Targeted epigenetic editing in melanoma: locus-specific manipulation of DNA methylation in the EBF3 promoter

Jim Smith1, Euan Rodger1, Robert J Weeks1, Michael Eccles1, Aniruddha Chatterjee1

1. Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand
*Corresponding author email: smiji730@student.otago.ac.nz

Introduction:
DNA hypermethylation of gene promoter regions is typically associated with transcriptional repression. Furthermore, dense promoter methylation within tumour-suppressor genes has been widely characterised as a hallmark of oncogenesis. However, there is an accumulating body of evidence which suggests, in specific contexts, that promoter hypermethylation may also be associated with gene activation. Our previous work has uncovered this phenomenon in cutaneous melanoma, where the gene EBF3 has been identified as a putative “epigenetic driver” of metastasis. Here, increased EBF3 promoter methylation was strongly associated with transcriptional upregulation. However, existing technologies have been inadequate to conclusively establish a causal relationship between promoter-specific methylation changes and gene expression. The emergence of Clustered, Regularly Interspaced Short Palindromic Repeats (CRISPR)-based editing technologies now offers scope to establish causality for these observations.

Materials and Methods:
A CRISPR-based system was constructed with the aim of inducing site-specific demethylation. Our CRISPR construct facilitated the recruitment of human TET1 dioxygenase effector proteins to actively demethylate target 5-methylcytosine residues within the EBF3 promoter region, directed by locus-specific guide RNA molecules. Methylation changes were evaluated using a methylation-specific next-generation sequencing assay.

Results:
We successfully constructed a CRISPR-based system for site-specific manipulation of DNA methylation, delivered into three human melanoma cell lines via lipofection. Targeted demethylation was induced using this system, with up to 58.8% absolute methylation difference between edited and control samples.

Conclusions:
Generating an elegant, CRISPR-based method for locus-specific manipulation of DNA methylation in human melanoma cells has laid a platform for further investigation into the direct relationship between DNA methylation and gene expression for the metastatic oncogene EBF3, and possibly other genes in future.

High altitude medicine: understanding the mechanism of acute mountain sickness

Holly K. Barclay1, Yu-Chieh Tzeng1, Jui-Lin Fan1

1. Department of Surgery and Anaesthesia, University of Otago, Wellington, New Zealand
*Corresponding author email: barhol69@student.otago.ac.nz

Introduction:
The present study was undertaken to determine the integrative physiology behind acute mountain sickness (AMS) susceptibility. We compared the respiratory, cardiovascular, renal, and cerebrovascular responses to acute hypoxia.

Materials and Methods:
The participants (28 total; 14 females; mean age 24 ± 7 years) were exposed to normobaric hypoxia (high altitude [HA]: FiO2 12.5%, PO2 88.6 mmHg) and normoxia (sea level [SL]: FiO2 21%, PO2 148.8 mmHg) for ten hours. Repeated measurements were made of AMS symptoms, respiratory, cardiovascular, renal, and cerebrovascular variables.

Results:
Compared to SL, HA induced an increase in ventilation (p <0.001). This caused a respiratory alkalosis (p <0.001) which was compensated for by an increased bicarbonate (p <0.001) and cation excretion (Na+: p = 0.048; K+: p <0.001). Fluid balance varied between individuals based on their level of renal compensation (p = 0.519). Cerebral blood flow increased (p <0.001), cerebral autoregulation was impaired (very low frequency [VLF] coherence: p <0.001; VLF gain: p = 0.026; VLF phase: p <0.001), and cerebrovascular CO2 reactivity was enhanced (p <0.001).

We calculated slopes of change in every variable over the HA exposure and correlated these to the change in AMS score. Those who were more susceptible to AMS showed a greater increase in ventilatory response (p <0.020). This caused a decrease in heart rate over time (p = 0.040), and a more severe alkalosis (p = 0.001). They had an increase in weight (p = 0.005), venous bicarbonate concentration (p = 0.047), and venous sodium concentration (p = 0.001), indicating an antidiuresis, intravascular fluid shift, and fluid retention. The flow through the vertebral artery increased more in those who were more susceptible due to an increased diameter (p <0.001). Cerebral autoregulation impairment was not greater in those with AMS. Cerebrovascular CO2 reactivity decreased over the day in susceptible individuals (p = 0.029) due to their increased pH and bicarbonate
Concentration. The change in vertebral artery diameter (p = 0.001) and venous pH (p = 0.001) were strong predictors of the change in AMS score (R² = 0.605).

Conclusion:
These results indicate that vertebral artery hyperperfusion, inadequate renal compensation, and the augmenting venous pH may play a key role in the pathogenesis of AMS during acute exposure to hypoxia.

The reliability of freehand 3D ultrasound and magnetic resonance systems in measuring the in vivo gastrocnemius muscle volume in infants

Ghaliya S. Al Masruri1*, S. A. Mirjalili
1. Department of Anatomy and Medical Imaging, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
*Corresponding author email: gahl65@aucklanduni.ac.nz

Introduction:
The muscle volume of the gastrocnemius is important in producing force around the joints to facilitate locomotion. Understanding the changes in the muscle volume of typically developing infants will allow clinicians to better interpret growth impairment in patients with musculoskeletal disorders. This study aims to assess the reliability of freehand 3D ultrasound (3DUS) and magnetic resonance (MR) in measuring the in vivo muscle volume of the medial gastrocnemius (MG) and lateral gastrocnemius (LG) in infants.

Materials and Methods:
MG and LG from both lower limbs of three infants, aged three months, were scanned using both freehand 3DUS and MR. Two processors segmented the images obtained from both modalities to measure the muscle volume. The intra- and inter-reliability of the segmentation method was assessed using the intraclass correlation coefficient (ICC).

Results:
The intra-processor segmentation reliability of both freehand 3DUS and MR in quantifying the in vivo muscle volume of the MG and LG was excellent (ICCs between 0.99 and 0.93). ICCs for the inter-processor reliability of 3DUS and MR were 0.84 and 0.27, respectively.

Conclusions:
This is the first study to assess the segmentation reliability of MG and LG in typically developing infants using freehand 3D ultrasound and MR. Freehand 3DUS is found to be highly reliable. However, our study demonstrated that MR reliability is moderate. MR is considered to be the gold standard for muscle volume measurements in adults. However, its reliability in segmenting the in vivo gastrocnemius muscle volume in infants needs further investigation.

Cardiac risk factors and biomarkers in the acute and convalescent phase of acute coronary syndromes: a sub-study of MENZACS

Alexandra Chaptynova1*, M. Legget1,2 S. Aish1, K. Bradbury1, M. Heath1, V. Thorpe1, K. Poppe1,4 N. Earle1, R. Doughty1
1. Department of Medicine, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
2. Greenlane Cardiovascular Service, Auckland District Health Board, Auckland, New Zealand
4. School of Population Health, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
*Corresponding author email: acha521@aucklanduni.ac.nz

Introduction:
Acute coronary syndromes (ACS) are influenced by several cardiovascular risk factors, including diet, physical activity, stress, and cardiac biomarkers, which are likely to change in the weeks following an ACS. This study aimed to explore changes in cardiac biomarkers (N-terminal pro-B-type natriuretic peptide [NT-proBNP]) and risk factors following ACS.

Materials and Methods:
Participants were those enrolled in a cohort study of patients with first time ACS (MENZACS). This sub-study incorporated assessment of acute phase and follow-up cardiac biomarkers, and repeated nutrition, physical activity, and stress assessment in the weeks post-ACS.

Results:
Sixty-nine patients with ACS were included: median age 64 (minimum 44, maximum 92) years; 33% women; 52% non-ST elevation myocardial infarction (NSTEMI), 42% ST-elevation myocardial infarction (STEMI), and 6% unstable angina. NT-proBNP was measured during admission: 124 (0.6, 2510) pmol/L overall, NSTEMI 86 (0.6, 2510) pmol/L. STEMI 158 (19, 1460) pmol/L. Nineteen patients were followed up for more than one month post hospital discharge: NT-proBNP decreased from 164 (35, 1080) during admission to 40 (7, 175) pmol/L. Rates of decline in NT-proBNP varied. Twelve patients had dietary reassessment at ten weeks, of which 11 (92%) reported dietary change. A reduction in median total energy intake (9640 kJ versus 8880 kJ daily) and fat (percentage of energy: 34.3% versus 31.5%); and increase in protein (85g versus 104g daily) and carbohydrates (222g versus 249g daily) were observed for the 12 participants. Higher levels of stress were reported at baseline, and moderate intensity recreational physical activity increased weeks after ACS.

Conclusions:
This sub-study demonstrated that NT-proBNP levels are elevated at the time of first ACS. Secondly, these levels decrease in the weeks post-discharge, although remain elevated above population normal levels. Thirdly, important changes in risk factor modification occur during the early recovery phase.

Pathogenesis of tonsillar hyperplasia

Ruyan Chen1*, K. Biswas1, S. Waldvogel-Thurlow1, J. Johnston1, M. Mahdevan1,2, R. Douglas1
1. Department of Surgery, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
2. Auckland District Health Board, Auckland, New Zealand
*Corresponding author email: rchen554@aucklanduni.ac.nz

Introduction:
Tonsillar hyperplasia is the most common cause of upper airway obstruction in children, and causes a spectrum of disorders ranging from recurrent tonsillitis to sleep-disordered breathing. The underlying pathogenesis remains unclear. Differences in bacterial community and local atopic responses have been implicated. This study aimed to determine the underlying pathogenesis of recurrent tonsillitis versus sleep-disordered breathing causing tonsillar hyperplasia.

Materials and Methods:
Both tonsils and a blood sample were collected from ten recent
E-screening for mental health and risky behaviours by high school guidance counsellors: is YouthCHAT a feasible and acceptable approach?

Hannah Clare*, M. Darragh†, F. Goodyear-Smith‡

1. School of Medicine, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
2. Department of General Practice & Primary Health Care, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

*Corresponding author email: hcl235@aucklanduni.ac.nz

Introduction:
Risky behaviours and mental health concerns commonly emerge during adolescence. Issues often go undetected by parents or school staff, therefore screening is important to facilitate early detection and intervention. YouthCHAT is a holistic digital questionnaire which screens for risky behaviours and mental health concerns. Schools are a high-yield locale for screening because they allow access to high-risk youths who may not regularly attend primary care. This study investigated whether it was (1) acceptable and (2) feasible for high school guidance counsellors to use YouthCHAT to screen students for mental health and risky behaviours.

Materials and Methods:
YouthCHAT was piloted in two Auckland high schools using a co-design approach to implementation. One school attempted schoolwide screening using YouthCHAT and the other used YouthCHAT for opportunistic screening. Semi-structured interviews were conducted with each of the guidance counsellors who used YouthCHAT and analysed using a general inductive approach to assess acceptability and feasibility.

Results:
All school guidance counsellors found YouthCHAT acceptable. In terms of feasibility, YouthCHAT was integrated easily into School A via the structured schoolwide screening protocol and well-used by participants, whereas it was integrated less easily into School B via an unstructured opportunistic screening protocol. Specific feasibility considerations identified included time, human resources, and potential language and comprehension barriers.

Conclusions:
YouthCHAT is an acceptable tool for use by guidance counsellors in Auckland high schools. Provided that feasibility considerations are acknowledged and addressed prior to implementation, it shows promise as a method of school-based screening for mental health and risky behaviour concerns.

Effect of maternal position on feto-placental blood flow and placental oxygenation in late gestation pregnancy: a magnetic resonance imaging study

Sophie Couper*, P. Stone, A. Clark, D. Flouri, R. Aughwane, A. David, A. Melbourne, J. Thompson, A. Mirjalili

1. Department of Obstetrics and Gynaecology, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
2. Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand
3. School of Biomedical Engineering and Imaging Science, King’s College, London, United Kingdom
4. Institute for Women’s Health, University College London, London, United Kingdom
5. Department of Paediatrics and Child Health, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
6. Department of Anatomy and Radiological Imaging, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

*Corresponding author email: scou918@aucklanduni.ac.nz

Introduction:
Maternal position in late gestation pregnancy is associated with an increased risk of stillbirth. Previous magnetic resonance imaging (MRI) studies quantified the effects of supine position on reduced maternal cardiac output and abdominal aortic blood flow. The aim of the current study was to use novel MRI techniques to assess the effects of maternal position on feto-placental blood flow and oxygenation.

Materials and Methods:
Eighteen healthy pregnant women, 34–38 weeks gestation, underwent MRI scans in both left lateral decubitus and supine positions. Phase-contrast MRI was used to scan the maternal internal iliac arteries and fetal umbilical vein, to calculate blood flow. A novel technique combining T2 relaxometry and DWI-IVIM (DECIDE®) was used to assess oxygenation in the maternal and fetal placental compartments.

Results:
Significant blood flow reductions in right and left internal iliac arteries were seen when women lay supine compared with left lateral. Right and left internal iliac arteries were found to reduce by 22.5% (p = 0.00044) and 29.2% (p = 0.0001) respectively when supine, with an 11.7% reduction in the umbilical vein which neared statistical significance (p = 0.0712). Fetal oxygen saturation (FO₂) reduced by 6.6% (p = 0.0705). Placental Flux, a measure of oxygen delivery across the placenta, was reduced by 10.6% (p = 0.0138) when supine compared to left lateral, as did Delivery Flux (product of FO₂ and umbilical vein flow), 16.4% (p = 0.056).

Conclusions:
In this novel study, maternal position in healthy late pregnancy affects not only placental blood flow, but fetal blood flow and oxygen diffusion across the placenta. The healthy fetus compensates for the positional hypoxia by umbilical flow reduction, which is likely to be a chemoreflex response. Effects of supine position in vulnerable, growth restricted pregnancies may be less well tolerated.
Development of a scalable body surface gastric mapping system and clinical workflow for aid in diagnosis of functional gastrointestinal disorders

Thomas Hayes1*, G. O’Grady1, AA. Gharibans1

1. Department of Surgery, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

*Corresponding author email: thay697@aucklanduni.ac.nz

Introduction:
Functional gastrointestinal disorders (FGIDs) afflict one in three New Zealanders. However, effective diagnosis of FGIDs remains difficult, relying on insensitive motility tests or non-specific subjective symptom questionnaires. One contributing factor proposed to underlie some FGIDs has been spatial gastric myoelectrical dysrhythmias. These have recently been recorded in experimental studies using high-resolution body surface gastric mapping (BSGM). We have developed a scalable BSGM system with integration into a clinical workflow for the aid in diagnosis of FGIDs.

Materials and Methods:
A novel BSGM recording system was developed as a clinical tool to evaluate gastric electrical dysrhythmias. Patients were given a wearable electrode array, an acquisition box and a symptom-logging app for correlating symptoms with electrophysiology. Propagation patterns from BSGM were compared to a symptom and meal timeline, and gastric emptying time course from the simultaneous scintigraphy study.

Results:
Current results have focused on development of the new clinical device. Benchtop testing validated device performance across a reference physiological frequency range. Tolerance of the wearable array was excellent. The device has commenced prototype builds and healthy control testing has shown the feasibility of accurate recording and analysis and ease of integration into a clinical workflow with high participant satisfaction.

Conclusions:
Ergonomic BSGM could fit smoothly into clinical practice to provide a more certain diagnosis for FGIDs, with or without abnormal gastric emptying, and without relying on symptoms alone. The sensitivity and specificity of the test awaits confirmation. Improved knowledge of gastrointestinal pathophysiology from this novel technology will hopefully allow for improved future treatments.

Using 3D-reconstruction to analyse trends in maxillary sinus growth

Sophie Lee1*, J. Fernandez2, M. Mahadevan1, A. Mirjalili1

1. Department of Anatomy and Medical Imaging, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
2. Department of Engineering Science, Faculty of Engineering, The University of Auckland, Auckland, New Zealand
3. Department of Surgery, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

*Corresponding author email: silee446@aucklanduni.ac.nz

Introduction:
Chronic rhinosinusitis commonly affects the maxillary sinus (MS), and when refractory to medical management it is often treated surgically in both adults and children. However, the developmental anatomy of the MS in children is poorly understood, despite the important implications it has in performing safe and effective surgery.

Materials and Methods:
Individuals 0–18 years old who had undergone a computed tomography (CT) head scan at Auckland District Health Board were examined retrospectively, and a total of 65 patients were selected for the study after careful review. After dividing them into two-year age groups (0–1, 2–3, 4–5 etc.), each MS was 3D-reconstructed from the CT images. Mean changes in size, volume, and shape with increasing age were analysed.

Results:
From 0–1 years average MS size was 1.03 x 1.82 x 1.27 cm (width x length x height) with a volume of 0.81 ± 0.69 cm³. By 16–18 years, average size increased to 3.39 x 4.30 x 4.63 cm and average volume became 21.63 ± 6.49 cm³. Growth increased linearly but most rapid expansion occurred in the first five years of life. Sinus morphology progressed from an oval shape at 0–1 years to a pyramidal shape from 14 years of age and onwards.

Conclusions:
The results of this study can be used as a reference for normal MS growth in healthy children. This may be clinically useful in better interpreting sinus imaging, diagnosing sinus pathology, and planning prior to surgery.

Patient understanding and perspectives of DNA testing in cancer – consideration in implementation of a genomic programme in Auckland

Nathaniel Li1*, Ian Hayes1, Cristin Print1, Cherie Blenkiron1, Mark Greenslade1, Helen Whong1, Paul Donaldson1, Kimberley Gamet1, Rob McNeill1, Michelle Wilson1

1 Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

*Corresponding author email: jli678@aucklanduni.ac.nz

Introduction:
Cancer remains the leading cause of mortality in New Zealand and is likely to be the defining health issue for the next decade. Treatment paradigms are evolving with the advent of personalised oncology. DNA testing is an integral part of this. Despite significant advances of genomic technologies, little is known about patient knowledge of and attitudes towards DNA testing, especially in the context of New Zealand.

Material and Methods:
This study involved a 33-item, self-administered survey assessing patient’s knowledge, attitudes, and expectations towards DNA testing. Eligible participants were patients with advanced disease who were receiving treatment or undergoing follow-up after cancer diagnosis. Logistic regression models were used to evaluate the association between baseline characteristics and patient understanding and perspectives of DNA testing. Additional comments on the survey were assessed using qualitative thematic analysis.

Results:
Results were reported from 202 patients who completed the survey, representing a response rate of 62.2%. Of these, 68.8% of patients were interested in learning more about DNA testing in cancer care, while only 29.7% of patients thought that they had sufficient knowledge to pursue DNA testing. The mean score on a six-item knowledge assessment was 4.29 (SD = 1.639). The knowledge score was...
associated significantly with level of education, gynaecological cancer diagnosis, and some ethnicities (p = 0.008, p = 0.004, p = 0.001 respectively).

Conclusions:
Further education is required for patients before they conduct genomic testing in cancer. This study can be used to help build the capacity and infrastructure to educate and support patients who are interested in accessing genomic testing.

Understanding national variation in preoperative therapy in rectal cancer: a survey of multidisciplinary teams’ decision making

Martin Lu1*, Ian P. Bissett2

1. School of Medicine, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
2. Department of Surgery, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
*Corresponding author email: mlu786@aucklanduni.ac.nz

Introduction:
There is wide variability between District Health Boards (DHBs) in New Zealand in the proportion of people with rectal cancer who receive preoperative radiotherapy. The aim of this study is to compare the management decisions made by different multi-disciplinary groups that treat rectal cancer across different DHBs and identify underlying causes for differences in the rates of preoperative therapy.

Materials and Methods:
All multidisciplinary teams (MDTs) nationally that treat rectal cancer were identified. They were tasked to decide the treatment for eight de-identified cases. They were able to choose one out of four outcomes: straight to surgery, short course radiotherapy, and long course chemo-radiation with or without chemo. Their choices were collected using an online survey along with the rationale and reasoning behind the decision.

Results:
Wide variation existed in the treatments decided by MDTs for all cases. There was no unanimous treatment outcome for any case in this study. Some form of radiotherapy was indicated in 65 out of the 80 responses (81.25%), of which 40 responses were for long course chemo-radiation and 25 responses opted for short course radiotherapy. Only 15 responses (18.75%) indicated surgery as their choice. Certain MDTs put more weight into different prognostic factors such as staging, mesorectal fascia involvement, height, or EMVI status.

Conclusions:
This differing value system may influence the variation in treatment choice. We further speculate that MDTs may even look into different evidence-based guidelines in order to inform their decision. There is no universal agreement between these guidelines, and this may explain variations in treatment choice.

Evaluation of the role of cyclic AMP response element-binding protein as a transcriptional regulator in glioblastoma multiforme

Rahul Makam1*, T. Park1, M. Dragunow1

1. Department of Pharmacology, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
*Corresponding author email: rmak106@aucklanduni.ac.nz

Introduction:
Glioblastoma multiforme (GBM) is the deadliest and most common primary brain cancer with poor prognosis and limited therapeutic options. Recent evidence has implicated the hub transcription factor, cyclic AMP response element-binding (CREB) protein as plausibly critical in the oncogenesis of several cancers including GBM. The role of CREB in malignancy was explored in primary human glioma cells.

Materials and Methods:
Immunohistochemistry was performed to validate the presence of CREB in primary glioma. CREB-targeted small interfering RNA was used to silence CREB in vitro in several cases of primary glioma under different media conditions in order to evaluate the role of CREB in glioma cell proliferation, differentiation, and survival.

Results:
CREB was highly expressed in glioma tissue and constitutively active in primary glioma cells. The kinetics of CREB silencing in fast proliferating glioma cells was explored and a consistently potent mechanism to achieve knockdown was established involving pre-plating delivery. Manipulation of CREB revealed that the gene played a moderate role in malignant cell proliferation. Most significantly, the silencing of CREB drove cells towards a more differentiated phenotype and rendered them sensitive to death through apoptosis inhibition using glutathione impeding mechanisms.

Conclusions:
Together these data suggest a role for CREB in the control of survival and differentiation in glioma cells and posit CREB or CREB-controlled genes as plausible targets for novel therapeutic agents in GBM.

Enteral tube feeding intolerance: current knowledge and pathways for future study

Holly Mashlan1*, V. Asrani1, S. Pendharkar1, B. Stokes1, A. Phillips1, J. Windsor1

1. Surgical and Translational Research (STR) Centre, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand
*Corresponding author email: hmas991@aucklanduni.ac.nz

Introduction:
Enteral nutrition (EN) is a common method of nutrient support in clinical and community settings but can be problematic. Approximately 1/3 of patients receiving EN develop enteral tube feeding intolerance (ETF1), with a spectrum of severity ranging from abdominal discomfort to peritonitis and death. The overall aim was to develop a method to evaluate the relationship between patient symptoms and ETFI.

Materials and Methods:
The first study was a systematic review of the literature exploring the relationship between ETFI and a range of physiological measures. The second study bench-tested the performance characteristics of a technique for measuring intestinal physiological states using an intestinal
phantom and motorized syringe to mimic the ETFI. Some pilot clinical studies were then undertaken using one method.

**Results:**
The systematic review identified several factors associated with ETFI, including altered intestinal motility, injury, disease, and intra-abdominal pressure. An absence of randomized controlled trials meant that we could not identify causes of ETFI. The review highlighted the knowledge gap regarding changes in physiological parameters during ETFI development. The bench experiments showed there were specific conditions under which the various sensor technologies were the most accurate and sensitive. Two techniques for measuring intestinal physiology were characterized.

**Conclusions:**
Several patient factors were found to be associated with ETFI; however, the link between measurements and ETFI remained unclear. Both physiological measurement techniques appeared satisfactory for undertaking future clinical studies to better define the relationship with symptoms of ETFI.

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**Stroke trends in New Zealand by ethnicity and deprivation from 2005 to 2016**

Susan Middleton*, C. Grey, R. Jackson

1. School of Population Health, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

*Corresponding author email: smd290@aucklanduni.ac.nz

**Introduction:**
New Zealand stroke trends stratified by ethnicity and deprivation are unknown. These data are valuable for planning health care services and analysis by ethnicity and deprivation can identify areas requiring further attention.

**Materials and Methods:**
The VIEW research team has a dataset which links multiple Ministry of Health databases and includes information on an individual’s age, ethnicity, deprivation level, and hospitalisation or death from stroke. Stroke hospitalisation, death, and incidence rates from 2005 to 2016 for Māori, Pacific, Indian, Asian, and European people, and rates by deprivation level, were calculated. Three age groups — 25–64 years, 65–74 years, 75–84 years — were studied.

**Results:**
Stroke rates for Māori and Pacific people were higher than for Europeans, Asians, and Indians. This was especially noticeable in younger age groups. There was also a clear correlation between deprivation level and stroke rates, with the more deprived having the higher rates. When examining stroke rates by ethnicity within each deprivation group, differences by ethnicity persisted. Similarly, differences in stroke rates between deprivation groups were present within each ethnic group. Trends in the youngest and oldest age groups were unclear, but in those aged 65–74 years, stroke rates declined throughout the study period.

**Conclusions:**
There is a clear disparity in stroke rates between ethnic and deprivation groups. Māori and Pacific people, and those living in poorer areas, have significantly higher rates of stroke death and hospitalisation. The findings suggest that ethnic differences are independent of deprivation differences and vice versa.

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**Reducing cataract complications by pre-operative risk analysis in a public teaching hospital**

Soobin Namkung*, J. Han†, C. McGhee

1. Department of Ophthalmology, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

*Corresponding author email: snam373@aucklanduni.ac.nz

**Introduction:**
The New Zealand Cataract Risk Stratification (NZCRS) is a preoperative risk stratification system previously designed to reduce intraoperative complications of phacoemulsification cataract surgery in New Zealand. The aim of this study was to further validate the NZCRS system, as it becomes standard of care at Greenlane Clinical Centre (GLCC), Auckland District Health Board (ADHB), New Zealand.

**Materials and Methods:**
A prospective cohort study of preoperative risk stratification of consecutive cataract cases (n = 300), using the NZCRS scoring system in an Auckland public teaching hospital, was conducted. Identification of higher-risk cases and recommendation to allocate these cases to senior surgeons — fellow or consultant — was recorded. The primary outcome was intraoperative complication rates relative to adherence to NZCRS recommendations.

**Results:**
Eighty-seven cases (29%) were identified by NZCRS as high-risk and recommended for fellows or consultants. Primary surgeons were registrars, fellows, and consultants in 47 (16%), 48 (16%) and 205 (68%) cases, respectively. Overall intraoperative complication rate was 6.7%. There was no statistical difference in complication rates between surgeon levels (p > 0.05). The NZCRS scoring recommendations were adhered to in 99% of cases. In the group of cases that adhered to the NZCRS recommendations (n = 296), the intraoperative complication rate was 6.4%. Postoperatively, the mean best-corrected visual acuity was 6/7. Postoperative cystoid macular oedema was reported in 4.7%. Re-scoring by an investigator revealed 30% of high-risk cases compared to 20% identified by the surgeons, due to differences in anterior chamber depth and cataract density scores.

**Conclusions:**
The NZCRS system helps identify high-risk cataract cases. Subsequent allocation to appropriate surgeon levels results in a reduction of intraoperative complications. To improve the consistency of NZCRS scoring, standardisation of cataract density grading may be explored in the future.

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**The anatomy of the thoracic duct lymphovenous junction in humans**

Lomani A. O’Hagan†$, J. A. Windsor‡, A. R. J. Phillips†, S. A. Mirjaliil

1. Department of Anatomy and Medical Imaging, School of Medical Sciences, Faculty of Medical and Health Sciences, The University of Auckland
2. Department of Surgery, School of Medicine, Faculty of Medical and Health Sciences, The University of Auckland
3. Applied Surgery and Metabolism Laboratory, School of Biological Sciences, Faculty of Science, The University of Auckland

*Corresponding author email: kha911@aucklanduni.ac.nz

**Introduction:**
The majority of lymph generated in the body is returned to the blood vasculature via the lymphovenous junction of the thoracic duct. A
lymphovenous valve is believed to guard this junction by regulating the flow of lymph to the veins and preventing blood from entering the lymphatic system. Despite these important functions, the morphology and mechanism of this valve remains unclear. The aim of this study was to investigate the anatomy of the lymphovenous junction and lymphovenous valve of the thoracic duct in humans.

Materials and Methods:
The thoracic duct and the great veins of the left neck were harvested from 16 formalin-embalmed human cadavers. After exclusion, the lymphovenous junctions from 12 cadavers were examined with stereomicroscopy, X-ray microtomography, and transmitted light microscopy.

Results:
The thoracic duct commonly branched before entering the veins. Thus, from 12 cadavers, 21 lymphovenous junctions were identified. Bicuspid valves were present at 15 of the 21 junctions (71%). Valves were absent at six junctions (29%). The lymphovenous valve cusps were typically semilunar, though their size and position varied. Microscopically, the cusps comprised of luminal extensions of endothelium with a thin core of collagenous extracellular matrix.

Conclusions:
The lymphovenous valve of the thoracic duct is typically bicuspid and semilunar in morphology. It may prevent blood from entering the lymphatic system, but its variable morphology and frequent absence remains an enigma. Further structural and functional studies are needed to understand how this part of the human body works in both health and disease.

The effect of Staphylococcus aureus and lactoferrin on the sino-nasal mucosal integrity

J. Park**, K. Biswas¹, W. Sharon¹, R. Douglas¹

1. Department of Surgery, Faculty of Medical and Health Sciences, University of Auckland

*Corresponding author email: jpar480@aucklanduni.ac.nz

Introduction:
Chronic rhinosinusitis (CRS) is a complex disease characterised by chronic inflammation of the sino-nasal mucosa. Its pathophysiology is not fully understood, especially the role of microbes on the sino-nasal mucosa. The aim of this study was to investigate Staphylococcus aureus, a microbe commonly found in the sino-nasal cavity of CRS patients; and lactoferrin, a glycoprotein that is nasally secreted with antibacterial properties on mucosal integrity.

Materials and Methods:
Sino-nasal tissue of six healthy control patients, eight CRS patients without nasal polyps (CRSsNP), and 12 CRS patients with nasal polyps (CRSwNP) were obtained in theatre. The biopsies were placed into four groups – (i) control “C” (growth medium), (ii) bacterial treatment “B” (S. aureus supernatant), (iii) bacteria and lactoferrin treatment “BLF”, and (iv) lactoferrin treatment “LF”. Mucosal integrity was measured with histology for cilia damage and mucosal collagen content. Immunohistochemistry and western blotting techniques were performed to detect and quantify the tight junctions occludin, zonula occludens-1, and claudin-1.

Results:
Significant damage to collagen and claudin-1, directly caused by S. aureus supernatant, was observed in the CRSwNP cohort. An increase in occludin content in the healthy cohort only was detected as a result of adding S. aureus supernatant. Lactoferrin demonstrated a potentially protective role on collagen content against S. aureus toxins in CRSwNP. However, lactoferrin had no significant impact on tight junctions.

Conclusions:
The role of lactoferrin against S. aureus toxins was inconclusive but showed activity in protecting against mucosal damage; this was demonstrated by revealing that lactoferrin might potentially inhibit S. aureus exotoxins that destroy collagen. Future work should focus on gene expression analysis of the tight junctions. This work is clinically relevant by providing evidence for S. aureus as a disease-modifying pathogen in CRS, and the introduction of lactoferrin as a potential therapeutic agent in CRS with nasal polyps.

“Goals give you hope”: a qualitative look into the usefulness and efficacy of goal setting in young people experiencing challenges to their mental wellbeing

Jamie L.T. Penno*

1. School of Medicine, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand

*Corresponding author email: jpen74@uaklanduni.ac.nz

Introduction:
New Zealand, although globally perceived as being an enviable place to live, is faced with devastatingly high youth suicide and mental illness statistics. We explored the therapeutic technique of goal setting, one that is both holistic as well as being a core element in many of our current psychotherapies.

Materials & Methods:
A qualitative view of young peoples’ lived experiences and perceptions of goal setting was investigated using semi-structured interviews and a contextualist model of thematic analysis.

Results:
Six key themes emerged: (1) motivation for goal setting and achievement; (2) barriers to goal setting and achievement; (3) strategies for goal achievement; (4) the role of self-efficacy; (5) the role of support; and (6) ways in which goal setting is helpful. Sub-themes were identified within these to create a thorough picture of what drove young people to make and achieve goals, the impacts of mental illness symptoms on goal setting, how positive and negative emotions both influenced and resulted from goal outcomes, and the impact that goal setting had on young peoples’ perspective of the future.

Conclusions:
Our findings suggest that goal setting as a therapeutic technique in youth experiencing challenges to their mental wellbeing, when used well, can have substantial beneficial impacts on their emotional state, productivity, and view of the future.

Factors associated with failure of anterior cruciate ligament reconstruction

Richard Rahardja**, M. Zhu¹, SW. Young¹∗²

1. Department of Surgery, Faculty of Medical and Health Sciences, University of Auckland

2. Department of Orthopaedic Surgery, North Shore Hospital

*Corresponding author email: rah466@uaklanduni.ac.nz
Introduction:
Surgical reconstruction of the anterior cruciate ligament (ACL) has a rising incidence in New Zealand (NZ). Despite failure rates as high as 20% in active patients, there remains a lack of consensus on the patient and surgical risk factors for treatment failure.

Materials and Methods:
A systematic review was performed on studies published by the current national and community ACL registries and was used to inform three subsequent studies involving prospective data collected by the NZ ACL Registry. Statistical analysis was performed via adjusted Cox proportional hazards regression to identify the risk factors for revision and contralateral ACL reconstruction.

Results:
Thirty-three registry studies were reviewed and over twenty different patient and surgical risk factors were identified. In the NZ ACL Registry, younger age, male sex (adjusted hazard ratio [HR] = 2.00, p < 0.001), and a shorter time from injury to surgery (adjusted HR = 2.15, p = 0.016) were associated with a higher rate of graft failure. Using a hamstring tendon autograft reduced the rate of ipsilateral graft failure (adjusted HR = 0.40, p < 0.001) but was associated with a higher rate of injury to the contralateral ACL when compared with a hamstring tendon autograft (adjusted HR = 1.91, p = 0.002). No difference was found in the rate of revision between anteromedial portal and transtibial femoral tunnel drilling techniques.

Conclusions:
Younger age, male sex, and a shorter injury to surgery time are patient factors associated with a higher rate of ACL re-injury. The surgeon’s choice of graft impacts the outcome of both the ipsilateral graft and contralateral ACL.

Stem cell therapy – the next corneal endothelial transplant

Jane Shi1*, J. Zhang1, D. Patel1, C. McGhee1

1. Department of Ophthalmology, New Zealand National Eye Bank, The University of Auckland, Auckland, New Zealand
*Corresponding author email: tshi642@aucklanduni.ac.nz

Introduction:
Currently there is a global shortage of donor corneas for corneal transplantation, the only definitive treatment for corneal endothelial disease. This project aims to further characterise the protein and genetic profiles of putative stem cells in the peripheral corneal endothelium, also known as the “transition zone” (TZ), at different stages of cell culture using western blotting (WB) and real-time polymerase chain reaction (rt-PCR). The hypothesis is that under optimal conditions, these cells would express increasing levels of corneal endothelial cell markers and decreasing levels of stem cell markers.

Materials and Methods:
TZ cells were cultured and harvested at passages three, six, and nine. WB and rt-PCR were carried out to assess the relative expression levels of various corneal endothelial markers and stem cell markers.

Results:
Western blotting showed increasing expression of the corneal endothelial markers GPC4 and Na+/K+ ATPase, and decreasing expression of the stem cell marker PITX2 with increasing passage number. Rt-PCR echoed the increasing expression of GPC4 and Na+/K+ ATPase with passage number. It also showed corneal endothelial markers to be the most abundant, and pluripotency markers to be the least abundant.

Conclusions:
These results support the hypothesis that TZ cells express increasing levels of select corneal endothelial markers, and decreasing levels of a stem cell marker with increased passage number under optimal conditions.

Behavioural determinants of visceral and ectopic fat deposition

Charlotte E.A. Stuart*, Maxim S. Petrov

1. Department of Surgery, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
*Corresponding author email: cstu666@aucklanduni.ac.nz

Introduction:
Obesity is widely acknowledged as a major public health concern. There has been increasing shift from using body mass index to body fat distribution — including visceral and ectopic — as a more precise marker of cardiometabolic risk. Lifestyle factors are frequently implicated with obesity; however, evidence for their association with more comprehensive fat phenotypes is lacking. Our study aimed to seek the association between common lifestyle factors and magnetic resonance imaging-derived fat phenotypes.

Materials and Methods:
Visceral, intra-pancreatic (IPFD), intra-hepatic (IHFD), and skeletal muscle fat depositions were measured using previously published protocols. Information on tobacco smoking, alcohol consumption, and cannabis use was acquired using a comprehensive standardised questionnaire. Generalised additive models, univariate and multivariate linear regression models, as well as relative importance analyses were conducted.

Results:
A total of 157 individuals were included in the cross-sectional study called ARIES. Tobacco smoking had a greater influence on IPFD than alcohol consumption. The amount of tobacco smoked was more important for IPFD than the number of years smoked. Regular cannabis use was most associated with IHFD, with minimal effect on the other fat phenotypes.

Conclusions:
Common lifestyle factors influence differentially visceral and ectopic fat deposition. Tobacco smoking has the strongest association with fat deposition in the pancreas and cannabis use with fat deposition in the liver. This information has the potential to inform future preventative and/or treatment strategies with a view to reducing the burden of excess adiposity.

Understanding the aseptic practices of the anaesthetic team: a potential new angle for preventing postoperative infection

Evelyn Talbot1*, D.A. Gargiulo1, T. Jowsey2, M.R. Moore1, S.J. Mitchell1, A.F. Merry1

1. Department of Anaesthesiology, School of Medicine, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
2. Centre for Medical and Health Science Education, School of Medicine, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
*Corresponding author email: etal420@aucklanduni.ac.nz
Introduction:
Previous work has shown extrinsic contamination of intravenous medications given during anesthesia and linked this to lapses in aseptic practice. This could contribute to postoperative infections. The effect of a bundle of guidelines on practice was evaluated, and knowledge and attitudes behind practice were explored.

Materials and Methods:
This project connected to the larger Anaesthetists Be Cleaner (ABC) study, in which a “bundle” of aseptic practices (including injecting medications through a 0.2 micron filter) were recommended in anesthesia for joint replacements. Anaesthetic teams’ aseptic practices, including filter use, were observed before (n = 18) and after (n = 20) implementation of this bundle to evaluate its effect on practice. Six tasks related to aseptic practices were evaluated on a five-point behaviourally anchored rating scale. Anaesthetists were also interviewed (n = 19) to explore knowledge and attitudes underpinning their practice, establish reasons for differences, and identify opportunities for future intervention.

Results:
Of the six evaluated tasks, only propofol handling (p <0.001) improved significantly from baseline. Filters were used in all post-intervention cases. Interviewees raised the importance of providing clinical evidence for guidelines, being sensitive to the busy and time-pressured nature of the operating theatre, allowance for clinical judgement, and the importance of quality improvement projects taking a wide-lens approach.

Conclusions:
ABC’s main goals around propofol handling and filter use were being met, while other hygiene practices had not significantly changed. These results mirror beliefs from interviewees that the bundle was simply about filters and propofol. However, these two changes alone have considerable potential to reduce postoperative infection.

The role of the innate immune system in the development of bone damage in gout

Dylan Van Lier1, N. Dalbeth1, D. Naot1
1. Department of Medicine, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
*Corresponding author email: dvvan37l@aucklanduni.ac.nz

Introduction:
Gout is an inflammatory arthritis caused by monosodium urate (MSU) crystal deposition in soft tissues. Localised bone erosion commonly occurs in affected joints. Recent studies exploring innate immune responses in gout found that MSU crystals induce formation of neutrophil extracellular traps (NETs) — complexes of DNA and proteins that can regulated bone erosion. This project tested the hypothesis that MSU-induced NETs affect bone-resorbing osteoclasts and contribute to regulation of gouty bone erosion.

Materials and Methods:
NETs were generated from isolated human neutrophils incubated with MSU crystals, and characterised and quantified using fluorescent DNA stains and anti-neutrophil elastase immunostaining. NETs were added to RAW264.7 cells that were treated with receptor activator of NFκB ligand (RANKL) to induce osteoclastogenesis. Cell viability was determined by alamarBlue™ and lactate dehydrogenase assays, and gene expression by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). RANKL was incubated with NETs and degradation measured by enzyme-linked immunosorbent assay (ELISA).

Results:
DNA- and immuno-staining were used to confirm the presence of NETs and optimise production and collection conditions. Gene expression analysis found that NETs induced expression of the osteoclast markers Dcstamp, Acp5, Ctsk, and Oscar. In contrast, NETs attenuated RANKL-induced osteoclastogenesis, reduced cell viability, and degraded RANKL in solution.

Conclusions:
Although NETs promote osteoclast-associated gene expression, their inhibition of RANKL-induced osteoclastogenesis and degradation of RANKL suggest that they may mitigate excessive bone resorption in gout-affected joints.

Non-invasive sacral neuromodulation in colorectal disease

Chris Varghese1, C. Wells1, B. Stokes1, C. Stinear2, G. O’Grady3
1. Department of Surgery, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
2. Department of Medicine, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
*Corresponding author email: cva0706@aucklanduni.ac.nz

Introduction:
Sacral nerves (S2-4) provide important autonomic and sensorimotor innervation to the bowel and bladder. Diagnostic and therapeutic sacral neuromodulation can be applied in postoperative ileus, inflammatory bowel disease, faecal and urinary incontinence. The objectives of this thesis were to: i) evaluate the state of implantable sacral neuromodulation for faecal incontinence in New Zealand; ii) evaluate the feasibility of non-invasive magnetic sacral neuromodulation in conjunction with high-resolution electromyography as a diagnostic tool; and iii) explore two novel methods of non-invasive sacral neuromodulation.

Materials and Methods:
A retrospective audit of clinical records and postal survey were conducted to evaluate efficacy, complications and costs of implantable sacral neuromodulation in New Zealand. In vivo porcine and human studies were used to evaluate the technical feasibility of trans-sacral magnetic stimulation and high-resolution electromyography in evaluating the external anal sphincter and pudendal nerve function. Benchtop experiments and software simulations were used to assess proof-of-concept for two novel non-invasive stimulation modalities.

Results:
While effective, current implantable sacral neuromodulation is invasive, expensive and has a high rate of complications. Magnetic sacral neuromodulation with high resolution electromyography is a feasible and patient-preferred method of evaluating pudendal nerve and external anal sphincter function. Motor-evoked potential morphology, neural latency and spatiotemporal maps were generated as diagnostic outputs. The concepts underlying electroultrasonic and interferential stimulation were deemed plausible, and proof-of-concept was established in benchtop experiments.

Conclusions:
The sacral nerves are an effective diagnostic and therapeutic stimulation target, however current methods are too invasive. Magnetic, electroultrasonic and interferential stimulation offers non-invasive modalities for diagnostic and therapeutic sacral neuromodulation. However, further refinements are required prior to clinical translation.
Optimising the management and measurement of post-operative pain

William Xu1*, Armen Gharibans1, Cameron I. Wells1, Greg O’Grady2

1. Department of Surgery, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
*Corresponding author email: wxu958@aucklanduni.ac.nz

Introduction:
Post-operative pain is a significant clinical problem following surgery. Current treatments rely on opiate medications associated with adverse effects and delayed bowel recovery. Optimal pain management requires timely assessment using a multi-modal and opiate minimising approach.

Materials and Methods:
A literature review and retrospective study were completed to investigate the role of opiates in delayed bowel recovery after surgery. A network-meta analysis (NMA) of local and regional analgesic techniques was conducted to determine the optimal analgesic adjunct to minimise pain and opiate consumption after surgery. A pilot trial of patient-controlled transcutaneous electrical nerve stimulation (TENS) as a post-operative analgesic adjunct was conducted. The feasibility of ambulatory physiological recordings for the assessment of post-operative recovery was examined.

Results:
Increased intravenous (IV) opiates and decreased oral (PO) opiates, although not independent predictors of the post-operative complication, are higher in patients with prolonged post-operative ileus (PPOI). Spinal analgesia and transverse abdominis plane (TAP) blocks optimise pain and opiate minimisation after colorectal surgery. Patient-controlled TENS is feasible and patient-tolerable as an analgesic adjunct after surgery. Ambulatory monitoring of physiological signals such as electrodermal activity (EDA) is feasible and may show associations with post-operative symptoms.

Conclusions:
Better pain management and opiate minimisation are important factors in optimising patient recovery after abdominal surgery. Several therapeutic options explored may be effective in clinical practice. Novel methods of pain monitoring are promising. Future studies should seek to confirm the efficacy of these methods.

We hypothesise that these haemodynamic changes may compromise cerebral perfusion, which may be risky in the setting of an ongoing occlusion.

Materials and Methods:
Normotensive, hypertensive, and treated-and-controlled hypertensive rats were instrumented with telemeters to remotely record blood pressure (BP). Rats were anaesthetised with isoflurane at baseline and the middle cerebral artery (MCA) was occluded via an intra-luminal suture. Anaesthesia was turned off and after two hours; rats were re-anaesthetised whilst the MCA remained occluded. Reperfusion was established with the removal of the suture and functional recovery and infarct size were determined. Data were reported as mean ± standard deviation (SD).

Results:
In normotensives with an ongoing stroke, the induction of anaesthesia resulted in a rapid fall in mean BP (~57.3 ± 15.1 mmHg) that was significantly greater (p <0.05) than the same rats without occlusion. Amongst groups with an ongoing stroke, the magnitude and rate of the hypotensive response was exaggerated in hypertensives (109.1 ± 40.3 mmHg, p <0.05; 19.7 ± 4.5 mmHg/minute, p <0.01), and treated-and-controlled hypertensives (−101.7 ± 25.5 mmHg, p <0.01; 19.7 ± 6.02 mmHg/minute, p <0.01) compared to normotensives.

Conclusions:
During ischaemia, the brain is thought to be vulnerable to BP variations due to impaired cerebral autoregulation. Our data suggests that hypertensives, making up most of the stroke population, may be more vulnerable to anaesthetic-induced hypotension. This provides evidence in favour of an individualized approach to active haemodynamic management during endovascular thrombectomy.

The challenge of anaesthesia during ischaemic stroke: haemodynamic observations in normotensive and hypertensive rats

Tracy Zhang1*, P. Thakkar1, T. Emans1, C. Barrett1, R. Billings2, F.D. McBryde1

1. Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand
2. Department of Anaesthesiology, School of Medicine, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand
*Corresponding author email: tzha339@aucklanduni.ac.nz

Introduction:
Endovascular thrombectomy has revolutionized the treatment of ischaemic stroke, yet the optimum physiological management during this procedure remains uncertain. In New Zealand, >90% of thrombectomy procedures are performed under general anaesthesia which can cause hypotension and impair cerebral autoregulation.