imposes a vicious cycle that may act to worsen poverty, as they often have poor access to health and social services, reduced employment opportunities and pension options, and fewer social security ‘safety nets’ than those in higher-income countries.

The World Health Organization (WHO) has compiled longitudinal data from over 44,000 respondents aged 50 years and older in six lower- and middle-income countries: China, Ghana, India, Mexico, Russian Federation and South Africa. Dr Brennan-Olsen and colleagues stratified respondent data from the WHO’s study on global AGEing and adult health (SAGE) by age, sex and socioeconomic factors, providing insight on the association between these factors and arthritis prevalence in lower- and middle-income countries. In the entire study population, for men and women respectively, they found that 20% and 14% had self-reported (lifetime) arthritis, while 5% and 3% had current symptom-based arthritis. The team also found that, similar to higher-income countries, arthritis prevalence increased proportionally with age. For both sexes and in all the countries studied, the researchers found that arthritis was more prevalent among those with the least education, and in women who were separated, divorced or widowed. After age-standardisation, men in five of the six countries were twice as likely as women to have arthritis, and in Ghana, men were three times as likely. The highest rates of arthritis were observed in Russia, in 38% of men and 17% of women.

These findings have important implications for resource-poor countries. The high prevalence of arthritis observed in lower- and middle-income countries emphasise the need for health authorities to direct resources towards arthritis prevention and treatment. Moreover, individuals living in poverty and with a low level of education may be predisposed to arthritis onset, as they are more likely to be employed as manual labourers. Dr Brennan-Olsen and her team are now investigating occupation types and occupational-related physical exposures as risk factors for arthritis.

Residents of higher-income countries are not immune to the impact of social and economic burdens on MSDs such as arthritis: just as in poorer countries, arthritis disproportionately affects those who are socioeconomically disadvantaged. Dr Brennan-Olsen and her colleagues investigated the social gradient of surgery related to osteoarthritis in her own country of Australia, by examining the association between socioeconomic status and uptake of total knee joint replacement (TKR). By analysing patient demographic data from the Australian Orthopaedic Association National Joint Replacement Registry, they found that there was a negative relationship between socioeconomic status and TKR uptake, with both men and women in the lowest 10% of socioeconomic status consistently more likely to undergo TKR than those in the highest 10%.

While these results are reflective of the well-documented social health gradient, the reality may be more complex. Individuals with lower socioeconomic status may wait in pain for longer than their less disadvantaged counterparts, most often due to having fewer economic resources on which to draw, less social support to rely upon during the necessary recovery period, and a reduced ability to take time away from work (if employed). Furthermore, individuals of lower socioeconomic status typically have a lower health literacy, which means they are less likely to seek, access, understand and implement health-related information. Patients with a higher socioeconomic status may also have greater financial resources and flexibility to access other modes of osteoarthritis management, such as private healthcare, physiotherapy, analgesics and early retirement.
The Social Gradient of Osteoporosis and Fractures

Bones are metabolically active organs, and undergo continuous remodelling across the lifespan, whereby mature bone tissue is removed (called resorption) and new bone tissue is formed (called formation). The remodelling process is both systemic and local – disturbances in this remodelling process can lead to deterioration of the bone matrix and a progressive decrease in bone mineral density, leading to increased risk of osteoporosis. Osteoporosis is the most common of the bone diseases: worldwide, osteoporosis affects 1 in 3 women and 1 in 5 men over the age of 50 years.

In recent years, there has been increasing interest in elucidating associations between socioeconomic disadvantage and low bone mineral density, and the subsequent risk of osteoporosis. Dr Brennan-Olsen and her colleagues recently used demographic data from patient records in Canada to examine the association between income and bone mineral density in more than 50,000 women aged 50 years and older. They found that there was a strong dose-response association between low income and low bone density, independent of known clinical risk factors. Women at the lowest income quintile were found to be 40% more likely to have low bone density (at the hip) than those at the highest income quintile – suggesting the existence of a social gradient in osteoporosis risk. Lower uptake of regular physical activity, lower dietary calcium intake, and a greater prevalence of smoking are all low income-related factors that may possibly contribute to this lower bone mineral density.

Bone fractures can happen due to high force impact or stress, such as playing football or rugby. But in patients with bone-weakening diseases such as osteoporosis, even minimal impact – for instance a fall from standing height or less – can result in fractures. Worldwide, an osteoporotic fracture is estimated by the International Osteoporosis Foundation to occur every 3 seconds.

Whether due to bone-weakening diseases or not, fractures can be financially, personally and psychosocially disastrous for individuals. They are more prevalent in the elderly, with higher incidence in women than men. However, fractures can happen to anyone, at any age and for many reasons. Nevertheless, the social gradient of health outcomes also appears to apply to fractures – and Dr Brennan-Olsen and her team have extensively studied this. They found that in Australians aged 50 years and over, disadvantaged men and women have increased fracture incidence compared to their less disadvantaged counterparts, represented by a six-fold and two-fold greater odds, respectively. The apparent disparity, not only across socioeconomic status but also sex, warrants further investigation.

Worldwide, indigenous populations experience disproportionately poorer health outcomes, greater morbidity and reduced life expectancy than their non-indigenous counterparts. Dr Brennan-Olsen and her team reviewed fracture rates in indigenous and non-indigenous populations, in a project that involved sifting through more than 3000 published articles containing real-world fracture data in PubMed, OVID, CINAHL and EMBASE databases. The only countries for which data were available were Australia, Mexico, USA and New Zealand. The findings of this study – thought to be the first of its kind – have been incredibly insightful.

The team found a greater incidence of trauma-related facial fractures in indigenous persons compared to non-indigenous persons. Compared to their non-indigenous counterparts, Canadian First Nations people had an almost five-fold greater risk of craniofacial fractures, New Zealand Maori people had an almost three-fold greater risk of facial fractures respectively, and Australian indigenous men had an almost eight-fold greater risk of jaw fractures. However, most shockingly, jaw fractures were 22-times greater in Australian indigenous women compared to non-indigenous Australian women. For hip fractures, however, the researchers found that indigenous people were at lower risk than their non-indigenous counterparts in all countries – with the exception of Canada and Australia where the opposite was observed.

This review also identified the risk factors associated with fractures that are disproportionately experienced by indigenous persons – comorbid diseases such as diabetes, and risk-taking behaviours such as substance abuse, and the reality that indigenous persons were more often victims of trauma resulting in fractures (for instance traffic accidents and interpersonal violence) compared to non-indigenous persons. This study provides insight into deep socioeconomic inequalities between indigenous and non-indigenous communities, and emphasises the priority of governments, global health initiatives, communities – and indeed wider society – to strive for equality for all.

Future Directions for Musculoskeletal Health

Dr Brennan-Olsen and her team are committed to investigating MSDs, as an outcome strongly related to social and economic disparities within lower-, middle- and higher-income societies across the world. Through their real-world observational studies, they demonstrate that musculoskeletal health is no exception to the observed social gradient in health: this gradient can be observed in all countries spanning the economic development continuum. Dr Brennan-Olsen’s more recent studies have focused on underpinning the biological mechanisms that underpin the social gradient of MSDs – especially biomarkers such as inflammation and epigenetic mechanisms.

Methylation of DNA influences the phenotypic expression of disease via a range of physiological processes – including bone development. A number of recent studies suggest that socially-related environmental factors can influence DNA methylation – providing a potential epigenetic explanation for the social gradient in musculoskeletal health. Dr Brennan Olsen, her team and colleagues have proposed a conceptual model that describes how social and environmental stressors – starting in utero, and occurring during early life, adolescence and adulthood – influence DNA methylation, which they hypothesise leads to inflammatory dysregulation and thus an increased risk of osteoporotic fracture.

In another future research direction, the team is investigating the social determinants of sarcopenia, a disease characterised by loss of muscle and loss of strength, and only added to the International Classification of Diseases in 2016. Combined with the presence of osteoporosis, both diseases together result in a condition referred to as osteosarcopenia. “Osteosarcopenia, an even less understood condition than sarcopenia, is also taking priority in my program of population health research,” states Dr Brennan-Olsen.
Meet the researcher

Dr Sharon Lee Brennan-Olsen
The University of Melbourne
Department of Medicine-Western Health
Australian Institute for Musculoskeletal Science (AIMSS)
Melbourne
Australia

Dr Sharon Brennan-Olsen gained her PhD in the Department of Epidemiology and Preventive Medicine, Monash University, Australia in 2010. She has since completed a number of Fellowships and research appointments, before starting her current position as Senior Research Fellow with the Department of Medicine-Western Health, University of Melbourne, and Program Director of ‘Population Health-Musculoskeletal Disease’ at the Australian Institute for Musculoskeletal Science (AIMSS). Her research interests are the social determinants of musculoskeletal diseases and related health service utilisation, and her more recent investigations have focused on biological mechanisms, such as inflammatory biomarkers and epigenetic processes, that she has posited may underpin the social gradient of osteoporosis, sarcopenia, osteosarcopenia and arthritis diseases. She is also committed to knowledge translation of research findings to healthcare professionals, policy makers and the general population, particularly disadvantaged communities. Her many outreach efforts include managing the Osteosarcopenia Roadshow (an accredited training workshop for General Practitioners), holding a position on the Women in Bone and Mineral Science Committee for the American Society for Bone and Mineral Research, promoting osteoporosis awareness on ‘World Osteoporosis Day’ and during ‘Healthy Bones Week’.

CONTACT
E: sbrennan@unimelb.edu.au
T: (+61) 8395 8114
W: http://findanexpert.unimelb.edu.au/display/person125267
W: aimss.org.au
@Brennan_Olsen

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As the large baby boomer generation of the United States ages, society is shifting social services to match the needs of the population. The number of elderly people living in rural areas is growing, where the public transit infrastructure they need is often lacking. Solving this problem requires interdisciplinary collaboration between researchers from multiple areas of social and applied sciences. In October of 2016, Dr James Mjelde and Dr Rebekka Dudensing of Texas A&M University, along with Texas A&M Transportation Institute colleague Jonathan Brooks, assembled a group of researchers and experts at the National Conference on Rural Public and Intercity Bus Transportation (RIBTC). The resulting white paper focuses on the economic issues surrounding public transportation in rural areas, along with technological alternatives and areas where more research needs to be conducted.

An Aging Rural Population

The United States, along with the rest of the world, is currently experiencing an increase in the proportion of elderly individuals in the population. With the advent of healthier lifestyles and advances in medical care, life expectancies for both men and women is on the rise worldwide. In the 2010 U.S. census, approximately 13% of the population (just over 40 million people) were over the age of 65, a number that is expected to increase to nearly 20% over the next decade as baby boomers continue to age while living longer, healthier lives. While this generation is staying healthier longer than past generations, they still are subject to the common problems of old age, such as loss of mobility and cognitive function, and require greater access to healthcare and social services. In particular, elderly members of disadvantaged and low income groups need access to healthcare, business services, and social interaction.

Roughly 83% of the land area of the United States is characterised as rural, sparsely populated areas. Populations of elderly Americans located in rural areas are increasing, due to many factors. Some American baby boomers move to a rural area from an urban one in retirement, and those moving to rural areas late in life tend to be financially sound. Those that have already established homes in rural areas earlier in life are less likely to leave, but are more likely to be lower income. Many rural areas also see an outmigration of young people, because of a lack of career and educational opportunities in most rural towns. People living in rural areas are more likely to be elderly, disabled, lower income, and/or from disadvantaged populations than those in metropolitan cities. There is often a greater need for access to health care and social services among these demographics, but these amenities are often more difficult to come by in rural areas.

Mobility and Quality of Life

Mobility is strongly linked with our quality of life. Easy access to employment, grocery shopping, healthcare appointments, recreation, and entertainment can be a major factor contributing to comfort and satisfaction throughout life. While these amenities are often abundant in urban areas, in rural areas there are typically fewer options that are located further from residents. To compound this issue, there are often fewer transportation options in rural areas. Thus, accessibility, the number of amenities at a given distance, and the availability and quality of transportation networks, are often limited in sparsely populated areas.
populated areas. When both mobility and accessibility of essential services are limited, it can put elderly individuals’ quality of life at risk.

The most common mode of transportation for elderly people in the United States is the personal vehicle, where often the convenience outweighs the potential costs, both financial and in terms of safety risks. A study in 2005 estimated that nearly 92% of recent trips taken by elderly people had been made by automobile. However, as people age, many factors decrease their ability to drive. The American Association of Retired Persons (AARP) estimates that on average, men usually live 7 years beyond driving comfortably, while women usually outlive their driving years by 10 years.

Individuals often give up driving for safety reasons related to cognitive and health problems. Elderly people that are still driving often start limiting their driving to avoid certain conditions, such as in the dark, in poor weather, at heavy traffic times, on highways, and on unfamiliar routes. When old age begins to limit or end a person’s driving years, they become much more reliant upon friends, family, and public transportation, and may require additional human assistance to use transportation of any kind (such as help getting in and out of a vehicle). However, family may not be nearby or available during work hours, and friends tend to be of a similar age, which may limit their driving ability as well. Mobility typically begins to drop at age 65 and the drop is even more pronounced for the rural poor. ‘As people are living longer, transportation becomes even more paramount,’ Dr Mjelde summarises.

Problems in Rural Transportation

Public transportation is often limited and difficult to access in rural communities. However, due to the growing elderly population in rural areas, the demand for access to transportation beyond personal vehicles is greater than ever before. Improving the availability and use of these services requires targeted research, but transportation data specific to rural areas is currently limited. ‘When we started looking at the literature, there was a lack of good economic studies on rural transportation for the elderly,’ Dr Mjelde explains.

There are numerous studies of urban transport, and that research is often broadly applicable, as the transportation problems in cities tend to be similar nationwide. However, unlike cities with metropolitan transit organisations, the party responsible for providing transportation in a rural area is often not well defined and varies by region. Responsibility may fall on the state, a rural planning organisation, or local government. Rural areas also vary dramatically in geography, infrastructure, and population distribution, making understanding the needs and best practices in rural settings a much more complicated endeavour. Many of the present studies in rural transportation are limited in their scope and applicability, and have not included experts from across the spectrum.

Dr Mjelde and Dr Dudensing recognise that collaboration between transportation researchers, economists, sociologists, and service providers is necessary to help facilitate positive practices and innovations in rural transportation. ‘We recognised the need to help the elderly, brought forth by living in rural communities and having personal experience with family and friends aging and struggling with transportation,’ Dr Mjelde describes. As resource and community development economists, respectively, Drs Mjelde and Dudensing posit that understanding the economic variables that determine not only if a transportation system is financially viable for a community, but if anyone will use it, can have meaningful
impacts on how rural communities handle transportation puzzles.

They organised a meeting of economists interested in rural transportation at the RIBTC to interact with transit planners and providers and develop a plan for addressing the problems of rural transportation. Conference participants were given surveys so they could offer their stance on many transportation issues, and Drs Mjelde and Dudensing worked with their colleagues to illuminate the best path forward in recommending economic research needed to develop effective transportation solutions.

Feedback and the Future of Rural Transportation

The RIBTC survey revealed that most respondents were concerned with rural transportation for the elderly and disadvantaged and expected these needs to rise in the next decade. They felt that public money should help fund this endeavour, but also expressed a willingness to pay a reasonable fee for services used. Most were unsure of how technological advances could help alleviate the situation.

Taking these responses into consideration, Dr Mjelde, Dr Dudensing and their colleagues formed research recommendations for the USDA and other agencies to consider. They agreed that research must be focused in five general areas: theoretical issues, innovative solutions, rural socioeconomic considerations, economic assessment and evaluation of rural transit, and information technology solutions. Research on theoretical issues underlying the economics of rural transit is lacking and could provide frameworks for each of the other areas. Innovative solutions, such as self-driving cars or self-correcting driving technologies, could help to alleviate some of the transportation issues that limit elderly people’s ability to drive and transport themselves, although some riders may need passenger assistance and others simply benefit from the interaction with their driver. Other innovative solutions, such as ride-sharing and volunteer networks also show potential.

Studying rural socioeconomic considerations helps policy makers hone in on how public transportation options affect the community as a whole. Translating the individual needs of community residents into a comprehensive understanding of broad reaching issues, such as liveability and sustainability of the community, is necessary to assess the broad need for transportation services. It is critical that rural communities with limited funds have appropriate economic assessments and evaluations of transit options, to understand if the benefits outweigh the costs to communities with limited funding. Little research in this area currently exists, and much of what has been done has followed questionable economic and social assumptions that could lead policy makers down the wrong path. Finally, pursuing information technology solutions with the potential to transform rural transit could dramatically improve both quality of service and reduce unnecessary costs. These solutions can help streamline the management of rural transportation systems, and help to ensure that vehicles are only being deployed if and when they will be used, reducing costs.

Rural transportation systems have not received the same research attention as urban networks, but through collaboration and innovation, Drs Mjelde and Dudensing are hoping to change that. The need for these services is expected to continue to grow over the next decade, and without a proactive search for solutions, rural communities could find themselves facing difficult problems as aging populations lose access to vital services. By following the recommendations of the RIBTC committee on rural elderly transit, they hope that communities will be able to make better informed decisions that benefit their residents and improve quality of life for everyone. As Dr Dudensing describes, ‘With this work, we are providing USDA and other agencies with guidance on economic issues associated with rural transportation for the elderly. Rural transportation providers are looking at innovative solutions.’

‘With this work, we are providing USDA and other agencies with guidance on economic issues associated with rural transportation for the elderly. Rural transportation providers are looking at innovative solutions.’ – Dr Dudensing
Meet the researchers

**Dr James W. Mjelde**  
Department of Agricultural Economics  
Texas A&M University  
College Station, Texas  
USA

Dr James W. Mjelde completed his Bachelor’s degree in Fish and Wildlife Management and his Master’s degree in Applied Economics at Montana State University. He went on to receive his PhD in Agricultural Economics from the University of Illinois in 1985. He has served in his current position as Professor of Agricultural Economics at Texas A&M University since 1985, during which he has mentored over 40 graduate students, and received numerous prestigious awards for his research in economics and excellence in teaching. He is a fellow of the Western Agricultural Economics Association and specialises in dynamic modelling of resource economics and economics of information.

**Contact**

E: mjelde@tamu.edu  
T: (+1) 979 845 1492  
W: http://agecon.tamu.edu/faculty-staff/faculty/mjelde-james/

**Dr Rebekka Martin Dudensing**  
Department of Agricultural Economics  
Texas A&M University  
College Station, Texas  
USA

Dr Rebekka Martin Dudensing began her collegiate studies at Kansas State University studying Agriculture, and went on to receive a Master’s in Agriculture & Applied Economics from Texas Tech University. She was awarded a PhD in Applied Economics from Clemson University in 2008, after which she joined the faculty at Texas A&M University, where she enjoys an appointment as Associate Professor in the Department of Agricultural Economics and Extension Economist with the Texas A&M AgriLife Extension Service. She specialises in rural and regional economic planning and the application of economic models to region-specific characteristics.

**Contact**

E: rmdudensing@tamu.edu  
T: (+1) 979 845 1719  
W: http://ruralcommunities.tamu.edu

**Key Collaborators**

Geoffrey Battista, McGill University  
Jonathan Brooks, Texas A&M Transportation Institute  
Maria Carrillo, Washington State University  
Blane Counsil, Texas A&M University  
Dr Anil Giri, University of Central Missouri  
Dr Man-Keun Kim, Utah State University  
Dr V. Dimitra Pyrialakou, West Virginia University  
Dr Stan Ullerich, Buena Vista University

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The ways in which human beings communicate have constantly evolved throughout the years. Yet, regardless of whether individuals communicate in person, or through phone, e-mail, text message or pigeon post, all verbal and written exchanges between them are made possible by the existence of languages.

Languages allow us to express complex thoughts, abstract ideas and feelings to one another, which would be difficult to convey using mere gestures and arbitrary sounds. Roughly 6,500 languages are spoken in the world today, but about a third of these have less than 1000 speakers. In addition to their practical value in our daily lives, when analysed, languages can help researchers map the history of populations and the geographical areas they inhabit. The field of historical linguistics does exactly this, by studying language as a system: in its grammatical structure, meanings, sounds and unique characteristics.

Dr Marian Klamer at Leiden University in the Netherlands is an expert in linguistics and has devoted her career to the complex task of researching Austronesian and Papuan languages, spoken in areas that have very few historical records. Dr Klamer's interest in these languages dates back to her childhood years in southwest Papua, a region of Indonesia populated by a vast variety of cultural groups. ‘Part of my roots lie in southwest Papua, where my parents worked as a missionary and a nurse,’ she explains. ‘From my parents, I learned that the colourful multitude of people, cultures and languages is a miracle that we must cherish.’

Dr Klamer spent her childhood in a small village in the jungle, inhabited by people from different clans. ‘Every clan had their own language, so several Papuan languages where spoken in the village, alongside Papuan Malay that was used as a lingua franca; at home we spoke Dutch. Perhaps because of this early multi-lingual environment I have always been curious about how people use languages, and how different languages are structured,’ she says.

Her early fascination with languages prompted her to study linguistics later in life, specialising in Austronesian and Papuan languages, many of which are at risk of extinction due to their limited number of speakers. Dr Klamer has conducted extensive linguistics research in Eastern Indonesia, trying to re-construct parts of its history by analysing its multitude of spoken languages. ‘I compare structures and words of languages to find out how they are related to each other, to reconstruct their common history and find out in which ways they have influenced each other through contact,’ she explains. ‘This provides information about the history of speakers and the possible migrations and types of contacts they had in the past.’

**Tracing History Through Language**

Travelling back in time through the laborious analysis of languages is not an easy task. Languages comprise complex patterns related to grammar, vocabulary and phonetic characteristics. Historical linguists use vast amounts of language-specific data to try and trace a language’s ‘family tree’, which points to other languages it
There are two main ways in which languages can influence each other: through vertical transmission when languages have a common ancestor, or through horizontal transmission, when languages borrow words from other languages they come into contact with. Even names of cities or villages can help to retrace the history of a geographical area – if we think of village names such as Katwijk in Holland: the wijk part of that name derives from the Latin word Vicus (i.e. village) and dates back to the Roman times.

Words derived from other languages can help to identify past connections between different clans or populations, as well as the nature of these connections. Linguistics research can be even more interesting when applied to languages spoken in places with very few traces of the past, shedding light upon their history and the relationships between different populations.

**Austronesian and Papuan languages**

Linguists have often studied Western languages, while languages spoken by minorities in other parts of the world have much less associated research. During her undergraduate studies, Dr Klamer became interested in the possible application of Western models of linguistics to the study of non-Western languages. ‘I learned a lot about formal theoretical models of ’universal’ structures of human language,’ she tells us. ‘I found it interesting to see that at the time these models were almost entirely based on Western languages and I was curious whether they could also be used to describe the structure of non-Western languages.’

The first time to test this was when Dr Klamer carried out fieldwork to study a language spoken on the island of Sumba, in Indonesia. ‘That was a short field study and I was still a student at the time, but it made me realise how little work was done on the hundreds of (unwritten) languages of eastern Indonesia, and that I wanted to contribute to documenting and describing some of them,’ she explains.

Languages spoken in Indonesia are Austronesian and Papuan languages. Austronesian languages are spoken in a variety of places, including Madagascar, the Philippines, Indonesia, New Zealand, Hawaii and Easter Island. Papuan languages are spoken in Papua New Guinea and other neighbouring Islands. In total, there are about 1200 Austronesian and 800 Papuan languages, which together make up one third of all languages spoken on Earth. They include big languages such as Indonesian, Malay and Javanese, as well as small ones, spoken by less than 1000 people. Over 90% of the languages spoken in Indonesia have no written tradition, and could hence leave no trace once they become extinct.

The first lists of Austronesian words were collected by Dutch explorers Willem Schouten and Jacob Lemaire. Dr Klamer is continuing this Dutch tradition, focusing her studies on languages spoken in eastern Indonesia. ‘I believe that theorising about the structure of human language will work better if we take into account data from a wide range of non-European languages,’ she says. ‘But also, these languages are fragile cultural heritages under pressure from Indonesian, and they need to be fostered.’

**Eastern Indonesia – a Multi-Language Region**

Languages spoken in Indonesia today amount to approximately 700. Most of these have no related historical records and have never been studied before. At the moment, Indonesia’s linguistic diversity is being threatened by the widespread adoption of

‘I compare structures and words of languages to find out how they are related to each other, to reconstruct their common history and find out in which ways they have influenced each other through contact. This provides information about the history of speakers and the possible migrations and types of contacts they had in the past.’
Indonesian, the national language. This is particularly true in eastern Indonesia, where many parents are choosing to teach their children Indonesian instead of their native language.

As part of her job, Dr Klamer travels to Indonesia, speaking with its inhabitants and trying to learn more about their language and cultural heritage. ‘I love studying languages, and I love working with people in the field,’ she says. ‘Indonesia is really a wonderful country with a truly amazing richness of peoples and cultures. People are always incredibly hospitable and welcoming, and always happy to share their knowledge.’

Speaking with local populations, Dr Klamer learnt that many children no longer speak their parents’ language. This means that in one or two generations many of these languages could become extinct. Linguistic studies, such as those conducted by Dr Klamer, might help to preserve them, by developing orthography and compiling dictionaries, so speakers can write in their own language and the language can be used in schools. This would allow children to have a better grasp of their parents’ language, while offering speakers of these languages a means through which to write down their traditional stories, songs and histories. The recordings that are made as part of the investigations are stored in an open access archive of language materials, so that records of these endangered languages will be available for future generations.

The Lesser Sunda Islands – Pantar and Alor

So far, most of Dr Klamer’s research focused on comparing languages of the Lesser Sunda islands – particularly the islands of Alor and Pantar. In 2014, she was awarded a prestigious 1.5 million euro VICI grant by the Netherlands Organisation of Scientific Research (NWO), for five years of further linguistic research focusing on the Lesser Sunda Islands.

Before the arrival of European explorers, Pantar and Alor were part of the trading route between Java, Timor, Moluccas, China, Vietnam and India. The Portuguese were the first to make agreements with local leaders, followed by Holland in the 1800s. The inhabitants on these islands were perceived as heathens, some of which with cannibalistic and aggressive tendencies. Initially, contact was made with the people living on the coasts, but in the 20th Century, travellers discovered that the islands have around 20 different population groups, each with its own language.

As indigenous written sources are lacking, Western visitors collected the only sources of history relative to these islands during Colonial times. Dr Klamer has carried out research analysing the language of the different populations residing in Pantar and Alor, discovering links between them that point to past interactions between some of these communities. For instance, she found that a middle area on the map appeared to be cut off from the rest of the language family for a certain period in the past, as the languages spoken there had more influence from outsiders than the languages elsewhere on the islands. She also discovered that the population speaking a language called Alorese, the only Austronesian language on the two islands, was likely to have settled on Pantar about 700 years ago, originally coming from Flores, an island further east. Her studies were able to retrace some of the history of these two islands and their communities. This is of great value for historical reconstruction purposes, and it also helps researchers create more detailed genealogical groupings and ‘language fingerprints’, which could help to compare similar languages in future.

A Glance at the Future

Over the past 15 years or so, Dr Klamer’s research has mainly focused on studying and comparing languages of the Lesser Sunda Islands, in order to find out more about the history of their people. She hopes to later broaden her studies to Papuan languages in other regions of Indonesia.

It is thanks to researchers like Dr Klamer that a variety of data was collected about non-documented languages in eastern Indonesia, which has provided valuable insight into the region’s history and could help simplify the study of these languages in future. There is now a significant amount of data related to minority languages in Pantar and Alor. In future, similar data could also be collected in other areas of Indonesia, where languages are still largely unexplored.

‘For the future, I hope that similar work can be done on more languages of Papua, for instance in the Bird’s Neck region. The languages there are small, highly endangered, and most of them have not yet been described,’ Dr Klamer tells us. In addition to her research, she would also like to provide local inhabitants with the knowledge and tools necessary to document their own languages, particularly endangered ones.
Dr Marian Klamer is a linguistics professor with a particular interest in Austronesian and Papuan languages, as well as historical linguistics. Dr Klamer spent part of her childhood in Indonesia, before returning to The Netherlands in 1973. She carried out her postgraduate studies at Vrije University in Amsterdam, where she attained both an MA and a PhD in general linguistics. After completing her studies, Dr Klamer has been a lecturer in linguistics at several different Dutch universities, wrote several books, and carried out extensive research resulting in numerous articles on the grammar, origin and history of Austronesian and Papuan languages. Her projects have received several grants throughout the years, the most recent being a VICI Grant awarded to her by the Netherlands Organisation of Scientific Research (NWO) in 2014. Her research sees language as a key to understanding a place’s socio-cultural history, exploring its historical significance using both quantitative and qualitative methods.

CONTACT

E: m.a.f.klamer@hum.leidenuniv.nl
T: (+31) 71 527 2783
W: http://www.marianklamer.org/

KEY COLLABORATORS

Dr Francesca Moro, Dr Gereon Kaiping, Dr Owen Edwards, Hanna Fricke, George Saad and Jiang Wu, The VICI-Research group, Leiden University
Dr František Kratochvíl, Nanyang Technological University, Singapore/
Palacký University, Olomouc, Czech Republic
Dr Gary Holton, University of Hawai‘i, Manoa, United States
June Jacob, MA, Artha Wacana Christian University, Kupang, NTT, Indonesia

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How do we understand our own behaviour and the behaviour of others? How do we come to represent and evaluate our own motives and perspectives, and recognise alternative motives and perspectives in other people? Philosophers and psychologists alike have marveled at this question for centuries. Austrian researchers Dr Josef Perner and Dr Beate Priewasser have devoted their careers to advancing our understanding of how we come to understand ourselves, using a novel experimental protocol with young children.

Why Are You Doing That? Three Theories

The prominent theory of how we understand both our own behaviour and the actions of other people is theory-theory. Theory-theory (sometimes called theory of mind) posits that we understand the actions of others through an estimation of their beliefs and desires. Under this theory, we expect that other people act to achieve their desires within the framework of their personal beliefs. This theory frames human behaviour entirely in terms of an internal mental state, and views this internal state as the sole source of intentional behaviour.

In order to understand others through theory-theory, we must infer their internal mental states like their beliefs and desires and how they generate action. It has no room for the idea that people act for good reasons. The theory of mental simulation suggests that when trying to explain others’ behaviour, rather than inferring what they might think or desire, we simulate others’ mental life that led them to act. Under simulation theory we pretend to be another person in order to determine why they have behaved the way they did.

Teleology theory posits that in the majority of cases we understand the behaviour of ourselves and others by assuming there are objective reasons we act the way we do. Teleology assumes that: (1) objective reasons are factual and publicly accessible, (2) if one has an objective reason to do something, they should act on it, and (3) if a person has an objective reason to do something and they are competent to do it, they will engage in that action. Teleology bypasses the complex need to understand the interplay of mental states in theory-theory, and avoids having to imagine oneself being in another person’s situation in simulation theory.

At the surface, competition in a game may seem like an odd way to study how humans understand one another, but Drs Priewasser and Perner argue that competitive games elegantly tease out how we perceive the actions of ourselves and others. Imagine two people playing tic tac toe. Player A only makes marks to build a row of 3, while player B strategically makes marks to both block Player A and build their own row of 3. Which player will win? Mostly likely Player B. Player A’s strategy thinks only of their own motives and perspective, while failing to account for the motives and perspective of Player B. Player B’s strategy encompasses both players’ competing motives, and thus is able to shift between blocking Player A’s moves and advancing their own.

Developing a Competitive Spirit

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Children under the age of 3 or 4 are like Player A in that they remain oblivious about the competing motives of others. As such, young children typically do not derive enjoyment from competitive play. Drs Priewasser and Perner and their research team have devised a series of competitive games that help illuminate the development of how we come to recognise alternative perspectives in young children, and highlight a teleological explanation for human understanding of one another.

The common test for whether or not a young child is aware of the perspective of others is the false belief test. During this test, a child observes Puppet A hide a treat somewhere in a room, and then exit. Puppet B then enters and moves the hidden treat to a new location. When the Puppet A returns, the child is asked where they will look for the treat. Older children will quickly identify that Puppet A will look for the treat where it was most recently hidden by Puppet B, indicating that they are not aware that the puppet’s perspective does not share the same information as their own. Young children cannot conceive that the puppet holds a false belief about the location of the treat.

The research team wished to demonstrate a link between understanding false beliefs and appreciation of competitive games. They devised a simple game in which the goal was to collect the most beads on a stick. Children take turns rolling dice and adding beads to their stick, with the option to take the beads from either a communal pile or another player’s stick. Competitiveness was measured by how often a child chose to poach beads from another player rather than the communal pile. According to theory-theory, children learn to understand desires prior to understanding beliefs, in which case desire-driven competitiveness could emerge independently of understanding other’s beliefs. If teleology theory held, one would predict the performance on the two tasks to be strongly associated – a child would have to recognise that another person held a different perspective to appreciate that another person’s motives ran counter to their own during the game. They found that performance on the false belief test was strongly correlated to competitive poaching behaviour in the bead game, supporting the teleological view.

Sabotage is All About Perspective

During the bead game, children were focused on the positive goals of finishing the bead stack but failed to see that they should also take steps to prevent others from reaching that goal. To take things a step further, Drs Perner and Priewasser and their colleagues needed to move beyond children simply engaging in competitive behaviour to demonstrating their ability to predict it. Sabotage is perhaps the most obvious case of incompatible desires – one person is intentionally preventing the success of another person’s actions. Following theory-theory, young children should be able to
recognise desires earlier than and independently of beliefs, that is, they can understand conflicting desires prior to understanding perspectives independent of their own. Under teleology theory the two go hand in hand, because action driven by contradictory desires or contradictory beliefs, both need an understanding of different perspectives. Therefore, children will not be able to understand that others have conflicting desires prior to understanding that others can have differing beliefs.

In a project that is still currently ongoing, the team designed a clever sabotage test to evaluate whether or not young children are able to understand conflicting desires prior to conflicting beliefs. Children were shown a simulation with two puppets. Puppet A wanted to drive a truck with food to a group of animals, while Puppet B did not want Puppet A to feed the animals. Puppet A had two options: drive the truck a short route that Puppet B had the ability to block with a gate, or drive the truck a long route with no gate. The children were then asked to predict which route Puppet A should take. Children that had passed the false belief test consistently selected the correct answer – the longer route with no gate – while children that had not passed the test selected the short route blocked by Puppet B. Since these children could not conceive of alternative perspectives, they could not understand or predict that Puppet B would block the gate when the goal was to feed the animals.

**Board Games Reflect Reality**

To corroborate their findings on early childhood enjoyment and understanding of competitive activities, the research team looked outside the lab to one of the most extensive purveyors of research on toddlers and competitiveness – board game manufacturers. In order to obtain recommended ages (and thus marketing demographic) for children’s board games, most go through extensive trials to determine which age groups will most understand and enjoy playing.

The researchers analysed a group of over 100 children’s games on four qualities: (1) cooperative or competitive goal orientation, (2) chance or strategy based demands on the player, (3) level of player interactiveness required to achieve the goal, and (4) possibility to sabotage other players. They predicted that competitive games that require sabotage would only be marketed to children over the age of 4 (the age at which the majority of children pass the false belief test). Indeed, they found that the manufacturer’s recommended ages correlated strongly with the skills predicted by their competition and sabotage developmental assays.

**Next Moves**

To carry on refining our understanding of the developing mind and build teleology theory, Drs Priewasser and Perner plan to continue their research into children’s ability to understand contradictory desires. Future work is aimed at investigating the abilities of children as young as 9 months, and extending understanding of how children’s perception of other people’s perspectives develops through early childhood, all within the framework of teleology theory. As Dr Priewasser describes, “Together with philosophers we plan to sustain our work on the teleological theory and elaborate the structure of understanding actions and reasons.”

‘When collaborating with Johannes Roessler, an expert in the Philosophy of Action, I realised that our field was based on misleading premises. There was the tacit assumption that children come to understand people’s minds as a system of mental states that cause behaviour, ignoring the fact that we see ourselves act for reasons.’

– Dr Josef Perner
Meet the researchers

Dr Beate Priewasser
Department of Psychology & Centre of Cognitive Neuroscience
University of Salzburg
Salzburg
Austria

Dr Beate Priewasser began her career in 1994 as a preschool teacher in Austria. Fascinated with the behaviour of her young students, she began the pursuit of additional degrees in 2003, graduating with a BA in Education in 2008, an MS in Psychology in 2009, and completed her PhD in Developmental Psychology in 2015, all at the University of Salzburg. Dr Priewasser currently serves as a Senior Scientist in the Department of Psychology and Centre of Cognitive Neuroscience at the University of Salzburg, where she is engaged in research into the development of theory of mind, perspective taking, and competition in young children.

T: (+43) 662 8044 5168
E: Beate.Priewasser@sbg.ac.at
W: https://ccns.sbg.ac.at/people/priewasser/

Dr Josef Perner
Department of Psychology & Centre of Cognitive Neuroscience
University of Salzburg
Salzburg
Austria

Dr Josef Perner received his PhD in Psychology from the University of Toronto in 1978. He went on to serve as a Professor of Experimental Psychology at the University of Sussex from 1979 to 1993, and after a period as a Visiting Professor at the Max-Planck Institute for Psychological Research in Munich, joined his current laboratory at the University of Salzburg as a Professor of Psychology in 1995. He holds an honorary doctorate from the University of Basel, and has been awarded both the William Thierry Preyer Award for Excellence in Research on Human Development by the European Society of Developmental Psychology (ESDP) and the Bielefelder Wissenschaftspreis for his interdisciplinary research.

T: (+43) 662 8044 5124 (-5105)
E: josef.perner@sbg.ac.at
W: http://www.uni-salzburg.at/psy/people/perner

KEY COLLABORATORS

Johannes Roessler, University of Warwick
Johannes Brandl, University of Salzburg
Frank Esken, University of Salzburg
Eva Rafetseder, University of Stirling

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WHO WE ARE

Trees for Cities is the only charity working on an international scale to create greener cities. Since 1993, we have engaged over 70,000 people to plant over 650,000 urban trees in parks, streets, schools and housing estates across the UK, as well as internationally, revitalising these areas and improving the lives of the people who live in them. We strengthen communities through volunteering opportunities and inspire children to grow and eat good food and to connect with nature.

WHAT WE DO AND WHY WE DO IT

We focus on planting trees and greening community spaces where the social and environmental impact on local people is greatest. In London this might mean planting trees to clean the air or transforming unused community spaces into vibrant green areas, making our communities happier and healthier places to live, whilst in Nairobi it’s planting fruit trees for food and sustainable livelihoods.

MISSION

- Planting trees and greening cities worldwide.

VALUES

- People-led: Although our reach is global, we value the importance of a local focus. We always work through and within local communities to strengthen them and empower their members.
- Quality-driven: Both the quantity and quality of the trees we plant are at the forefront of our planning so that we constantly strive to maximise the impact of our projects to the environment and society.
- Delivery-focused: We are an organisation that gets things done. What we talk about, we do – effectively, efficiently and on-time.

WHY TREES MATTER

- Trees help our environment and the impact of climate change:
- They remove 4m tonnes of carbon from the UK atmosphere each year (Forestry Commission 2010)
- They can cool the air by 2 - 8 degrees C
- Trees absorb water, lowering stress on storm water drains and mitigating flood risk
- A single mature oak tree can host up to 423 different species of invertebrates that support birds and mammals
- Each year Trees for Cities plant around 65,000 trees in cities worldwide, revitalising cities and enhancing the lives of the people that live in them.

HELP US PLANT A MILLION URBAN TREES BY 2020

To date we have planted over 650,000 trees in cities. We have now set ourselves an ambitious new target to strive to plant 1 million urban trees by 2020. Help us meet this exciting new milestone...

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