# RESEARCH REVIEW 2021

Research For Life

WELLINGTON MEDICAL
RESEARCH FOUNDATION

# RESEARCH REVIEW 2021

COVER PHOTO:

Kathryn Hally, Postdoctoral Fellow, University of Otago, Wellington. Kathryn received a Research For Life grant to undertake research to determine the clinical utility of a monocyte inflammatory score for predicting infarct expansion in patients with Acute Myocardial Infarction (AMI). Kathryn's interim report of findings is on page 22 of this report.

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Research For Life
The Wellington Medical Research Foundation Incorporated

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# Editorial

BY ASSOCIATE PROFESSOR REBECCA GRAINGER

## I find myself writing this editorial after 24 hours "AFK"; "away from keyboard" for those of you who do not get updated on the latest lingo by people under 20.

I wasn't just AFK, I was offline. Even the accessible parts of Fjordland seem to only have 3G and after using 3G for a day or so, being offline entirely seems a better option anyway. A visit to Doubtful Sound is a wonderful salve for the demands and difficulties of managing the COVID-19 related challenges of the last 18 months. I didn't miss the news at all; or case numbers, double-dose vaccination rates, and the latest opinions of experts on the next steps for Aotearoa New Zealand. Instead, the natural beauty and rugged remoteness generated a calmness and effortless in-the-moment mindfulness that leaves me refreshed. I recommend some offline time to all of you over the summer period ahead.

Although the instant connectedness that internet provides can be demanding and tiring, it has enabled us to work and continue research activities when COVID-19 might have otherwise prevented us. Most Research For Life board meetings have been online in the last year. Zoom has become a ubiquitous business tool. How would we have managed the pandemic without it? Many of the researchers Research For Life supports have worked via zoom as teams and had to change their ways of laboratory work or data collection to be compliant with COVID-19 alert level restriction requirements. Many have been able to continue and Research For Life has or will be supportive of projects that may need more time to be completed. Researchers have also been reliant on technology for dissemination of their research, with peer-reviewed publications and presentation at "virtual" conferences. In some ways these virtual conferences have had some upsides.

Virtual conferences are delivered via an online web-portal. These portals have become increasingly sophisticated over time, often now with virtual meeting rooms (zoom within the portal) for networking meetings between attendees, chat functions and all the programme easily accessible from your device of choice. Often, speakers pre-record their talks, and then watch in real-time with questions popping up from the audience via a chat window. The speakers then have the question-and-answer session in real-time. This approach allows ANYONE regardless of relative career stage to have a chance to interact with global experts, an approach which supports inclusivity and breaks down hierarchies in science.

Presentations are provided for viewing at a later date, which means one can fully engage with all the content. While the excitement of international travel is gone, so is the jet-lag, time away from home and loved ones, high costs, and carbon emissions of the jet travel. Registration is also much cheaper, often only a few hundred dollars, compared to up to $1500 New Zealand dollars for a larger international conference in the USA or Europe. This means the conferences have often maintained their participation numbers and even attracted a more diverse range of participants than previously. Almost all the recipients of "travel grants" from Research For Life have been able to disseminate their research via the virtual version of the conference they previously hoped to attend in person. The predictable downside for New Zealanders is that northern hemisphere conference timing means one is often attending overnight. This can have the advantage of watching others talk while in whatever is your version of comfortable clothes but can also mean changing into business attire in the middle of the night if you are required to present in real-time. The other challenges of virtual conferencing are the additional effort for networking via booking online meetings or sending follow up emails. I suspect that even when international travel becomes easier again many conferences will provide hybrid events with in-person and virtual options. We will get better at making the most of these new types of events. The bottom line is that the talented scientists supported by Research For Life have been able to disseminate their research to an international audience and make connections with others working in similar areas.

While we find ourselves facing many challenges during the pandemic, we have also seen innovation and creativity. Virtual conferences are just one example. Our researchers have innovated, re-invented, and pivoted to continue to do their work and disseminate it widely. As Chair of the Research Advisory Committee I look forward to the applications for funding and (virtual) conference attendance in the coming years. I, and the other members of the advisory committee, always learn a lot from the applicants and it is such a privilege to support talented medical researchers in Wellington through Research For Life. To all our medical researchers, please keep being innovative, creative and resilient as we need you to do this more than ever. Oh, and if you need a break to recharge, get away from your computers and out to the wilds of Aotearoa New Zealand. There's nothing better in the world.

## RESEARCH ADVISORY COMMITTEE

Associate Professor Rebecca Grainger (Chair)

Dr Peter Bethwaite (until June 2021)

Dr Lisa Connor

Professor Anne La Flamme

Associate Professor Peter Larsen

Professor John H Miller (until June 2021)

Dr Jeremy Owen

Dr Michelle Thunders

Dr Olivier Gasser

# RESEARCH REPORTS

# Victoria University of Wellington

## Why is Reelin signalling dependent on its oligomerization?

D COMOLETTI, L TURK



### KEY FINDINGS

Using mass spectrometry, we confirmed the *in vitro* pattern of Reelin cleavage, such that the three previously described cleavage products, namely the N-terminal, central and C-terminal fragments have been identified. In addition, we confirmed the new fragment previously identified in our preliminary experiments. This new fragment, corresponding to N-RR1 of Reelin, is smaller than any of the other previously identified fragments and suggests further Reelin degradation or activation. No new degradation products were identified.

The generation of a protocol to purify a novel heterodimeric Reelin constructs with one or two receptor binding sites published (Turk et al, 2020) and the proteins were used to understand how Reelin initiates the signal activation in neurons (Turk et al., 2021). Of note, both papers acknowledge the generous support of the Wellington Medical Research Foundation.

### OBJECTIVES

Reelin controls and regulates a variety of mechanisms underlying brain development and plasticity. Reelin is a secreted glycoprotein composed of 8 Reelin repeats (RR1 to RR8) which undergoes dimerization followed by time dependent cleavage. However, it is unclear why dimerization is necessary for reelin activity and whether cleavage degrades or activate reelin's activity. Our working hypothesis was that dimerization is key to cluster and activate both ApoER2 and/or VLDL receptors in pairs and that cleavage generates additional fragments currently unrecognized by epitope tagging.

**Objective 1:** To determine the *in vitro* pattern of Reelin cleavage using Mass Spectrometry (MS) to provide precise experimental details of its fragmentation over time.

**Objective 2:** To use distinct Reelin constructs with one or two receptor binding sites to solve the mechanism of activation of the Reelin signaling pathway.

### METHODS

A large variety of methods were used for the duration of this award. Given the limited space available here we refer to them in these publications: Turk et al., 2020 and Turk et al., 2021.

### RESULTS

**Objective 1:** We have obtained data on the in vitro pattern of Reelin cleavage using MS, confirming the known published pattern (D'Arcangelo et al., 1995) as follows: the full-length protein is degraded in fragments containing the N-terminal, central and C-terminal fragments. In addition, we have confirmed a new N-terminal fragment that was not previously published (corresponding to N-RR1, a smaller fragment than the canonical C-terminal). In the future, the signal transduction of this new N-RR1 fragment will be assessed to understand whether new signalling pathways are activated by this fragment or N-RR1 simply represents a degradation production devoid of any function.

Due to FTE and time constraints, we could not advance this aim as we hoped. Instead, we have devoted all our resources to Objective 2, which led to the publication of two papers.

**Objective 2:** We have successfully purified the heterodimer of the central fragment of reelin (Turk et al., 2020) and we have completed the signaling pathway experiments using live primary neuronal cultures. The results of these experiments indicate that, despite containing only a single binding site (i.e. similar to the monomeric form), reelin heterodimer still induces canonical pathway signalling in dissociated neurons (Turk et al., 2021).

## CONCLUSION

With the availability of new homo and heteromeric constructs we showed that: as expected, the purified dimeric Central fragment is sufficient to activate the canonical Reelin signalling pathway, whereas the purified monomeric Central fragment, even at 200-fold higher concentrations, does not induce measurable signal transduction. The dimeric Reelin Central fragment binds to its receptors, ApoER2 and VLDLR, with affinities that are only 2- to 8-fold higher than those of the monomeric fragment. To our surprise, the heterodimer, despite containing only a single binding site (i.e. similar to the monomeric form) induces canonical pathway signalling in dissociated neurons. These findings support a model where dimeric Reelin alone has the necessary conformation to engage a single lipoprotein receptor in a manner that leads to signalling events (Figure 1).

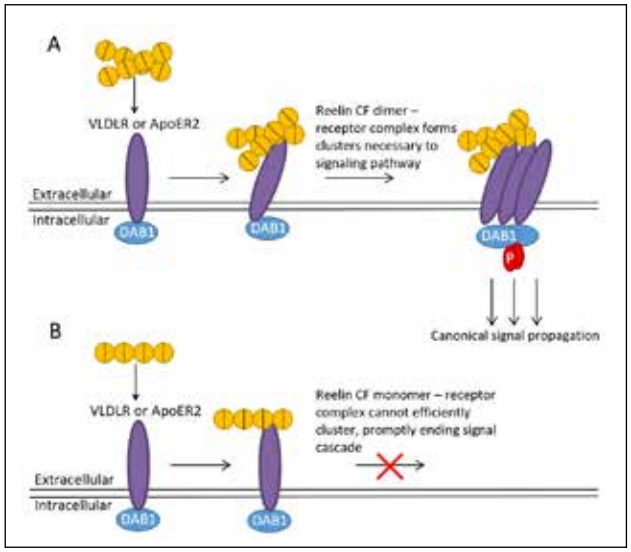


**FIGURE 1:** Suggested mode of dimeric activation. (A) X-like architecture enables the Reelin CF dimer to bind to either ApoER2 or VLDLR, forming a distinct Reelin-receptor complex in a conformation that favors receptor clustering to initiate canonical pathway signalling. (B) Reelin CF monomer binds to VLDLR or ApoER2, but is unable to form an active Reelin-receptor complex that can efficiently cluster and activate the signal cascade (Adapted from Turk et al., 2021)

## LITERATURE CITED


D'Arcangelo G, Miao GG, Chen SC, Soares HD, Morgan JI, Curran T. (1995) A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature. 374(6524):719-23.

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Turk LS, Mitchell D, **Comoletti D.** (2020) Purification of a heterodimeric Reelin construct to investigate binding stoichiometry. Eur Biophys J. 49:773-779.

# Victoria University of Wellington

# Identifying the Molecular Messages Shared Between Dendritic Cells and T cells

K BUICK, T BIRD, L TURK, C MORGAN, D COMOLETTI AND L CONNOR



## KEY FINDINGS

In this Research For Life project we established an ELISA screening platform to identify novel interacting proteins between Th2 CD4 T cells and dendritic cells (DC). The ELISA screen involved 31 individual proteins with a possible 231 interactions tested. The resulting screen identified 43 positive interactions, 40 were unknown, novel interactions that had not previously been reported. Using flow cytometric assays, we show that Fc-fusion proteins for known positive binding pairs can bind to corresponding proteins on the surface of T cells and provide evidence this approach can be used to assess the function of novel interactions. In addition, the data generated in this project supported L Connor's successful application of a Marsden fast-start grant.

## OBJECTIVES

DCs provide the key signals necessary to activate T cells and are pivotal in the generation of adaptive immune responses. Communication between DCs and T cells is typically driven by receptor-ligand interactions. As a result, receptor-ligand interactions are important drug targets however, to date only a small number of DC-T cell interactions have been successfully targeted, none of which are known to influence the development of allergen associated Th2 CD4 T cells.

There are still many DC receptors with unknown function, thus this project aims to identify novel protein interactions at the DC-T interface.

**Objective 1:** Identify novel T cell interacting partners for surface proteins expressed by Th2 primed DC.

**Objective 2:** Establish and validate the use of Fc-fusion proteins to elucidate the function of DC:T cell interacting pairs.

## METHODS

A recombinant protein library of 31 extracellular regions from DC and T cell surface receptors/ligands were generated using our custom designed expression plasmids (Fig 1a-c).

The extracellular domains of DC and T cell surface receptors were expressed as secreted fusion proteins, by HEK293F mammalian cells (Fig 1b-c). Ectodomain-Fc fusion protein expression was confirmed by western blot (Fig 1a-d). AP-fusion proteins are quantified by dephosphorylation activity, using calf intestinal alkaline phosphatase activity as a standard.

Novel protein-protein interactions were identified using an ELISA based approach (Fig 1e). All assays were carried out using automated multichannel pipettes and robotic liquid handling equipment. 384-well plates were coated with mouse anti-AP antibody in duplicate. AP-fusion protein, ectodomain-Fc fusion protein, and detection antibody anti-human IgG1-HRP were added sequentially.

Flow cytometry-based methods were used to detect protein-protein interactions with our ectodomain-Fc fusion proteins (Fig 3). Lymphocytes isolated from lymph nodes of immunized/non-immunized mice were incubated with ectodomain-Fc fusion proteins. Protein-protein binding was detected after staining with a fluorescently conjugated anti-human Fc IgG antibody. The cell population bound by ectodomain-Fc fusion protein will be determined by labelling cells with CD11c, MHCII, XCR1, SIRPa, CD326, CD11b, Ly6C, B220, TCRb, antibodies.

## RESULTS

T cell (11) and DC (20) surface genes of interest were identified as proteins involved in interactions between T cells and DCs during Th2 immunity via previous RNA-sequencing projects (Table 1). Expression of surface proteins encoded by selected genes was confirmed by western blot (Fig 1d) and alkaline phosphatase activity. An ELISA-based assay was set up to identify 231 potential interactions between T cells and DCs. The positive control proteins PD1-PDL-1, PD1-PDL-2, CTLA4-CD86, CD137:41BBL (Fig 1F) confirms expected interactions and validates the ELISA assay. Positive reactions could be clearly detected by colorimetric assessment (Fig 2a). Among 231 potential interactions, 43 positive reactions were detected. Interestingly, most proteins bound to more than one protein binding partner (Fig 2b). A literature search was carried out to predict functional role of positive gene interactions, cell adhesion and immune response function were the top functional groups representing the positive interactors (Fig 2c). Finally, we provide evidence that Fc fusion proteins expressed in our lab are capable of binding to cell surface proteins on specific immune cells populations known to express high levels of the corresponding binding partner (Fig 3a-c).

| T cell gene | DC gene |
|---|---|
| CD44 | Ccl6 |
| Il6st | Ccl12 |
| Tmem154 | Ebi3 |
| Rell1 | Lif |
| Igfbp7 | Il27 |
| Nrp1 | Ccl22 |
| CD274 PDL-1 | Ccl17 |
| CD80 | Cxcl9 |
| Havcr2 | Il34 |
| Timd4 | Icam5 |
| Cd27 | Htra1 |
| | Nrp1 |
| | Col23a1 |
| | Pgf |
| | Timd4 |
| | CD80 |
| | Cd274 |
| | Havcr2 |
| | CD70 |
| | CD40 |

**TABLE 1:** T cell genes (11) left, DC genes (20) right.



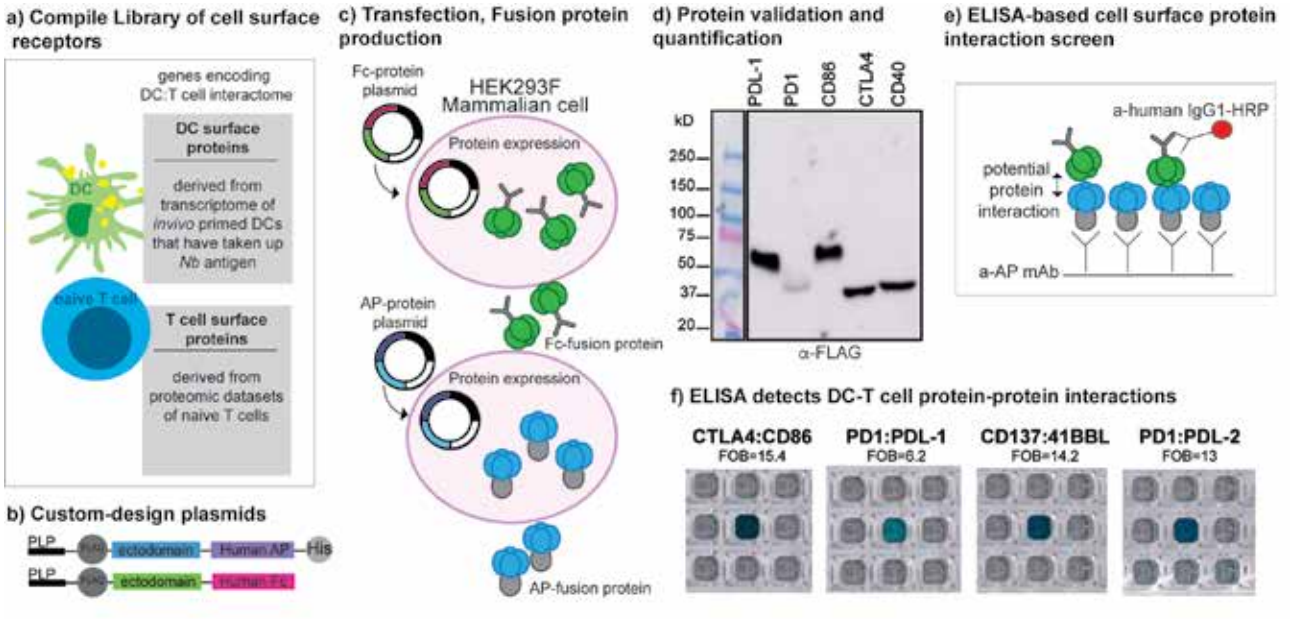


**FIGURE 1:** Large-scale screen of DC-T cell interactions a) Library of cell surface proteins from our RNAseq datasets and proteomic resources b) Plasmid design. c)extracellular domain of gene cloned into two plasmids, secreted products expressed in HEK293F cells. d) Validation via Western blot. e) ELISA assay, plates are coated with a-AP capture mAb and probed for interaction with fusion proteins. ahIgG1-HRP is added to detect captured Fc-proteins. f) Representative images of positive wells (blue) from plates coated with T cell surface protein and probed with DC surface proteins.

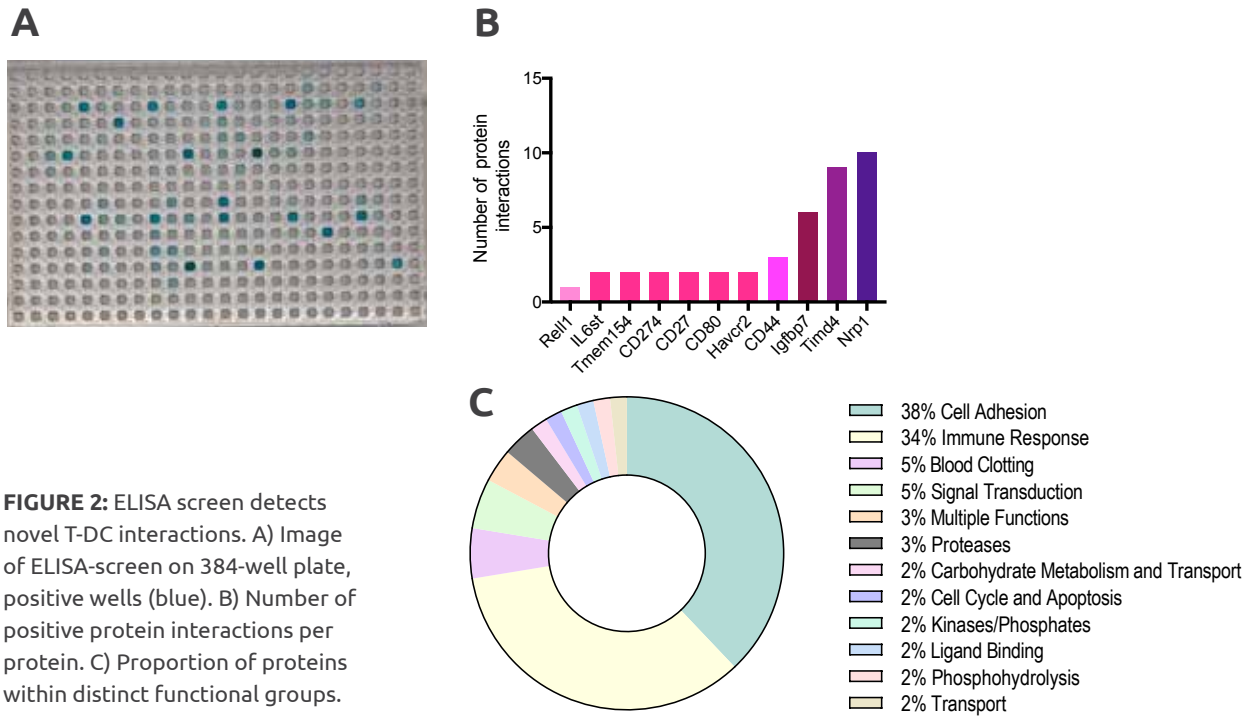


**FIGURE 2:** ELISA screen detects novel T-DC interactions. A) Image of ELISA-screen on 384-well plate, positive wells (blue). B) Number of positive protein interactions per protein. C) Proportion of proteins within distinct functional groups.



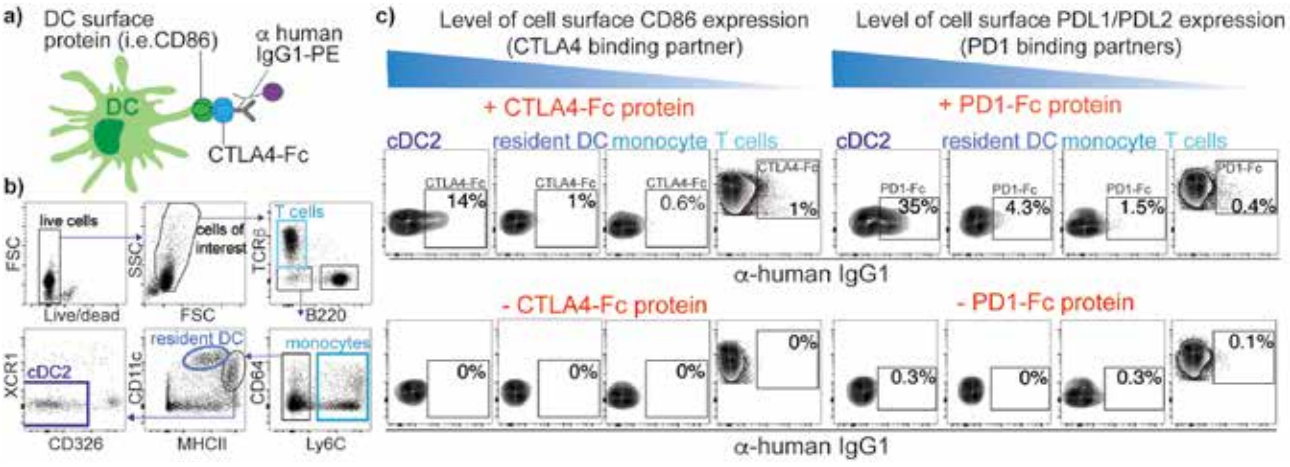


**FIGURE 3:** Fc fusion proteins bind to their binding partner on immune cells. a) Flow cytometry-based method to visualise Fc-fusion protein binding to immune cell surface proteins. b) Gating strategy used to identify distinct immune cell populations. c) Fc-protein labelling: CTLA4 Fc (left), PD1-Fc (right). Lymph node cells from naïve mice were incubated with or without 100uL of supernatant from HEK293F cells transfected with custom designed plasmids. To detect Fc-fusion protein binding, cells were labelled with a α-human IgG1 antibody and cell surface antigens to specific immune cell lineages.

## CONCLUSION

With support from Research For Life we have successfully established and validated a high-throughput ELISA-based screen to identify novel protein interactions between T cells and DC. We also demonstrate that proteins expressed in the lab can interact with their binding partner on the cell surface. Thus, we can take advantage of the fusion proteins that will be generated as a component of the ELISA assay to assess the function of novel receptor-ligand interactions. We are currently developing in vitro assays to faithfully report immune outcome when specific protein interactions are blocked to identify potential therapeutic targets for allergy.

# Victoria University of Wellington

## The serotonergic system intracellular signalling in an animal model of depression

S ALLEN, D CHAJI, B ELLENBROEK, DJ DAY

### KEY FINDINGS

Genetic differences that reduce the expression of the serotonin reuptake transporter (SERT) lead to a reduction in the number of synaptic connections between neurons.

The biochemical pathway that leads to the formation of neuronal connections is altered in animals showing reduced SERT expression.

High serotonin levels lead to serotonylation of proteins but from current data it is not possible to determine the physiological relevance.

An in-house synthesis of a serotonin analogue (5-PT) can be used for monitoring serotonylation in living cells.

### OBJECTIVES

Naturally occurring human polymorphisms (genetic variants) with the promotor region of SERT lead to a low expression variant (S/S) compared with the common (L/L) variant. Using a rat model we sought to determine whether rats that express low levels of SERT through haploinsufficiency of SERT (SERT heterozygotes) differed in their ability to make synaptic connections (synaptic plasticity) and whether any detected differences were correlated with the extent of covalent linking of serotonin to proteins (serotonylation).

A key regulator of synapse formation are members of the Rho family of monomeric GTPase. We sought to determine whether the different SERT haplotypes showed alterations in Rho family GTPase expression or its regulators.

To determine whether mass spectroscopy methods are suitable for detecting serotonylation of GTPase and if they can determine the ratio of serotonylated to non-serotonylated forms.

### METHODS

Primary cell cultures from rat frontal cortex were cultures from wild-type (WT), heterozygous (HET) and homozygous knockout (HOM) SERT rats. Immunocytochemistry was used to identify neurons and dendritic spines by staining for MAP2 and debrin respectively. The number of dendritic spines on a 20 mm section of primary dendrite was enumerated for cells prepared from each SERT genotype.



Serotonylation of proteins was investigated in cultured cells using mass spectroscopy of protein extracts separated on SDS PAGE gels. The region of interest corresponding to the molecular mass of Rho family GTPases was excised and processed for mass spectroscopy analysis. Proteomics analysis of the MS data was undertaken to identify protein fragments and their post-translational modification with serotonin. Additionally, the entire soluble cell contents were subjected to SDS PAGE and the gel divided into molecular weigh bands between 10kDa and 150kDa for further MS analysis to identify serotonylated proteins.

To directly visualize serotonylation in living cells a serotonin analogue, propargylated serotonin (5-PT) was chemically synthesised and characterised. SY5Y cells were treated with 5PT and uptake visualised by fluorescence microscopy. Covalent linkage to proteins akin to serotonylation was determined by SDS PAGE of cell extracts and transfer to low fluorescence PVDF membrane. Labelled proteins were visualised by laser scanning.

### RESULTS

Low SERT expressing animals have fewer dendritic spines than the normal SERT expressors. These data support our hypothesis that serotonin is a regulator of synaptic plasticity in the brain and is consistent with the idea that the depressive phenotype displayed is due to decreased spine formation in the frontal cortex.

Human differentiated SY5Y and U87 MS were cultured and grown in media containing serotonin in the range 1 to 10 µM. Protein extracts were prepared and analysis of proteins between 23 -27kDa identified multiple small GTPase protein including Rac1, RhoA, Cdc42 which are well reported regulators of synaptic plasticity. No serotonylation of these proteins was detected in the range of serotonin concentrations tested. Higher concentrations of serotonin resulted in cell stress followed by cell death upon extend exposure. These data do not support serotonylation as being a physiological relevant regulator of GTPase activity. Further analysis of proteins within the extracts by MS identified minor (<10%) of proteins associated with regulation ribosome function and proteasomal degradation, which is consistent with cellular stress following serotonin exposure.

SY5Y cells were treated with 5-PT in the range 1 – 25 µM. Uptake of 5-PT after 30 min treatment could be detected at all concentrations tested. Internal 5-PT was visualized using click-chemistry to a biotin-azide and detection with streptavidin-Texas red. Figure 1 shows red cytoplasmic fluorescence of internal 5-PT. Separation of the cytosol contents by SDS-PAGE and transfer to PVDF membrane, followed by laser densitometry did not detect any labelled proteins. These data suggest that while 5-PT is taken up into the cells via SERT it is not crosslinked (serotonylated) onto proteins at detectable levels. These results are in concordance with the MS findings in that while serotoynlation can be detected, the products are of low abundance.



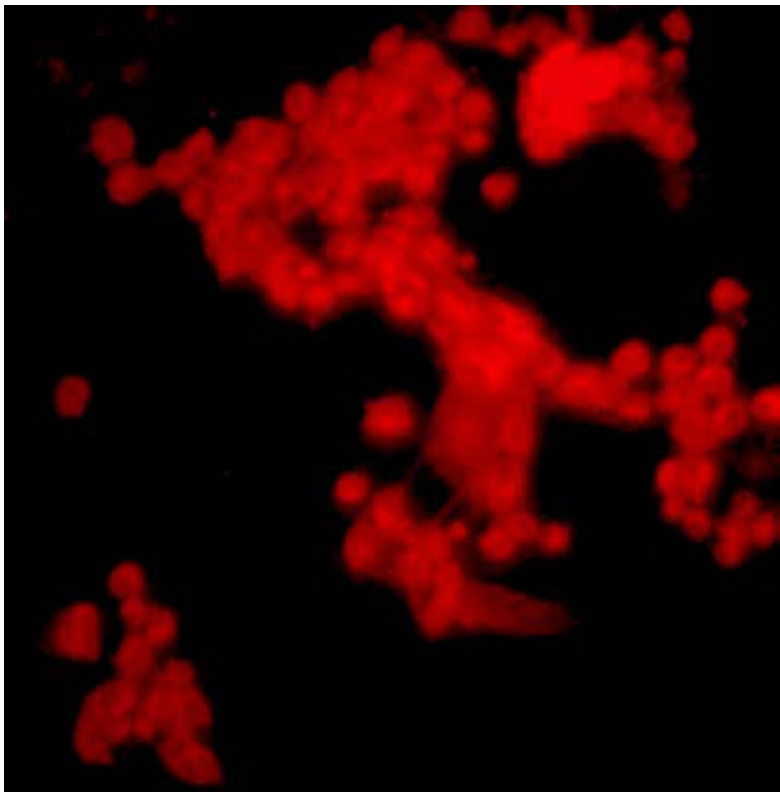

**FIGURE 1:** Uptake and incorporation of propargylated serotonin. Cells were incubated with 10 mM 5-PT for 30 minutes then fixed and 5-PT detected with biotin-azide and streptavidin-Texas red. Cytoplasmic staining of cells indicates uptake via SERT activity.

## CONCLUSIONS

Serotonylation of proteins can be detected using mass spectroscopy methods in cells treated with high concentrations of serotonin as may be encountered at the synapse but not at circulating serum levels of serotonin. LC MS M was able to identify small GTPase protein including Rac1, RhoA, Cdc42 robustly but no serotonylated peptides were found. Our data does not support serotonylation as being physiologically relevant for regulating small GTPase function in neuroplasticity.

# Victoria University of Wellington

## Determining population-level genetic regulators of sphingolipid metabolism that contribute to the diversity of cancer progression in individuals

A MUNKACSI

### KEY FINDINGS

With this support, we were able to generate gene deletion libraries in yeast strains that contain genetic variation comparable to that varies between unrelated human individuals. These libraries were then used to systematically understand the contribution of each gene, one at a time, to the molecular regulation of sphingolipid metabolism. Genes identified as being essential in yeast will be evaluated in future studies for their importance in human cells.

### OBJECTIVES

Sphingolipids are critical structural and signalling molecules in all eukaryotic cells, that when defective in humans, are involved in the onset and progression of many human diseases including neurodegenerative diseases, heart disease, diabetes and many types of cancer. However, the molecular regulation of sphingolipid metabolism is not fully understood. As sphingolipid metabolism is highly conserved from yeast to human cells and the genetic, structural and signalling aspects of sphingolipid are complex, the tractable genetic model yeast (*Saccharomyces cerevisiae*) has provided most of what is known about sphingolipid metabolism. However, the characterisation to date is limited to one genetic background (i.e., one yeast strain equivalent to one human individual). Here we proposed genome-wide analyses to systematically identify major genetic regulators of sphingolipid metabolism in diverse backgrounds.

Here we provide a report on the methods and results for the one aim in our research grant: To identify processes that regulate the response to sphingolipid metabolism perturbation conserved in genetically diverse individuals.

### METHODS AND RESULTS



We screened a curated collection of 1,011 genetic backgrounds for sensitivity to myriocin (an inhibitor of sphingolipid synthesis and thus global disruptor of sphingolipid metabolism), and then selected five genetic backgrounds that showed a large degree of variability relative to the commonly used lab strain S288C that has provided the basis for much of what is known about eukaryotic sphingolipid metabolism. Here, using methods developed in our lab and the five selected strains (CBS1252, CBS7765, CBS1387, CBS3093 and DBVPG1617_5A), we have made progress towards creating gene deletion libraries in these genetic backgrounds. Initially, we sought to use backcrossing methodology developed in our lab (Busby et al, *NPJ Syst. Biol.*, 2019) to generate (4,800 strains in each deletion library). Unfortunately, this methodology has not been able to be used on all five genetic backgrounds; many experiments were conducted to get this method to work in all strains but (ironically) it was variation in genetic markers required for the backcrossing that prevented this method from working in all genetic backgrounds. The backcrossing methodology has only worked for CBS1252 and CBS7765. Currently we have completed three of the six backcrossing steps required to generate each deletion library; subsequent steps are essentially repeats of backcrosses that we have already completed, hence we do not anticipate any problems for CBS1252 and CBS7765. Gene deletion libraries for three other genetic backgrounds (Y55 x S288C, UWOP87 x S288C, S288C x S288C) are being generated by an alternative method (i.e., crossing the non-S288C background with the S288C deletion library to generate a homozygous diploid

deletion library). Via the inclusion of the two deletion libraries made by the backcrossing method (CBS1252, CBS7765), the three deletion libraries made by the crossing method, and also two deletion libraries previously made in our lab by the backcrossing method (UWOPS87, Y55), we will have a diverse collection of deletion libraries with variable sensitivity to myriocin. For example, CBS1252 and CBS7765 are resistant to myriocin, while Y55 and UWOPS87 are sensitive to myriocin. All deletion libraries (a total of 45,000 strains) will be screened with or without myriocin and the growth of all strains will be quantified in treated media compared untreated media. Results will be used to identify genes that buffer the effects of sphingolipid homeostasis perturbation. We will analyse the genes using enrichment and network analysis tools to identify enriched processes that buffer the effects of sphingolipid metabolism perturbation in each background.

## CONCLUSION

We hypothesised that primarily the same cellular processes are required, independent of background, in response to sphingolipid metabolism perturbation, with the complexity that different genes accomplish these same processes. We will test this hypothesis upon completion of the additional crossing and backcrossing to generate the two new deletion libraries that will be evaluated in a viability-based assay. Future research will be conducted in human cells where we investigate homologous genes and pathways for their importance in cellular homeostasis upon sphingolipid stress (e.g., cancer cell lines).

# Institute of Environmental Science and Research (ESR)

## Investigating the mRNA expression of genes differentially methylated in the livers of guinea pigs born prematurely, or at full-term.
(INTERIM REPORT)


**DONIA MACARTNEY-COXSON**

Science Leader. Human Genomics. Institute of Environmental Science and Research (ESR)

**MAX BERRY**

Consultant neonatologist and Senior Researcher, CCDHB and University of Otago, Wellington


### KEY FINDINGS PENDING

Aiming for completion by end of December 2021.

### OBJECTIVE

To investigate the mRNA expression level of candidate genes for which we observed significant differential DNA methylation in the livers of adult guinea pigs born either at full-term, or prematurely.

### METHOD

Samples: Liver from the same 12 animals on which we performed the DNA methylation analyses was available and RNA has been extracted from ~ 10mg of snap frozen sample using a Qiagen RNeasy kit. We have obtained good quality RNA (Agilent Bioanalyser RNA integrity number ≥ 7.5).

RT-qPCR: 500ng total RNA will be reverse transcribed and RT-qPCR performed 2µl 1/5 dilution cDNA. β2 microglobulin (B2M), Beta-actin, and GADH will be used as endogenous controls. The combined mean expression (Ct, cycle threshold) for these controls will be used to normalise target mRNA expression using the ΔCt method (ΔCt = $Ct_{targetmRNA}$ – $meanCt_{controls}$).

Gene selection for RT-qPCR analysis: We decided to focus our mRNA expression analysis on genes: a) in which we had observed significant differential DNA methylation (FDR < 0.05, ≥ 10% absolute DNA methylation difference across a number of CpGs within the gene b) where this differential methylation occurred within or near the transcription start site and/or c) where the literature revealed a potential role in metabolism. We also checked for known expression of the gene in liver (GTeX portal The Genotype-Tissue Expression (GTEx) project, https://gtexportal.org/home/).

### RESULTS

The RT-qPCR analysis is pending therefore there are no results to report at this stage. However, we hope to be able to submit a final report on this to Research for Life by end of 2021.

A manuscript containing the background and rational for this research along with the DNA methylation analysis has been drafted. Once the RT-qPCR data is generated we hope to proceed swiftly to submitting the manuscript for publication.


We are very grateful to Research for Life for providing the funding ($5165.00) for the consumables (RT-qPCR reagents and primers) to fund this research. All reagents have been purchased and analysis is pending.

# University of Otago, Wellington

## Improving Adherence to a Reduced Carbohydrate Diet for Women with Gestational Diabetes

T AMATAITI, F HOOD, J KREBS, M WEATHERALL, R HALL



### KEY FINDINGS

Many women diagnosed with Gestational Diabetes Mellitus (GDM) are reducing their intake of carbohydrates (CHO) on their own accord to improve blood glucose control. However, the acceptability, safety, and effectiveness of reduced CHO intake on pregnancy outcomes is uncertain. This feasibility study demonstrated that women with GDM can follow a reduced CHO diet of 136g/day over a short period of 8 weeks without compromising dietary intake of fibre, vitamin, and minerals.

### OBJECTIVE

The aim of this feasibility intervention study was to provide information to support a full study to look at: (1) does providing fruit and vegetables to women with GDM at no cost, increase adherence to a reduced CHO diet and (2) is adequate vitamin and mineral dietary intake maintained when following a reduced CHO diet.

### METHODS

Women were included in the study if they had been diagnosed with GDM according to national criteria NZ Screening Diagnosis and Management of GDM and presented to one of the Diabetes in Pregnancy clinics in the Wellington Region at less than 32 weeks' gestation, a singleton pregnancy and aged >18 years. Potential participants were excluded if they had Type 1 or Type 2 Diabetes.

The intervention was integrated into usual clinical care to avoid excess burden on participants. As per standard care, at the first clinic visit, all women received diet and lifestyle advice delivered by the Dietitian that followed the NZ Food and Nutrition Guidelines for Pregnancy and Breastfeeding. Women were also encouraged to keep physically active and monitor their blood glucose levels. As part of standard care women were routinely reviewed by Obstetricians, Diabetes Physicians, Diabetes Specialist Nurses and Midwives.

Women were screened and eligible participants were approached during their first appointment by recruiters. Once informed consent was obtained, the baseline 4-day food diary and a pregnancy specific physical activity questionnaire was completed. Women were then given personalised dietary advice on how to follow a reduced CHO diet of 120-135g per day, while continuing to follow a healthy diet for pregnancy. Following this, participants were randomised to either (i) a weekly fruit and vegetable bag provided at no cost, (ii) a weekly supermarket voucher of similar value provided at no cost or (iii) no additional intervention beyond standard care.

On a weekly basis woman completed a 24-hour food diary that was reviewed by a research dietitian to provide support to follow a reduced CHO diet. Additional clinical measures collected were part of standard care, this included height, weight, ketones, HbA1c and blood pressure. At 36 weeks' gestation participants were asked to record a 4-day food diary, complete a pregnancy specific physical activity questionnaire and a questionnaire on the acceptability of following a reduced CHO diet.

The study was performed following the Declaration of Helsinki and ethical approvals were obtained from the NZ Health and Disability Ethics Committee (Ref18/NTB/236 AMO1), and the research ethics groups at the hospital sites. The study was registered with the Australia, NZ Clinical Trials Registry (ACTRN12619001406190).

### DATA ANALYSIS

Food diary data was analysed using FoodWorks (Professional version 10.0 Xyris Software package, Brisbane, Australia).

ANCOVA generalised linear mixed model analysis was used to compare between the three intervention arms and paired t test to compare within groups. SAS version 9.4 (SAS Institute, Cary, NC, USA) was used for all analyses.

## RESULTS

Of the 76 women enrolled in the Diabetes in Pregnancy clinics from the 4th of March to the 3rd of June 2020 and screened for the study, 42 women were eligible and invited to join the study and 20 women consented to take part in the study.

For all 20 participants the mean (range) age of the women was 34.7 (24 to 44) years and the mean gestational age of women when randomised to the study was 30.9 weeks. The mean BMI was 28kg/m2. Self-reported ethnicity comprised of Māori (5%), Pacific (10%), Indian/South Asian (15%), Other Asian (20%) and European (45%). This reflected the ethnicity profile of our clinic. In total 40% of women lived in households within the least deprived areas (score 1 to 2), and 25% of women lived in households within the most deprived areas (score 9 to 10). There were no cases of COVID-19 virus reported in this cohort of women, with 1500 confirmed cases COVID-19 virus in NZ, during the same period.

Figure 1 shows dietary intake of carbohydrate (p = 0.01) and sugar (p = 0.05) was significantly reduced when women with GDM followed a reduced CHO diet, compared to baseline. There was no significant change in dietary intake of protein, fat, and fibre.

Figure 2 shows women with GDM were able to follow a reduced CHO diet of 136g per day and providing weekly fruit and vegetables or supermarket vouchers at no cost made no difference to adhering to the reduced CHO diet. In addition, compared to baseline there was no significant difference in vitamin and mineral dietary intake and no increased episodes of ketones when following a reduced CHO diet. Findings from the survey suggested overall women with GDM found it moderate to very easy to follow a reduced CHO diet.

## CONCLUSION

This feasibility study demonstrated that women with GDM found it moderate to very easy to follow a reduced CHO diet of 136g per day over a short period of 8 weeks without compromising dietary intake of fibre, vitamin, and mineral intake. Future research is required to establish the effect of women with GDM following a reduced CHO of 136g per day on pregnancy and baby outcomes.



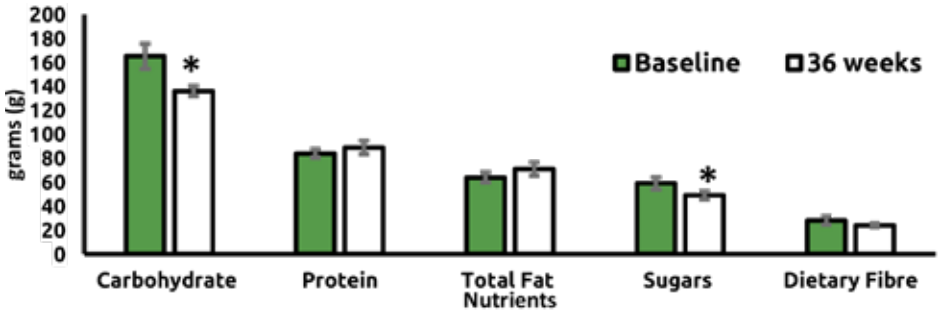


**FIGURE 1: Dietary intake of nutrients at baseline compared to during the intervention.**

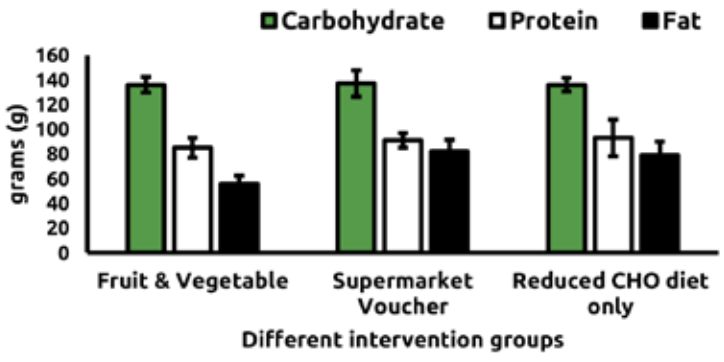


**FIGURE 2: The average intake of carbohydrate, protein and fat when following a reduced CHO diet for all 3 intervention groups.**

# University of Otago, Wellington

## The Extracellular Vesicle Profile of Cardiac Fibroblasts Stimulated with a Pro-inflammatory or Pro-fibrotic Stimulus *in vitro*



M BRUNTON-O'SULLIVAN, A HOLLEY, K DANIELSON, P LARSEN

### KEY FINDINGS

- Extracellular vesicles produced by cardiac fibroblasts comprise an array of unique microRNAs under control (no-treatment), pro-inflammatory (TNF-α stimulation) and pro-fibrotic (TGF-β1 stimulation) conditions.
- When stimulation conditions were compared using differential gene expression analysis, 16 significant differences in microRNAs were observed in extracellular vesicles from cardiac fibroblasts.
- These findings suggest that cardiac fibroblast synthesis of extracellular vesicles can be influenced by pro-inflammatory and pro-fibrotic stimulation *in vitro*.
- The extracellular vesicle microRNAs identified in this study require further investigation into the functional significance of their differential synthesis in the cardiac microenvironment.

### OBJECTIVES

Cardiac fibroblasts are an important cellular component of the heart, and regulate optimal repair following a heart attack. Early cardiac repair is described by upregulation of pro-inflammatory factors within the cardiac microenvironment followed closely by upregulation of pro-fibrotic factors. Cardiac fibroblasts are sensitive to this dynamic microenvironment and have been shown to alter their activity under these conditions. Extracellular vesicles are small particles secreted by all cell types, and contain unique genetic material, such as microRNAs, which enable cells to participate in crucial cellular signalling processes.

Cardiac fibroblasts secrete extracellular vesicles, but how the microRNAs comprised in extracellular vesicles change under early cardiac repair conditions remains unknown. Understanding the genetic make-up of cardiac fibroblast extracellular vesicles under pro-inflammatory conditions versus pro-fibrotic conditions will provide important information regarding the role of the cardiac fibroblast in co-ordinating repair processes following a heart attack.

Thus, we aimed to identify how the microRNA comprised in extracellular vesicles of cardiac fibroblasts is altered under pro-inflammatory and pro-fibrotic conditions in cell culture.

### METHODS

Cardiac fibroblasts were isolated from human heart tissue that was generously donated by patients undergoing heart surgery at Wellington Regional Hospital. Cardiac fibroblasts were grown in cell culture conditions and were stimulated with either tumour necrosis factor-alpha (TNF-α; pro-inflammatory), transforming growth factor beta (TGF-β1; pro-fibrotic) or no-treatment (control). Extracellular vesicles were isolated from cell culture supernatant following stimulation, and RNA was extracted. RNA sequencing libraries were prepared from extracellular vesicle RNA, and RNA sequencing was performed using Illumina Hiseq 2000. RNA sequencing analysis was performed by the Australian Genome Research Facility (AGRF). The unitas pipeline was undertaken for feature annotation and quantification, and differential gene analysis was performed using edgeR.

### FINDINGS

Cardiac fibroblasts were successfully isolated from cardiac tissue and were grown in cell culture for short term testing (Figure 1A). In total, 1,050 unique microRNAs were identified across all of the samples (Figure 1B). Specifically, 492 microRNAs were identified in extracellular vesicles from

control cardiac fibroblasts, while 475 and 618 microRNAs were identified in extracellular vesicles from TNF-α (pro-inflammatory) and TGF-β1 (pro-fibrotic) stimulated cardiac fibroblasts, respectively. To assess the microRNA diversity between stimulation conditions, we created a Venn diagram to explore microRNA composition (Figure 1C). Of the total 1,050 microRNAs, the expression of 164 was shared between stimulation conditions. Extracellular vesicles from cardiac fibroblasts stimulated with TGF-β1 had the largest number of exclusively expressed microRNAs (n=307), while a similar number of microRNAs were observed in extracellular vesicles from control (n=185) and TNF-α (n=187) stimulated conditions. Control and TGF-β1 conditions shared 81 microRNAs, control and TNF-α stimulation conditions shared 60 microRNAs, and 66 microRNAs were shared between TNF-α and TGF-β1 conditions.

Differential gene expression analysis was performed to identify which of these microRNAs were significantly different between stimulation groups. Three analyses were performed: TNF-α versus control (Figure 2A), TGF-β1 versus control (Figure 2B) and TGF-β1 versus TNF-α (Figure 2C). MicroRNAs are denoted by letters, as ongoing analysis is still taking place ahead of publication. In total, 16 microRNAs were shown to be significantly altered between stimulation groups. Twelve microRNAs were unique, and four microRNAs were shared across stimulation groups (microRNAs A, D, F & G).

## CONCLUSION

These novel findings are the first of their kind and suggest that cardiac fibroblasts release extracellular vesicles differentially under pro-inflammatory versus pro-fibrotic conditions in vitro. How these differences in microRNA composition influence the cardiac microenvironment is the next step for this research. Additionally, further research is required into how these extracellular vesicles could be harnessed as liquid-based biomarkers in the setting of cardiovascular disease.

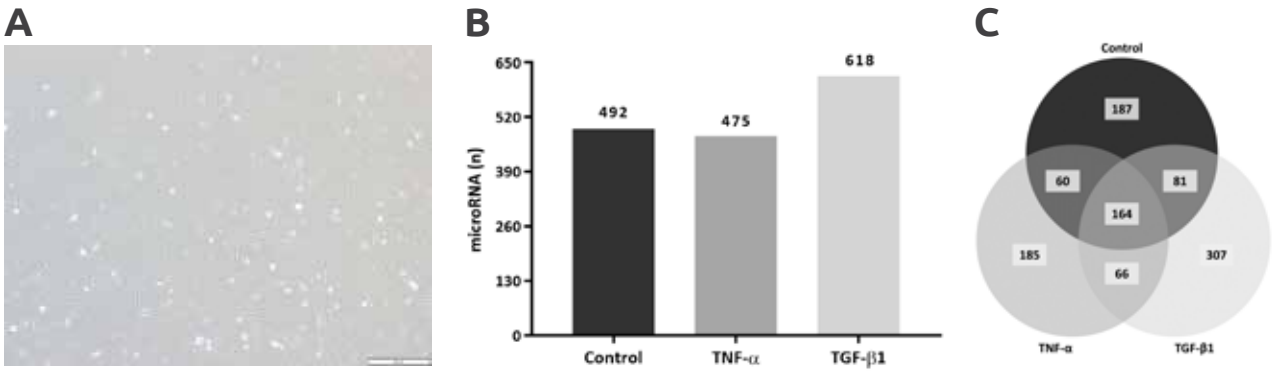


**FIGURE 1: Cardiac fibroblasts express extracellular vesicles which contain microRNAs.**
Phase contrast microscopy of cardiac fibroblasts under experimental conditions (A), The number of microRNAs (n) identified in cardiac fibroblast extracellular vesicles under each condition (B), a Venn diagram of microRNA targets shared between, or exclusively expressed, under each condition.

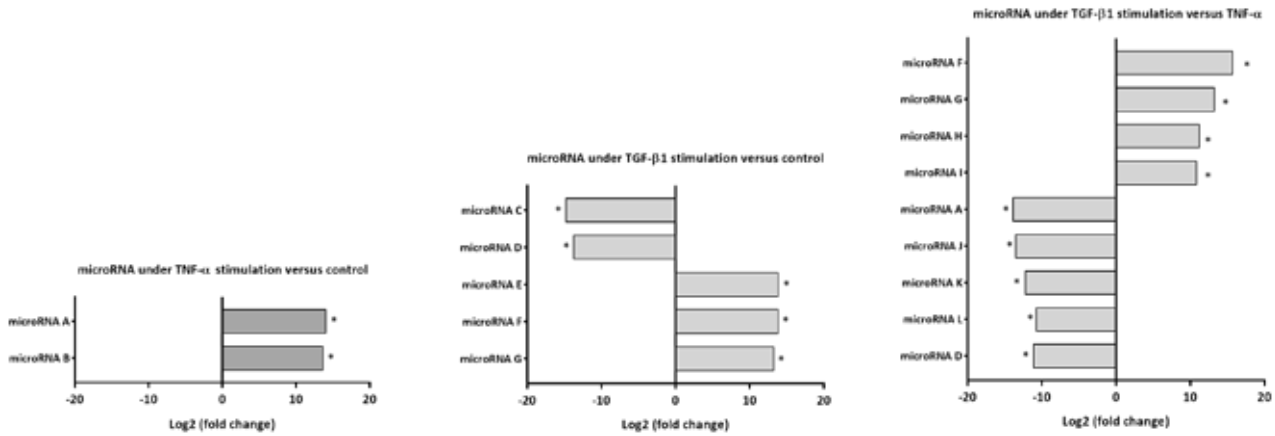


**FIGURE 2: Sixteen microRNAs were differentially expressed between stimulation conditions.**
Log2 fold-change of significantly different microRNAs using edgeR differential gene expression analysis under TNF-α versus control conditions (A), TGF-β1 versus control conditions (B) and TGF-β1 versus TNF-α conditions (C). Asterisks represent microRNAs with a false-discovery rate (FDR) of ≤ 0.05.

# University of Otago, Wellington

## Determining the clinical utility of a monocyte inflammatory score for predicting infarct expansion in patients with Acute Myocardial Infarction (AMI)

### (INTERIM REPORT)

**K HALLY[1,2], A HOLLEY[1], L FERRER-FONT[3], S HARDING[4], A SASSE[4], P LARSEN[1,2]**

[1] University of Otago, Wellington;
[2] Victoria University of Wellington;
[3] Malaghan Institute of Medical Research;
[4] Department of Cardiology, Wellington Regional Hospital



### KEY FINDINGS

Within this study, we aim to identify novel inflammatory biomarkers that can identify patients that are at-risk of adverse heart repair after experiencing a heart attack. We are particularly interested in measuring the activation status of monocytes, which are a subset of blood inflammatory cells that are involved in driving inflammation during a heart attack.

### PROJECT OVERVIEW AND AIMS

Heart attacks are a leading cause of death and disability in New Zealand and, for those that survive, life after a heart attack is far from free of health complications. The heart starts to repair soon after a heart attack but, in some patients, the quality of this repair process is compromised. This can result in growth or expansion of the region of the heart which is damaged during a heart attack (known as the 'infarct'). Currently, we do not know how to identify, and therefore treat, patients whose heart does not repair optimally.

We think that monitoring inflammation may be an effective way of identifying these high-risk individuals, because high levels of inflammation can drive infarct expansion. More specifically, we are interested in monitoring the activation status of monocytes, which are important blood immune cells that participate in inflammation during a heart attack. Moreover, we can monitor these monocytes within blood, which is a relatively non-invasive way of collecting this information (a 5 mL venous blood draw).

Within this study, we aim to identify particular features of monocytes which, if present within an individual, indicates infarct expansion. We will proceed to validate these biomarkers in an independent cohort of patients, with the ultimate goal of providing an inflammation-based biomarker score to direct appropriate treatment courses to patients suffering from a heart attack.

### METHODS

In order to achieve this aim, we have broken this study into three parts:

1. We are recruiting patients admitted to Wellington Regional Hospital with a heart attack. We aim to recruit 60 patients in total. We are drawing blood from these patients each day from enrolment to discharge (on average, 2-3 blood draws per patient). We are measuring monocyte immune status in these blood samples using full spectrum flow cytometry, which is a technique used to measure the physical characteristics of a cell type of interest. Each of the physical characteristics of the monocyte that we measure represents a potential biomarker of infarct expansion.
2. We are also asking our enrolled patients to return to hospital at between 7-10 days after their admission for a cardiac MRI. Within each patient, we will use the MRI data to measure the extent of infarct expansion, which

is outcome that we are using to indicate compromised heart repair.

3. We will then test which individual biomarkers, or combination of biomarkers, best predicts those patients that have significant infarct expansion.

## FINDINGS TO DATE AND PLAN FOR COMPLETION

Recruitment of our patient group is underway, and we are projecting that recruitment will continue through to a finish date in December 2022. From the patients recruited so far, we have successfully: 1) designed a laboratory protocol for measuring and analysing monocyte immune status using full spectrum flow cytometry, and 2) designed a cardiac MRI protocol for measuring infarct expansion. We continue to research and optimise a statistics protocol, which will allow us to successfully analyse the data generated from this study. Some aspects of this statistical analysis will be ongoing during patient recruitment, and we aim to complete analysis within the first quarter of 2023.

## CONCLUSION

We have successfully designed a series of protocols which allow us to assess monocyte activation status in blood samples and analyse the degree of infarct expansion in patients with a heart attack. We are currently in the patient recruitment phase of this project (due for completion December 2022), and aim to complete statistical analysis project within the first quarter of 2023. We would like to acknowledge both Research For Life for funding the blood processing portion of this study, and Lottery Health Research for funding the cardiac MRI portion.

# University of Otago, Wellington

## Biomarkers and their Relationship to Acute Brain Injuries in the Emergency Department. The BRAIN Study.



A ROGAN, A SIK, D MCQUADE, P LARSEN

### KEY FINDING

When blood is drawn within 6 hours of head injury, S100B has good diagnostic ability to rule out significant intracranial pathology (bleeding, swelling, skull fracture) seen on head CT scans in patients that present to the emergency department (ED) with traumatic brain injury (TBI). The negative predictive value was 96.9%.

### AIM

To investigate if clinical biomarkers (S100B) can safely rule out significant intracranial injuries in adult patients who present to ED with TBI.

### METHODS

**Study Design and Setting**

This was a prospective pilot observational study conducted in Wellington Regional and Hutt Hospital EDs. Patients were recruited using a consecutive sampling method.

**Eligibility**

Inclusion Criteria: Able to give informed consent; patients ≥ 18 years of age; presenting within 24 hours of suspected acute brain injury and a non-contrast CT head was performed to exclude TBI.

Exclusion criteria: Patients <18 years of age; patients presenting >24 hours of suspected injury or with an unknown time of onset predicted to be >24 hours; pre-existing acute brain injury at time of presentation, such as previous traumatic brain injury or recent stroke; pre-existing neurological condition such as dementia, acute psychosis, MS, MND or Unable to give informed consent.

**CT Head Scan**

Patients presenting within 24 hours of acute TBI were assessed by ED clinicians in line with current best clinical practice and standards. If a CT head was deemed appropriate by the treating clinician patients were screened for eligibility and informed written consent was obtained by the research investigators. Patients proceeded to their CT head scan according to local ED guidelines. A CT scan was recorded as positive in the presence of any of the following findings: skull fracture, pneumocephalus, intracranial haemorrhage or contusion, traumatic infarction, diffuse axonal injury, signs of herniation or any other acute traumatic intracranial pathology that requires admission to hospital for observation or surgical intervention.

**Blood Sampling**

A venous blood sample was drawn at the time of study enrolment into an SST tube and stored in a fridge below 4 degrees Celcius. Within 24 hours blood was centrifuged at 3000rpm for 10 minutes. Serum was then pipetted into aliquots and stored in four Eppendorf tubes and frozen at -80 degree Celcius in as the University of Otago Wellington CTP laboratory.

**S100B Testing**

S100B was batch tested in the SCL laboratory in Wellington Regional Hospital using a Cobas Elecsys S100 module. Remaining serum samples have been stored in a biobank. Regarding the results, an S100B >0.1ug/ml is the validated threshold level to indicate a positive S100B test. So ideally patients with an S100B <0.1ug/ml would not have a significant intracranial finding on CT head scan.

**Data Collection**

Data was collected to capture baseline demographics, co-morbidities, medications, TBI mechanism, time of injury, presenting features, clinical findings, CT head results, ED length of stay and ED disposition. CT head reports were obtained from the clinical reporting systems as reported by

the duty or on call radiologist to replicate the true clinical setting. Data was inputted to a REDCap database hosted by the University of Otago. Data was analysed using SPSS software.

**Primary Outcome**

To determine the diagnostic accuracy (sensitivity, specificity and negative predictive value) of S100B in patients with mild TBI who meet the criteria for CT head, using acute pathology on head CT (positive vs negative CT) as the primary endpoint.

**Secondary Outcome**

To compare the diagnostic accuracy of S100B patients with mild TBI who meet the criteria for CT head, using acute pathology on head CT as the primary endpoint, when S100B is obtained within 6 hours compared to 24 hours of injury.

## RESULTS

To date 281 patients out of a target 300 have been recruited to the pilot study. There are 216 patients that have been recruited from Wellington ED and 65 patients that have been recruited from Hutt ED.

**CT Head Results**

Of those recruited 35/281 (12%) have a positive CT head result. Regarding the two EDs, 28/216 (13%) and 7/65 (11%) had positive CT head results from Wellington and Hutt respectively.

**S100B Results**

From the samples obtained, 270 were suitable for S100B analysis; 206 from Wellington and 64 from Hutt. The median S100B level for those with positive CT head scans was 0.172ug/L (IQR 0.106-0.440) compared to 0.114ug/L (IQR 0.069-0.222), p=0.005. Regarding diagnostic accuracy of S100B as a rule out test for significant intracranial injury, sensitivity was 79.4%, specificity was 43.4% and negative predictive value was 93.6%.

**S100B Results within 6 hours**

There were 118/270 (44%) samples collected within 6 hours of injury, 84 (41%) from Wellington and 34 (52%) from Hutt. The median S100B level for those with positive CT head scans was 0.444ug/L (IQR 0.164-0.633) compared to 0.147ug/L (IQR 0.090-0.305), p=0.002. Regarding diagnostic accuracy of S100B as a rule out test for significant intracranial injury, sensitivity was 92.3%, specificity was 29.5% and negative predictive value was 96.9%.

## CONCLUSION

This pilot data demonstrates that S100B has potential to be used clinically to exclude intracranial injury seen on CT head scans in patients that present to ED with TBI. Blood drawn within 6 hours of injury had much better diagnostic accuracy compared to blood drawn within 24 hours. Further statistical analysis of the data is required to explore how other factors relating to patients and their presenting features affect the diagnostic ability of S100B. A larger prospective clinical trial is now underway to further explore the use of S100B as a rule out test in ED.

# Malaghan Institute of Medical Research
# Victoria University, Wellington

DEPARTMENT OF PATHOLOGY
# University of Otago, Dunedin


# Δ133p53: A driver of prostate cancer through inflammation


A W BRAITHWAITE, C DRUMMOND, M-S FABRE




## KEY FINDINGS

Cloned genes for mouse and human p53 isoforms, engineered cell lines to make these isoforms and identified some genes that are regulated by the isoforms.

## BACKGROUND

In contrast to the tumour suppressor functions of p53, we have shown that an isoform family of p53, called Δ133p53, actually promote cancer (1). In addition, studies, using unique transgenic mice expressing a mimic of D133p53, known as Δ122p53, showed that tumour progression is driven by inflammation (2). Furthermore, studies on human prostate and colorectal cancers showed that those with high Δ133p53 levels also had infiltration of tumour-promoting immune cells (2,3). Thus, the goal of our project was to test the hypothesis that Δ133p53 promotes immune cell infiltration into tumours thereby increasing their aggressiveness.

## AIMS

1. To generate mouse prostate cancer cell lines over-expressing Δ122p53
2. Identify genes regulated by Δ122p53/ Δ133p53
3. To make syngeneic grafts of these tumour cells in mice
4. Analyse the tumours for immune cell infiltration (and determine if the tumours were more aggressive compared to controls; this was a supplementary goal).

## RESULTS

1. We successfully generated the constructs making both the mouse Δ122p53 and human Δ133p53 isoforms that also contained fluorescent markers so we could track the cells after engraftment.
2. We successfully generating several mouse prostate cancer cell lines with the Δ122p53 and analysed them looking for expression of downstream immune genes as potential Δ122p53/ Δ133p53 targets genes. We also did some preliminary growth assays on these cell lines to show they proliferated faster than the controls as we had shown this in previous publications.
3. In parallel we also generated human prostate cancer cells expressing two Δ 133p53 isoforms Δ133p53α and Δ133p53β) and began looking at the effects of potential targeting agents/collaboration with Dr Joanne Harvey at Victoria University to start thinking about designing novel inhibitors.

## CONCLUSION

Not long after that work was done, the lab ceased to operate due to the current SARS-CoV2 pandemic, so we were not able to do any animal work. We still have the cell lines and have done some further characterization in the lab in Dunedin. Dr Harvey has done some *in silico* modelling of the Δ133p53 protein structure. We have submitted joint grant applications to the HRC to continue the work begun under the Research for Life grant and further the modelling studies.

## REFERENCES


1. Slatter TL, Hung N, Campbell H, et al. Hyperproliferation, cancer, and inflammation in mice expressing a Delta133p53-like isoform. Blood 2011;117:5166-5177.
2. Campbell H, Fleming N, Roth I, et al. 133p53 isoform promotes tumour invasion and metastasis via interleukin-6 activation of JAK-STAT and RhoA-ROCK signalling. Nature communications 2018;9:254.
3. Kazantseva M, Mehta S, Eiholzer RA, et al. The Δ133p53β isoform promotes an immunosuppressive environment leading to aggressive prostate cancer. Cell death & disease 2019;10:631.

# RESEARCH PROJECT & TRAVEL GRANTS

# Research Project Grants 2021

**THE FOLLOWING PROJECTS HAVE BEEN APPROVED FOR FUNDING IN THE FINANCIAL YEAR ENDED 30 JUNE 2021, AND WILL BE REPORTED ON IN SUBSEQUENT RESEARCH REVIEWS.**

| | |
|---|---|
| ## Whole exome sequencing in primary hyperparathyroidism: prevalence of predisposing germline mutations and application in clinical practice<br>**DR RICHARD CARROLL** | Dr Carroll, an endocrinologist at Wellington Regional Hospital and Hutt Valley Hospital, was granted up to $5,565 to undertake, with colleagues, a study assessing the prevalence of inherited genetic mutations predisposing to primary hyperparathyroidism using whole-exome sequencing in New Zealanders. Patients with primary hyperparathyroidism are at increased risk of osteoporosis and fractures, kidney stones, and cardiovascular disease. The condition is typically treated with surgical removal of the abnormal parathyroid gland. Up to 60% of selected patients with primary hyperparathyroidism will have a predisposing genetic mutation, and identification of these patients significantly changes management strategies. Early detection of these predisposing variants in New Zealand hyperparathyroidism patients will be extremely important for determining clinical treatment and type of surgical intervention, potentially saving patients an invasive operation. Results from this project have the potential to impact on clinical practice in New Zealand and overseas. |
| ## The immunomodulatory role of tumour-derived extracellular vesicles in colorectal cancer<br>**OLIVIA BUCHANAN** | Olivia Buchanan, a PhD student at the Department of Surgery and Anaesthesia at the University of Otago, Wellington, was granted up to $18,889 to investigate the immunomodulatory role of tumour-derived extracellular vesicles (tEVs) in colorectal cancer (CRC), with a specific focus on antigen presenting cells (APCs). EVs have been identified as significant mediators of carcinogenesis and are an attractive novel therapeutic target for disrupting tumour signalling. Continued research to elucidate the immunomodulatory role of tEVs could provide avenues for therapeutic intervention, potentially through interference of EV-mediated signalling. Such therapies could include targeting or re-educating macrophages or dendritic cells towards an anti-tumorigenic phenotype. This is particularly important for colorectal cancer, as New Zealand has one of the highest incidence rates of colorectal cancer worldwide and it is essential to explore novel therapies to minimise the global burden of this disease. |

## I get by with a little help from my friends: how do mesenchymal stromal cells enhance survival of cancer cells after therapy?

**DR MELANIE MCCONNELL**

Dr McConnell, a Senior Lecturer in Biology and Genetics at Te Herenga Waka – Victoria University of Wellington, was granted up to $19,050 to investigate a new mechanism by which cancer cells can resist therapy. Dr McConnell's group have observed that cancer cells treated with chemotherapy can survive that therapy if they physically interact with a type of non-cancer cells commonly found in their environment. If research can understand how cancer cells 'co-opt' these normal cells to enhance their own survival, then researchers can work out how to prevent this from happening. This would then improve the efficacy of anti-cancer therapy.

## A sticky situation: investigating meningococcal adherence during carriage

**DR JOANNA MACKICHAN**

Dr MacKichan, a Senior Lecturer in Medical Microbiology at Te Herenga Waka – Victoria University of Wellington, was granted up to $20,000 to investigate meningococcal adherence during carriage. *Neisseria meningitidis* is a bacterial pathogen that is usually carried asymptomatically in the upper airway tissues, but occasionally can cause severe, invasive meningococcal disease. Early interactions between the bacteria and the host mucosal airway tissues remain poorly understood, even though it can lead to invasive disease or pathogen transmission to new hosts. Our previous work identified a meningococcal protein important for the adherence of the bacteria to host airway cells. While this protein was previously thought to only play a role in acquiring host iron, we have demonstrated that it is important for adherence in the host. We have further shown that this protein manipulates host cells, impairing their ability to migrate during wound healing, which may further predispose toward the development of invasive disease. The aims of our study are to determine how this protein mediates attachment to the host, identify the parts of the protein that play a role in adherence, and determine host proteins it is binding to. These findings will be relevant for understanding how harmless carriage of meningococci can progress to invasive disease and will lay the foundation for the development of improved vaccines to prevent asymptomatic carriage of meningococci.



## Novel biomarkers for endometrial cancer

**DR CLAIRE HENRY**

Dr Henry, a research fellow in the Department of Obstetrics, Gynaecology & Women's Health at the University of Otago Wellington was granted up to $27,840 to investigate novel biomarkers for endometrial cancer. New Zealand Aotearoa has one of the highest rates of endometrial (uterine) cancer, and to our detriment, this is rising in young women. The Mirena is a long-acting reversible contraceptive device that has gained traction as a possible alternative treatment option to surgery for women with endometrial cancer. However, early evidence shows 1 of 3 women will have progression of their disease whilst using the Mirena. Our research will investigate whether a blood test using novel biomarkers can determine who will benefit most from this or who will still need surgery as a first line of care.

## Characterizing B Cells Within Epicardial Adipose Tissue of Patients with Cardiovascular Disease

**GEORGINA BIRD**

Victoria University of Wellington PhD Student, Georgina Bird was granted up to $15,318 from Research for Life to help advance our understanding of the responses of the immune system in heart attack patients. Heart disease is the leading cause of death and morbidity in New Zealand. Georgina's PhD research explores how a patient's immune system responds and reacts in the heart tissue following a heart attack. Specifically, she focuses on how the B cell, an important central immune cell, may be involved in changing the immune response and how this effects healing and long-term clinical outcomes following a heart attack. This research is a collaborative project with the Wellington Cardiovascular Research Group and The University of Otago, Wellington.

## Assessing the extent of genomic instability in cell lines that model malignancies

**ANDREW WILSON**

Andrew Wilson, a PhD student at University of Otago, Wellington, was granted up to $10,900 for his project to assess the extent of genomic instability in cell lines that model malignancies. Cell lines that model cancer are commonly used tools in a researcher's arsenal to help uncover the mechanisms behind the disease, enabling the discovery of novel cancer therapies. As cell lines can supply a researcher with an infinite supply of biological material for their experiments, it is imperative that these cancer cells are comprised of a genomic landscape comparable to their human cancer counterpart. Therefore, there is a need to assess the extent of genomic instability in cell lines that model cancer with the hope that they do not deviate considerably from their initially observed genetic landscape reported by suppliers.



## Safely improving outcomes for babies after birth planned by caesarean section in Wellington

**DR JUDY ORMANDY**

Dr Ormandy, an Obstetrician and Gynaecologist at CCDHB, was granted up to $9,275 to support research aimed at safely improving outcomes for babies born by planned caesarean section (CS). Each year, 7500 babies are born by planned CS (around 1 in every 10 births) including 450 babies in Wellington. Planned CS increases the risk of baby being admitted to the neonatal unit for breathing support. Antenatal corticosteroids may reduce this, but evidence is limited. The C*STEROID Trial will obtain high-quality evidence on the use of corticosteroids before planned CS, informing future care in New Zealand and around the world.

## Explore the Immunomodulatory Effect of Nalfurafine on Mast Cell Activation and Effector Functions In Vitro

**HASANAH HAMIZAN**

Hasanah Hamizan was granted up to $14,086 to unpack the potential benefits of the drug nalfurafine for Multiple Sclerosis (MS) treatment. Hasanah's research will explore the little-known mechanisms and immune response (particularly mast cell activity) involved during nalfurafine treatment of MS in animal models. Hasanah is a first year PhD student working under the supervision of Prof Anne La Flamme in the School of Biological Sciences and Centre for Biodiscovery at Victoria University of Wellington.

## Involvement of FoxP3+ Regulatory T Cells in Nalfurafine-Mediated Remyelinatoin of the Central Nervous System

**MACKENZIE KIERNAN**

Mackenzie Kiernan, a PhD candidate in Immunology at Victoria University of Wellington, was granted up to $14,518 to undertake research to help find a treatment for multiple sclerosis (MS). MS incidence in New Zealand continues to increase. MS results in the breakdown of nerves leading to significant pain and paralysis in individuals affected. The aim of Mackenzie's research is to determine the mechanisms promoting restoration of nerve conduction, aiding in the production of safe and effective treatment for this disease.

# Travel Grant Report

## CLAIRE HENRY

### NEW ZEALAND GYNAECOLOGICAL CANCER GROUP (NZGCG) SCIENCE SYMPOSIUM AND ANNUAL MEETING, CHRISTCHURCH

This two-day event brought together a range of NZ scientists and clinicians specialising in gynaecological cancer to discuss updates of current translational research that may improve the lives of women with these diseases. I was invited to present my research on the molecular profile of endometrial (uterine) cancer and showcase the programme of research I lead at the University of Otago Wellington.

I am very grateful to the Wellington Medical Research Foundation for their support and the opportunity to attend this event, which has been invaluable in building relationships in the field, learning from our country's experts and establishing my research group as an early career scientist.

Because of the overwhelming need for collaborative translational scientific research in gynaecological cancer, and strength of many researchers in Aotearoa, Dr Mak Sawar at the University of Otago Christchurch formed an additional scientific meeting to support the NZGCG annual meeting this year in May. Therefore, the concept was to highlight, network and discuss important clinical translational research around our country at the scientific meeting (6th May), and then discuss clinical practice the following day (7th May).

I was invited to present my research, 'molecular profile of endometrial cancer in NZ women' at this meeting. Whilst the molecular profile of endometrial cancer has been extensively published on, we do not know the distribution in NZ women, nor do we know how this can be feasibly incorporated into our clinical pathway and personalise treatment options. Furthermore, identifying *POLE* mutated tumours (1 of 4 molecular profiles) is difficult as the only way we can do this is through DNA sequencing, an expensive and time-consuming task. My research has found that in Aotearoa, there are a number of *POLE* tumour variants that are probably pathogenic yet have not been previously identified. In addition, the phenotype of *POLE* tumours in our cohort do not display usual patterns of tumour infiltrating lymphocytes as previously described in the literature; that is, we cannot tell which tumour is *POLE*-mutated by eye. Therefore, incorporating these molecular profiles into clinical practice, even though there is mounting evidence to do so, may not be immediately feasible in Aotearoa until more research is conducted.

I also highlighted our new research projects which are in development and discussed with colleagues about recruitment of women to these projects from different DHBs.

There were 63 attendees at the subsequent NZGCG annual meeting held on the 7th. Topics included endometrial cancer rates rising in young women, ovarian cancer advocacy and awareness, tautoko in medical school systems, and working towards equity. Overall, the two-day event was extremely collaborative and informative, with many new connections created and important discussions around improving outcomes for women with gynaecological cancer.

I thank Research For Life for the opportunity to attend and present at such a forum, which has been invaluable as I establish myself as an early career researcher in this field.



*Claire Henry*

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