# Research Review 2017

Research For Life

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# Research Review

2017

ISSN 1175-1053

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# Editorial

## Predatory publications: The new plague

We are all used to receiving spam e-mails. Many of these are filtered by firewalls maintained by institutional information technology systems, although a substantial number slip through. Prominent amongst this group are invitations to submit articles or join the editorial board of little-known journals. These journals have a number of features in common. They are generally in establishment or have been published in a few volumes only. They are open access and require a fee for publication, while the manuscripts are of quality and content that would always struggle to gain acceptance in mainstream established journals. Revealingly many of the e-mails soliciting manuscript submission are poorly written and yet, despite this limited command of the English language, purport to come from addresses in the United States or the United Kingdom. These invitations are usually based upon a request to write a follow-up article to one already published in a reputable journal. The requests frequently specify an impossible time-frame for submission – although one such journal did attempt to mollify this with the observation that "only a few pages are required which should not be a problem for an eminent researcher like yourself". Even more disturbing is the promised turnaround time which, including the full peer review process, can be as short as one week from submission to publication.

A number of studies have attempted to provide guidelines to permit identification of what have now become known as predatory open access publications or pseudo-journals. Until recently suspected predatory publishers/journals were listed on-line in a catalogue compiled by Jeffrey Beall, an academic librarian at the University of Colorado. The list has been cited by various authors and in the most recent accession was reported to contain 1113 journals (as at 5 October 2016). Unfortunately this list ceased to be accessible from January 2017 and it has been claimed that this was in response to the threat of 1 billion dollar lawsuit. Other investigators have indicated that the number for suspect journals may be as high as 11,873.

Beall defined 25 criteria to identify journals which are predatory in their behaviour and other authors have refined these criteria and added others. Of these the most important features that permit identification of these journals are that they are open access and charge a fee for publication. In the main the fees are not advertised up front and are usually claimed following submission of the article. Open access is a valuable and growing component of publishing; however, these fees are levelled by mainstream journals to cover legitimate costs. These costs do not apply to predatory journals which focus upon profit rather than quality. Interestingly the charges levied by predatory journals are usually less than the open access charges of legitimate journals and it is likely that this is a reflection of their largest market, which is predominantly developing countries with limited resources. Further identifying features of predatory

open access journals are a lack of scientific rigor in the review process, editorial boards populated by researchers unknown in the field, published manuscripts of poor quality, excessive use of spamming e-mails to solicit manuscripts, lack of standard authors and publisher guidelines, and publishers and/or editorial manager's addresses that are suspect. This latter feature can be difficult to detect as many journals appear to be based in the United States and the United Kingdom. In reality many of predatory open access journals are "published" from Asia or Africa and investigations have shown that, in some instances, the cited addresses are highways or vacant lots. If there is any doubt reference to Google Maps should provide evidence of authenticity (or otherwise).

Legitimate journals are ranked according to a variety of metrics with Web of Knowledge Impact Factor being the most widely employed. The lack of an impact factor or indexing of a journal by mainstream indexing organisations such as Scopus, Medline, PubMed and Web of Knowledge should be a cause for concern. Some predatory journals do quote impact factors and the credibility of these data require careful investigation. A journal currently publishing its second volume claims an impact factor which is clearly not possible as an impact factor is derived by quantifying the number of citations received in the third year from manuscripts publishing in the two preceding years. As a consequence an impact factor cannot be established until the fourth year of publication. It is recognised that a number of organisations are prepared to certify spurious impact factors and as such the source of the data should be carefully determined. A further disturbing trend that appears to give the veneer of legitimacy to pseudo-journals is the utilisation by publishers of titles that are no longer published. In some cases this has not been recognised and (albeit lower) impact factors continue to be assigned to these previously higher ranked journals. It has also been noted that impact factors of predatory journals may be increased by auto-citation or cross-citation by other journals within the publishers stable of titles.

Several studies have been undertaken that emphasise the misconduct associated with predatory journals and the most celebrated of these is that of Bohannon. In 2013 Bohannon authored a series of similar manuscripts relating to the anticancer properties of a chemical extract of lichen. The manuscripts contained both methodological and factual errors and as Bohannon later noted, were so hopelessly flawed that the results were meaningless. Over a ten month period the manuscripts were submitted to 304 open access journals at a rate of 10 per week. The manuscript was accepted by 157 journals, rejected by 98, while the remainder either failed to respond or in follow-up correspondence noted that the paper was still under review.

The study of Bohannon is not the only study to question the refereeing process. A group of Serbian authors submitted a clearly sub-standard manuscript to a suspect journal. The manuscript was accepted 2 hours and 40 minutes later without amendment and the acceptance was accompanied by a poorly worded invoice

that stated publication was contingent upon receipt of payment. In 2005 Stribling submitted a computer generated manuscript to the 9th World Multi-Conference of Systemics, Cybernetics and Informatics. Described by the author as totally spurious, the manuscript was accepted. In response the conference organisers noted that they did not receive reviews for some papers, but out of fairness accepted them!

While these three investigations clearly highlight deficiencies in the reviewing processes of suspect journals and conferences, the study by Pisanski et al. focussed upon the peer reviewers themselves. Utilising a fabricated curriculum vitae with fake degrees and containing published book chapters but no referred journal articles, they applied for appointment to the editorial board of 360 journals. The selected journals were suspected predatory journals, open access journals or journals indexed by Journal Citation Reports (JCR). Of the 120 suspected predatory journals contacted, 40 made appointments to their editorial board, with four as Editor-in-Chief. Pleasingly all JCR indexed journals rejected the application. The authors of the study reported an associated disturbing trend with some of the predatory journals offered a profit-sharing arrangement for recruitment of authors, the ability to host a conference – again on a profit sharing basis, or the invitation to establish a new journal as Editor.

Should we be concerned about the proliferation of pseudo-journals? These requests, which are designed to flatter and may cause amusement, are in reality a dangerous development that could impact on the credibility of science. Not only are these journals and the avalanche of spam they generate a major waste of time for the academic community, they represent an attempt to facilitate fraud. This is particularly important for the scientists from lower decile countries who would normally have difficulty in having manuscripts accepted by mainstream journals, as, contrary to expectations, publication of work in predatory open access journals will not provide evidence of scientific credibility. Beyond this they also have the effect of perpetuating scientific misinformation as non/poorly reviewed articles could be cited as legitimate findings. For many of the journals publication changes are not advertised and are levied once a manuscript has been accepted. If a journal requires transmission of copyright at the time of submission (as is usually the case), it becomes the property of the journal. It has been reported that once authors have become aware of the true nature of the journal that accepted their manuscript, requests for retraction were made and rejected. Once accepted there can be no certainty that even legitimate studies will remain on-line once published. Another disturbing feature of some predatory journals is that they add academics to editorial boards without permission and this underscores the need for constant vigilance.

The most effective way of countering the influence of pseudo-journals is the knowledge of their existence and an understanding of their practices. This is especially important for novice researchers as it has been shown that 77% of new researchers

were ignorant of the existence of predatory journals. This problem is compounded by the observation that some journals have titles similar to those of respected mainstream publications.

It has been recommended that ethics committees should produce guidelines to distinguish between legitimate and predatory journals. It could also be suggested that a declaration as to the choice of journal to which any resulting manuscript would be submitted, should be part of the ethical approval process.

**Professor Brett Delahunt**
**Editor**

Accepted for publication in PATHOLOGY Vol. 49 No. 7 (December 2017). Reproduced with permission of the publisher

## Research Advisory Committee Membership

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Dr Peter Bethwaite
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Associate Professor Peter Larsen
Professor Graham Le Gros
Professor John H Miller
Dr Robert Weinkove

# Reports of research work funded by grants prior to the current year

## Institute of Environmental Science & Research Ltd

### Dissecting the New Zealand meningococcal B epidemic using whole genome sequencing

**X Ren**
**Invasive Pathogens Laboratory**

In this study we set out to use whole genome sequencing to understand the emergence and adaptation of the New Zealand serogroup B epidemic meningococci strain (NZMenB). The aim of пthe study was to expand on our previous studies to sequence more isolates from key periods during the epidemic, to understand emergence and possible vaccine adaptation. This project started in late October 2015, so far, we spent most of our time selecting, extracting DNA and sequencing meningococci isolates. We have finished the first batch of whole genome sequencing and are in the process of analysing the data generated to see if more targeted sequencing are needed.

Thanks to funding from the Research for Life for this project, we were able to generate the preliminary data needed for a successful HRC project grant application. The summary of the project is linked here: http://www.hrc.govt.nz/funding-opportunities/recipients/dr-philip-carter

***Aim 1: Use WGS to understand NZMenB emergence***
We proposed to sequence and analyse an additional 30 NZMenB isolates from 1991 to 1993 as well as 18 pre-1991 serogroup B isolates. We also proposed to use MLST and porA PCR to analyse isolates from 1980s (excluding those identified as the Serogroup A epidemic type) to identify those from the ST-41/44 clonal complex and investigate whether the epidemic porA type existed before the epidemic.

So far we have concentrated in analysing and sequencing pre-1991 isolates. A summer research student organised and attempted to identify porA allele from 100 meningococci isolates from 1987 to 1991. Of these reactions only 42 were successful and epidemic porA was not found. For whole genome sequencing, we also tried to grow 82 isolates from 1988 to 1991 for genomic DNA extraction however only 16 grew. Apparently a freezer failure in 1991 may have affected the viability of these isolates. We also organised previously extracted DNA samples and selected 11 more for WGS based on previous typing information. We extracted fine typing and MLST from these 27 isolates (Table).

**Table. Typing of 27 cases**

| | Isolate number |
|---|---|
| NZMenB strain | 8 |
| Clonal Complex 41/44, not epidemic | 7 |
| Other STs, not epidemic | 6 |
| New Sequence type, not epidemic | 6 |

Detailed analysis of the sequences is still ongoing. So far increasing the number of 1991 isolates has strengthened our initial finding that the diversity of the NZMenB was already present early in the epidemic suggesting the strain has been circulating long before the epidemic started.

It has been hypothesised that the NZMenB isolate came from the Netherlands but during the 1980s Norway also had an outbreak caused by a similar strain.
To understand where the NZMenB may have originated we sequenced 10 isolates from Norway and the Netherlands from the 1980s and early 1990s. We found that the NZMenB isolate is more closely related to the isolates from Norway.

***Aim 2: Adaption of the NZMenB strain to vaccination***

We proposed to sequence 48 more isolates from 2002, 2006, 2007 and 2010. We sequenced 13 isolates from 2002, 12 from 2006, 13 from 2007 and 10 from 2010. Among these isolates, there were 31 ST-42, 12 ST-154, 2 new STs and 4 other STs. Adding these isolates to previous analysis resulted in 32 pre-vaccination and 39 post-vaccination ST-42 isolates. Recently a prokaryote specific GWAS tool named bugwas was released (https://github.com/sgearle/bugwas). We tried this programme on our data set. We analysed the data in two ways, based on the core single nucleotide polymorphisms and indels (insertions and deletions) and based on the whole genome using a kmer approach. The programme did find the principal component that can separate pre-vaccination vs post-vaccination isolates and about 10 SNPs that were above the noise, but failed multiple test correction. This suggests that the number of samples is still insufficient. With other less stringent corrections, several SNPs did pass significance. These SNPs were the same ones that were found before using the smaller dataset. Currently we are working with epidemiologists at ESR to identify isolates from individuals that have been vaccinated. These isolates will be used in future studies. Unfortunately we were not able to obtain statistical significance for any of the SNPS identified.

## Identifying a Small Non-coding RNA Signature for T2DM in obesity

D Macartney-Coxson, S Das, M Benton, K Danielson

### *Objective*

Type two diabetes mellitus (T2DM) and obesity are diseases of epidemic proportions. We will investigate if there is a specific signature of plasma small non-coding RNA molecular associated
with T2DM.

### *Background*

By 2030 it is predicted that 366 million people will have T2DM. The NZ health system is significantly impacted by the disease with projections suggesting that costs will be $1 billion by 2021. T2DM is strongly associated with obesity. Obesity is also a major public health problem and risk factor for T2DM and other diseases such as hypertension, dyslipidaemia and cardiovascular disease. NZ has the third highest prevalence of obesity worldwide; 63% of adults are over-weight and 27% are obese. The significantly higher rates of T2DM and obesity observed in people of Māori and Pacific island descent compared to those with European ancestry further heighten the national significance of these diseases.

MicroRNAs (miRNAs) are small, non-coding RNA molecules (19-23 nucleotides in length) that inhibit translation and/or direct mRNA degradation; they can be key regulators of cell fate and are involved in the pathogenesis of complex diseases including obesity and T2DM. MiRNAs are the most studied group of small non-coding RNAs and the role/function of these and other small RNAs (piwi-acting (piRNA), tRNA fragments and small nucleolar (snoRNAs)) is an area of intense research. These small non-coding RNAs are also present in biofluids including blood, where they are packaged in extracellular vesicles, associated with lipoprotein (LDL/HDL) or bound by RNA-binding protein Argonaute2. As such, their potential as disease biomarkers has been increasingly recognized and interrogated. *In vitro* studies have shown that miRNAs transported in association with exosomes or HDL can be delivered to recipient cells in their active form and modulate target mRNAs, altering cell function and key cell signalling processes. Adipose derived miRNAs have been shown to mediate cross-talk with circulating macrophages, hepatocytes and muscle tissue. Therefore, while much research is still required to understand the functional role of circulating miRNAs *in vivo*, they may well represent a novel cell-cell communication network, mediating cross-talk between organs, and implicated in the pathogenesis of complex multi-faceted diseases.

Since the first report of circulating miRNA associations with T2DM, which antedated disease development, by Zampetaki et al. in 2010 a number of studies have reported associations between circulating miRNA levels and obesity and T2DM. However, the majority of these have utilized QRTPCR arrays or microarray technologies to focus on known miRNAs, and as such very little is known about the role other circulating RNA molecules play in obesity and T2DM.

RNA sequencing provides the opportunity to explore the profile of additional circulating small RNA molecules to those assayed by the various microarray technologies; notably to gain information on the circulating levels of piRNAs and snoRNAs. In this study we will investigate potential associations of these small non-coding RNAs with T2DM (for which, to the best of our knowledge, there are no current reports in the literature). Our US collaborators are part of the NIH Extracellular RNA Communication Consortia that has been working to optimize methods for sequencing RNA molecules from blood, with the ultimate goal of detecting both miRNAs and other novel small RNAs in plasma as potential functional biomarkers. Work from this group has already identified small RNAs, other than miRNAs, present in the plasma in a large cohort study. Unpublished data from the group has shown significant differences in the circulating small RNA profile in patients with adverse or beneficial remodelling after myocardial infarction (n=10 each group). These data suggest that, although challenging, RNA sequencing for small RNAs in plasma is feasible, and can lead to the discovery of novel potential biomarkers (for details of small RNA profiles in different diseases see exRNA ATLAS at www.exRNA.org).

We propose to utilise this expertise and aim to identify additional circulating small-RNA molecules (e.g. piRNAs and snoRNAs, potentially previously unrecognized miRNAs) that are associated with T2DM. Obesity is a significant risk factor for T2DM and the majority of individuals with T2DM are obese, Focusing on obese patients will allow us to identify differences between the T2DM and non-T2DM groups in a very relevant patient group without the confounder of obesity. As such any candidate small-non-coding RNA identified for further analysis may provide novel disease biomarker(s), at least for obese individuals; and reveal avenues of research which inform additional therapeutic interventions, and/or provide new insights in to disease development

*Method*

**Objective:** To investigate if there is a specific signature of plasma small non-coding RNA molecular associated with T2DM.

Study Cohort: We analysed the small non-coding RNA in fasted plasma samples (stored at -800C) from individuals with severe obesity. Samples were available from The Wakefield Tissue Bank and all individuals gave their written informed consent and the study was approved by HDEC. Plasma from 19 individuals, matched for gender (female), age and BMI, and with and without T2DM was analysed by small RNAsequencing. Individuals with T2DM were selected based on previous diagnosis (oral hypoglycemics n=3, insulin n=5, diet controlled n=1)), or previously unrecognised (n=10) with HbA1c ≥ 48 mmol/mol, and/or fasting glucose ≥ 7.0 mmol/L. To provide maximum separation between cases and controls individuals without T2DM were selected based on fasting glucose ≥ 5.7 mmol/L, fasting insulin ≤ 79 pmol/L and HbA1c ≤ 5.7 mmol/mol.

**Clinical and anthropometric data is provided in Table 1.**

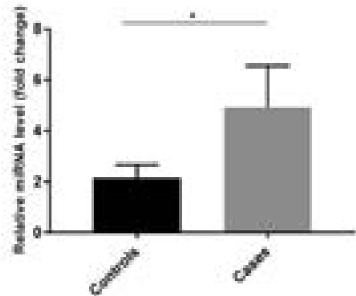
Table 1. Clinical and Anthropomteric Data for Individuals with and without T2DM

| | Individuals without T2DM | Individuals with T2DM |
|---|---|---|
| Age (years) | 45 (± 8) | 46 (± 8) |
| BMI (kg/m2) | 44.0 (± 8.6) | 45.4 (±9.4) |
| Fasting Glucose (mmol/L) | 4.9 (± 0.5) | 9.6 (± 3.4) |
| Fasting Insulin (pmol/L) | 62.4 (± 14.5) | 141.3 (± 80.4)* |
| HbA1c (mmol/mol) | 34.6 | 64.3 |
| Values shown are Mean ± standard deviation.<br>* fasting insulin influenced by medication for some T2DM | | |

RNA extraction and sequencing. RNA was extracted using an Exiqon miRCURYTM RNA isolation kit and assessed for quality using a Bioanalyser. Library preparation was performed using the NEBNext Multiplex Small RNA Library Preparation Set (Illumina) and 75 cycles of single-end sequencing performed on an Illumina NExtSeq.

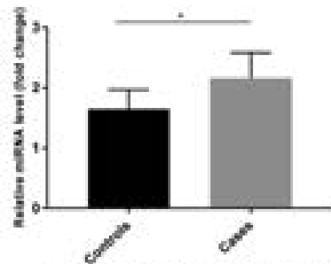
*Results*

The Das Lab has been optimising its approach for small non-coding RNA sequencing (from extraction through sequencing) as part of The NIH Extracellular RNA Communication Consortium and this project has benefited as a result. However, because of this there has been a delay in obtaining the sequencing data. Sequencing has now been completed and we are awaiting the final set of raw sequence data before performing the bioinformatic and statistical analyses. We anticipate that the analysis will be completed in the next 6 months, and plan to perform some validatory analyses before writing a manuscript.




*Finances*

We are very grateful to Research for Life for providing the funding for the RNA sequencing and will shortly be submitting our invoice, now the sequencing is complete. Finances: $17,839 was requested and granted. The sequencing was US$15,193 (~NZ$20,494) slightly more than anticipated.



## Validation of White Blood Cell Lineage Specific DNA methylation markers

MC Benton[1,2], A Cameron[3], J Clapham[1], D Macartney-Coxson[1]

1 Kenepuru Science Centre, Institute of Environmental Science and Research, Wellington, New Zealand
2 Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
3 Malaghan Institute of Medical Research, Wellington, New Zealand

Using publicly available data we identified a series of genes with cell specific DNA methylation that differentiates between white blood cell types. We hypothesised that cell specific DNA methylation influences the gene expression profiles of cell populations in different manners. Our project aimed to investigate epigenetic regulation of cellular lineage via the potential effects on cell specific mRNA expression profiles.

The original application which was funded by Research for Life in 2015 had four main stages:

1. Sample (blood) collection and cell sorting [completed];
2. Pyrosequencing of identified DNA methylation markers [completed];
3. QRTPCR to investigate mRNA expression of these genes [completed];
4. Analysis and integration of the data [completed].

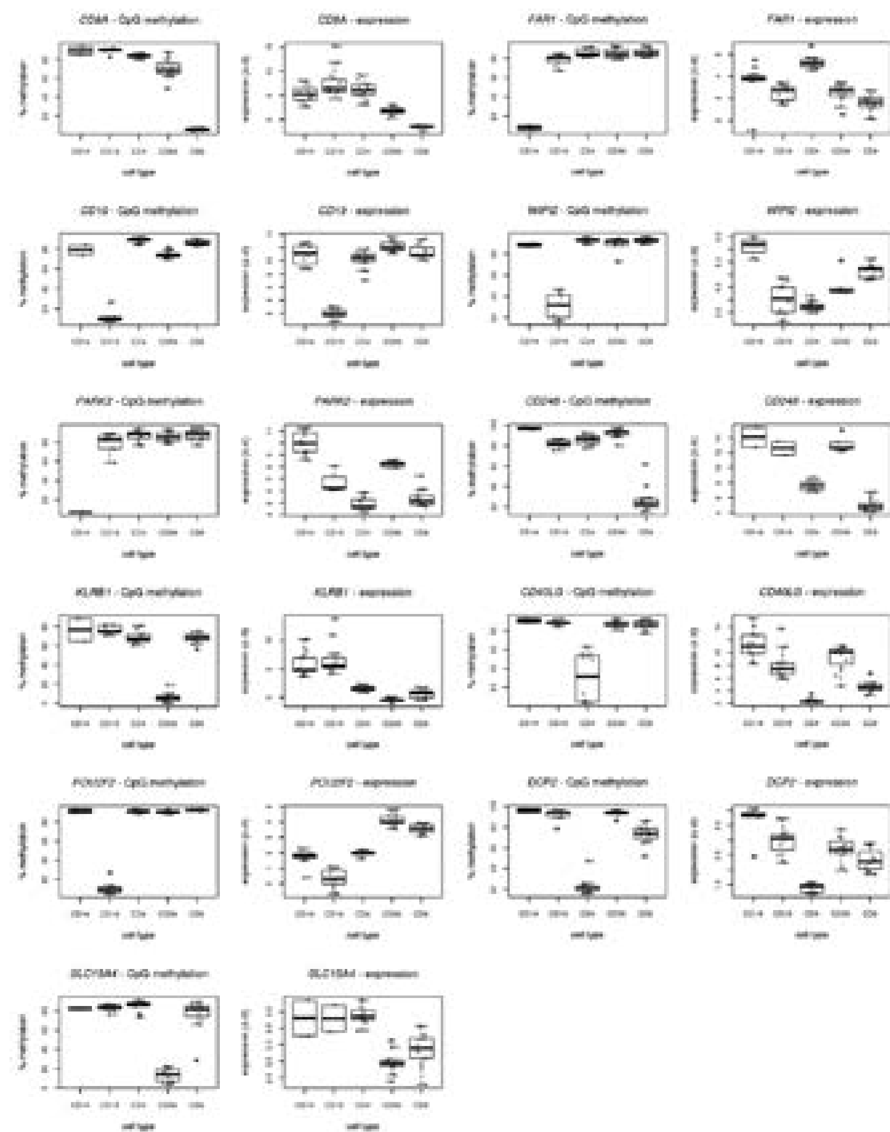
Our successful application was granted in May, starting mid-2015. At the time we proposed a 12 month period for completion. Due to several unforeseen delays (documented in the 2016 report) progress was slowed; however, we have now completed all analysis and are in the final stages of drafting a manuscript to be submitted to the journal Blood. The working title for this manuscript is "DNA methylation in blood - potential to provide new insights in to immune cell biology".

The key points described within the manuscript:

- We identified excellent cell-type discriminatory markers using a bioinformatics approach in cell-sorted public data, these validated in blood cells extracted from 12 healthy individuals.
- Points of cell-specific differential methylation clearly correlated with gene expression (Figure 1), indicating an important developmental/biological role.
- We illustrate the potential to identify new and useful cell-specific markers

This new information about the epigenetic regulation of specific immune cell lineages, has the potential to deepen our understanding of these immune cell lineages and thus potentially impact knowledge of immune cell biology and provide new avenues of research. For instance, identification of such markers (epigenetic and expression) may facilitate the discovery of 'new' sub-cellular lineages.

Figure 1. Methylation and gene expression boxplots for all genes investigated. Methylation is presented as percentage, gene expression as normalised Δ beta.



## Identification of Novel Candidates that can Influence th2 Induction by Dendritic Cells

L Connor, S-C Tang, K Hilligan, E Roussel, F Ronchese

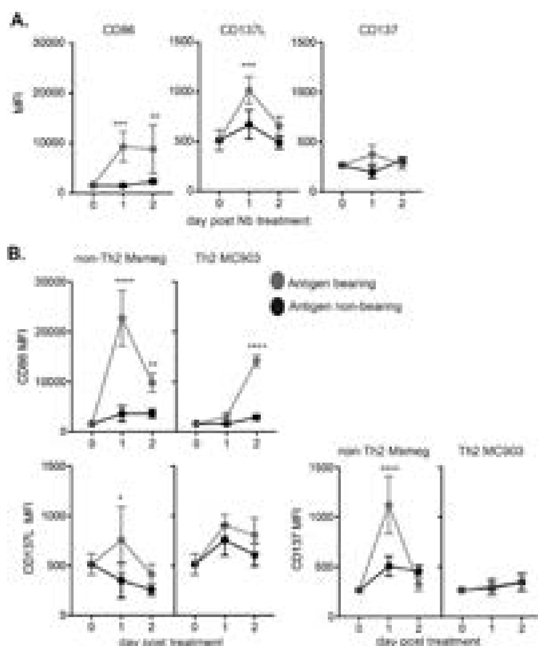
T helper type 2 (Th2) immune responses mediate allergic diseases. Understanding the mechanisms involved in the initiation of allergic Th2 responses is an important goal in the development of effective therapies to treat allergies. Dendritic cells (DC) are known to be critical for the development of Th2 Immunity; however, the mechanisms underpinning DC instruction of Th2 differentiation are not known.

To identify molecules and signalling pathways that could be involved in priming Th2 responses by DCs, we performed RNA sequencing on DC subsets isolated from Lymph nodes (LN) of mice treated with Th2-inducing stimuli. The aim of this current research project was to validate whether selected candidates could be measured by flow cytometry over time, whether the nature of expression was unique to DC primed by Th2 stimuli and whether these candidates play a role in priming Th2 responses.

DCs isolated from Th2 conditions failed to upregulate transcripts for the costimulatory molecule CD137-ligand (Tnfsf9) and Intracellular Adhesion Molecule (ICAM)1, which have been reported to favour Th1 differentiation by supporting IL-12 production by DC and strengthening DC-T cell interaction. To confirm whether mRNA transcripts reflect protein expression, flow cytometry on DC primed with Th2 stimuli, *Nippostrongylus brasiliensis* (Nb) was measured. DCs that have directly encountered antigen can be identified after treating mice with fluorescently labelled antigen. The expression of CD137-ligand, CD137 and ICAM1 was measured on antigen-bearing and non-bearing DC on day 0, 1 and 2 post treatment. ICAM1 expression on DC was stable across time and was not regulated by Th2 treatment (data not shown). Notably, RNA sequencing was performed on Nb-primed DCs isolated from LNs at day 2, at this time point, cell surface expression of CD137 and CD137L was similar on DC from treated and untreated mice, however at day 1, while CD137L was slightly upregulated on antigen-bearing DC, CD137 expression was not regulated by Nb treatment. To determine whether the nature of expression was unique to Th2-conditioning or shared across different immune stimulations, expression of co-stimulatory markers was measured by antigen-bearing and non-bearing DC isolated from Mycobacterium *smegmatis* (Msmeg) (non-Th2 stimuli) and MC903 (Th2 stimuli) treated mice. While CD137L expression pattern was similar on all antigen-bearing DC regardless of treatment, CD137 expression was only upregulated on DC by Msemg and not by the Th2 stimuli.

**Figure 1.** Upregulation of CD137 expression on DCs only occurs after stimulation with non-Th2 stimulation. C57BL/6 mice were treated with fluorescently labelled Nb or Msmeg by intradermal injection or topical application with MC903. LN were

isolated from treated mice at day 0, 1, 2 post treatment and expression of CD86, CD137L and CD137 on XCR1$^{lo}$CD11b$^{lo}$ dermal dendritic cells was measured by flow cytometry. Data are representative of two experiments with 4 mice per group. P values were calculated by two-way Anova and a Bonferroni post-test. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05.




CD137 is more well known to be expressed by T cells, which engages with CD137L provided by DC to induce T cell activation. Therefore, the role of CD137 on the DC is not well understood and will need to be investigated further. Due to the lack of difference in CD137L expression between Th2 and non-Th2 primed DC, experiments planned to investigate the role of CD137L using monoclonal antibodies was not applicable. Instead further analysis of DC primed by different stimuli was carried out.

The transcriptional profiles from DCs isolated from Nb treated mice also revealed a reduced proinflammatory signature. To determine whether this was unique to Th2 inducing stimulation we performed RTqPCR on sorted antigen-bearing and non-bearing DC from Nb and Msmeg treated mice to measure the levels of IL-12a, IL12b and IL-6. In support of the RNA-sequencing data, transcripts for the proinflammatory genes failed to increase in DC isolated from mice treated with Nb. In contrast, Msmeg treatment induced a significant increase in proinflammatory cytokines by antigen-bearing DC.

In summary, DC isolated from Th2-inducing environments are functionally and phenotypically distinct from DC isolated from non-Th2 environments. Overall Th2-stimuli such as Nb and MC903 do upregulate the co-stimulatory marker CD86, however, they fail to induce a proinflammatory response. Further studies will be carried out to determine whether the lack of pro-inflammation by DCs is key for the development of Th2 CD4 T cells.

## Antipsychotic Induced Gastrointestinal Hypomotility

**S Every-Palmer**
**Department of Psychological Medicine**

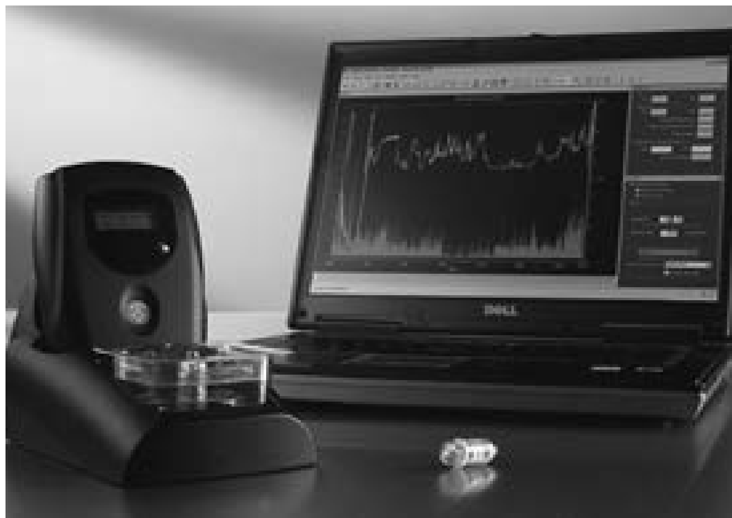
Our study concerned gastrointestinal hypomotility ("slow gut") caused by antipsychotic medication, a common, important and poorly understood side effect spectrum that causes distress and even sudden death amongst those with serious mental illnesses. Up to 50% of patients prescribed antipsychotics (especially those on clozapine) suffer chronic constipation as a side effect, reducing their quality of life. Some of these patients experience such profound gut motility problems that they develop obstruction which can lead to their bowel perforation. In Australia and New Zealand dozens of mental health patients have died from these complications. Using new technology we wanted to better understand the condition, allowing us to identify those at risk and subsequently look at targeted treatment. The study had ethical approval through the Health and Disability Committee.

In 2014 we completed a pilot study, the first of its kind, investigating how long it took for food to pass through the gut (termed transit time) in antipsychotic-treated patients. In normal healthy adults transit time is usually just over 24 hours. We found some antipsychotics virtually paralysed the bowel. For example, all subjects on clozapine had profoundly abnormal gut function, with average transit times being over four days. Some of the patients hadn't passed any bowel motions for over a week, but had become so accustomed to this they did not perceive it as abnormal.

These results clearly warranted further investigation, particularly to determine whether medications like laxatives are helpful in improving gut function and reducing the risk of serious side effects such as obstruction and bowel perforation. Surprisingly this had never been studied before, although antipsychotics are some of the most commonly prescribed medications in the world. The principal investigator conducted a comprehensive review on this topic for the Cochrane Library and failed to find any high quality trials addressing management of this problem.

It took us a while to obtain sufficient funding to start the study. The equipment necessary to conduct wireless motility capsule studies (WMC) is expensive, with the cost per study exceeding $1000. However, there is no alternative technique that can provide similar data without radiation exposure. Alongside funding from Research for Life, we also received additional funding from the Maurice and Phyllis Paykel trust and a grant-in-aide from the University of Otago, which collectively meant the project became viable in mid-2015. We are also grateful to Massey University for lending us some of the WMC equipment.

The WMC system (SmartPill Corporation, Buffalo, NY, USA) consists of an ingestible single-use capsule, a receiver, and display software.



The WMC or 'smartpill' is a polyurethane capsule measures 26mm x 13mm – about the size of a vitamin pill – and contains a solid-state pressure sensor, an ion-sensing field effect transducer, a pH sensor, a solid-state temperature sensor, and electronic subassemblies supporting the pressure and pH sensors, as well as a radiofrequency transmitter and an antenna. The WMC is activated then ingested by the subject and its sensor technology allows it to continuously monitor pH, temperature and pressure as it passes through the gastrointestinal tract.

Three days prior to ingestion of the WMC, participants discontinued medications that could potentially alter gastrointestinal motility and pH three days before the study (e.g. laxatives). They also were asked to avoid tobacco and alcohol in the 24 hours prior to the study.

Demographic and diagnostic information was collected including age, gender, ethnicity, BMI and smoking status. Current medication was transcribed from the participants' drug charts. Psychiatric diagnoses, clozapine levels, commencement dates and date of admission were collected from the electronic health records.

As well as readings from the WMC we also assessed the subjective awareness of constipation as assessed by Modified ROME III constipation criteria measured at day five of the WMC test.

We used SPSS version-21 (SPSS Inc Chicago, Illinois, USA) to analyse the data. Descriptive statistics (medians with IQR, and plotted distributions of transit times) provide data summaries for transit times. For demographic and clinical covariates, continuous variables were compared between medication groups using t-tests for normally distributed variables (age, BMI) or Mann-Whitney U tests for skewed

distribution variables (e.g. anticholinergic load). Categorical covariates were compared using Pearson chi-squared tests, or Fisher's exact tests when one or more expected count was less than five.

Linear regression was used to examine the relationship between the outcome variables and other covariates such as age, gender, ethnicity and medication dose. While transit times displayed a skewed distribution, we also calculated means and standard deviation for comparison with population normative values. For hypothesis tests, differences were considered statistically significant when $p<0.05$.

Between 2015 and 2017, we continued to recruit at a steady rate (this took time as the possible pool of participants was small; the inclusion criteria being mentally stable inpatients residing on an inpatient psychiatric rehabilitation campus, competent to consent and having achieved a constant clozapine dose with the appropriate leave).

We recruited 17 participants to complete the study. The median age of participants was 38.7 years, Eleven (65%) were male and 6 (35%) were female. Eight patients (47%) identified primarily as New Zealand European, seven (41%) as Maori, and two (12%) as Pacific Islander. Most participants (n=15, 88.2%) had diagnoses of schizophrenia while two had diagnoses of schizoaffective disorder. They were all treated with clozapine.

Our key results are as follows:

1. The gastric emptying times, small bowel transit time and colonic transit times of clozapine-treated participants are significantly longer than population norms.
2. Hypomotility in at least one region of the gastrointestinal tract was evident in 88% of clozapine-treated patients, and 59% of participants had multiregional dysmotility.
3. Participants displayed this pattern of hypomotility independent of age, ethnicity, gender and duration of clozapine treatment.
4. Subjective reporting of constipation symptoms had low sensitivity in predicting hypomotility.
5. The WMC does not measure oesophageal motility. This requires further exploration.
6. Using gender matching and protocol specific normative data ultimately made little difference to our significance testing, so marked was the difference between transit times of clozapine-treated patients and normative values.

In conclusion, this study used new technology to confirm that clozapine has a significant effect on gut motility, which accounts for the associated morbidity and mortality of gastrointestinal complications in this population. The results have helped us progress further research proposals to look at how we can successfully mitigate these significant adverse effects. The study is currently being written up for publication in an international journal.

Many thanks once again to Research for Life for your generous support.

## Effect Of Short-Term Temperature Changes On Energy Expenditure

**T O'Donnell, M Fan, J Krebs, S Tzeng**
**Centre for Translational Physiology and Department of Surgery and Anaesthesia**

This PhD study is an investigation into the effect ambient temperature has on the amount of energy expended in humans. In modern society, large proportions of the population spend the vast majority of their time exposed to controlled air-conditioned environments. This is designed to maximise comfort however there is a risk that this may be limiting daily energy expenditure and predisposing this large proportion to obesity. Whilst this is concerning given our rising rates of obesity, it also presents an opportunity to use modern technology to modulate our environments to create ideal conditions to not only be comfortable but also conducive to healthy living.

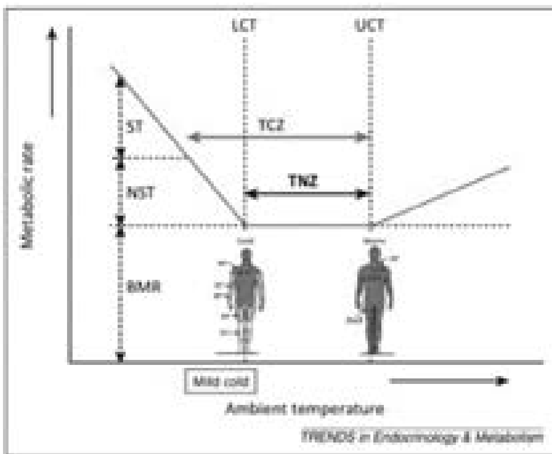



**Figure 1. The thermoneutral zone as it is traditionally depicted.**

The thermoneutral zone is a range of temperature in which resting energy expenditure is not required to increase to warm or cool the body, (Figure 1). This traditional figure was established in the 1960's with low subject numbers and simple measurement tools, so confirming such a curve and its properties still exist is pertinent. Previous research suggests that variance outside the thermoneutral range results in elevated energy expenditure and presents the possibility of an interventional target for raising daily energy expenditure. In overseas populations this range has varied between 17-26 degrees and we have sought to establish if such a range existed for our unique local population in NZ.

Using the Global Energetics and Environmental Simulation Suite (GENESIS) at the Centre for Translational Physiology, University of Otago Wellington we are able to alter and control temperature whilst accurately measuring energy expenditure. Participants underwent two separate protocols which started at 23 degrees for 30 minutes and over 90 minutes the temperature was either decreased down to

11 °C or increased up to 35 °C. Participants were seated in a semi-reclined position wearing standardised scrubs and their expired air continuously collected with a hood calorimeter to quantify energy expenditure.

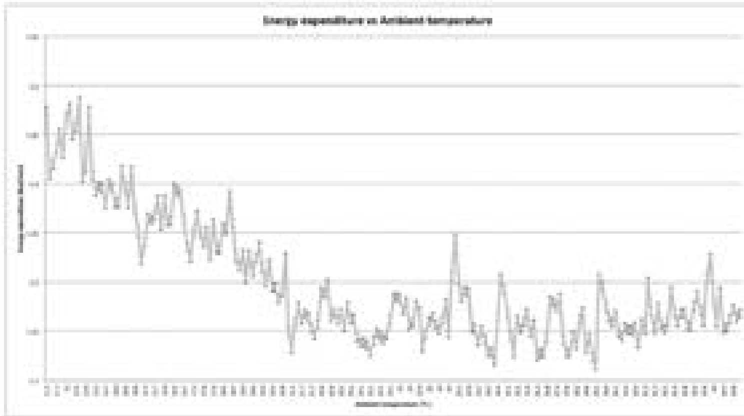


**Figure 2. Energy expenditure changes as ambient temperature is modulated.**

This study is still in progress, however we present interim data in Figure 2, collected from 21 participants. This shows energy expenditure on the vertical axis and ambient temperature on the horizontal axis. Of note is that, cooler temperatures seem to have a much more pronounced effect on energy expenditure and the variability of the data is much higher than depicted in the stylised versions of the thermoneutral zone shown in Figure 1.

We expect to finish data collection shortly with manuscript submission by the end of this year. We would like to thank the Research for Life for their generous and incredibly valuable support.

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## MicroRNA and Left Ventricular Remodelling after Myocardial Infarction

**A Holley**
**Department of Surgery and Anaesthesia**

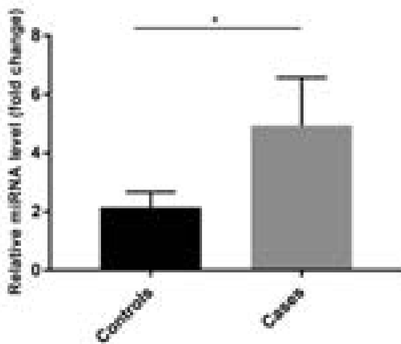
Following an acute myocardial infarction (AMI - more commonly known as a heart attack), the development of congestive heart failure is a significant clinical problem for our patients. The left ventricular (LV) remodelling process that ultimately leads to heart failure is a complex and dynamic process, normally occurring progressively in AMI patients. The degree of remodelling that takes place post-infarction remains one of the main determinants of long-term survival. Early prediction of pathological LV remodelling and subsequent risk of heart failure remains challenging, and the gold standard marker, N-terminal pro-brain natriuretic peptide (Nt-pro-BNP), is only elevated once there is a change in cardiac output i.e. after significant remodelling of

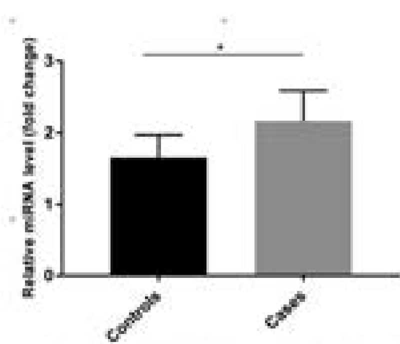
the left ventricle has already occurred. An earlier marker of adverse remodelling might allow for initiation of heart failure therapy prior to significant remodelling, and could therefore be protective.

MicroRNAs are negative regulators of gene expression and dysregulation of these small molecules have been implicated in various pathological conditions, including cardiovascular disease. The objective of this study was to examine the relationship between levels of circulating miR-30d and miR-146a and the development of heart failure in AMI patients.

We examined circulating levels of miR-30d and miR-146a in the plasma of 40 AMI patients who developed new onset of heart failure within 1-year of their index event, and compared to two matched controls each who did not develop heart failure. The following clinical characteristics were controlled for: age, hypertension, prior AMI and diabetes. We found that levels of miR-30d and miR-146a0 were significantly higher in those patients who developed heart failure. miR-30d was 2.3 fold higher in heart failure cases relative to the controls (Figure 1), and miR-146a was 1.4 fold higher in heart failure cases relative to the controls (Figure 2).

Patients who developed heart failure were also found to have higher levels of the inflammatory marker, C-reactive protein (CRP), than those who did not develop heart failure [3.8 (1.8-16.3) vs 1.9 (0.9-5.2) mg/ml respectively, **p<0.01]. Previous studies have suggested microRNAs play a role in regulating inflammatory processes. Although post-AMI inflammation is essential for the healing process, excessive inflammation plays an important role in the development of cardiac fibrosis and subsequent LV remodelling. The future of this project is to further understand potential inflammatory targets of these miRs and whether they can be used as potential therapeutic targets for modulating inflammatory levels. Methods to control inflammation, in order to potentially suppress pathological remodelling, would be important in developing new treatments for heart failure.



## New treatment options to prevent the late neurological and metabolic costs of preterm birth

**M Berry**
**Department of Paediatrics and Child Health**

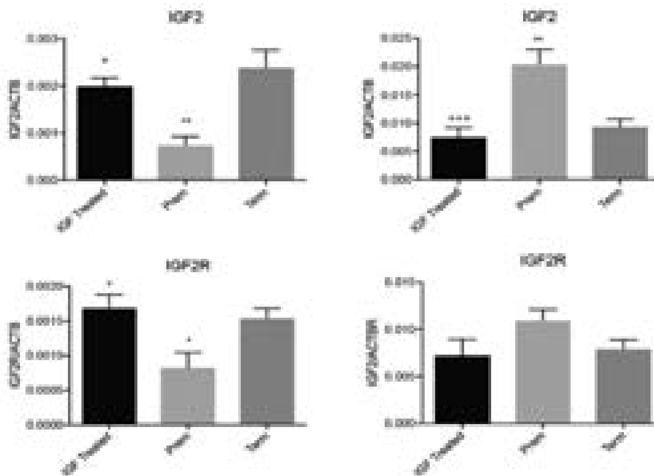
To date, 20 term (ten male, ten female), 16 preterm control (eight male, eight female), and 16 IGF-treated preterm (eight male, eight female) animals have been delivered and undergone behavioural and metabolic assessment. Tissues were collected at postnatal age 28d (corrected) for detailed analysis.

*Specific project objectives*

### Neurodevelopmental Assessment

The behavioural tests undertaken show that juvenile animals born preterm have an ADHD-type phenotype that appears to be 'rescued' by neonatal treatment with IGF-1. Detailed assessment of changes in neuroanatomy, gene expression, and other key measures of brain injury is underway in with one of our collaborators (Dr Andrew Clarkson) and will form a significant component of Mr Josh Houlton's PhD thesis.

Preliminary data (6/sex/group) show that there are, amongst other changes, significant, sex and gestation-specific differences in central gene expression for IGF 1 & 2, and their receptors, in which there appears to be restitution of a term-phenotype following neonatal IGF1 treatment in preterm-born animals.




### Metabolic Function Assessment

Similar to our pilot data, it appears that the preterm animals have reduced glucose tolerance compared to term-born animals and that neonatal IGF treatment, in males, 'rescues' this metabolic dysfunction and returns the preterm phenotype to a more term-like phenotype.

The insulin assay needed to continue to progress this work is under development in our collaborators laboratory (A/P Mulhausler University of Adelaide) and is expected to become available towards the end of the year; all biological samples have been banked pending analysis.

Another complication of preterm birth is the development of non-alcoholic fatty liver disease. Our Honours student (Ms Heather Barnes) is currently working through these tissues to both quantify preterm-associated liver disease, and the effect of neonatal IGF1 treatment.

*Supplementary comment*
The degree to which both neurological and metabolic improvements have been seen in these experiments is remarkable. So much so, we are repeating the entire study to ensure that the results are truly reproducible as the potential clinical implications are significant.

The compound that we used in this series of experiments, (Pegylated IGF1) is under patent with Roche and as such, all data relating to the use of pegylated IGF-1 for the treatment of preterm-associated morbidity is under embargo.

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## Predictions of Resting Energy Expenditure in Maori & Pacific Populations: The PREEMPt Study

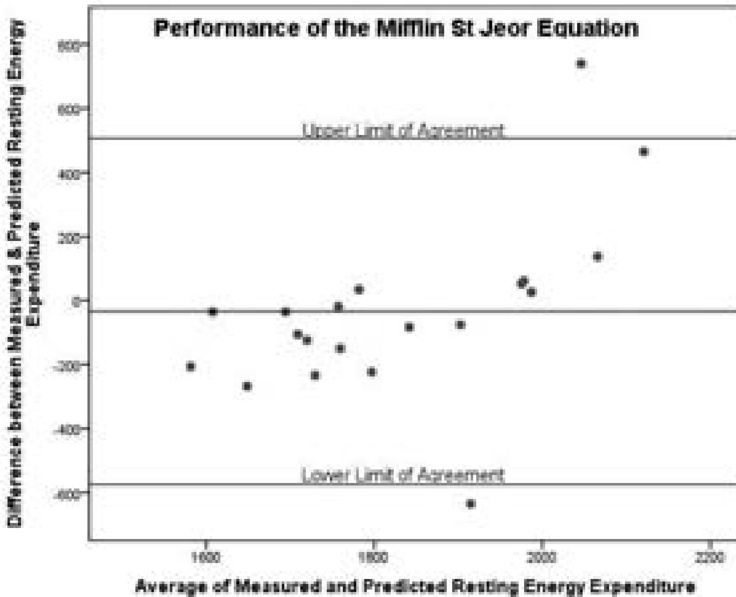
**B Corley, J Krebs, R Hall, R Carroll, M Weatherall**
**Centre for Endocrine Diabetes & Obesity Research**

*Background*
Predictive energy equations are used to estimate an individual's basal metabolic rate and thus, their daily calorie requirement. These equations relate age, gender, weight and height, to direct measures of energy expenditure. All the equations currently in use such as Schofield, Harris Benedict & Mifflin St Jeor, were developed outside of New Zealand in populations and social contexts that are different from the New Zealand contemporary experience. The Schofield equation, for example, was developed predominantly in lean Italian and American men in the 1940's. With obesity prevalence at epidemic proportions, accurate estimates of calorie requirements and national evidence based energy intake guidelines have never been more important.

*Objectives*
The aim of this pilot study was to compare estimated versus measured energy expenditure in a small pilot sample of New Zealand Maori and Pacific participants. This would provide an initial estimate of the error of the most commonly used predictive energy equations and provide the means to estimate the sample size required to develop a New Zealand specific resting energy equation. The pilot would also provide the means to estimate sample sizes for any study of resting energy expenditure.

*Results*

Ten Maori and ten Pacific participants with a body mass index over 25 were recruited within the Wellington region. The mean age was 43 years, 75% were female. Average resting energy expenditure was 1814 (SD 256) kcals/24hours, resting energy expenditure per kg lean mass was 18.14 (SD 3.18) kcals kg-1 day-1. Body fat percentage ranged from was 28 – 47.7%. The Mifflin St Jeor Equation had the poorest predictive ability, whilst Harris Benedict performed best, though all equations performed poorest at higher resting energy expenditure levels.




*Discussion*

Results from the PREEMPt study allowed us to accurately generate sample size estimates for several new energy expenditure studies, including the CREEDS study. The findings confirm the limitations of existing commonly used predictive energy equations in estimating daily calorie requirements, particularly in the higher range of resting energy expenditure.

*Conclusion*

We are continuing to collect resting energy expenditure and body composition data with a view to a NZ based predictive equation that can be tested against currently available predictive algorithms and used to inform dietary recommendations for New Zealanders.

## Victoria University of Wellington

### Aptabiotics: Aptamer Targeted Nanomedicines For Treating Bacterial Infections

**D Day, School of Biological Science**

This project has developed a series DNA aptamers specific for the pathogen *Pseudomonas aeruginosa* that have then been functionalised with nanosilver to allow the targeted treatment of infections.

*P. aeruginosa* is an opportunistic bacteria, commonly infecting the immunocompromised, including cystic fibrosis suffers, diabetics, burns victims and patients with medical implants. In 2017, the World Health Organization placed it in the Priority 1 category of pathogens in need of new antibiotics, within which it ranked as the second most threatening to human health. *P. aeruginosa* infections withstand current antibiotic treatments because of the organisms low membrane permeability and expression of broad specificity multidrug efflux pumps that extrude a wide variety of antimicrobial drugs. Additionally, coordinated communal behaviour of growing cultures mediated through quorum sensing (secreted signalling chemicals that regulate behaviour) allow adaptations such as the formation of biofilms and metabolic changes that facilitate survival for extended periods of time in the presence of drugs.

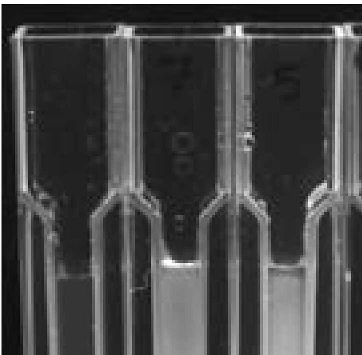


**Figure 1.** Tuneable fluorescence of DNA scaffolded silver nanoclusters. The sequence of the DNA scaffold for silver nanocluster synthesis plays a role in the emitted fluorescence of the clusters. Displayed in this figure is red, orange, yellow and green fluorescence of silver nanoclusters.

They are similar to silver nanoparticles in having antimicrobial properties, but are much smaller and more potent antimicrobials, because of their greater surface area-to-volume ratio. Additionally, we have conjugated our aptamers with an unusual DNA structure called an i-motif, which is able to bind silver ions ($Ag^+$) with high affinity. Aptamers conjugated with silver nanoclusters or $Ag^+$ chelated i-motifs have strong antimicrobial activity with minimal toxicity when tested in an invertebrate model of microbial infection.

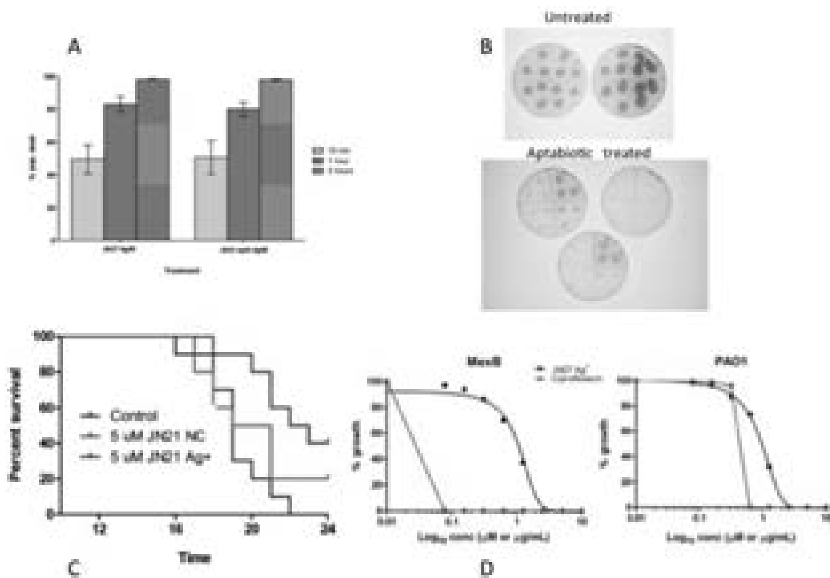



**Figure 2.** Aptabiotics are potent antimicrobials. Panel A, treatment with I mM aptamer conjugated i-motif causes approximately 50% cell death a growing *P. aeruginosa* within 10 min and more than 99.9% within 3hr. Panel B, shows plates displaying the number of viable colonies after 20hr exposure to aptamer-silver nanoclusters. Plating of 10-fold serial dilutions of the untreated samples (top) grow as a dense bacterial lawn. The treated cultures show no, or minimal, growth compared with the equivalent untreated samples (comparable dilutions are top right). Panel C, shows survival of *G. mellonella* larvae after treatment with aptamer-silver nanoclusters or aptamer-silver i-motif compared with the infected but not drug-treated larvae. Panel D, the efflux pump mutant MexB (left) is equally sensitive to aptamer-silver i-motif as the wild type strain (right), whereas the MexB mutant is highly sensitive to ciprofloxacin (red line) compared with the wild type that has functional efflux pumps.

DNA aptamers can be considered to be in *vitro* evolved DNA molecules with the same functionality of selective binding as conventional antibodies. They are fast to produce compared to antibodies and show low immunogenicity. Additionally, drug molecules including nanomaterials can readily be incorporated into their structure to allow aptamer mediated delivery to target cells. The targeted delivery of antimicrobials to bacterial pathogens improves the efficacy of the drug by enhancing exposure to the pathogen while reducing off-target effects on host cells.

Our laboratory has developed a technology that uses DNA aptamers for the targeted delivery of antimicrobial silver to *P. aeruginosa*; in its general form this approach is applicable to any pathogenic microorganism. Silver acts through a diverse number

of mechanisms to kill both Gram negative and Gram positive bacteria, resulting in exceedingly low levels of natural resistance to treatment, while having a much lower toxicity to mammalian cells than bacterial. We have utilised recent advances in nanotechnology to add structures able to form silver nanoclusters to our aptamers. Silver nanoclusters are small clusters of silver atoms less than 2 nm in size that display molecular-like properties and are brightly fluorescent (Figure 1).

**Figure 2** shows that the aptamer silver-conjugates that we call Aptabiotics, act to kill *P. aeruginosa* rapidly (Fig. 2A) and have potent antimicrobial action (Fig. 2B). Using the invertebrate Gallaria mellonella model of infection our aptabiotics are able to cure an otherwise lethal *P. aeruginosa* infection, with both the aptabiotic nanoclusters and i-motifs having strong bactericidal activity (Fig. 2C). Importantly, unlike the antibiotic ciprofloxacin that is clinically used to treat PA infections, aptabiotics are not substrates for the efflux pumps that make PA multidrug resistant (Fig. 2D).

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## Assessing Early Immune Cell Inflammatory Responses to MIS416 in Secondary Progressive Multiple Sclerosis (MS)

**C Beyers**
**Centre for Biodiscovery**

MIS416 is being investigated as an innate immune modulator therapy for patients with secondary progressive MS (SPMS) in a Stage 2B clinical trial. The initial trial results show a significant variation within the MIS416-treated patient population for the clinical measures used. These data, coupled with previous anecdotal evidence of improvement in disease symptoms from a cohort of patients receiving MIS416 for compassionate use, eludes to the presence of a subset of patients who are putatively responders to therapy.

These findings further substantiate the need to investigate the effect of MIS416 therapy by looking at the activation state of peripheral blood immune cells, which is the aim for our experimental work funded by this Research for Life grant. In our application, we outlined three specific aims for the work we propose to undertake:

1. Compile and validate a custom fluorescent antibody panel to detect intracellular cytokine (ICS) production in monocytes in whole PBMC by flow cytometry, and validate experimental conditions for resting and stimulating PBMC in culture to detect early cytokine production by monocytes.
2. Evaluate the cytokine bias of monocytes from SPMS patients, contrasting untreated SPMS participants to a healthy control population and determine the impact of MIS416 therapy in SPMS patients in response to ex vivo PBMC stimulation.
3. Determine if genetic variation in the immunoregulatory NFκBIA gene modulates the immune outcomes of MIS416 treatment in patients with SPMS.

We are well on track with this project and have complete the first aim from our original application. Our validation data for a custom designed antibody panel of ICS production in monocytes, using control material, shows proof of principal and has advanced to the stage where we have finalised our laboratory protocols and optimisation testing for this work.

Ongoing work for this project is now centred on completing our experimental work using PBMC from the stage 2B trial participants and our healthy cohort. Stratification of patients through genetic analysis and clinical response measures will allow us to conclude the project and will ensure a high quality publication of our findings supported by Research for Life.

The investigators would like to thank Research for Life and its donors for their continued support of this research.

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## Comparing the effect of Nasal High Flow (nhf) Therapy with Non-invasive Ventilation (niv) on PACO2 in COPD Patients with Chronic Respiratory Failure

**S McKinstry, J Fingleton**
**Capital and Coast District Health Board, Medical Research Institute of New Zealand, Victoria University Wellington**

*Synopsis*

The aim of this randomised controlled trial is to compare the AIRVO device (Nasal High Flow therapy) against Non-invasive Ventilation in stable chronic obstructive pulmonary disease (COPD) with chronic respiratory failure, with regards to reducing hypercapnia (elevated arterial carbon dioxide tension).

Eligible subjects with a diagnosis of COPD and chronic hypercapnia will undergo two separate interventions in a randomised order. Each subject will receive 60 minutes of AIRVO 2 use at 45L/min and 60 minutes of NIV via Bi-level Positive Airways Pressure (BiPAP) at a pressure setting of 15/4cmH2O. The subjects will be monitored with a transcutaneous carbon dioxide (PtCO2) monitor continuously and will undergo a 15 minute washout period between interventions. The primary outcome is comparison of PtCO2 between groups, adjusted for baseline. Physiological parameters will be noted every five mins (oxygen saturations, heart rate, respiratory rate) during the investigations and subjects will be asked to complete a tolerability questionnaire and BORG dyspnoea score following each intervention.

*Literature review and background*

Chronic obstructive pulmonary disease (COPD) is a significant health problem in New Zealand (NZ) and worldwide. COPD affects 14% of adults aged over 40 in NZ and is responsible for over 12,000 hospital admissions every year. Māori and Pacific people carry a higher burden of disease. Acute exacerbations of COPD (AECOPD) requiring hospitalisation are associated with high healthcare costs reduced quality of life, and shorter survival. Around 20% of people presenting with AECOPD develop acute hypercapnic respiratory failure (AHRF), which is a rise in arterial carbon dioxide tension ($PaCO_2$) due to the inability of the patient to expire carbon dioxide. Patients who develop AHRF are more likely to need to go to the intensive care unit (ICU) and are more likely to die. The hospital mortality of patients with AECOPD and ARHF is 12%.

Although there are no NZ guidelines on management of AECOPD, British guidelines on the ventilatory management of AHRF recommend that patients with AECOPD and AHRF should have an initial trial of 60 minutes with steroids, titrated oxygen and nebulised bronchodilators. About 20% of patients will improve with this treatment however about 80% will require more intensive forms of treatment such as non-invasive ventilation (NIV). NIV is airway and ventilatory support that does not use an endotracheal tube. Appropriate use of NIV reduces need for subsequent intubation, hospital length of stay and mortality in patients with AECOPD. However, although less invasive than intubation, NIV requires its own set of equipment, trained staff, close patient supervision, and regular arterial blood gas testing to monitor progress and appropriate adjustments to NIV machine settings. In addition NIV is very poorly tolerated by many patients and there is an unmet need for a well-tolerated, easy to administer treatment to reduce the need for NIV.

Nasal High-flow (NHF) therapy is a gas delivery system that provides heated and humidified air by nasal cannulae. Air administered in this way can be supplemented with oxygen as required. NHF oxygen therapy reduces mortality in comparison to standard oxygen therapy in ICU patients with hypoxic respiratory failure although in the study reporting this the research participants did not have AHRF. NHF therapy is also reported to reduce the risk of re-intubation after ICU therapy and after abdominal surgery. To date NHF therapy has not been investigated in a controlled trial in COPD patients with AHRF.

NHF therapy may reduce hypercapnia and the work of breathing and could reduce the need for NIV in AECOPD. Three clinical trials in patients with lung disease show that NHF therapy with oxygen can reduce CO2 levels compared to standard oxygen therapy. Further research at the Medical Research Institute of New Zealand (MRINZ) shows that NHF therapy is well tolerated, reduces PaCO2 in patients requiring supplemental oxygen, and in stable COPD causes a modest reduction in PaCO2 and a significant reduction in work of breathing.

*Progress to date*

With the aid of generous funding support from Research for Life, we have made excellent progress conducting a 24 patient randomised controlled two-way cross over trial in patients with COPD who have chronic hypercapnic respiratory failure. The trial directly compares NHF for 60 minutes, via the myAIRVO 2 device, with NIV in the form of bi-level positive airways pressure (BiPAP) support for 60 minutes, in line with current BTS guidelines regarding recommended settings. We used the funding to purchase: 1) a state of the art NIV device (Respironics BiPAP AVAPS-ST 60 Series); 2) NIV consumables (masks and tubing) 3) Sentec (transcutaneous measuring device) consumables (gas cannisters, ear clips, probe membranes, gel).

We have currently recruited 23 of the required 24 participants, envisaging completion in the next month. We look forward to presenting the results in the very near future and once again thank the Research for Life for their contribution to our important medical research.

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## Evaluating immune cell phenotype and function in healthy subjects and in secondary progressive multiple sclerosis (SPMS) patients treated with clozapine or risperidone

**A La Flamme, C Beyers**
**Centre for Biodiscovery**

The CRISP clinical trial is on-going at Wellington hospital and the first participant was recruited in May 2016. In total, we aim to recruit 36 progressive multiple sclerosis (MS) patients. Currently, we have recruited nine participants and have performed an initial immunological analysis on the baseline samples to validate our flow cytometric plan.

The Research for Life grant supported the recruitment and analysis of a healthy cohort of subjects as a comparator for the CRISP trial participants. The research has three specific aims:

1. Validate the use of Cyto-Chex BCT (Streck, Omaha) whole peripheral blood (PB) tubes as compared to heparinised whole blood tubes (BD, New Jersey) for the analysis of blood leukocytes in healthy controls.

2. Optimise an immunophenotyping protocol to detect variations in leukocyte cell surface marker expression, intercellular cytokine production and immune cell function in SPMS.
3. Recruit a healthy control (HC) cohort and assess immune function over six-months.

We have successfully completed these three specific aims. We confirmed that the Cyto-Chex tube, which has FDA approval for use in clinical immune phenotyping, was best suited for our methods (aim 1). Using these tubes, we have optimised of our method for immunophenotyping (aim 2). Our validation data for a custom designed antibody panel showed proof of principal early on, and we have had great success with this method allowing us to implement the use of four 8-colour antibody tubes to monitor whole blood immune phenotype.

For the final aim, we recruited the planned 12 individuals for our healthy cohort, and performed PB immune phenotyping at multiple time points. Additionally, PB mononuclear cells and plasma have been stored for future functional analyses.

Given that the CRISP trial participants have been randomised as to treatment group, our analysis of the data so far has focussed in comparing the immune phenotype of HC with those 9 CRIPS participants at their baseline measure (i.e. before treatment). This interim analysis shows insights into the immune composition and profile of progressive MS patients and how they differ to HC. Because our data are composed of absolute counts for leukocytes, we have an added degree of accuracy where many older publications rely on percentage values for subset profiling. From these data, we identified an absolute shift in some immune subsets for MS patients. These findings indicate that progressive MS patients form a distinct cohort not only by their clinical and diagnostic criteria but also their immunophenotypic features, with putatively more localised CNS pathology. From these data, we can infer that progressive MS pathology does not appear to greatly impact the absolute numbers of circulating PB leukocytes. The impact on the immune system may be confined to local sites of pathology, or disease processes. Future work will compare how treatment with risperidone or clozapine impacts the composition of progressive MS PB immune phenotype over time.

The investigators would like to thank Research for Life and its donors for the support of this work.

## How do Platelets Change the Immune Response to Infectious Agents?

K Hally
School of Biological Sciences

*Introduction*

Platelets are key regulators of intravascular thrombosis and inflammation, and emerging research indicates that platelets have dual roles in activating and also limiting host immune responses. Toll-like receptors (TLRs) are the first-line defence against infectious and injurious 'danger' signals, and we hypothesize that platelets may limit inflammation by regulating aspects of leukocyte function in response to TLR stimulation.

The generous research grant given by Research For Life in September 2016 has allowed us to pursue the following aim: to examine how platelets change leukocyte activation and leukocyte production of pro-inflammatory molecules in response to TLR stimulation. Specifically, we aimed to:

1. Examine how neutrophil, monocyte and T cell activation in response to TLR stimulation changes following platelet co-culture.
2. Examine how granulocyte elastase production in response to TLR stimulation changes following platelet co-culture.
3. Examine how patterns of PBMC cytokine production change in response to TLR stimulation following platelet-co-culture.

*Methods*

1. ***Cell isolation and culture.*** PBMCs and granulocytes from 10 healthy volunteers were either cultured alone or co-cultured in a 1:250 ratio with platelets. Cultures were left unstimulated or stimulated with 1-100 ng/mL of LPS (TLR4 agonist), Pam3CSK4 (TLR2/1 agonist) and FSL-1 (TLR2/6 agonist). Granulocytes ± platelets were cultured for 4 hours and PBMCs ± platelets were cultured for 24 hours at 37°C/5%CO2. Following stimulation, cell culture supernatant was collected and stored.
2. ***Flow cytometry***. Neutrophil activation (CD66b expression), monocyte activation (HLA-DR expression) and T-cell activation (CD38 expression) was assessed by flow cytometry.
3. ***Analysis of cell culture supernatant.*** As a marker of activation, granulocyte elastase production was measured with ELISA. The following cytokines and chemokines were measured in PBMC supernatant by Luminex: TNF-α, IL-6, IL-10, IL-1ra, MCP-1, MIP-1β. Cytokine measurements are currently being assessed.
4. ***Statistical analysis.*** Data assessing the effect of platelet co-culture are presented as relative change. Differences between measurements were examined using paired t-tests.

*Results*

1. ***Platelets suppress neutrophil and monocyte activation, but enhances CD8+ T cell activation, in response to TLR stimulation.*** Following co-culture with platelets,

neutrophil CD66b expression decreased by 11% in response to low-dose LPS ($p<0.05$) by 8% in response to high-dose LPS ($p=0.06$), by 8-15% in response to Pam3CSK4 (both $p<0.05$) and by 15-19% in response to FSL-1 (both $p<0.001$; Figure 1a). With platelets, monocyte HLA-DR expression was modestly reduced by 4% ($p=0.06$) and 5% ($p<0.05$) in response to low- and high-dose LPS, respectively (Figure 1b). CD8+ T-cell CD38 expression was modestly increased by 5-8% in response to low-dose Pam3CSK4 and high-dose FSL-1 (all $p<0.05$). Under all conditions tested, no change in CD4+ T-cell CD38 expression was seen in platelet co-cultures.

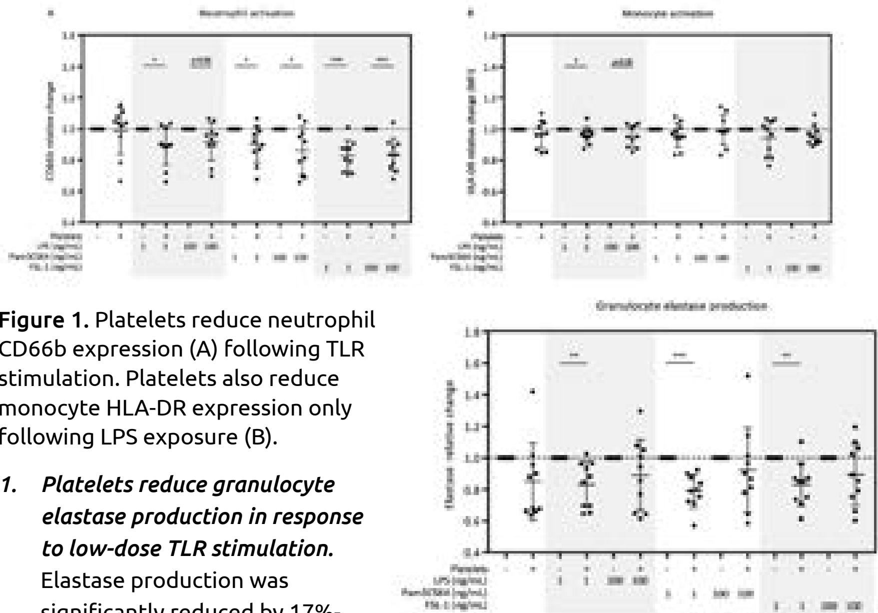

**Figure 1.** Platelets reduce neutrophil CD66b expression (A) following TLR stimulation. Platelets also reduce monocyte HLA-DR expression only following LPS exposure (B).

1. ***Platelets reduce granulocyte elastase production in response to low-dose TLR stimulation.*** Elastase production was significantly reduced by 17%-21% in response to a low dose of LPS, Pam3CSK4 and FSL-1 (all $p<0.01$; Figure 1b). Elastase production was non-significantly reduced in unstimulated cultures, and in response to high-dose stimulation of all agonists tested (Figure 2).

**Figure 2.** Platelets suppress granulocyte elastase production following low-dose TLR stimulation.

*Conclusion*

Platelets suppress neutrophil activation in response to a range of TLR agonists and suppress monocyte activation in response to LPS. In modulating certain leukocyte activation patterns, platelets may limit inflammation and, ultimately, limit host damage. Interestingly, platelets enhanced CD8+ T cell activation. Combined, these results suggest a dynamic platelet effect on leukocyte activation in response to TLR stimulation.

*Acknowledgement:*
We would sincerely like to thank Research For Life for the opportunity to conduct this research. This research is currently in draft manuscript form and has been submitted for the Australasian Society for Immunology (ASI) Annual Scientific Meeting in November/December 2017.

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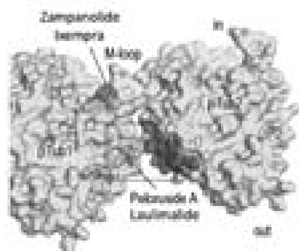
## Peloruside, Laulimalide, and Zampanolide Interactions with Tubulin

**J Miller, P Northcote**
**School of Biological Sciences and Centre for Biodiscovery**

The main aim of this project was to investigate the interactions of three potential anticancer drugs – peloruside, laulimalide, and zampanolide – with their protein target, tubulin. Tubulin is a protein that polymerises into 12 protofilaments that form the microtubules of the cytoskeleton. All three compounds prevent cancer cell growth by stabilizing and reducing the dynamic behaviour of microtubules required for cell division. Their mode of action is similar to the taxanes (Taxol® and Taxotere®) and Ixempra®, a major class of anticancer drugs used in the clinic against solid tumours of the breast, ovary, and lung. Unfortunately, these drugs have adverse side effects, and cancer cells can acquire resistance to the drugs after long-term use. Recent protein structural studies on tubulin using mass spectrometry, X-ray crystallography, and cryo-electron microscopy have highlighted potential hydrogen bonds or covalent bond formation between tubulin and these compounds that are important in their interactions with amino acids in tubulin.



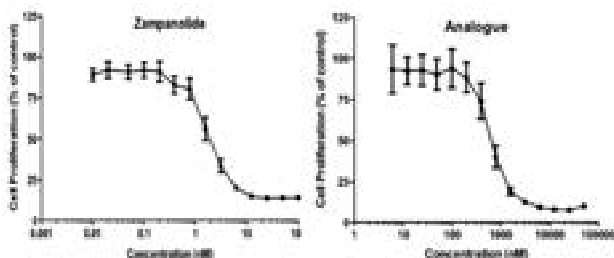

Aim 1 of this project was to determine the importance of interprotofilament binding in the action of peloruside and laulimalide by mutating specific amino acid residues in βI-tubulin believed to be important in their binding to tubulin.

Aim 2 was to establish the importance to microtubule stabilisation of covalent bond formation between zampanolide and a specific amino acid in tubulin, histidine 227, again by mutation of the amino acid. In addition, we also planned to carry out a structure-activity relationship using a synthetic zampanolide analogue synthesised by Karl-Heinz Altmann in Zürich.

For aim 1, two graduate students at VUW, R Gordon and B Jones, investigated the ability of peloruside, a New Zealand marine sponge compound, and laulimalide, another compound from a Pacific marine sponge, to inhibit the growth of cancer cells in culture. Peloruside and laulimalide are active against various drug-resistant cancer cell lines and bind a site different to the taxane binding site on β-tubulin where the taxanes, zampanolide, and Ixempra bind. Structural studies in other labs suggested

that there was a hydrogen bonding interaction between peloruside or laulimalide and the adjacent protofilament in the microtubule. To test this hypothesis, human embryonic kidney (HEK293) cells were transiently transfected with the gene for a mutant tubulin that would prevent this interprotofilament binding. The mutant tubulin, however, proved to be equally effective to wild type tubulin at both producing functional microtubules and inhibiting the transfected cell's growth and proliferation. Thus, there was no evidence that interprotofilament binding of peloruside and laulimalide plays a major role in the molecular action of these compounds.

For aim 2, the question of whether covalent binding was important in the mode of action of zampanolide, another Pacific marine sponge compound that targets the taxoid site on tubulin, was investigated using a similar approach to aim 1. Graduate students M Storey, M Parker, and S Nobel carried out the experimental studies. Histidine residue 227 was mutated, and the gene for mutant tubulin was transfected into HEK-293 cells. The mutation, however, had no effect on the ability of zampanolide to inhibit the growth and proliferation of the cells, suggesting that the covalent bond was not important in the action of the compound. Since zampanolide may also be able to form a covalent bond with the adjacent amino acid, asparagine 226, it was possible that a different covalent bond may form with this amino acid when histidine is not present. A double mutant in which both histidine 227 and asparagine 226 are mutated may better answer this question. Washout experiments designed to prove that a covalent bond didn't form with the mutant tubulin were unsuccessful, leaving the question unanswered of whether the covalent bond is important in the action of zampanolide.




A second approach used was to investigate whether a synthetic analogue of zampanolide lacking a carbon double bond required for covalent bond formation could still inhibit cancer cell proliferation. C Davies carried out these experiments and found that the analogue was 400 times less active than the parent compound, suggesting the covalent bond was critical in the action of zampanolide. With the two approaches giving opposite answers, additional experiments are required to establish for certain whether the covalent bond formed between zampanolide and histidine 227 of tubulin is critical for its inhibitory activity on cancer cell proliferation.

## Phosphatidic Acid-mediated Autophagy as a Mechanism of Disrupted Mammalian Development

**A Munkacsi, P Pfeffer**

Niemann-Pick type C (NPC) disease is a fatal, paediatric neuro-visceral disease due to lysosomal accumulation of cholesterol and sphingolipids. Currently, there is no effective therapy to treat NPC disease. Direct screens of compound libraries have not identified any candidates that have progressed through clinical trials, thus highlighting the need to understand the genes and cellular processes that modify disease severity. The objectives of our Research for Life grant were to characterize a phosphatidic acid hydrolase (*Lpin1*) as a modifier of NPC disease. We initially identified this modifier using genomic analyses of the yeast model of NPC disease and sought to further characterize this modifier. We have completed a significant portion of all three aims with Research for Life funding and anticipate publishing these results in the next six months. Specifically, our progress is the following:

***Aim 1: To define the developmental defect due to simultaneous loss of Npc1 and Lpin1.*** Prior to the funding of the grant, we demonstrated that the *Lpin1* mutation significantly reduced the birth of *Npc1*$^{-/-}$ pups at the expected Mendelian ratios when pups were genotyped at P21. Specifically, we recovered only 8 of 299 *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ mutants when we crossed *Npc1*$^{+/-}$ mice with *Lpin1*$^{+/-}$ mice. Of the 8 *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ mice that survived, these animals died between 3-8 weeks, a lifespan that was significantly shorter than the 10-12 week lifespan of the *Npc1*$^{-/-}$ mouse and the 44-48 week lifespan of the *Lpin1*$^{-/-}$ mouse. This result was not significantly consistent with the expected Mendelian ratio of recovering one double mutant every 16 births (x2 test, P = 0.0107), suggesting that *Lpin1* deficiency causes a developmental defect in *Npc1*$^{-/-}$ mice. To determine if this result was a consequence of neonatal development, we crossed *Npc1*$^{+/-}$ mice with *Lpin1*$^{+/-}$ mice and genotyped all animals at P0 (birth), a time point that was not be confounded by cannibalization of pups and was achievable without sacrificing breeders to collect embryos.

We genotyped 94 pups for 9 possible genotypes at P0 (Table 1). With 9 degrees of freedom and a chi square value of 5.79 (Table 1), we fell short of the chi square value (x2) of 16.92 required to reject the hypothesis that the observed genotypes of mice were born according to the expected Mendelian ratio. We also investigated birth weight of the pups as an additional assessment of viability. To account for variables such as litter size and time (hours) since birth, all pup weights were normalized to the average pup weight in their litter. The *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ mice weighed significantly less than *Npc1*$^{+/+}$*Lpin1*$^{+/+}$ and *Lpin1*$^{-/-}$ mice, albeit this was largely a contribution of the *Npc1*$^{-/-}$ mice. There was not a significant difference between the birth weight of *Npc1*$^{-/-}$ and *Npc1*$^{-/-}$*Lpin1*$^{-/-}$, which was consistent with the previous result that it was the *Npc1* mutation contributing to the phenotype in *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ mice. These results suggest NPC disease may actually be classified as a developmental disease; however, this will require further investigation of animal models and human patient at birth.

| Possible Genotype | Observed | Expected | Observed -Expected | $(O-E)^2$ | $(O-E)^2/E$ |
|---|---|---|---|---|---|
| N-/-L-/- | 3 | 5.875 | -2.875 | 8.265625 | 1.406915 |
| N+/-L-/- | 11 | 11.75 | -0.75 | 0.5625 | 0.047872 |
| N+/+L-/- | 6 | 5.875 | 0.125 | 0.015625 | 0.00266 |
| N-/-L+/- | 10 | 11.75 | -1.75 | 3.0625 | 0.260638 |
| N+/-L+/- | 23 | 23.5 | -0.5 | 0.25 | 0.010638 |
| N+/+L+/- | 13 | 11.75 | 1.25 | 1.5625 | 0.132979 |
| N-/-L+/+ | 4 | 5.875 | -1.875 | 3.515625 | 0.598404 |
| N+/-L+/+ | 18 | 11.75 | 6.25 | 39.0625 | 3.324468 |
| N+/+L+/+ | 6 | 5.875 | 0.125 | 0.015625 | 0.00266 |
| SUM | 94 | 94 | 0 | | 5.787234 |

**Table 1.** *Npc1Lpin1*$^{-/-}$ mice are born according to Mendelian ratios of expected and observed genotypes. With 9 degrees of freedom and a chi square value of 5.79, the chi square value of 16.92 was not obtained to reject the hypothesis that the observed genotypes of mice were born according to the expected Mendelian ratio. N=*Npc1*; L=*Lpin1*.

***Aim 2: To determine whether the development of Npc1*$^{-/-}$*Lpin1*$^{-/-}$ *mice is defective due to toxic levels of phosphatidic acid***

Prior to the funding of the grant, we demonstrated that phosphatidic acid (PA) levels were increased in the liver of *Npc1*$^{-/-}$ mice at the onset of disease (P50) compared to WT mice. This result suggested that PA homeostasis contributes to disease severity at the onset of ataxia and weight loss in the *Npc1*$^{-/-}$ mouse. To determine if PA homeostasis contributes to the developmental defect underlying the insufficient recovery of double mutants, we measured PA as well as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylglycerol (PG) in WT, *Npc1*$^{-/-}$, *Lpin1*$^{-/-}$, and *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ mice at P0 using liquid chromatography and mass spectrometry (LC/MS). Given that lipin proteins catabolize PA for the synthesis of triglycerides, we hypothesized that PA levels would be increased in the *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ liver at P0.

In the liver, there were no significant changes in PA or PC levels in any mutant compared to WT (Figure 1). There were significant reductions in PE, PS, PI and PG in *Npc1*$^{-/-}$ compared to WT mice, although these changes were not further exacerbated in *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ mice. In contrast, there were not any significant changes detected in these lipids in the brains of WT, *Npc1*$^{-/-,}$ *Lpin1*$^{-/-}$, and *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ mice at P0 (data not shown). These results suggest that PA levels are kept at a regular level in utero and in the neonatal stage of life, perhaps due to the placental connection to the *Npc1*$^{+/-}$ *Lpin1*$^{+/-}$ mother. It is thus plausible that there is a demand for PA during embryonic development that delays toxic accumulation of PA until post-partum (e.g., the PA accumulation observed *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ mice at P50).

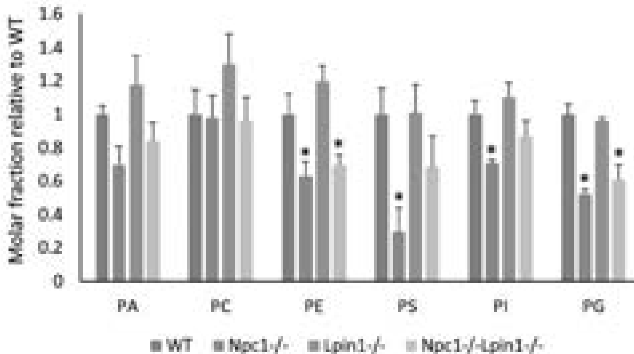



**Figure 1.** Phospholipid levels in the liver are not significantly altered in *Npc1Lpin1*$^{-/-}$ mice compared to parental *Npc1*$^{-/-}$ and *Lpin1*$^{-/-}$ mice at P0. Levels of phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylglcerol (PG) were quantified by normal-phase HPLC with Applied Biosystems Triple Quadrupole 3200 QTrap. Bars represent molar fraction of total lipid relative to wild-type (WT) mice. *, P < 0.05, Student's t-test comparison to WT.

***Aim 3: To determine whether the development of** Npc1*$^{-/-}$*Lpin1*$^{-/-}$ ***mice is defective due to disrupted autophagy***

Since PA regulates autophagic flux and the yeast lipin PAH1 regulates vacuole homeostasis, we determined if the deletion of *Lpin1* regulated autophagy in the *Npc1*$^{-/-}$ mouse. Autophagy is a fundamental cellular process in which cytosolic proteins and organelles are engulfed by an autophagosome in the cytoplasm and degraded in the lysosome. Given our previous observation of an autophagic defect in *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ mice at P50, we hypothesized that autophagy is a mechanism underlying the lipin-mediated defect in the development of *Npc1*$^{-/-}$ mice. To determine if this disrupted flux at P50 was conserved during the neonatal period, we measured LC3-II using Western blot analysis. Levels of LC3-II were consistent in WT, *Lpin1*$^{-/-}$, and *Npc1*$^{-/-}$, and *Npc1*$^{-/-}$*Lpin1*$^{-/-}$ animals at P0. These results are consistent with autophagy being a crucial cellular process immediately after birth. Disconnection of the neonate from placental nutrients initiates a state of immediate starvation before the first milk meal, and autophagy thus is a necessary means of nutrient scavenging between feeding. For example, a mouse model deficient in ATG5, a protein necessary for autophagosome formation, will die within the first day of birth. It is thus plausible that the loss of *Npc1* and *Lpin1*, genes that have wide-ranging effects on lipid metabolism, is compensated by alterations in mechanisms other than autophagy.

*Summary*

In summary, our research has determined that lipin-1 deficiency exacerbates NPC disease severity by disrupting the degradation of intracellular cargo via the process of macroautophagy in mice after weening. More specifically, our research has determined that macroautophagy is not a mechanism that underlies developmental defects of $Npc1^{-/-}Lpin1^{-/-}$ mice. At P0, the $Npc1^{-/-}Lpin1^{-/-}$ mice generally reflect the $Npc1^{-/-}$ profile. From this it could be interpreted that the impact of *Lpin1* knockout is of a lesser magnitude, or is downstream of, the effects that result from *Npc1* deficiency. With our results in neonatal and pediatric mice, we now have results necessary to write a paper reporting our characterization of the *Lpin1* modifier of NPC disease.

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## Search for New Drug Addiction Therapies – A Molecular Genetic Approach

**BA Ellenbroek, D Day**
**School of Psychology**

The serotonin transporter (SERT) plays a significant role in aetiology of drug addiction. The SERT knockout rat available in our lab is a highly exciting model because, while the rats have no SERT expression they have not displayed a significant difference in other genes we have tested. We have shown that these rats have a significantly increased sensitivity to the reinforcing properties of MDMA ("ecstasy") and cocaine, but not heroin.

In the present project we aimed to investigate the molecular mechanisms that may be responsible for these differences in sensitivity. To that end we are looking at gene expression using RNA sequencing. First we will investigate baseline differences between wild type rats and rats heterozygous and homozygous for the SERT knock-out mutation. Next we will investigate changes in gene expression in animals that have self-administered MDMA for a prolonged period of time.

So far, we have successfully established a collaboration with the lab of Dr Larisa Haupt at Queensland University of Technology. This has enabled us to maximise the generous donation of the Research for Life foundation by allowing us to sequence more samples efficiently.

In the previous months we have designed the experimental parameters and selected the samples to be sequenced. No RNA sequencing work has been performed on this model, as such, we chose to send multiples of each genotype to ensure an appropriate analysis of the model can be made which will contribute significantly to the field.

The appropriate regions of the brains have been excised and these were transported to and are awaiting sequencing in the Lab of Dr Haupt. Dr Haupt has been performing some initial optimisations on rat brains prior to using these important samples.
We expect the sequencing results to be with us in the coming weeks. During this time, in collaboration with our bioinformatics advisor Dr Miles Benton we have begun to

establish an analysis pipeline including sourcing the appropriate computing resources here in Victoria University. Finally preparation of a manuscript has begun in advance to publish the data.

It is our belief that examining the genetic difference in these rats will enable us to further understand the mechanisms of drugs of abuse. We thank the Research for Life for the generous donation and hope to update you with more final results in the coming months.

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## What factors influence sexually transmitted infection testing behaviour?

**H Denison, E Dennison**
**School of Biological Sciences and Centre for Public Health Research, Massey University**

Sexually transmitted infections (STIs) are a significant public health problem; globally, more than a million people acquire a STI every day (WHO 2012). If left untreated, STIs can cause serious health problems such as pelvic inflammatory disease in women, which can be painful and result in infertility in some cases. Among men, there is also some evidence that untreated STIs can lead to infertility. Early detection is crucial, so that the infection can be treated before causing long-term health damage. Early detection and treatment can also prevent the infection from being passed on to new people. Therefore, increasing the number of people testing and the speed at which people test after potential STI exposure has the potential to reduce STI rates over time. Complicating matters is the fact that STIs like chlamydia usually don't cause any obvious signs or symptoms, so individuals need to choose to go for a test even in the absence of a physical reason. Identifying and understanding the factors which influence STI testing behaviour, is crucial to developing effective sexual health programmes and services.

The funding provided by the Research for Life enabled us to conduct 24 interviews with university students from New Zealand to explore the unique complex social behavioural factors influencing STI testing behaviour. Resulting data were thematically analysed employing a qualitative content analysis method to produce a set of themes relating to our research question.

We found that there are many barriers to seeking an STI test, and individuals may experience any number of these. First of all, underestimating the personal risk of contracting an STI was common. This led people to erroneously believing that they do not need to have a test. This thought-pattern has also been shown in other countries. Some people, especially males, were afraid of the STI test procedure itself, imagining it to be very invasive and painful. In actual fact, for a regular check-up where a male does not have any symptoms, a urine test is usually all that's required. Other barriers to testing included the misconception that most STIs are not serious, embarrassment about having a physical examination, or being too busy to go for a test.

Structural barriers to testing were also apparent, including the financial cost for the individual of getting a test. Although many participants in our study expressed a preference for a same-sex health professional when going for an STI test, most said they would not be deterred from having an STI test if a health professional of their preferred gender was not available.

Finally, a pervasive barrier to STI testing is STI stigma. Being diagnosed with an STI suggests a violation of social norms and values, specifically that an individual has chosen to engage in behaviour often viewed as immoral, such as unprotected sex, sex with multiple partners, or sex with disreputable partners. This stigma may cause people to avoid going for an STI test because they are afraid that if they are seen seeking an STI test, people may assume that some deviant behaviour has occurred, which presents a threat to their social identity.

In addition to barriers to STI testing, we also identified factors which motivated testing behaviour. We found that there were five main drivers for testing: crisis, partners, clinicians, routines, and previous knowledge. The first barrier, crisis, refers to when an STI test was prompted by an event the participant experienced as a crisis, such as developing symptoms, finding out that a previous sexual partner had been diagnosed with an STI, or having engaged in a sexual encounter which they perceived to be high-risk. A second driver was the perspective of, or advice from, sexual partners. Another important driver to having an STI test reported by the participants was the opportunistic advice of a doctor or nurse who suggested an STI test while the individual was attending the clinic for a different reason. This often occurred when attending for sexual health related matters, such as a cervical smear, and was more commonly reported by female participants. A fourth driver was routine; some participants went for STI tests regularly, as part of their routine health care. The final driver, previous knowledge, intersected with the previous four, particularly in relation to routines. Many participants acknowledged that the more they knew about STIs the more likely they were to undertake regular tests. However, at the same time, many participants felt they did not have a good knowledge base and that their school sex education had been lacking.

The findings of this research, which have now been published, provide relevant information for clinicians, service-providers, educators, and policy makers, which can be used to find ways to improve testing and subsequently reduce STI transmission in New Zealand. Locally, the results of this project have been made available to relevant health providers, resulting in new approaches to service provision.

# Wellington Hospital

## Understanding the utilisation of protein in a restricted carbohydrate environment in Type 1 diabetes

AP Strong
Centre for Endocrine, Diabetes and Obesity Research, Wellington Hospital

*Aim:*
This exploratory study aims to understand the relationship between protein and insulin requirements in differing carbohydrate environments in participants with type 1 diabetes. The hypothesis: once habituated to a low carbohydrate diet, an insulin bolus for protein intake is required to maintain glycaemic control.

*Current Position:*
This study has finished recruiting and has the last visits scheduled through August and September. We aimed to recruit 16 participants and have currently recruited 17 in case of a further drop out or incomplete data. Once final visits are completed in September, blood will be sent to Dunedin to be analysed in October. We expect analysis results to be available before Christmas and the study presented at NZSSD in Hamilton May 2018. Publication will be sought soon after results are obtained.

## Project Grants 2017

The following projects were approved for funding in May 2017 and will be reported on in subsequent Annual Reports of the Foundation.

**Dr Kirsty Danielson**

**University of Otago, Wellington**

***Small non-coding RNAs as early biomarkers for colorectal cancer***
Colorectal cancer is the most common form of cancer in New Zealand and kills 1200 per year. Early diagnosis of CRC is a critical factor in reducing mortality and the development of novel, non-invasive biomarkers for disease screening is of paramount importance.

**Professor Dawn Elder**

**University of Otago, Wellington**

***Intermittent hypoxia in preterm infants in the first year of life: effects on growth, and measures of heart rate variability***
It is anticipated that this study will provide a better understanding of the natural history of intermittent hypoxia in preterm and term infants, and contribute to the knowledge base available to clinicians making decisions about whether to intervene clinically while the preterm infant is in hospital.

**Dr Bronwyn Kivell**

**Victoria University of Wellington**

***Investigating the therapeutic potential of MP1104 for the treatment of neuropathic cancer pain***
This preclinical study will evaluate a new therapeutic target for its ability to attenuate chemotherapy induced neuropathic pain.

**Varun Venkatesh**

**Victoria University of Wellington**

***The role of the apelinergic system in glioblastoma multiforme***
Glioblastoma is a deadly brain cancer disease, and more knowledge of its pathological processes will be beneficial to present and future patients.

**Dr Ayesha Verrall**

**University of Otago, Wellington**

***Development of a mycobacterial growth inhibition assay***
This grant was to fund the purchase of a cell counter that allows standardisation of the amount of donor cells in a given sample. The project seeks to identify host susceptibility and pathogen virulence factors that underpin the high burden of infectious disease in New Zealand and globally.

# Travel Grants

## 11th World Immune Regulation Meeting (WIRM), 15-18 March 2017 and laboratory visits in Davos, Switzerland

**K Naidoo**

The generous travel funding provided by the Research for Life in 2016, contributed towards my attendance of WIRM. In addition, I was able to visit the Swiss Institute of Asthma and Allergy Research (SIAF) in Davos, Switzerland.

This conference was a consortium of immunologists, clinicians, and experts from pharmaceutical companies such as Novartis. Most of the conference was focused on allergy and hence relevant to my current PhD project. The talks were extremely insightful, in particular the focus on where the field of allergy was headed. Dr Marc Rothenberg presented his latest research on eosinophilic esophagitis while Prof Thomas Bieber illustrated the progression of the current clinical trials for the treatment of atopic dermatitis. Furthermore, WIRM was an excellent platform for networking and the more informal poster sessions provided opportunities to meet renowned scientists. Notably, I was able to arrange a meeting with a principle investigator, Prof Claudia Traidl-Hoffmann in Augsburg, Germany after discussions with her at WIRM. I presented my PhD project in the form of a poster and during these poster sessions, received valuable feedback which will assist in the next phase of my project.

During the time in Davos, I met with Prof Cezmi Akdis and Dr Liam O'Mahony to discuss potential post-doctoral opportunities at SIAF and visited their institute. These meetings enabled me to have a good understanding of the different projects these principal investigators (PIs) are working on as well as gave me a chance to showcase my work to them. Discussion with the PIs and those during the conference will help me during the write-up of my thesis and manuscript for publication.

It is with absolute gratitude that I thank the Research for Life committee for making this journey to Davos possible. It has opened a world of possibilities for me as a scientist and will ensure further growth of my scientific career. Moreover, as this is my final year at the Malaghan Institute, I would like to express my appreciation to the Research for Life who have supported my work and travel over the past four years.

## 14th International Symposium on Dendritic Cells Shanghai, October 2016

**K L Hilligan**

I am currently in the third year of my PhD study at the Malaghan Institute of Medical Research. My research aims to improve current vaccination strategies by understanding how a population of innate immune cells, known as dendritic cells, initiate and regulate protective immune responses against bacterial, fungal and parasitic infections.

With the generous support of Research for Life, I attended the 14th International Symposium on Dendritic Cells that was held in Shanghai in October of this year. The conference was attended by many world-renowned dendritic cell biologists who presented their latest data and offered opinions about other work in the field. One of the many standout talks was presented by Prof Caetano Reis e Sousa from the Francis Crick Institute, UK. He presented some unpublished data gathered using elegant microscopy techniques that allows them to "fate map" or track the development of dendritic cells from bone marrow stem cells.

In addition to the formal presentations, there was a "Meet the Editor" session that was attended by the senior editors from high impact immunology journals such as Nature Immunology and Immunity. This was a highlight session for me as it was structured as an open discussion and specifically targeted to early career researchers. Topics covered included tips on getting your research published in a high impact journal and ways to address reviewer's comments on your work.

I also presented my own work in the form of a poster during the "Dendritic Cells and T cell subsets" session. Poster sessions are highly interactive and I had the opportunity to discuss my work with researchers from many different countries, specialties and backgrounds.

I would like to offer my sincere thanks to Research for Life for their support which allowed me to have this fantastic opportunity. The knowledge and skills that I have gained from attending this conference are invaluable for the further development of my research. Thank you very much.

## 40th Australasian Society for the Study of Brain Impairment (ASSBI) Annual Brain Impairment Conference 2017 at the Grand Hyatt, Melbourne

**S Lillas**

The travel grant that I received from the Research for Life allowed me to attend the 40th Australasian Society for the Study of Brain Impairment (ASSBI) Annual Brain Impairment Conference in Melbourne. This annual conference is a multidisciplinary conference focusing on learning about the most recent evidence in relation to brain impairment. This particular conference had a theme of "Looking back to look ahead: 40 years of science, practice and education in brain impairment".

At this conference, I had the opportunity to present a data blitz oral presentation. This is a five-minute session where you aim to introduce data that is later presented during the poster session. The purpose of the data blitz session is for poster presenters to entice delegates attending the conference to visit their posters.

My presentation and poster, 'Gimme a Break', focused on my master's thesis where I addressed the question "How do people manage fatigue, that they attribute to Traumatic Brain Injury (TBI), to enable them to conduct paid work". I specifically presented part of my results focusing on the types of breaks that people implemented in their workplace to enable them to remain productive in their jobs, yet manage their TBI related fatigue. I found presenting at this conference increased my confidence in preparing and presenting information in a short period of time. I was really happy with my ability to keep to the timeframe yet still present everything I aimed to. I also found it interesting to present my data in a visual way on a poster to allow people to get a good description of my work. The conference also gave me a lot more information that I found useful both for the completion of my master's thesis and networking with people in a similar field.

I also attended a concurrent session where there was a talk on strategies to increase the likelihood that Māori will participate in research. I found the strategies suggested interesting including utilising Kaumatua, having hui's at the Marae and ensuring Māori customs and traditions were included in the research recruitment process. I was able to reflect on this in relation to my own research where I had not recruited any Māori and consider possible options for future research to ensure Māori were involved.

At the conference I met a few individuals who had also completed some research on fatigue management after TBI. One in particular, had looked at a fatigue management programme in the UK as part of her master's thesis. She had based the programme on ones that had been developed for multiple sclerosis. Overall, attendance at this conference was successful in terms of exposing myself to oral and poster presentations, gathering information that contributed to my thesis, and networking with other like-minded individuals.

## International Conference on Motivational Interviewing, Hilton Penn's Landing, Philadelphia, USA, 18-21 June 2017

**S Dean**

Title of oral presentation: Engaging Adolescents in Mental Health Treatment

Attending this conference has allowed me to network with International researchers, clinicians, and leaders in the field. Together with the pre-conference workshop I had the opportunity to learn about implementing high quality motivational interviewing practices in research and/or clinical settings. I enjoyed listening to the experiences of clinicians, academics, and researchers, and exploring recent research relevant to training, coaching, and supervising for MI-consistent interventions. Importantly, the uptake and sustainability of evidence-based practice was attended to in a number of sessions.

My presentation seemed well received. Discussion with conference attendees relating to motivational interviewing for engagement followed; with much interest in the study design and clinical application of the intervention with adolescents.

Overall, this conference allowed me to discuss my research and consider possibilities for dissemination and implementation of motivational interviewing in mental health settings. I am excited about the possibilities for further research on motivational interviewing.

## International Society of Aptamers Annual Symposium, Aptamers 2017 in Oxford, UK

**J Soundy**

On 10-12 of April this year I was able to attend the International Society for Aptamers annual symposium, Aptamers 2017 in Oxford, UK, with the generous help of a $2000 travel grant from the Research for Life. At the symposium I presented a poster about my research showing how you can use aptamers linked with silver nanoclusters as a novel antimicrobial against Pseudomonas aeruginosa; a clinically important, multi-drug resistant bacterium. Through this poster presentation and designated networking breaks I had the opportunity to speak to a number of very knowledgeable aptamer scientists, with a few being useful future contacts for me. While I was not doing an oral presentation myself I did listen to every talk given over the three days, and was able to take notes on ideas and concepts that were relevant for my research.

# Notes

Research For Life

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