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Recycling of the epithelial sodium channel requires SNX1 and SNX2

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The epithelial sodium channel (ENaC) is located at the apical membrane of polarised epithelial cells. ENaC's function involves sodium absorption and regulation of salt and water homeostasis making it crucial for determining blood volume and, therefore, blood pressure. Regulation of ENaC number at the cell surface by delivery to and removal from the cell membrane is strictly controlled and increased surface ENaC causes Liddle's syndrome, a form of hypertension. A potential novel candidate involved in ENaC recycling to the cell membrane is retromer, an endosome-localised protein trafficking complex. This study aimed to investigate whether retromer is involved in the recycling of ENaC, focusing specifically on the SNX1 and 2 (sorting nexin) heterodimer retromer sub-complex.

To examine whether the SNX1 and SNX2 proteins are involved in ENaC recycling, siRNA knockdown (KD) was used and the effects on ENaC trafficking measured. Using transiently transfected FRT (Fischer rat thyroid) cells, western blots were used to visualise efficiency of the SNX protein knockdown. Ussing chamber experiments measured changes in ENaC's amiloride-sensitive short circuit current (I_{sc}–Amil), in control and KD cells.

Significant protein knockdown was obtained for both SNX1 (P<0.0001, n=3) and SNX2 (P<0.0001, n=3) in FRT cells. The electrophysiological data demonstrated a ~50% reduction in I_{sc}–Amil with SNX1 KD (P<0.001, n=6), SNX2 KD (P<0.002, n=8) and SNX1/SNX2 double KD epithelia (P<0.001, n=8). Treatment of SNX1/SNX2 double KD cells with Brefeldin A (inhibits Golgi trafficking) had no significant effect on I_{sc}–Amil. This suggests that both SNX1 and 2 are required for recycling, but not synthesis or forward trafficking of ENaC.

This project signifies an important step towards understanding the mechanism of ENaC recycling, contributing to the understanding of and prevention of hypertension.

Modelling the climate impacts of meeting the NZ Eating & Activity Guidelines

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Under the 2015 Paris Climate Agreement, New Zealand committed to reducing greenhouse gas emissions: all major government policies should consider effects on the climate. Like other Western countries, the New Zealand eating pattern has large health and environmental impacts. This study modelled the climate impact of different dietary scenarios that conform to the Ministry of Health's Eating & Activity Guidelines (EAGs).

As there is currently no New Zealand-specific database of foods and their associated production emissions, a reference database from abroad was selected and emissions estimates for each food item were modified according to the New Zealand context. Diet-related emissions were estimated by combining the modified food emissions database with consumption data from the most recent New Zealand Adult Nutrition Survey. Dietary scenarios meeting the EAGs were developed in consultation with the Ministry of Health; each scenario's impact on emissions was modelled by scaling consumption of individual food groups according to EAG recommendations.

Whole plant foods, including vegetables, fruits, legumes and whole grains, were found to be less emissions-intensive than most animal-based foods, particularly red and processed meats. Daily diet-related emissions for the average New Zealand adult were estimated to be 6.6kgCO_2e: equivalent to 11% of New Zealand's annual emissions on a population level. New Zealand adults could reduce their diet-related emissions while meeting the EAGs by as much as 50%, mainly by reducing meat, fish, egg and dairy intake, along with food waste.

There is significant overlap between health and environmental considerations as they relate to eating pattern choices. Increasing consumption of whole plant foods and reducing consumption of both animal-based and highly processed foods presents a significant opportunity to mitigate the impacts of climate change, while improving health outcomes. There is good evidence to support the inclusion of sustainability considerations within the EAGs.

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Genetic variants in the *SLC2A9* locus confer risk for hyperuricemia in Māori and Pacific Island individuals

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Hyperuricemia, elevated levels of serum urate, is a prerequisite for gouty arthritis. The solute carrier family 2 member 9 (*SLC2A9)* gene that encodes a urate transporter tops the list of hyperuricemic genes. It is a key genetic determinant of serum urate levels and explains about 3% of urate variance. Gout is highly prevalent in the New Zealand Māori and other Polynesian populations. As an attempt to understand the reason for this increased prevalence, this study focused on the identification and characterisation of Polynesian-specific genetic variants within the *SLC2A9* locus conferring susceptibility to hyperuricemia, using the rare variant analysis approach.

The *SLC2A9* locus was resequenced in 809 individuals comprising hyperuricemic cases and normouricemic controls. Based on self-reported ancestry, the cohort was split into two subsets (Polynesian, n=440 and European, n=369). All Polynesians were from New Zealand while Europeans were from New Zealand and the US. Association analysis was carried out to identify risk variants within the *SLC2A9* locus that confer risk for hyperuricemia. Multiple adjusted logistic regression analysis was carried out using R version 3.4.1.

A total of 3,964 variants were identified within the locus, with 100 variants found to be significant in the Polynesian population (OR [95% CI] = 0.10 [0.01;0.88] to 5.43 [1.93;15.33], P_{OR} = 0.00028 to 0.049, $MAF_{controls}$ = 0.014 to 0.535, MAF_{cases} = 0.002 to 0.546). Twenty-five of these variants were found to be Polynesian-specific, among which 14 were found to be novel. These variants will be further analysed, replicated and functionally annotated in a larger cohort as a continuation of this study.

This research would provide a greater insight into the genetic causes of gout. More importantly, the identification of penetrant variants could be applied in precision medicine and public health genomics to improve health outcomes for the target population.

Modulating the immune response to colorectal cancer in mice using a cancer vaccine

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Vaccines modulate the host's anti-tumour immune response and represent an area of emerging immunotherapy research for the treatment of cancer, including colorectal cancer (CRC). Murine subcutaneous injections of tumour cell lines are often used to test cancer vaccines for the treatment of CRC. However, we have shown that CRC can also be modelled by a microsurgical intracaecal injection of the tumour cell CT26, a murine colon adenoma carcinoma.

To determine if the immune response to CRC could be modulated with a cancer vaccine, mice were vaccinated with chitosan hydrogel gel alone, gel and the endogenous tumour peptide AH1, or PBS for the control. Mice were then challenged either subcutaneously or intracaecally with CT26 colon adenoma carcinoma. The immune cells: dendritic cells, macrophages (F480+ and CD11b+), T cells, (CD4+ and CD8+) and B cells were identified via flow cytometry at the tumour site (local immune response) and in the spleen (systemic immune response). Splenic T cell phenotype (antigen experience, memory/regulatory phenotype, cytokine production) was also analysed via flow cytometry.

The chitosan gel vaccine provided protection against tumour growth in both subcutaneous (not significant) and intracaecal models (n=8–9, One-way ANOVA with Tukey post-hoc, P<0.01). In the subcutaneous model, there was no difference in the frequency of infiltrating macrophages, dendritic cells or CD4+ and CD8+ T cells; nor any differences in T cell phenotype; although these experiments need to be repeated. However, in the intracaecal model, protection was correlated with an increase in splenic tumour-antigen specific and IFNγ-producing T cells (n=4–5, One-way ANOVA with Tukey post-hoc, P<0.0001). These cells have also been shown to be important in human CRC.

This work will help link animal models and human data, and potentially translate cancer therapeutics into treatments for human patients.

Secreted amyloid precursor protein-alpha and active peptide fragments regulate neuronal morphology

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Neurodegeneration in Alzheimer's Disease is thought to arise both from an excess of pathogenic amyloid-beta and a deficiency of neuroprotective secreted amyloid precursor protein-alpha (sAPPα). To understand more about the biology of sAPPα and two putative active peptide fragments, RER (Arg-Glu-Arg) and 16mer, on normal tissue, we characterised their effects on dendritic complexity and dendritic spine density (two measures of neuronal connectivity).

Primary cultures of rat hippocampal neurons were transduced with green fluorescent protein via lentivirus to enable visualisation, and treated with 1nM sAPPα, RER, 16mer or control peptides for 24 hours, or sAPPα for two hours. Phosphate-buffered saline (PBS) was used as a control. Cultures were treated, then fixed with paraformaldehyde after 21 days *in vitro*. Confocal imaging analysed dendritic complexity

and spine density. Immunofluorescence allowed quantification of functional synapses.

Dendritic complexity was significantly increased by 24-hour treatment with sAPPα ($F(1, 70)=4.22$, $P=0.044$), and 16mer and the control peptide scrambled 16mer ($F(34, 1717)=2.01$, $P<0.001$), compared to PBS. RER and two-hour sAPPα treatment did not affect dendritic complexity. Dendritic spine density (specifically of thin spines) was decreased after two-hour sAPPα treatment ($t(68)=2.21$, $P=0.03$), but matched control levels after 24-hour sAPPα treatment. RER and 16mer did not affect spine density. The proportion of postsynaptic densities forming functional synapses (defined as colocalised synaptophysin and PSD-95) was unchanged by any treatment.

Given 24-hour sAPPα-treated neurons had higher dendritic complexity but equal spine density compared to control neurons, it is likely the total spines per cell were increased. The sAPPα-mediated transient decrease in spine density may have been due to actin recruitment for subsequent dendritic outgrowth. Taken together, these data suggest that sAPPα and 16mer have potential as therapeutic agents for restoring or preventing loss of neuronal connectivity in Alzheimer's Disease.

Post-hoc epigenome-wide association analyses validating differential DNA methylation patterns related to age, diabetes and smoking

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Increasing evidence indicates that significant components of disease aetiology may lie in complex epigenetic interactions between an individual's genome and environment. Epigenetic patterns, particularly DNA methylation, have significant potential to: broaden understanding of pathogenesis; identify diagnostic biomarkers; facilitate development of novel pharmacological interventions. Consequently, many recently conducted epigenome-wide association studies (EWAS) have investigated risk factors including age, type 2 diabetes mellitus (T2DM) and smoking exposure. We undertook an opportunistic post-hoc analysis of an Otago-based cardiovascular disease EWAS cohort in order to validate previously reported differential methylation at particular cytosine/guanine dinucleotide (CpG) sites purportedly associated with specific phenotypes.

Genome-wide DNA methylation profiles of 487 males were analysed using BeadChip assay, which assessed 456,279 CpG sites. Post-hoc case-control EWAS were conducted for a range of risk factors, including age, T2DM and smoking. Methylation data was analysed using principal component analysis and multivariate regression.

The age EWAS included individuals between 40 and 94 years. Concordant with published analyses, the most significant CpG sites associated with genes *ELOVL2* (cg16867657, $P<4x10^{-37}$; cg24724428, $P<9x10^{-25}$) and *FHL2* (cg22454769, $P<6x10^{-22}$). In the T2DM EWAS, which compared only 50 cases with 436 controls, our second top association (cg19693031 in *TXNIP*, $P<6x10^{-7}$) also matched the top association in all published diabetes-specific EWAS. Finally, numerous previous analyses have consistently reported a large number of differentially methylated CpG sites associated with smoking. We observed a highly concordant set of associations, including cg05575921 (*AHRR*, $P<6x10^{-52}$), cg03636183 (*F2RL3*, $P<2x10^{-23}$) and cg19859270 (*GPR15*, $P<5x10^{-15}$).

Our dataset was not specifically designed to interrogate age, T2DM and smoking exposure, but we were able to show remarkable reproduction of previously reported results from EWAS. Such post-hoc EWAS validation of these published associations demonstrates the potential robustness of CpG site methylation as disease biomarkers.

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Attitudes and barriers to physical activity and levels of physical activity in adults with obesity consuming very low calorie diets

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Physical activity for prevention and management of non-communicable diseases (NCDs) is supported by evidence, and is a core component of physiotherapy interventions. High BMI, associated with most NCDs, is the leading modifiable risk to health in New Zealand. A very low calorie diet (VLCD) is an effective and widely used weight loss intervention for adults with obesity. However, adverse effects include loss of lean tissue, strength and aerobic capacity. Regularly engaging in physical activity can attenuate these adverse effects and contribute to weight maintenance following VLCD. The level and perceptions of physical activity in New Zealand adults with obesity, consuming a VLCD, have not previously been investigated. This mixed-methods study aimed to identify attitudes and barriers to physical activity in adults with obesity consuming VLCDs and measure their physical activity levels.

Ten adults (mean age 44.3y, SD 12.3; BMI 40.8kg/m^2, SD 6.8) participated. Physical activity levels over seven days were measured with ActiGraph wGT3x-BT accelerometers and the Global Physical Activity Questionnaire (GPAQ). Participants' attitudes and barriers to physical activity were explored through thematic analysis of semi-structured interviews.

Responses to the GPAQ indicated that six participants engaged in less than 150 minutes of moderate activity/week. Seven days of accelerometric data were captured for eight of the 10 participants; five of these participants accumulated less than 150 minutes of moderate intensity activity. Themes identified included psychological and physical barriers, motivators, facilitators and positive feedback cycles. VLCD was perceived to be a facilitator of physical activity.

The majority of adults with obesity who were consuming a VLCD engaged in less moderate intensity physical activity than is recommended by the World Health Organization (150 minutes/week). VLCD was not a perceptible barrier: it facilitated physical activity. Physiotherapy interventions utilising physical activity, and VLCD may be mutually beneficial interventions in adults with obesity.

Curcumin derivatives as a novel strategy for overcoming crizotinib resistance in EML4-ALK+ lung cancer cell lines

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Lung cancer is an aggressive disease and is the most lethal cancer worldwide. Approximately 20–30% of lung cancers are caused by oncogenic receptor tyrosine kinases (RTK). The fusion of echinoderm microtubule associated protein like 4 (EML4) with anaplastic lymphoma kinase (ALK) produces an oncogenic RTK. Crizotinib is the current first-line treatment for this subtype of cancer, however, resistance usually develops after 12 months. One strategy to overcome resistance is to test existing compounds with anticancer properties. Curcumin has been previously shown to produce cytotoxic effects in other cancers, however, it has not been examined in this subtype of lung cancer. Curcumin has a poor pharmacokinetic profile, so derivatives have been developed. This project tested if curcumin derivatives (RL66 and RL118) were potent in crizotinib-sensitive and crizotinib-resistant EML4-ALK+ cancer cells.

The sulforhodamine B assay was performed to investigate cytotoxicity and non-linear regression analysis was conducted to calculate the IC_{50} ± **SEM**. The extra sum of squares F test was used to test for differences in IC_{50}s.

EML4-ALK+ H3122 cells exhibited significantly greater sensitivity to RL66 and RL118 (IC_{50}=0.97±**0.05** and 0.70±0.02μM, respectively), when compared to EML4-ALK- A549 cells (2.65±**0.12** and 1.15±**0.10**μM, respectively, *P*<.0001). The addition of an ALK agonist, pleiotrophin, did not significantly alter the cytotoxicity of the compounds (*P*>0.05). In the resistant cells (CR-H3122), RL66 and RL118 retained potency (IC_{50} of 2.15±**0.26 and 1.44±0.13**μM, respectively) and produced smaller fold changes (2.2- and 2.1-fold increases) compared to crizotinib (13-fold increase).

We conclude that the mechanism of RL66 and RL118 toxicity does not involve direct effects on ALK. The potent cytotoxic effects of these compounds may be a promising treatment in crizotinib-resistant EML4-ALK+ lung cancer.

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