Scientia

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September 2015

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BIOTECHNOLOGY

Today, we are at the forefront of biotechnology, a multi-billion dollar industry. Biotechnology encompasses the wide range of technological applications of biological compounds and processes. It has been utilized by humans for thousands of years for agriculture, medicine, and food processing. For an example, microorganisms and biological processes have been employed to produce beverages (e.g. beer and wine) and dairy products for centuries. More recently, biotechnology has been used to harnesses resources from nature to provide us renewable energy and to reduce pollution. It is used to improve crop yield and resistivity to insects. Furthermore, it has expanded to the development of novel compounds and techniques for biomedical research, enabling the examination of various biological processes and the molecular mechanisms underlying many diseases.

In the following section, we are going to focus on the more recent advancements in the field and their application in biological and medical research.
Novel compounds and biological organelles are continuously being developed and harnessed to integrate into both modern and more traditional laboratory techniques such as microscopy, western blotting, and immunoprecipitation. The utilization of these novel compounds and biological processes has been an essential part of the recent advancement in medical research as well as in other fields such as manufacturing.

Two recent innovations will be highlighted. Professor Ed Bayer, an expert in avidin-biotin system and a pioneer of cellulose research, will discuss his “designer cellulose technique” and its various biotechnology applications. In addition, the mysterious magnetotactic bacteria, which can navigate the oceans using a structure called the magnetosome, will be discussed, focusing on Dr. Raz Zarviach’s work on the magnetosome and its potential application in protein purification.

One discovery. Two discovery. Three discovery? More?

Professor Ed Bayer seems to constantly make new discoveries, starting during his PhD with avidin-biotin interactions (a technology used by thousands of scientists today) and continuing with the discovery of the tiny factories known as cellulosomes. Here he discusses the twists and turns of his career.

Could you describe your research background? What brought you into this field of study?

It is true I've had a varied path, and in retrospect, I probably did everything wrong. I started in Zoology, but was bored with anatomy (and other such topics) and wanted to know more about mechanisms. So I studied Biology. Same thing! I wanted to go deeper, so I went into Biochemistry and then Molecular Biology and Protein Chemistry. It probably would have been better to do it in the reverse, i.e., from the physical to the chemical to the biological. In any case, here I am! My PhD was on "The Biotin Transport System in Yeast", during which time I became interested in the avidin-biotin complex. Together with my mentor, Meir Wilchek, we invented, (as it were), avidin-biotin technology, which is still broadly used in biological and clinical research, and in industry. During my postdoctoral studies I worked on oil-degrading bacteria, developing various genetic and molecular technologies to address the interaction of microbes and insoluble substrates. Towards the end of my postdoc I connected with another young scientist, Raphael Lamed, who had been working on bacterial degradation of cellulose. We put the technologies I had developed in my postdoc to good use, and the rest has been documented in the literature.

What do you believe is the most significant outcome from your research career?

I would probably have to divide my research career into two major discoveries: The avidin-biotin system and the cellulosome. The first, the invention of the avidin-biotin system, has evolved to be a widely used tool in the biological sciences, and the second, the discovery of the cellulosome, embodies a major paradigm of plant cell wall degradation with a host of significant implications – environmental, biotechnological and for alternative energy.

You were one of the pioneers of cellulosome research – was there ever a 'breakthrough' moment?

There was a "Wow!" moment. When we started, we weren't even looking for a multi-enzyme complex at all! We started with a simple observation: the bacterium binds to the cellulose substrate before it starts to degrade it, and we were actually looking for what we then called a "cellulose-binding factor", or CBF. We had two things going for us: the unconventional approach that I had previously developed precluded predetermined opinions – we could simply let the research lead us; and we ignored dogma – when research led us in a unique direction we didn't just throw the results down the sink! (I suspect that others who tried to study this system before us might have done so…).

There were two surprising defining moments. The first occurred when we discovered that the CBF contained a multiplicity of proteins, (at the time we counted 14), and so realised that the CBF was a multi-protein complex. The second was when we discovered that most, but not all, of these proteins exhibited cellulose-degrading activity – thus we inferred that the CBF was a multi-enzyme complex. Fortunately we were astute enough at the time to propose a generic term, and so the "cellulosome" was born.

Have you thought about scaling-up the designer cellulosome system to develop into an industrial enzyme mix?

Despite that fact that over 30 years have elapsed since the discovery of the cellulosome, its use in industry currently is infeasible… As opposed to the free cellulase systems, produced by aerobic fungi and bacteria, cellulosomes are produced by anaerobic bacteria. Anaerobes just don't have enough energy to compete with aerobes, and the amount of protein produced is comparatively little. To circumvent these problems we developed 'designer cellulosome' technology, based on the principles of synthetic biology, to produce cellulosomal components in large quantities in aerobic systems and allow them to self-assemble into designer cellulosomes. The development of this technology has thus far taken 20 years and is ongoing. We have now produced designer cellulosomes that rival the activity levels of the native complex, nevertheless scaling-up of the system is still premature. We still need to address numerous other aspects of the technology before we can consider its broad application as a competitive strategy for cellulosic biomass conversion to biofuels.

Where would you like to take your research in future? Have you a 'dream goal', as it were?

I have a lot of dreams! It is true that a breakthrough in the development of renewable energy and viable solutions to the energy crisis would be admirable long-term objectives – it would be exceptionally gratifying to have contributed to these lofty goals for the betterment of human society. Nevertheless, as a scientist, my goals are much less lofty, more down-to-earth perhaps, and more science-oriented. My dream goals are more connected to the biochemistry and genetics of cellulosome production and assembly. I'd like to know more about how these multi-enzyme complexes are formed on the molecular level, and the secret to their highly efficient function. These are the topics that greet me when I wake up in the morning, occupy my daydreams, and penetrate my thoughts as I fall asleep at night…

The smallest factory in the world

The Dept. of Biological Chemistry at the Weizmann Institute of Science is home to over 24 research groups, each tied together by a common thread: a focus on the biochemistry of life. Here we look at Professor Bayer's work on natural, Nano-scale factories – cellulosomes.

Imagine a factory. Not as you usually would, there is no giant boxy shed full of machinery here. Instead you need to picture a long stretch of LEGO blocks, each manufacturing a certain product, clicking into place in a way that is both highly ordered and yet immensely flexible. Sounds like nothing you've ever heard of? That's because this particular factory is built at the molecular scale, comprised of enzymes which are simply invisible to our eyes. Although tiny, we can still build, modify and use them for our own purposes.

Paper, wood, cotton. Many industries rely on one vital, humble molecule: cellulose. New research may help us to do even more with this plentiful, renewable resource.

Where do these factories come from? And why do they look so strange? To understand this, we need to look at one of the most ubiquitous energy sources available – plants. Plants, trees, grasses, shrubberies, etc. All of these convert energy from sunlight into sugars (carbohydrates) which are then used both for energy storage and to provide the structure for the plant itself. The most common structural carbohydrate in plants is known as cellulose, and it plays an important role in the lives of organisms ranging from humans to bacteria.

A WORLD OF OPPORTUNITY IN A SINGLE PLANT

As an exceptionally common molecule, cellulose is a valuable potential energy source for many different organisms. However, due to the particularly intractable nature of cellulose, it is often too difficult to degrade into the component sugars and thus provides very limited nutritional value. It instead passes through the intestines effectively untouched, providing us with the dietary fibre which our body needs. For those species which can degrade cellulose, however, a whole new world of carbohydrate energy opens up. This is a particular advantage for fungi which colonise dead and decaying wood, allowing them to feed off the energy stored within the structure itself. Many bacteria have also evolved the ability to degrade cellulose, allowing them to colonise otherwise unavailable niches (cow stomachs, for example, where they are vital for energy extraction from plant matter).

Enzymes which degrade cellulose are known, generally, as cellulases. The action and format of these enzymes vary, as does the amount of enzyme which is produced by any one bacteria. Aerobic bacteria live in oxygen-filled environments, such as on the wooden log outside your door, and tend to produce vast amounts of cellulases. Anaerobic bacteria survive in the absence of oxygen, such as within a cow's rumen, and due to the generally low-energy environment tend to be more efficient in how they produce these enzymes. In particular, they usually produce lower numbers of enzymes but organise these enzymes together into clusters known a cellulosomes. This was first noted in an anaerobic bacterium known as *Clostridium thermocellum,* which produces an entire enzyme complex which is capable of, given time, degrading even tough cotton fibre into soluble sugar.

How does this actually work in practice? The first stage, the LEGO baseplate as it were, is made from a protein known as scaffoldin, which attaches itself to the cellulose fibre at one end via the aptly named carbohydrate-binding module, or CBM. Scattered along the protein are cohesin modules, which act as attachment points for other parts of the growing complex. Cellulase enzymes then bind to these cohesin locations via their own dockerin modules, each of which allows strong and specific binding at one or more locations. The earliest observed cellulosomes were very simple

constructs, with a single base and 5-9 cellulase 'blocks'. However, the complexes can become immensely complicated as further scaffoldin proteins attach themselves to the initial scaffold, each of which can then branch further and attach even more enzymes to the complex. Bacteria such as *Clostridium clariflavum* can have up to 160 enzymes in a single complex, all working together to form a giant cellulose-degrading machine.

The major advantage of this rather intricate approach is that it brings together a number of diverse enzymes together into one common location.

How do we actually know this? Predominantly due to the pioneering work of Professor Edward Bayer, who discovered the cellulosome system with Professor Lamed in the early 1980's. Following on from this discovery he began to focus on characterising the multitude of parts involved in the system, thus beginning a long and successful career in cellulosome research which continues to this day. One of the areas in which his lab is focused is in the development of 'designer cellulosomes' – whereby target-binding domains are modified to create a specific complex for each possible need. As each cohesin/dockerin pair has a set binding specificity, it is possible to construct a human-defined molecular factory. Professor Bayer's group has already designed novel cellulosomes and shown that they can be produced at small scales – improved yields could help with the growing problem of cellulose waste.

"HOMO CELLULOSIS"

The strength and stability of cellulose are vital to a number of human endeavours. As Professor Bayer comments "Cellulose has been closely woven into the fabric of our society through the development of the wood, paper and textile industries". The oldest of these is construction, with wood providing one of the earliest building materials and one which is still in constant use today. It is also vital for the textile industry (cotton being almost pure cellulose, for example) and paper industry (paper being, essentially, cellulose fibres stuck together). While its stability is the driving force behind the utility of cellulose, it is precisely this stability which leads to problems in disposal. While paper and cloth recycling systems currently exist, they are not applicable to a number of industrial processes which involve the production of large amounts of plant waste (as for example in fruit juice production, which already involves the heavy use of less-effective cellulase enzymes).

Efficient degradation of cellulose to produce free sugars also allows it to be used as feedstock for further biotech processes, such as enzyme production or bioethanol manufacture. Current technology involves the use of free-floating cellulases, a process which is not yet truly cost effective. Professor Bayer sees potential in the incorporation of designer cellulosome systems: "the two systems – free versus cellulosomal – act in very different, but complementary ways" he comments. "Perhaps the secret to high efficiency lies in a combination of the two". Now that the initial steps have been taken, the next step is to scale the system up, to see how well it can compare to current technology used by industrial producers such as DuPont and Novozymes. While promising, Professor Bayer is cautious, "the development of this technology has thus far taken 20 years and is ongoing… We still need to address numerous aspects of designer cellulosome technology before we can consider its broad application".

Despite thousands of years' experience in working with cellulose-containing substances, we remain puzzled by efficient degradation – the sheer toughness that we prize often returns to haunt us. Plant matter, full of cellulose, remains a plentiful, renewable resource. Recent research into the cellulosome by groups such as Ed Bayer's may allow us to gain the maximum amount of value from each tree.

Researcher Profile

Professor Edward A. Bayer
The Maynard I. and Elaine Wishner Chair of Bio-Organic Chemistry
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Professor Edward Bayer received his PhD in Biophysics from the Weizmann Institute of Science. His career has encompassed two major discoveries; the existence of the avidin-biotin interaction system and the discovery of the cellulosome. He has authored almost 400 publications and serves as an editor-in-chief, editor, and/or on the editorial board of several journals in the fields of biotechnology and microbiology. For his work in pushing the boundary of knowledge he has been awarded both the Sarstedt Award and the Ulitzky Prize, as well as being elected to the Fellowship of the American Academy of Microbiology and the European Academy of Microbiology.

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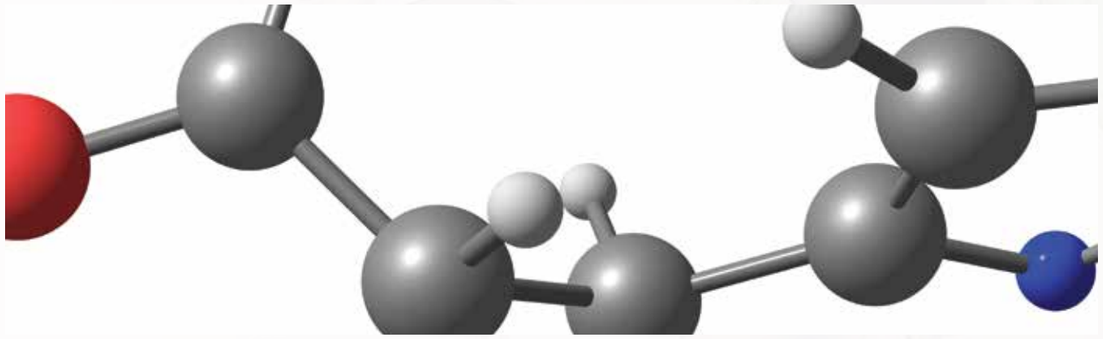
European Commission
US Department of Energy

The maker of magnets

Dr Raz Zarivach is a leading expert in the structural biology of magnetosome-associated proteins. We ask him about his current research.



How did you end up in your current role?

I got here via a conventional route: I started as a chemist during my first degree, then went into doing a PhD in Structural Biology, which I had previously really liked as a topic. By mistake or by chance I did my PhD in the lab of Ada Yonath, the group that determined the molecular structure of the ribosome and in doing so won the 2009 Nobel Prize. I was actually working on this topic as a PhD student, continued in structural biology during my Postdoc, and then afterwards I was selected for the position here in Ben-Gurion University of the Negev.

What brought you into the magnetosome world?

Moving into the magnetosome world was different, because this is a very specialized topic. I had already heard about biomineralization during my first year as a PhD student – and it somehow always stayed with me, including hearing about the magnetosome and magnetic / magnetotactic bacteria. It intrigued me as to how things were being done, but of course I was already doing my PhD on another, very specific field. When I finished my Postdoc on bacterial pathogenicity mechanisms, with a strong focus on bacterial secretion systems, I really wanted to move my lab onto a new topic. At this point I recalled my excitement upon hearing of work with magnetic bacteria – and this is taking into account that even after 10 years I was still interested.

You have had quite an international career; do you think it has shaped the way you perform research?

I'm not sure it's actually because it was an international career, so much as that I was exposed to good researchers. I learned many 'best practices' – how one should conduct research, how work should be done patiently and correctly, how to look at things in a different way. I don't think it's the location of where I have worked; it's more who I have met and worked with.

What do you think will be 'the' big finding in magnetosome research?

I think there are many big findings. One direction is always towards biotechnology, one that many groups are taking – this is what brings money into the field. However there are also very big questions regarding protein-mineral interaction, how do you control a mineral using biological components? We use bacteria as they are much easier to study and simply see how it goes.

Is it possible to transfer the entire magnetosome production system to a foreign host?

It has been tried many times. One of my collaborators has actually managed to do that in a bacterial strain that is very closely related to magnetotactic bacteria. His name is Dr. Dirk Schuler and his work was actually published in Nature Nanotechnology, but that was the only time that someone has managed to transfer the magnetosome into another organism.

Do you think it will be possible to integrate foreign proteins into magnetite crystals?

There are currently many attempts to cover magnetite crystals with proteins. In a way this is not hard, once you find an appropriate protein from the magnetotactic bacterial genome (one that can bind biomagnetite), then you can use that to cover magnetite with your desired protein. This is something we do, and this is something that our collaborators are doing, so we are all heading in this direction.

Can this replace the current techniques using labelled magnetic-beads?

I think so. The idea is not just to cover magnetite, you can also do this using current techniques. Instead the idea is to control the size and shape of magnetite, then process them to obtain uniform, controlled, and tightly-bound protein surface coatings.

Do you see yourself moving some of these discoveries into the commercial sphere? Are you encouraged by your experience with companies such as SmartZyme?

I'm a member of the National Institute for Biotechnology in Israel, and we are looking in the direction of patenting and spin-offs, i.e. commercialising our ideas. As a shareholder of SmartZyme, and as someone on the Scientific Directive Board, I'm always exposed to the 'industrial' world. Having these connections is really important, allowing me to take my work in other directions. However I do see a distinction between my life as a scientist (where I'm looking at the very basic scientific questions), and that involvement in the industrial world (in which I act as an advisor, as someone with ideas, as someone with different opinions that can be taken). Scientists in pure research are really looking at these very fundamental questions, which cannot necessarily be easily translated across.

The race to the Pole

The Department of Life Sciences at Ben-Gurion University conduct cutting edge research into a wide range of biological fields. It is located within Beer Sheva, the largest city in the Negev desert of southern Israel.

There is a moment in every child's life when they take a couple of magnets off the fridge and hold them close to each other. They then marvel as those magnets somehow push or pull back and forth without any visible sign that something is happening. At least, no visible sign to our eyes. But what if you could detect magnetic fields, see them curving through space and follow them as you wanted to? When people hear this idea their thoughts normally jump straight to homing pigeons, but bacteria have been perfecting this skill for millennia.

Magnetotactic bacteria sense magnetic fields to navigate the chaotic depths of the ocean. How? Read on…

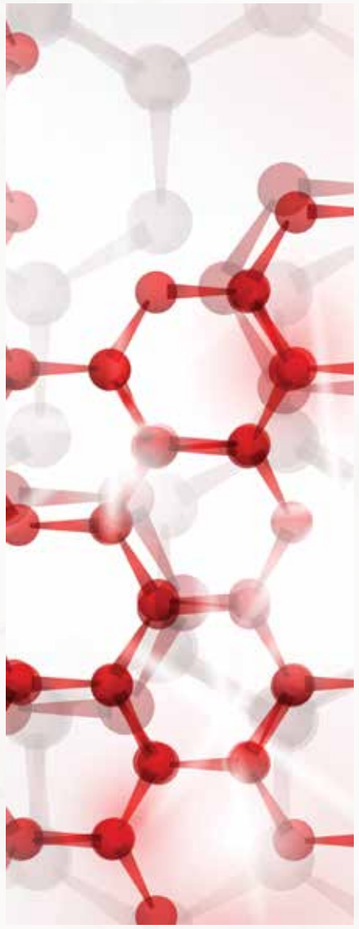
This seems a rather odd ability for a single-celled microorganism to have, but in fact aquatic 'magnetotactic' bacteria can use magnetic field lines to determine 'up' and 'down', no small feat when you are floating in the middle of the ocean. But how can you detect a magnetic field? The answer lies in a specialised organelle known as the magnetosome, which consists of a lipid bilayer surrounding an iron crystal known as magnetite. By producing a number of these magnetosomes and lining them up, end to end, the bacteria essentially become a single (very small) magnet, which then lines up alongside the Earth's magnetic field. Held in this orientation, magnetotactic bacteria simplify the chaotic ocean movement into a simple Go Up/Go Down choice (as the magnetic field dips in towards the earth's core). They then use this knowledge to choose their goal: the oxygen-filled surface zone or the anaerobic depths.

While this seems simple enough, actually forming the magnetosome is a remarkably complex process requiring the formation of the organelle from cell membranes, filling it with iron precursors, nucleating, and then controlling crystal growth. As such, a number of specialised proteins known as magnetosome-associated proteins (MAPs) are involved in the magnetosome production process. The majority of these proteins were identified using proteomic approaches – while they are known to play a role in magnetosome formation, just what that role is often remains a mystery.

MYSTERIOUS MAGNETISM

Understanding these roles is where scientists such as Dr. Raz Zarivach from Ben-Gurion University come in. As a structural biologist, his expertise lies in determining the shape of proteins at the molecular level, using that to identify just how they perform their designated jobs. Skills honed during a PhD in the lab that successfully determined the structure of the ribosome (which eventually brought the lab head a Nobel Prize) provided him with the basis for a long, multinational career in structural biology.

The research goals of the Zarivach group focus on identifying the roles these MAPs play, one at a time, by examining their molecular



A MAGNET FOR CASH

While this kind of research sounds quite abstract, it has some unexpectedly important outcomes for biotechnology. Of the many examples, let's look at the purification of a single protein or biomolecule from a large mixture, a process which often uses magnetic beads. Polymer beads have long been used as a solid support for biomolecule capture, usually by attaching an antibody, mixing everything together, and then centrifuging it all – the relatively heavy beads will form a pellet at the bottom of the tube, hopefully with your biomolecule attached. The downside is the need to centrifuge, which is slow, hard to scale up, and cannot be used in continuous flow systems. Enter the magnetic bead, which can be directly pulled from the solution by applying a magnetic field, no centrifugation required.

Ok, but where do magnetosomes come in? Currently, magnetic beads are produced chemically, a process that can lead to a mixture of a variety of shapes and sizes. By contrast, magnetosomes produced by bacteria are the same size and shape every time – a huge boost in quality, if only it can be applied at an industrial scale. First, however, scientists need to figure out what each protein does, and whether the whole system can be moved into a simpler bacteria, such as the E. coli beloved by biotech groups everywhere. This is where the research performed by Professor Zarivach, his group, and his wide ranging collaborators come in.

Dr. Zarivach is no stranger to commercialisation. He has served for several years on the advisory board of SmartZyme, a biotech company in the field of enzyme development, based just outside Tel Aviv, which is itself a known hotbed of biotech start-ups. He credits this experience with showing him the myriad possibilities for expanding scientific developments, possibilities which sadly pass by the majority of research focused academic groups.

Despite this, however, his heart remains in pure research, in the chase after knowledge. As he says "this is what really powers me – the structure of biology, and how you connect that knowledge to so many different things: from pure analysis, to creating new drugs, to modifying the protein's function". Luckily for him, the field of magnetosome research has enough unknown connections to keep even a researcher of Dr. Zarivach's calibre busy for a while.

structure. Proteins often contain typical 'motifs' or 'domains', combinations of structure and chemistry that provide a particular effect, for example a Zinc Finger is involved in binding DNA, while a GxxxG region promotes binding of proteins within membranes. By examining the structure of MAPs they intend to identify magnetosome-specific domains, then determine how they work.

This process is harder than it sounds, naturally. The gene for each MAP needs to be cloned and expressed in a simpler bacteria, such as E. coli (yet more complications, E. coli use different coding systems for their DNA-protein translation process, requiring the use of specially modified strains). These MAPs then need to be purified in large amounts and turned into crystals, a slow process full of trial and error – made even worse by the need to crystallise in the presence of magnetite. However, once all of this is done, the researchers can see the structure of the protein – right down to the atomic level.

Researcher Profile

Dr. Raz Zarivach
Associate Professor, Chair of the Macromolecular Crystallography Research Centre
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Dr. Zarivach is the head of the Zarivach Laboratory for Structural Biology, as well as the Chair of the Macromolecular Crystallography Research Centre (MCRC) at Ben Gurion University. His work involves X-ray crystallography, which his group uses to determine the structures of proteins involved in magnetosome formation, as well as those involved in effector secretion by pathogenic bacteria. A PhD completed in a Nobel-Prize winning lab led to a successful career, and he has been part of over 45 publications, including several in Nature and Cell.

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FROM BENCH TO BEDSIDE

The importance of the role that basic science plays in advancing medical research through translational studies cannot be missed when browsing medical articles published in the past 30 years. Quite simply, translational research aims to translate basic science findings to applications that can improve medical care. To use an old example, the discovery of the green fluorescent protein has contributed to the identification of novel targets for therapeutic intervention by leading to the development of visualization techniques by attaching fluorophores to antibodies and nucleic acids that can be used to detect various biological molecules. Using fluorophore-conjugated detection tools, we can now diagnose tumors and neurological diseases by examining tissue sections and identify biological mechanisms. We can also discover novel drug targets and have a better understanding of disease mechanisms by visualizing various components of the cell including proteins, DNA/RNA, lipids, small molecules, and many others. Like this example, translational research can transform basic science findings to develop intervention techniques, drugs, and diagnostic tools that can be directly applied to improve patient diagnosis, disease management and even discover cures. Translational research has been recently garnering much attention in many different medical fields including cancer research, cardiology and neurology, which will be discussed in greater detail in this section. In fact, much research funding in North America and Europe are focused on translational research as the rewards of a successful study can influence medical care in a significant way that can be applied in the foreseeable future. Indeed, with the aging population introducing new challenges for healthcare where diseases that become more prominent with aging, such as cancer, neurodegeneration and diminishing cardiac health, are major contributors to morbidity and mortality, we are in great need of more effective tools to treat such conditions. To do this, translational research can use a wide variety of tools and techniques. As findings are developed from basic science studies, researchers often start by examining simpler systems, such as cell lines to identify a potential drug target or pathway that can be generalized into humans. However, this is not always the case as targets can also be directly identified in humans by finding molecular alterations >

> in patients against the healthy population using peripheral samples such as blood, tissue extracted during surgery, and/or post-mortem samples. Once the target is identified, existing or newly generated compounds can be tested for efficacy in changing this target or pathway of interest to produce certain effects that are related to the disease mechanism. This is then tested in more complicated systems, such as animal models, to see if such interventions change physiological or behavioral variables of interest. The findings that will be discussed here highlight the diversity by which translational research can be conducted, and the powerful effect such studies can have in improving healthcare. More specifically, studying magnetoferritin allowed Dr. Cao to identify a method by which these molecules can be used to enhance tumor detection. Furthermore, by studying the pathways involved in telomere maintenance, Dr. Wong discovered mechanisms behind maintenance of tumors, which can be used to identify targets for treatment. By studying the mammalian orthologue of Sir2, a longevity protein in yeast, Dr. Wang identified molecular alterations that can be used to characterize and perhaps treat aged arteries. In examining the link between the mitochondria and adrenomedullin receptors of the heart, Dr. Yoshizawa identified these receptors as a promising target for treating heart failure. Lastly, Dr. Pomp's work with KATNB1 and its role in cortical growth and development may have significant therapeutic implications for microencephaly.

Biomimetics Builds Better Nanoparticles for Biomedicine

Dr. Changqian Cao uses biomimetic approaches to synthesize high-quality magnetic nanoparticles. Here he discusses his development of a novel magnetic nanoparticle with enhanced capability for detecting early stage tumours.

To begin, could you describe your research background and how you became interested in studying magnetic nanoparticles?
I came into contact with the magnetic nanoparticles when I became a graduate student of professor Yongxin Pan. Professor Pan is a pioneer involved in the research of biogeomagnetism. When I first came into his laboratory, he told me some stories related to geomagnetism and magnetic nanoparticles that I have never seen. For example, many organisms such as magnetotactic bacteria, pigeons, bees, salmon etc. could use the Earth's geomagnetic field for navigation. There were also many magnetic nanoparticles (magnetite, Fe3O4) found in these organisms. These biological magnetic nanoparticles are characterized by narrow size distribution, distinct crystal morphorlogy, and chemical purity. My initial idea for synthesizing magnetic nanoparticles in a biomimetic way was inspired both by biomineralization of magnetic nanoparticles in organisms and the current requirement for high-quality magnetic nanoparticles in biomedicine.

How are microscopic or pre-angiogenic tumours currently detected in the clinic? What limitations do these technologies have?
Pre-angiogenic tumors could not be detected in the clinic until now. This is because the primary tumors at an early stage usually exhibit very small size (microscopic level), no angiogenesis, they have biological barriers, and express low level of tumor-associated antigen. Current clinical imaging techniques still cannot sensitively and specifically detect them. Nuclear imaging modalities such as positron emission tomography (PET), single photon emission computed tomography (SPECT) and computed tomography (CT) show a high sensitivity, but they have problems of ionizing radiation, low resolution and anatomic localization of the small tumor lesion. MRI is a very promising clinical tool to diagnose tumors thanks to its higher resolution, noninvasive nature and tomographic abilities, but its applications have been limited by intrinsic low sensitivity and non-specificity.

What advantages do magnetoferritin nanoparticles offer over current in vivo tumour detection methods?

The magnetoferritin nanoparticles show two advantages over current in vivo tumor detection methods. First, magnetoferritin nanoparticles exhibit much higher relaxivity compared with current clinical Gd-based or iron oxide-based magnetic resonance contrast agents, which will significantly enhance the sensitivity of MRI. Second, magnetoferritin nanoparticles have intrinsic tumor targeting ability with no need of conventional surface coating and targeted ligand modification. The magnetoferritin nanoparticles can specifically bind to the transferrin receptor 1 (TfR1) overexpressed on cancer cells. When these nanoparticles were intravenously injected, they could intrinsically cross serial biological barriers (endothelium, epithelium and blood-brain barrier), specifically target to tumor cells and enhance MRI of microscopic (<1-2 mm) breast and brain tumors in vivo.

Did you run into any challenges from conception to synthesis of the magnetoferritin nanoparticles?
I have spent nearly three years from conception to successful synthesis of high-quality magnetoferritin nanoparticles because the synthesis is really tricky. Ferritin shell should be expressed and purified with intact structure and biomineralization ability. The synthesis condition should be strictly controlled. Otherwise, it is very easy to form aggregated magnetic nanoparticles outside of protein shell. Fortunately, we successfully explored a simple process for synthesizing magnetoferritin nanoparticles in these three years, which guarantee our further investigation of their practical application in biomedicine.

You found that a larger M-HFn core size enhanced its performance in MRI and immunoassays. How else might you enhance M-HFn functionality?
Magnetoferritin nanoparticles have three distinct interfaces for modification to enhance their functionality: the interior, the exterior, and the interface between subunits. We can enhance their functionality through not only controlling the size and structure of interior core, but also modifying the exterior and the interface though chemical conjugation or genetic engineering. For example, we are now conjugating the exterior of the protein cage with polyethylene glycol (PEG) and we find that circulation time in blood and relaxivity are significantly enhanced.

Are there any limitations or barriers to using this technology in the clinic? How long do you anticipate before this technology is implemented in clinic?
Our recently developed magnetoferritin enhanced MRI technology shows high promise for in vivo detection of cancer at the earliest stage. However, we still need a long time to extensively study phamacokinetics, nano-toxicity and to perform clinical trials of magnetoferritin nanoparticles. Usually, drug development is a very complicated process that takes an average of 10-15 years from the time it is discovered to when it is available for treating patients. But if we find any company who is interested in developing this technology, it will be a brilliant future for this technology implemented in clinic.

Biomimetic Magnetic Nanoparticles Enable the Detection of Microscopic Tumours

Early detection is key for treating most cancers. Now, research by Dr. Changqian Cao and colleagues has led to the development of a novel magnetic nanoparticle capable of detecting microscopic tumours with high sensitivity and specificity.

CHALLENGES IN EARLY TUMOUR DETECTION

Without a blood supply tumours cannot grow beyond a couple of millimeters. To sustain their growth, tumours stimulate the formation of new blood vessels, a process known as angiogenesis. If tumours can be detected and treated before they undergo angiogenesis then cancer progression and metastasis can be prevented. But detecting pre-angiogenic tumours is challenging. In addition to their small size, they do not produce significant amounts of tumour-associated antigens, biomarkers that could be used to detect the cancer or target drugs to the site of the tumour. Pre-angiogenic tumours may also possess biological barriers that prevent efficient drug delivery. While currently used nuclear imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) are very sensitive, their resolution is poor and they require the use of ionizing radiation. As such, there is a significant need for new technologies capable of sensitively, specifically, and safely detecting and treating early-stage tumours.
Magnetic resonance imaging (MRI) is an attractive tool for tumour detection because it has higher resolution than nuclear imaging techniques and does not require the use radiation. However MRI is limited in its sensitivity and specificity and thus is not suitable as a standalone technology for detecting microscopic tumours. To enhance the sensitivity of MRI magnetic nanoparticles have been used as a contrast agent. With further functionalization magnetic nanoparticles are capable of specifically being targeted to cancer cells for imaging or drug delivery. One of the major challenges of developing magnetic nanoparticles is their synthesis. For biomedical applications, magnetic nanoparticles need specific properties that can be difficult to control through physical and chemical synthesis. To address this problem Dr. Changqian Cao, Associate Professor at the Institute of Geology and Geophysics, Chinese Academy of Sciences, and colleagues have taken a lesson from nature, enabling the synthesis of high-quality magnetic nanoparticles with intrinsic tumour-targeting ability.

BIOMIMETIC SYNTHESIS – BORROWING FROM NATURE

Ferrimagnetic iron oxide nanoparticles are widespread in nature, used by many animals and bacteria for navigation using the Earth's magnetic field. These biological nanoparticles exhibit properties that are highly desirable for biomedical applications including uniform size, shape and chemical purity. However, attempts to synthesize such magnetic nanoparticles by chemical and physical means have failed to achieve the same elegant precision as nature. As an alternative approach, Dr. Cao, in collaboration with Professors Weifeng Liu and Guanjun Chen from Shandong University, used the ubiquitous iron-storage protein ferritin as a biotemplate for the synthesis of a novel magnetic nanoparticle.
Nearly all organisms, including humans, synthesize ferritin for the storage of free iron. It is comprised of 24 subunits that form a spherical protein shell with an outer diameter of 12 nm and an inner cavity of 8 nm where the iron is stored as ferrihydrite. The ferritin synthesized by Dr. Cao and colleagues, known as magnetoferritin, contains a magnetic iron oxide core that can be used as an MRI contrast agent. In the past, other groups have used demetalized ferritin purified from horse spleens to form magnetoferritin, but these nanoparticles suffered from aggregation and nonuniform shape and size. The approach taken by Dr. Cao and colleagues is unique in that they used genetically engineered, recombinant human heavy-chain ferritin as a biotemplate for the formation of magnetoferritin. This resulted in monodispersed nanoparticles with uniform shape, size and high crystallinity. In addition to their high-quality structure, Dr. Cao found that biomimetically-synthesized magnetoferritin has several other advantages over traditionally synthesized magnetic nanoparticles.

IMPROVED TUMOUR TARGETING AND DETECTION

Ferritin-based nanoparticles are highly amenable to biomedical applications because they are made from a molecule that is naturally produced by human cells. This means that unlike chemically synthesized magnetic nanoparticals, which require special coating to make them biocompatible, magnetoferritin is inherently nontoxic to cells. Another advantage of using magnetoferritin for tumour detection is that it requires no modification to be targeted to cancer cells. It was recently shown that cancer cells overexpress a receptor for ferritin known as transferrin (TfR1). Dr. Cao showed that magnetoferritin specifically binds to TfR1 on cancer cells and that this interaction was sufficient to target the molecule to tumours in vivo. This was true even in the case of microscopic tumours and brain tumours, indicating that magnetoferritin is capable of crossing biological barriers, a limit of many imaging and therapeutic agents. In addition to showing high specificity towards tumour cells, magnetoferritin also displays high sensitivity. Compared to current clinical MRI contrast agents, magnetoferritin has greatly increased relaxivity, thereby improving the detection limit of MRI. Indeed, Dr. Cao showed that magnetoferritin could be used to detect tumours as small as 1 mm by MRI.
As with any new biomedical tool, it will be some time before magnetoferritin sees the clinic, but the initial work conducted by Dr. Cao and colleagues indicates that magnetoferritin holds great potential for improving the detection and treatment of tumours. In the meantime, Dr. Cao and others are investigating other uses for magnetoferritin, including targeted drug delivery, treatment of hyperthermia, magnetic separation and biosensor applications.

Researcher Profile

Dr. Changqian Cao
Associate Professor, Institute of Geology and Geophysics
Chinese Academy of Sciences

Dr. Changqian Cao is an associate professor at the Institute of Geology and Geophysics, Chinese Academy of Sciences. He received his B.Sc. in Veterinary Medicine from Shenyang Agricultural University and his Ph.D. in Geobiology from the Institute of Geology and Geophysics, Chinese Academy of Sciences. In 2011 he received the honour of Excellent Doctor of Chinese Academy of Sciences. His research focuses on genetic engineering of biomineralization for the biomimetic synthesis of magnetic nanoparticles with highly desirable properties as well as their use in nanomedicine and nanodevices.

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The Biology of Telomere Chromatin in Stem cells and Cancers

Dr. Lee Wong is a molecular biologist at Monash University, who has been studying the mechanisms regulating the role of specific chromosomal structures known as telomeres in enabling stem cells and cancer cells to develop and replicate. The work has implications for anti-cancer and stem-cell based therapies.

First, what is your academic background, and how did you start your career in biomedical research?

I have obtained both my undergraduate and postgraduate degrees from Monash University, Australia. In 1995, I have completed a Bachelor of Science Degree and I undertook an Honours year in the Interferon Laboratory, Department of Biochemistry and Molecular Biology under the supervision of Dr. Stephen Ralph. Later, I started my PhD studies on the same project. The focus of my PhD generally involved the interferon treatment in Cancers. After obtaining my PhD in 1999, I worked as a postdoctoral fellow with Prof. Andy Choo at the Murdoch Childrens Research Institute to study chromosome biology. I have recently established a new research group at the Department of Biochemistry and Molecular Biology. Our vision is to identify new molecular players and fundamental mechanisms that control chromosome structural stability through research on stem cells and cancer cells. This is important as chromosome function is vital for proper inheritance of genetic material and has significant implications on human health.

Your recent publications address the role of the cellular protein H3.3 in the regulation of telomere stature and integrity in stem cells and cancer cells. What are the most important findings and what are their possible implications for human medicine?

A continual maintenance of telomere length is required for cell growth as critically short telomeres induce replicative senescence and cell cycle arrest. Cancer cells bypass this arrest mostly by activating telomerase (the enzyme responsible for synthesis of telomeres). However, a subset of cancers (~15%) uses a DNA recombination based Alternative Lengthening of Telomeres (ALT) mechanism. ALT is prevalent in cancers such as bone and brain cancers.

H3.3 is highly evolutionary conserved protein and normally localises to telomeres, but very little is known about the molecular functions associated with this chromatin modification. Our studies show that H3.3 distribution in the genome and its specific modification are severely disrupted in ATRX null cancers. This is one of the few chromatin modifications which is known to distinguish ATRX null cancers from normal cells.

Throughout your research have you collaborated with other research groups? If so, what has been their role and how did they contribute to the progress of your research?

I have collaborated with Professor Philippe Collas from the University of Oslo. Collas heads a lab with strong expertise ranging from cell imaging, chromatin studies to bioinformatics analysis. He has ~160 publications in the chromatin field, including in highly ranked prestigious journals. Collas has a strong interest in studying the role of H3.3 in gene regulation, particularly in cancers and has a long-term collaboration with our group. Collas and his team will provide their expertise in chromatin studies using advanced technologies.
I have also a long-term collaboration with Dr. Jeffrey Mann from Monash University, who is a co-author of several of my publications. Mann has vast experience in gene-editing and producing mouse models using modern molecular technologies. Mann has and will continue to help us in the establishment of stem cells with specific H3.3 mutation, which is a key tool in our research.

Are you planning to continue your research on the regulation of telomere function by chromatins? What might be the next step?

One of the next immediate steps is to perform a series of experiments to determine the molecular role of H3.3 in both normal and cancerous cells. This will contribute to an understanding of core chromatin pathways, and reveal how defects in these pathways can lead to cancer. This knowledge will be instrumental to the development of effective and targeted therapies for these cancers, which have proven to be refractory to standard chemotherapeutic interventions.

The other step we will take is to define the non-genetic factors involved in ALT suppression. It is unclear how ATRX mutation leads to ALT. We will perform methodical and targeted disruptions of factors which contribute to telomere chromatin formation in order to identify the components which act in concert with ATRX to suppress ALT. This work will yield a better understanding of the non-gene defects associated with ALT activation and thus, potentially lead to new diagnostic and treatment targets for ATRX mutation-driven ALT cancers.

Principles and Biomedical implications of the Chromatin Regulation of Telomere Integrity

Telomere length is directly linked to the ability of stem cells and cancer cells to live and replicate infinitely. Here we discuss the principle in light of the findings of Dr. Wong on the role of H3.3 histone protein and its chaperone ATRX on maintaining telomere integrity, and their possible therapeutic implications.

TELOMERE FUNCTION IN CELL BIOLOGY

The DNA held within the nuclei of our cells is packaged and arranged in biological structures known as the chromosomes. The latter carry all the genes necessary to control the various functions performed by the cell. During cell division, our chromosomes undergo duplication, a process undertaken by specialized DNA-building enzymes to ensure that both of the daughter cells receive equal sets of new chromosomes. However, in each time a cell divides these enzymes do not copy the chromosomes to their full length, making the daughter chromosomes of the new cells shorter than their predecessors (a phenomenon known as chromosome truncation). To avoid the loss of essential genes due to chromosome truncation, the terminal ends of the chromosomes possess a non-gene-coding region of repetitive DNA units known as 'telomeres'. Upon subsequent cell divisions, a number of these telomeres are abraded, accounting for shorter daughter chromosomes. Adult body cells (referred to as somatic cells) lack efficient mechanisms for telomere replenishment. Thus, at some point in the cell lifespan where the telomeres reserve is fully consumed (after 50-70 divisions), the cell stops dividing and eventually dies. Indeed, scientists have established a link between telomere chromosomal truncation and cell ageing. On the other hand, some types of eukaryotic cells, namely embryonic cells (include stem cells) and cancer cells, possess a specific enzyme termed telomerase, which is capable of replenishing the chromosomal telomeres, allowing a nearly infinite ability of division. Although telomerase is also present in somatic cells, its activity is regulated to very low or even undetectable levels under normal circumstances.

Although telomerase is a primary biological factor that drives telomere lengthening, other factors also influence the capacity for telomere renewal. Chromosomes are not typically found on their own in the nucleus, but are rather complexed with structural proteins. The chromosomal DNA-protein complexes are termed chromatins. One of the chief proteins that are found in close association with chromosomal DNA is known as histones. The latter has been found to play a major role in controlling the integrity and stability of telomeres. Dr. Wong and her research team have been studying the role of chromatins in telomere biology, in terms of maintaining structure and length. These studies will help the scientific community to better understand the basis of the diseases associated with telomere mutation, malformation or dysfunction.

DISEASE IMPLICATIONS OF TELOMERE BIOLOGY

As discussed, there is a well-established correlation between the lifespan of cells with telomere shortening. Shorter telomere lengths have been linked to human health conditions, many of which are age-related. They have been reported in patients suffering from age-related diabetes, cardiovascular disease, migraines and increased risk of neurodegenerative diseases. There are also direct evidences for the importance of telomere length in human disease derives from patients with mutations in the genes encoding the functional components of the telomerase enzyme. These diseases are characterized by rapid telomere attrition, and thus have shorter telomeres and exhibit compromised regenerative capacity of tissues particularly in highly proliferative tissues such as bone marrow, epithelial cells and liver. One example of such human diseases is Dyskeratosis congenital, a premature aging syndrome linked to mutations in the telomerase complex resulting in decreased telomerase stability and shorter telomeres. Patients with Dyskeratosis congenital develop numerous different disease conditions, including short stature,

bone marrow failure, skin defects, blood-cells regeneration defects, infertility and premature death. These patients are also susceptible to develop cancer. Another example of a human disease involving telomerase mutation is Aplastic Anaemia. Individuals with Aplastic Anaemia also show accelerated telomere shortening and die young. Consistently, mouse models of telomerase deficiency also show affected maintenance and regeneration of tissues that undergo extensive proliferation, further implicating the impact of the short telomere length on health.

TELOMERE CHROMATIN AND STEM CELLS

Stem cells are undifferentiated cells that retain an exceptionally high capacity for unlimited replication and the ability to differentiate into specialized organ cells, such as muscle or liver cells (a characteristic referred to as pluripotency). The capacity for continual telomere replenishment is important to the

maintenance of pluripotency in stem cells, but neither the detailed telomeric chromatin structure nor the mechanism for regulating the continual telomere length renewal have been defined. This knowledge is particularly valuable for better understanding of the pluripotent nature of stem cells, which can be the basis of therapies for many diseases related to telomere length and integrity. For instance, research on a class of stem cells, known as induced pluripotent stem cells, which are artificially obtained from somatic cells, show that upon their development, a telomerase-dependent telomere elongation occurs, which continue post-reprogramming until reaching an embryonic cell telomere length. Interestingly, induced pluripotent stem cells generated from dyskeratosis congenital patient cells, which have short telomeres and suffer from premature senescence, could restore telomere integrity.
In somatic cells, telomeres are mostly enriched with histone modifications characteristic of 'silenced chromatin', which remains in a permanent strong association with telomeres. However, Dr. Wong and her fellow researchers have shown for the first time that embryonic stem-cell telomeres are enriched with an 'active' histone variant termed H3.3, which gradually dissociates from telomeres upon cell differentiation. Moreover, depleting embryonic stem cells of H3.3 resulted in telomere-dysfunction and deregulation of telomere chromatin, indicating that H3.3 plays important role in maintaining telomere chromatin integrity. These findings raise the question of whether telomere chromatin remodelling might be a requisite for telomerase-dependent telomere elongation during cellular differentiation.

TELOMERE CHROMATIN AND CANCERS

As discussed above, somatic cells in absence or insufficiency of telomerase can stop dividing and die. However, if telomerase is re-activated, cells escape telomere attrition crisis and can become immortalized. The majority of human cancers reactivates telomerase expression, which makes it a potential biomarker for most cancers. Human ALT (Alternate Telomere Maintenance) cancer cells, cells characterized by remarkable high telomere length, do not contain any telomerases, but rather adopt other mechanism to maintain telomere length. ALT mechanism is observed in at least 15% of human cancers, ALT is a common phenomenon in tumours including those from the brain/central nervous system and bones. Brain and bone cancers represent the leading cause of cancer related mortality respectively, in young people (15-29). In addition, brain cancers are the leading cause of cancer mortality in all age groups under 40. .A recent study has linked ALT cancers to a common mutation of ATRX, an enzyme recently identified by Dr. Wong and other researchers to be essential to the H3.3 histone assembly and deposition. The frequency of ATRX mutation is as high as 90% in human ALT tumours. Thus, currently the work of Dr. Wong is focused on determining whether mutations of ATRX affect establishment of chromatin marks at telomeres in ALT cancer cells. The loss of a proper inheritance of chromatin marks at telomeres in ALT cancers may drive indefinite telomere elongation, hence promoting an unlimited cellular lifespan in these cancers.

The existence of ALT for telomere maintenance in cancers raises the possibility that telomerase-positive tumours might escape anti-telomerase therapies by activating the ALT mechanism. For these reasons, it is important to delineate the ALT mechanism to have a clearer picture of the tumorigenic process and the development of specific chemotherapeutic interventions targeting ALT-specific cancers. The work of Dr. Wong will unveil a novel telomere maintenance mechanism that is central to the replication of ALT cancer cells. It will have an impact on our understanding of the behaviour of ALT cancers in terms of the underlying mechanism for telomeric alterations accompanying malignant transformation, and potential target for the clinical treatment of these cancers.

THE PROMISES OF THE TELOMERE CHROMATIN RESEARCH

In the future, more detailed studies of the dynamic chromatin changes at telomeres that occur as cells undergo differentiation, nuclear reprogramming, or cancer development will be key to understanding how telomeres and their epigenetic maintenance are crucial to human health. Furthermore, many questions endure about the role of telomeres and the aging process. A full understanding of how telomeres impact the proliferative capacity and pluripotency of embryonic stem cells and induced pluripotent stem cells holds great promise for stem cell therapies of disease. Thus, the complete understanding of telomere biology will have broad-ranging implications for human health.

Researcher Profile

Dr. Lee Wong
Research group leader, Monash University

Dr. Wong is a group leader at the Department of Biochemistry and Molecular Biology, Monash University. She leads an internationally competitive group of researchers in the fields of chromosome and epigenetic research in stem cells and cancers. Her research is mainly focused on understanding the mechanisms underlying the establishment and regulation of centromere and telomere chromatin in stem cells and cancers, and the epigenetic reprogramming during stem cell differentiation and early embryo development.

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MONASH University
Medicine, Nursing and Health Sciences

Preventing Vascular Ageing as a **Means of Life Extension**

Dr. Yu Wang is a cardiovascular medicine research scientist interested in the ageing processes occurring in mammals. Her research has been focused on understanding how the blood vessels age, and specifically on the role played by SIRT1 protein, a longevity regulator.

To aid our readers better understand your work, please tell us how your research background led to your interest in vascular ageing?

It was my strong curiosity of research- and technology-based discovery led to my current interest in vascular ageing. Eighteen years ago I started a research project in the University of Auckland focusing on metabolic hormones and their potential in the treatment of type two diabetes. I identified a novel phosphoprotein, P20, involved in the signalling pathways of two important metabolic hormones, and a glycosylated adiponectin, which we identified as an important hormonal factor for regulating lipid homeostasis. Although we did not discover a drug so far, the experience of these pioneering results convinced me to follow my own true and reproducible results.

The results obtained at the University of Hong Kong set the foundation for my current interest in vascular ageing. They showed the endothelial dysfunction occurs much earlier than those of the metabolic defects in various animal models with metabolic syndrome. For example, vascular insulin resistance can be detected as early as at three weeks of high fat diet whereas metabolic insulin resistance appears at around two month of the same dietary treatment. This indicated that vascular abnormality may represent a major culprit for many ageing-related diseases. Thus, it is crucial to understand the fundamental etiopathogenesis for early vascular ageing.

The role and functioning of the SIRT1 protein are central to your research. Why this specific enzyme and why now – what role does SIRT1 play in current biomedical research?

SIRT1 (Sirtuin 1) is a longevity regulator and plays a unique role in the prevention of endothelial senescence and vascular ageing. As an enzyme, its activity can be modulated by pharmacological approaches. SIRT1 differs from other sirtuins in that it contains extra-long NH2- and COOH-terminal domains that are dynamically regulated by posttranslational modifications, which affects the enzyme activity. Identification of the signalling pathways responsible for modifying the termini will uncover more specific biological process for targeted therapy against early vascular ageing. For example, the CDK5 as an upstream kinase of SIRT1 phosphorylation may be a potential drug target, if a detailed functional relevance of this regulation within the vasculature wall becomes available.

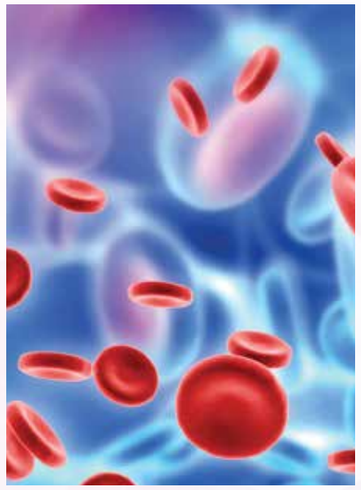
Does SIRT1 hold any promise from a therapeutic standpoint? How might a potential SIRT1-based treatment be implemented?

As mentioned above, SIRT1 has already been identified as a promising anti-ageing candidate. However, more investigations are needed to understand its signalling and functional mechanisms, in order to identify drugs that can modulate a specific signalling pathway within a fully-defined tissue/cell type for modulating SIRT1 activity.

How do you expect this study to affect your future work? Might it contribute to healthcare, society and/or policy?

Life expectancy has been creeping upwards in most countries for many years. The portion of people aged over 80 years is growing faster than any other age groups and will exceed the number of young (under the age of 15) people by 2050 for the first time in history. Population ageing will pose a great challenge to global healthcare system in 21th century, as ageing-associated organ dysfunctions are the major culprits for the world's leading causes of death. To develop therapeutics for preventing early vascular ageing will not only help to reduce the healthcare burden for chronic diseases but also increase the workable population beyond the current retirement age.

Have you worked with any other researchers for this study? If so, how did they contribute?



I feel very lucky to have met Prof Paul M Vanhoutte, whose passion in vascular biology has greatly inspired me. In addition, our achievement in the field should be credited to the friendly and stimulating research environment in the Department of Pharmacology and Pharmacy, the University of Hong Kong, and the hard working students and research fellows.

Will you extend this research further? Which directions are you interested in exploring further and why?

Our research has been expanding and extending from endothelial senescence to endothelial dysfunction, atherosclerosis and vascular ageing. Since the actions of SIRT1 are tissue- and organ-specific, our goal is to focus on the endothelial SIRT1 and elucidate endothelial-selective pathways for targeted drug discovery and development. During the next few years, we will also concentrate on the molecular events and signalling mechanisms underlying early vascular ageing.

Promising prospects for novel anti-ageing treatments

Ageing and especially anti-ageing treatments are topics that have fascinated mankind from very early on. Current anti-ageing research, such as Dr. Yu Wang's work on vascular ageing prevention offers new perspectives on curing illnesses associated with old age and hopes of slowing down the aging process.

LIFE EXTENSION BY CALORIC RESTRICTION

Researchers have shown as early as 1930's that a low calorie intake (caloric restriction, CR) without malnutrition can as much as double laboratory rats' life spans. Since that seminal experiment the hypothesis that CR can extend life has been tested on a number of species. The results showed that a caloric restrictive diet can results in an increase (sometimes by as much as 10-fold) in the lifespan of many, but not all species.

Despite the mixed results on interspecies effects and the relatively few studies on primates (two randomised control studies on rhesus monkeys and one study on human volunteers started in the 1980's; and two studies on humans started in 2007 and 2010, respectively), life extension has been a topic of interest to sufficiently many people that CR has been quick to enter the pop culture. Of those who have already subjected themselves to CR in the hopes of increased longevity or improved health, staying on the diet has proved challenging. In reality, the long-term health effects of moderate CR with sufficient nutrients are as of yet unknown in humans.

Identification of the signalling pathways responsible for modifying the unique regions of SIRT1 will uncover more specific biological process for targeted therapy against early vascular ageing.

ANTI-AGEING: A GENETIC APPROACH

In parallel with research efforts such as the primate and human studies mentioned above, which measure the effects of CR, a significant body of work exists that attempts to understand why CR leads – at least in some cases – to greater longevity and/or fewer or less severe illnesses associated with the old age. A genetic approach led to the discovery of the sirtuins in early 1980's. Sirtuins are a family of NAD+-dependent protein deacetylases that exert multiple cellular functions. Sir2 (Silent information regulator 2), was the first gene discovered in this family in the budding yeast, but its role in caloric restriction-dependent lifespan extension was not known until 2000, when it was referred to as the "longevity protein Sir 2". Sirtuins are highly conserved during the evolution from bacteria to humans and genetically modified yeast cells overexpressing Sir2 showed 30 per cent longer lifespan, while those lacking the Sir2 gene showed 50 per cent reduction in lifespan. Similar effects of Sir2 on lifespan were subsequently observed in other lower organisms.

Sirtuins are hypothesised to play a key role in an organism's response to stresses (such as heat or starvation) and to be responsible for the lifespan-extending effects of calorie restriction. In mammals, the family is represented by seven members named SIRT1-7, which share the catalytic domain with Sir2. SIRT1 is the mammalian orthologue most highly related to Sir2 and Dr. Wang's research programme focuses on understanding the role played by SIRT1 in mammals.

MAMMALIAN SIRTUINS

Sir2 plays a direct role in anti-aging in species as varied as yeast, worm and fruit flies. In higher organisms SIRT1 works by a more complex mechanism and appears to operate in an indirect manner, targeting the cellular energy metabolism in ways that benefit normal physiology. To that end, an energy-sensor network model has been put forward recently, which proposes that SIRT1 adjusts cellular responses to the energetic state of the cell. By deacetylating transcription factors, cofactors, and histones, SIRT1 has been shown to promote resistance to metabolic, hypoxic, and genotoxic stress, thereby controlling cell metabolism, survival, proliferation, and cell fate. SIRT1 achieves this formidable task by not being specifically localised in the cell, but instead shuttling between the cytoplasm and the nucleus.

More recent studies highlighted important homeostatic functions of SIRT1 in the vascular endothelium, where it modulates vascular growth, shape and function. Prof. Paul M Vanhoutte, a medical doctor specialising in vascular biology has played a significant role in Dr. Wang's research efforts to understand SIRT1's role specifically in vascular ageing. By studying the thin layer of cells on the interior surface of blood vessels, which separate the circulating blood from the rest of the vessel wall (the endothelium), Dr. Wang observed that endothelial dysfunction occurs much earlier than those of the metabolic defects in various animal models with metabolic syndrome. Vascular abnormality may represent a major culprit for many ageing-related diseases. Dr. Wang and Prof. Vanhoutte's recent work suggests that in aged arteries, SIRT1 expression and activity is blunted, which contributes to the development of atherosclerosis and abnormal vascular responses. A recent study suggests that cyclin-dependent kinase 5 (CDK5) is responsible for the phosphorylation of SIRT1, which blocks the anti-senescence activity of SIRT1 and plays a critical role in the loss-of-SIRT1 function during vascular ageing. Thus, by inhibiting CDK5, SIRT1 function can be improved, in turn preventing the development of atherosclerosis and slowing down the process of vascular ageing.

HEALTHCARE APPLICATIONS

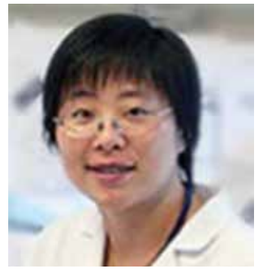
Life expectancy has been creeping upwards in most countries for many years. The portion of people aged over 80 years is growing faster than any other age groups and will exceed the number of young (under the age of 15) people by 2050 for the first time in history. Population ageing will pose a great challenge to global healthcare system in 21th century, as ageing-associated organ dysfunctions are the major culprits for the world's leading causes of death. To develop therapeutics for preventing early vascular ageing will not only help to reduce the healthcare burden for chronic diseases but also increase the workable population beyond the current retirement age.

As mentioned above, SIRT1 has already been identified as a promising anti-ageing candidate. However, more investigations are needed to understand its signalling and functional mechanisms, in order to identify drugs that can modulate a specific signalling pathway within a fully-defined tissue/cell type for modulating SIRT1 activity.

As an enzyme, SIRT1's activity can be modulated by pharmacological approaches, especially in endothelial cells - the direct contact with the blood stream. Unlike the other members of sirtuins, SIRT1 contains extra-long NH2- and COOH-terminal domains that are dynamically regulated by posttranslational modifications, leading to enhanced or decreased enzyme activity. Thus, identification of the signalling pathways responsible for modifying these unique regions of the protein will uncover more specific biological process for targeted therapy against early vascular ageing. The identification of CDK5 as one of the upstream kinases of SIRT1 has uncovered an important regulatory pathway and provides a new strategy for combating vascular ageing to inhibit hyperphosphorylation of SIRT1 by antagonising CDK5 activity. This, and other approaches, may effectively slow down the vascular ageing process in line with the pace of chronological ageing, in turn reducing the risks of ageing-induced cardiovascular events.

Dr. Wang's research has been expanding from endothelial senescence to endothelial dysfunction, atherosclerosis and vascular ageing. During the next few years, she is planning to concentrate on elucidating the molecular events and signalling mechanisms underlying early vascular ageing, especially how these contribute to the alterations of individual components within the arterial wall.

Researcher Profile



Dr. Yu Wang
Associate Professor
Department of Pharmacology & Pharmacy
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Dr. Yu Wang is a researcher in the Department of Pharmacology and Pharmacy at the University of Hong Kong. Her research interests have been led by various abnormalities within the field of cardiometabolic syndrome, since the initial encounter with biological research at the University of Auckland about eighteen years ago. Currently she is primarily working on vascular ageing and specifically the mechanisms of action of SIRT1, a protein deacetylase involved in caloric restriction-dependent life extension. While not in the laboratory, Dr. Wang enjoys spending time with her husband and three children, as she places equal importance of having a happy family and a successful career.

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New developments in **heart disease research**

Dr. Takahiro Yoshizawa is interested in unveiling the role of homeostasis in health. Here he discusses the mechanisms of homeostasis regulation and his work on the role of a small protein, Adrenomedullin, in heart health.

As a starting point, can you discuss how your research background led to your interest in healthcare and more specifically in Adrenomedullin's role in regulating homeostasis?

I have always been interested in human health and disease treatment and prevention. In a healthy organism, all organs and cells work in harmony to sustain the life. In an unhealthy one, the organism is attempting to return to homeostasis.

Consequently, maintaining homeostasis is essential for health, with Adrenomedullin (AM) playing a key role. AM is a peptide involved in anti-hypertrophy, anti-oxidant stress, anti-inflammation and organ protection. Our work focuses on RAMP2, a regulating factor of the AM-receptor system in the heart. Understanding the AM-receptor system may shed light on cardiac homeostasis regulation.

Your research interests are highly focused on exploring the role and functioning of the 52-amino acid Adrenomedullin. Why this specific protein and why now?

We are generally interested in the AM-receptor system. AM is secreted by many organs and cells where it participates in autocrine and/or paracrine signalling by receptor binding. Receptor-activity-modifying protein (RAMP) is one example of AM receptor-related molecule, and three subtypes of RAMPs were identified. Our work suggests that RAMP2 (whose genetic deletion was embryonic lethal with abnormality of cardiovascular development in knockout mice) is essential for AM function.

Has your work revealed any significant findings to date, and if so, what are the potential implications?

Prior reports indicate that AM or RAMP2 homozygote knockout mice are dead in utero, which hinders analysis of AM and RAMP2's cardiac function. To address that, we engineered drug-induced cardiac-specific RAMP2 knockout mice (C-RAMP2 KO). Prior work suggested AM-dependent regulation of NADPH oxidase was the cause of AM's organ protective effect. Our work showed instead that AM-RAMP2 system regulates a range of mitochondrial functions, thereby maintaining cardiac homeostasis. This finding may help understand the mechanisms of mitochondrial regulation in the heart (a common dysfunction in heart failure patients). This could in turn help the development of new treatments for heart failure.

Much of your work uses transgenic mice as a model organism. What is the degree of genetic and functional similarity between Adrenomedullin in humans and mice? In other words, how likely is your research to help understand and possibly treat human disease?

AM is produced in many mammals including humans and mice. While AM shows many structural differences across species, on the functional level there are many similarities. In humans, AM blood levels increase in hypertension, heart failure, kidney diseases and myocardial infection, which suggests involvement in organ damage. Similar to the effect observed in humans, transgenic mice overexpressing AM show lower blood pressure and resistance to organ damage. However, AM knockout mice can show embryonic lethality with abnormal cardiovascular development (homozygote KO) or higher blood pressure and cardiac hypertrophy (heterozygote KO).

A RAMP2 homozygotic knockout is lethal in utero and is phenotypically similar to an AM knockout, suggesting that the AM-receptor system is essential for AM functions and homeostasis. In humans, naturally-occurring variations of RAMP2 gene may induce hypertension. However, almost of the functions of RAMPs are still unclear in humans.

This study focused on understanding the AM-RAMP2 system may help develop a therapeutic or diagnostic method for cardiac disease based on the AM-receptor system.

Given Adrenomedullin's role in a range of illnesses, do you envision any type of Adrenomedullin-based treatments and how they might be implemented?

Since AM levels in the blood are increased in hypertension, heart failure and myocardial infarction in humans, AM is likely involved in cardiovascular disease. AM may be useful as a biochemical marker and even as a therapeutic agent. In this latter role it has been tested with heart failure patients by Dr. Kitayama (Miyazaki University).

AM binds two types of receptors (CLR-RAMP2 and CLR-RAMP3), which may explain AM's versatility. The drawback is that non-specific effects may cause unwanted side effects. Investigation of the AM-receptor system is important to provide safe and effective cure for a range of diseases.

What implications might this study have on future work and potentially on healthcare, society and policy?

To elucidate the mechanisms of receptor specificity is essential to discovering new drugs. RAMPs regulate AM receptor activity and specificity. Our studies on the newly-discovered function of RAMP2 in the heart show that the AM-RAMP2 system is essential for cardiac metabolism and homeostasis. This makes the AM-RAMP2 system a promising therapeutic target of heart failure.

New Cures for the Aching Heart?

Heart disease is the single deadliest illness of our times, responsible for nearly a quarter of human losses worldwide. Research in the biomedical field by Dr. Takahiro Yoshizawa may help develop new treatments for heart failure.

21ST CENTURY MEDICINE

Improved standards of living and advances in medicine have led to an unprecedented increase in life expectancy from about 30 years just a century ago to a 2010 world average of 67 years. We are no longer dying from the epidemics of early 20th century, but from old-age diseases such as cancer and heart disease.

Heart disease alone is responsible for 17 million deaths worldwide. Healthy diets, exercise, and reducing smoking and alcohol are well-publicised preventative strategies. Blood clot prevention, medicines that lower cholesterol levels, and various surgical procedures are but a few of the treatments available. In addition, biomedical researchers make great efforts to shed more light on the causes of the illness, in the hopes of new and possibly improved cures.

This finding may help understand the mechanisms of mitochondrial regulation in the heart (a common dysfunction in heart failure patients). This could in turn help the development of new treatments for heart failure.

HEART DISEASE AND THE ADRENOMEDULLIN SYSTEM

A long standing interest in health and disease treatment and prevention is the motivation of Dr. Takahiro Yoshizawa's work to understand the Adrenomedullin system. An Assistant Professor at Shinshu University and recipient of several Japanese and international Young Investigator Awards and a Research Fellowship for Young Scientists, Dr. Yoshizawa is specifically interested in how a healthy organism maintains homeostasis and what goes wrong when that balance is upset. Adrenomedullin (AM) is a 52-aminoacid peptide which plays a key role in the maintenance of homeostasis and Yoshizawa's research focuses on the RAMP2 protein, a component of the AM-receptor system in the heart.

According to Dr. Yoshizawa, understanding the AM-receptor system may shed light on cardiac homeostasis regulation. His hypothesis is based on recent studies showing that AM plays an important role in vasodilation, depression, anti-hypertrophy, antioxidant stress, anti-inflammation, and in organ protection, suggesting that AM may be secreted to compensate organ damage. Dr. Yoshizawa collaborated extensively in this work with Professor Takayuki Shindo of Shinshu University Graduate School of Medicine.

A STORY OF MICE AND MEN

Although coming from a country known for its healthy diet and consequent low rate of heart disease, Dr. Yoshizawa is interested in better understand the AM-RAMP2 system as that knowledge may help develop a therapeutic or diagnostic method for cardiac disease based on the AM-receptor system. His work shows that the AM-RAMP2 system maintains cardiac homeostasis by regulating a range of mitochondrial functions. A dysfunctional mitochondrial regulatory system is often encountered in heart failure patients; therefore, this finding may help develop new treatments for heart failure.

AM is produced in many mammals including humans and mice, and as much of the biomedical research, this work is done in mice.

While AM shows many structural differences across species, on the functional level there are many similarities. AM's involvement in organ damage is shared between humans and mice. Genetically-engineered mice that do not express AM can show embryonic lethality with abnormal cardiovascular development (homozygote KO) or higher blood pressure and cardiac hypertrophy (heterozygote KO).

The AM-receptor system exists in many organs and cells where it participates in autocrine and/or paracrine signalling. Receptor-activity-modifying protein (RAMP) is one example of AM receptor-related molecule, and RAMP2 (whose genetic deletion was embryonic lethal with abnormality of cardiovascular development in knockout mice) is essential for AM function. A RAMP2 homozygotic knockout is lethal in utero and is phenotypically similar to an AM knockout, suggesting that the AM-receptor system is essential for AM functions and homeostasis. In humans, naturally-occurring variations of RAMP2 gene may induce hypertension. However, almost of the functions of RAMPs remain unclear in humans. Dr. Yoshizawa set out to address important questions about the role of RAMP2 in heart function, using modern genetic engineering and the latest techniques in the heart described below.

Dr. Yoshizawa and Professor Shindo's research team solved the function of RAMP2 by generating drug inducible cardiac myocyte-specific RAMP2 knockout mice (C-RAMP2-/-) using the *α*MHC-MerCreMer-lox system. The Cre-lox system is a sophisticated tool for conditional knockouts, which uses the Cre recombinase, an enzyme that catalyses recombination between two lox sites. The alpha myosin heavy chain (*α*MHC) promoter regulates the place of gene expression (cardiac motet-specific) on the downstream side. In C-RAMP2-/-, cardiac motet-specific deletion of RAMP2 can be caused in embryonic stage to senility stage.

C-RAMP2-/- mice showed dilated cardiomyopathy-like heart failure with cardiac dilatation and disarray of myofibrils after induction of the gene-deletion. Abnormality of mitochondrial structure was also found by electron microscopy. Using real-time PCR, the mitochondrial regulatory factor PGC-1 was also found to be down-regulated. Primary-cultured cardiac myositis showed reduced mitochondrial-membrane potential and increased mitochondrial reactive oxygen species (ROS) production in RAMP2-deletion dependent manner. C-RAMP2-/- showed the down-regulated activation of CREB, one of the regulator of PGC-1. Finally, forskolin treatment recovered the cardiac remodelling, CREB activity, and the expression of mitochondria-related genes.

The complex set of experiments, accessible only to specialists in the field, demonstrated that the AM-RAMP2 system is essential for cardiac metabolism and homeostasis.

HEALTHCARE APPLICATIONS

Commenting on his results in a 2013 article published in the reputable medical journal Hypertension, Dr. Yoshizawa concludes that the AM-RAMP2 system is a promising therapeutic target of heart failure. AM's involvement in hypertension, heart failure and myocardial infarction in humans makes the peptide a major player in cardiovascular disease. Many researchers propose to use AM as a biochemical marker for early detection of heart health problems and even as a therapeutic agent. Ongoing work with heart failure patients by Dr. Kitayama at Miyazaki University already explores that possibility.

Dr. Yoshizawa nonetheless warns that the clinical applicability of AM, like that of other bioactive endogenous peptides, has two major limitations: AM has a very short half-life in the blood, and the cost of the recombinant protein is very high, which together make the use of AM impractical for treatment of chronic diseases. However, his findings show that the cardiac activity of AM can be affected by modulating RAMP2. Medicines targeting the AM-RAMP2 system, if developed, would directly promote both energy production and free radical suppression in cardiac myocytes. Since elucidating the mechanisms of receptor specificity is essential to drug discovery, Prof. Shindo's research team recommend further investigation of the AM-receptor system to provide safe and effective cure for a range of diseases.

Genetic studies and in vivo studies such as Dr. Yoshizawa's aimed at understanding the causes of heart disease add to the repertoire of biomedical research undertaken at world's medical and academic centres. Additional concerted efforts are, by way of examples, ongoing R&D by pharmaceutical companies who develop new medicines targeting cholesterol levels, heart inflammation, etc. or new developments in imaging techniques such as computed tomography, positron emission tomography, and magnetic resonance imaging. It would appear that while the cure for the aching heart is just about as complex as the problem itself, we are getting closer to finding it by the day.

Researcher Profile

Takahiro Yoshizawa
Assistant Professor

Dr Takahiro Yoshizawa obtained his Ph.D. in medical sciences at Shinshu University where he is now an Assistant Professor and recipient of several Japanese and international Young Investigator Awards and a Research Fellowship for Young Scientists. His research focuses on homeostasis maintenance in healthy organisms and what goes wrong when that balance is upset. A major component of his work unveiled that the AM-RAMP2 system maintains cardiac homeostasis by regulating a range of mitochondrial functions, thereby proving that the AM-RAMP2 system is essential for cardiac metabolism and homeostasis. This finding can form the basis for developing new treatments for heart failure.

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SHINSHU UNIVERSITY

Expanding Our Knowledge of Brain Expansion

Dr. Oz Pomp discusses his involvement in a team study on Katanin p80

As humans, we owe our superior intelligence to our large brains, in particular our large cerebral cortex. The cerebral cortex is responsible for higher-order brain functions such as reasoning and language, and has undergone rapid expansion in the human lineage in the past three to four million years. However, the genes responsible for this expansion remain largely unknown. Now, by sequencing the genomes of three families affected with microcephaly, or small brain size, an international team of researchers from Harvard Medical School and the A*STAR Institute of Singapore have identified a novel regulator of cortical growth and development known as KATNB1. Their findings are a significant contribution to our understanding of brain development. Not only do their results have potential applications in genetic testing and therapeutic development, they also expand our knowledge of the pathways that may have been important for the evolution of the impressive size of the modern human brain.

A familial story

Often the best way to gain insight into how a process works is to look at what happens when that same process stops working. One approach to this, known as forward genetics, is to identify a phenotype that one is interested in, such as small brain size, and to then investigate the cause of that phenotype. To study how brain expansion occurs, the authors of this study looked to families with individuals whose brains failed to grow and develop normally, a condition known as microcephaly. Microcephaly is typically diagnosed shortly after birth and is characterized by very small head size. The condition can stem from defects in a number of neurodevelopmental pathways but is usually accompanied by serious cognitive impairments and reduced life expectancy no matter the cause. To determine the underlying genetic cause of the microcephaly in the families identified in this study the team sequenced the exons, or protein-coding DNA sequences, of the affected individuals and their unaffected family members. By comparing which genomic regions were shared between affected family members but not with unaffected members the researchers were able to pinpoint mutations in a single gene, KATNB1, as the likely culprit for microcephaly in these families.

KATNB1, a novel regulator of brain development

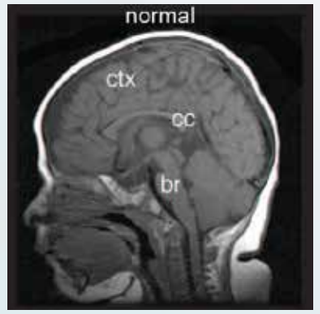
The KATNB1 gene encodes the p80 subunit of the katanin complex. Named from the traditionally made Japanese sword known as a 'katana', the katanin complex severs microtubules, structural proteins important for dynamic cellular process such a cell division, motility and axon outgrowth. The katanin complex is comprised of a p80 regulatory subunit and a p60 catalytic subunit. While the catalytic activity of the complex has been well studied, less is known about its role in vivo. This work identifies a novel function for katanin in brain growth and development. To further investigate how mutations in KATNB1 lead to microcephaly, the researchers used a combination of cell culture and animal models of neuronal development.

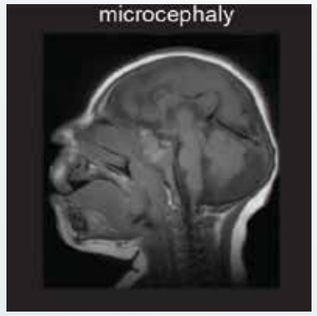
Building brains big and small

To study the function of katanin p80 in a developing organism the investigators genetically engineered a mouse line lacking functional Katnb1. Mice lacking Katnb1 show even more severe neurological defects than their human counterparts and none of the embryos survived past 15.5 days. As an independent approach the team used TALEN genome editing to create zebrafish harboring truncated forms of katnb1. Similar to the Katnb1 gene-trap mice, the zebrafish showed a range of developmental defects before dying. Interestingly, the zebrafish lacking katnb1 were viable so long as the maternal fish possessed a functional copy of gene. This can be explained by the maternal contribution to embryo development. Before the zygotic genome is transcriptionally active, maternal mRNA supplies the genetic material necessary for development. This finding emphasizes the importance of the developmental timing of Katnb1 function and its role in early embryogenesis. In addition, the severity of defects observed in the mouse and zebrafish lines compared to the affected humans suggests that Katnb1 is likely to be at least partially functional in these individuals.

Next, to determine how mutations in KATNB1 lead to the neurodevelopment defects, specifically in human patients, the team examined neuronal development on a cellular level. Since primary brain cells from patients are not available, the team reprogrammed patient's skin cells into induced pluripotent stem cells (iPSCs) and next differentiated the cells into brain cells. Examining these disease relevant cells, the team found that mutation of KATNB1 leads to defects in microtubule organizing centre known as centrosomes. These defects ultimately resulted in perturbation of the cell cycle and cells with extra chromosomes, leading to fewer proliferating cells and increased cell death. The approach taken is a classic example of the tremendous potential of using iPSCs technology to model human diseases in a dish which highlights the utility of naturally occurring human mutations for providing insights unavailable in animal models. "Similar to the situation in patients, mutation in KATNB1 induced mitotic abnormalities in human brain cell precursors but not in other iPSCs derivatives, thus, this in vitro system may be used to address one of the fundamental questions in the field is - why is it only the brain size that is affected?" says Dr. Oz Pomp.

This work adds significantly to our understanding of cortical growth and development by identifying katanin p80 as a novel regulator of brain expansion.





Researcher Profile

Dr. Oz Pomp
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Dr. Oz Pomp received his Ph.D. from Bar Ilan University, Israel in 2008. Currently, he is a Senior Research Fellow in Bruno Reversade's lab at the Agency for Science, Technology and Research (A*STAR), Singapore. His research interests include neuronal diseases and X-chromosome dynamics.

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CLINICAL TRIALS

Once a drug or a therapeutic intervention has been demonstrated to be effective in cell and animal models of disease at the preclinical research stage, it is critical to validate these findings in humans in the population of interest. These studies, also called clinical trials, are performed for the purpose of a) demonstrating safety of the drug/intervention, b) demonstrating efficacy against its proposed target in specific human populations, and importantly, c) to obtain regulatory approval for marketing the drug/intervention to clinicians and ultimately, to patients. While it is important to remember that clinical trials are by no means the final stage to prove a drug's efficacy against a certain indication (it is necessary to monitor the drug for long periods of time to demonstrate its efficacy and safety against rare populations that may have not constituted a significant portion of the selected patients during clinical trials), clinical trials are a critical step in drug discovery. This section will discuss novel interventions against oral mucositis in patients receiving chemotherapy and patients with angina/ischemia that are in or have passed clinical trials, respectively. First, Dr. Donini and colleagues' discovery of SGX94, which stimulates innate immunity to ameliorate infections in patients receiving chemotherapy who have a compromised immune system, will be discussed. This compound is now under clinical trials and is a novel and exciting method to use an innate defense regulator to modulate the immune system in place of antibiotics. The second part of this section examines a creative novel intervention to treat refractory angina, Reducer ™, by Dr. Banai and colleagues, which improves blood flow to cardiac muscles by narrowing the coronary sinus.

Managing Refractory Angina in Absence of Options

Professor Shmuel Banai is an interventional cardiologist at the Tel Aviv Medical Center, affiliated with the Tel Aviv University Sackler School of Medicine, Israel. He is interested in vessel wall biology and physiology and in exploring therapies and interventions to obstructive diseases of the heart blood vessels. Professor Banai has recently validated a new heart device for the treatment of refractory angina.

To begin, can you tell the readers how did you start your career in medical research and why did you choose Cardiology as a domain?

During the years 1988 and 1992, I was a visiting research fellow at the Cardiology Branch of the National Heart, Lung and Blood Institute of the National Institutes of Health in Bethesda, USA. There, under the guidance of Dr. Stephan Epstein and Dr. Ellis Unger, I pursued the early stages of my career in Cardiology research. Since I was a medical student, I have been fascinated by the physiology of the heart, the amazingly engineered organ that is in constant movement to pump the blood throughout our bodies. Understanding the mechanistic events underlying heart diseases and developing solutions to circumvent them has been gratifying to me. In addition, I have a particular empathy for heart disease patients and their families, who are fighting some life-threatening disease conditions.

As we will come to later, you have recently demonstrated the benefits of a new heart device in the treatment of refractory angina. But first, can you explain what is refractory angina and to what extent it affects the patient's health and lifestyle?

Angina is a common clinical problem, which results from the insufficient blood flow to some regions of the heart muscles (ischemia). This often occurs as a complication of coronary artery disease, in which one or more of the coronary arteries, which nourish the heart, become occluded. However, a growing number of patients who suffer from coronary artery disease continue to experience severe angina, despite optimal medical therapy, and despite the surgical interventions aimed to overcome the coronary occlusion. This resistant condition is referred to as refractory angina, while the patients suffering such a type of angina are often labeled as 'no option' patients.

Patients with refractory angina are usually severely disabled, experience marked limitation in their ability to perform ordinary physical activities, and have poor quality of life. In contrast to what was previously reported and believed, the life expectancy of patients with refractory angina is not significantly inferior to that of other patients with stable/chronic ischemic heart disease. Therefore, the goal of refractory angina therapy is mostly directed at improving these patients' quality of life rather than extending their lifespan.

According to your publications, the new heart device is described as a 'coronary sinus reducer'. What is the nature of this device, and how does it alleviate refractory angina?
The coronary sinus reducer is a balloon-expandable hourglass-shaped metal mesh designed to be implanted in the coronary sinus, which drains blood out of the heart muscles. Placement of the Reducer creates a controlled narrowing of the lumen of the coronary sinus, which leads to an increase in backward pressure and dilatation of small blood vessels supplying blood to the heart muscle. As a result, more blood is forced into the areas of the heart muscle which lack sufficient blood supply.

What about the safety of the Reducer™? Were there any major complications or adverse effects of the implantation or the operation of the stent?

There are no safety issues with the Reducer. In the COSIRA trial, which was designed to evaluate the safety and efficacy of the Reducer, no difference in the rate of adverse events was observed between the treatment and the sham-treatment groups.

Does the implantation of the coronary sinus reducer require special care prior administration or long period of hospitalisation afterwards?

Implantation of the Reducer is a very simple and straightforward procedure. It does not require any special care. Patients can go home on the same day of the procedure or on the next morning. Implantation of the Reducer is done under local anaesthesia through a vein in the neck.

Throughout your therapeutic studies on the coronary sinus reducer, have you collaborated with other research groups or institutes?

Absolutely, the earliest clinical trial (First-In-Human) was conducted in one medical centre in Germany and two medical centers in India. The COSIRA trial was conducted in 11 medical centers in western Europe and Canada. The two principal investigators of the COSIRA trials were Dr. Stefan Verheye from Antwerp Cardiovascular Centre, Belgium, and Dr. Marc E. Jolicoeur, from the Montreal Heart Institute, Canada.

Are you planning to extend your research on the management of refractory angina? What might be the scope of the next step?

Currently, the Reducer™ is routinely in use in several clinical centres in Europe. A multicentre observational clinical study is currently undergoing in Europe. Patients with severe chronic refractory angina, confirmed for heart ischaemia, and have 'no options' for other surgical interventions are being enrolled.

Reducer™: A New Treatment For Refractory Angina

A new heart device (Reducer™) for the treatment of refractory angina has been recently validated in a multicenter international clinical trial. Reducer™ provides a safe and effective treatment option for patients who are neither responsive to medical therapy, nor admissible to surgical or other interventions.

REFRACTORY ANGINA & THE 'NO OPTION' PATIENTS

Angina pectoris – better known as angina – is a clinical symptom in which the patient suffers chest pain accompanied by a feeling of tightness in the chest and shortness of breath. Angina symptoms typically manifest in patients suffering shortage in the supply of oxygen-rich blood to a considerable area of the heart muscles (cardiac ischemia). This shortage is often a direct result of a partial occlusion of one or more of the coronary arteries that nourish the heart muscle, a condition most commonly encountered by patients with atherosclerotic coronary artery disease. The latter involves the accumulation of atheroma (a waxy substance composed of cholesterol and cells) within the walls of the coronary arteries, leading to a significant narrowing of the lumen. Patients with atherosclerosis of the coronary arteries are at a high risk of experiencing heart attacks, which is potentially life-threatening. Patients with angina experience marked limitation in their ability to perform ordinary physical activities and have a poor quality of life. Those patients continue to have debilitating symptoms that keep them from climbing stairs or even walking longer than 100 feet, Professor Banai said.

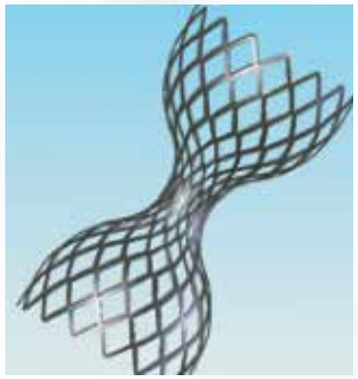
The majority of patients with angina are responsive to medical treatment or to revascularization with by-pass surgery or with stent implantation. However, a significant number of patients continue to suffer from angina, despite the proper medical care and despite revascularization. Moreover, some of these patients might not be amenable to surgical or interventional procedures. 'These patients continue to suffer from angina, commonly referred to as refractory angina, and are clinically labelled as 'no option' patients', says Professor Banai. According to some studies, patients with refractory angina constitute around 25% of total angina patients. The number of these patients is currently growing, especially in aging populations. For example, in the United States, 1,000,000 people are estimated to suffer refractory angina. With the current lack of curative options, managing cases of chronic refractory angina has been a tough challenge for cardiologists.

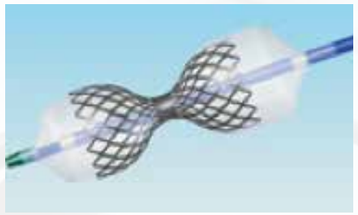
REDUCER™: THE CORONARY SINUS REDUCER TECHNOLOGY

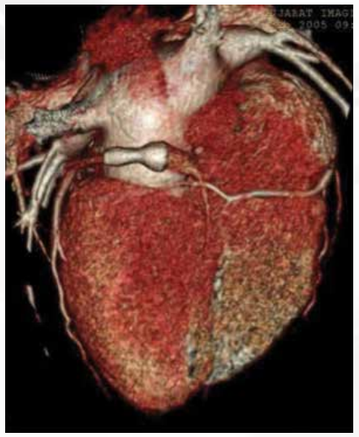
The Coronary Sinus reducer (Reducer™) is a balloon-expandable hourglass shaped stainless-steel mesh, developed by the specialty medical device company, Neovasc Inc. The Reducer™ is designed for implantation in the coronary sinus, the terminal vein through which the blood is drained out of the heart, to create a controlled narrowing in the sinus. As a result, coronary sinus pressure is elevated. These events favour the redistribution of oxygenated blood through the coronary arteries towards ischemic areas of the heart muscles, according to Professor Banai. The implantation of the Reducer™ is performed via a catheter, which is introduced through a large vein at the right side of the neck by a painless procedure under local anesthesia.

THE COSIRA TRIAL TO VALIDATE THE CONCEPT

The earliest study exploring the validity of the Reducer™ was conducted by Professor Banai and his research team in 2007, on a limited number of refractory angina patients who were not suitable candidates for revascularisation procedures. The study gave the first evidence on the safety and efficacy of Reducer™, which resulted in no adverse reactions and relieved the angina symptoms in the majority of the patients over a six-month period of follow up. However, despite the promising outcomes, a subsequent study was required to confirm these preliminary results in a larger group of patients, while taking stronger control measures to ensure their reliability. Hence, Professor Banai, together with a panel of international collaborators has conducted a large clinical







Reconstruction of the back of the heart and the Reducer in the coronary sinus

trial to evaluate the safety and efficacy of the Reducer™ in 14 clinical centres in Canada, Belgium, England, Scotland, Netherlands, Sweden and Denmark. The study, which is better known as COSIRA, lasted from April 2010 to April 2013 involved a total number of 104 patients of with refractory angina. Selected patients were >18 years old, and belonged to class III or IV angina, according to a four-class scale of physical disability adopted by the Canadian Cardiovascular Society (CCS). Importantly, these patients suffered from angina despite optimal medical therapy and were not amenable to revascularization procedures ('no option' patients).
During the COSIRA study, strict measures were taken to circumvent biases related to the patient or the staff's prior knowledge of the group assignments. The participants were randomly divided into two study groups, one of which was assigned the Reducer™ implantation (treatment group), while the other was assigned the same procedures, with no Reducers being placed (sham control group). None of the patients were aware with the group allocation throughout the study. 'Participants were offered either headsets playing music or conscious sedation to mask the conversation in the room regarding the randomisation and the procedure', says Professor Banai. Similarly, except for the cardiologists performing the implantation procedures, none of the investigators responsible for assessing the angina at follow-up, the laboratory staff and the statisticians analysing the data were aware of the group assignments.

The patients were assessed on various measures reflecting both the safety and efficacy of the Reducer™. Efficacy measures included the proportion of patients with an improvement of two or more CCS angina classes from baseline to 6 months after implantation, as a primary measure, while the proportion of patients with an improvement of one or more CCS classes from baseline to 6 months and exercise tolerance was set as a secondary measure. Safety measures included any adverse effects during a six-month follow-up.

The Reducer™ device offers a safe and efficacious therapeutic option for alleviation of refractory angina in patients who are neither responsive to therapy nor admissible to revascularisation procedures

REDUCER™ IS A PROMISING TREATMENT OPTION

At 6 months, 35% of the patients in the treatment group had improved by at least two CCS classes versus only 15% of the control group. In addition, 71% of the patients in the treatment group versus 42% in the control group improved by at least one CCS class. Quality of life, has improved by 17.6 points in the treated group versus 7.7 points in the control group, as measured by a validated questionnaire.

Overall, 76 adverse events were reported in the treatment group and 93 in the control group. Three cases have suffered complications of cardiac ischemia, and one death (due to multiorgan failure) in the control group, and there was one case of ischemic complications and no deaths in the treated group.

'The COSIRA trial demonstrated that implantation of the Reducer™ device to narrow the coronary sinus significantly improved symptoms and quality-of-life in refractory angina patients', says Professor Banai.

THE FUTURE OF THE REDUCER™ TECHNOLOGY

Parallel to the evidence provided by COSIRA on the validity of Reducer™ as a curative option in cases of refractory angina, the device has already obtained a CE mark in Europe in 2011. Professor Banai and his team have also recently reported the clinical results of the first 23 patients treated with the Reducer implantation under the CE mark. This report combines the results from 2 medical centres in which the Reducer is currently used for the treatment of such 'no option' refractory angina patients. The results show that the implantation of the Reducer™ was associated with a significant improvement in the severity of angina and ischemia, while no associated adverse effects were observed. Currently, Neovasc Inc plans to conduct a trial to obtain approval of the US food and in the United States.

Currently, the Reducer is used in selected medical centers in the United Kingdom, Belgium, The Netherlands, Italy, Switzerland and Germany.

Researcher Profile

Neovasc
Neovasc Inc. is a specialty medical device company that develops, manufactures, and markets products for the rapidly growing cardiovascular marketplace.

Its products include the Tiara™ technology in development for the transcatheter treatment of mitral valve disease, the Neovasc Reducer™ for the treatment of refractory angina and a line of advanced biological tissue products that are used as key components in third-party medical products including transcatheter heart valves.

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Innate Defense Regulators: Novel Therapeutics for Emerging and Antibiotic-Resistant Diseases

Dr. Oreola Donini is the Chief Scientific Officer of Soligenix Inc, a late-stage biopharmaceutical company developing products that address unmet medical needs in the areas of inflammation, oncology and biodefence. Dr. Donini is the inventor of Soligenix's anti-infective technology, SGX94, which offers an alternative option for treatment of antibiotic-resistant infectious diseases.

First, what has been your primary motivation as a scientist and what is your academic and professional background?

I have always been focused on the application of science at the crossroads of disciplines. For my PhD program, I joined a research group working at the interface of chemistry and neurology to understand potential treatments for epilepsy and Alzheimer's disease. There, my interest in computational chemistry as a discipline started to grow, because it operates at this interface, integrating knowledge from many disciplines.

I received my PhD in chemistry from Queen's University and went on to complete a post-doctoral fellowship with one of the leading computational chemists, Dr. Peter Kollman of the University of California, San Francisco. After completing my fellowship, I moved into biotechnology, attracted by the cross-disciplinary research and the desire to apply my skills to developing drugs to improve patients' lives.

During my career, I have always focused on working with biotechnology companies with novel solutions to unmet medical needs. These included the former Inimex Pharmaceuticals Inc, where I and my colleagues invented SGX94, and the current Soligenix Inc, where we have established a late-stage development pipeline focused on treatments for rare diseases with unmet medical needs.

SGX94 is the name of a novel drug that you have invented as a therapy to bacterial infections. What is the nature of SGX94 and how does it work?

SGX94 is representative of a new class of small peptides (essentially small molecules) which we call 'innate defence regulators' (or IDRs). These peptides interact with and activate a specific component of our immune system, known as the innate immune system, which comprises a number of first-line cells and molecules that instantly respond to infections. IDRs basically harness our body's innate immune responses to control infection.

Most of us are familiar with antibiotics as the most common treatment for bacterial infections, so what is the difference between innate defence regulators and conventional antibiotics?

Antibiotics kill bacteria directly, while IDRs engage your body to use its normal mechanisms to kill and clear bacterial infections. This is extremely important to understand because there have been no other attempts to develop drugs with this type of mechanism. IDRs do not kill or even interact with the bacteria directly, but rather they change the innate immune response so that more bacterial killing mechanisms operate at the site of infection, while reducing inflammation.

Why are new approaches for emerging and antibiotic resistant diseases so important?

Antibiotics are probably the most powerful drugs known to mankind, and have greatly reduced illnesses and deaths due to infections over the past six decades. However, with the wide-scale use of antibiotics to treat varieties of infections, a growing number of bacterial species and strains have evolved mechanisms to adapt to antibiotic toxicity. It is not a myth to think of a coming era when antibiotics are no longer effective against bacterial diseases. There is a wide consensus among medical and public health communities on the pressing need to develop potential alternatives to antibiotics.

Throughout the stages of the SGX94 development, has Soligenix collaborated with any academic research groups? If so, what has been their role and how did they contribute to the progress of the research?

Innate immunity has been a very under-appreciated aspect of our immune system for many years and it is only with the ground-

breaking work of our collaborators, as well as others in the field, that a treatment approach like SGX94 is possible. The original idea of modulating the innate immune system instead of directly targeting the bacteria was developed at the University of British Columbia by Dr. Brett Finlay and Dr. Robert Hancock. As the program advanced, other collaborators lent their expertise in molecular biology (Dr. Leonard Foster, University of British Columbia) as well as specific animal models of infection (Dr. Steve Opal, Brown University; Dr. Lisa Morici, Tulane University). As with any scientific endeavour, the outcome is always built upon the efforts of many individual scientists and this is true of the SGX94 program as well.

Is there anything else you would like to add?

While we believe that the impact of IDRs in the treatment of antibiotic resistant and emerging infectious disease is a breakthrough, we cannot lose sight of the fact that the innate immune responses to both pathogenic invasion and tissue damage has applicability in many other indications. Given the broad applicability of IDRs, we are also investigating the utility of IDRs in oral mucositis and other indications such as macrophage activation syndrome. At Soligenix, we pursue these and other indications as part of our overarching goal to develop treatments for rare diseases, where there remains an unmet medical need.

Innate Defence Regulators as Alternative Anti-infectives: **Principles and Current Status**

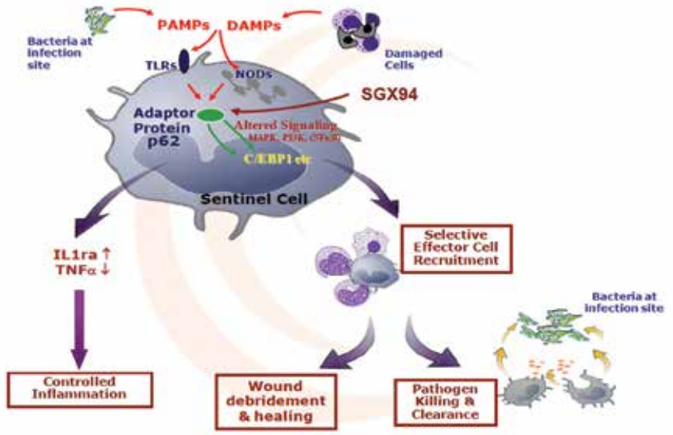
Antibiotic resistant and emerging diseases represent an alarming public health problem, with a growing number of diseases being difficult to treat with conventional antibiotics and anti-infectives. Innate defence regulators constitute a late-stage technology that offers promising therapeutic alternatives to antibiotics for the treatment of a variety of infections and inflammatory conditions.

THE ALARMING THREAT OF ANTIBIOTIC RESISTANCE

Antibiotic resistant and emerging infectious diseases represent constant and growing threats to public health, both in the developed and the developing countries. A number of the world's most dangerous diseases are caused by pathogens that are not only difficult to treat, but also antibiotic resistant, making the treatment options very limited. Antibiotics have been the gold standard in the treatment of infectious diseases and since their discovery in the 1940's they have greatly reduced illnesses and deaths due to infections. However, with the wide use of antibiotics over the past decades, an increasing number of bacterial species and strains have become adapted to their action. According to the US Center of Disease Control and Prevention, approximately two million people become infected with bacteria that are resistant to antibiotics each year, with at least 23,000 annual deaths occurring as a direct result of these infections. Therefore, with the gradually diminishing efficacy of the currently used antibiotics, and with only few discoveries being made on new antibiotic generations, the importance of identifying alternative methods to treat infections has been highlighted by the World Health Organization (WHO) and major American public health bodies including Centers for Disease Control and Prevention, the National Institutes of Health (NIH), and the Biomedical Advanced Research and Development Agency (BARDA). Moreover, the US White House released a 'National Action Plan for Combating Antibiotic-Resistant Bacteria' in March 2015, with the search for new treatment alternatives being a key goal.

INNATE IMMUNITY TO COMBAT INFECTIONS

The immune system is the biological system that defends our body and organs against invading pathogens and microbes. The protection provided by the immune system is achieved through two functional components, termed adaptive and innate immunity. The



former involves the production of antibodies and killer cells that are specifically generated to neutralize or destroy a certain individual microbe, while the latter comprises a set of killer and scavenger cells that recognize common factors among microbes and thus reacts generically to all of them. Activation of innate immunity typically involves inflammation, the reaction in which innate immune cells aggregate at the site of infection to destroy and clear the causative microbe. The generation of adaptive immunity requires days or weeks as it involves a sequence of time-consuming processes. By contrast, innate immunity is pre-programmed to respond very quickly, constituting the first line of defence against infections. The majority of the research done on the immune system has been directed to adaptive immunity as it constitutes the main biological target for vaccines, but recently innate immunity is gaining growing interest from the perspective of exploring a rapidly acting alternative to antibiotics. Several classes of molecules have been investigated for their ability to stimulate or enhance innate immune responses to kill and clear pathogens during infection. It is noteworthy that this anti-infective mechanism differs from that of antibiotics, which act by directly targeting and killing the bacterial cells. However, most of these innate immune-stimulants are unable to differentiate inflammatory and pathogen-clearing pathways of the innate immunity, which can bias the outcome either towards aggravated inflammation (potentially harmful) with high pathogen-clearing activities or towards diminished inflammation and insufficient infection clearance. In 2004, Dr. Donini and her research fellows discovered a clinical candidate in a novel class of small molecules known as innate defence regulators (IDRs), which have the potential to selectively modulate infection-clearing and inflammatory pathways so as to reduce inflammation, while enhancing the infection-clearing responses. Therefore, IDRs offer a unique and safe therapeutic approach that harnesses innate immunity to treat infectious diseases as well as other inflammatory disorders.

THE IDR PROTOTYPE SXG94 AS AN ANTI-INFECTIVE

SGX94 is the lead representative of IDRs that binds to a highly evolutionarily conserved protein of the innate immune system known as p62. This leads to stimulation of innate immune cells like monocytes and macrophages, which engulf both bacteria and other damaged cells and clear them from the body, while mitigating the associated deleterious inflammatory responses. Since the discovery of the IDR concept, SGX94 has been shown to improve the disease outcome in mouse models of both local

and systemic infections with a broad array of bacterial pathogens. The anti-infective power of SGX94 was evident upon either preventive or therapeutic administration (i.e. prior to or during infection), and either as a stand-alone agent or in conjunction with suboptimal antibiotic treatment. Despite these promising therapeutic effects, Dr. Donini and her research team do not propose SGX94 to totally replace antibiotic treatment, but to rather be the drug of choice in cases of antibiotic-resistant infections or in cases where antibiotic use is discouraged or contraindicated. 'Antibiotics are true "miracle drugs" and we would not consider replacing them with IDRs, however, the latter can be the drug of choice in cases where antibiotics are ineffective or contraindicated', said Dr. Donini. For instance, with the ongoing concerns over the growing problem of antibiotic resistance, the empirical use of broad-spectrum antibiotics to prevent infection in individuals under high risk (e.g., patients suffering immune-deficiencies) or to blindly control infections until the causative bacteria is identified in the laboratory is highly discouraged. In these cases, SGX94 can be used instead of antibiotics to prevent or treat infections. Moreover, antibiotics in many instances can increase inflammation, because as they kill the bacteria, the contents of the bacterial cells further activate the inflammatory pathways of the innate immune system. Thus, combining SGX94 to antibiotic treatment will not only enhance the infection clearance, but also mitigate antibiotic-induced inflammation. This is of significant importance, because most other anti-inflammatory approaches can delay pathogen clearance as well as tissue healing.

Innate defence regulators (IDRs) offer a promising alternative to antibiotics for treatment of antibiotic-resistant infections, for prevention of infections in highly susceptible individuals, and for empirical treatment of yet-undiagnosed infections.

THE PRESENT AND FUTURE OF SGX94

Dr. Donini and her research team have already characterized the majority of the therapeutic and pharmacological attributes of the SGX94 action in a variety of animal models, as well as in laboratory cell and organ culture systems.

These studies demonstrate the value of the drug in enhancing the clearance of bacteria and increased survival after acute infections with a wide array of bacterial species. This work has led to a phase I clinical trial with SGX94 to evaluate the safety and tolerability of the molecule in human subjects. The lead clinical IDR, SGX94, was found to be well tolerated in 84 healthy volunteers under single and multiple ascending dose administration. In addition, there were no serious or severe side effects and there was no dose-limiting toxicity or maximum tolerated dose identified. Importantly, although the trial was conducted to primarily evaluate safety, secondary studies on isolated blood cells from the treated participants showed similar innate immune responses as obtained from mouse models, indicating consistency of the SGX94 action across species. SGX94 is currently being evaluated in a phase II clinical study of approximately 100 patients as a potential treatment for the reduction of oral mucositis in patients receiving combined chemo- and radiation therapy for head and neck cancer. Oral mucositis is non-infectious disease, but a condition of serious inflammation, ulceration and damage of the mouth cavity as a side-effect of chemoradiation. In such a case, SGX94 may potentially reduce the inflammation while enhancing clearance of the dead/dying cells, reducing the severity and the duration of oral mucositis in these patients.

Dr. Donini and her research team are currently pursuing the SGX94 technology platform (USAN: dusquetide) in a number of other unmet medical needs including emerging and antibiotic resistant diseases. For instance, they have been evaluating SGX94 in preclinical models of melioidosis, a disease caused by the antibiotic-resistant, gram-negative, intracellular bacterium Burkholderia pseudomallei, which is broadly endemic in areas of northern Australia and southeast Asia and is considered a high priority biothreat agent.

Researcher Profile

Dr. Oreola Donini
Chief Scientific Officer
Soligenix, Inc.

Dr. Donini has more than 15 years experience in drug discovery and preclinical development with start-up biotechnology companies and has been instrumental in leading early stage development of several novel therapies into the clinic. She is an inventor of Soligenix's SGX94 innate defence regulator technology, which is currently undergoing a phase II clinical study in oral mucositis. Since the discovery of the IDR technology, Dr. Donini has been responsible for overseeing all aspects of SGX94 development, including its manufacture and preclinical testing, which demonstrated efficacy in combating bacterial infections and mitigating the inflammation and tissue damage caused by trauma or infections.

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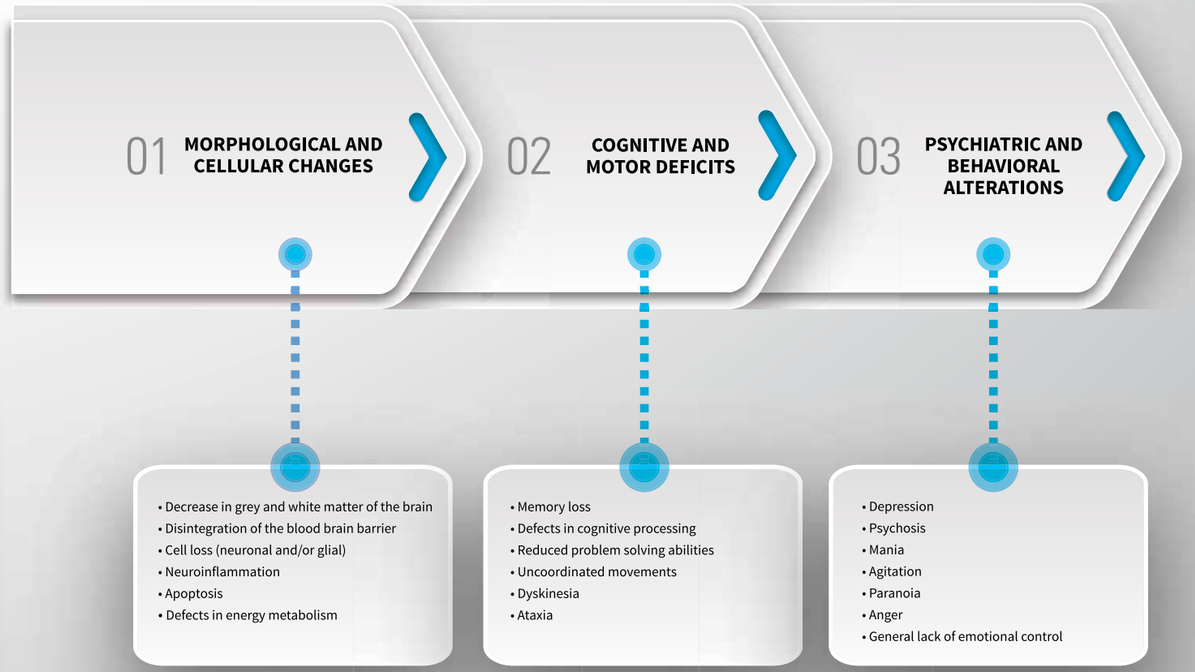
BRAIN DISORDERS

The brain is a highly complex organ that functions to regulate our mobility, behavior, emotions, memory, homeostatic mechanisms and survival. It is amazing that a single organ can carry out such a range of different functions through its different areas that are segregated and yet work together to maintain a functional organism. The human brain is by far the most complex and developed, allowing us to carry out emotional and cognitive processing that is unseen in any other organism. As such, the brain is a highly protected organ that is shielded from most agents outside of the blood brain barrier which protects the central nervous system, including from endogenous components such as immune cells, giving it the term "privileged organ". The brain is also very carefully regulated, both by internal and external mechanisms as disruption of its functioning can have devastating effects that can range from emotional deficits, loss of cognitive abilities, inability to perceive or sense accurately, dysregulation of bodily functions, and even death. Brain disorders is a term that has been recently used to describe disorders that occur due to altered functioning of the brain, either due to acute causes, such as traumatic brain injury, or chronic alterations, such as neurodegenerative disorders. Recently, this term has been expanded to include psychiatric disorders that are strongly hypothesized to have >

> biological contributions, such as schizophrenia, major depression and bipolar disorder. This is of particular importance, as patients with neurological disorders often experience psychiatric alterations, to the extent that they are diagnosed with a psychiatric illness, such as depression, and patients with psychiatric disorders show neurological alterations when their brains are examined post-mortem or through imaging techniques. To understand the incredibly broad range of changes that patients with brain disorders experience, we can think about the different effects a single brain disorder can have. An individual with traumatic brain injury to the frontal lobe may experience global effects, which could involve inflammation of the brain, characterized by infiltration of immune cells to the site of damage. While this occurs to protect the brain from invasion by pathogens at the site of injury, neuroinflammation has been consistently show to cause cell death due to the release of cytotoxic cytokines and impairment of neuronal and glial functioning, leading to cognitive damage. It may cause effects specific to the site of injury as well, where the patient may experience deficits in functions that are regulated by the brain area that has been damaged, such as working memory, emotional regulation, fine motor control and comprehension. Brain disorders can also be studied using different tools at different levels of alterations. For example, as much of the brain function depends on its morphology, examining alterations in the shape of the brain, either globally by examining its mass and diameter, or specifically by looking at differences in grey and white matter volume in brain regions of interest may yield important pathological information. One can also examine morphological changes at the cellular level, such as alterations in the number and shape of synapses, organelles such as the mitochondria, and neuronal projections. The investigators can also examine brain disorders at the molecular level, where they can examine apoptotic factors, cytokines, metabolites and/or changes in nucleic acids. The three researchers covered in this section approached brain disease/injury from three unique angles. Dr. Imamura's group studies how the blood brain barrier is damaged in septic encephalopathy and how it further contributes to the pathophysiology of sepsis in the brain. Dr. Zupan's group takes a very different approach to brain injury, where they study how traumatic brain injury affects the ability to perceive emotions in patients. Lastly, Dr. Duennwald and his group studies brain disorders at a very molecular level, where they examine protein misfolding and the molecules responsible for this in neurodegeneration.

ALTERATIONS SEEN IN BRAIN DISORDERS



Challenges and opportunities for understanding septic encephalopathy

Dr. Yukio Imamura studies the molecular mechanisms of septic encephalopathy, enabling the development of better treatments and prevention.

To begin, could you describe your research background and how you became interested in studying the molecular mechanisms of the brain?

When I was Master's student in engineering I was engaged in biological chemistry. I was particularly interested in how living organisms are controlled by a lot of molecules. At this time (around 1995), since molecular neuroscience contained a large mystery and was becoming a major topic worldwide I was strongly interested in the molecular mechanisms and started the research.

How did you come to study septic encephalopathy in particular?

Although I moved labs in Japan and Canada, when I went to the brain science institute, RIKEN, one of my colleagues (Dr. Matsumoto, Osaka Univ.) had a strong interest in my research background. He is a physician and neuroanatomist at Osaka University Hospital in Japan. He introduced me to septic encephalopathy as a very important topic in the medical intervention field including intensive and critical care. Especially the brain dysfunction that follows sepsis (i.e. septic encephalopathy) still remained obscure and he thought my research background should be useful to tackle the molecular mechanisms behind it.

Why do you think progress has been relatively slow regarding understanding the molecular causes of septic encephalopathy?

Patients of septic encephalopathy often showed coma, delirium and cognitive dysfunction. Clinical studies and preclinical research using animal models have been challenged to uncover the pathophysiological mechanisms and therapeutic potentials. Although a large variety of pathological molecules and physiological conditions affect the outcome of septic encephalopathy, their individual dynamics are not totally clear. Therefore, many candidate pathophysiological molecules associated with septic encephalopathy have been tested for their association with a better outcome for septic encephalopathy without success.

You discovered that sepsis causes an increase in interleukin-1ß(IL-1ß) in the brain resulting in decreased synaptic function and that inhibiting IL-1ß alleviated this effect. Could IL-1ß be a potential target for therapeutic development? What challenges might that pose?

IL-1ß will be a therapeutic target for septic encephalopathy. In fact, increased IL-1RN, an inhibitor of the IL-1 ß receptor, was linked with a better outcome for septic patients. However, septic encephalopathy sometimes shows complicated symptoms including vascular dysfunctions that lead to ischemia and edema. Therefore, in addition to molecular targeting therapy, morphological alteration should be carefully considered.

Quantum dots are an exciting new tool with potential uses in biomedical imaging. How can quantum dots be used to better understand septic encephalopathy?

Quantum dots show fluorescent intensity and employ a higher signal to noise ratio than the conventional fluorescent probes. In addition, we are currently working on developing a novel quantum dot in the second near-infra red wavelength (900-1300nm). The light in this wavelength shows highly permeability and less absorbance by the body. If this novel technology is successfully applied, the understanding of septic encephalopathy will be better.

Could quantum dots be used for therapeutic delivery in addition to imaging?

Q-dots can be useful for the delivery of drugs. Of course, to do so, Q-dots have to be coated with a hydrophilic polymer. Our research group is currently trying to produce novel Q-dot probes for drug delivery.

You mention vagus nerve stimulation as a potential method for mitigating sepsis. How long before we might see something like this in the clinic? What are the current barriers to its use?

Currently, pre-clinical research groups stimulate the vagus nerve with electrical stimuli directly. This process includes surgical operation. I think that the surgical operation is hard on patients with severe sepsis. Therefore, we are currently working on developing a novel method for non-invasive stimulation of the vagus nerve without surgical operation.

How will your findings inform and shape future studies or treatment of septic encephalopathy? Where should future work on septic encephalopathy focus?

Although our research studies were performed using a rodent model of septic encephalopathy, these lines of research suggest two potential therapeutic approaches for future development. First is the development of novel therapeutics targeting the antagonist of molecules involved in inflammation (e.g. interleukins, matrix metalloproteinases etc.) and neuronal function (e.g. neuronal synaptic plasticity, population activities of neurons). Second is the possibility of vagus nerve stimulation. To apply these methods to human patients we should very carefully test the validity and potential side effects in humans. Therefore, future works of septic encephalopathy will be needed to tackle these issues.

Tackling sepsis head on

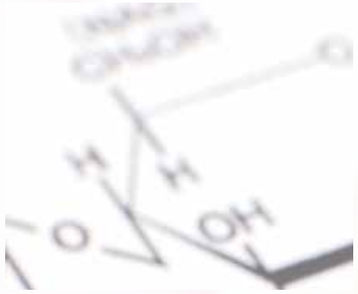
Systemic inflammation caused by some bacterial infections often leads to lasting brain damage. Dr. Yukio Imamura is working to figure out how this happens on a molecular level and how it can be prevented.

TOO MUCH OF A GOOD THING

In general, the inflammatory response is a good thing; it is the body's way of protecting itself from excessive cellular damage. Upon pathogen infection, or other forms of cellular wounding, the body mounts a coordinated response that involves a multitude of cellular players and signals. Typically, the inflammation is localized to the site of infection or injury and is relatively short-lived, resolving itself without need for serious intervention. However in the case of widespread infection, a systemic inflammatory response can be triggered. This condition, known as sepsis, is an over activation of the innate immune system. The response overwhelms the body and if left unchecked can lead to organ failure, neurological dysfunction and death. Sepsis affects millions of people each year and is one of the leading causes of death in intensive care units. Between 28 and 50 percent of patients admitted with sepsis will die.

For a condition that affects so many, surprisingly little is known about how sepsis-induced brain damage occurs.

While the immunological cascade leading to sepsis is well characterized, efforts to halt the overactive immune response have been largely unsuccessful. As such, treatment of sepsis primarily focuses on clearing the responsible infection from the body by administering antibiotics, and preserving organ function by managing symptoms such as fever and low blood pressure. Unfortunately, early symptoms of sepsis are easy to mistake for other, less deadly diseases such as the flu and treatment may not be sought until sepsis has done severe damage to the body. Even for patients who undergo successful treatment, sepsis can have lasting effects including impaired liver, kidney and brain function.



SEPTIC ENCEPHALOPATHY

The brain is especially sensitive to the effects of sepsis. Septic encephalopathy, brain damage caused by sepsis, is one of the most common complications of sepsis and is the leading predictor of death. Despite the critical role that septic encephalopathy plays in determining outcomes of sepsis, little is understood about how it arises in septic patients. There are no conclusive molecular markers of septic encephalopathy. A diagnosis is usually made by testing cognitive function. Without a more complete understanding of the pathophysiology of septic encephalopathy, successful treatments and preventative measures will be difficult to develop.

Dr. Yukio Imamura, a researcher in the laboratory of nano-bio probes, quantitative biology center, RIKEN, is working to elucidate the molecular mechanisms of septic encephalopathy. With a multidisciplinary research background that includes chemical electronics, biochemistry, and molecular oncology, Dr. Imamura is well poised to tackle this multifaceted problem. Using animal models of sepsis, in combination with biochemical assays and electrophysiological techniques, Dr. Imamura has identified several key factors in the development of septic encephalopathy, as well as some promising approaches for prevention and treatment.

MOUSE MODELS: TOWARDS A MOLECULAR MECHANISM

Postmortem examination of the brain following sepsis shows brain swelling, bleeding, and

cell death due to lack of oxygen. It is known that during sepsis the blood brain barrier is disrupted. This allows chemical substances to access the brain that would normally be excluded. Inflammatory cytokines, for example, are released during sepsis and are known to alter synaptic function by inhibiting the expression of excitatory receptors and enhancing the expression of inhibitory neurotransmitters. Other data suggests that sepsis might alter brain function in part by altering the abundance of several amino acids that function as neurotransmitters. These findings are consistent with the observation that septic encephalopathy disrupts neurotransmission, but there is still a lot that is unknown about the interactions of these molecules and others, and how they are involved in the progression of septic encephalopathy.

Dr. Imamura uses an animal model of sepsis to study the molecular mechanisms of septic encephalopathy. Septic conditions are stimulated in the mouse using a surgical procedure known as "cecal ligation and puncture" (CLP), wherein the cecum of the large intestine is closed off, punctured, and then returned to the abdomen. This allows a large amount of bacteria to be released into the body, triggering systemic inflammation very similar to that seen in septic humans. To study what happens in the brain following sepsis, the mice are sacrificed and very thin brain slices are prepared. Abundance and localization of specific protein can then be performed by immunohistochemistry. In one such assay, Dr. Imamura found that occludin, a marker of the blood brain barrier, was significantly reduced in mice that underwent CLP. He also found that the proinflammatory cytokine interleukin-1ß(IL-1ß) accumulated in microglial cells that its receptor was upregulated on neurons. These results are consistent with the pathology of septic encephalopathy and known mechanisms of the inflammatory response.

To enable better treatments of septic encephalopathy, it is important to understand how molecular changes in the brain lead to impaired brain function. To measure changes in brain function Dr. Imamura uses an electrophysiological technique known as field excitatory post-synaptic potential (fEPSPs) recording. In this method, an electrical pulse is applied to a brain slice, stimulating neuronal activity and the amount of time the neurons remain active following stimulation, termed long-term potentiation (LTP), is recorded. If neurons are damaged or inhibited, LTP will be short or nonexistent. Indeed, Dr. Imamura found that LTP could not be induced in brain slices from septic mice. This was consistent with his finding that IL-1ß was increased in the brain of septic mice, as previous research has suggested that IL-1ß inhibits LTP. To test whether this was the case, Dr. Imamura preincubated the brain slices with an antagonist of the IL-1ß receptor before performing fEPSPs recording. Remarkably, LTP was restored. These results suggest that during sepsis, increased IL-1ß in the brain disrupts normal neurotransmission. It also raises the possibility that the IL-1ß receptor may serve as a target for mitigating the effects of sepsis on the brain.

NEW TREATMENTS AND TECHNOLOGIES

Inhibition of neuronal activity caused by sepsis also plays a role in the body's ability to quell the systemic inflammation. The vagus nerve, part of the autonomic nervous system, is a modulator of the innate immune response. During sepsis, the vagus nerve is weakened and fails to signal to the brain to suppress the overactive immune response. However, researchers have found that electrical stimulation of the vagus nerve during sepsis activates the cholinergic anti-inflammatory pathway, inhibiting the immune response and preventing organ damage in the lungs, gut and spleen. Currently, stimulation of the vagus nerve is done directly and requires a surgical procedure. However, such a procedure would be hard on patients already suffering from the symptoms of sepsis. Therefore, to bring the promising treatment to septic patients, Dr. Imamura and colleagues are working on developing a noninvasive method of vagus nerve stimulation.

There is still a lot to be learned about septic encephalopathy. Only a handful of molecular players contributing to the pathophysiology of septic encephalopathy have been identified and their effects have only been studied in a small region of the post-mortem brain. Quantum-dots (Q-dots) are a novel technology for single molecule imaging that show potential for tracking molecules in the living brain with high spatial and temporal resolution. In the future, Q-dots may even be used for drug delivery. Currently, Dr. Imamura and his colleagues on working on developing novel Q-dots for just such purposes. If they are successful, our understanding of septic encephalopathy will be greatly increased.

Researcher Profile

Dr. Yukio Imamura
Research Scientist, Laboratory of Nano-bio Probes, Quantitative Biology Center, RIKEN

Dr. Yukio Imamura has a multidisciplinary research background. He holds a B.A. in Chemical Electronics and an M.S. in Biochemistry from Tohoku University in Japan, and most recently a Ph.D. in Molecular Oncology from Kyoto University, Japan. Dr. Imamura has held research positions at the Mitsubishi Kagaku Institute of Life Sciences, Ottawa Health Research Institute, Okinawa Institute of Science and Technology, the RIKEN Brain Science Institute, Department of Human Health Sciences, Unit for Liveable Cities at the Kyoto University Graduate School of Medicine. Currently he is a research scientist in the laboratory of nano-bio probes, quantitative biology center, RIKEN. His research has focused primarily on synaptic transmission and the pathophysiology of the brain. His current work focuses understanding and developing treatments for septic encephalopathy.

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QBiC RIKEN QUANTITATIVE BIOLOGY CENTER

Listening to Emotions

Dr Barbra Zupan uses her background in linguistics to understand how traumatic brain injuries (TBI) affect people's ability to process the facial expressions and auditory cues of emotion. She and her collaborators developed a new training program for therapists working with TBI patients.

To begin, how did your background lead you to study communication disorders?

When I started my undergraduate program, I enrolled in Child Studies and Teacher's Education. My goal was to work as a teacher of children with special needs. By the end of my first year in my undergraduate program, I realized the teaching program wasn't really for me. At the time, I was working with a number of children with special needs, and had the opportunity to work alongside the speech language pathologist for one session, for one child. That's all it took. At the end of my first year, I changed my major to Applied Linguistics, and never looked back. After completing my Masters degree in Speech Language Pathology, I worked for five years with children 0-5 years of age with various speech and language disorders, including children with hearing loss. I recognized a lack of research in therapy for children with hearing loss, and decided to pursue my PhD so I could investigate the topic further. I did this to some degree, but ultimately focused my work on how we perceive auditory and visual cues of emotion, generally in people with traumatic brain injury.

You stated that your interest moving forward lies in establishing better norms for how emotional cues are processed. Do you have any specific plans to research this as of now?

I submitted a grant to the Social Sciences and Humanities Research Council in the fall 2014 outlining a very detailed plan to establish these norms. Unfortunately, I found out this month that my scores were just outside the funding range so while I was placed in the funding category, the grant was not actually funded. I plan to re-submit next fall but am also looking at other options for pursuing this work. These norms are integral to moving the field of emotion recognition forward. The importance of emotion recognition to one's overall emotional well-being, self-esteem, mental health, and academic and employment success has been increasingly gaining a lot of attention, so much so, that education ministries across Canada are beginning to prioritize emotional competency in their curriculums. However, since we don't yet fully understand how emotion perception develops and changes across the lifespan, I have difficulty grasping how these skills can be adequately nurtured and targeted in our youth, and how we expect teachers (and various other professionals) to identify when there is a problem, or implement appropriate strategies to assist children (or adults) at improving their emotion recognition skills.

You worked with international collaborators to find and screen a large number of people with TBI. Did this present any significant challenge?

Overall, we worked very well as a team. Scheduling conference calls could be a challenge due to the significant time difference between us, but we did meet regularly via conference calls. We even completed some video taping of mock sessions that we then each scored to ensure we were all scoring responses the same way. However, I would say that the times we managed to meet in person (generally once per year over the course of the grant), we were far more productive. Having open discussion versus sticking to a very scripted, tight agenda via phone, resulted in much more fruitful analysis of our progress and allowed us to better evaluate next steps and discuss the project and data in more detail. Now that the funding has ended, we are unable to arrange meetings such as these, which I think has contributed to us not pushing further development and use of the program. Discussing how to move this forward will require extensive discussion and planning, which we've found challenging to do via conference call (due to the time difference, and the fact our research time is mostly committed with other projects).

Were there any other roadblocks in testing or developing this system?

Initially, we struggled with how best to train the various therapists, doctoral students, and research assistants who would be carrying out the therapy at each site. Training was extensive and we wanted to be sure that each individual involved would be competent in the therapy and delivering all aspects of it as similarly as possible. Since it was impossible for Dr Neumann, Dr Babbage, and I to all train all members of the team, we created consistency through videos and follow-up sessions. Dr Neumann and I held a two-day training workshop for the therapists who would be carrying out the program here in Ontario and we recorded those sessions. We then sent copies of those sessions to Dr Babbage in New Zealand and his doctoral student (who was carrying the program out there) used those for training. I then flew out to New Zealand to observe her first few sessions with participants and provide extensive feedback. I also observed the first few sessions of each of my therapists here in Ontario. Dr Neumann trained her research assistants herself following the same format that we had used on the videos, and also observed their initial sessions.

Emotion Recognition Can Be Recovered

People who have suffered traumatic brain injuries (TBI) often have difficulty identifying other people's emotions. Dr Barbra Zupan and her colleagues have developed several new tools to help people with TBI recover their ability to recognize emotions in others.

A LINGUISTIC APPROACH PROVIDES NEW INSIGHTS

When people with TBI are released from the hospital and return to normal life, their ability to understand how another person feels does not return with time. This ability, known as affect recognition, must be targeted by specific therapies in order to improve it. Dr Barbra Zupan has studied affect recognition and people with TBI over the course of her career as a communicative disorders scientist. She began her education by earning a bachelor's degree in linguistics; approaching communicative disorders this way gives her the capacity to acoustically analyse both speech and emotion expressions. Since linguistics focuses on how acoustic cues, or tone of voice, and the words used in everyday speech can alter perception, she is able to study how both these signals interact with each other, and whether one is more important to perceiving emotion.

The importance of emotion recognition to one's overall emotional well-being… has been increasingly gaining a lot of attention. Since we don't yet fully understand how emotion perception develops and changes across the lifespan, I have difficulty grasping how these skills can be adequately nurtured and targeted

Over the course of their research, Zupan and her colleagues have found that certain emotions are easier for people with TBI to recognize, and that people with TBI don't recognize emotions equally in the face and voice. Zupan and her colleague, Dr. Neumann, designed a study in which 60 people with TBI and 60 controls were asked to identify emotions from static pictures, sound clips, and film clips. They found that for facial expressions, happiness is more easily identifiable than negative emotions such as sadness or anger. Many scientists studying facial emotion recognition argue that the ability to recognize happiness in the face is preserved in patients with traumatic brain injuries; however, Zupan and Neumann, found that if facial affect recognition is impaired, it is at least somewhat impaired for all emotions, including happiness. Unlike facial expressions, happiness is one of the most difficult emotions to recognize in the voice; even people who have not suffered TBI find it challenging. However, Zupan and Neumann found that people with TBI find it equally difficult to recognize happiness in the voice as they do negative emotions in the voice. When viewing film clips, people with TBI did not significantly differ from controls, suggesting that the redundancy provided by cues from both the voice and the face makes it much easier to identify the emotion.

NEW STRATEGIES TO HELP PATIENTS

Zupan, who lives and works in Canada, collaborated with an international team located in Buffalo, NY; Charlotte, NC; and Wellington, New Zealand. Together, they created two new training programs that help people with TBI recover affect recognition, and then tested their programs to ensure that they were effective. The international nature of their work allowed them to collect data from a large number of patients. In total, 203 people with TBI participated; 34% of these had facial affect recognition impairment, 22% found it difficult to recognize vocal cues, and 15% were found to struggle with both. Most of the literature on this subject focuses on facial affect recognition, so the team's finding that vocal cues are similarly difficult to identify is a unique contribution of their work. The results indicate that facial affect recognition can be recovered using the training programs developed by the team. Participants' injuries occurred, on average, 10.27 years previously, and the longest time since injury was 42 years. This suggests that facial emotion recognition is recoverable at any time. All of the data the team was able to collect also made it possible to develop an emotional inferencing test; a tool not previously available to therapists. This tool could potentially highlight other areas of difficulty in emotion recognition for people with TBI.

MOVING FORWARD

These training programs are designed for therapists who work with people with TBI. Many therapists have already provided positive feedback to Zupan and her collaborators. However, Zupan would like to develop the program further by adding moving faces to treatment. Coordinating schedules across time zones and securing funding with an international team makes it difficult for these researchers to introduce therapists to their work. Despite these setbacks, they continue to discuss how to move the project forward. Sympathising with loved ones and responding to their feelings is a skill that vastly improves quality of life, and its importance in rehabilitation should not be overlooked.

Researcher Profile

Dr Barbra Zupan
Associate Professor, Department of Applied Linguistics
Brock University

Dr Barbra Zupan studied linguistics at Brock University. She has received many academic awards during her time in school, and was awarded the American Congress of Rehabilitation Medicine Early Career Best Poster Award. She currently teaches at Brock University and researches emotion recognition in TBI patients.

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A Niche in Neurodegenerative Disease

Dr Martin Duennwald is an Associate Professor in the Department of Pathology at the Schulich School of Medicine and Dentistry. Here he discusses his latest research project, in which he studied protein misfolding in neurodegenerative diseases.

To start, what inspired you to study protein misfolding and neurodegenerative diseases such as Huntington's disease?

I have always been interested in the mechanisms by which basic biological processes, such as protein misfolding and cellular stress response programs, modulate human diseases. Understanding the underlying basic biological mechanisms motivates my research and keeps me enthralled. Another major reason for my interest in neurodegenerative diseases is the tremendous personal suffering they afflict on patients, their loved ones, and our entire society. At present, there is no real cure for any neurodegenerative disease. I am convinced that by deciphering the basic biological principles underlying these diseases, such as protein misfolding, we will find therapeutic strategies to delay, hold, or even cure these devastating diseases.

The number of patients suffering from neurodegenerative diseases in Canada has reached 700,000 and is expected to double over the next 20 years. Are there any numerical estimates of how much of a burden these diseases place on society in terms of money or human resources such as man hours?

The Alzheimer's Society of Canada estimates that the total cost associated with dementias and other neurodegenerative diseases, combining medical expenses and lost earnings, amounts to a staggering CA $ 33 Billion per year. Notably, the medical expenses alone will exhaust almost the entire Canadian health budget in one generation from now. Certainly, the situation in Canada is not an exception as the World Health Organization deemed neurodegenerative diseases and dementias the most significant health crisis of the 21st century world-wide. Clearly, we have to work on this problem and find solutions soon.

You enlisted a team of experts in each necessary field to conduct your research. What fields were involved and did you involve a number of different institutions? Did coordinating this team present any particular challenges?

In my experience, research is most interesting, most productive, and most fun when conducted in a team of people with different expertise and different ways to think about the same problem. It would be impossible for one researcher to become an expert in the many powerful yet complex experimental techniques of modern biomedical research. However, each of these different approaches adds tremendous value to the study of a particular problem, particularly protein misfolding. In my view, it is only the synthesis of these different approaches that will allow us to answer even the most intricate questions in biology, which will ultimately result in finding effective therapeutic strategies for the treatment of human diseases.

Of course it can sometimes be challenging to coordinate these different approaches or even translate the different languages that each expert speaks. One way to overcome these challenges for me is to permanently read publications in many different areas of research and communicate frequently with colleagues, if possible in person. Therefore it is sometimes easier to collaborate with researchers close by but fortunately modern electronic communication also enable collaborations across vast distances.

Do you have any ideas about where you will take your research next or will you need to wait for the results of your current research?

I am eager to expand our research on the connection between protein misfolding and aging. I find that this area of research is mostly unexplored and deserves more attention and innovative experimental initiatives, such as ours using yeast as a model.

Is there anything else you would like to add?

I lately observe with much concern that public opinion and many governments regard basic research as a rather wasteful and elitist pastime with little noticeable impact on our society. Yet it seems evident to me that basic research, which sometimes is not even directed towards a commercially measurable goal, will eventually help to solve humanities most pressing problems, including the dramatically growing number of people affected by neurodegenerative diseases. I therefore try to remind myself that as a researcher it my duty to share what we do and why we do it in an open dialogue.

Unravelling the Cellular Network

Protein misfolding is a key marker of many neurodegenerative diseases (ND) such as Huntington's disease (HD). Dr Martin Duennwald researches the underlying cellular mechanisms that result in protein misfolding, and therefore ND.

PROTEINS AND NEURODEGENERATIVE DISEASE

Proteins, like any molecule, must have a specific shape and size to function properly. Since proteins are very large molecules, specific shapes occur as a result of protein folding in which certain biochemical reactions within cells dictate how proteins are built. These reactions can be disrupted by a number of different factors, including toxic environmental substances, aging, and cellular energy production. These disruptions cause the proteins to fold into the wrong shape and size, and function improperly. Improperly functioning proteins can be toxic. In healthy humans, the cells in the body enact another set of biochemical reactions, called cellular stress programs, to counteract the damage. Proteins that have been made incorrectly will either be corrected or destroyed by the cellular stress programs. Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease are characterized by protein misfolding coupled with a failure of cellular response pathways to properly eradicate or correct protein misfolding. ND is one of the greatest health care burdens faced around the world today, as these diseases are often associated with aging and many countries will soon or already experience a growing elderly population. Duennwald's research is significant not only for the fundamental information it will generate, but also for its potential for developing new treatments for ND that may have a very direct, positive effect on society.

A COMPLEX DESIGN FOR A COMPLEX SUBJECT

Duennwald's proposed research ambitiously plans to explore protein folding from several different aspects. He uses an innovative approach that combines microscopic studies and cell biological experiments. He also studies cellular processes with transcriptome (RNA molecules), genome (DNA molecules), and proteome-wide studies. Proteome refers to the vast number of biological pathways that control how proteins are made and where they are sent within the cell. The data will be analysed using computational algorithms in order to discover the complex networks that result in protein misfolding.

It seems evident to me that basic research, which sometimes is not even directed towards a commercially measurable goal, will eventually help to solve humanity's most pressing problems, including the dramatically growing number of people affected by neurodegenerative diseases

To study protein misfolding in Huntington's disease (HD), Duennwald hypothesizes that the genetic mutation that causes HD significantly compromises the cellular stress programs of specific brain cells. Impaired cellular response in neurons would explain the brain damage seen in HD patients that contributes to the detrimental symptoms of HD. Therefore, Duennwald is focusing on HD mouse models and HD human brains to determine the role of stress response programs in the pathogenesis of HD. He will also evaluate whether targeting stress response programs can effectively be used as a treatment for HD.

In order to study the cellular mechanisms that cause protein misfolding, Duennwald used the heat shock response. This cellular stress program is activated by high temperatures that induce protein misfolding. The heat shock response can be induced by other conditions that result in protein misfolding, such as exposure to heavy metals and other environmental toxins. Much research has been conducted to determine the cellular processes involved in the heat shock response, which allows Duennwald to use it to study which biochemical reactions occur during protein misfolding in patients with ND. Research also demonstrates that the heat shock response may be linked to the development of many diseases and specifically ND. Duennwald would like to use this research to explore how the heat shock response differs between different types of tissues and cells, and figure out why cellular stress response programs fail in those cells afflicted with ND. This research will also help the medical community understand precisely how cells sense protein misfolding and activate the heat shock reponse. He also hopes this research will elucidate how aging affects the heat shock response.

Another significant aspect of Duennwald's study design is his choice to use yeast models to explore these cellular pathways. Yeast is cheap and easy to work with, saving both time and money. Yeast also grows quickly and is easy to manipulate and analyse in the laboratory. More importantly, the protein misfolding and cellular quality control programs Duennwald wishes to research are very similar between the yeast models and humans. Since it is both a simple organism and easy to manipulate, it is possible to conduct experiments that would be impossible using fruit flies, mice or humans. Yeast models allow the researchers to study the effects of entire genomes in rather simple experiments. Yeast can also be used as a living test tube to screen for molecules that may be further developed into treatments.

Duennwald also collaborates with other researchers, consulting biochemists and structural biologists to add insight regarding the molecular details of protein misfolding. He works with researchers who have expertise studying mouse models of neurodegenerative diseases, since they have a deep understanding of how particular neurons in the brain are involved in protein misfolding diseases. Finally, pathologists help validate the findings for human conditions.

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WIDE-REACHING RESULTS

Duennwald hopes that his research methods will be applied to other diseases, such as cancer, diabetes and cardio-vascular disease. Protein misfolding is a hallmark of these diseases as well. This means that cellular stress programs may be similarly failing to correct or destroy misfolded proteins in these diseases. Duennwald's experimental tools will also elucidate why certain tissues and cell-types are affected in one disease, but not others. For instance, those suffering from cardio-vascular disease experience protein misfolding in the heart and blood vessels, while those suffering from diabetes will experience it in a wide variety of tissues and organs related to metabolic disease. Interestingly, cancer results from the opposite problem, cellular stress programs are hyperactive in cancer, allowing cells to divide. An understanding of how cellular processes are disrupted by failing stress response programs may lead to better therapies for a wide range of very common diseases.

The possible application of these research methods to other diseases demonstrates an important belief of Duennwald's. While this research project, specifically, does have the potential to generate money by creating intellectual property or through collaboration with bio-tech companies to provide jobs and lessen the burden of ND on the Canadian population through new treatment programs, this research is important for its own sake. It is often the case in scientific research that the outcome of any given research project may not produce results that are immediately economically viable or even applicable in real-life scenarios. However, since all research builds on past information, any seemingly impractical results will lead to better research in the future by providing information about the fundamental processes that shape the natural world. In this case, even if these new experimental tools do not prove to be useful for treating ND, researchers will have a deeper understanding of these diseases and new experimental tools to apply to other ailments. By focusing his research at the systems-level, Duennwald is contributing very important, fundamental information to the medical research community and may positively affect the lives of millions of people.

Researcher Profile

Dr Martin L. Duennwald
Department of Pathology
Schulich School of Medicine and Dentistry

Dr Martin Duennwald received his education from the Max-Planck-Institute for Breeding Research and the University of Cologne in Germany. He was awarded with a postdoctoral fellowship from both the German Research Association and the Huntington's Disease Society of America, and received an award on a talk about protein folding at the Federation of American Societies for Experimental Biology conference. He currently teaches at the Schulich School of Medicine and Dentistry where he researches protein misfolding, protein aggregation, neurodegenerative diseases, cellular protein quality control, protein-protein interactions and yeast models.

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PERSONALIZED MEDICINE

Medicine has gone through incredible changes in the past 20 years with the advancement of technology, to the extent that students entering medical school now may find out that the application of the information they are currently learning is almost entirely different by the time they are out of residency for specialization. Personalized medicine began with the development of high throughput technologies, particularly in genomics, allowing us to examine alterations in genes and proteins rapidly at a relatively low cost. Using this data, one can examine if a particular patient is more likely to be vulnerable to certain metabolic changes, diseases, pathogens and also if they are more or less likely to respond to certain treatments or experience side effects. A commonly used example is found in cancer treatments. Patients with the same cancer could have multiple origins of the disease meaning that they will respond to treatments differently. Instead of giving everyone the same treatment, the affected tissue is biopsied and examined using immunohistochemistry to see if it has certain markers indicative of the pathway that is being altered. Once the pathway is identified, the drug that specifically targets this pathway is then given to the patient to allow for more effective treatment. Like other areas of medicine, personalized medicine has gone through tremendous developments in the past twenty years. Personalized medicine was first largely enabled through the popularization of pharmacogenetics, which examines specific genes or the entire genome to identify drug response. Using pharmacogenetics, clinicians were able to improve the safety profile and efficacy of various drugs including warfarin. Personalized medicine has now expanded into multiple fields, including psychiatry, which has recently shown an exponential increase in examining individual variations within patients with the same disease to improve therapeutic efficacy. RDoC, or research domain criteria implemented by NIMH (National Institute of Mental Health), aims to do this by dividing behavioral manifestations of different psychiatric disorders into further categories beyond the diagnostic categories. In the future, they aim to be able to match specific biomarkers, or biologically measurable factors indicative of a certain pathology/disease, to each of these subcategories, allowing for more personalized and specific treatment of psychiatric diseases. Personalized medicine also includes measurement of individual factors to make accurate prognosis to guide patient-specific treatment plans. Indeed, while metabolic disorders such as diabetes, cardiac dysfunction, and hyperlipidemia may seem to follow a similar course in most individuals, the drug response profile of these patients and the speed in which the illnesses progress may differ quite extensively from one patient to another. Therefore, the use of monitoring techniques that measure various aspects of the disease allow us to customize treatment plans that cater to the predicted prognosis of each patient. With the wide spread of personalized medicine, establishments of research laboratories that can measure various biomarkers, genetic alterations and other factors used for diagnosis and disease management in a cost-effective, timely >

> and reliable manner becomes critical. Indeed, institutions as well as individual patients are now seeking to identify more information about their pathophysiological profile outside of the hospital to be better able to manage their condition. In this section, three research groups will be discussed that aim to expand the application of personalized medicine in psychiatry, metabolic disorders, and genetic sequencing. First group led by Dr. Kennedy is examining the proteomics, genetics, imaging, and treatment data in patients with major depression to identify biomarkers that could guide disease diagnosis and treatment in psychiatric disorders. Second group led by Dr. Eguchi and Dr. Takahashi has developed a method to measure lipid content in skeletal muscle using CT imaging, which can be used to predict prognosis. Lastly, Dr. Al-Mulla has established a company for sequencing genes allowing for patients to obtain data regarding individual pathophysiological alterations and potential drug response profiles.

Beating the Blues

Dr. Sidney H Kennedy is one of the world's leading depression researchers. Here he discusses his latest project, CAN-BIND, a program that aims to revolutionize the way doctors view depression.

To start out: why depression research? How did you become interested in your area of study?

Depression affects more than 350 million people around the world and yet research to explore the basis of depression and provide adequate treatment services has been dramatically underfunded.

I've always been interested in the relationship between our environment and our genetic makeup and depression is a prime example of genetic risk interacting with multiple adverse life events. In fact, the first research study I ever published showed that people with Bipolar Disorder, at that time, considered to be highly genetically determined, experienced a marked increase in life events before episodes of mania. In many ways, our current research investigates these relationships using more sophisticated tools and approaches.

Depression is a growing issue in many developed countries. As an expert in the field, do you have any insight into why that may be?

Depression is not just a concern for developed countries. In 2010, authors of the Global Burden of Disease Study concluded that depression is the second leading cause of disability worldwide, with growing awareness that presenteeism accounts for even more of the economic burden of depression than absenteeism. Although some would argue that depression is a "disease of modernity or affluence" due to a larger prevalence in high income countries, it remains one of the top 5 leading causes of Disability-Adjusted Life Years (DALYs) in all regions of the world, regardless of GDP per capita, with the exception of Africa where the burden from communicable, maternal, perinatal and nutritional conditions is greater.

At the societal level, some would say the widening gap in income distribution, the breakdown of social cohesion, increasing isolation in many urban centres and the growing competition to secure employment have led to increased rates of depression.

You argue that current depression treatments are inadequate. Could you elaborate on the problems with the way depression is treated today?

Diagnosing a Major Depressive Episode (MDE) is a different process from identifying most medical disorders. For example, a physician is able to detect arthritis by checking for swollen joints, reviewing X-rays and blood levels for the presence of antibodies 'rheumatoid factors' in the case of Rheumatoid Arthritis. In contrast, there are no laboratory tests or brain imaging scans that can confirm a subtype of 'clinical depression': we are still reliant on clinical interview and self-report measures to confirm a diagnosis of depression.

Many genetic, biological and environmental risk factors interact to produce the symptoms of depression. Each person experiencing depression has a unique genetic background, personality and neurobiological makeup as well as distinct early childhood experiences, life events and social support systems. These variables not only trigger the onset of depression, but contribute to the on-going risk for relapse and recurrence. Current treatments which include antidepressant medications, psychotherapies and neurostimulation, work for some individuals but not others. The challenge is to predict treatment outcomes and tailor it according to what works best for the individual.

CAN-BIND will be a major step forward in personalized healthcare. What is the benefit of CAN-BIND? What impact do you expect it to have?

The CAN-BIND research program uses a standardized platform of clinical and biological measures which include measures of brain structure (MRI) and function (fMRI and EEG), genetic and clinical information for each individual. Many previous research studies have focused on one or two of these measures, but since depression may result from an interaction among many factors, the comprehensive and integrated CAN-BIND approach should provide unique patient data. Our core hypothesis is that there are numerous underlying pathways (subtypes) to the final presentation of depression.

We have an unprecedented level of sustained funding over 5 years from the Ontario government, through the Ontario Brain Institute (www.braininstitute.ca). With this funding support, we are able to maintain a high level of scientific rigor and quality control across sites, and carry out critical follow-up studies to validate the findings from initial studies. We have designed several sequential projects that will use the same platform to identify biomarkers using different treatments (pharmacotherapy, psychotherapy, brain stimulation devices), different populations (adult MDD, youth at-risk, geriatric) and different types of mood disorders (unipolar and bipolar).

A Bold New Approach to Treating Depression

Depression is a growing problem throughout the world, and addressing it is one of modern medicine's biggest challenges. Dr. Sidney H Kennedy and his team are taking treatment in a bold new direction.

Depression affects more than 350 million people worldwide, in both developed and developing countries. It's correlated with a host of health problems, ranging from weight gain to cancer. It's also associated with rapidly increasing medical costs in the UK, US, Canada, and a number of other countries.

Why are people so unhappy? Scientists and sociologists have cited numerous and varied reasons for rising depression rates. More aggressive job competition, economic hardship, genetic predisposition, technology-driven social isolation, increased environmental toxins—all of these factors have been implicated in depression. Times are tough. But with increasing awareness of depression and decreasing stigma surrounding it, more and more people are identifying with the symptoms of depression and are looking for help.

Why is depression treatment so ineffective? Dr. Sidney H Kennedy, one of the foremost experts in the field, has an answer.

Even after decades of research, the challenge of finding successful treatment for depression remains the biggest barrier in helping patients. Scientists still aren't sure how different factors interact to cause it. In addition, they are not able to pinpoint the possible biological subtypes and optimal treatments based on those subtypes. What is known about the disorder is its complexity, far more than what has been previously imagined. A range of techniques have been used to address it, including talk therapy, antidepressant medication, and electrical stimulation, among others. But the results across all of these treatments have shown that the success of each type of treatment can vary across the different groups of patients.

Depression is more common than ever, but finding the right treatment for a particular individual can be very challenging. Happiness, it seems, is in short supply these days.

THE COMPLEXITY OF DEPRESSION

Dr. Sidney H Kennedy, one of the foremost experts in the field, has found an answer to the biggest question: Why is depression treatment so challenging? "Diagnosing a Major Depressive Episode (MDE) is a different process from identifying most medical disorders", he says. "For example, a physician is able to detect arthritis by checking for swollen joints, reviewing X-rays. In contrast, there are no laboratory tests or brain imaging scans that can confirm a subtype of 'clinical depression'. We are still reliant on clinical interview and self-report measures to confirm a diagnosis of depression".

And therein, Kennedy argues, lies the big problem. Depression presents itself in many different ways. Some cases are severe, others less so. Depressive bouts may last only weeks or they may last years. Even the symptoms themselves change from person to person, and these variations require different methods of treatment. With such a complicated and varied disorder, analysis of an individual's depression requires much more sensitivity than a clinical interview provides.

"Each person experiencing depression has a unique genetic background, personality, and neurobiological makeup. Add to that the complexity of an individual's distinct early childhood experiences, life events, and social support systems", Kennedy explains. Each depressed person experiences the disorder differently, due to different underlying causes. Because this is the case, no cure-all exists. A single treatment will not work for everyone. What the healthcare community needs is a more individualized approach to combatting depression – one that considers each person's case using a comprehensive set of tests.

CAN-BIND: LOOKING AT THE BIGGER PICTURE

Kennedy and his colleagues have developed such an approach to treatment. After years of planning and research, they have created the Canadian Biomarker Integration Network in Depression (CAN-BIND), a research program that views the treatment of depression in a bold new way. The CAN-BIND program works two ways. First, it will systematically evaluate outcomes using different types of treatments (medication, neurostimulation, psychotherapy). Second, CAN-BIND will collect data on three standardized platforms: 1) symptoms (i.e. hopelessness, sadness, weight gain, etc.), and other factors unique to the individual, such as environmental stress, quality of life, personality, childhood trauma, lifestyle factors, 2) structual and functional neuroimaging and EEG and 3) a comprehensive proteomic and genomic analysis of blood samples.

By analysing so many variables, Kennedy and his colleagues aim to find biomarkers – biological measures like specific proteins, genes, or brain abnormalities – that influence outcome in depression. In addition, Dr. Kennedy and his colleagues have developed complex

mathematical models that can analyse these biomarkers to look for patterns in large populations of depressed individuals. The large-scale approach aims to use CAN-BIND to help researchers in identifying common subgroups of depression. By identifying people who have certain biomarkers, or combinations of biomarkers, Kennedy and his team aim to find what kind of treatment plans typically work for individuals dependent on the depression sub-group they are associated with. On top of specific treatments, the researchers are also looking at different demographics—men and women, young and old—for a more encompassing method.

CAN-BIND's big picture approach doesn't stop there. Its size and scope alone could cause the project's progression to take years before completion. But Kennedy and his colleagues have developed a solution for that issue as well. More than 10 different major Canadian academic centres are working together to make the venture a reality. Each institution is implementing CAN-BIND and sharing its results with all the others.

This collaborative approach is far from the norm. Researchers are infamous for guarding their results and protecting novel findings until they are ready to publish their work. This competitive approach limits sharing of expertise and productivity. CAN-BIND has avoided this problem with the generous financial support of players in both industry and government. The Ontario government has provided an unprecedented five-year funding commitment to this program through the Ontario Brain Institute. Together with significant contributions from Lundbeck, institutions like Pfizer, Bristol-Myers Squibb, and Servier have all come together to support the project.

A project of this scope is a benchmark for the scientific world. In a field that all too often sacrifices progress due to competition with colleagues or lack of funding, CAN-BIND shows that science and business can come together, forming a united push to end one of the most pressing medical concerns of the 21st century.

A HAPPIER FUTURE

According to Kennedy, the preliminary results of the first CAN-BIND projects will be published by the end of 2015. Hopefully with it will also come a flood of new information to further understand depression. The program doesn't end with research, though. CAN-BIND is also developing a worldwide network of healthcare providers that will help spread results, allowing them to be incorporated into treatment practices around the world quickly and effectively.

There's a final benefit. By elucidating the biomarkers of depression, CAN-BIND aims to change the way the world looks at this powerful disorder. Too often, depression is trivialised and stigmatised. Because there are no biological tests for it – unlike for, say, heart disease – depression is frequently seen as mental weakness, or something that can and should be overcome. Kennedy hopes to change that.

"Viewing depression in the same light as other medical conditions", he says, "will help de-stigmatise mental illness and improve treatments through a targeted and personalised medical approach". With decreased stigma and better treatment, CAN-BIND aspires to provide something that sufferers of depression desperately need: hope. By spreading awareness, identifying causes, and personalising treatment, the CAN-BIND project is taking a brave step toward making the world a brighter place.

Researcher Profile

Dr. Sidney H Kennedy
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Dr. Sidney H Kennedy is Professor of Psychiatry at the University of Toronto and a Scientist at Li Ka Shing Knowledge Institute and Toronto Western Research Institute, Toronto, Canada. Dr. Kennedy is the lead investigator for a large depression biomarker initiative. He has published extensively on new drug evaluation, neuroimaging and neurostimulation therapies, personality factors in depression, antidepressant effects on sexual function and treatment guidelines for Major Depressive Disorder and Bipolar Disorder. Dr. Kennedy is the Immediate Past President of the International Society for Affective Disorders, a former President of the Canadian College of Neuropsychopharmacology and the founding chair of the Canadian Network for Mood and Anxiety Treatments (CANMAT). He has published more than 350 peer reviewed publications and 11 books on depression and related topics.

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Lundbeck
Bristol-Myers Squibb
Pfizer
Servier

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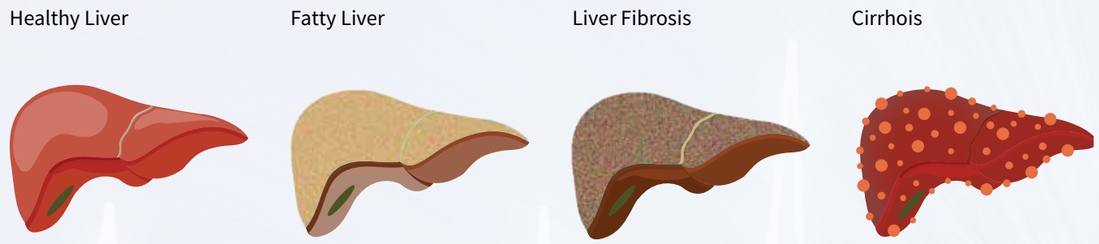
CAN-BIND Canadian Biomarker Integration Network in Depression

Hidden Connections

Professor Yuichiro Eguchi teaches for the Division of Hepatology at the Saga Medical School. Dr Hirokazu Takahashi is now a research fellow at the Joslin Diabetes Center in Boston, MA. The two have worked together over a period of years to understand the relationship between liver disease and fat accumulation in skeletal muscle.



A Hepatic Manifestation of Metabolic Disease

Non-alcoholic fatty liver disease (NAFLD) has long been associated with visceral fat accumulation, insulin resistance, and type II diabetes mellitus. The work of Eguchi, Takahashi, and their colleague, Dr. Yoichiro Kitajima, began in 2003, when both still worked at Saga University. The researchers grew concerned when the rise in rates of metabolic disease and obesity led to more patients developing NAFLD. It is well-known that aging and obesity cause lipid accumulation in muscle. Skeletal muscle is an important organ in regulating glucose metabolism, and 70% of glucose is absorbed into these tissues. Adipose tissue interbedded in skeletal muscles is thought as "ectopic fat". Generally, the primary function of adipose tissue in visceral fat and subcutaneous fat is to store lipids, but it also plays an important role in the endocrine system because it secretes enzymes that regulate tissue function across multiple organs. When adipose tissue becomes dysfunctional, the enzymes it excretes lead to the formation of metabolic disorders like type II diabetes and insulin resistance. Adipose tissue's ability to communicate with many different organs results in something called organ crosstalk.

Organ crosstalk is a concept that is currently being extensively researched, and describes systems within the body that require the involvement of multiple organs. So far, research on visceral and subcutaneous fat has identified crosstalk between fat and multiple organs in NAFLD, but the relationship between the ectopic fat including lipid accumulation in skeletal muscle is not yet well known. The research described here may demonstrate another instance of organ crosstalk and provide insight into how it works. Eguchi, Takahashi and Kitajima consider NAFLD the hepatic manifestation of metabolic disease and have conducted four experiments and published two papers elucidating the relationship between NAFLD and fat accumulation in skeletal muscle. Dr. Kitajima, a Radiological Technician, also developed a novel for measuring lipid accumulation in muscle without which the research presented here would not have been possible. Dr. Kitajima's methods will allow researchers to finally fully explore the relationship between visceral fat accumulation and NAFLD, and may provide profound insight for the study of organ crosstalk.

Understanding the Language

Fatty liver disease can also be called liver steatosis, and refers to an abnormal retention of lipids in the liver. In addition, 20-30% of NAFLD is thought as non-alcoholic steatohepatosis (NASH) which shows histological inflammation and/or liver fibrosis, and can develop to liver cirrhosis and hepatocellular carcinoma, or liver cancer. The first paper by Eguchi and Takahashi was published in 2009 and primarily uses the term NAFLD, while their second paper was published in 2013 and uses the terms NAFLD and NASH. The two papers refer differently to the skeletal muscle being studied. The researchers specifically chose multifidus muscles, a type of skeletal muscle lining the spine, because its location in the abdomen allowed for the measurement of liver and visceral fat accumulation; therefore, using this tissue also made it possible to estimate the effects of exercise on adipose tissue in skeletal muscle. The 2009 study refers to fat accumulation in multifidus muscles as the ratio of the multifidus muscle attenuation ratio over the subcutaneous fat attenuation ratio (MM/F ratio). Attenuation refers to a reduced level of either the multifidus muscles or subcutaneous fat, and so a higher MM/F ratio indicates that muscle tissue is being lost at a greater rate than subcutaneous fat. In their 2013 study, Eguchi and Takahashi refer to this ratio as intramuscular adipose tissue content, or IMAC. IMAC levels and MM/F ratios, however, refer to the same thing.

The Long and Short of It

Finding a way to demonstrate the relationship between these two different organs required two cross-sectional studies and two longitudinal studies. Cross-sectional studies are designed to be "snapshots" of a population at a certain moment in time, while longitudinal studies collect data over a longer period in order to understand changes over time. In the 2009 paper, 333 NAFLD/NASH patients suspected of suffering from NAFLD/NASH were enrolled in a cross-sectional study. 75 healthy participants were also included as controls. Potential participants whose liver disease could be attributed to alcohol or medications were excluded. The patients' level of obesity as well as other metabolic indicators such as blood glucose levels were recorded, and MM/F ratios were measured. Measurements of the MM/F ratios were taken using computed tomography (CT), which takes pictures of the inside of the body using x-rays. Eguchi and Takahashi credit the lead author of the papers, Dr Yoichiro Kitajima, with developing the procedure to measure lipid content in skeletal muscle using CT imaging. The results showed that MM/F ratios were much higher in the NAFLD/NASH patients compared to the controls. MM/F ratios also increase with age, height, and are higher in women.

The longitudinal study published in the 2009

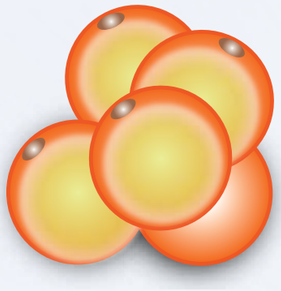
paper prescribed a diet and exercise program for patients to examine how visceral fat accumulation and obesity relates to NAFLD/NASH. The patients were then divided into two groups: those that reduced their weight by at least five percent, and those that did not lose weight or lost less than five percent. To evaluate whether weight reduction effected the MM/F ratios, changes in the ratio were compared between the two groups. Over three months, 20 patients achieved the required five percent reduction in weight, while 22 did not. A comparison of the two groups revealed that MM/F ratios were reduced in both men and women who had achieved significant weight loss. Taken together, the cross-sectional study and the longitudinal study together demonstrate that NAFLD/NASH and MM/F ratios are correlated. Lower MM/F levels are also correlated with improved insulin resistance and reduced visceral fat. The results also indicated that MM/F ratios could reflect the severity of fat retention in the liver, and therefore could also indicate whether a treatment for NAFLD/NASH was working or not.

The most important finding is the correlation between liver and skeletal muscle in pathogenesis of NAFLD. It is possible organ cross-talk

The 2013 paper also presented two analyses, one of which was cross-sectional and one of which was longitudinal. The short-term study enrolled 208 patients. All of these patients had undergone a liver biopsy to diagnose NAFLD/NASH, and again, those that demonstrated excessive alcohol intake or were taking certain medications were not included in the experiment. IMAC levels were again measured using abdominal CT. Bodyweight, height, BMI and insulin resistance were also calculated for each participant, and liver biopsies were also evaluated and used to determine the severity of NAFLD/NASH for each patient. Comparison of all these factors revealed that the severity of NAFLD/NASH is strongly linked with BMI, insulin and IMAC.

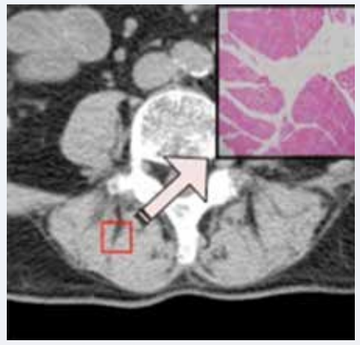
21 patients with NAFLD/NASH were followed for the longitudinal study, and after an average time of 24 months on a prescribed diet and exercise plan, they were divided into two groups. Some patients were also given a combination of drugs designed to treat diabetes and high cholesterol in order to test whether the current treatments for these diseases also result in lower IMAC levels. This time, the groups were divided based on whether their

Adipose tissue

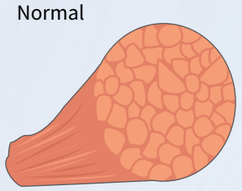


IMAC was reduced. 11 patients improved their IMAC levels; these were the patients who also showed a significant decrease in bodyweight, BMI, and insulin. The results of this second paper strengthen the evidence for a correlation between IMAC levels and pathogenesis of NAFLD/NASH. However, it should be noted that Eguchi , Takahashi and Kitajima do not believe that the research they have conducted so far is extensive enough to conclude that IMAC levels are correlated with NAFLD/NASH severity. In order to prove their hypothesis, they must demonstrate that the measurements they have taken using CT are accurately reflecting fat accumulation in muscle. They are currently working with Boston University to perform both CT and magnetic resonance imaging on mice in order to compare their ability to measure the amount of fat in muscle. Magnetic resonance imaging is considered the gold standard for making these kind of measurements.

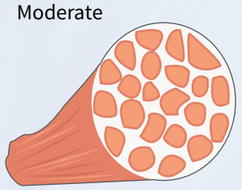
The results of the longitudinal lifestyle change experiments demonstrated that exercise reduced IMAC levels in NAFLD patients independent of body weight reduction. However, exercise regimens also result in whole-body improvements to glucose tolerance and metabolism, and not just in the reduction of IMAC levels in skeletal muscle. Further in-vivo/in-vitro studies will be able to verify whether improvement of lipid accumulation independently contributes to the amelioration of NAFLD/NASH.



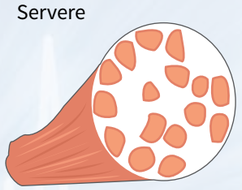
Normal



Moderate



Servere



Application of Results in Treatments

Once CT can be demonstrated to be an effective way to measure adipose tissue content in skeletal muscle, it will be an effective marker to indicate severity of NAFLD/NASH and evaluate whether a treatment is effective or not. CT has the benefit of being a low-cost, easy and minimally invasive procedure for both patients and medical providers. In addition to measuring IMAC levels, abdominal CT can be used for visceral fat analysis, to compare spleen fat levels to liver fat levels, and to screen for hepatic tumours and other liver diseases. CT images can also screen for biliary diseases. The biliary ducts are responsible for carrying bile produced by the liver to the bile duct, so it is easy to image this area while also imaging the liver.

In the future, skeletal muscle may one day be the target of treatments for NAFLD/NASH. IMAC was significantly correlated to liver fat, and improvements in IMAC reduced liver fat along with weight reduction. Therefore, it is possible that directly reducing IMAC levels will help ameliorate NAFLD/NASH.

Helping Other Researchers

The work done by Eguchi and Takahashi was very influential in research related to the liver, skeletal muscle and metabolism, and has been cited in many other studies. The results of the experiments published in 2009 were considered in a study by Bertolotti (2014) and his collaborators strategizing how best to treat elderly patients with NAFLD/NASH. Farshad (2013), along with several other authors, used the work on multifidus muscle to examine whether its deformation correlates with nerve root compression, a painful spinal condition. The paper published in 2013 has been cited by metabolic and diabetes researcher Wiernsperger (2013), who used the work done by Eguchi and Takahashi to study how liver sensitivity to insulin levels can contribute to the formation of diabetes. It was also referenced by Del Rocio Ibarra-Reynoso (2014) and her collaborators in their study of hepatic insulin resistance and visceral fat accumulation in school children. The work by these researchers also supported the relationship between fat accumulated in the abdomen and whole-body as well as hepatic insulin resistance.

Both studies were cited by Hamaguchi (2014) and his collaborators. The research conducted by Eguchi and Takahashi helped this group find that patients with high IMAC levels were less likely to survive a liver transplant than those that had lower IMAC levels.

A Long-Term Partnership

Professor Eguchi and Dr Takahashi have been working together for a long time, and now collaborate while separated by both the Pacific Ocean and most of the North American continent. Despite the distance, when asked if they plan to continue working together, they answer “absolutely yes”. In fact, they are currently preparing another article for submission. This new research expands on their previous work and explores the effect of the glucagon-like peptide-1 receptor (GLP-1) on IMAC improvement. GLP-1 agonists, or chemicals that bind to receptors, have already been used as drugs to increase the uptake of insulin and improve glucose regulation. This work strengthens evidence for a possible relationship between insulin resistance and high IMAC levels. Eguchi and Takahashi are also already planning their next research project. The lipid content in skeletal muscle is comprised of both intramyocellular lipids and extramyocellular lipids. They plan to focus on the differences between the two types and would like to clarify the mechanism of correlation between NAFLD/NASH and skeletal muscle in further detail.

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Uncovering the role of **genes in disease**

Professor Fahd Al-Mulla divides his time between his leading genomics research unit at Kuwait University and his private genomic medicine centre Genatak. Here, he discusses how gene sequencing technology improves patient outcomes.

To start with, could you please tell us what inspired you to get into genomic research?

Two events shaped my life and career direction. First, I suffered from poliomyelitis at seven-months-old, only to learn later in life that it was simply because of medical negligence.

The second event happened after gaining my medical degree from Glasgow University in 1993 and during my residency in Ayr Hospital in Scotland. I clearly remember attending to this nice middle-aged lady in her hospital bed. She told us about how she had repeatedly informed her general practitioner that many of her family members died of breast cancer.

Now, after many years, we have come to know that the family was a carrier of a genetic defect in the BRCA2 gene, which leads to hereditary breast, ovarian or other cancers. These events made me realise that many diseases are preventable, just by knowing the genetic code. In fact, this knowledge may be the key to changing one's future.

How did you come to start a private genomic medicine clinic and how do you balance your research and business responsibilities?

In late 2013, I approached Dr. Jamal Al Ghanim, an eminent entrepreneur in the medical field. He listened to my introduction about genomic medicine, and 10 minutes into the conversation, he immediately invited me into a joint venture with him.

As a visionary, he realised the potential of my proposal primarily in improving the health and wellness of our people. We have now performed thousands of genetic tests and have gained the trust of almost all the physicians and patients who have used our services.

There is no doubt that research is the engine that drives entrepreneurship. During the last 10 years, I have received more than $3 million in funding to perform research on identifying diagnostic and prognostic factors in cancer. Research has always been the backbone of my strength.

Is personalised medicine beginning to live up to its hype and offer practical benefits to patients?

The premise of personalised medicine is to tailor treatment to overcome abnormally activated or inhibited pathways specific to a patient due to their private genetic mutations or epigenetic events. This knowledge benefits even cancer treatments, as it contributes to extending the disease-free survival rate of patients tremendously.

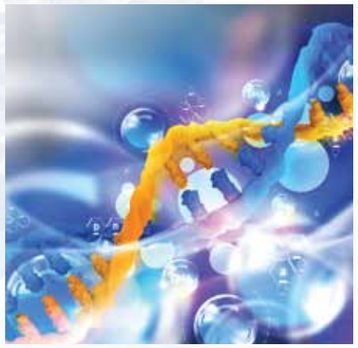
Today, we are sequencing whole genomes from cancers and informing clinicians of the disrupted pathways found in their patients. This would then allow them to tailor therapies accordingly. We have a long way to go in understanding many of the genetic changes in a particular cancer, but this is a good start.

You provide both exome and full-genome sequencing. Why is it important to have both approaches?

Exome sequencing looks at the tip of the iceberg—the 1% of variations in the genome that occur in exons or areas of the genome that are translated to mRNA and protein. The whole genome examines all three billion bases that constitute the human genome and thus represents a window through which you see almost the whole iceberg.

Exome sequencing is less expensive and may be useful in cases where you have a good idea of what is going on with the patient. Whole genome sequencing is perhaps the most effective technology we have in personalised therapy and prevention. We no longer have to rely on traditional techniques where we're guessing what could be wrong.

The lack of a well-documented Arab genome means you sometimes get variants of unknown significance. How do you explain these to patients?



The model we currently follow at Genatak is to report pathogenic or actionable mutations. Of course, our knowledge of pathogenicity varies perhaps daily, where new research changes a variant of unknown significance to pathogenic and *vice versa.*

Therefore, Genatak adopted a dynamic reporting mechanism, which is introduced into the clients' counselling process so they understand this complexity. The client is invited back every six months after their genome has been reanalysed with the updated information.

You established the office for Technology Transfer and Patenting at Kuwait University. How important do you think it is for researchers to develop their commercial skills?

Kuwait and the Gulf states generally rely heavily on oil as the sole source of income. Moreover, Kuwait dedicates only 0.1% of its GDP to research and development.

The ability to commercialise research is vital to any economy. Our attempt was intended to highlight the capability of local researchers to commercialise their research. The office managed to patent at least 42 inventions in record time and at least two of them address diabetic wound healing, an industry worth $2 billion dollars annually.

Tailoring medicine for the Arab world

Societal pressures have resulted in a unique public health landscape in the Middle East, which holds both opportunities and obstacles for personalised medicine. Professor Fahd Al-Mulla is working to overcome its hurdles while championing the benefits.

INDIVIDUALISING INTERVENTIONS

The last great revolution in healthcare came at the end of the previous century when the adoption of evidence-based medicine became widespread. Using knowledge gained from well-conducted, large-scale clinical trials to inform public health policy has helped to optimise patient outcomes and promote consistency of treatment.

Despite the success of the approach, however, the broad view it takes on the efficacy of various medical interventions can make it imprecise when dealing with individual patients. A treatment might be the best on average for a population as a whole, but biological variations between individuals can make it ineffective for certain patients.

Prevention is vital. Our vision stems from the fact that genomics may be able to pinpoint individuals who are prone to these chronic disorders and an early intervention could be useful in their prevention.

Professor Fahd Al-Mulla is the head of Molecular Pathology at Kuwait University where he investigates the impact of genetic variation on people's propensity for certain diseases and their reactions to different treatments. "Traditionally, all cancer patients are treated with the same effective therapy that has been validated and verified in randomised clinical trials. However, we know that some patients respond well – about 20% – while others do not do so well," he says.

THE NEXT MEDICAL REVOLUTION

The reasons for this mixed response can be environmental, but most of the time, it is the genetic variation between patients that determines the treatment's effectiveness. With the completion of the Human Genome Project in 2003 and the rapidly falling cost of

sequencing an individual's genome, predicting the variations' influence on a patient's medical outcomes is finally becoming feasible.

These advances mean that the prospect of personalised medicine is moving from science fiction to science fact. This promises to be the next great revolution in healthcare, and Al-Mulla has made it his mission to pioneer the approach in the Gulf States.

His first foray into the area came when he was urged by colleague Dr. Issam Francis, Director of the pathology laboratory at Kuwait Cancer Control Center, to introduce the fluorescent in situ hybridisation (FISH) test to Kuwait. This test ascertains whether breast cancer patients have amplified levels of the HER2 gene. High levels of the gene are an indicator of particularly aggressive cancer types, but they are responsive to Herceptin, a new monoclonal antibody therapy produced by drug maker Roche.

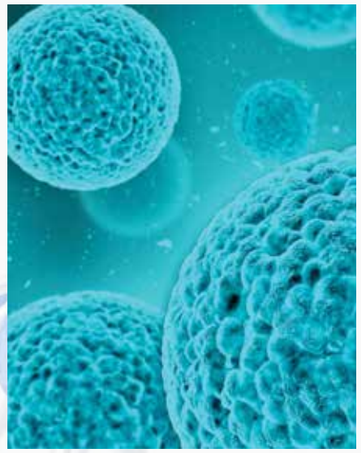
After establishing his lab's test locally in 2000, the drug was introduced to Kuwait in 2004. Since then, mortality rates for advanced breast cancer patients with amplified HER2 have dropped from 80 per cent to 20 per cent. "This reality reflects the success of personalised medicine and how its proper implementation can improve outcome," says Al-Mulla.

CHAMPIONING ARAB GENOMIC MEDICINE

The success spurred Al-Mulla to open his own genomic medicine clinic in Genatak last year,

under the umbrella of Dr. Jamal Al Ghanim's Global Med Clinic Services group. Through the company's partnerships, it has access to 21 next-generation DNA sequencing machines, including 10 HiSeq X-TEN, six Illumina HiSeq 2500 systems, two Illumina MiSeq instruments and three of Life Technologies Ion Torrent Personal Genome Machines.

The clinic provides genome and exome sequencing services for a variety of patients, such as patients with cancers or undiagnosed disorders, who may be looking for appropriate treatments specific to their genetic make-up. The clinic's service may even be beneficial to individuals who want to know the risks, prevention, and management of chronic diseases.



Al-Mulla believes there is high demand for these kinds of services in his part of the world. "With 30% of the population of the Gulf States suffering from obesity and diabetes, the local economies will struggle in treating these diseases and their chronic complications," he says. "Therefore, prevention is vital. Our vision stems from the fact that genomics may be able to pinpoint individuals who are prone to these chronic disorders and an early intervention could be useful in their prevention."

Another factor driving demand for genomic medicine in the Gulf States is the prevalence of consanguineous marriage – marriage within the extend family – which results in higher frequencies of Mendelian genetic disorders. "A simple approach we are taking at Genatak is called pre-marital compatibility testing. This tests for about 600 to 1,500 genetic diseases in the future bride and groom, who may carry a single gene defect that will not be expressed in them. It also involves counselling on how to avoid having a child affected with a particular genetic disease," says Al-Mulla.

LOCAL CHALLENGES

But there are both technical and cultural challenges to making personalised genomic medicine widespread in the Gulf States. One issue that has preoccupied Al-Mulla is the fact that the majority of genome-wide-association studies on detection of disease-causing genetic variations and the Human Genome Project have been carried out on DNA from individuals in the West. While many of the lessons learned from these projects can be applied to Middle Eastern societies, Al-Mulla's work on Arab genomes has revealed that the population exhibits major differences in polymorphisms and background mutation rates.

This has prompted him to take a leading role in the Genome Arabia project A better picture of the Arab genome could help improve the targeting of treatments in a population with significantly different genetics to those in the West.

As an example, genomic screening for breast cancer in the West typically looks for mutated pathogenic BRCA1 and BRCA2 genes, which are a strong indicator that the patient will develop breast or ovarian cancer. While there is a strong genetic component to breast cancer cases in the Gulf States, mutations in these two genes are uncommon within families with a strong history of breast or ovarian cancer. "Sequencing only BRCA1 and BRCA2 in affected members with a strong family history of cancer is a common mistake made by doctors not specialised in genetics," says Al-Mulla. "We really need to sequence and examine small structural gene changes in at least 65 cancer causing genes in women with a family history of cancer, not only two."

On top of the technical challenges there are also significant cultural hurdles that need to be overcome before the deeply conservative societies of the Gulf States fully embrace personalised genomic medicine. According to Al-Mulla many physicians and patients remain sceptical about genetic testing, equating it with playing God. "This is why Genatak is focused on educating policymakers, the public and doctors about the strength and caveats of genomic medicine. Education is key," says Al-Mulla.

Researcher Profile

Professor Fahd Al-Mulla
Director of Genatak
Head of Molecular Pathology
Kuwait University

Professor Fahd Al-Mulla is the head of the Molecular Pathology Unit in the Faculty of Medicine at Kuwait University and also founder and director of genomic medicine center Genatak. He attended Glasgow University, where he received his medical degree in 1993 and a PhD in molecular genetics of cancer metastasis in 1999. A fellow of the Royal College of physicians of Edinburgh, his research focuses on cancer and metabolism. His work led to the identification of two novel metastasis suppressors, namely carbonyl reductase and Raf kinase inhibitory protein. He holds patents on a method for prediction of metastasis by determining RKIP expression levels and another on a method of treating diabetes-related vascular complications by administering patients with a therapeutic dose of alpha-lipoic acid. As a professor at Kuwait University, he is responsible for teaching molecular pathology to medical students and MSc and PhD Pathology student

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SCIENTIA

IMPROVING PATIENT CARE

Despite recent enormous advances in patient care, much improvement is still required. For instance, there are still long patient waiting times and inadequate treatments of some patients. In addition, although numerous technical advances in medical research are released everywhere, many of such findings have yet to be applied to clinical practice. Outside the hospital, there is a pressing need to improve outpatient care and primary care, which are both important aspects of healthcare and are essential for enhancing the quality of life of the patients.

In countries with public health care systems, such as the UK, Canada, and many European countries, access to healthcare is seen as a right. There are various positive aspects to this system, such as reduced inequality in access to care. However, unlike countries with privatized healthcare practices, there are also some negative aspects to the public healthcare system, including the presence of lowered competition, which has been argued as a major contributor to long patient waiting times. Furthermore, limited resources and availability of doctors are also key contributors to long waiting times, which could contribute not only to unsatisfactory care but to worsening of symptoms. In particular, it is not a rare occurrence for many patients to be seen sitting in the ER waiting to be seen by doctors for hours. There are also times that patients coming through ambulances with critical conditions, where time is of essence, are not able to be seen properly in a timely-manner. Sometimes, even if the patients are seen, they are not able to receive the most adequate treatment, which could negatively impact their rate of survival >

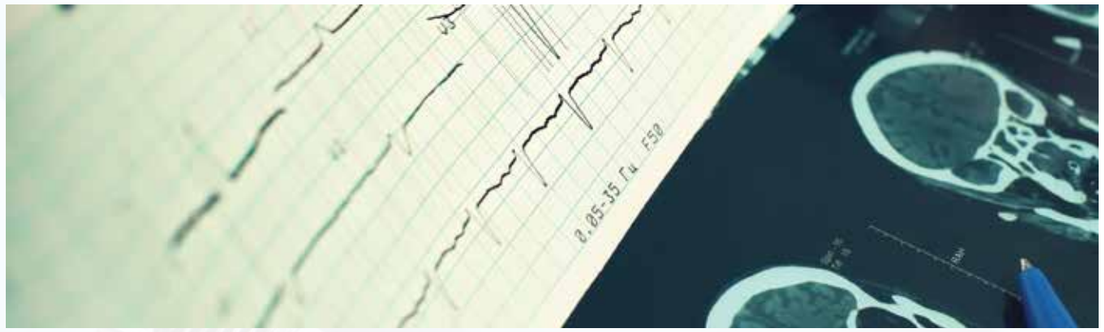
> and rate of recovery. As highlighted in this section by Dr. Andrew Munro and Dr. Dion Stub, it is particularly important to ensure that patients with cardiac diseases are treated in a timely and effective manner both at the ambulatory setting and inside the hospital. One method to improve the timely treatment of patients is the development of better biomarkers to accelerate diagnosis, as discussed by Dr. Martin Than.

Patients with chronic conditions are often cared at home instead of being hospitalized due to various reasons such as personal will, limited financial resources, and hospital bed availability. In addition, although the presence of comorbidities of various illnesses and their severity tend to increase with age, the possibility of having mobility difficulties also increase in this population, hindering elderly patients from frequenting local hospitals/clinics, rendering lay caretakers particularly important for palliative care. Therefore, the ability of the caretaker outside the healthcare system is essential in the quality of life of these patients. However, there are no current assessment modalities available to ensure that these caretakers are properly trained. By ensuring the proper training of lay caretakers, we can dramatically improve the quality of care that these patients are receiving. In this section, registered nurse Sue Healy and Prof. Liz Reymond specifically discuss the importance of lay carer education in delivering high quality care for palliative patients.

Primary care is often referred as family medicine. It is a fundamental part of the healthcare system and is often the first point of contact for majority of the patients, in which most of the health concerns are addressed. In this section, Dr. Patricia Li discusses the implementation of family medicine groups to facilitate continuous care to patients and how its role impacts children in particular.

Evidence-Based Medicine

Dr Andrew Munro works to make clinical practice more efficient and effective. He recently developed new protocols to determine when emergency room patients experiencing chest pain can be safely discharged. Using data from this population, he then conducted a research study demonstrating the usefulness of these protocols.



How did your background lead you into medicine and why did you decide to focus your efforts on evidence-based clinical practice?

I was led to medicine by my family doctor, a true old-style rural GP, who often visited my family home for a cup of tea or end-of-day scotch. He often regaled the cases of the day—all de-identified, I am sure! This captured the imagination of the youngster that I was at the time. I subsequently developed a fascination for scientific enquiry, leading me to study towards a BSc. During this period, [my] father's ill health and premature cardiovascular death compelled me to further understand this field.

Whilst at medical school, I spent some time as a junior researcher in the vascular lab. This rekindled an interest in research and publication.

During my house officer years, I realized that acute and critical care medicine was for me. Shortly after embarking on Emergency Medicine specialist training, it became clear to me that evidence-based medicine for emergency medicine was, at best, vestigial. I feel it's important to contribute to the evidence as best I can without losing focus on clinical practice. My research focus recently has been about pragmatic medicine and POEM (Patient Oriented Evidence that Matters).

You suggested that since research published by Goldman became standard in the field 1982, efficiency as reflected by the discharge rate has not increased. Can you go into more detail about Goldman's work and explain why it does not address discharge rates?

ACS is a frightening and life-threatening event that can be missed. Admission rates are largely driven by the fear of inappropriate discharge.

Ultimately, Goldman's work signalled the birth of rapid assessment pathways. However it has been a circuitous route.

Goldman's study established that a hierarchical clinical decision tool could work to produce safe discharge in almost 40% of patients with chest pain. Disappointingly, the intervening period has seen higher admission rates and the development of pathways that require additional beds such as chest pain units. The cause is multifactorial and largely associated with the availability of advanced diagnostics and treatments as well due to published consensus guidelines.

Were there any significant roadblocks that you encountered while conducting this research?

No funding, and we are all full-time clinicians. The work was done in non-clinical and 'free time'.

We were fortunate to work in a hospital where the cardiology department and its staff were fully supportive of the project

Why do the existing acceleration decision protocols result in over-estimates of risk and too few patients with chest pain being discharged?

This is primarily due to risk-scoring tools. It should be noted that in our study (and others), ED physician gestalt [was] used to over-rule discharge [as suggested] by the protocol. [Gestalt is a decision-making process that uses pattern recognition to come to a sound conclusion]. We found that applying the serum cut-offs retrospectively improved risk estimation with addition of SMO gestalt. The only instances that yielded different results is in two potential misses for the price of a number of unnecessary admissions. The study was not designed to tease this out. However the general feeling is that clinical experience remains a key element in a functional risk assessment pathway.

Are you planning to build on this research or are you headed in a new direction?

Currently, we are completing a twelve-month follow-up for all non-high risk patients. Some additional materials from the original data have been used to analyse the specificity of a negative initial troponin or a troponin measures at <5ngm/l.

We await external validation of this protocol.

We are also involved in a multi-centred study looking at improving finding clinically-important pulmonary emboli [arteries in the lungs blocked by blood clots].

Recently, it was announced that the NZ Emergency Medicine Network, a patient-centred emergency care research collaborative has been launched. As an inaugural member, I hope to continue to provide research used in daily clinical practice.

New Research Improves Hospital Staff Decision-Making

Hospital staff who work in emergency departments must quickly decide whether a patient needs to be discharged or admitted to the hospital for further treatment. Dr Andrew Munro of the Nelson Hospital created new decision-making tools to aid hospital staff in making these crucial decisions.

AN EMERGENCY IN THE EMERGENCY DEPARTMENT

It is a problem when emergency department (ED) staff do not have good tools to help them make decisions quickly. Working in such a chaotic, high-intensity environment requires clear guidelines for how to manage patients. One decision made constantly by ED staff is the discharge or admission of a patient. Hospital resources are crucial, which means that every treatment and test should be designated to patients who are in dire need or with serious illnesses. The wrong decision would result to either wasted resources on a relatively healthy patient or the harm or death of a patient who was misdiagnosed and discharged. Because of the risk , many patients who visit EDs with chest pain are given more care than is necessary.

Shortly after embarking on Emergency Medicine specialist training, it became clear to me that the evidence-based medicine for emergency medicine was, at best, vestigial. By contributing to patient-oriented evidence that matters, it helps us maintain the focus on clinical practice and gives us confidence in some of the necessarily pragmatic decision-making required in busy and often overcrowded Emergency Departments.

This is because of the difficulty of quickly determining whether that patient may be at risk of having a heart attack. Specifically, patients who visit the ED with chest pain of a possible cardiac nature make up more than five percent of the total number of patients who present to the emergency department. However, only a small portion of this five percent actually have Acute Coronary Syndrome (ACS). Consequently, the number of patients admitted from the ED without getting a high-risk discharge diagnosis is still too high.

In recognition of the important role that decision support in the ED plays, the New Zealand Ministry of Health requires all EDs to utilise a chest pain pathway or accelerated decision protocol (ADP). These tools are flow charts that guide hospital staff through the process of deciding whether or not to admit a patient. Most chest pain pathways divide patients into three or more categories of risk: low, intermediate and high. Still, Munro believes that these terms are not clear enough to give hospital staff the confidence to discharge patients who are not at high-risk to develop ACS over the next 30 days.

NEW INSIGHTS FROM OTHER RESEARCHERS

Despite L. Goldman's research in 1982, which suggested that implementation of rapid assessment pathways would increase discharge rates, Munro noted that many emergency departments still struggle with waste as a result of non-ACS patients being admitted by EDs. Munro hoped to use new medical tools that would make rapid assessment of ACS risk possible. Work done by Tobias Reichlin offered one such tool. Reichlin had previously studied high sensitivity cardiac troponin-T (hs-TnT) and its usefulness in predicting myocardial infarction, also known as heart attacks. Troponins are regulatory proteins that assist in the contraction of cardiac and skeletal muscle. Hs-TnT is specifically associated with cardiac muscle, and a measurement of the levels of hs-TnT present in cardiac muscle can help reveal whether chest pain may be caused by ACS.

Raised levels of hs-TnT are due to cardiac necrosis (cell death). However, cardiac necrosis alone is not always symptomatic of ACS. Relatively high levels of hs-TnT are also caused by many other diseases such as pericarditis, an inflammation of the sac housing the heart, pulmonary embolism or arterial clotting in the lungs, a tachyarrhythmia or abnormal increase in heart rate, and Takotsubo cardiomyopathy, a temporary weakening of the heart muscle.

Chronic elevations can also occur in conditions such as renal impairment, which is a kidney disease. Congestive cardiac failure, a disease that prevents the heart from circulating blood efficiency, also raise hs-TnT levels over a long period of time. Previous work suggested that the best way to use measurements of this protein in a clinical setting is to monitor the change in hs-TnT over an hour. The change over time will be able to target whether chest pain is specifically caused by ACS.

FINDING A NEW PATH

Munro and his team developed a new set of chest pain pathways that classify patients as "high risk" or "non-high risk" based on the change in their hs-TnT levels over time. To test the effectiveness of the new protocols, the team designed study capable of being executed by hospital staff alongside their various job-related duties. If there was a possibility that a patient's chest pain was caused by cardiac problems, he or she was asked to be included in the study. Any patient with chest pain clearly unrelated to heart conditions were excluded. Ultimately, 452 chest pain patients agreed to participate. This gave the study the power to detect if a previously calculated miss rate of <1% was due to chance or not. (The miss rate is the chance that a patient whose chest pain is of a cardiac nature will be discharged.)

The specific criteria for assigning risk status to patients involved three different considerations. First, ECG wave patterns are examined to look for a specific pattern. Second, an initial troponin level is recorded. If indicated by the protocol, a second level is taken at two hours. A change in troponin-T over time is then used to assign the risk type. Finally, a senior emergency department doctor is required to sign off on disposition (admission vs. discharge). The emergency department doctor could override the ADP at any point in the pathway. If the senior ED physician on staff felt that the patient was at a high risk of experiencing a heart attack over the next 30 days, they were free to admit them even if the protocol indicated they should be discharged.

The research team followed up with patients via phone interviews a month after the trials. This was conducted to determine whether the discharged patients had experienced any cardiac episodes over the past 30 days. They discovered that the new pathways demonstrated 100% sensitivity, meaning that not a single patient discharged had experienced a heart attack or died in the intervening time. The Nelson Hospital ED also saw an unprecedented discharge rate of 70%. The protocols also identified all patients who were at high risk of having heart attacks. Additionally, the average length of time spent in the ED was four hours and five minutes. Several other EDs have already expressed an interest in using the new chest pain pathways developed by Munro and his team, since they will dramatically improve the care that patients receive. Not only that, the protocols will help hospital staff do their jobs quickly in less stressful conditions.

Researcher Profile

Dr Andrew Munro
Nelson Hospital

Dr Andrew Munro received his BSc from the University of Canterbury and his MBChB from the University of Otago. Apart from being a full-time clinician, he is also a part-time researcher working in the Emergency Department of Nelson Hospital, New Zealand, as well as a fellow of the Australasian College for Emergency Medicine. A known regular contributor to Best-BETS in the Emergency Medicine Journal, Munro maintains his focuses on the research to improve clinical practices for better patient care.

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Changing the way we diagnose heart attacks

Dr. Martin Than is the Director of Emergency Medicine research at Christchurch Hospital, Canterbury, New Zealand. Here he describes both his colourful history and the work begun at the Canterbury District Health Board in New Zealand and now being disseminated internationally on accelerated diagnoses for coronary failure, which promises to slash the time spent having investigation.

First a bit about yourself. How did your career path bring you to your current position?

Having attended medical school in the UK, I completed Emergency Medicine training in the UK, Australia and New Zealand. While I was in the UK I also worked with the Centre for Evidence-based Medicine in Oxford, acting as course tutor. Having returned to New Zealand in 2001, I have worked as Emergency Medicine Specialist at Christchurch Hospital ever since. In 2003-2006 I set up and co-directed the Centre for Evidence-based Medicine at the Christchurch School of Medicine. I am currently a Health Research Council of New Zealand Fellow for "Assessment of cardiovascular disease in the Emergency Department".

You have had a very international career in NZ, the UK, and Australia. What do you feel was the best experience during that time?

This is not really linked to our current research themes in any way, but the best experience was working for two years on the rescue helicopter in Sydney. I really enjoyed the comradery and teamwork, and was awarded a citation for bravery by the commonwealth of Australia for a rescue that we carried out in 2006.

How else do you think the ADP method can be improved for speedier diagnosis? Are there other biomarkers you would be interested in examining?

Our accelerated decision-making pathway is comprised of a structured risk assessment, electrocardiographic findings and biomarkers combined together. I expect there will be some refinements that will occur in the way that we use such risk assessments and improvements of the other parameters used. At the moment we use 12-lead electrocardiography. Promising research has taken place on multi vector ECG analysis and if there are advances in the versatility of such devices such that they become economical and easier to use, then they may provide useful extra diagnostic information.

There is of course a constant search for improvements in biomarker analysis. High sensitivity cardiac troponin assays are now extremely advanced and I cannot see them being replaced in the short term. Increasingly patients who would previously have been diagnosed as having unstable angina are now categorised as having had an acute myocardial infarction (AMI) because of raised troponin levels. However I still believe there is a need for a biomarker that can reliably identify patients with either myocardial ischaemia prior to, or in the absence, of myocardial cell death occurring. If after ruling out AMI using cardiac troponins there was a biomarker that could reliably predict which patients did or did not require further investigation then that would be a major step forward.

Adherence to 'best practice' is often tricky in the stressful situation of the ED. How easy do you believe it will be to successfully implement the ADP within hospitals?

In Canterbury we have managed to increase the number of people discharged within 6 hours from 5.4% in 2008 to approximately 33% in 2014. The median length of stay has changed from 51 hours to 8 hours.

Thus in Canterbury it has been relatively easy to implement the ADP into clinical practice because the Canterbury District Health Board is very good at supporting integrated clinical pathways; both by releasing clinicians' time to work on them and by providing appropriate funds when necessary to facilitate project development. This sort of support from the Canterbury District Health Board was instrumental in allowing us to make this a truly translational project which was rapidly implemented into clinical care rather than stand-alone research. It would have been impossible without their help.

The new protocol is based on proven results from research – including a large randomised controlled trial, and the subsequent accelerated diagnostic pathway was trialled within Christchurch Hospital and hospitals in Australia and Hong Kong. The next logical next step is to integrate this research into clinical practice, which is directly aligned with a Ministry of Health (MOH) plan to introduce 'accelerated chest pain pathways' for suspected acute ischaemic heart disease (IHD) into every District Health Board.

Change is not always 'easy', but the long term benefits of implementing a pathway that facilitates safe early discharge are clear. We have strong clinical support and are finding that the process of adopting the new standards of best practice is, on the whole, very successful across the country. Additionally the pathway has been rolled out across Queensland, and other locations in Australia and we are collaborating with researchers at several other international sites.

Is there a desire from physicians to hold patients in the department, 'just in case', despite the ADP identifying them as low risk?

That has certainly been our experience at the time of initial research and early implementation. I think the best way to overcome this problem is repeated education and positive data feedback demonstrating good outcomes. In general the biggest barrier is familiarity but we have found this is not too difficult to overcome. I believe that the pathway we are implementing will offer a safe, consistent and reliable way to most effectively manage chest pain patients to offer better outcomes for patients and healthcare systems.

Spotting an unhappy heart

The Emergency Care Foundation is a local charity which supports research into the care of patients in the hospital Emergency Departments.

The Canterbury District Health Board is the health funding and healthcare provider organisation for the city of Christchurch and its surrounding region which is strongly engaged in the process of creating effective cross-system healthcare pathways.

You wake up one morning, far earlier than you would like, with an unpleasant pain in your chest. Although the pain is manageable, it slowly appears to be spreading into your arm. This is a classic symptom of acute coronary syndrome, or ACS, and so you naturally do the right thing and get yourself to hospital. So far, so good. However now the physicians at the hospital have a problem – many patients turn up every day with these symptoms, up to 10% of the people that come into the emergency room. Not everyone has cardiovascular problems, not everyone needs urgent attention, and indeed almost 80% will eventually be diagnosed as not having ACS. For the hospital doctor, the real problem lies in distinguishing emergency cases from low-risk patients.

Unfortunately this is very difficult. The medical profession sets a very high standard for ruling out ACS – in fact the benchmark is 100% accuracy – therefore a prolonged observation period is often required and it can take from 6-12 hours to properly diagnose a case of coronary distress. During this time the patient needs to wait in the ER, their families must sit around getting more and more worried, and the emergency department itself becomes more and more crowded. Even worse, the number of patients who can be safely sent home early distracts clinicians from tending to those who urgently need their help. There is thus a strong requirement for a faster diagnostic method, one which allows doctors to identify the high-risk patients, focusing their scarce time on those who are in the most danger.

In Christchurch a group of physicians facilitated by Martin Than and strongly supported by resources and funding from the local Canterbury District Health Board, have been

leading the way in solving this problem.Bringing the same focus to this task which he has applied to each of his preceding 15 years of research experience, his work has focused on improving the sensitivity and speed of current ACS diagnostics. Thanks to their work developing an accelerated diagnostics program they have managed to slash the time required to identify low-risk patients. This process utilises a combination of factors to identify patients in minimal danger, allowing them to be safely sent home far earlier than previously possible.

Diagnosing acute coronary syndrome can take over 6 hours, a long time for patients. Newly developed methods can cut this down to only 2 hours.

HEARTBEATS AND BIOMARKERS

A major facet of this system is the use of a biomarker called Cardiac Troponin which is a biological indicator of heart damage. These proteins are present within the muscle cells of the heart and are released into the bloodstream when heart cells die – as often occurs during a heart attack. Comparing Troponin levels between the time of admission and two hours afterwards allows for accurate identification of high/low risk patients. This method is quick, simple and very sensitive.

Despite these advantages, diagnostics can

be improved by the addition of other tests. The results from Cardiac Troponin tests can be enhanced by these additional diagnostic methods. These include further tests, such as electrocardiography (ECG – a recording of the heart's electrical activity), but also statistically modelled decision aids for disease risk to predict these risks and help clinical decision-making. Examples of such decision aids are the Thrombolytis in Myocardial Infarction score (TIMI), the HEART score and the Emergency Department Assessment of Chest Pain Score (EDACS) which take numerous factors into account in assigning risks. A combination of these decision aids with the results from biomarkers and ECGs can facilitate accelerated diagnostic pathways in safely identifying which patients do not have an acute coronary syndrome using results from tests ordered within two hours of arriving in hospital. This is a significant improvement in terms of speed and reliability.

After an initial trial of the accelerated diagnostic pathway - funded by the Health Research Council of New Zealand at a single hospital in Christchurch, the team has expanded their testing to numerous locations throughout the Asia-Pacific region. Dr. Than emphasises that the local research was made possible through the Health Research Council having a research funding stream focussed on service delivery, which has enabled this sort of research to take place not on a supernumerary basis but integrated into clinical practice. He adds, "We were also fortunate to have strong support from the Canterbury District Health Board who facilitated the integration of the research into clinical practice by assisting with funding and making key personnel available to make internal arrangements and modify processes as needed."

These international and local trials have been able to successfully show identification of 'low risk' groups from as little as 2 to 4 hours after arrival at the ED, a significant improvement when compared to the 6-12 hours commonly needed. The next logical next step is to integrate this research into clinical practice nationally, which is directly aligned with a Ministry of Health (MOH) plan to introduce 'accelerated chest pain pathways' for suspected acute ischaemic heart disease (IHD) into every District Health Board.

"We are also currently working with a number of collaborators in Singapore, the UK and USA regarding possible trials" comments Dr. Than, who is looking forward to spreading these improved practices around the world.

SPREADING THE WORD

Implementation of new research findings can often be a difficult challenge – many doctors continue with the processes they are familiar with, and there is a natural desire not to send patients home if there is any chance the diagnosis may be wrong. Dr. Than recognises these challenges: "Overall the feedback I have received has been very positive," he comments, "as with any new process, uptake has been gradual while clinicians become familiar and learn to trust the new approach." Thus the next stage of rolling out the new procedure, currently underway, requires optimising the process of changing over from standard diagnostic techniques to the accelerated diagnosis program. This requires talking to local hospital clinicians, studying their current processes to see where improvements could best be made, choosing an ADP (there are several varieties to choose from), implementing the ADP, and then reviewing how implementation has progressed and what lessons have been learnt.

Once implemented into hospitals, the ADP takes its place amongst a vast number of processes making up the standard care of an emergency department, Dr. Than's goal is to combine them such that "assessment of the low risk chest pain patient is a part of a larger overall assessment." His aim is to standardise the process as much as possible, with defined time-points for each test, a well-documented pathway to guide clinicians to perform the work, clear reasons to perform follow-up diagnostics on patients, and robust quality testing to ensure that everything works as it should. Together, this should provide "safe, consistent and reliable way to most effectively manage chest pain patients to offer better outcomes."

The ultimate aim of this work? A reproducible process for implementing an accelerated diagnostic process, quickly, easily, and reliably. If successful, this could lead to the spread of the ADP across the globe, with corresponding decreases in ED wait times for many patients. This would provide physicians with more time to focus their limited resources on treating the life threatening emergency cases – in turn saving lives. In the end, for physicians such as Dr. Martin Than, there are few better goals to strive for.

Researcher Profile

Dr. Martin Than
Director of Emergency Medicine Research
Christchurch Public Hospital

Dr. Than received his medical degree from the University of London with a strong interest in emergency medicine. He was awarded the Beaven medal for Excellence in Translational Research by the Health Research Council and has been recognised by the receipt of the Commonwealth of Australia Decoration for Bravery, the Surf Life-Saving Australia Meritorious Award for Bravery and the Royal Humane Society of Australia Bronze Medallion. Dr. Than's current research focuses on faster diagnosis of potential acute coronary syndrome from unclear symptoms such as chest pain, and the implementation of these improved techniques into hospitals.

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Oxygen in heart attacks: a long-standing belief changes

Dr. Dion Stub is interested in studying treatments for emergencies related to heart attacks. Here he discusses his research interests and his recent scientific input to the controversy between the benefits versus adverse effects of supplemental oxygen therapy in heart attack cases.

To start, can you tell the readers how did your career start in cardiology and what are your research interests?

I graduated from Monash University, Australia, in 2003 and undertook cardiology training at The Alfred Hospital. In 2013, I was awarded a PhD for my research on developing a unique treatment pathway for patients with refractory cardiac arrest, through combined research at the Baker IDI Heart and Diabetes Institute and The Alfred Hospital. Afterwards, I spent two years in the US and Canada as a post-doctoral fellow, working alongside international leaders in researching cardiac emergency care and transcatheter heart interventions. I have a particular interest in researching and treating emergencies related to coronary disease and cardiac arrest, whilst specializing in coronary and structural heart interventional procedures.

Your research is mainly focused on cardiovascular diseases, what are their impacts on public health and patient's lifestyle?

Cardiovascular diseases account for almost 30% of the disease burden around the world. In Australia, heart diseases and stroke cause over 50,000 deaths per year, which is only expected to increase over the coming decades due to our ageing population. The major risk factors for cardiovascular diseases include high blood pressure, high cholesterol, diabetes and smoking. Other key lifestyle features increasing the risk of cardiovascular disease are obesity and a sedentary lifestyle. Accordingly, patients suffering cardiovascular diseases or individuals at high risk are required to adapt their lifestyle habits such as diet, smoking, alcohol consumption and physical exercise in order to avert or reduce the critical lack of blood supply to the heart and the brain. Failure to do so might cause life-threatening outcomes.

Given the old-age belief in the therapeutic benefit of supplemental oxygen in cases of heart attacks, do you expect the findings of your 'AVOID' study, which recommend the opposite, to change the current international treatment guidelines?

The AVOID trial specifically aimed to investigate the effects of supplemental oxygen therapy on heart injury, in patients with suspected heart attack. Although oxygen is beneficial in complicated cases where patients develop hypoxia, evidence supporting its routine use in patients with normal blood oxygen levels is not strong. Recent mechanistic studies have highlighted the potential adverse effects of supplemental oxygen to the heart. Simply, our bodies respond to excess of oxygen by decreasing the blood flow to the various organs. During a heart attack, this response may further reduce blood flow through the coronary arteries. Additionally, excessive oxygen can promote the generation of destructive molecules known as free radicals in the cells, which can further aggravate the damage of the heart muscle.

The AVOID study, together with these recent physiological studies, does not demonstrate any significant benefit of routine oxygen therapy in alleviation of heart attacks or their symptoms. Instead, the AVOID trial identified a signal for increased heart muscle injury with the routine use of supplemental oxygen in patients with normal blood oxygen levels. Oxygen should be treated like all other medical therapies, balancing efficacy versus side effects. Based on the findings of the AVOID trial - the largest so far - we would recommend that pre-hospital and hospital care providers review their current practice regarding supplemental oxygen. Until larger studies are available, international guidelines should consider updating recommendations, highlighting the lack of benefit of oxygen therapy and its potential harm in cases of heart attacks, unless blood oxygen saturation is below 94%.

Was the AVOID study done in collaboration with other research groups or institutes?
The study was led and conducted by Ambulance Victoria in collaboration with nine metropolitan hospitals that provide 24 hour cardiac intervention services in Melbourne, Australia, between October 2011 and July 2014. Important contributions were also made by Baker IDI Heart and Diabetes Institute and Monash University.

Are you planning to extend your research on oxygen therapy further? What might be the scope of the next step?

Further trials are needed to confirm the potential harmful effects of excess oxygen during acute heart attacks and more importantly, its effect on patient survival. Currently a trial is running in Sweden, based on registered records of patients with acute heart attack to investigate the possible adverse effects of oxygen therapy. The trial is statistically designed to provide evidence for the effects of supplemental oxygen on cardiovascular survival. Also, the AVOID investigators together with the Australian Resuscitation Outcomes Consortium is planning a similar trial in patients with out-of-hospital heart arrest to investigate the potential cardiac and neurological outcomes of high flow oxygen following heart arrest.

Oxygen for Heart Attack Victims: Friend or Foe?

Dr. Stub and his fellow researchers have recently completed a clinical study that addresses the concerns over the adverse effects of supplemental oxygen therapy in patients with heart attack who exhibit normal blood oxygen levels.

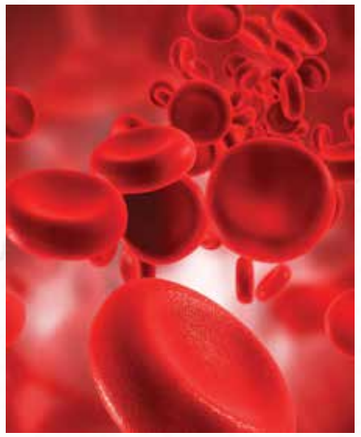
THE STORY OF HEART ATTACKS AND OXYGEN

Each year some 19 million people around the globe experience cardiac emergencies such as heart attack and heart arrest. The majority of heart attacks occur as a result of disease conditions causing severe occlusion of one or more of the coronary arteries that nourishes the heart. These conditions commonly include coronary artery disease and coronary thrombosis. As a result of coronary occlusion, some parts of the heart musculature (myocardium) suffer a lack of blood flow, leading to heart muscle injury, a condition known as myocardial infarction. At this point, the victim might suffer symptoms of a heart attack, which include pain and discomfort in the chest and upper body parts such as the jaws, neck, arms and shoulders and shortness of breath.

Following the first report of supplemental oxygen for heart disease in 1900, oxygen therapy has been commonly used in the initial treatment of patients with heart attack. This was based on the belief that supplemental oxygen may increase oxygen delivery to the heart muscles lacking blood supply and hence, reduce myocardial injury. 'Until recently giving supplemental oxygen to all patients with suspected heart attack was considered a fundamental first aid response and was part of all international medical guidelines, taught to paramedics, medical and nursing staff', Says Dr. Stub.

ALARMS AGAINST ROUTINE OXYGEN THERAPY

Oxygen therapy can in fact be beneficial in complicated cases of heart attacks, where a patient concurrently suffers a significant decrease in blood oxygen saturation levels. However, a combined analysis of the data obtained from three independent small clinical trials suggested a possible increase in adverse outcomes of supplemental oxygen administration in patients with normal blood oxygen. Similarly, a combined analysis of the



findings from two clinical trials where novel techniques were used for additional oxygen delivery to the parts of the myocardium lacking sufficient blood supply indicated that this treatment caused a significant reduction in coronary blood flow, an increase in coronary resistance to blood flow (coronary constriction), and a significant reduction in myocardial oxygen consumption. 'These data appear to suggest that supplemental oxygen may be harmful', says Dr. Stub.

THE 'AVOID' STUDY

With the growing concerns over the potential adverse effects of supplemental oxygen therapy in patients with heart attack showing normal blood oxygen levels, Dr. Stub and his research team have conducted a larger clinical trial to compare supplemental oxygen therapy with no oxygen therapy in these patients to determine the effects of the treatment on the size of myocardial damage. The trail which has been given the title The Air Versus Oxygen in Myocardial Infarction is better known in the scientific community as 'AVOID'.

The AVOID trial was conducted by Ambulance Victoria and nine metropolitan hospitals across Melbourne, between October 2011 and July 2014. The study involved 441 adults of ≥18 years of age, who complained chest pain commencing less than 12 hours prior to assessment, and who were later confirmed to suffer a serious type of heart attacks known as ST-segment elevation myocardial infarction. Importantly, all of the selected patients showed normal blood oxygen saturation levels, while patients with saturation levels of ≥94% were excluded from the study since withholding oxygen therapy may be unsafe for them. Supplemental oxygen therapy was randomly assigned to half of the patients (218), while the other half received no oxygen (referred to as Air). Paramedics were given a 'randomisation envelope' to open at the scene, informing them to provide or withhold oxygen therapy in each case. Patients assigned to supplemental oxygen therapy continued to receive oxygen during the in-hospital care.

In order to evaluate the possible adverse outcomes of supplemental oxygen therapy in patients with heart attack, a set of primary and secondary clinical measures relevant to cardiac health was assessed. The primary measure was the size of the myocardial damage, as indicated by the blood levels of two enzymes, troponin and creatine kinase. These enzymes are known to be synthesized in the muscle cells of the heart and are found in the blood in high concentrations when a significant myocardial damage occurs. Secondary measures included abnormal heart rhythm, as indicated by electrocardiogram and repeated heart attacks. Also, after 6 months, the researchers reevaluated the size of myocardial damage in about one-third of the participants by cardiac magnetic resonance imaging.

The findings of the AVOID study provide evidence for an increase in myocardial damage in patients with heart attacks as a result of supplemental oxygen therapy

A CENTURY-OLD PRACTICE DOING MORE HARM THAN GOOD

The findings of the AVOID study have further confirmed the suggested adverse effects of supplemental oxygen therapy in heart attack patients, who exhibit normal blood oxygen saturation. Dr. Stub and his colleagues have found that supplemental oxygen therapy can significantly contribute to increasing the size of the myocardial damage. According to the abstract presented to the American Heart Association Scientific Sessions in November of last year, a 25 percent increase in blood levels of troponin and creatine kinase was recorded in the patient group receiving oxygen compared to the no oxygen group. Even more, a significantly higher percentage of the oxygen-treated patients developed more dangerous heart rhythms or experienced subsequent heart attacks compared to the patients received no oxygen. Among the subgroup of patients who returned after 6 months for magnetic resonance imaging of the heart, the oxygen-treated patients showed a 30 percent increase in the size of myocardial damage compared to the no oxygen group. Additionally, supplemental oxygen therapy made no difference in symptoms felt by the patients during the study, which is rather surprising since supplemental oxygen has been commonly believed to help relieve the chest pain.

TIME TO AVOID SUPPLEMENTAL OXYGEN?

Although the findings of the AVOID study clearly demonstrate the potential adverse outcomes of supplemental oxygen therapy in cases of heart attacks, it does not give a full insight on the influence of the practice on patients' survival. In the course of the AVOID study, no statistically significant differences in mortality were found among oxygen-treated and non-oxygen-treated patient groups. However, with the relatively low overall mortality observed during the study, such differences might have been missed. Therefore, larger studies are necessary to circumvent these limitations. 'An outcomes-based study would require much larger numbers of patients', says Dr. Stub.

So far, AVOID is the largest available study, in terms of the number of participants. Therefore, in light of the evidences provided by AVOID, the community of cardiologists and cardiac care providers should revise the current practices in treatment of heart attacks patients, particularly those with normal blood oxygen saturation.

In response to the recommendations of the AVOID trial, a growing number of hospitals, not only in Australia, but also in other countries, instruct the ambulance crew to check the blood oxygen levels of patients suspected with heart attacks before any supplemental oxygen is given. Additionally, the findings of Dr. Stub and his research team have been applauded by independent cardiology experts.

Researcher Profile

Dr. Dion Stub
Alfred Hospital and Western Health

Dr. Dion Stub is a cardiologist investigating new ways of managing people with cardiac arrest and heart attack. He was awarded his PhD in 2013, through his work at Monash University and the Baker IDI Heart and Diabetes Research Institute. He is also a coronary and structural interventional cardiologist at the Alfred Hospital and Western Health in Melbourne. Dion spent 2 years overseas as a post doctorate fellow, with the support of the prestigious Victoria Fellowship and Australian Cardiac Society Award. He worked first at the University of Washington, Seattle and then spent 12 months at St Paul's Hospital, Vancouver working alongside Prof. John Webb, an international expert at transcatheter aortic valve replacement. His ongoing research is supported by joint NHMRC and National Heart foundation grants.

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LINKS

http://globalnews.ca/news/1690465/watch-study-could-change-how-heart-attack-patients-are-treated/
http://www.medscape.com/viewarticle/835297

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Delivering Quality Care for Palliative Care Patients

Registered Nurse Sue Healy is passionate about end-of-life care, and Professor Liz Reymond specializes in palliative care. Here, they discuss a joint project aimed to aid those who care for palliative patients at home.

As a starting point, can you tell us what led you to your respective interests in palliative care?

[SH] Caring for the dying has always been an interest of mine. My passion for palliative care began early in my nursing career. Working as a community nurse, I was exposed to many home deaths. It was an absolute privilege to be invited into someone's home and to be involved in their care, as we prepared the family and friends for the journey ahead, especially as end of life for the patient approached. It was here that I recognised that palliative care was about holistic care supporting the family unit, delivering regular and ongoing assessment, and importantly administering effective pain and symptom management.

[LR] I started my career as a General Practitioner [and], during this time, developed a real interest for palliative care. I have been part of a team that successfully established a large palliative care service that encompasses dedicated hospice beds across several hospitals within the Metro South district. As well as a consultative service at a large tertiary hospital in Brisbane and three community palliative care sites. I established the Brisbane South Palliative Care Collaborative, which is the research and service development component of the service. The collaborative has been successful in gaining funding to pursue research in the area of palliative care, and, the Caring Safely at Home Project was one of the fruits from this endeavour.

Were there any significant roadblocks to completing this project?

Not really. Ethics approval was granted from several ethics committees. One ethics committee initially challenged the practice of teaching laycarers to administer opioid injections, as they were concerned about the interface between effective pain management and euthanasia. The Guidelines for the Handling of Medication in Community Based Palliative Care Services in the Queensland document endorsed by the appropriate peak bodies assisted in the process.

Another issue was trying to access a community pharmacist to prepare the medications for the patients that were assigned to the pharmacy arm of the project. Very few community-based pharmacies had the capacity to prepare injections under sterile conditions. Eventually, a private hospital pharmacy agreed to prepare and label the medications. The project coordinator collected the medications daily and delivered them to the patients as required.

You received funding for this project from the Australian Government Department of Health and Ageing. Are the needs of laycarers a new concern for the Australian government and have they tried to address these issues in the past? Periodically, the Australian Government allocates funds for projects that may specifically address laycarers concerns. However, the needs of this group are increasing significantly and funding is difficult to find especially in the area of community services that aims to support laycarers (domestic support, respite – especially as end of life approaches, personal care, packages of care, and the like). In the future, this may prevent laycarers from being able to stay at home.

The randomised controlled trial found that laycarer confidence increased regardless of who prepared the medications. Did this result surprise you?

Yes. It was considered that the RCT may show that laycarers who prepared their own injections would be less confident than if the registered nurse or pharmacist prepared the injection. However, it was identified that laycarers' confidence was not influenced by who prepared the injection and, as the laycarers gained experience with injecting their level of confidence increased. This shows that, if laycarers are given standardised and appropriate information and training, they can acquire the confidence and skill to safely prepare, store, and administer subcutaneous injections to manage symptoms as they arise.

What is the next best step to continue addressing problems in home palliative care in Australia?

Support Palliative Care peak bodies to continually lobby the Government for improved funding. The aim is to increase funding for palliative care services across Australia so that there is equity and access to high quality palliative care for all patients, as they require it.

Do the two of you plan to continue working together?

Yes, we will continue to work together to promote the use of the resources to support laycarers. Part of our advocacy is to allow patients and their caregivers easier access to palliative care service with similar programs as this.

Educational Package Improves Home-Based Care Palliative Care Patients

Australians who live in regional and rural areas often have difficulty accessing palliative care services. To address this issue, Prof. Sue Healy and Prof. Liz Reymond have recently developed a suite of new tools to teach at-home caregivers to administer subcutaneous injections to those palliative patients whose symptoms need control.

PALLIATIVE PROBLEMS

The choice to receive care and die at home is an important freedom for patients. It gives them the chance to maximise the quality of the time they have left and spend it with their families and friends in the environment of their choice. However, this choice often means that a family member, or another person close to the patient, is put in charge of care that can extend to symptom control.

…as the laycarers gained experience in administering injections, their confidence level also increased, as statistically demonstrated by the experiment. This proves that if laycarers are given standardised information and training, they can acquire the confidence and skill to safely prepare, store, and administer subcutaneous injections as symptoms emerge.

This person is known as the laycarer. Often, the laycarer may lack background knowledge to provide extensive medical care to the patient. While this situation can be distressing the stress can be further exacerbated by lack of a supportive infrastructure. Sue Healy, RN, and Prof. Liz Reymond have worked to fix this problem in Queensland, Australia by creating an educational package called Caring Safely at Home. The goal of the project is to teach laycarers how to properly administer medications by subcutaneous injection to relieve patient's symptoms if they emerge. They also trained RNs to use the educational package to ensure laycarers received consistent information in their areas of medication management.

As in many other parts of the world, laycarers in Queensland, particularly those who live in the regional and rural areas, have difficulty getting access to medical professionals and institutions. Medical centres in regional and rural locations may be too far from their residence to access in times of emergency. Some, but not all, of these centres offer over-the-phone services 24 hours a day. However, there is also the issue of some centres being understaffed or closed at night, despite the need for round-the-clock care for palliative patients.

Laycarer health education is also a concern among medical professionals in Queensland. Laycarers can often be misinformed about aspects of health care such as the benefits of opioids in palliative care. During their research, Healy and Reymond experienced resistance from laycarers to administering opioids because they believed they were too dangerous. Many believed that opioids were inescapably addictive if taken every day, even if used as prescribed. As a result, patients maybe undertreated. With the proper education, however, Healy and Reymond hope to provide laycarers with the tools to provide high-quality care autonomously.

EDUCATING OTHER NURSES

Education for health professionals working with laycarers is just as crucial to the success of the Caring Safely at Home project. While palliative care specialists have been unofficially teaching laycarers how to properly administer subcutaneous injections for a long time, some healthcare professionals and organisations believe that subcutaneous injections should not be administered by laycarers at all. When Healy and Reymond invited palliative care sites to participate in their trial, they were met with some resistance from healthcare professionals. These professionals required

clarity when it came to the legal ramifications of teaching laycarers to administer subcutaneous injections at home. The researchers recognized the need for official documentation outlining the legality of managing medication in the community. This is the reason why the "Guidelines for the Handling of Medication in Community-Based Palliative Care Services" was developed. Luckily, specialist palliative care nurses had already begun drafting these guidelines, which contributed greatly to the project. The guidelines were approved by the Nurses Registration Board of Queensland, the Safety Medication Management Unit, and the Environmental Health Department within Queensland Health.

Even when the randomised controlled trial to examine the effectiveness of the program had commenced, some service providers were still reluctant to ask laycarers to participate in the trial. They felt that subcutaneous injections were too difficult a task for non-medical professionals. Healy and Reymond point out that this attitude only exacerbates the problem they are trying to solve. If a laycarer runs out of a medication at night or on a weekend, the patient will likely be unable to receive any care at all. That may result in hours or days of excruciating pain or other symptoms or an unwanted admission to hospital. It is less stressful for laycarers if they are prepared for this situation by learning how to manage symptoms by administering medications themselves.

CARING SAFELY AT HOME EMPOWERS LAYCARERS

The program itself includes helpful charts, lists, and other tools to help laycarers at home. There are five illustrated, step-by-step charts, which exhibits the different medical techniques in the program. The first chart demonstrates how to open and draw up from a glass medication ampoule to measures dosage. The other charts guide laycarers through four different techniques including both blunt needle and needle-less injections. Laycarers also received a colour-coded medication labelling system to easily track which medications are appropriate for different symptoms and a diary to document each injection to make patient treatment monitoring easier for RNs. A medication booklet provides laycarers with information on symptom control and addresses frequently asked questions and common myths. Also included is a DVD giving laycarers a visual guide for how to properly prepare, store, and inject medications. RNs used the practice demonstration kit included in the package to physically show patients how to give subcutaneous injections during the face-to-face training session. The included competency checklist allows RNs to check laycarer competence.

One important goal of this project was to ensure that laycarers felt capable and competent to provide quality care. To test their confidence, Healy and Reymond implemented the program in 24 sites that provided palliative care. Scattered across southeast Queensland, these sites were located in urban, regional, and rural settings. Two hundred seventeen RNs were trained to educate laycarers about how to use the resources in the package. To gauge the results, 106 laycarers completed two questionnaires: one after the training session and one after they had more experience giving injections. The questionnaires asked participants to rate their satisfaction with various aspects of the program on a seven-point scale. The results showed laycarers being satisfied with the program, with each question receiving an average of 5.9 out of 7 for both sets of questions. Fifty-three RNs completed the questionnaires at the conclusion of the study. Nurses also responded positively to the program, believing that it explains all the necessary information well and addresses the needs of laycarers.

A randomised controlled trial examined whether laycarer confidence was affected by who prepared the medications to be injected. Ninety-four laycarers were randomly assigned to one of three groups. In the first group, medications were prepared by laycarers. In the second, they were prepared by RNs. For the third group, the medications were prepared by a pharmacist. While laycarers in all three groups became more confident in their ability to administer injections over time, there was no significant difference between these three groups. Of the 1306 injections analysed, the correct medication was given each time. Implementation of the package and analysis of the RCT reveal that laycarers are capable of confidently administrating subcutaneous injections at home. For those who care for loved ones as they reach the end of their lives, the benefits of this program are tremendous.

Researcher Profile

Sue Healy

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Sue Healy earned her Bachelor's degree in Health Science Nursing, and her Masters of Nursing Chronic Disease and Palliative Care. Prof. Liz Reymond began her education by earning a Bachelor of Medicine, Bachelor of Surgery, and received her PhD from the Australian National University.

Healy and Reymond continue their careers in the pursuit of finding new ways to improve care for patients with chronic and end-of-life illnesses, conducting researches that combine and utilise both of their specialisations.

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BRISBANE SOUTH PALLIATIVE CARE COLLABORATIVE
Queensland Government
Griffith UNIVERSITY

Primary Care: Ensuring A Strong Foundation For Child Health Services

Dr. Patricia Li is a pediatrician who recently received funding to begin work on a project that will evaluate health care reforms in Quebec and discover the best way to improve primary care for children in Canada.

As a starting place, how did you come to focus your work on primary care, specifically for children?

Primary care is pivotal in every child's health. It is the cornerstone of our healthcare system. An extensive body of research supports the positive correlation between having a source of primary care and better overall health. Over a decade ago, government-commissioned reports exposed numerous problems with attributes of primary care in Canada, drawing attention to fragmented and inaccessible care. Recognising the vital need to improve primary care services, the federal government established the Primary Health Transition Fund and later the Health Reform Fund providing millions of dollars to assist provinces and territories in reform efforts.

As I was finishing my master's degree in clinical epidemiology and health care services at the University of Toronto and the Institute for Clinical Evaluative Sciences, publications evaluating reform efforts were starting to come out, with some demonstrating improved outcomes. As I am a general pediatrician and none of these evaluations focused on children, this topic naturally became the focus of my research program.

You involved parents and clinicians, the end-users of your research, in your projects. How did their perspective change the project, if at all? Did it provide any unique challenges?

Having end-users—in particular, decision- and policy-makers, health administrators, clinicians, parents—on our team has ensured that our projects and objectives stay relevant to the health of children and their families, as well as the health system. In our projects thus far, they have not changed the perspectives of projects as much as they have added perspectives and insights. Our end-users will also be important in helping us disseminate our results to the appropriate audiences, in appropriate formats (i.e. in ways that are accessible and understood by knowledge users). Still, there are challenges in having a bigger and more diverse team, such as being unable to address all the questions and viewpoints raised by every member in a single project – in some cases, they may help fuel another study.

The literature review gathered in support of this project found that there is almost no information relating to the primary care of children in Canada after the reforms were passed. Do you have any hypotheses as to why this is the case?

A lot more focus is put on evaluating and improving the care of adults and the elderly with more costly chronic diseases. There is more impact on costs when improving the quality of care in the elderly/adult population, which is a large and growing percentage of the population in Canada and around the world. The capacity for health services research is also not as great for children as it is for adults. For instance, there are just not as many researchers focused on child health services as adults. The Canadian Institutes of Health Research (our main federal funding agency) has documented this dearth in child health services research capacity and has made strides to support the development of researchers in this field in the past few years as well as support more studies related to child and maternal health.

Did you experience any significant setbacks to gaining funding?

Apart from having a strong team, I have been fortunate in having successfully obtaining grants from our provincial and federal funding agencies (Fonds de Recherche du Québec – Santé, and Canadian Institutes of Health Research, respectively). The strength in Canada (as in some Nordic countries) is that we have longitudinal data for all publicly-funded health care services and providers. This allows us to study the care of the entire population (using anonymised data), across all health care settings (such as clinics, hospitals, emergency departments, etc.).

Improving Children's Health through Primary Care

Dr. Patricia Li of McGill University is heading a research team whose goal is to improve primary care for children in Canada. Recently, her research efforts has received funding from the Canadian Institutes of Health Research and the Fonds de Recherche du Québec - Santé.

HEALTH CARE REFORMS IN QUEBEC, CANADA

Access to primary care is crucial for all members of society. Primary care services are those related to health promotion and frontline management of injury and illness including prevention, diagnosis and treatment. Primary care interventions may reduce future anxiety by addressing potential health problems early. More importantly, primary care can help avoid tremendous morbidities and costs. These high costs are paid by individuals, but also by society at large.

Primary care is pivotal in every child's health and is the cornerstone of our healthcare system. An extensive body of research supports the positive correlation between having a source of primary care and better overall health.

In 2000, the Canadian government turned its efforts and attention to improving primary care, investing millions of dollars in primary care reforms through the Primary Health Transition Fund and the Health Reform Fund. Each province and territory allocated these federal health care money into primary care reforms as they saw fit. Reforms across Canada share common features with other international primary care initiatives (such as those in the United States and the Quality Outcomes Framework in the United Kingdom), and aimed to improve primary care accessibility, comprehensiveness, coordination, continuity, as well as quality of care.

While some aspects of the reforms in the province of Quebec are unique, other Canadian provinces are using very similar structures to implement primary health care. Quebec's new system established entities known as family medicine groups (FMGs) and network clinics that enable physicians or clinics to work together in providing care to a group of patients.

Through the FMGs, access to physicians is now improved by making them more available on-call to patients who are in their given network. In addition, this extends working hours for physicians. The physicians are also often compensated for long-term care of patients with chronic diseases. Continuity of care is also provided since the registered patients are able to access and often see the same group of doctors within the network. This further improves the coordination between patient and doctor and establishes comprehensiveness of care. On the doctors' part, the FMGs are designed to incentivise doctors through pay to provide quality care to patients. Some FMGs have also implemented electronic medical records that make it easier to share patient information among doctors.

In Canada, the state of primary care services for children after the reforms is still not widely studied. The reforms instituted by Canada's provinces have shown some positive outcomes in adults, including reduced emergency department visits and reports of better experiences with health care overall. Dr. Patricia Li's research plan works with doctors to analyse whether FMGs are providing the same benefits to children. They will examine whether primary care reforms have improved the quality of care for children with chronic diseases, such as asthma and diabetes, and if FMGs reduced the disparity in health outcomes for children in different socioeconomic classes. The research team will also look at emergency department visits to see if visitation rates are at all affected by access to primary care as in adults.

In order to assess whether FMGs have affected children's primary care in Canada, the research team must consider many different aspects of health care. When there is a concern, primary care physicians are often the first doctors to see patients and recommend treatment or other specialists, so the research outcomes for this project have the potential to transform health care for the next generation. It is an ambitious goal that needs qualified researchers to be successful. As a paediatrician, Dr. Li's expertise with children's health issues and the involvement of doctors and parents of patients as members of the research team allows the study results to be more accurate and useful.

AN INTEGRATED KNOWLEDGE APPROACH

The study will be conducted using health administrative data in Quebec. Canada has a publicly-funded universal healthcare system, and Quebec (as in other provinces) has population-based data which can be used to link health care utilization across settings (such as emergency departments, hospitals, and outpatient clinics). A cohort of children will be studied from 2010 to 2013. Each child will be assigned to a type of primary care provider (for example, paediatrician vs. FMG vs. non-FMG general practitioner vs. no primary care) and quality of care outcomes will be studied, including emergency department visits and hospitalizations. The research should be complete within the next two years.

The research makes use of an integrated knowledge approach, whereby end-users of the research (such as decision-makers, clinicians, parents/patients) are involved throughout the study. The research team includes people from across multiple disciplines, providing feedback and input to the study to ensure that the findings will be informative for health care policy and practices. Specifically, the team includes stakeholders who will advise government and health care agencies about the health care needs of children and how to plan future resources. The primary stakeholders in this project are leaders in the department of maternal-child health and health services at the Ministry of Health and Social Services in Quebec.

MAKING A DIFFERENCE IN THE COMMUNITY

As a paediatrician, Dr. Li is part of a group of clinician-researchers dedicated to studies that aid doctors in providing the best care for patients. Together with collaborator Dr. Evelyn Constantin of the Montreal Children's Hospital, Dr. Li is setting up a clinical site in Montreal for a primary care research network in Canada called TARGet Kids! which was co-founded by doctors Catherine Birken, Jonathon Maguire, and Patricia Parkin. It is one of the largest ongoing prospective cohorts of children under six years old in Canada that is recruiting patients and conducting studies within a network of primary care clinics. In Ontario, TARGet Kids! already encompasses over 5,000 children and eight paediatric and family medicine clinics. The clinics recruit children for longitudinal studies to research primary and preventative care practices such as nutrition, physical activity, growth and cardiometabolic disease. TARGet Kids! also involves clinicians and parents as members of the research team.

The work of Dr. Li and her collaborators aim to improve primary health care for children and shed light on the current system in Canada so that the benefits can be used by other health care systems. The researchers hope to see their research improve the well-being of patients and their families. But in the long run, by improving primary care for children, there may also be great benefits to society as well. A healthy start in childhood increases the chance of a healthy, productive adulthood. Quality preventive and primary care also reduces the costs of health care for everyone, and so there are potentially several significant outcomes of this research.

Researcher Profile

Dr. Patricia Li
Montreal Children's Hospital, McGill University Health Centre
Department of Pediatrics, Faculty of Medicine, McGill University

Dr. Patricia Li completed her medical doctorate at McMaster University (Ontario, Canada), paediatrics residency at McGill University (Montreal, Quebec), and master's in epidemiology and health care research at the University of Toronto and the Institute for Clinical Evaluative Sciences (Ontario, Canada). A certified paediatrician, she is also assistant professor in the Department of Pediatrics, Faculty of Medicine at McGill University in Montreal. She is a clinician-scientist funded by a new investigator salary award from the Canadian Institutes of Health Research. Her research interests include the organization and delivery of health care services to children, specifically primary care and common childhood illnesses. Her work has been included in publications, such as the Archives of Paediatrics & Adolescent Medicine, Journal of Pediatrics, Cochrane Reviews, Canadian Family Physician and Surgery.

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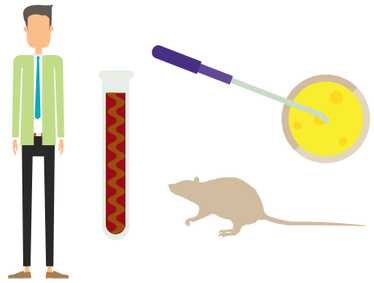
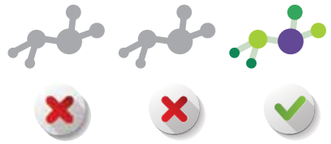
Canadian Institute of Health Research
Fonds de Recherche du Québec – Santé

FROM BEAKERS TO PATIENTS

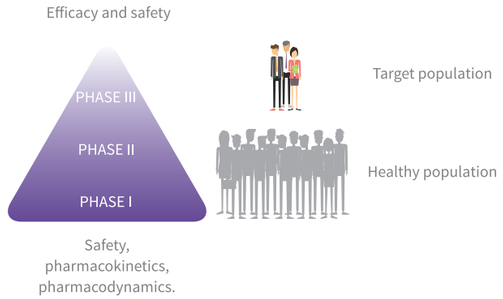
TARGET IDENTIFICATION:
drug target is identified in the patient OR animal/cell models of disease using various basic science research techniques.



PRECLINICAL STUDIES:
compounds, biologics, or therapeutic interventions are tested against the identified target



PHASE I, II AND III CLINICAL STUDIES: identified therapeutic compound or intervention is tested in healthy individuals and the target patient population to obtain safety data, drug response profile and examine efficacy.



CLINICIANS: the newly approved drug now must be marketed to clinicians for them to learn about the drug and make prescription decisions based on the therapeutic effects of the drug.

PATIENT RESPONSE AND CONTINUED MONITORING: once the drug is released into the market, safety and drug efficacy must be monitored for many years to firmly establish its positive and negative effects in the target population.

EMPLOYMENT AND DISABILITY

It has been reported that the majority of individuals with disabilities are capable of working and desire to continue working in the labour force. Although it is against the law for employers to discriminate against employees/potential employees because of disabilities, it is still common for individuals with disabilities to have more difficulty obtaining employment opportunities. In this section, Dr. Lisa Nishii will discuss the potential factors underlying these issues.

Many psychology studies have reported the positive effects of prosocial behaviours and the sense of control for psychological wellbeing. Enabling more individuals with disabilities to continue working may be essential for increasing their sense of control, improving their quality of life both financially and psychologically as a result. Furthermore, various socioeconomic benefits can result as more individuals with disabilities continue to contribute to the labour force. For instance, in many aging communities, such as the UK, Canada, and US, a large portion of the current skilled labour force will be retiring in the near future, posing a potential for labour force shortage in various fields, which would make the latter point particularly important. In this section, Professor Josephine Wilson will discuss his team's experience in working with individuals with disabilities and the online service that they provide in aiding individuals with disabilities in obtaining the appropriate training and assistance with finding employment.

Enhancing Employment Outcomes

Professor Josephine Wilson currently serves as the Director of the Substance Abuse Resources and Disability Issues Program. Here, she answers questions about her latest research project, which aims to make vocational rehabilitation available online to those with disabilities.

To start off, what inspired you to work with disabled persons? Do you focus your work on vocational rehabilitation (VR), or do you work with disabled persons in other capacities as well?

Even before we became SARDI—or the Substance Abuse Resources and Disability Issues Program—the observation from my staff who included rehabilitation counsellors was that there were no substance abuse and mental health treatment programs that were willing or able to treat individuals with disabilities in our geographical area. We began first by studying this issue and then opened the first clinical unit in our area to offer substance abuse and mental health treatment for individuals with disabilities. These groups included those who are deaf or have conditions like traumatic brain injury or developmental delays. Our work has focused on VR (including supportive employment), mental health and substance abuse treatment and recovery services, and online resources for individuals with disabilities.

This project requires that a number of counsellors participate. How did you select the counsellors you will be working with? Will they be communicating both with you and the other counsellors frequently throughout the course of this study? Beyond that, do you have other collaborators, and if so, has that complicated the project in any way?

We will be working with counsellors in two states who work for State VR offices. Our plan also includes recruiting counsellors who are with private VR firms. We will be allowing the state VR agencies to recruit the counsellors for us.

Apart from this, the procedure includes the production of a webinar series about online VR services. The goal of the webinar series is to provide continuing education credits to VR counsellors. The expectation at the end is to have these counsellors be interested in trying out our online services after viewing our webinars. We will use the same tactic with private VR agencies, which is to allow the administrators to recruit volunteers for us. Beyond the organizations that are supplying VR counsellors to our project, we have no outside collaborators on this field-initiated project. The state agencies are anxious to work with us, given cuts to their budgets, particularly in the area of counsellor travel.

Your research plan includes following up with patients for up to a year as they receive VR. Are you concerned about whether the patients will continue the treatment for the duration of the study, and do you expect a certain percent of patients to drop out?

The amount of time a VR consumer spends with the VR counsellor depends on a number of factors. We will continue to make our online services available to any consumer until they have been discharged from VR counselling services. If they receive VR counselling services for longer than one year, they will be dropped from our study (i.e., we'll stop collecting data on them), although online services will continue to be available for them.

Of course, we do expect a certain number of consumers to drop out, but most of them do remain because this is how they stay eligible for benefits/income. On the other hand, any consumer who asks to be returned to traditional (a.k.a. face-to-face) VR services will be transferred immediately – this is one piece of data we will be tracking.

Why did you choose Kentucky, Illinois and Ohio as the locations to conduct your research?

We chose these three states because we have worked successfully with them on other grant-funded VR projects related to assessment and supported employment. Since receiving the grant, Kentucky has asked not to participate because of another unrelated project that they have implemented. We've decided that, rather than enlist another state to work with us (and I am certain many states with large rural populations would have loved to join on), we might try out the feasibility of these services in a private VR setting.

Did you have any difficulty securing funding for this project?

It took us two tries to NIDRR (now NIDILRR) to land a grant. Our first proposal was too unrealistic and grandiose, as it involved too many states and too many consumers, but we pared our project down and received funding the second time around.

Online Job Services for the Disabled

The Substance Abuse Resources and Disability Issues Program (SARDI) recently received funding from the National Institute on Disability and Rehabilitation Research to conduct a research project that will provide online vocational rehabilitation (VR) services to persons with disabilities.

INCREASED ACCESS FOR PERSONS WITH DISABILITIES

Many persons with disabilities (PWDs) face numerous challenges when looking for employment. While many states have counselling services designed to help with training and the job search process, these services may not be easily accessible to those with severe disabilities. For some, lack of mobility or transportation makes it physically impossible for them to travel to a counselling centre. For others, their access to counselling services may be limited due to cultural and social problems, such as a reduced ability to communicate. Professor Josephine Wilson works to alleviate this problem by developing online programs for PWDs who need assistance finding jobs.

Wilson's online platform can provide nearly all the necessary services to those who need them, with the exception of a few tasks that must be performed on-site, such as on-the-job training or fittings for assistive devices. Consumers using the online platform will be able to access pre-employment assessments, career exploration advice, job-seeking skills training, job search assistance, guidance and counselling, vocational training, assistive technology, job placement assistance and benefits planning. Post-employment VR consumers have access to supported employment services, job coaching and support and transport services. The online programs will be evaluated to determine in three ways: ease and effectiveness of use, contribution to the improvement of employment outcomes and satisfaction with the service for both VR counsellors and consumers.

By creating an Individualized Plan for Employment (IPE) together with the counsellors, the online services cater to an individual's needs. The skills, work history and natural ability of consumers will help counsellors determine the specific course of action that will benefit a particular customer. The online platform is comprised of web portals, which are personal web pages that can be accessed using a unique username and password. Each web portal has buttons which direct the user to online counselling sessions, homework assignments, online job listings, VR assessment tools or social networking sites. Counsellors can assign consumers to complete certain tasks using the portals.

The observation…was that there were no substance abuse and mental health treatment programs that were willing or able to treat individuals with disabilities in our geographical area. We began first by studying this issue and then opened the first clinical unit in our area to offer substance abuse and mental health treatment for individuals with disabilities.

Some of these assignments may include reviewing web pages that will help them pick the right career through links to articles, websites and videos. They can gain even more information about potential jobs through informational interviews and job shadowing, which are also available online. Counsellors may also request that the consumer complete the O*Net Online Skills Search Assessment so that they can discuss the results together. After reviewing the resource materials available, the counsellor and consumer may discuss a plan of action so that the consumer can continue career exploration on their own. The counselling sessions will also focus on educating the consumer about what to expect during their career exploration. This may eliminate the cost of authorizing career exploration services or reduce the number of hours for the job search process. Increasing consumer independence is

one of the major goals of this project, since the online platform allows them to be more active in their own vocational rehabilitation, be more accountable for their job search outcomes, and have a better understanding of the services that are available to them.

SUPPORT FOR COUNSELLORS, TOO

Another major goal of this project is to train VR counsellors about the potential benefits and proper use of online tools for vocational rehabilitation. The online platform benefits counsellors by allowing them quick and easy access to consumers via video conferencing technology. This will increase face-to-face time with consumers and strengthen the counsellor-consumer relationship. The use of these online services will also reduce the need for counsellors to travel to meet with their consumers. By eliminating unnecessary travel time, they will be able to spend more time working directly with consumers. The online service also benefits counsellors by giving them access to a 24-hour call centre with trained IT support staff to help them address a particular consumer's needs. This is beneficial for counsellors who are not included in the original online content.

ANALYSING EFFICACY

To compare the effectiveness of the newly-developed online VR program with traditional on-site VR, Wilson will conduct a randomised controlled trial. There will be a total of 540 consumers in the study, of the sample, 270 patients will use the online services and 270 patients will use traditional VR services. It should be noted that consumers who are assigned to traditional VR services will likely utilise online services as well. The extent to which each individual uses online resources will be tracked and taken into account during analysis. Those assigned to the online service will receive all services online. It is also important to note that the same counsellors will provide online and face-to-face counselling so that differences among counsellors are accounted for. Comparing online to off-site services allows the researchers to evaluate the process as well as the outcomes for each method. Data will be collected at three, six, nine and twelve months after VR is initiated in both groups. Employment outcome will be determined by consumer retention in VR services, the number of hours of training completed, training completion rates, university enrolment, community college enrolment, hours of employment completed per week, duration of employment and wages received.

The researchers will compare the success and cost-effectiveness of the programs, and assess whether these online programs are feasible, easy-to-use for consumers and easy-to-adopt for VR counsellors. The number of minutes counsellors need technical assistance will also be considered when deciding whether an online program is more time-efficient. Throughout the course of this project, researchers also aim to ensure that the services are optimised for any platform, including computers, smartphones and tablets. The effectiveness of the programs will be evaluated using variables such as the number and types of VR services received, the number and types of accommodations required, the total cost of services and employment outcomes. Consumer and counsellor attitudes toward the online program will also be surveyed and reviewed, since it is hypothesised that users will regard it favourably.

Ultimately, the researchers at SARDI hope to enhance employment outcomes for many individuals. The ability to use these services anywhere, at any time and using any device is critical. Ease of access to these resources will likely significantly speed up the VR process to the benefit of both counsellor and consumer. An efficient process for PWDs and their counsellors means that more work will be accomplished, and increased productivity has potential socioeconomic benefits.

Researcher Profile

Professor Josephine Wilson
Substance Abuse Resources and Disability Issues Program
Boonshoft School of Medicine at Wright State University

Professor Josephine Wilson' began studying psychology at SUNY Fredonia. Later on, she received her Ph.D. in Psychology at Columbia University. She also completed training as a dentist at SUNY Buffalo. Currently, she teaches community health at the Boonshoft School of Medicine at Wright State University. Wilson has received numerous awards, including the Omicron Delta Kappa Distinguished Teaching Award for New Faculty, the Wittenberg University Alumni Association Distinguished Teaching Award, and the Outstanding Achievement Award from SUNY Fredonia. She serves as a reviewer for National Science Foundation (NSF) and National Institute on Disability, Independent Living, and Rehabilitation Research (NIDILRR) grants and is the faculty representative for Wright State University on the Federal Demonstration Partnership.

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Boonshoft School of Medicine
WRIGHT STATE UNIVERSITY

Improving the working lives of people with disabilities

Dr. Lisa Nishii studies and conducts research that is focused on how to improve diversity and inclusion in the workplace. She has conducted multiple studies that focus on workplace outcomes for persons with disabilities (PWDs).

To begin, what attracted you to researching disability issues?

The Cornell University School of Industrial and Labor Relations (ILR) has a long history of working with organisations, government agencies and communities to enhance the employment outcomes of individuals with disabilities. About 10 years ago, I was approached by researchers working at the Employment and Disability Institute about collaborating on a research project. I had already begun my research on diversity and inclusion but, like many other diversity researchers, had focused primarily on issues related to race and gender in organisations. I knew nothing about disabilities and was interested in expanding my lens.

At the time (and this is still largely true), much of the published research focused on the individual employment experiences of people with disabilities working across a wide variety of organisations. What we planned to do together was somewhat different – we wanted to do a "deep dive" into organisations and collect data not just from individuals with disabilities, but also their co-workers, managers and senior leaders so that we could develop a more nuanced understanding of how the work experiences of individuals with disabilities are impacted by the social context and relationships at work.

Were there any major roadblocks to gaining funding for this project?

People might be surprised to learn that social science research can be expensive. We would not have been able to conduct our ongoing research projects without the generous funding from the U.S. Department of Health and Human Services.

Were there any other complications you experienced while conducting your research?

Unfortunately, we often face complications while conducting research. For instance, although we always assure respondents of the confidentiality of their responses, we usually prefer to be able to track survey responses to particular individuals. However, in one of our studies, we ended up needing to conduct an anonymous survey in which we could not identify individual respondents. We prefer to be able to identify respondents not because we care which individuals have said what, but because this is the only way we can then link survey data to other data sources once we start analysis. By having to make the survey anonymous (based on our partner organisation's requests), we lost our ability to link employee responses to those of their specific supervisors as well as with HR data, which was unfortunate.

Another common complication that we face relates to survey response rates. Low response rates can bring a number of limitations to the research. It becomes difficult to know whether the respondents who have completed the survey are representative of the broader workforce. In addition, survey response rates also dictate the type of analyses that are possible. In the case of one of our studies which involved social network analyses, we were unable to construct complete models of the social networks within the organisation due to low response rates. Instead, we focused our analyses on egocentric networks or people's reports of their own social networks.

This means that respondents answered questions about the individuals at work they consider to be friends, key sources of information and/or mentors, as well as the demographic background of these individuals. They also shared information about the quality of their relationships with each of these individuals. Although we couldn't tell from this egocentric network data which individuals were most central in the social networks of the organisation, we were still able to learn how people's relationships at work are affected by the inclusiveness of their work environment. Individuals working in inclusive groups not only have more demographically dissimilar individuals in their networks, but they also have fewer negative interactions with dissimilar others. Being able to show this in our social network data is really important because it is these negative interactions that are particularly harmful when it comes to people's satisfaction and engagement at work. When, in diverse work contexts (which almost all are nowadays), managers fail to foster inclusive norms, people tend to experience more conflict particularly with dissimilar others. This conflict drives down the engagement and performance of groups and increases turnover.

You used a complicated and many-faceted study design in order to adequately assess what is a complex topic. Was the planning and preparation for this project more arduous than for others you have done in the past?

This type of study design is the norm for the kind of research that I do in which I link information about the adoption of specific organisational practices and the social context of work (i.e. workgroup climate, group norms, leadership, etc.) with employee experiences and employment outcomes. These research models necessarily involve data collected from multiple sources: HR information systems, organisational leaders, HR representatives, line managers and employees, often at multiple points in time.

Sophisticated Research Method Provides New Insights

The Employment and Disability Institute of Cornell asked Dr. Lisa Nishii to use her training as an organisational psychologist to research ways to improve the workplace experience of individuals with disabilities.

INEQUALITY IN THE WORKPLACE

Managing workplace relationships is a daunting task for many people. For persons with disabilities (PWDs), this task is even harder. PWDs are chronically un-employed and under-employed. According to the U.S. Census Bureau- In the year 2012, an estimated 34% of people with disabilities were employed, compared to 72% of people without disabilities. PWDs who are employed earn, on average, 72 cents to the dollar, and face higher risks of being fired. They are also less likely to be given job-related training. The Americans with Disabilities Act was passed in 1990 to provide equitable access to the workplace for people with disabilities. Despite this, many managers do not understand the policies and practices in place for helping employees who have disabilities. Some disabilities are not readily apparent, and this often leaves PWDs in the position of deciding whether or not to disclose that information to an employer. They worry that disclosure will cause others to expect less of them, not take them seriously or isolate them from social networks in the workplace. Some may simply not disclose because their disabilities and their tasks at work are often unrelated. Although not disclosing might protect individuals with disabilities from being discriminated against due to their disabilities, not disclosing can come at a cost, in particular the emotional toll of constantly and consistently hiding their disabilities. The work of Dr. Nishii provides new information that can address the many factors that complicate the lives of so many.

A NEW ERA OF DISABILITY RESEARCH

One challenge faced by researchers who study the workplace relationships of PWDs is the current body of research that is available. Most previous research conducted on this topic consisted of small-scale studies that primarily used other people's perceptions of PWD instead of PWDs' own experiences in the workplace. There are several reasons for this. First, employees who do not report their disability status may be excluded from the research. Since disability status can change over time with the aging process or progressive conditions, people don't necessarily see themselves as being a person with a disability and also disability status can change from year to year. Lastly, the vast diversity of disabilities should be included in research for accurate representation in addressing the issues of workplace equality.

What we planned to do together was somewhat different – we wanted to do a "deep dive" into organisations and collect data not just from individuals with disabilities, but also their co-workers, managers and senior leaders so that we could develop a more nuanced understanding of how the work experiences of individuals with disabilities are impacted by the social context and relationships at work.

Organisations that work to improve diversity and inclusion in professional environments have long focused on race and gender, and only now are they turning their attention to PWDs. This has led to new opportunities for researchers like Nishii. Nishii addresses this dearth of information by conducting multi-level studies, a type of study design with which she is very familiar and has used frequently in the past. Multi-level analyses are capable of elucidating the relationships among higher-level constructs and lower-level constructs. In this case, high-level constructs include managerial behaviour or workgroup climate, and low-level constructs include the individual-level experiences of employees working in these workgroups. These research methods are complex, but are becoming more common with the development of increasingly sophisticated statistical software packages that can accommodate the computational requirements involved. Using this method, Nishii was able to examine the individual relationships

between different people and workgroups in an organisation and how those relationships are affected by workplace practices and culture, as well as how those relationships affect outcomes for individual employees and the workplace as a whole.

FINDING THE ANSWER

In a study for the Employer Practices Related to Individuals with Disabilities Rehabilitation Research and Training Center (see www.employerpracticesrrtc.org), Nishii and her colleagues conducted interviews with senior managers in various HR departments as well as with managers and supervisors. These interviews gave her insight into the barriers to success that senior managers feel those with disabilities face. In addition, she conducted focus groups with those with disabilities about their workplace experiences in order to determine what barriers they thought were detrimental to their success. Researchers sought opinions on how well their organisation recruits, engages, and retains employees with disabilities, and what other complications

they faced on a daily basis. They also obtained information from employees without disabilities who work closely with those with disabilities. Nishii found that co-workers' reaction to requesting accommodations was a large factor in how inclusive employees with disabilities perceive their work environments to be. Separate focus groups were also conducted to identify how supervisor attitudes can facilitate or create barriers to effective accommodations for people with disabilities.

Using the information obtained from interviews and focus groups, Nishii then developed a survey. Examining the results of individual-level employee experiences, Nishii found that, overall, employees with disabilities report less favourable experiences at work: for example, in terms of being treated fairly, feeling supported, having a high quality relationship with one's supervisor, receiving adequate performance feedback, and perceiving opportunities for advancement. The survey also revealed that only 58.6 per cent of employees with disabilities shared their disability status with supervisors. Rates of disclosure to HR departments are even lower at a mere 12.2 per cent. Employees with emotional and mental disabilities are the least likely to disclose that information, with only 38.5 percent disclosure to supervisors. Individual-level responses also provided information as to when and why accommodations are requested, and more importantly, when and why they are not requested. Those with physical and vision impairments are the most likely to request accommodations, with slightly fewer requests being made by those with chronic health, mental and emotional, cognitive and hearing disabilities. The accommodations requested most frequently were changes related to work tasks and job structure or schedule. Other requests included physical changes to the workplace, new or modified equipment and changes in policy or communication practices. Few employees with disabilities did not request an accommodation that they needed, out of fear that it might affect their future opportunities or change their manager's opinion of them.

The experiences of PWDs, as well as their willingness to disclose their disability status, vary significantly depending on their immediate work environment. The most important contributions from Nishii's research involve insights about the factors that predict the quality of PWD's work experiences. Surprisingly, nearly three-quarters of managers are not aware of the disability policies and practices in place within their organisations, and yet this awareness is critical. The more managers are aware of their organisation's disability practices and think that they are effective, and the more they perceive that senior leaders are committed to disability issues, the better they are at fostering work environments that are inclusive of PWDs. Their data show that managers who believe in the strategic benefits of disability initiatives implement disability practices in ways that promote better outcomes for PWDs than managers who think that disability initiatives are adopted purely for legal compliance purposes. These better outcomes include having a positive experience when requesting an accommodation, feeling more comfortable about disclosing one's disability, and being treated well and fairly by co-workers even after disclosing one's disability.

Nishii also finds that PWDs who have a good working relationship with their supervisor, or have a mentor within the organisation, experience less disability-related bias within their work groups. Also, to the extent that PWDs have what they need to be effective in their jobs, the less bias they experience. PWDs whose skills are well matched to their job, receive adequate socialisation when they first enter a job, and receive adequate performance feedback feel less socially isolated because of their disability. These important, novel contributions to the literature will enhance future research on the work experiences of persons with disabilities as well as vastly improve the work lives of many people.

Researcher Profile

Dr. Lisa Nishii
Human Resources Studies Department
Cornell University School of Industrial and Labor Relations

Dr. Lisa Nishii was educated at Wellesley College and received her Ph.D. from the University of Maryland. Currently, she teaches in the Human Resources Studies department at Cornell University. It is here that she also pursues her research interest in the field of organisational diversity and inclusion, employee perceptions of organisational practices, and cross-cultural/international issues within organisations. Nishii received the Best Dissertation Award from the Society for Industrial and Organisational Psychology and five "best paper" awards for papers that appeared in Science, the Journal of Applied Psychology, and Personnel Psychology. She also serves on the editorial boards of five different psychological and organisational research journals, and is the incoming Chair of the Gender and Diversity in Organisations Division of the Academy of Management.

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Cornell University ILR School

RATIO BIAS

What is bias? Bias is the tendency to favor one side to another that is not based on “good judgement”, or rational reasoning. Bias is an important component of understanding decision making processes and making predictions of human behavior. Indeed, study of bias is used in many fields including medicine, biotechnology, economics, and psychology. There are many different types of biases, including conformational bias, which is one’s tendency to choose whichever side that confirms his or her preconceptions. This is why it is necessary to “blind” all researchers of the treatment conditions the patients are assigned to in order for the drug trial to produce reliable results. Another example is the mere exposure effect, where we tend to prefer options that we have been exposed to more frequently. This is one of the main drivers of advertisements for different drugs or therapies; patients may ask their physician for a certain drug simply because they have seen it multiple times in different advertisements. While bias may be a non-quantitative concept that can be used to make qualitative assumptions, several researchers have attempted to create mathematical algorithms to make accurate predictions of bias. These studies can be highly useful in correcting for biases in how people process and/or interpret information. In the following piece, Dr. Yamagishi’s research in identifying and utilizing a mathematical formula to calculate people’s tendency to erroneously prefer one representation of a ratio over another is discussed. In examining how individuals interpret ratios, we can gain a better understanding of how people might correctly or incorrectly make judgements based on quantitative data.

In Search of Logical Bases beneath **the Superficially Irrational**

Dr. Kimihiko Yamagishi studies the way in which people incorrectly interpret statistical information. Here he discusses the concept of ratio bias and how it relates to a new psychophysical model he has developed which accurately predicts irrational behaviour when analysing statistical information.

To begin, what is your research background and what attracted you to studying ratio bias?

My interest in people’s handling of statistical information dates back to high school. In high-school mathematics, my favorite subject was probabilities (I’ll admit, I was an oddball). Learning probabilities and statistics made me aware that well-educated people casually make statistical mistakes. In one example, I was talking to an architect with a Ph.D. and a faculty position at a university about the test-taking tactics of students taking the TOEFL. He remarked, if a test-taker has no clue as to which is the correct answer out of 5 choices, fill out the bubble such that the test-taker’s choices would make a zigzag line over the series of answer columns. His point was that this is the optimal answer tactic in the case of ignorance, because the correct answer columns in the TOEFL test are distributed randomly. I thought, “His point is not wrong, but inadequate. Assuming that the correct bubbles appear in the random positions, deciding upon any row, left-end, right-end, or middle, wherever, and filling all the bubbles in that row should suffice. His zigzag tactic requires some extra effort to make a zigzag line, involving wasteful cost.” Thus as a high-school student, I made an early preparation for the study of statistical intuitions by observing that mistakes may occur.

Your work unifies ratio bias literature by introducing a simple formula to explain a widely reported phenomenon. Considering you drew from other areas of study within psychology when formulating your theory, are there other areas of psychology to which it could be similarly applied? If so, what areas?

Social psychology comes to my mind as the quickest example. When I presented this idea at a conference, I had a good conversation with a social psychologist who worked on self-esteem. We talked about how the kind of thinking which results in ratio bias would differ when applied to different types of personalities. Consider a person who took a standardized test and received a score in the 70th percentile. A high self-esteem individual would frame the result as “I am ahead of 70% of people” whereas a low self-esteem person would think, “I am worse off than 30% of people.” Now what would happen if the total number of test takers were 100 or 10,000? A high self-esteem person would experience elation in the 10,000 case, knowing their score was better than 7,000 test takers rather than 70. In contrast, a low self-esteem person would experience depression in the 10,000 case, realizing that s/he is behind 3,000 competitors rather than merely 30.

Your previous research suggested that base rate neglect resulted in ratio bias. What are the primary differences between base rate neglect and psychophysical phenomenon? Are they mutually exclusive?

I do not necessarily regard the “base rate neglect” and the psychophysical formulation as mutually exclusive. Rather, I would like to characterize the Dual Weber-Fechner theory as a process articulation of the “base-rate neglect” or “insensitivity to base rates.” The literature uses the expressions in the quotation without describing the cognitive processes behind the phenomena. Thus I am happy to characterize that the common expressions, “base-rate neglect” or “insensitivity to base rates,” as labels attached to the observable phenomena, and the Dual Weber-Fechner theory as a possible description of psychological processes that produce the observed phenomena.

Clearly, ratio bias is an important concept for anyone working to educate the public. Are there industries you would like to see apply this knowledge?

I would welcome positive contributions of the notion of ratio bias studies in risk education. Since the 2011 Tohoku Earthquake and Tsunami incident, Japanese researchers, politicians, activists and such are keenly aware of the relevance of risk education.

The Power of Small Modifications

Ratio bias is an oft-cited but little understood psychological phenomenon. Dr. Kimihiko Yamagishi of the Tokyo Institute of Technology modifies a previously-existing psychophysical model and is now able to predict irrational statistical reasoning in cases of ratio bias.

A COGNITIVE MYSTERY EXPLAINED

Logically inconsistent behavior in humans has always been recorded and studied by psychologists. Humans are clearly logical creatures; developmental psychology demonstrates that infants continually try to identify the logical relationships between every object they encounter. However, humans consistently use irrational decision-making processes, and the work of Dr. Kimihiko Yamagishi attempts to define when and how micro-logical thinking processes go awry in the context of the human logical system as a whole.

Understanding the way in which humans assess risk and make decisions has many implications in the psychological field. Ratio bias is frequently demonstrated in the literature, but little research had been conducted to develop a model that would explain the phenomenon. Yamagishi's ability to predict intuitive and irrational risk assessment calculations deepens the scientific community's understanding of the thought processes which result in ratio bias.

I realized that tweaking a textbook psychological notion, the Weber-Fechner Law, could well explain the base-rate neglect phenomena after minor modifications.

UNDERSTANDING RATIO BIAS

Ratio bias has long perplexed psychological researchers. This phenomenon occurs when comparing ratios and statistical information. In an often-cited study conducted by Yamagishi in 1997, he presents two different experiments in order to demonstrate ratio bias. In the first experiment, he presented test subjects with eleven well known causes of death. Subjects were also presented with an estimation of yearly deaths for each cause in the form of a ratio. The estimates were the result of a previous study in which test subjects were asked to guess the number of deaths due to each cause listed based on their intuition. There were four types of ratios: small numerators over a narrow range (denominator), large numbers over a narrow range, large numbers over a wide range, and small numbers over a wide range. Each participant viewed only one type of ratio, and the type of ratio received was randomized across test subjects. Participants were then asked to rate the riskiness of that particular cause of death on a scale of zero to 25 where zero represented no risk and 25 was the maximum amount of risk. The results indicated that larger numbers in the denominator, no matter what percentage is represented, are judged as riskier. Large and small numbers over wide ranges were consistently given higher ratings than small and large numbers over narrow ranges.

In the second experiment, the design was essentially the same as the first, but used only ratios with small numbers over a wide range and large numbers over a narrow range. This design was used so that subjects were only presented with small percentages represented with large numbers or large percentages represented with small numbers. For example, one group of individuals were asked to assess the phrase "cancer kills 1,286 out of 10,000 people" while other participants assessed the phrase "cancer kills 24.14 out of 100". The first statement describes a percentage of 12.86% while the second statement denotes a 24.14% chance of developing cancer. The results corroborated the results of the first experiment; test subjects rated the first phrase representing a 12.86% chance as riskier than the second phrase representing a 24.14% chance.

At the time, Yamagishi attributed this inability to accurately compare ratios as a case of "base-rate neglect", or a tendency to emphasize certain information and ignore equally important facts when making judgments. For example, in this experiment, subjects only consider the magnitudes of the numerators and ignore its relation to the magnitude of the denominators. While base-rate neglect is still relevant to the study of ratio bias, in 2007, Yamagishi was able to develop a new model to accurately predict ratio bias. This model describes the psychological process used in decision making.

A NEW PSYCHOPHYSICAL MODEL

The Weber-Fechner Law, a classic principle in psychology, states that changes perceived in subjectively-experienced stimuli are proportional to the stimulus magnitude. For instance, the addition of one-kilogram to a person holding a five-kilogram weight will feel the same to a person who is holding a ten-kilogram weight and adds two kilograms. The Weber-Fechner Law also applies to changes in noise, light, or any time human perception inaccurately calculates the magnitude of a stimulus. Mathematically, the calculation consists of taking the logarithm of the ratio of the perceived intensity over the actual, measured intensity of the phenomena.

The Dual Weber-Fechner Theory developed by Yamagishi modifies the formula to calculate the psychological magnitude separately for both the numerator and the denominator. With this new model, he accurately predicted the behavior of the study subjects in his work produced ten years before in 1997. The Weber-Fechner Law, applied to Yamagishi's previous research, would be calculated as log(1,286/10,000), which results in an answer of 0.8, and log(24.14/100), which results in an answer of 0.9. A larger number denotes the ratio most psychologically significant in the human mind, so the Weber-Fechner Law does not account for this irrational decision-making. Now, with the Dual Weber-Fechner Theory, the calculations become log(1,286)/log(10,000) and log(24.14)/log(100). These calculations give answers of 0.78 and 0.64, respectively, and now account for the odd behavior displayed by test subjects.

TESTING THE THEORY

When asked if a meta-analysis across ratio bias literature would be an appropriate way to test his theory, Yamagishi says: "Having to test all the existing cases of ratio bias phenomena seems like a form of "Probatio diabolica," or asking for the virtually impossible", as the cases of ratio bias in the literature are so extensive. However, the Dual Weber-Fechner theory accurately describes the outcomes of key studies and influential research conducted by others. Yamagishi applied the Dual Weber-Fechner theory to a study published in 1989 by researchers Miller, McFarland and Turnbill in which participants were asked to rate the chances of winning a game in which the odds were either 2/20 or 20/200. While these ratios have the same probability, analysis by the Dual Weber-Fechner theory results in the calculations log(2)/log(20) and log(20)/log(200). These calculations give answers of 0.167 and 0.565, respectively, explaining how participants would find odds of 20/200 more attractive.

In another well-known demonstration conducted in 1994 by Denes-Raj and Epstein, test subjects were offered a choice between the odds "1 in 10" and "9 out of 100", where the odds represented the chances of winning a cash award. Participants irrationally chose the phrase "9 out of 100" as being more likely to result in winning the money than the phrase "1 in 10". The Dual Weber-Fechner theory explains this result as well. In this case, the psychophysical function log(Q+0.01) must be applied in order to account for the fact that log(1)=0, and would thus strip the calculation of its usefulness. The odds "1 in 10" would therefore be calculated as log(1.01)/log(10.01), resulting in an answer of 0.004. The odds "9 in 100" are calculated as log(9.01)/log(100.01) and give an answer of 0.477. The Dual Weber-Fechner theory, again, accounts for the irrational choice of preferring a 9% chance of winning money to a 10% chance. Yamagishi's work and its ability to explain multiple cases of ratio bias in the literature demonstrates the amazing explanatory power of this simple model.

Researcher Profile

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Dr Kimihiko Yamagishi received his Ph.D. from the University of Washington. He has been recognized by the Japan Psychological Association with the Distinguished Paper Award in 2003 and 2006, and received the Special Award at the 2012 Annual Conference of the Japan Cognitive Science Society. Dr Yamagishi aims to understand how humans make decisions and when and how human perception does not reflect reality. He has published papers in the areas of risk perception, decision making, and probability judgement, and primarily uses controlled experimentation, surveys, and statistical and mathematical modelling to conduct his research.

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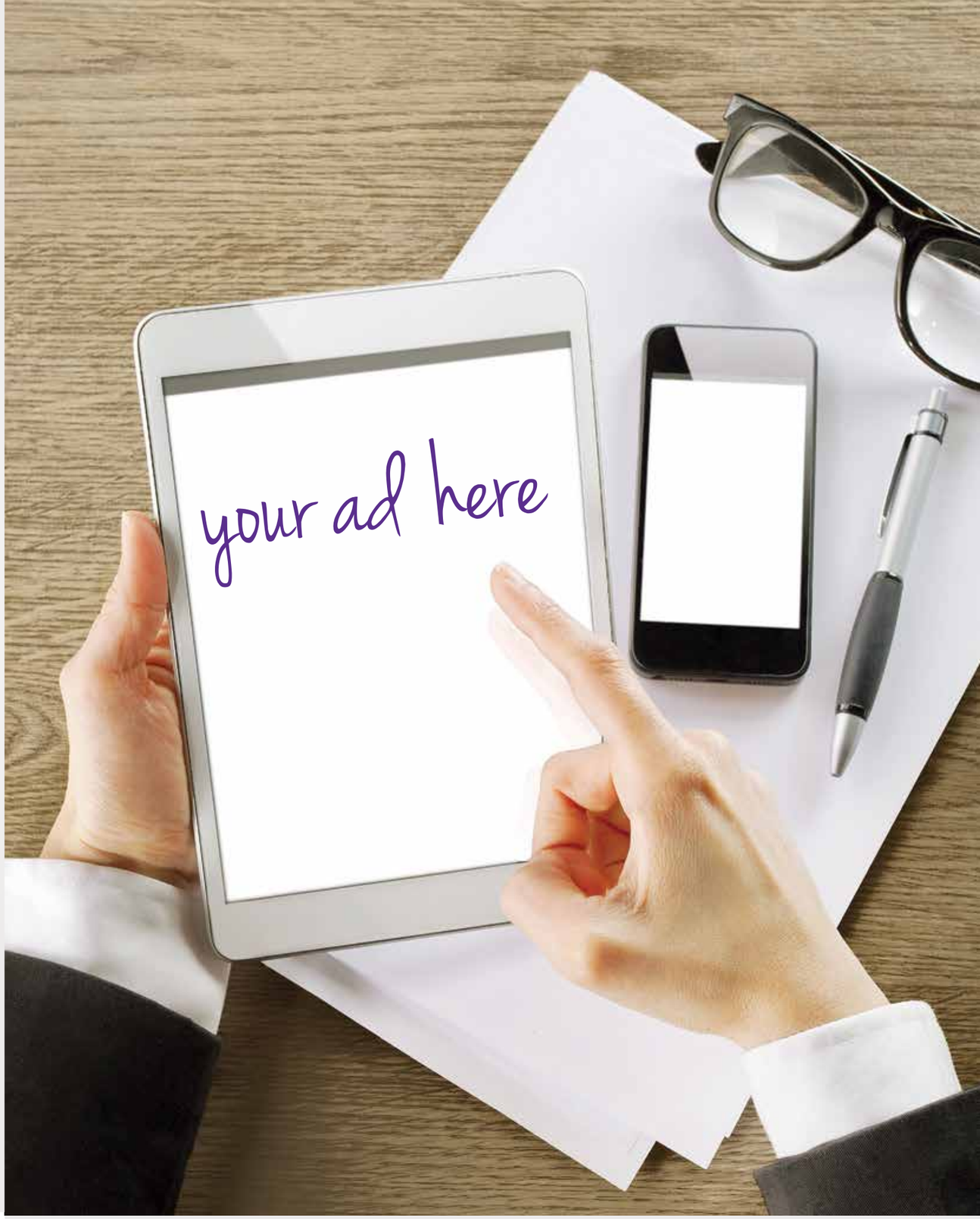

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