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Theoretical reduction in the anticholinergic burden in older adults with dementia in New Zealand

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A high prevalence of prescribing potentially inappropriate medications, particularly medications with anticholinergic properties (MAP), has been observed in older adults (>65 years of age) with dementia in New Zealand. MAP have been associated with several adverse outcomes in this vulnerable population, specifically the risk of accelerating cognitive decline in patients with pre-existing dementia. The purpose of this study was to compile a comprehensive list of therapeutic alternatives to MAP prescribed for comorbidities in individuals with dementia. Further, the list was used in a cohort of patients with dementia, to determine theoretically if a reduction in the anticholinergic burden (ACB) could be achieved.

An extensive literature review of ACB scales and serum anticholinergic activity of numerous medications was conducted. The list was applied to the individuals who had a standardised comprehensive clinical assessment, the International Resident Assessment Instrument Home-Care (interRAI-HC), to determine the reduction in the ACB. Using a Paired-Samples Test, we compared the results of the ACB before and after the theoretical intervention of the pharmacological alternatives to MAP.

The 2015 interRAI dataset constituted 75,410 community-dwelling older adults, of which 12,984 (17.2%) were diagnosed with dementia. Of these, 49.5% (6,430) individuals were prescribed at least one MAP. By incorporation of the recommendations, we observed a mean reduction of the ACB by 0.49 (95% CI, 0.47-0.51). We could theoretically reduce the prescription of MAP by suggesting therapeutic alternatives in 2,006 older adults with dementia (31.2%).

The list of alternatives to MAP is intended to be a useful tool for health professionals that manage individuals with dementia. The implementation of the recommendations for prescribing therapeutic alternatives to MAP in this vulnerable population along with an awareness created among prescribers has the potential to reduce untoward effects associated with the prescription of MAP.

Building a brain – from genes to phenotypes.

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Cortical malformations arise from *in utero* disruption of neurogenesis, the process of proliferation, differentiation, and migration of neurons in the developing brain. Finding attributable genetic causes for these malformations will sharpen diagnosis and prognostication for patients. However, the heterogeneous nature of these abnormalities creates challenges for assigning pathogenicity. The aim of this study was to apply high throughput sequencing methods to a cohort of 205 patients with periventricular nodular heterotopia (PVNH), a cortical malformation characterised by grey matter nodules abutting the lateral ventricles of the brain due to a failure in neural migration.

Aligned sequence data from patients was processed using a range of custom pipelines designed to capture short genetic variants, as well as large structural events and somatic mutations. Data for parental samples was available for 137 patients, permitting filtering based on inheritance models, along with identification of *de novo* events and causal biallelic genotypes. A filtering method based on recurrence with known and suspected disease genes was adopted for patients without complete parental data.

Pathogenic variants were identified for 17 patients with parental data, along with several candidate variants of

uncertain diagnostic significance. Of these variants, only two genes were recurrently mutated, *FLNA* (n = 4, $P < 0.001$, binomial) a known PVNH gene, and *SON* (n = 5, $P < 0.001$, binomial), associated with the multisystem developmental disorder ZTTK syndrome. Using known and candidate disease-gene patterns, another 12 pathogenic variants were found in patients without parental data, including an additional *SON* variant.

The identification of six patients with *SON* variants heavily implicates it in the pathogenesis of PVNH, increasing our understanding of the molecular pathways critical for neurogenesis. Further, the association between PVNH and ZTTK syndrome is underappreciated and may provide a distinctive radiological marker pointing to this diagnosis in the absence of genetic testing.

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Repurposing the hypoglycemic agent, metformin, for targeted lung cancer.

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Lung cancer accounts for the highest incidence of cancer mortality worldwide. The EML4-ALK chromosomal rearrangement is involved in 2-7% of lung cancer cases. Crizotinib, the first-line treatment for ALK+ lung cancer is an effective treatment, however, resistance develops usually after one year. To prevent/overcome resistance, novel strategies are being explored. Epidemiological studies show a reduction in the risk of cancer with the use of a hypoglycemic agent, metformin. We aimed to test a combination of metformin and crizotinib for toxicity and efficacy in a xenograft model of lung cancer.

For toxicity analysis, balb/c mice (n = 6) were administered (P.O.) vehicle, metformin (100 mg/kg), crizotinib (25 mg/kg) or the combination once daily for 14 days. On the 15th day serum was extracted for ALT and creatinine analysis. For efficacy testing, Nu/J mice (n = 6) were subcutaneously injected with ALK+ H3122 cells in the flank region. Mice were administered the same dosing regimen as in the toxicity study. Tumor volumes were weighed daily and full necropsies were performed on day 15.

The serum markers of liver (ALT) and kidney (creatinine) toxicity both remained under the normal threshold for all treatment groups. When examining the efficacy of the drugs; metformin, crizotinib and the combination significantly decreased tumor volume compared to vehicle (612, 424 and 552 vs. 943 mm^3, respectively, $P < 0.001$). However, the combination produced no added benefit when compared to crizotinib alone.

The combination produced no additional toxicity and while metformin alone was able to slow tumor growth, the combination did not have any greater therapeutic benefit compared to the monotherapies. We hypothesize that crizotinib is limiting the entry of metformin into cells via an inhibition on organic cation transporters. Nevertheless, metformin alone produced a significant difference in tumor growth compared to the control. This provides justification to further examine the effect and mechanisms of metformin in cancer.

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An investigation into dysfunctional feed-forward inhibition within the cortico-thalamocortical network on absence seizure generation.

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Brain function depends on a balance between excitation and inhibition. Feed-forward inhibition (FFI) within the brain controls the firing of principal excitatory neurons and prevents runaway excitation. It is primarily mediated by parvalbumin-expressing (PV+) inhibitory interneurons. Abnormal functioning of these interneurons leads to neurological disorders, including epileptic seizures. In the cortico-thalamocortical (CTC) network, dysfunctional FFI has been implicated in absence seizure generation. The hallmark of absence seizures is spike-wave discharges (SWDs) measuring 3-4 Hz on an electroencephalogram (EEG) with concomitant behavioural arrests termed absences. Our laboratory has previously reported defects in the activation of PV+ interneurons in the stargazer mouse model of absence epilepsy, which could underlie hypersynchronous excitation leading to seizures. The aim of the current study was to investigate the impact of dysfunctional FFI within CTC network on absence seizure generation and behaviour.

We used Designer Receptors Exclusively Activated by Designer Drug (DREADDs) technology to silence/excite PV+ interneurons. To target these interneurons, mice expressing Cre recombinase in PV+ interneurons (PV-Cre) were bred with inhibitory Gi-DREADDs or excitatory Gq-DREADDs mice. We confirmed selective expression of DREADDs in PV+ interneurons via confocal microscopy. Simultaneous video/EEG recordings were made after silencing/exciting PV+ interneurons within CTC microcircuits.

Silencing PV+ interneurons generated absence-like SWDs and reduced ambulation in Gi-DREADD animals. The mean duration and frequency of SWDs were 2.9 ±0.3 sec and 5.5 ±0.5 Hz, respectively. SWDs were blocked by administrating the anti-absence epileptic drug ethosuximide. Conversely, activating PV+ interneurons

during pentylenetetrazole (PTZ) induced seizures in Gq-DREADD animals delayed the latency, decreased mean duration and total number of PTZ-induced absence-SWDs.

These data indicate that loss of FFI within the CTC network is one causative mechanism for pathological SWD oscillations. This could be a target for future improved treatment strategies since current anti-epileptic drugs are ineffective or cause serious side-effects in one-third of patients.

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Inhibitory receptor expressing T cells produce functional cytokines and are enriched in the tumour compared to the non-tumour bowel and peripheral blood of patients with colorectal cancer.

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Expression of inhibitory receptors (iRs), such as CTLA-4 and PD-1, are associated with impaired immune cell function and can limit anti-tumour immune responses. Therapies that block inhibitory receptors (immune checkpoint blockade, ICB) can enhance the anti-tumour immune response and improve patient survival. A high infiltrate of iR+ T cells are present in the tumours of patients with colorectal cancer (CRC); however, ICB is not effective in many CRC patients. The aims of this study were to determine iR expression on T cells in CRC and associate iR expression on T cells with T cell functionality *ex vivo* and *in vitro*. We hypothesised that expression of iRs does not correlate with impaired immune cell function and iR+ T cells are important in the anti-tumour immune response.

High dimensional analysis of mass cytometry data from patient samples (n = 8) identified heterogeneous populations of iR+ T cells. Two populations of T cells were significantly enriched in the tumour compared to blood of CRC patients: CD4+FOXP3+BLIMP-1+ "effector Tregs" (P = 0.0009, Dunn's multiple comparisons); and CD4+PD-1+CTLA-4+CD39+ T cells (P = 0.035, Dunn's multiple comparisons). These iR+ T cells, enriched in the tumour, secreted anti-tumour molecules (Granzyme B, IFNg, TNF; n = 8). Therefore, iR expression does not indicate impaired function within CRC tumours. PD-L1 ligation *in vitro* did not significantly alter cytokine expression (IL-2) or proliferation (Ki67) in PD-1+CD4+ T cells. However, PD-1 ligation decreased cytokine production and proliferation in CD4+FOXP3+ T cells (n = 4, P = 0.0625, Wilcoxon sign-rank test), suggesting these two T cell populations respond differently to PD1 signalling. Ongoing *in vitro* tumour conditioned media experiments will determine the effect of the tumour microenvironment on these populations.

This study identifies populations of functional iR+ T cells in CRC tumours, which may explain a lack of clinical efficacy of ICB in these patients.

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Arrhythmogenic and inotropic effects of long-chain acylcarnitines in the human heart.

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Long-chain acylcarnitines (LCACs) are metabolites essential for lipid metabolism in the heart. Recent metabolomic studies have revealed that high plasma levels of LCACs, especially the 18:1 species, are associated with increased risk of cardiovascular diseases, including atrial arrhythmias. This study aimed to address whether LCAC 18:1 directly alters the susceptibility for arrhythmias and contractility of human heart muscle.

Human heart muscles (N = 32) were mounted in a tissue superfusion bath and stimulated to contract were at 1 Hz. The propensity for spontaneous muscle contractions in the absence of external stimulation was used to assess *ex vivo* arrhythmias. Relative to baseline conditions, exposure to LCAC 18:1 at a 25 µM dose for 45-minutes (n = 8) increased the proportion of spontaneously active muscles by 50% (Fishers exact test, P < 0.05). Furthermore, LCAC 18:1 concurrently increased the contraction force of the muscles in a dose-dependent manner (1, 5, 10, and 25 µM, n = 8 for each), with the 25 µM dose inducing a 1.5-fold increase (P < 0.01, repeated measures one-way ANOVA). Both the arrhythmogenic and inotropic effects of LCAC 18:1 were reversed following LCAC wash-out. To gain mechanistic insight, recombinant HEK293 cells were loaded with the cytosolic Ca^{2+} indicator, fluo-4, and were superfused with 25 µM LCAC 18:1. LCAC 18:1 induced a marked cytosolic Ca^{2+} overload in this cardiac cell model, as well as a reduction in the intracellular Ca^{2+} store size. These effects were augmented

by increasing extracellular Ca^{2+} concentrations, suggesting that LCAC 18:1 enhances Ca^{2+} flux across external and internal membranes.

This study is the first to show that LCAC 18:1 can promote arrhythmias and contractility changes in human cardiac muscle, which are linked to cytosolic Ca^{2+} overload. This first use of human heart tissue is a critical step in improving the translatability of LCAC pathophysiology and metabolomics to a clinical setting.

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Calcium calmodulin dependent protein kinase II δ in the pathogenesis of atherosclerosis.

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Atherosclerosis is the leading cause of death in the developed world. The build-up of atherosclerotic lesions within the arterial wall are responsible for life threatening events including stroke. The vascular wall is exposed to a number of stresses that require cellular responses to maintain homeostasis. The calcium/calmodulin-dependent protein kinase II (CaMKII) isoform family has important roles in maintaining vascular homeostasis but more recently emerged in the context of vascular dysfunction.

Previously, we showed that systemic inhibition of CaMKII leads to a reduction of atherosclerosis in the brachiocephalic artery of a mouse model of atherosclerosis ($ApoE^{-/-}$). The next critical step in translating this work to a clinical intervention is to investigate which isoform of CaMKII contributes to atherogenesis.

The aortic tree of 13- and 20-week $ApoE^{-/-}$ mice was dissected and the aortic arch and carotid artery isolated. In addition, human umbilical vein endothelial cells and human coronary artery smooth muscle cells were cultured. PCR and Western blotting was carried out in all samples for the two primary vascular isoforms of CaMKII (δ and γ). Results showed calcium calmodulin-dependent protein kinase II (CaMKIIδ) as the predominant isoform. We next employed a genetic approach by crossing $ApoE^{-/-}$ mice with $CaMKII\delta^{-/-}$ to generate an $ApoE^{-/-}CaMKII\delta^{-/-}$ (dKO) mouse model. Analysis of the aortic sinus at 20 weeks showed extensive atherosclerosis in female groups. Female dKO mice had a 162 ± 31 μm^3 reduction in aortic sinus lesion volume compared to female $ApoE^{-/-}$ (mean ± SEM, n = 11, $P < 0.05$, t-test). Finally, adeno-associated viral vectors (AAVs) expressing either CaMKIIδ-mCherry or control-mCherry were introduced to ligated carotid arteries of dKO mice and 4-weeks later the extent of lesion development was assessed to show the contribution of CaMKIIδ in atherogenesis.

Collectively, our results show that CaMKIIδ is a major promoter of atherosclerosis lesion development. Importantly, we have identified a specific therapeutic target that could provide impetus for drug development.

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