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The clinical course, therapeutic responses, and outcomes in relapsing MOG antibody-associated demyelination

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Background: There is increasing recognition of patients with demyelination who have antibodies targeting myelin oligodendrocyte glycoprotein (MOG), a protein on the external layer of the myelin sheath. These patients can exhibit a relapsing clinical course. Data regarding the efficacy of immunosuppressive agents in patients with MOG antibodies is limited.

Objective: We characterised the clinical course, treatment, and outcomes in 59 patients with relapsing MOG antibody-associated demyelination.

Design Methods: We evaluated clinical phenotypes, annualised relapse rates (ARR) prior and on immunotherapy, and expanded disability status scales, in 216 demyelinating episodes from 33 paediatric and 26 adult patients, with an average follow-up of five years.

Results: The most common initial presentation was optic neuritis (ON) in 54% [bilateral (BON) 32%, unilateral (UON) 22%]. Patients were steroid responsive but 70% of episodes treated with oral prednisone relapsed, especially at doses <10 mg and within two months of cessation. Immunotherapy, including prednisone, intravenous immunoglobulin (IVIg), rituximab, and mycophenolate, all reduced median ARRs on-treatment. Treatment failure rates were lowest with maintenance prednisone. 59% of patients experienced residual deficits. >3 episodes were associated with disability.

Conclusions: Relapsing MOG antibody-associated demyelination is strongly associated with ON, is steroid responsive but vulnerable to relapse on withdrawal, and responsive to maintenance corticosteroids, IVIg, or immunosuppression. A subgroup of patients with MOG antibodies can have poor outcomes. Increasing relapses lead to sustained disability.

The Efficacy of Psychosocial Interventions for People with Multiple Sclerosis: A meta-analysis of specific treatment effects

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Background: Psychosocial interventions are often used as an adjunct to the medical management of Multiple Sclerosis (MS). However, the efficacy of such approaches for a range of psychosocial indications remains unclear.

Objective: The objective of this meta-analysis was to determine the efficacy of psychosocial therapies for people with MS (PwMS).

Design Methods: We searched Medline, Embase, PsycINFO, Cochrane Central Register of Controlled Trials and CINAHL from database inception until April 21, 2016 using systematic review methodology. We included randomized controlled trials that reported the effects of psychological interventions for depressive symptoms, anxiety, pain, fatigue, or health-related quality of life (HRQoL) in PwMS.

Results: The search yielded 356 articles with 13 included studies (n=1617). Benefits of psychological interventions were found for depressive symptoms (Cohen's $d=0.281$), anxiety ($d=0.285$), fatigue ($d=0.228$), mental ($d=0.398$) and total HRQoL ($d=0.444$), but not physical HRQoL. Interventions were more effective for HRQoL for patients with relapsing-remitting MS and when treatment doses were larger. Cognitive behavioural therapy (CBT) was less efficacious than other interventions. However, this may be due to smaller treatment doses in CBT studies.

Conclusions: Psychosocial interventions have a significant, positive impact across a range of outcomes for PwMS with small, yet consistent effect sizes. Alternative approaches such as mindfulness-based interventions appeared to be more promising than CBT in moderator analyses, particularly for HRQoL. Further research to confirm this finding is needed.

Assessing the risk of cervical dysplasia in women with MS compared to women without disease using a data linkage approach

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Background: Human papillomavirus (HPV) infection is the most common sexually transmitted disease worldwide and the underlying cause of pre-malignancies and cancer of the cervix. Control of HPV infection relies upon host local immune response. Long-term immunosuppressive therapies can increase risk of persistent HPV infection and related diseases.

Objective: The risk of persistent HPV infection, cervical dysplasia and HPV-related cancers is unknown in women with MS. Given their long-term immunomodulatory and immunosuppressive treatments, it is important to determine the prevalence of cervical dysplasia and consequently help inform guidelines for the highly effective HPV vaccine for this MS cohort.

Design Methods: We identified women aged 18-70 with a primary diagnosis coded at hospital separation as MS through the Victorian Admitted Episode Database. Their cervical screening history was identified using probabilistic data linkage to women with cervical screening episode(s) between 2009–2013 on the Victorian Cervical Cytology Registry. Cervical dysplasia outcomes identified were: cytological low- and high-grade abnormalities (LGA, HGA) and histologically confirmed abnormalities (HisA). Results were stratified by age and intergroup comparisons were performed.

Results: 2382 MS patients were compared with 929,670 women in the general population. There were similar proportions of cytological and histological abnormalities between cohorts. In the MS cohort, 7.18% had LGA, 2.35% had HGA, and 1.68% had HisA; these were 6.84%, 2.46% and 2.31%, respectively, in the general population. The younger MS cohort had higher rates of all abnormalities, but these rates dropped below that of the general population in the later age groups.

Conclusions: Data demonstrates similar rates of cervical dysplasia for women with MS and the general population. While the results are reassuring, the lack of data on DMT-exposure makes it difficult to assess risk on an individual basis. Physicians should remain vigilant and recommend screening and vaccination to all patients with MS.

A new way of looking at MS. Does the fact that myelin proteins are old and degraded explain why MS develops?

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Background: Demyelination, which is characteristic of MS, is caused by an abnormal immune response against a component of central nervous system myelin. Myelin proteins are long-lived and

degrade over time. If decomposition is significant, they become effectively a novel antigen. This could induce an untoward immune response that manifests as MS.

Objective: To determine if myelin proteins in the human brain, in particular MBP, undergo post-translational modifications that are indicative of them being long-lived. If so, how abundant are these changes, and are there consistent differences between controls and MS patients?

Design Methods: MBP was isolated from the cerebellum of control human brains and from MS patients suffering from Relapsing-Remitting, Secondary-Progressive, Primary-Progressive and Progressive-Relapsing MS. The degree of protein degradation with age and MS was determined using proteomics.

Results: Proteomic analysis revealed that, by the age of 18, a high degree of MBP modification had occurred in both MS and control brain samples. Transformation of aspartate/asparagine residues to isoaspartate was common. This spontaneous process involves both racemisation (i.e. conversion of an L- to a D-amino acid) plus the insertion of a CH₂ group into the main polypeptide chain. Deimination of arginine to citrulline was also abundant. Such processes lead to changes in protein structure.

Conclusions: MBP is long-lived in the human brain and in adults it has been extensively degraded. MBP from MS patients displayed significantly higher amounts of modification at some sites compared to age-matched controls. Such MS-specific alterations to MBP may be particularly antigenic and could act as a trigger for MS.

Identifying human regulatory B cells activated by IL-4-treated mast cells or their microvesicles by mass cytometry

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Background: The ultraviolet (UV) component of sunlight can protect from multiple sclerosis. In mice, UV-protection is partly due to immune suppression via activation of regulatory B cells. Mast cells also play an important role in UV-immunosuppression. Because mast cells are known to influence the activation of B cells, our hypothesis is that these events are linked.

Objective: Early experiments in mice demonstrated IL-4-treated mast cells (or their microvesicles) activate B cells that were functionally immune suppressive in vivo. These regulatory B cells were phenotypically similar to those found in lymph nodes that drain UV-exposed skin. Our work investigates whether human mast cells could activate regulatory B cells.

Design Methods: Human mast cells derived from peripheral blood stem cells were treated with IL-4 before being co-cultured with autologous B cells. There is currently no standard marker for identifying human regulatory B cells. To expand our discovery scope, we adopted a mass cytometry approach to characterise the phenotype of the B cells emerging from this co-culture. Using a combination of 35

antibodies, we could identify memory, naïve and transitional B cell subsets and analyse marker expression within each group.

Results: B cells emerging from the mast cell cultures displayed a predominantly transitional phenotype. There were marked changes in a number of immune-related molecules, including an increase in IL-10.

Conclusions: Human IL-4-treated mast cells are capable of activating B cells with a regulatory phenotype. This interaction may be an important mechanism by which UV can suppress the immune response, and in turn grant protection from multiple sclerosis. This could provide a new avenue of therapeutics for those suffering from multiple sclerosis.

No association between antioxidant intake and risk of a first clinical diagnosis of central nervous system demyelination

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Background: Although diet is potentially a modifiable risk factor for multiple sclerosis, the components of dietary intake that may relate to multiple sclerosis are unknown. The evidence for the role of antioxidants in reducing the risk of multiple sclerosis is inconclusive.

Objective: We tested associations between antioxidant intakes (vitamin C, vitamin E, beta-carotene, alpha-carotene, beta-cryptoxanthin, lutein/zeaxanthin, lycopene) and risk of a first clinical diagnosis of central nervous system demyelination (FCD), a common precursor to multiple sclerosis.

Design Methods: We used food frequency questionnaire data from the 2003-2006 Ausimmune Study (272 cases, 519 controls), an incident case-control study examining environmental risk factors for FCD. Intakes of vitamin C, vitamin E, beta-carotene, alpha-carotene, beta-cryptoxanthin, lutein/zeaxanthin and lycopene from food were calculated per 1000 kcal of energy intake per day. Logistic regression models were used to test for associations with FCD, adjusting for age, sex, region, smoking, vitamin D status and history of infectious mononucleosis (n=750).

Results: We found no associations with FCD for vitamin C (AOR=1.00; 95%CI 1.00,1.01; p=0.17), vitamin E (AOR=1.07; 95%CI 0.89,1.28; p=0.49), beta-carotene (AOR=1.00; 95%CI 1.00,1.00; p=0.29), alpha-carotene (AOR=1.00; 95%CI 1.00,1.00; p=0.59), beta-cryptoxanthin (AOR=1.00; 95%CI 1.00,1.00; p=0.62), lutein/zeaxanthin (AOR=1.00; 95%CI 1.00,1.00; p=0.30) or lycopene (AOR=1.00; 95%CI 1.00,1.00; p=0.15).

Conclusions: Our results suggest there is no protective effect of higher antioxidant intake and risk of FCD. However, intake of polyphenols was not assessed, and we measured antioxidant intake from food only; the contribution of dietary supplements to antioxidant intakes may be substantial.

Racemisation of Long-Lived Proteins in Human Myelin: Implications for Multiple Sclerosis

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Background: The trigger for multiple sclerosis is currently unknown. Recently, it has been found that myelin proteins such as myelin basic protein (MBP), proteolipid protein and 2',3'-cyclic-nucleotide 3'-phosphodiesterase are long-lived. Over time long-lived proteins degrade and accumulate post-translation modifications (PTMs). This can have major consequences for both protein structure and function.

Objective: To determine if myelin proteins from human brains, in particular MBP, undergo post-translational modifications that are typical of long-lived proteins, such as racemisation and isomerisation. These modifications have previously been shown to be immunogenic.

Design Methods: MBP was isolated from cerebellum, hippocampus, prefrontal cortex, and entorhinal cortex from control human brains. Additionally, MBP was isolated from the cerebellum of MS patients suffering from Relapsing-Relapsing, Secondary-Progressive, Primary-Progressive and Progressive-Relapsing MS. The amount of racemisation with age and MS was determined by a combination of proteomics and amino acid analysis.

Results: Amino acid and proteomic data revealed at an early age a high degree of modification had already occurred to MBP. Racemisation was abundant in all brain regions tested. By age 18 between 8-11% of all Asn/Asp residues in MBP had been racemised. This equates to the insertion of approximately 1 D-Asp residue in every MBP polypeptide from adult brain. Interestingly, MS MBP displayed a significantly higher amount of racemisation in comparison to age-matched controls.

Conclusions: From these results we conclude that MBP is long-lived in the human brain. Since racemisation in MS patients was significantly higher, it raises the possibility that these post-translation modifications may be a potential trigger for MS.

Genotype-phenotype correlations in relapsing-remitting multiple sclerosis: preliminary results from a multinational study

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Background: To-date efforts to identify genetic associations with MS phenotype have been largely unrewarding. One possible explanation for this is that past genotype-phenotype association studies have relied on cross-sectional definitions of disease severity. Despite their limitations, these studies have identified 109 genetic variations putatively associated with disease severity.

Objective: To validate previously reported putative genetic correlates of disease severity using a robust relapse-onset MS (RMS) disease severity phenotype based on longitudinally acquired outcomes data.

Design Methods: Data were obtained from MSBase. We created and validated an algorithm that identifies patients at the extremes of RMS outcomes, based on minimum 5 years prospective follow-up, and minimum 3 expanded disability status scale (EDSS) scores recorded in the absence of a relapse. DNA from 1236 consented individuals at outcome extremes underwent genotyping using the Illumina MegaEx platform. Our MegaEx chip was customised with additional content to ensure past suggestive severity associations could be tested.

Results: The mild RMS cohort as selected by the algorithm (25.9% of our population) had a median symptom duration of 11.6 years, 14 EDSS scores assessed, and median observation follow-up MSSS of 1.50. The severe RMS cohort constituted 18.4% of our population. Severe RMS cohort characteristics comprised: median symptom duration of 19.0 years, 15 EDSS scores assessed, and median observation follow-up MSSS of 7.21. Here we report validation of one previously published putative severity variant.

Conclusions: We have successfully defined a robust RMS phenotype using longitudinal, prospectively acquired clinical outcomes data validated against the MSSS in which we assessed genotype-phenotype associations. We have validated one of the previously published disease severity variants making this the first replicated genetic variant associated with MS disease severity.

Pregnancy incidence, therapy exposure and post-partum relapse risk: a 12-year retrospective multicentre analysis

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Background: The treatment armamentarium for relapsing MS has changed significantly over the past decade. Few disease modifying therapies (DMT) have indication for use during pregnancy in MS. Outside of pregnancy registries, little is known about how women of childbearing potential interact with these DMT. Further predictors of post-partum relapse require clarification.

Objective: To retrospectively determine the incidence of pregnancy and therapeutic exposure in women with relapsing MS between 2005-2016. Additionally, to identify predictors of time to first relapse post-partum.

Design Methods: We conducted a retrospective cohort analysis. We identified all women with relapse-onset MS who were of childbearing potential (aged 15-45) in the MSBase Registry between January 2005 and October 2016. Annualised pregnancy incidence rates were calculated. Demographic, clinical information, and disease-modifying therapy (DMT) exposure during pregnancy were assessed. Predictors of time to first post-partum relapse were determined using survival analysis.

Results: 9,098 women of childbearing potential were identified. 1,178 women recorded 1,521 pregnancies. Annualised pregnancy incidence rate was 0.042. 635 (41.7%) pregnancies were conceived on therapy: 76.7% on injectables, 6% on orals, 16.7% on monoclonal antibodies, 0.6% on azathioprine. Annualised incidence rates of pregnancy were similar by DMT class. Pre-pregnancy relapse rates (HR 1.2), EDSS 2 at pregnancy (HR 1.52), and lack of DMT re-initiation (HR 7.98) were significantly associated with increased risk of post-partum relapse.

Conclusions: Pregnancy incidence was low in our cohort. A large proportion of pregnancies were conceived on DMT; a significant number were conceived on class C/D drugs, with comparable annualised incidence by therapeutic class. Post-partum relapse risk was associated with high pre-pregnancy disease activity, and slow re-initiation of DMT post-partum.

Genetic variation in the gene LRP2 increases relapse risk in multiple sclerosis

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Background: Due to the lack of prospective studies with longitudinal data on relapse, past genetic studies have not attempted to identify genetic factors that predict relapse risk (the primary endpoint of many pivotal clinical trials testing the efficacy of multiple sclerosis (MS) disease-modifying drugs) at a genome-wide scale.

Objective: We conducted a genome-wide association analysis (GWAS) to identify genetic variants that predict MS relapse risk, using a three stage approach.

Design Methods: Firstly, GWAS was conducted using the Southern Tasmania MS Longitudinal Study with 141 cases

followed for a mean of 2.3 years. Secondly, GWAS was conducted using the Ausimmune Longitudinal Study with 127 cases having a classic first demyelinating event followed for 5 years from onset. Thirdly, the top hits with $p < 5.0 \times 10^{-6}$ from the first two stages were combined with a longitudinal US pediatric MS cohort with 181 cases followed for 5 years after onset.

Results: In the pooled results using these three unique longitudinal MS cohorts, we discovered one novel locus (LRP2; most significant SNP rs12988804) that reached genome-wide significance in predicting relapse risk (HR=2.18, $P=3.30 \times 10^{-8}$). LRP2 is expressed on the surface of many CNS cells including neurons and oligodendrocytes and is a critical receptor in axonal guidance.

Conclusions: The finding of a genetic locus that has extensive effects on neuronal development and repair is of interest as a potential modulator of MS disease course.

X wide association analysis identifies a novel FRMPD4 locus associated with the differential sex risk for multiple sclerosis

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Background: Multiple sclerosis is characterised by a significant sex imbalance with an increasing female to male ratio of 2.3-3.5:1 for relapse onset MS. Characterising the role of the X chromosome in MS will enable a unique biological perspective on the MS aetiology.

Objective: The availability of a recently developed software (XWAS) that is specially designed for X chromosome analysis has allowed us to assess the role of X chromosome genetic variation in MS onset.

Design Methods: We therefore conducted an X-wide association analysis using 2482 MS cases and 4304 controls as a discovery sample involving 6,518 SNPs and replicated using independent MS exome array data (583 MS cases and 410 controls).

Results: We identified a significant association in FRMPD4 (rs6641026) that showed different effects by sex. In the discovery stage, the MS risk OR for males was 2.06 ($P=1.84 \times 10^{-6}$), while the OR for females was 0.94 ($P=0.35$). In the validation stage, the OR for males was 5.09 ($P=0.03$) while the OR for females was 0.83 ($P=0.38$). The combined test for differentiated effect size between sexes was significant after multiple testing ($P=3.9 \times 10^{-6}$).

Conclusions: This gene FRMPD4 functions as a positive regulator of dendritic spine morphogenesis and density, and is required for the maintenance of excitatory synaptic transmission. The finding of an association with MS risk only in males may suggest a significant parent of origin effect.

Ultraviolet irradiation of the skin alters sphingosine-1-phosphate levels in distant lymph nodes

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Background: Exposure to the ultraviolet (UV) component of sunlight contributes to the latitude-gradient effect seen in multiple sclerosis. How UV protects from CNS-autoimmunity is not well understood. Studies in mice have revealed an important role for UV-induced immune suppression, in part via the activation of regulatory B cells.

Objective: UV manipulation of normal lymphocyte recirculation by “trapping” them in skin-draining lymph nodes may be an important mechanism. As this phenomenon is also observed with fingolimod treatment, we hypothesised that UV was sequestering lymphocytes within lymph nodes by altering sphingosine-1-phosphate (S1P), a lipid chemoattractant critical to the recirculation of lymphocytes.

Design Methods: C57BL/6 mice were exposed, or not, to a single dose of solar simulated UV irradiation. 24 hours later, lymph node and plasma lipids were extracted and quantified by liquid chromatography tandem-mass spectrometry. Cells from the lymph nodes and peripheral blood were also stained for flow cytometry analysis.

Results: UV exposure significantly increased S1P levels within skin-draining lymph nodes. This corresponded to a decrease in cell-surface expression of the S1P receptor, S1P1, indicating an inhibition of cell egress. Although plasma S1P was unaffected, exposure to UV decreased the numbers of naïve and effector memory T cells in peripheral blood with a concomitant increase in skin-draining lymph nodes.

Conclusions: These results show that UV manipulates the S1P pathway which may explain the lymphocyte sequestration in the skin-draining lymph nodes, and subsequent protection from CNS-targeted autoimmunity.

Environmental risk factors in Multiple Sclerosis: Epigenetics at work?

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Background: Multiple sclerosis is a debilitating condition of the CNS where both genetic and environmental factors are associated with increased disease risk. We propose that the effect of environmental factors, particularly latitude of childhood, is mediated through epigenetic mechanisms.

Objective: We propose that unfavourable gene methylation, set in childhood/adolescence, predisposes individuals to multiple sclerosis, and is transmitted from haematopoietic stem cells to progeny. We therefore examined the methylomes of CD34+, CD14+ and CD56+ cells from multiple controls to determine, whether methylation settings were recapitulated from CD34+ to progeny cells.

Design Methods: CD34+, CD14+ and CD56+ cell subsets were isolated from peripheral blood of healthy controls. Libraries enriched for CpG islands and promoter regions were generated using modified reduced representation bisulfite sequencing and subjected to next generation sequencing. Site specific methylation profiling of genome wide CpG islands, including MS susceptibility genes was conducted using Methpipe software.

Results: Genomic coverage was consistent with published methylomes using modified reduced representation bisulfite sequencing. The methylation signature of peripheral blood derived subsets showed greater differences in methylation compared to buccal cells than with each other. Individuals displayed differences in CD34+ methylomes, and these were recapitulated in progeny CD56+ and CD14+ cells for those individuals.

Methylation of specific genes regions (e.g. PRF1), were consistent with the biological function of these genes and potential contribution to MS risk.

Conclusions: The vast majority of CpG islands recapitulated their methylation signature from CD34+ to progeny. Our results are consistent with the establishment of an epigenetic signature in early life. The physiological basis underlying this unclear, but may involve UV light and/or vitamin D, and may provide novel therapeutic targets.

Autologous haematopoietic stem cell transplantation (aHSCT) in multiple sclerosis (MS) a phase II trial at St Vincent's Hospital, Sydney.

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Background: Multiple sclerosis (MS) is an inflammatory condition of the central nervous system (CNS) marked by demyelination and axonal loss that results in chronic neurological disability. aHSCT has been shown to suppress inflammatory activity in MS and subsequently disease progression.

Objective: To evaluate the safety and efficacy of autologous hematopoietic stem cell transplantation with high-intensity immunosuppression in patients with multiple sclerosis (MS).

Design Methods: A Phase II clinical trial of aHSCT using a BEAM + ATG chemotherapeutic regimen for patients with relapsing remitting and secondary progressive MS commenced at St Vincent's Hospital in December 2010. Patients eligible for aHSCT had an Expanded Disability Status Scale [EDSS] of 2.0–7, had failed two prior disease-modifying therapies and displayed evidence of ongoing disease activity manifesting as clinical relapses and/or new MRI lesions in the year prior to aHSCT.

Results: 24 patients with RRMS and 12 SPMS completed aHSCT, with a median follow-up of 23.77 months (range 2.73–63.16). 55.6% of patients received >4 disease modifying therapies prior to aHSCT with 63.8% having received Natalizumab. Progression-free survival, clinical relapse-free survival, and MRI activity-free survival for the entire cohort are 77.8% (91.7% in RRMS), 91.7% and 91.7% respectively. Treatment related mortality was 0%. 14/36 patients demonstrated a sustained improvement in EDSS. 8/36 had worsening of disability.

Conclusions: aHSCT effectively induced sustained remission in the majority of active RRMS and SPMS patients within our heavily pre-treated cohort. Age <35 yrs and baseline EDSS score <4 had the highest rates of EFS. The results support revision of aHSCT criteria to identify patients that will derive greatest benefit from aHSCT.

Identification of Blood-Brain Barrier Disrupting Factors in Multiple Sclerosis.

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Background: The dysregulation of the blood brain barrier (BBB) and recruitment of activated leukocytes across the BBB into the brain are critical in relapsing multiple sclerosis (MS)

pathogenesis. The triggers of the BBB breakdown are not fully understood, but studies have suggested the involvement of cytokines and chemokines on endothelial regulation.

Objective: Assessment of MS serum for BBB disrupting activity using real-time biosensor technology.

Design Methods: 30 serum samples from relapsing-remitting MS patients with active lesions were acquired from the Brain and Spinal Fluids Bank in the USA. The effect of serum on the integrity of an in vitro brain microvascular endothelium BBB model was measured using Electric Cell-substrate Impedance Sensing (ECIS) biosensor.

Results: 3 of the 30 MS serum samples majorly disrupted the integrity of brain microvascular endothelium. All samples were obtained from relapsing MS patients. We hypothesized that BBB breakdown is caused by non-cellular soluble factors (e.g. inflammatory cytokines, antibodies, lipids or enzymes) in the serum of some patients with relapsing remitting MS. The mechanism of the serum-mediated barrier disruption is still unknown, and identity of the soluble factors is being investigated.

Conclusions: We propose that the BBB compromise caused by these soluble factors could potentially contribute to relapses. Ongoing study aims to extend these findings in larger cohort to discover the molecular identity of these BBB disrupting non-cellular factors.

The impact of multiple sclerosis on life expectancy, quality-adjusted life years and total lifetime costs: evidence from australia

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Background: Multiple Sclerosis (MS), a long-term neurodegenerative disease that has a substantial effect on morbidity and mortality, as well as being a major economic burden to patients and society.

Objective: Our aim was to quantify life expectancy (LE), quality adjusted life years (QALYs) and total lifetime costs for a typical cohort of Australians with MS.

Design Methods: A 4-state Markov model simulated progression between mild, moderate and severe disability and death for a cohort of 35year olds starting with mild disability over a lifetime horizon. Risks of death were adjusted by increasing disability severity. State-dependent relapse probabilities and their associated disutilities were accounted for. The probabilities of MS progression and relapses were estimated. Direct and indirect costs (2016 Australian dollars) and health state utility values for each state were derived from AMSLS.

Results: We have developed a health economics model of the progression of MS, allowing us to calculate the impact of MS on life expectancy, QALYs and lifetime costs in Australia. Mean (95% confidence interval) LE from age 35 years was 43.3 (42.2–44.2) years. This was 6.9 years less than for the Australian population. QALYs were 28.7 (26.7–30.4) years, a loss of 12.6 years compared with the Australian population. Discounted mean lifetime costs were AUD 954,419 (349,875–2773,570).

Conclusions: LE and QALYs were substantially decreased in comparison to general population norms due to relapses and progressive disability associated with MS, with correspondingly huge lifetime costs. This model will form the basis for future cost-effectiveness analyses of interventions that reduce relapses and progression of disability resulting from MS.

Altered regulatory T-cell fractions and Helios expression in Clinically Isolated Syndrome

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Background: Disturbances in immunoregulatory T cell function is a common finding in multiple sclerosis, and may be present from the earliest phase of the condition (Clinically Isolated Syndrome). However, the particular aberrations underlying the impaired suppressive capability of regulatory T cells in multiple sclerosis remain to be elucidated.

Objective: The objective of this study was to determine whether aberrations in the proportion of functionally delineated regulatory T cell “fractions”, expression of the transcription factor Helios, or frequency of circulating follicular regulatory T cells, are present in Clinically Isolated Syndrome.

Design Methods: Peripheral blood mononuclear cells from n=18 treatment-naïve individuals with recently diagnosed Clinically Isolated Syndrome (<120 days) and n=19 healthy controls were examined by flow cytometry. Regulatory (CD3+CD4+FoxP3+CXCR5-) and follicular regulatory (CD3+CD4+FoxP3+CXCR5+) T cells were analysed for the proportion of suppressive Fraction I (CD45RA+FoxP3lo) and Fraction II (CD45RA-FoxP3hi) cells, and non-suppressive Fraction III (CD45RA-FoxP3lo) cells. Expression of Helios, a transcription factor required for stable regulatory T cell function, was determined by mean fluorescence intensity.

Results: Clinically Isolated Syndrome was associated with lower proportions of Fraction I regulatory and follicular regulatory cells (p=0.003 for both), and greater proportions of Fraction III regulatory (ns) and follicular regulatory cells (p=0.013). Clinically Isolated Syndrome was also associated with lower Helios expression across all regulatory and follicular regulatory T cell fractions (p<0.001 for all). Supporting its role in regulatory function, Helios expression correlated positively with the frequency of CD4+IL-10+ T cells (subset n=23; rho>0.6, p<0.01).

Conclusions: This study is the first to report a disturbed balance between suppressive and proinflammatory regulatory T cell

fractions early in the course of multiple sclerosis. Impaired Helios expression may underscore the disturbed regulatory T cell function in multiple sclerosis and represents a target for future mechanistic and therapeutic research.

Narrowband UVB phototherapy for Clinically Isolated Syndrome: Delivering the benefits of all UVB-induced molecules to early MS patients

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Background: In light of no definitive outcomes after trials of vitamin D supplementation for MS patients and the ability of narrowband UVB to induce vitamin D, as well as other immune-regulatory molecules in skin, the PhoCIS trial (Phototherapy for Clinically Isolated Syndrome) was established in Perth, Australia (32 degrees S).

Objective: To investigate the benefits of narrowband UVB phototherapy on MS development in individuals with high-risk Clinically Isolated Syndrome. Phototherapy was an adjunct to supplementing all 20 participants with vitamin D to 25(OH)-vitamin D levels of approximately 80 nmol/L. No participant was taking any disease modifying drugs at recruitment.

Design Methods: Twenty individuals with CIS have been recruited with 50% of them given narrowband UVB phototherapy (3 sessions per week for the first 8 weeks), by a protocol similar to that given to people with psoriasis. MRI has been performed after 3, 6 and 12 months, and extensive blood cell phenotyping 1 week, 1, 2, 3, 6 and 12 months after recruitment.

Results: After 12 months, 9 of 9 (100%) of those in the No phototherapy group had new lesions on their MRI. In the phototherapy group, 7 of 10 had converted to MS by McDonald criteria. The slower/delayed conversion to MS by those receiving phototherapy was supported by a tight prevention by UVB of decreasing levels of CD56loCD16hi NK cells.

Conclusions: UVB phototherapy may slow the progression of individuals with high-risk Clinically Isolated Syndrome to MS. As all participants were supplemented to ‘sufficient’ vitamin D status, the outcomes suggest that molecules other than vitamin D that are produced in UVB-irradiated skin are immunomodulatory and may regulate the progression to MS.

Vitamin D regulates expression of MS risk genes IRF8 and STAT3 in dendritic cells

Authors

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Background: Multiple Sclerosis risk is affected by latitude and UV production of vitamin D. Investigating how vitamin D controls expression of genes will enhance understanding of its mechanism of action. We recently identified that IRF8 and STAT3 have Vitamin D receptor (VDR) binding peaks in their regulatory regions in monocyte-derived cells.

Objective: Monocytes and dendritic cells represent crucial elements for a balanced immune response. We studied the RNA expression levels of IRF-8 and STAT3 in monocytes and dendritic cell models. Differences in gene expression due to single nucleotide polymorphisms (SNPs) of these genes, and differences resulting from vitamin D treatment, were examined.

Design Methods: Blood samples (Red Cross Blood Bank) were genotyped for SNPs of STAT3 and IRF8, two transcription factors important in monocyte and dendritic cell development and identified in Genome-Wide Association Studies. Monocytes were isolated and cultured with, or without, vitamin D. Monocytes were also used to generate non-differentiated, inflammatory and tolerogenic dendritic cell states, with vitamin D treatment begun at the time of differentiation. Messenger RNA levels of IRF8 and STAT3 were measured after 24 hours.

Results: Culture conditions and vitamin D treatment, but not the SNPs, influence expression of these genes. Differentiation of monocyte-derived dendritic cells, in itself, resulted in increased IRF8 and STAT3 expression. Vitamin D treatment significantly ($p = 0.018$) decreased IRF8 expression in the monocyte-derived dendritic cells differentiating to a tolerogenic state. Vitamin D caused a significant increase in STAT3 expression in both inflammatory ($p = 0.0008$) and tolerogenic dendritic cells ($p < 0.0001$) compared to undifferentiated cells.

Conclusions: Culture conditions and vitamin D significantly influence expression of these transcription factors, which are important for monocyte and dendritic cell differentiation. No genotype effects were detected. The degree and direction of change in IRF8 and STAT3 suggest dampening of an autoimmune response can be achieved in the course of differentiation.

Do Epstein Barr virus microRNAs affect Multiple Sclerosis pathogenesis?

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Background: Epstein Barr virus (EBV) infection is ubiquitous in MS, suggesting it is necessary, but not sufficient, to cause the disease. Regulation of infection may provide a new therapeutic approach in MS. EBV miRNAs may be especially tractable for this purpose.

Objective: We aimed to investigate (i) the EBV microRNAs (miRNA) targets among host MS risk genes and (ii) the effect of host MS risk single nucleotide polymorphism (SNPs) on the EBV miRNAs binding sites.

Design Methods: From in silico sources, we identified EBV miRNAs, including variants from different strains of EBV. To find putative gene targets in MS risk genes for the 44 known EBV miRNAs; we used the computational algorithms TargetScan, PITA and RepTar. We sought MS risk variants, including SNPs in linkage disequilibrium with them that might affect binding to these targets.

Results: We found that 39 EBV miRNAs have potential to bind to almost half of all known MS risk genes, 138 genes. Furthermore, we found 59 different EBV miRNA variants in 87 different EBV strains that could influence EBV miRNA function. One, BART2-3p, normally doesn't have any targets in MS risk genes but we found the variant of this miRNA that can bind to one of MS risk genes (DDX6).

Conclusions: Host regulation of EBV infection may be dependent on the function of the EBV miRNAs that target MS risk genes, either directly or indirectly, here identified. Experimental validation of these implicated miRNAs may provide novel therapeutic targets to improve immune regulation of this virus in MS.

The reliability and sensitivity of a Wii Balance Board® to measure balance in people with MS

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Background: Individuals with multiple sclerosis (MS) often experience difficulty with balance and walking. The current best available assessment for balance requires laboratory equipment, which is not readily available outside university settings. As a result, little is known about potential effects on balance following physiotherapy in people with MS.

Objective: The aim of this pilot feasibility study was to use the Wii Balance Board (WBB) to measure balance in people with MS, and to investigate the reliability and sensitivity to detect change using force and centre of pressure measures. The measures were taken at baseline and following six physiotherapy interventions.

Design Methods: A repeated measure design protocol assessed ten individuals with stable MS and Expanded Disability Status Scale (EDSS) range 4–6.5. Participants performed 8 tasks while standing on the WBB. Assessments were conducted on Day 1 and Day 2 to determine reliability, and then following six physiotherapy sessions over 3 weeks to detect a change in performance. Additional clinical measures of balance and gait were measured before and after the interventions.

Results: Feasibility was demonstrated by all participants being able to attempt the tasks on the WBB. Preliminary evaluation of the reliability data showed significant correlations in WBB measures. The baseline data were compared to the post treatment performance to evaluate sensitivity in measuring a treatment response. Force and COP variability was significantly different following the intervention in 4 and 3 tasks respectively following the intervention. There was no significant difference in clinical measures following the interventions.

Conclusions: The results indicate the Wii balance board is reliable and more sensitive in detecting a change in balance performance

than the clinical measures used. WBB technology may be a useful tool for investigating effects of physiotherapy interventions in individuals with MS in clinical trials.

Understanding how voltage-gated calcium signalling regulates oligodendrocyte progenitor cell function in the mature central nervous system

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Background: During development and following demyelination, neuronal activity can be detected by oligodendrocyte progenitor cells (OPCs) through the synaptic connections they form with axons. These synapses are lost in the healthy adult central nervous system (CNS), but OPCs still increase their proliferation and differentiation in response to increased neuronal activity.

Objective: OPCs express cell surface receptors and channels that allow them to respond to neuronal activity, such as the voltage-gated calcium channel Cav1.2. The deletion of Cav1.2 from OPCs in the developing mouse CNS, impairs oligodendrocyte production and reduces myelination. We aim to determine whether Cav1.2 also regulates adult OPC behaviour.

Design Methods: Pdgfra-CreERT2 :: TaumGFP (control) and Pdgfra-CreERT2 :: Cav1.2fl/fl :: TaumGFP (Cav1.2 deleted) mice received Tamoxifen in adulthood. Acute brain slices were generated and OPCs targeted for whole cell patch clamp analysis. Additional mice were given EdU in their drinking water to quantify cell division. Control and gene deleted mice were perfused at various time points after gene deletion to examine the generation and myelinating morphology of TaumGFP+ newborn oligodendrocytes by immunohistochemistry.

Results: By performing whole cell patch clamp analysis of OPCs ex vivo, we determined that OPCs have voltage activated calcium channels that are rapidly downregulated as these cells differentiate into oligodendrocytes. Furthermore, Cav1.2 is the predominant channel, as its conditional deletion from OPCs significantly reduced the size of the voltage-activated calcium current. In the absence of Cav1.2 OPCs survive, and they incorporate EdU, indicating that they are still capable of proliferation.

Conclusions: We have shown that OPCs in the adult brain have functional Cav1.2 channels. Furthermore, we can selectively delete these channels from OPCs without causing OPC death. We are now undertaking a detailed histological analysis of determine the effect that Cav1.2 deletion has on OPC proliferation and oligodendrocyte differentiation over time.

Protocadherin signalling and store operated calcium entry are key regulators of the oligodendrocyte progenitor cell cytoskeleton

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Background: Oligodendrocyte progenitor cells (OPCs) have highly motile structures at the end of each process (Hughes et al.,

2012). These structures are highly reminiscent of neuronal growth cones (Fox et al., 2006) and would be predicted to perform a similar sensory role, acting to interpret signals from the extracellular environment.

Objective: The reestablishment of synaptic connections between neurons and OPCs is important for remyelination outcome. However, the signals that mediate OPC process extension and retraction and OPC growth cone motility are largely uncharacterised. We aim to understand the signals that are important for stationary OPCs, with processes navigating their nearby environment.

Design Methods: We consulted microarray and RNA sequencing databases and determined that mRNA for protocadherin 15 (Pcdh15), a cadherin superfamily member that mediates calcium-dependent cell adhesion, and STIM proteins that are associated with store operated calcium entry (SOCE) and are critical for axon guidance, were highly expressed by OPCs. We utilized siRNA and shRNA to knockdown gene expression in cultured OPCs and examined the role of each signaling pathway in regulating OPC process motility and cytoskeletal reorganisation.

Results: Decreased PCDH15 expression significantly increased OPC proliferation and extracellular signal-related kinase (ERK) activation in vitro. This effect could be negated by the blockade of ERK phosphorylation. Time-lapse imaging revealed that PCDH15 knockdown also increased filopodial contact time between OPCs and reduced the number of veiling events, and this was independent of ERK. Knocking down STIM1 and STIM2, which reduced SOCE, also decreased the number of veiling events made by OPC growth cones.

Conclusions: These data suggest that PCDH15 acts as a negative regulator of OPC proliferation through ERK signaling pathway. PCDH15 and SOCE are also critical regulators of OPC growth cone motility in vitro.

The effects of Vitamin D analogues in modulating Immune function

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Background: Increasing latitude is associated with increased MS risk, implicating vitamin D in MS pathogenesis. Further, many MS risk genes are regulated by vitamin D, notably in dendritic cells (DCs). Vitamin D clinical trials in MS have been equivocal. Novel analogues of vitamin D may make DCs more tolerogenic.

Objective: 1. Determine the effects of vitamin D analogues in modulating dendritic cell differentiation and state. 2. Evaluate the effects of these vitamin D analogues on expression of the vitamin D modifying enzymes (CYP27B1 and CYP24A1). 3. Determine their effect on regulation of key monocyte differentiation transcription factors (IRF8 and IRF4).

Design Methods: CD14+ cells were isolated from peripheral blood and seeded into wells with GM-CSF and IL-4. Cells were then treated with the vitamin D analogues, Calcipotriol or Paricalcitol on Day 4, Day 5, or untreated for the control group. On Day 5 media containing (1) only LPS or with LPS and (2) IFN- (DC-1) or (3) IFN- (DC-2) is added. Cells were harvested at 24hrs or 48hrs. Gene expression analysis was performed using RT-PCR.

Results: Treatment of the DC subtypes with vitamin D analogues affected expression of the vitamin D pathway regulators and MS risk genes CYP27B1 and CYP24A1. Further, DC subtype markers IL-12 and IL-10, and MS risk genes IRF8 and IRF4, were affected.

Conclusions: Dendritic cell differentiation was affected by both vitamin D analogues, with effects dependent on time of addition and type of vitamin D. These data support further investigation into alternative analogue use to increase vitamin D benefit in the treatment of MS.

Repetitive Transcranial Magnetic Stimulation Drives Oligodendrocyte Addition in the Healthy Brain

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Background: Increased neuronal activity has been shown to increase oligodendrogenesis and myelination. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive form of brain stimulation that can induce electrical currents in the underlying central nervous system (CNS).

Objective: To determine whether rTMS can promote oligodendrocyte generation in the CNS.

Design Methods: Adult Pdgfra-CreERT2 :: Rosa26-YFP and Pdgfra-CreERT2 :: TauGFP transgenic mice received Tamoxifen to fluorescently label oligodendrocyte progenitor cells (OPCs) and their progeny. They then received 600 pulses of sub-threshold, non-invasive intermittent theta burst (iTBS), 10Hz or continuous theta burst (cTBS) rTMS (120mT coil) or sham stimulation for 14 days. Mice were perfused and immunohistochemistry performed to examine new oligodendrocyte addition to the brain, and the myelin internodes they produce.

Results: We found that iTBS, but not cTBS or 10Hz increased new oligodendrocyte addition to targeted cortical regions relative to sham stimulation (M1: $P < 0.0001$, Sham 15.61 ± 1.8 , iTBS 23.92 ± 1.8 ; V2: $P = 0.012$, Sham 13.53 ± 2.7 , iTBS 23.59 ± 2.3 , %OLs), but this difference was not sustained two weeks after the cessation of stimulation (M1: $P > 0.05$, Sham 18.88 ± 3.2 , iTBS 23.64 ± 3.5 ; V2: $P > 0.05$, Sham 19.81 ± 1.0 , iTBS 24.06 ± 3.7 , %OLs). Additionally, iTBS increased the length but not the number of internodes generated by new oligodendrocytes.

Conclusions: Non-invasive rTMS applied in an iTBS pattern can effectively drive oligodendrocyte addition and increase the myelin profile of newly born oligodendrocytes. However, removal of stimulation attenuates new oligodendrocyte addition in mice that previously received iTBS, but not sham stimulation, suggesting homeostatic regulation of oligodendrocyte addition in the healthy brain.

Using genetics to investigate the involvement of myeloid cells and the vitamin D pathway in the pathogenesis of MS

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Background: The risk of MS is known to increase over six fold between latitude extremes in Australia, with both ultraviolet light and Vitamin D pathways thought to contribute to the observed

protection. However, neither phototherapy nor Vitamin D treatment have conferred similar protection.

Objective: Of immune cell types, myeloid cells have the highest expression of the two MS-risk genes which control the production and catabolism of active Vitamin D. In these cells we sought to identify the key molecular actions of Vitamin D to derive improved approaches to exploit the pathway for therapeutic benefit.

Design Methods: Earlier we identified Vitamin D Receptor (VDR) binding sites in monocytes, tolerogenic and inflammatory dendritic cells for MS risk genes, notably the transcription factors ZMIZ1, ZMIZ1-AS1, STAT3 and IRF8. These genes have been previously implicated in maintaining tolerance. We have now assessed the effect of risk genotype and 1,25D3 treatment on expression of these genes in myeloid cell subsets, and if their expression is correlated with methylation differences, or response to various Vitamin D analogues.

Results: ZMIZ1 and ZMIZ1-AS1 expression and response to Vitamin D was cell subset dependent. ZMIZ1 was most highly expressed in monocytes, ZMIZ1-AS1 was most highly expressed in tolerogenic and inflammatory dendritic cells. Expression of ZMIZ1 was increased in tolerogenic dendritic cells cultured with 1,25D3, whereas ZMIZ1-AS1 was increased in both tolerogenic and inflammatory dendritic cells cultured with 1-25D3. Similarly, IRF8 and STAT3 expression was regulated by 1-25D3. The effect of risk genotype on expression was established.

Conclusions: These data support manipulation of myeloid cells using agents that target these MS risk genes, including Vitamin D analogues, to promote immune tolerance in latitude dependent autoimmune diseases including MS

Test-retest reliability of two falls specific outcome tools, the PPA and the choice stepping reaction time test in people with Multiple Sclerosis

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Background: Falls intervention programs are important to reduce falls in people with multiple sclerosis (MS). Although falls-specific tools are available, they are infrequently used to assess the efficacy of interventions. Two valid falls-specific tools in people with MS are the Physiological Profile Assessment (PPA) and simple Choice Stepping Reaction Time (CSRT).

Objective: The test-retest reliability and therefore, usefulness of these tests as outcome tools has not been established. Thus, our aim was to examine the test-retest reliability of the Physiological Profile Assessment falls risk score and its individual components and simple Choice Stepping Reaction Time in people with MS.

Design Methods: 15 participants with MS aged 35-62 years (mean $\pm$ SD, 51 ± 8 years), recruited as part of a larger trial, took the PPA and CSRT on two days with a minimum of 7 days separating the two days. Test-retest reliability was evaluated by intraclass correlation coefficient (ICC2,1). For the PPA, falls risk and its components (i.e. visual contrast sensitivity (VCS), proprioception, quadriceps strength, reaction time, and postural sway) were assessed. For CSRT, mean stepping time was compared.

Results: Test-retest reliability for falls risk PPA was excellent at 0.85 ($p < 0.05$). ICCs for quadriceps strength, reaction time, and postural sway were 0.86, 0.81, 0.90, respectively ($p < 0.05$). However, reliability for VCS and proprioception was poor (ICC=0.30 and 0.07, respectively. $p > 0.05$) despite the means being similar on the two days (mean $\pm$ SD; VCS: 21.73 $\pm$ 1.33, 21.60 $\pm$ 1.86; proprioception: 2.68 $\pm$ 0.94, 3.16 $\pm$ 2.33). Test-retest reliability for CSRT was good (ICC=0.79, 95% CI=0.37–0.93, $p = 0.004$). Mean difference for the two days was 1.2 $\pm$ 6.0s ($p = 0.4$). **Conclusions:** Both the Physiological Profile Assessment and the simple Choice Stepping Reaction Time, which are falls-specific and easy to use clinically, are reliable tools for assessing falls risk and thus, could be used as effective outcome measures for falls reduction intervention programs in people with MS.

Selective and discontinuous reduction of B and T lymphocytes by cladribine tablets in patients with early and relapsing multiple sclerosis (ORACLE-MS, CLARITY and CLARITY Extension)

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Background: Efficacy of cladribine tablets (CT) 3.5mg/kg has been demonstrated in patients with early MS (ORACLE-MS) and in patients with relapsing multiple sclerosis (RMS) in CLARITY/CLARITY Extension studies.

Objective: To evaluate lymphocyte subtypes over time in patients treated with cladribine tablets (CT).

Design Methods: Longitudinal (48 weeks) evaluation of peripheral blood lymphocyte subtypes from patients treated with CT3.5mg/kg (N=274) or placebo (N=140) was conducted. CLARITY Extension patients received placebo in CLARITY and CT3.5mg/kg in the Extension phase. Lymphocytes were measured at baseline, and Weeks 5, 13, 24 and 48. Changes in absolute counts and changes in relative proportion of the lymphocyte subtypes were evaluated.

Results: The baseline distributions of absolute lymphocyte counts (ALC) were similar across studies. Profiles of CD19+ B lymphocytes and CD4+ and CD8+ T lymphocytes were generally consistent across studies. The most rapid reduction in cell numbers occurred in the CD19+ B cell compartment (approximately 75% at Week 5 in each study). Nadir was reached at Week 13 with reductions between 81% to 84% for patients treated with CT3.5 mg/kg in CLARITY (N=97), CLARITY Extension (N=136), and ORACLE-MS (N=47). Reconstitution of CD19+ B cells towards baseline occurred from Week 24 to 48. CD4+ and CD8+ T cells were also markedly reduced in numbers, but to a lesser degree than CD19+ B cells (at most 55% for CD4+ cells and 48% reduced for CD8+ cells in patients treated with CT3.5 mg/kg in ORACLE-MS). CT3.5 mg/kg had a stronger suppressive effect on CD4+ than CD8+ T cells.

Conclusions: There was only incomplete reconstitution of T cells at Week 48. Changes in relative proportion confirmed the effect on CD19+ B cells at ALC nadir. **Conclusions** CT3.5 mg/kg achieved an early and discontinuous reduction of peripheral blood B cells with a rapid reconstitution to baseline, and a moderate reduction in T cell counts.

Year-by-year lymphopenia rates in patients with relapsing multiple sclerosis (RMS) treated with cladribine tablets 3.5mg/kg in CLARITY and re-treated in CLARITY Extension

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Background: CLARITY and CLARITY Extension demonstrated the efficacy of cladribine tablets (CT) in patients with RMS. The most common adverse event was lymphopenia, consistent with the mechanism of action of CT.

Objective: To evaluate whether lymphopenia persists following treatment with CT.

Design Methods: Lymphopenia by grade (Common Terminology Criteria for Adverse Events v3.0) for patients who were randomised to CT 3.5mg/kg in the two-year CLARITY study and re-randomised to CT 3.5mg/kg in the two-year CLARITY Extension (N=186) are reported. Patients with Grade 0 lymphopenia ($\geq 1.0 \times 10^9$ cells/L) before the first course and Grade 0/1 ($\geq 0.8 \times 10^9$ cells/L) prior to administration of all subsequent courses in Years 2/3/4 were included in the analysis, according to re-treatment guidelines.

Results: 176 patients were Grade 0 at the start of CLARITY and 167 patients were Grade 0/1 at the start of CLARITY Extension. By Week 13 in Year 1 and Week 12 in Years 2/3/4, 18–55% of patients developed Grade 2/3 lymphopenia. By Week 48 in each of Years 1/2/3/4, Grade 2/3 was observed in 11–14%. Occurrence of Grade 3 lymphopenia at any time was reported in <18% of patients; no patients had Grade 4 lymphopenia.

Conclusions: In patients with lymphocyte counts $\geq 1.0 \times 10^9$ /L before the first course and $\geq 0.8 \times 10^9$ /L before ≤ 3 subsequent annual courses of CT, no patients experienced Grade 4 lymphopenia at any time. Approximately 86% of patients recovered to Grade 0/1 by the end of each treatment year. There was no reduction in the proportion of patients recovering towards baseline with additional CT courses.

Absolute lymphocyte count recovery in patients with relapsing-remitting multiple sclerosis (RRMS) treated with cladribine tablets 3.5 mg/kg in CLARITY and CLARITY Extension

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Background: In CLARITY, cladribine tablets (CT) significantly reduced relapse rates, disability progression and MRI measures of disease activity over 2 years. The most commonly reported adverse event was lymphopenia, consistent with the mechanism of action for CT.

Objective: To investigate the absolute lymphocyte count (ALC) in patients with RRMS receiving 2 years of treatment with CT 3.5 mg/kg (CT3.5).

Design Methods: Data from patients randomised to CT3.5 for 2 years in CLARITY or CLARITY Extension including time spent in the PREMIERE registry (N=685) were pooled to provide long-term follow-up. Data from patients randomised to placebo in CLARITY and followed up in CLARITY Extension and PREMIERE are also reported (PBO; N=435).

Results: At baseline (start of CLARITY or CLARITY Extension), median (Q1–Q3) ALC was $1.86 \times 10^9/L$ (1.50–2.29) for CT3.5 and $1.91 \times 10^9/L$ (1.54–2.32) for PBO. During Year 1, ALC in CT3.5 reached a nadir at 9 weeks post-treatment [$1.00 \times 10^9/L$ (0.80–1.30)]. At the end of Year 1 (48 weeks), median ALC had increased to $1.21 \times 10^9/L$ (0.95–1.50). During Year 2, ALC in CT3.5 reached a nadir 7 weeks after re-treatment [$0.81 \times 10^9/L$ (0.60–1.04)], increasing to $1.03 \times 10^9/L$ (0.80–1.30) at the end of Year 2 (96 weeks). At the end of Years 3 and 4 (144 and 192 weeks), ALC in the CT3.5 group (with no further treatment) increased to $1.36 \times 10^9/L$ (1.02–1.65) and $1.40 \times 10^9/L$ (1.10–1.81), respectively, reaching a final median ALC of $1.76 \times 10^9/L$ (1.17–2.65) after 6.5 years (312 weeks). In PBO patients, median ALC values were between $1.69 \times 10^9/L$ (1.45–2.05) and $1.95 \times 10^9/L$ (1.59–2.38).

Conclusion: Rapid reductions in ALC after CT3.5 treatment in Years 1 and 2 were followed by gradual returns toward baseline. Median lymphocyte counts were within normal range beyond Year 2 (96 weeks) in all patients for whom follow-up data are available.

Pharmaceutical-Grade High-Dose Biotin improves outcome in Non-active Progressive Multiple Sclerosis: Phase 3 placebo-controlled results

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Background: MS is a disabling neurological disease characterized by multifocal destruction of central nervous system myelin. Most patients initially experience relapsing-remitting disease (RRMS) followed 15 to 20 years later by a progressive course (secondary progressive; SPMS). Approximately 15% of patients initially experience a progressive (primary progressive MS; PPMS).

Objective: Using a placebo-controlled, multi-center, randomised approach (n=154), we sought to confirm the benefit/risk profile of high-dose biotin (MD1003). Patients with baseline EDSS score of 4.5–7 and disease worsening within the previous 2 years were randomised to 12-month MD1003 (100mg biotin) or placebo thrice daily.

Design Methods: Once randomized, non-active patients (no relapse in last year) were followed for 12-months, and all patients were switched to MD1003 after the double-blind period. The primary endpoint was the proportion of patients with disability reversal at month 9, confirmed at month 12, defined as an EDSS decrease of ≥ 1 point (≥ 0.5 for EDSS 6–7) or a $\geq 20\%$ decrease in timed 25-foot walk time compared with the best baseline among screening or randomisation visits.

Results: Non-active progressive MS patients on MD1003 (high-dose biotin) achieved sustained reversal of MS-related disability (12.6% in the active arm vs 0% in the placebo group, $p=0.005$). In addition a decreased risk of disease progression (4% in the active arm vs 13% in the placebo group, $p=0.07$) and better clinical impression of change assessed by the clinician ($p<0.0001$) and the patient ($p=0.0094$) were observed after 1 year in the patients treated with MD1003.

Conclusions: High-dose biotin, a critical co-factor for ATP and fatty acid synthesis, supports improvements in disability in patients with progressive MS, either from increased production of ATP or myelin precursors. An additional Phase 3 trial is currently underway to support global registration of this unique metabolic approach in progressive MS.

Accounting for gender and disease type in the Multiple Sclerosis Severity Score (MSSS)

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Background: The Multiple Sclerosis Severity Score (MSSS) was developed in order to adjust for disease duration in assessments of disability, and is a widely used metric of disability progression. However, it is a general measure and does not account for potential differences in disability progression between onset types or between genders.

Objective: We aim to evaluate the impact of gender and disease onset type (relapsing: RRMS or SPMS, or progressive: PPMS or PRMS) on the construction of an MSSS table, in order to determine if it may be beneficial to use a gender- or onset type-specific table in assessments of disability progression.

Design Methods: Using a dataset from MSBase, an international database of >30,000 MS patients, we have constructed several MSSS tables: a general table, including all patients for which we have data, and gender- and onset type-specific tables. We used interpolation to fill any cells left vacant, giving us a series of complete tables. We then compared these using permutation analysis with 20,000 replications, in order to assess the likelihood of the differences between the tables being random.

Results: Our final dataset included 30,359 MS patients. Of these, 8,797 (29.0%) were male and 1,962 (6.5%) had a progressive onset type. Preliminary results indicate that the progressive onset-specific table differs significantly from the general MSSS table ($p<0.001$) and the relapsing onset-specific table ($p<0.001$). The

difference in MSSS may result from the higher EDSS of progressive onset patients. The male-specific table differs marginally from the general MSSS table ($p=0.07$) and from the female-specific table ($p=0.06$).

Conclusions: Our results suggest that the substantial clinical differences between male and female, and progressive and relapsing MS cases have a strong effect on the MSSS. These differences should be accounted for in the measurement of disability progression for people with MS.

Complementary and alternative treatments of multiple sclerosis: a review of the evidence from 2001-2016

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Background: People with multiple sclerosis (PwMS) commonly use complementary and alternative medicines (CAM), non-invasive therapies used in addition to or in lieu of the standard pharmacological treatments, but an understanding of their efficacy is lacking.

Objective: The aim of this review was to quantitatively assess the most recent high quality evidence of efficacy for CAM treatments of MS, in order to evaluate their utility for PwMS.

Design Methods: We conducted a thorough search of the PubMed database from January 2001 to January 2017, using a search strategy of appropriate MeSH terms and key words. This search returned 1,916 articles. These were evaluated against a series of inclusion criteria, including study outcomes and quality of evidence. Only class I and class II studies of treatment efficacy for MS with at least 50 total participants (control and treatment groups) were included.

Results: The 38 studies included in this review are divided across five CAM types (cannabis, diet, exercise, psychological approaches, and other). We found little evidence to support CAM efficacy. The studies contained little replication in intervention, primary outcomes or study design. Six of the sixteen included CAM were each assessed in a single study. The CAM with the strongest evidence supporting them, cannabis extract, physical activity, and cognitive-behavioural therapy, were each investigated in seven studies.

Conclusions: There is currently little evidence of CAM efficacy in MS treatment. Future work should cultivate methodological consistency. We advocate for the establishment of a CAM trial group within the MS research community. CAM treatment is an area of significant interest to the MS community and should be a research priority.

Treating progressive multiple sclerosis

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Background: We showed that the available immunotherapies do not modify disability outcomes when used in secondary progressive multiple sclerosis. However, these therapies are effective in advanced active multiple sclerosis. Primary progressive multiple sclerosis may present with or without superimposed relapses. Significance of these relapses for disability accumulation and treatment remains contested.

Objective: To examine the effect of the available immunotherapies in primary progressive multiple sclerosis.

Design Methods: 1427 eligible patients with primary progressive multiple sclerosis from MSBase were studied. Confirmed disability progression of disability was compared between treated and untreated propensity score-matched cohorts. Multivariable regression models were used to compare disability accrual between primary progressive multiple sclerosis with and without superimposed relapses. Finally, the effect of therapy on disability accrual in cohorts with and without superimposed relapses was analysed.

Results: 173 treated and 373 untreated patients were matched. No differences in the risk of disability progression ($p=0.79$) and improvement ($p=0.98$) were observed over the median 3-year follow-up. The likelihood of disability progression was relatively lower in patients with superimposed relapses (hazard ratio=0.83, $p<0.01$). We observed an association between the proportion of time on immunotherapy and the hazard of disability progression in active (hazard ratio=0.96, $p=0.01$) but not in the inactive primary progressive disease ($p=0.21$).

Conclusions: Superimposed relapses in primary progressive multiple sclerosis represent a favourable prognostic marker, associated with slower disability accrual. This is most likely attributed to the effectiveness of immunotherapy in active primary progressive disease. Relapse activity, therefore, is a treatable modifier of disability accrual in primary progressive disease.

The Role of MERTK in Multiple Sclerosis

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Background: Clinical examination and magnetic resonance imaging (MRI) are currently used to assess MS relapse. A biomarker to supplement these modalities would have high clinical utility. We have established that mutations in the MERTK gene confer increased MS risk and that MERTK expression correlates with disease activity in MS animal models.

Objective: In thirty patients, we are comparing levels of the receptor tyrosine kinase MERTK on monocytes during MS relapse and disease quiescence. This will allow us to determine if MERTK is a biomarker of MS activity. In addition, we are comparing levels of MERTK expression in patients and healthy controls.

Design Methods: Peripheral blood mononuclear cells (PBMC) have been isolated from MS patients experiencing relapse of sufficient severity to warrant treatment with methylprednisolone. Patients will also have PBMCs isolated after three months, during clinical remission. Paired samples will be analysed using flow cytometry to assess MERTK protein expression. RNA will be isolated and qPCR for the MERTK gene performed. A comparative analysis of MERTK expression and immune cell phenotype during relapse and remission will be undertaken.

Results: To date, we have recruited 18 patients with MS relapse, along with five healthy controls. We have established that PBMCs from patients can be successfully isolated, frozen and thawed with preserved detection of MERTK expression. By the time of presentation in October 2017, we will have preliminary data from paired samples for approximately 12 patients and five controls. We will have an early indication of potential correlations between disease activity and MERTK expression.

Conclusions: We have previously established that Mertk expression in innate immune cells is increased in animal models of MS and then normalises with remyelination. If we establish a similar pattern in humans, we will have identified a potential biomarker of MS activity with substantial clinical utility in accurately detecting disease relapse.

Polymorphism in the serotonin transporter gene polymorphisms (5-HTTLPR) modifies the association between significant life events and depression in people with Multiple Sclerosis

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Background: In the general population, variation in the serotonin-transporter-linked polymorphic region (5-HTTLPR) has been shown to modify the association between stressful events and depression/anxiety. This has not been examined in people with MS, which has a higher prevalence of anxiety and depression when compared to the general population.

Objective: We aimed to examine the interaction between significant life events (SLE), 5-HTTLPR and depression/anxiety in people with MS.

Design Methods: A population-based longitudinal cohort of 198 people with MS was followed 6-monthly for 2.5 years. Depression and anxiety symptoms were measured at each review using the Hospital Anxiety and Depression Scale. SLE were assessed using a questionnaire based on the Social Readjustment Rating Scale. The 5-HTTLPR polymorphism was genotyped using 50 ng of genomic DNA.

Results: We found an interaction between SLE load in the previous 12 months, functional variation in the 5-HTTLPR allele type and depression, with the association between SLE load and depression being stronger for those with S/S allele type ($\beta=0.21$ (0.09, 0.33) per 10 unit increase) and S/L ($\beta=0.14$ (0.05, 0.24)) compared to L/L allele type ($\beta=0.04$ (-0.05, 0.24)) ($p<0.001$ for interaction). No convincing evidence of an interaction was found with anxiety.

Conclusions: We found that the association between SLE load and MS depression severity was stronger among those with one or two copies of the short allele of the 5-HTTLPR. The identification of a gene-environment interaction between SLEs and depression in a population where depression is partly disease-driven is novel.

Expression of MS risk genes in whole blood of CIS patients treated with narrow band UVB phototherapy

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Background: Low vitamin D and/or sun exposure are risk factors for the development of Multiple Sclerosis (MS), potentially through their effects on the immune system. Such effects might be detected through changes in expression of MS risk genes in the immune cells of the blood.

Objective: Determine if the expression of MS risk genes known to be dysregulated in MS blood, and associated with season and vitamin D functions, is altered in response to treatment with narrow band UVB in patients with clinically isolated syndrome (CIS) in the PhoCIS trial.

Design Methods: RNA was extracted from whole blood PAXGene tubes from 18 CIS patients either treated ($n=8$) or untreated ($n=10$) with narrow band UVB phototherapy. Blood from healthy controls ($n=10$) and patients with other neurological diseases was also tested. Expression of EOMES, ZMIZ1, PTGER4, CYP24A1 and CYP27B1 were assessed by Taqman RT-PCR.

Results: As in MS, expression of EOMES, ZMIZ1, and PTGER4 was lower in CIS. It was also stable over time and seasonal in controls. There was no significant difference between baseline and subsequent time points for those treated or untreated with narrow band UVB phototherapy in CIS patients; and no association with serum Vitamin D levels in patients or controls. Expression of these genes was correlated with immune cell subsets.

Conclusions: Expression of these MS risk genes was reduced in disease and associated with season, corresponded with immune cell subsets, but did not alter on therapy, and was not associated with vitamin D levels.

Estimating MS-related work productivity loss and factors associated with labour force participation in a representative Australian sample of people with multiple sclerosis

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Background: Many people with multiple sclerosis (MS) leave the workforce early due to their MS. Less is known about MS-related work productivity loss with a combination of absenteeism (time missed from work) and presenteeism (reduced productivity while working).

Objective: To quantify and estimate associated economic costs of MS-related work productivity loss; to compare factors associated with labour force participation and work productivity in people with MS by a nationally representative sample from participants in the Australian MS Longitudinal Study.

Design Methods: From December 2015 to July 2016, participants completed three surveys. Labour force participation was assessed in 1,471 respondents of working age while work productivity loss was assessed in 740 respondents who were working. Total MS-related work productivity loss was defined as absenteeism plus presenteeism. Work productivity outcomes were expressed as equivalent percent productive time lost. Data were analysed by cluster analysis, log binomial and linear regression. All costs were in 2016 Australian dollars (AUD).

Results: Among workers, 56% experienced work productivity loss due to MS with mean loss due to absenteeism and presenteeism being 3.4% vs. 10.8%, leading to 14.2% total work productivity loss and AUD\$7,331 loss per person annually. Work productivity loss was determined most strongly by “fatigue and cognitive symptoms”, “pain and sensory symptoms”, “problems with walking, balance and spasticity” while older age, and lower education level were also associated with not being in the labour force.

Conclusions: MS-related presenteeism productivity loss were three times higher than absenteeism, highlighting the importance of including presenteeism in employment outcomes. Factors associated with labour force participation and work productivity are different. Symptoms being strong predictors of both work participation and productivity emphasises the need for improved management of symptoms.

Epigenome-wide association study of CD19+ B Cells in MS patients reveals distinct methylation profiles compared to T cells

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Background: DNA methylation provides an epigenetic interface for environmental factors to influence the genome. Our previous work has identified a major differentially methylated region at HLA-DRB1 in the CD4+ T cells of RRMS patients. B cells have been found in the brains of MS patients and may also contribute to disease.

Objective: The objectives of this study were to 1) compare the DNA methylation profile in CD19+ B cells in RRMS patients to healthy controls and 2) compare DNA methylation profiles of CD19+ B cells to CD4+ and CD8+ T cells.

Design Methods: DNA was extracted from purified CD19+ B cells collected from 24 RRMS and 24 healthy controls, and for which T cell analysis was also previously conducted. DNA was bisulphite converted and hybridised to Illumina 450K arrays. Raw fluorescence data were processed using a combination of R/Bioconductor and custom scripts. CpGs that met the criteria of

a false discovery rate of <5% and had a median difference of >5% were considered associated with MS.

Results: Ninety-seven differentially methylated CpGs were associated with MS. Of these the majority (80%) were hypermethylated in MS cases compared to controls. The largest difference was seen in the Interleukin 21 Receptor (IL21R) gene, which showed 27% hypermethylation in MS cases compared to controls. The data did not overlap with our data in CD4+ or CD8+ T cells from the same cohort including the major CD4+ signal found at HLA-DRB1.

Conclusions: CD19+ B cells have a unique methylation profile in MS cases versus controls compared to CD4+ and CD8+ T cells from the same cohort. This suggests that epigenetic mechanisms of B cells may mediate disease risk independently and highlights the need to investigate different cell subtypes when investigating MS.

Dimethyl fumarate changes the methylation profile in CD4+ cells of MS patients

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Background: The MS disease modifying therapy Dimethyl fumarate (DMF) is known to influence the prevalence of circulating CD4+ cells in MS patient. The mode of action of DMF is unclear but may act via the nuclear factor-like 2 pathway, leading to transcription of anti-inflammatory and cytoprotective genes.

Objective: We have recently described significant differences in the methylation profile of CD4+ T-cells of MS patients compared to age and sex- matched controls. The objective of this study was to explore if DMF can change the CD4+ methylation profile in patients and has potential as an epigenetic medicine.

Design Methods: CD4+ T-cells were purified from 7 relapsing remitting MS patients (3 males and 4 females) before and 6 months after treatment with DMF. DNA was bisulfite converted and hybridized to Illumina EPIC methylation arrays and analysed using well established bioinformatics pipelines. Change in methylation was calculated using paired Tests of M-values. Point-wise FDRs and magnitude of change was used to rank CpGs and identify those associated with exposure to DMF.

Results: In total 1347 CpGs showed a change in methylation over time (FDR<0.05) with 97% showing an increase in methylation. 216 CpGs had a larger than 10% difference in methylation. In particular tumour necrosis factor (TNF) on chromosome 6 showed increased methylation associated with DMF treatment in this patient group. Gene set analysis of the 1347 CpGs highlighted pathways involved in Wnt-signalling, axon guidance, focal adhesion and MAPK enriched.

Conclusions: This study suggests that DMF potentially alters the methylation profile of CD4+ T-cells in MS patients. We have previously shown that TNF is differentially methylated in MS patients compared to healthy individuals. This effect might to be corrected by DMF treatment suggesting DMF may act as an epigenetic medicine.

Nogo receptor 1 (NgR1) deletion in axons halts axonopathy and demyelination during experimental autoimmune encephalomyelitis (EAE)

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Background: We have previously shown that deletion of the *ngR1* allele limits experimental autoimmune encephalomyelitis (EAE) severity by preserving central nervous system (CNS) axons. What is unknown is whether this preservation is governed by myelin being intact thereby protecting axons, or in fact axonal degeneration is limited, thereby preventing demyelination.

Objective: We investigated how neuronal deletion of *ngR1*, may prevent axonal degeneration during EAE. In a parallel study, we investigated whether neuronal over-expression of *ngR1* in *ngR1*^{-/-} mice potentiates axonal degeneration during EAE. This approach may differentiate the role of NgR1 in promoting axonal damage during EAE.

Design Methods: Conditional deletion of *ngR1* in axons was produced by intraocular injection of AAV2 encoding Cre (AAV2-iCre-eGFP) in *ngR1*^{flx/flx} mice. Conversely, conditional re-introduction of NgR1 in axons was produced by intraocular injection of AAV2 encoding full-length mouse NgR1 (AAV2-NgR1-eGFP) in *ngR1*^{-/-} mice. Animals were induced with EAE and culled at the peak stage of disease. The degree of axonal degeneration and demyelination were assessed by immunohistochemistry. Molecular mechanisms of axonal degeneration were verified with immunoblot.

Results: Axonal degeneration is limited in AAV2-iCre-eGFP injected *ngR1*^{flx/flx} whereas, significant axonal damage is found in AAV2-NgR1-eGFP injected *ngR1*^{-/-} optic nerves during EAE. As a corollary, the preservation of myelin integrity was a prominent feature in AAV2-iCre-eGFP injected *ngR1*^{flx/flx}, whereas significant demyelination was found in AAV2-NgR1-eGFP injected *ngR1*^{-/-} optic nerves. Furthermore, the interaction between the axonal motor protein, kinesin-1 (KIF5) and collapsin response mediator protein 2 (CRMP-2) was reduced in AAV2-NgR1-eGFP injected *ngR1*^{-/-} optic nerves.

Conclusions: Our data suggest that NgR1 governs axonal degeneration in the context of inflammatory-mediated demyelination through phosphorylation of CRMP-2, abrogating axonal vesicular transport. Moreover, the axon-specific deletion of *ngR1* preserves axons blunting the induction of demyelination during EAE, thereby suggesting that NgR1-dependent neurodegeneration maybe a primary mechanism during neuroinflammation.

Healthy & unhealthy dietary patterns significantly change over five years follow-up: results from the AusLong Study

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Background: Dietary intake may be relevant in multiple sclerosis (MS) onset and progression.

Objective: To examine dietary patterns in a cohort of participants in the first five years following symptom onset, including change over time and how the patterns correlate with demographic and behavioural covariates.

Design Methods: Food consumption, reported by a cohort of participants in the early stages of MS (the AusLong Study) was used to evaluate the distribution of dietary intake at baseline and five-year review. Using iterated principal factor analysis, patterns of diet were extrapolated from food intake questionnaires, and determinants of individual-level pattern scores were evaluated using linear regression.

Results: Three reproducible dietary patterns we labelled 'Western', 'Mixed' and 'Prudent' were independently found at baseline and five-year review. Western dietary pattern score was significantly lower among females and older participants. Prudent pattern was higher among older, more physically active, and more educated participants, but lower among smokers. The cohort increased their Prudent and decreased their Western and Mixed pattern scores during follow-up, with marked & significant differences by sex, BMI, smoking behaviour and employment.

Conclusions: While all three dietary patterns persisted over five years of follow-up, there was evidence that some participants improved their diet while others did not. These findings have implications for the health improvement efforts of people in the early stages of living with MS.

Investigating the diversity and immunoreactivity of recombinant anti-MOG antibodies associated with central nervous system demyelinating diseases

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Background: Certain neuromyelitis optica spectrum disorders, including bilateral optic neuritis, have been associated with autoantibodies against myelin oligodendrocyte glycoprotein (MOG). However, it is unknown whether the anti-MOG immunoreactivity is the only one present in anti-MOG antibody-positive demyelinating patients, or if immunoreactivities to other autoantigens also exist.

Objective: The overall aim of my project is to characterise the autoantibody repertoire in anti-MOG antibody-associated demyelinating patients.

Design Methods: Paired heavy and light chain variable regions will be amplified from single B cells to be subcloned into expression vectors and transfected into mammalian cells to produce recombinant monoclonal antibodies (rAbs), which can then be analysed for their immunoreactivity and pathogenicity.

Results: Variable light chains were amplified from single plasmablasts at low efficiencies with one-step RT-PCR. The Igλ chain was amplified from 4 of 18 (55.6%) single plasmablasts, which was 4 times more frequent than the Igκ chain (1/18; 9.3%), even though kappa to lambda chain expression is 60:40. We were not able to amplify the variable heavy chain from a single plasmablast, but a two-step RT-PCR is currently being optimized to increase the sensitivity of reaction.

Conclusions: The production of rAbs will allow the characterisation of MOG-specific antibody response in patients with CNS demyelinating disorders. Furthermore, the generation of abundant rAbs can be used to model functional pathogenic effects in *in vitro* cellular models.

Using a parasite derived peptide to prevent immune-mediated demyelination

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Background: The prevalence of MS in human populations has reportedly been significantly reduced once a critical threshold of infection with parasitic worms (helminths) was exceeded. Such geo-epidemiological distribution of helminths and MS implies that helminth parasites are a protective factor against the development of MS.

Objective: We have discovered that a novel peptide (FhHDM-1) secreted by the helminth *Fasciola hepatica* has beneficial therapeutic effects without the adverse side effects of current treatment options. The objective of this study was to determine the mechanism by which the peptide inhibits the infiltration of immune cells to the CNS

Design Methods: Using the relapsing remitting experimental autoimmune encephalomyelitis (RR-EAE) mouse model of human MS, we have shown that a short treatment course of synthetic FhHDM-1 reduces the incidence of relapse and ameliorates clinical symptoms of disease. Absence of perivascular macrophages prevents the progression of T cells across the glia limitans and blocks EAE progression. Therefore, we analysed whether treatment with FhHDM-1 modulated the activation of macrophages and thus inhibited the progression of cells into the CNS.

Results: Our preliminary analysis showed that treatment of RR-EAE mice with FhHDM-1 had no effect on the cytokine profile of peripheral T cells. However, treatment with FhHDM-1 inhibited the activation of pro-inflammatory peripheral macrophages, which consequently altered the ratio of cytokines within the perivascular cuff. This in turn, prevented the infiltration of inflammatory immune cells into the brain parenchyma thus halting the progression of disease.

Conclusions: These findings suggest that the parasite peptide FhHDM-1 selectively targets macrophages to prevent the development of immune-mediated demyelination. Importantly, no adverse events have been recorded for the peptide treatment and disease prevention occurs in the absence of global immune suppression.

Exosomal microRNA signatures in multiple sclerosis reflect disease status

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Background: There is currently no single definitive test for multiple sclerosis (MS). Circulating exosomes represent promising candidate biomarkers for a host of human diseases. Exosomes contain RNA, DNA and proteins can cross the blood-brain barrier, and are secreted by almost all cell types including cells of the CNS.

Objective: We hypothesized that serum exosomal miRNAs could present a useful blood-based assay for MS disease detection and monitoring.

Design Methods: Exosome-associated microRNAs in serum samples from MS patients (n=36) and matched healthy controls (n=11) were profiled using small RNA next generation sequencing.

Results: We identified differentially expressed exosomal miRNAs in both relapsing-remitting MS (RRMS) (miR-15b-5p, miR-451a, miR-30b-5p, miR-342-3p) and progressive MS patient sera (miR-127-3p, miR-370-3p, miR-409-3p, miR-432-5p) in relation to controls. Critically, we identified a group of nine miRNAs (miR-15b-5p, miR-23a-3p, miR-223-3p, miR-374a-5p, miR-30b-5p, miR-433-3p, miR-485-3p, miR-342-3p, miR-432-5p) that distinguished relapsing-remitting from progressive disease. Eight out of nine miRNAs were validated in an independent group (n=11) of progressive MS cases.

Conclusions: This study is the first demonstration that microRNAs associated with circulating exosomes are informative biomarkers not only for the diagnosis of MS but in predicting disease subtype with a high degree of accuracy.

Associations between diet and health outcomes in two international samples of people with Multiple Sclerosis

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Background: Increasing evidence links healthy dietary habits with improved health outcomes in people with multiple sclerosis (MS).

Objective: To explore the associations between diet and health outcomes in two international samples of people with MS.

Design Methods: Using Diet Habits Questionnaire (DHQ) in two independent samples of people with MS online, participants completed the survey at baseline and 2.5yrs (N=1,441) (longitudinal), or only at baseline (N=573) (cross-sectional). Physical and mental health-related quality of life (HRQOL) were measured with the Multiple Sclerosis Quality Of Life-54 and depression risk and fatigue with Patient Health Questionnaire-2 and Fatigue Severity Scale. Multivariable linear and logistic regression models explored associations between diet and outcomes, adjusting for confounders.

Results: Cross-sectionally, every 10-point increase in DHQ score was associated with increasing physical HRQOL (Mean Difference(MD):5.43, 95% Confidence Interval(CI):4.17-6.69) and mental HRQOL (MD:4.93, 95% CI:3.50-6.36) and decreasing depression risk (Odds Ratio (OR):0.65, 95% CI:0.53-0.79) and fatigue (OR:0.62, 95% CI:0.52-0.75). Longitudinally, a 10-point increase in DHQ score was associated with significantly increased physical HRQOL (MD:2.36, 95% CI:1.31-3.41) and mental HRQOL (MD:2.00, 95% CI:0.71-3.29) and lower depression risk (OR:0.77, 95% CI:0.60-1.00), but non-significant fatigue reduction (OR:0.86, 95% CI:0.69-1.07).

Conclusions: These results suggest that a healthy diet can potentially improve physical and mental HRQOL and decrease depression risk and fatigue in people with multiple sclerosis.

Cognitive function screening tools in MS: Performance comparison of patients using the Audio Recorded Cognitive screen (ARCS) and the Brief International Cognitive Assessment for Multiple Sclerosis.

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Background: Cognitive impairment is a common and debilitating symptom of MS affecting up to 65% of patients. To enable successful monitoring of cognitive function, a number of screening tools have been developed to overcome long testing times, the need for specialist input and to increase routine cognitive assessment in clinical practice.

Objective: The major aim of our study is to assess the clinical usefulness of the Auditory Recorded Cognition Screen (ARCS) to detect and monitor cognitive function in MS patients, compared to the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) as the gold standard.

Design Methods: Thirty MS participants (76% females) underwent the BICAMS battery and ARCS consecutively. Test scores were explored by correlation analysis using Pearson's (r) testing and subsequently Spearman's (rho) testing - only variables that were significant in both tests were considered robust. ROC diagnostic analysis was then performed on the relevant ARCS variables to estimate AUC as an index of accuracy to predict the gold standard BICAMS outcomes. A p-value of <0.05 was considered as statistically significant.

Results: The study cohort was aged 49.6±2.47 years, with mean EDSS 3.1±0.36 and disease duration of 13±1.9 years. The ARCS memory, fluency, visuospatial and attention scores were correlated with raw scores for the SDMT (r=0.54 to 0.62, p<0.01), BVMTR (r=0.37 to 0.67, p<0.05) and CVLT-II (r=0.50 to 0.78, p<0.01). ROC analyses demonstrated good accuracy between visuospatial, attention and total ARCS with SDMT, and between the memory, attention and total ARCS with CVLT-II (AUC=0.76 to 0.87, P<0.05).

Conclusions: Preliminary analysis of our comparison between the BICAMS and ARCS cognitive screening tools suggests that ARCS provides good accuracy for measuring cognitive function of MS patients. Unlike BICAMS, ARCS provides an overall performance score and information on language function and does not require a technician to be present during testing.

Clinical implications of IL7R genotype: from disease risk to disease management

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Background: MS risk gene IL7RA encodes a subunit of the receptor for the immune cell growth factor interleukin-7. Common

genetic variations (single nucleotide polymorphisms; SNPs) in IL7RA not only confer susceptibility to MS, but influence recovery following anti-retroviral therapy for HIV infection, and outcome of haematopoietic stem cell transplant.

Objective: To deconvolute the complex biological effects of IL7RA genotype and investigate clinical associations. The MS protective SNP (rs6897932; tagging haplotype 2, Hap 2) reduces expression of the inhibitory soluble receptor isoform; effects of an IL7RA SNP associated with transplant outcome (rs1494555, tagging Hap 1) are unknown and will be investigated.

Design Methods: Association of IL7RA genotype with: 1. serum soluble IL7R (ELISA) and CD4 cell counts following antiretroviral therapy in HIV-infected cohorts from Malaysia, and the USA (CNICS; composed of white, Hispanic and African-American); 2. treatment-related mortality following matched unrelated donor stem cell transplants in an Australian cohort (ABMDR; 1999-2016); 3. CD4 cell phenotype by flow cytometry in MS and controls will be determined.

Results: The MS-protective SNP was also associated with improved CD4 recovery and lower serum soluble IL7R following antiretroviral therapy in HIV-infected patients in both cohorts examined. Hap 1 was associated with higher treatment-related mortality following unrelated donor stem cell transplant in an Australian cohort; and with reduced proportions of inflammatory Th1 and Th17 subsets (with a trend to reduced cell-surface IL7R expression) in a combined MS and control cohort.

Conclusions: IL7R genotype has highly significant effects on immune recovery and clinical outcome in settings of extreme immune perturbation, however the direction of association may be environment-dependent. Potential translation to personalised management on the basis of genotype, such as initiation of IL7 therapy and donor selection, will be discussed.

Regional Quantitative Susceptibility Mapping with Clinical Correlation in Mild Relapsing-Remitting Multiple Sclerosis: An Ultra High Field MRI Study

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Background: Brain iron accumulation is a novel biomarker and potential therapeutic target in a number of neurodegenerative conditions, including multiple sclerosis. Quantitative susceptibility mapping is an emerging MRI technique which strongly correlates with tissue iron concentration. This may allow the assessment of disease-related iron changes in MS patients.

Objective: We performed a study using QSM acquired with ultra high field MRI, to assess relationships between QSM value, in a number of brain regions, and both clinical and other MRI parameters, in mild (Expanded Disability Status Scale <or=2) relapsing-remitting MS patients.

Design Methods: 3D gradient-echo (TEs/TR/FA:5-21ms/24ms/13°, 0.75mm isotropic), T1-MP2RAGE and FLAIR scans were acquired in 19 RR-MS patients on a Siemens 7 Tesla research scanner. Volumes were co-registered and processed using Freesurfer 6 software. QSM maps were calculated using standard algorithms (V-SHARP and iLSQR). Mean regional values from Freesurfer segmentations were correlated to clinical EDSS and MR parameters.

Results: A significant positive correlation was seen between QSM value in deep grey matter of the globus pallidus and patient EDSS score (Pearson $R=0.65$, $p=0.007$). Additionally, a significant negative correlation was observed between thalamus QSM value and EDSS (Pearson $R=-0.67$, $p=0.002$).

Conclusions: Higher globus pallidus susceptibility with clinical score indicated increased iron deposition in this area of high iron turnover. Additionally, we observed lower thalamus susceptibility with EDSS, suggesting iron loss in this architecturally complex structure. Our work supports the notion that regional QSM values may offer information on MS disease state.

Cold fluid ingestion prolongs time to exhaustion in people with multiple sclerosis exercising in the heat

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Background: Heat intolerance commonly affects the exercise capacity of people with multiple sclerosis (MS) during bouts of hot weather. Cold-water ingestion is a simple cooling strategy but its efficacy for prolonging exercise capacity with MS remains undetermined.

Objective: To identify whether cold-water ingestion blunts exercise-induced rises in body temperature and improves exercise tolerance in heat-sensitive individuals with MS.

Design Methods: On two separate occasions, 20 participants (10 relapsing-remitting MS (EDSS: 2.8 ± 0.9 ; 82.5 ± 15.7 kg; 1.7 ± 0.1 m; 2.4 ± 1.4 L·min⁻¹) 10 age- and fitness-matched healthy controls (76.1 ± 15.7 kg; 1.7 ± 0.1 m; 2.9 ± 0.9 L·min⁻¹) (CON)) cycled at 40% VO₂max at 30°C, 30%RH until volitional exhaustion (max. 60 min). Every 15 minutes, participants ingested 3.2 mL·kg⁻¹ of either 1.5°C (CLD) or 37°C (NEU) water. Rectal (Tre) temperature, mean skin (Tsk) temperature, and heart rate (HR) were measured.

Results: All 10 CON, but only 3 of 10 MS participants completed 60 minutes of exercise in NEU. The remaining 7 MS participants all cycled longer ($P=0.006$) in CLD (46.4 ± 14.2 min) compared to NEU (32.7 ± 11.5 min), despite similar elevations in Tre (NEU: 37.32 ± 0.34 °C; CLD: 37.28 ± 0.26 °C; $P=0.44$), Tsk (NEU: 34.48 ± 0.47 °C; CLD: 34.44 ± 0.54 °C; $P=0.82$) and HR (NEU: 114 ± 20 bpm; CLD: 113 ± 18 bpm; $P=0.38$) for the same exercise volume.

Conclusions: Cold-water ingestion enhanced exercise tolerance for MS participants by ~30% despite no differences in core and skin temperatures. These findings support the use of a simple cooling strategy for mitigating heat-intolerance with MS, and demonstrate the potential role of cold-afferent thermoreceptors in modulating exercise tolerance with MS in the heat.

Elevated circulating microparticle counts in multiple sclerosis are regulated by platelet P2X7 receptor function

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Background: The purinergic receptor P2X7 is best known to promote the assembly of NALP3 inflammasome and secretion of IL-1b by immune cells. We have shown an increased number of activated platelets in MS compared to normal and have found that MS plasma contains two-fold increase in platelet-specific microparticles compared to control.

Objective: We measured P2X7 expression on platelets and platelet-derived CD41+ microparticles by flow cytometry and by western blot analysis. We also explored the function of platelet P2X7 by quantitating the release of microparticles from gel-filtered platelets (GFP) with a specific P2X7 agonist, BzATP with or without the antagonist AF27139 at 100nM.

Design Methods: Expression of P2X7 was found on the platelet surface and confirmed in lysates of human platelets by western blot analysis using sheep anti-human P2X7 antibody. Microparticle release was quantified by flow cytometry of platelet poor plasma using either particle tracking analysis (Nanosight 300) or forward and side scatter analysis with a CytoFlow cytometer.

Results: Microparticles derived from gel-filtered platelets (GFP) expressed both P2X7 receptors and the platelet integrin CD41. The specific P2X7 agonist BzATP gave a large increase in CD41+ microparticles from GFP with linear time course over 30min and this increase was abolished by the specific P2X7 antagonist, AF27139. While monocyte P2X7 function is inhibited in high Na⁺ media, the P2X7-mediated release of microparticles from platelets occurs in both Na⁺ and K⁺ media.

Conclusions: The release of platelet-derived microparticles is regulated by platelet P2X7 receptor. Since P2X7 stimulates formation of NALP3 inflammasome and IL-1b, microparticles may play a role in the permeabilisation of the blood brain barrier at relapse in MS. Hence, antagonists targeting the P2X7 receptor may be beneficial in MS.

Reduced cerebral Th17 cell migration confers protection to EAE in DOCK8 deficient mice

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Background: Experimental autoimmune encephalomyelitis (EAE) is a murine model of multiple sclerosis mediated by CD4+IL-17a producing (Th17) cells. Mice lacking functional dedicator of cytokinesis 8 (DOCK8) have elevated Th17 cells but do not develop EAE. How DOCK8, a guanine exchange factor expressed by immune cells confers protection to EAE is unknown.

Objective: We assessed the migration and functional capacity of Th17 cells in vivo in DOCK8 mutant mice. We also further examined the role of DOCK8 on Th17 cell function ex vivo in both the steady state and in a neuroinflammatory setting.

Design Methods: Dock8^{-/-} mice, lacking functional DOCK8 protein were immunised with a neural peptide to induce EAE. CNS infiltrating lymphocytes were quantitated using flow cytometry. Adoptive cell transfer was used to assess T cell migration. T cells isolated from protected mice were tested for their ability to undergo cell differentiation in vitro using cell culture and flow cytometry. Western blot and qPCR were used to analyse protein and RNA expression by cell subsets of DOCK8 mutant mice.

Results: In vitro T helper cell differentiation confirmed that the elevated Th17 cell population was not based on a T cell intrinsic differentiation bias or altered apoptosis. Th17 cells expressed normal Th17 cell specific CCR6 levels and migrated towards chemokine gradients in transwell assays. Adoptive transfers of Th1 and Th17 cells in EAE mice indicated a Th17 cell specific migration defect in the absence of functional DOCK8.

Conclusions: Mice lacking functional DOCK8 are protected from EAE by reduced CNS infiltration of CD4 T cells. Despite increased resting Th17 cells and normal in vitro differentiation, DOCK8 deficient mice have dysfunctional Th17 cell migration in EAE. DOCK8 thus appears to function by regulating Th17 cell migration into inflamed CNS tissue.

The evaluation of neutrophil activation as a potential prognostic indicator in multiple sclerosis

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Background: Neutrophils are innate immune cells that can expel their DNA and form extracellular traps (NETs) to kill bacteria. Neutrophils contribute to the development/progression/pathology of murine experimental autoimmune encephalomyelitis. The role of neutrophils and NETs in MS and their prognostic value in detecting active relapsing remitting MS (RRMS) is unclear.

Objective: We aim to longitudinally analyse neutrophil activation and evaluate associations between acute sub-clinical demyelination events in RRMS patients and neutrophil activation. We further aim to quantitate NETs, identify the source (mitochondrial or nuclear) of the neutrophil extruded DNA and establish methods to allow routine assessment of neutrophils/NETs in clinical samples.

Design Methods: Neutrophil production of antimicrobial agents (reactive oxidant species, myeloperoxidase) with and without stimulation will be quantified in whole blood. Flow cytometry will phenotype neutrophils in cryopreserved whole blood. Plasma/serum markers of neutrophil activation will be measured by ELISA and LUMINEX. NETs will be quantified in whole blood

smears using fluorescence microscopy. Quantitative PCR will be used to distinguish the genomic origin of NET DNA and plasma cell free DNA will be measured using Sytox.

Results: We are developing and verifying methods to understand neutrophil activation in RRMS by measuring soluble biomarker production, quantifying neutrophil activation and NETs and determining ROS and MPO activity. Preliminary PCR data indicate that NET DNA contains a relatively higher copy number of nuclear than mitochondrial DNA.

Conclusions: Our research will contribute towards an improved understanding of the role of neutrophils and NETs and will help determine whether either or both are potential prognostic indicators in MS.

Investigating the shared genetic risk factors for the autoimmune diseases Type 1 Diabetes Mellitus and Multiple Sclerosis.

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Background: Multiple Sclerosis (MS) and type 1 diabetes (T1DM) are both autoimmune diseases with inflammatory, auto-antigen-specific T-cell, and decreased T-cell suppressor components, but they affect different organs and have marked differences in pathogenesis and clinical manifestations. Despite this, they co-occur more often than expected by chance, pointing to shared susceptibility factors.

Objective: We sought to better define the risk-loci shared between MS and T1DM that must override the known differences in HLA association including protection against T1DM by the MS risk genotype, HLA-DRB1*1501. We also sought rare variants that may be contributing to disease risk in individuals with both T1DM and MS.

Design Methods: We compared linkage disequilibrium (LD) regions around the MS/T1DM associated SNPs using the rAggr program to better define shared risk genes. We screened 7 individuals with both T1DM and MS for rare variants that may be contributing to disease risk using whole genome sequencing (WGS). Algorithms used to filter the variants for impact on the genome included CADD, polyphen and SIFT. The HLA type of each individual was also determined using the WGS data.

Results: Over 20 genes were identified as risk genes for both MS and T1DM. Immune cell subset RNAseq indicates that the shared genetic risk across MS and T1DM likely involves multiple arms of the immune system. The WGS identified several high impact rare variants in each of the individuals including variants in the identified shared T1DM/MS risk genes. All 7 individuals with MS and T1DM were negative for the HLADRB1*1501 allele (Chi-Square $p < 0.003$).

Conclusions: These data implicate shared, specific immune dysregulation between T1DM and MS. Downstream analysis of the common risk genes may point to therapeutic interventions with utility for both conditions. Absence of HLADRB1*1501 in T1DM+MS individuals may provide a link between the HLA-type and the differences in pathogenesis in the two diseases.

Patient-reported effects of disease-modifying therapies on employment outcomes in people with multiple sclerosis

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Background: The employment rate of Australians with multiple sclerosis (MS) has increased from 2010 to 2013 and the increased availability of more potent disease-modifying therapies (DMTs), especially high efficacy DMTs may be an important contributor. However, few studies have evaluated effects of DMTs on employment outcomes in people with MS.

Objective: To examine whether the use of DMTs in a 5-years period maintained or improved work ability for people with MS and which DMTs are more effective in improving work attendance, work amount and work productivity by a nationally representative sample from participants in the Australian MS Longitudinal Study.

Design Methods: In December 2015, respondents reported DMTs treatment since 2010 and that whether the use of a DMT contributed to changes in employment. We classified 11 DMTs into 3 categories (β -interferons and glatiramer acetate as mild efficacy; fingolimod, teriflunomide and dimethyl fumarate as moderate efficacy; natalizumab, alemtuzumab and mitoxantrone as high efficacy). Each DMT used by a participant was treated as one observation. Data were analysed by random effects logistic regression based on observations.

Results: Of 879 respondents included, 1,475 observations were generated. We found significant dose-response relationships between the efficacy category of the DMTs and improvements in the three employment outcomes (test for trend $p < 0.001$). Compared to mild efficacy DMTs, the adjusted OR (95% CI) of perceived increases in the work amount, work attendance and work productivity for high efficacy DMTs were 20.01 (5.72 to 69.98), 31.99 (9.46 to 108.19) and 16.61 (6.08 to 45.37) respectively.

Conclusions: Our finding suggests that higher efficacy DMTs are contributing to improvements in work attendance, the amount worked and work productivity for people with MS. Future clinical trials and cost-effectiveness studies should include potential benefits of DMTs on different employment outcomes.

Factors influencing the use of smartwatch technology for symptom self-management in people with multiple sclerosis

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Background: Smartwatches, which synchronise with applications on personal mobile devices, provide an innovative means of delivering rehabilitation strategies whilst enhancing self-management of symptoms associated with a chronic condition.

Objective: This pilot study aimed to investigate user confidence and satisfaction in using smart technology for self-management of symptoms associated with multiple sclerosis. Specifically, to determine if demographic and disease variables were predictive of baseline confidence and satisfaction in smart technology use and the effects of time and training.

Design Methods: This 2x2 mixed between-within subjects study involved 8 community-dwelling adults with multiple sclerosis. The treatment group received smartwatch training and used the device for a 4-week period with ongoing support from an occupational

therapist. All participants completed a newly-developed questionnaire, the Smart Technology Satisfaction and Confidence Scale at baseline and post intervention to examine perceptions of satisfaction and confidence in using smart technology.

Results: Findings suggest that age, marital status, living situation, education, work status and disease severity were significantly related to baseline confidence and satisfaction ratings. There was a significant within-subjects effect of time on confidence scores but this was not affected by treatment condition. There was also a significant change in satisfaction of usability scores from baseline to post intervention, which was significantly associated with receiving training.

Conclusions: Smartwatches present a novel platform for assisting with self-management of symptoms. Exposure to the latest smart technology may improve user confidence. Training in its application should be tailored based on user demographics, disease severity, baseline confidence and aspects of usability.

Factors associated with the age at symptom onset of first clinical diagnosis of central nervous system demyelination

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Background: Age at symptom onset (ASO) is a prognostic factor that could affect disability development in multiple sclerosis (MS) patients. A number of factors are now known to influence the risk of MS, but their influence on the ASO is less well-investigated.

Objective: Examine the associations between known or emerging MS risk factors and ASO.

Design Methods: Multicentre study; cases ($n=279$) aged 18–59 years with a first clinical diagnosis of central nervous system demyelination (FCD), recruited at four Australian centres (latitudes 27°S to 43°S), from 1 November 2003 to 31 December 2006. Linear regression was used to assess the association between risk factors and ASO.

Results: A history of tobacco smoking, higher offspring number (≥ 2 children) before age 31-years, progressive-onset and cerebral impairment were associated with 3.05-years ($p=0.002$), 3.61-years ($p<0.001$), 5.61-years ($p=0.001$) and 4.37-years ($p=0.006$) later ASO, respectively. A history of marijuana use was associated with 2.80-years earlier ASO ($p=0.004$). All significant variables explained 38% of the total variance in ASO. Those with ≥ 4 risk factors for earlier onset had a 16.45-years earlier ASO ($p<0.001$) than those with ≤ 1 risk factor.

Conclusions: We found a novel association between higher offspring number and later ASO. History of tobacco smoking was associated with later onset, whereas marijuana use was associated with earlier onset. These factors are important drivers of MS onset timing and may aid in our understanding of MS pathogenesis.

Body temperature regulation in MS patients performing physical activity in the heat

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Background: The impact of heat intolerance and fatigue among people with multiple sclerosis (MS) are well documented. However to date it is unclear whether an impaired thermoregulatory capacity is evident in people with MS. It is also unknown whether any such impairment is manifested in the sudomotor system or vasomotor system.

Objective: To determine whether thermoregulatory responses are independently altered in MS patients relative to healthy controls during physical activity in a warm/hot environment.

Design Methods: Sixteen participants with relapsing-remitting MS (EDSS:2.8±0.9; 47±8y; 77.6±14.0kg) and 14 healthy controls (43±11y; 78.6±17.0kg) cycled at a heat production of 4 W~kg⁻¹ for 40-min at 30°C, 30%RH. A subset of 8 MS (EDSS:2.6±0.5; 44±8y; 82.3±18.2kg) and 8 healthy controls (44±12y; 81.2±21.1kg) also completed exercise at 35°C, 30%RH. In both trials, rectal temperature (Tre), mean skin temperature (Tsk), and local sweat rate (LSR) and cutaneous vascular conductance (CVC) on the upper back was measured throughout.

Results: At 30°C, end-exercise Tre (MS:37.2±0.3, CON:37.3±0.4°C; P=0.31), Tsk (MS:34.5±0.5, CON:34.6±0.5°C; P=0.43), LSR (MS:0.44±0.22, CON:0.47±0.21 mg cm⁻² min⁻¹; P=0.71), and CVC (MS:344±256, CON:268±157% of baseline; P=0.35) were similar between groups. Likewise, at 35°C, end-exercise Tre (MS:37.2±0.4, CON:37.3±0.3°C; P=0.70), Tsk (MS:35.5±0.5, CON:35.5±0.5°C; P=0.87), LSR (MS:1.26±0.44, CON:1.34±0.38 mg cm⁻² min⁻¹; P=0.61), and CVC (MS:425±163, CON:378±236% of baseline; P=0.68) were not different.

Conclusions: Individuals with relapsing-remitting MS do not demonstrate any clear impairments of sweating or skin blood flow control during moderate levels of physical activity at air temperatures as high as 35°C.

Patient-reported outcomes are worse for progressive-onset MS than relapse-onset MS, particularly early in the disease process

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Background: Past decades have seen dramatic progress in risk factors and therapies for people with relapse-onset MS. However, for the small proportion of progressive-onset MS cases, knowledge is limited. To make progress and provide better disease management for progressive-onset MS, understanding the differences between two MS onset types is vital.

Objective: To examine differences between progressive-onset MS and relapse-onset MS in relation to disability, disability progression, quality of life and MS symptoms on the basis of patient-reported outcomes.

Design Methods: The Australian Multiple Sclerosis Longitudinal Study (AMSLS) database of 1,985 cases was used in this

cross-sectional study. The exposure was MS onset type, and the outcomes were Patient-Determined Disease Steps (PDDS), progression over the last year, Fatigue Severity Scale (FSS), Hospital Anxiety and Depression Scale (HADS)-Anxiety & Depression, European quality of life (EQ-5D) and severity of thirteen common MS symptoms. Negative binomial regression was used to analyse associations.

Results: The severity of progressive-onset MS patients was significantly higher than relapse-onset MS patients for 17 of the 19 outcomes (adjusted mean ratio ranging from 1.11 to 1.52, p<0.05). The difference between the two onset types was most pronounced early in the disease process and reduced with increasing MS duration, this interaction with MS duration being significant for disability, progression over the last year, walking difficulties, bladder and bowel symptoms and spasticity (tests for interaction p<0.05).

Conclusions: People with progressive-onset MS were significantly worse off on nearly all patient-reported outcomes compared to relapse-onset MS. The differences were most pronounced early in the disease course. The findings highlight the urgency of identifying early interventions for progressive-onset MS.

Suitability of the Behavioural Assessment of Dysexecutive Syndrome for assessing the impact of executive dysfunction associated with multiple sclerosis

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Background: The nature and impact of executive dysfunction for people with multiple sclerosis is not thoroughly understood. Traditional neuropsychological measures often lack sensitivity to detect milder cognitive problems and few ecologically valid assessment tools are available in practice.

Objective: This study explored the sensitivity of the Behavioural Assessment of the Dysexecutive Syndrome (BADS) for classifying executive dysfunction in people with multiple sclerosis and its ability to predict impact on daily functioning.

Design Methods: Performance on the BADS was compared between people with multiple sclerosis (n = 67) and healthy controls (n = 67) using independent t-tests. Correlational analysis was used to determine whether performance on the BADS was associated with functional impacts on instrumental activities of daily living (IADL) using the Frenchay Activities Index.

Results: 19.4% of people with multiple sclerosis were categorised as "impaired" on the BADS. There were significant differences between groups on overall BADS performance (p < 0.001) and for all subtests (ps < .01), except Temporal Judgment. Executive dysfunction was significantly associated with poorer everyday functioning in outdoor (r = .43), leisure (r = .46), and domestic activities (r = .26).

Conclusions: Performance of people with multiple sclerosis was significantly poorer on tasks requiring cognitive flexibility, planning, problem solving, behaviour regulation, monitoring and organisation, but not self-awareness. BADS performance was associated with engagement in IADL, supporting the suitability of the BADS as an assessment tool for use with people with multiple sclerosis.

Acceptability and concurrent validity of the MSReactor cognitive assessment in people with multiple sclerosis

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Background: Cognitive impairment is common in multiple sclerosis, and practical and acceptable tests that are sensitive to change are needed. Computerized batteries can fill this gap. Concurrent validity is a measurement tools ability to vary with another measure of the same construct and is one aspect of determining criterion-related validity.

Objective: To determine the acceptability and concurrent validity of the psychomotor, attention and working memory tasks of the MSReactor computerized cognitive battery, when compared to the Symbol Digit Modalities Test and the Audio Recorded Cognitive Screen.

Design Methods: Twenty-four relapsing remitting multiple sclerosis patients (19 female) were enrolled from a larger longitudinal MSReactor cohort over 2 months for the cross-sectional pilot study and completed the MSReactor psychomotor, attention and working memory tasks, the written version of the Symbol Digit Modalities Test and the Audio Recorded Cognitive Screen in a single testing session at the Royal Melbourne Hospital. An acceptability survey was completed following the MSReactor tasks.

Results: From the longitudinal cohort, 70% of participants were happy to repeat the MSReactor tasks and 1.5% did not enjoy the tasks. MSReactor psychomotor, attention and working memory tasks correlated ($p < 0.05$) with Symbol Digit Modalities Test scores ($r = -0.45, -0.62, -0.56$ respectively), and memory tasks of the Audio Recorded Cognitive Screen. MSReactor tasks measuring attention (attention and working memory tasks) correlated with the attention domain task of the Audio Recorded Cognitive Screen (-0.37 and -0.47 respectively).

Conclusions: The MSReactor platform is acceptable and valid when compared to existing cognitive tests. Concurrent validity is a weak measure of criterion validity, and predictive validity will be measured in future experiments. These results provide preliminary support for the integration of MSReactor into clinics and community to efficiently monitor cognition.

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Identification of the immune phenotypes associated with haematopoietic stem cell transplantation for Multiple Sclerosis

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Background: Autologous haematopoietic stem cell transplant (HSCT) is an effective treatment for selected patients with severe autoimmune diseases providing a unique opportunity to study immune deregulation in humans. This study aims to elucidate the mechanisms through which HSCT achieves clinical improvement of Multiple Sclerosis (MS) in transplant recipients.

Objective: We hypothesised that autologous HSCT eliminates the pro-inflammatory disease-causing T cells to halt disease progress and promotes tolerance via multiple mechanisms to sustain disease control and reconstitution of immunoregulatory suppressive cell subsets.

Design Methods: We aimed to characterise changes in these subpopulations in the peripheral blood lymphocytes of eighteen MS patients, being treated with HSCT at St Vincent's Hospital using four custom-designed complex flow cytometry panels with 9-14 colours at pre-HSCT, 3, 6, and 12 months post-HSCT time points. The panels included differentiation markers, chemokine receptors, trafficking markers and cell surface integrins, recently reported to be relevant to HSCT for autoimmune diseases. Analysis included t-test, ANOVA and tSNE.

Results: We found that the pro-inflammatory disease-causing T cells, CCR6+CD161hi mucosal-associated invariant T (MAIT) cells (a Th17 subset with brain-homing abilities) and CD49d+CCR6+ CNS-homing cells significantly decreased following treatment. Additionally, immunoregulatory subsets such as regulatory T cells, cytotoxic CD8+CD57+ T cells and natural killer cell subsets (CD56hi) initially increased post-HSCT and stabilised by 12 months.

Conclusions: These panels successfully identified characterised subpopulations including pro-inflammatory disease-causing subsets and suppressive disease-stabilising subsets. These findings have the potential to allow us to track disease progression and treatment outcomes by monitoring the presence of MS-causing and MS-suppressive cell types, which may help improve the management of patients living with MS.

Exploring the importance of antigen conformation in MOG autoantibody detection

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Background: Detection of autoantibodies targeting brain antigens have become a powerful diagnostic tool. Autoantibodies which recognise conformational myelin oligodendrocyte glycoprotein (MOG) have been recently associated with subsets of CNS demyelinating disorders, and are now used to delineate patients from Neuromyelitis Optica Spectrum Disorders (NMOSD).

Objective: Extensive literature highlights that rodent MOG autoantibodies must recognise conformational MOG to induce demyelination. Current commercial MOG autoantibody detection systems require fixatives to preserve MOG-expressing cells/tissues. As fixation methods chemically alter protein structure, we examined the influence of fixatives, and whether human MOG autoantibody binding was sensitive to target conformation.

Design Methods: Using live MOG-expressing eukaryotic cells in a flow cytometry cell-based assay, we have reliably identified 252 MOG autoantibody-positive patients, the largest cohort worldwide. We assessed by flow cytometry the binding of sera from controls (n=28 children, n=32 adults), MOG autoantibody-negative (n=28 adults, n=28 children), and MOG autoantibody-positive patients (n=28 adults, n=27 children) to paraformaldehyde- and methanol-fixed human MOG-expressing cells.

Results: Only 37% (10/27) of seropositive children and 32% (9/28) of seropositive adults could bind to paraformaldehyde-fixed MOG-expressing cells, whereas 7% (2/28) of MOG autoantibody-negative children bound and all control (60/60) and MOG autoantibody-negative adult sera (28/28) did not bind. When MOG-expressing cells were fixed with methanol, all adult control patients (32/32) and MOG autoantibody-negative sera (28/28) remained negative, whereas only 14% (4/28) of MOG autoantibody-positive adult sera bound to the cells.

Conclusions: Modifying the conformation of human MOG significantly influences MOG autoantibody binding, and can lead to higher risk of false-positive and negative patient diagnosis. We shed critical light on the binding sensitivity of human autoantibodies, hence, antigen conformation must be carefully considered in diagnostic and pathogenic assays involving human autoantibodies.

Cytometric Analysis of T-Regulatory Cell Phenotypes in Multiple Sclerosis

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Background: T-regulatory cells (Treg) are key mediators of immunoregulation, and protective in multiple sclerosis (MS). Th-like Treg have been identified, which phenotypically mirror pro-inflammatory Th1, Th2 and Th17 subsets. Compared to thymic-derived Treg, in vitro generated murine Th1-like Treg have 100-times higher suppressive ability in vivo and 1000 more in vitro.

Objective: To compare Treg phenotypes in healthy humans and MS patients to provide a foundation for our future studies to examine the effect of currently approved MS therapies on Treg with a focus on assessing Th-like Treg in MS.

Design Methods: Blood was collected from MS patients and healthy donors and peripheral blood mononuclear cells (PBMC) were isolated using Ficoll. PBMC were subjected to flow cytometry to identify CD4+CD25+CD127lowFoxP3+Treg, and to assess naïve (CD45RA+) and memory (CD45RA-) Treg. Chemokine

receptor expression specific to Th1-like Treg (CXCR3), Th2-like Treg (CCR8), Th17-like Treg (CCR6) and circulating Treg (CCR7) was examined. Chemokine receptor expressing naïve and memory Treg were compared within Treg and CD4+ populations.

Results: Analysis of CD45RA and FoxP3 expression within CD4+T-cells and Treg identified three populations: CD45RA+Foxp3+, CD45RA-Foxp3+ and a third CD45RA-Foxp3hi population. CD45RA-Foxp3hi population frequency was significantly increased in MS patients when gated on CD4+T-cells or Treg and was believed to contain antigen-specific Treg. CXCR3+ Treg, but not CCR6+Treg, were significantly lower within the CD45RA+Foxp3+ and CD45RA-Foxp3+ populations in MS patients compared to healthy controls. CCR6 expression in MS patients was lower in the CD45RA-Foxp3hi Treg population.

Conclusions: Flow cytometric analysis of PBMC showed altered Treg phenotypes in MS patients in terms of CD45RA expression and chemokine receptor expression. These findings may suggest a deficiency of Th-1 and Th-17 like Treg in MS, and hence, may have further implications in the assessment of effectiveness of MS therapy.

Activation of antigen specific T regulatory cells from human naïve T regulatory cells

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Background: Human T-Regulatory cells (Treg) are CD4+CD25+CD127loFoxp3+T cells. Treg are shown to be functionally deficient with little consistency in Treg phenotype in MS patients. Our rodent studies in allotransplantation and autoimmunity demonstrate in vitro activation of antigen-specific Treg expressing specific cytokine receptors with enhanced suppression. These have potential in MS therapy.

Objective: We aim to extend our studies on antigen-specific Treg to humans, and ultimately explore the therapeutic potential of antigen-specific Treg in MS. As a first step, here we activated antigen-specific human Treg by culture of thymic Treg with antigen and IL-2 or IL-4 to characterize their phenotype.

Design Methods: Peripheral blood was collected from healthy donors. Treg cells were isolated as CD4+CD25+CD127lo cells using a Treg isolation kit (Stemcell Technologies) and cultured with IL-2 or IL-4 for 3-4 days. Their phenotypes were examined pre-and post-culture by multicolour flow cytometry using mouse anti-human antibodies, specifically CD45RA to assess naïve and activated Treg, and PCR for cytokine receptors expression.

Results: FACS analysis showed an increase in CD45RA-, CD45RO+ and CD45RB+ population. CD62L expression remained similar on all cells. Culture with IL-4 appears to induce polyclonal activation of tTreg as well. Similar to our rat studies, RT-PCR of cultured Tregs showed induction of IL-5R α , the specific chain of IL-5 receptor, only in tTreg cultured with IL-4 and alloantigen and not other cells.

Conclusions: Human CD4+CD25+CD127loTreg can be activated in vitro to express the IL-5 receptor, mirroring results from murine models. Increased CD45RA-/CD45RO+ population is consistent with activation of antigen-specific Treg. This suggests culture with IL-4 as a potential pathway for activation of tTreg in producing potent antigen-specific Treg for MS therapy.

A pro-inflammatory diet is associated with increased risk of a first clinical diagnosis of central nervous system demyelination in women

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Background: Diet plays a role in chronic inflammation whereby certain nutrients have known pro- and anti-inflammatory effects. While a number of studies have examined the association between dietary intake and multiple sclerosis, little is known about the dietary intake of inflammation-modulating foods and risk of multiple sclerosis.

Objective: To test associations between dietary inflammation index (DII) and risk of a first clinical diagnosis of central nervous system demyelination (FCD), a common precursor to multiple sclerosis. The DII is a recently devised tool that categorises individuals' diets on a continuum from maximally anti-inflammatory to maximally pro-inflammatory.

Design Methods: Dietary data were collected using a food frequency questionnaire from the 2003-2006 Ausimmune Study (272 cases, 519 controls matched on age, sex and region). Intakes of pro-inflammatory and anti-inflammatory foods were calculated per 1000 kcal of energy intake per day. Conditional logistic regression models were run separately by sex (n=530 women; n=164 men) due to a statistically significant sex interaction. Models were adjusted for smoking, serum 25-hydroxyvitamin D concentrations, history of infectious mononucleosis and education.

Results: In women, every one-unit increase in DII (corresponding to 13% increase in DII) increased the risk of FCD by 18% (AOR=1.18; 95%CI 1.04, 1.35; p=0.01). There was no statistically significant association between DII and risk of FCD in men (AOR=0.88; 95%CI 0.72, 1.07; p=0.19).

Conclusions: These findings suggest that a pro-inflammatory diet is a risk factor for FCD in women only. Given that diet may be a modifiable risk factor for multiple sclerosis, further research is necessary to elucidate the mechanisms by which diet may influence FCD risk and to understand the sex differences.

Positive stressful life events are associated with lower hazard of relapses in early multiple sclerosis

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Background: Despite significant evidence that psychological factors may influence disease course in many chronic disorders, few studies have investigated the association of stressful life events (SLEs) both negative and positive with the clinical course of multiple sclerosis (MS), especially the effect of positive SLEs.

Objective: We evaluated the association between SLEs and clinical course in demyelinating disease patients at the early stages of disease, in particular, whether there are differential effects between positive and negative SLEs.

Design Methods: The study included 251 cases, followed prospectively for around 5 years post FDE. Conversion to MS and relapses for each person was assessed at annual reviews and EDSS was assessed at the 5-year review.

Results: A greater number (p=0.035), higher perceived severity (p=0.010) and load (p=0.052) and a longer duration of the events (p=0.042) that were perceived as positive were consistently associated with a reduced hazard of relapses in a dose-dependent manner. Associations were less strong for conversion to MS. No associations were seen between perceived negative SLEs and MS conversion or relapse. There were no associations between any SLEs variables and annualised change of expanded disability scale score.

Conclusions: We found consistent evidence that positive perceptions of SLEs were associated with a reduced risk of relapses, suggesting that positive SLEs and possibly an overall positive psychological well-being may reduce disease activity.

Modifiable lifestyle factors at baseline could not influence the clinical course of multiple sclerosis

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Background: Emerging evidence suggested that modifiable lifestyle factors could potentially influence the development and progression of multiple sclerosis (MS), but the associations were still illusive currently.

Objective: We evaluated associations between tobacco smoking, alcohol/coffee consumption, physical activity, offspring number and the hazard of subsequent conversion to MS, relapses and annualised change of Expanded Disability Status Scale (EDSS) following a first demyelinating event (FDE).

Design Methods: The study included 279 cases, followed prospectively for around 5 years post FDE. Conversion to MS and relapses for each person was assessed at annual reviews and analysed with survival analysis. EDSS was assessed at the 5-year review and analysed with linear regression.

Results: Tobacco smoking behaviours before or after MS onset were not associated with any clinical course outcome. Alcohol (aHR 0.59, p=0.019) or coffee consumption (aHR 0.58, p=0.004) was associated with a lower hazard of conversion to MS, but not associated with relapses or disability progression. No associations

were seen for physical activity and offspring number for and any clinical course outcome.

Conclusions: There was little convincing evidence that modifiable lifestyle factors influenced the clinical course of MS after a first diagnosis of demyelination.

The CLEC16A locus and susceptibility to multiple sclerosis

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Background: The CLEC16A gene region has been found to be associated with multiple sclerosis (MS). Little is known about the function of CLEC16A and the effects the MS associated CLEC16A SNPs may have on the expression of CLEC16A and/or its 3 neighbouring genes CIITA, DEXI and SOCS.

Objective: Identify potential drivers of the association of the CLEC16A locus with MS risk. Determine immune cells the genes are expressed in, their co-regulation in these cells and the genotype effect of MS associated CLEC16A SNP rs12708716 on the expression of the CLEC16A locus genes in different immune cell contexts.

Design Methods: Gene expression data from whole blood (RNAseq) and immune cell subsets (RT-PCR) was used to determine whether expression of the 4 genes were correlated with each other and also to determine other genes whose expression was highly correlated with this locus. Immune cell subsets were also cultured with interferon-gamma, interferon-beta or dexamethasone to determine the effects of these stimuli on the CLEC16A locus. Gene expression data was analysed in relation to CLEC16A SNP rs12708716 genotype.

Results: Genes in the CLEC16A locus are expressed in number of immune cell subsets and their expression correlated in a cell subset-dependent manner. In whole blood subjects homozygous for the MS protective allele (GG) exhibited higher expression of DEXI. There was a trend to higher expression of DEXI in CD4 T cells (highest DEXI-expressing cell subset) of subjects bearing the protective allele and this association was more pronounced when CD4 T cells were treated with interferon-gamma.

Conclusions: We have shown that MS risk SNP rs12708716 confers higher expression of DEXI, encoding the dexamethasone-induced protein of unknown function; and that CD4 T cells in an interferon-gamma-stimulated inflammatory environment may represent one functionally relevant context in which this locus contributes to increased MS risk.

Overcoming monocarboxylate transporter 8 (MCT8)-deficiency to promote human oligodendrocyte differentiation and myelination

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Background: Thyroid hormone (TH) plays a critical role during oligodendrocyte differentiation. Cell membrane TH transport can be facilitated by the monocarboxylate transporter 8 (MCT8). Human mutations of the gene, SLC16A2, result in the X-linked-inherited psychomotor retardation and hypomyelination disorder, Allan-Herndon-Dudley syndrome (AHDS).

Objective: We posited that abrogating MCT8-dependent TH transport limits oligodendrogenesis and myelination and the

provision of DITPA (an MCT8-independent TH analog) upon knockdown of SLC16A2 in hESC-derived oligodendrocyte precursor cells (OPCs) may potentiate their proliferation, differentiation and myelination.

Design Methods: We utilised the NKX2.1-GFP human embryonic stem cell (hESC) reporter line to derive an enriched homogeneous population of oligodendrocytes. We tested the functionality of these hESC-derived OPCs by myelination assay where they co-cultured with rat retinal ganglion cells. hESC-derived OPCs were transduced with psi-LVRU6MP vectors carrying either scrambled shRNA or shRNA sequences for SLC16A2 to stably knockdown MCT8. Then these cells were treated with DITPA to rescue the OL survival and myelination.

Results: We show that our hESC-derived oligodendrocytes express MCT8. Moreover, treatment of these cultures with DITPA, up-regulates OL differentiation transcription factors and myelin gene expression. DITPA promotes hESC-derived OL myelination of retinal ganglion axons in co-culture. Pharmacological and genetic blockade of MCT8 induces significant OL apoptosis, impairing myelination. DITPA treatment limits OL apoptosis mediated by SLC16A2 down-regulation primarily signalling through AKT phosphorylation, driving myelination.

Conclusions: Collectively, our results highlight the potential role of MCT8 in TH transport for human OL development and may implicate DITPA as a promising treatment for developmentally-regulated myelination in AHDS.

Multiple sclerosis: a disease in a dish

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Background: The data generated from this investigation offers an analysis of adipose stem cell neurogenic capacity, and establishes a disease in a dish model that provides insight on multiple sclerosis itself through the quantification of the cellular proteome, quantification of secreted cytokines and morphological changes observed throughout neuronal differentiation.

Objective: This model compares adipose stem cells from multiple sclerosis and non-multiple sclerosis patients that have undergone neuronal differentiation. The cellular protein abundance changes were quantified by liquid chromatography tandem mass spectrometry, while quantification of secreted cytokines was performed using a multiplex immunoassay, with the objective of better understanding multiple sclerosis.

Design Methods: This proteomic analysis highlighted the functional complexity of patient derived stem cells, as protein abundance differ both spatially and temporally, consequently providing substantial insight into the phenotypic characteristics of the disease itself. The 27 cytokines that were examined are associated with immune signalling and contribute to neurological functions such as synaptic plasticity. As multiple sclerosis is an autoimmune disease, it is vital to understand the role inflammation has on the fate of stem cells.

Results: Cytokine expression in multiple sclerosis patients had greater homogeneity than that of non-multiple sclerosis patients, which was expected as this cohort could have undiagnosed and undetectable health conditions. Cytokine secretions significantly

differed between patient cohorts and suggested that multiple sclerosis patient adipose stem cells have a reduced neurogenic capacity. This was further supported through phase contrast microscopy, as the multiple sclerosis patient adipose stem cells did not demonstrate morphological changes that are characteristic of differentiation.

Conclusions: The data generated from this investigation offers an analysis of adipose stem cell neurogenic capacity, and establishes a disease in a dish model that provides insight on multiple sclerosis itself through the quantification of the cellular proteome, quantification of secreted cytokines and morphological changes observed throughout neuronal differentiation.

Laquinimod as a regulator of Kynurenine Pathway in Multiple Sclerosis

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Background: Our research has uncovered that Kynurenine pathway (KP), the canonical route of amino acid tryptophan metabolism, is dysregulated in MS, producing neurotoxic metabolites in increasing levels corresponding to MS severity. Quinolinic acid (QUIN) is one metabolite which we showed potentially kills oligodendrocytes as well as neurons at even nanomolar levels.

Objective: Laquinimod (LAQ) is a drug currently in phase III clinical trials for the treatment of relapsing-remitting MS and other neuropathologies. Its effects on the KP are currently unknown and the focus of this study. We aimed to investigate if LAQ reduces expression of KP and levels of QUIN.

Design Methods: LAQ was tested on both cultured human monocytes and cultured mouse primary subventricular zone mouse neural stem/precursor cells. Monocytes were cultured on fibronectin with/without interferon- γ , the canonical inducer of KP enzymes including the rate-limiting IDO-1. SVZ cells grown as neurospheres were dissociated and plated on Poly-L-ornithine/laminin coated dishes for experiment. A Multi-caspase kit was used to analyse apoptosis on a Muse cytometer. A p value <0.5 was regarded as statistically significant.

Results: LAQ reduced basal IDO-1 expression by 18.44+6.1% compared to control. In IFN- γ -treated monocytes, LAQ increased levels of neuroprotective Kynurenic acid and significantly increased it in monocytes pre-treated for 24 hours with LAQ prior to 72 hours IFN- γ exposure. LAQ significantly reduced neurotoxic QUIN levels with IFN- γ -treatment, and completely rescued QUIN levels in LAQ-pretreated monocytes. Rescue of apoptosis was also seen in mouse SVZ cells treated with LAQ and 4 μ M QUIN, restoring to control levels.

Conclusions: Monocytes highly upregulate IDO-1 in response to IFN- γ , secrete QUIN, cross the blood-brain barrier and localise to lesions. QUIN secretion may further compromise oligodendrocyte survival and neuronal atrophy. LAQ showed protective effects on neural stem cells. Enhancing survival of resident stem cells and modulating monocytes maybe an effective therapeutic strategy.

The kynurenine pathway of tryptophan metabolism modulates neural stem cell proliferation

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Background: The search for molecules which critically regulate neural stem cell (NSC) proliferation is ongoing, underpinning future production of cell lineages for therapy, while helping understand why innate repair in neurodegenerative diseases fails. Our ongoing research has investigated a role of the kynurenine pathway (KP) in healthy metabolism and neurodegenerative diseases.

Objective: The KP critically regulates bioavailability of the essential amino acid tryptophan. In MS the KP is dysregulated, producing high levels of metabolites like neurotoxic Quinolinic acid. We investigated if modulating the KP altered NSC proliferation. In particular, if interferons (IFNs) activate KP and drive changes in the proliferation of NSCs.

Design Methods: Developing mouse NSCs from E14 neurospheres were cultured. Agonists, antagonists or siRNAs to KP enzymes were used to dissect the pathways.

Results: IFN- γ activates indoleamine-2,3-dioxygenase (IDO-1) expression, the initial rate-limiting enzyme metabolising Tryptophan, and indeed significantly induced IDO-1 in NSCs. NSCs express all KP enzymes, and IFN- γ lead to impaired proliferation and an alteration of metabolic state of NSCs including their NAD⁺/NADH ratio (cell energy levels) via Trp depletion (required for protein biosynthesis), rather than through effects of KP metabolites. IFN- β negligibly effected IDO-1 levels, but induced IDO-2, and significantly decreased proliferation and downstream enzyme kynurenine-3-monooxygenase.

Conclusions: We show that KP enzymes play a specific role in the biology of NSCs and tryptophan metabolism, including the dominant regulation of the KP by interferons e.g. IFN- γ and IFN- β . Selective KP inhibition could minimize cell death during inflammatory episodes and optimize NSC proliferation and differentiation with direct therapeutic applications.

Increased brain activity during a cognitive eye movement task in patients with clinically isolated syndrome

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Background: Cognitive impairment is a common symptom in MS, including patients with clinically isolated syndrome (CIS). However, the underlying neural processes driving cognitive impairment in CIS is largely unknown. Saccadic eye movement is a validated approach used to probe the neural processes of cognitive function in a sensitive and objective manner. Integrating functional magnetic resonance imaging (fMRI) with a saccadic eye movement paradigm provides the opportunity to assess the neural activation associated with cognitive dysfunction in patients with CIS.

Objective: We sought to examine the neural processes of cognitive impairment in CIS patients using a saccadic eye movement task

Design Methods: 18 CIS patients and 17 healthy controls completed an fMRI eye movement task across four runs. The task involved switching between interleaved pro-saccades (direct eyes towards the target) and anti-saccades (divert eyes away from the target). For each trial, saccadic response time, error rate, and spatial accuracy were recorded. Contrast images for antisaccade vs. prosaccade between patients and controls were examined and thresholded with FDR of $z > 2.3$ and cluster significance of $p = 0.05$.

Results: Saccadic measures did not differ significantly between groups. However, patients demonstrated increased neural activation in the right parietal operculum, and postcentral and supramarginal gyrus compared to controls for antisaccade > prosaccade.

Conclusions: Despite comparable saccade performance, CIS patients reported stronger neural activation relative to healthy controls during the eye movement task. This change in neural activation suggests that there are neuroplasticity changes occurring in the earliest stage of MS to enable CIS patients to achieve the same cognitive outcomes. Furthermore, this study demonstrated that the saccadic eye movement task is an approach to examine the neural correlates of cognition in early MS.

Inter-subject variation in intrinsic connectivity of the ocular motor network in patients with clinically isolated syndrome

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Background: Cognitive impairment in MS has been associated with functional connectivity changes in the attentional frontoparietal network (FPN) and the default mode network (DMN). However, there are conflicting findings, particularly in patients with clinically isolated syndrome (CIS). Assessment of saccadic eye movements is an approach to probe the neural processes of cognitive function.

Objective: We sought to examine whether inter-subject variation in resting-state connectivity was associated with cognitive dysfunction in CIS patients.

Design Methods: 18 CIS patients and 17 healthy controls completed a (1) saccadic eye movement task during a functional MRI (fMRI) acquisition, and (2) resting-state acquisition. The eye movement task consisted of interleaved pro-saccade (direct eyes towards the target) and anti-saccade (divert eyes away from the target) trials. Region of interests (ROIs) were generated from the

task fMRI anti-saccade vs. pro-saccade contrasts. Significant regions for (1) anti-saccade > pro-saccade agreed with the FPN and (2) pro-saccade > anti-saccade with the DMN. For each ROI, the resting-state time-series was extracted, and a correlation analysis was performed between each ROI. For each patient, the connectivity correlation matrix was converted into a z-score matrix. The averaged z-scores for intra and inter connectivity within and between the FPN and DMN were calculated and correlated against three saccade measures of cognitive dysfunction - saccadic response time, error rate, and spatial accuracy.

Results: Saccade measures did not differ significantly between groups. For CIS patients, anti-saccade directional error rate correlated with (1) intra connectivity within the FPN ($r = -0.50$, $p = 0.04$) and (2) inter connectivity between the FPN and DMN ($r = 0.56$, $p = 0.02$).

Conclusions: In CIS patients, we have shown that cognitive dysfunction is associated with a change to functional connectivity, particularly within the FPN. This suggests that there may be an underlying functional mechanism driving cognitive function in people with CIS.

Objective analysis of speech correlates with disease severity in Multiple Sclerosis and differentiates groups with and without upper limb tremor.

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Background: Dysarthria is highly prevalent in Multiple Sclerosis. The relationship between dysarthria, MS disease severity and other cerebellar manifestations (such as tremor) is poorly understood.

Objective: To examine the relationship between objective markers of speech, disease severity and upper limb tremor in relapsing-remitting and secondary progressive Multiple Sclerosis.

Design Methods: An experienced neurologist determined A) the presence of upper limb tremor, B) the Expanded Disability Status Scale (EDSS) score and C) the degree of dysarthria (from 0, no disturbance to 4, unintelligible). Through acoustic analysis of speech, we investigated: 1) stability of vocal pitch, in sustained utterance of the vowel /a/; 2) stability of loudness, in the same sustained vowel; 3) diadochokinetic speed, in fast repetition of the meaningless word /pa/ta/ka/ and 4) maximum speed of vocal tract movement (i.e. change in pharynx and mouth cavity shape), measured in the word "always" (from a standard reading passage). After adjustment for multiple comparisons, $p < 0.0125$ was considered for statistical significance.

Results: We assessed 24 participants with Multiple Sclerosis and upper limb tremor (47.2 ± 12.3 years, 75% female, EDSS = 3.7 ± 1.6) and 24 matched patients with Multiple Sclerosis without tremor (51.2 ± 10.7 years, 75% female, EDSS = 3.6 ± 1.7). Clinical dysarthria scores (median = 0, mean = 0.375 ± 0.76) correlated with all acoustic variables measured: diadochokinetic speed Spearman's

rho=.561 ($p<.001$); pitch stability rho=.37 ($p=.011$); loudness stability rho=.37 ($p=.01$); and maximum speed of vocal tract movement rho=.363 ($p=.012$). Diadochokinetic speed strongly correlated with EDSS (rho=.529, $p<.001$). Speed of vocal tract movement correlated with tremor and differentiated tremor and non-tremor groups (2-tailed t-test $p=0.002$, rho=.418).

Conclusions: In a typically non-to-mildly dysarthric cohort, acoustic speech measurements correlate with disease severity and can differentiate overt cerebellar dysfunction in Multiple Sclerosis. Further study is needed to understand the significance of this relationship longitudinally.

Pathophysiology of MS tremor: an fMRI study

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Background: Tremor is a disabling movement disorder in Multiple Sclerosis (MS) that has no effective treatment and poorly understood pathophysiology. Understanding the brain regions involved in MS tremor could advance treatment development.

Objective: To understand the functional brain abnormalities associated with upper limb tremor in MS.

Design Methods: Twenty-three MS patients with unilateral upper limb tremor (MST) and twenty MS patients without tremor (MSC) underwent 3T magnetic resonance imaging (MRI) (MPRAGE, DIR and task functional MRI (fMRI)) and a clinical tremor assessment. Tremor was quantified using the Bain score (0 to 10) for overall severity, writing and Archimedes spiral drawing. Cerebellar function was quantified using the Scale for the assessment and rating of ataxia (SARA). The fMRI tasks included a 'brick-breaker' joystick game that aimed to isolate tremulous movement by contrasting two conditions: 'play' (playing the game) and 'move' (rhythmic left/right movement of joystick without game). Task fMRI was analysed using FSL FEAT. Lesions were automatically segmented using LST toolbox version 2.0.15 for SPM. Regions of interest (ROI) were identified from FEAT analyses for correlation with tremor scores. Statistical analyses were performed in SPSS (24).

Results: MST (47.6±12.9y, Expanded Disability Status Scale (EDSS) 4.1±1.7) and MSC (46.2±11.0y, EDSS 3.4±1.6) groups were well matched. MST showed significantly higher activation in 'play' vs 'watch' in the bilateral sensorimotor cortex compared to MSC. Furthermore, activation within bilateral sensorimotor

cortex significantly correlated with Bain tremor severity ($p<0.001$), handwriting ($p<0.000$), Archimedes spiral ($p<0.000$), and SARA ($p<0.005$). Ipsilateral sensorimotor cortex activation correlated with lesion load ($p<0.010$).

Conclusions: This study demonstrates a strong involvement of the sensorimotor cortex in the pathophysiology of upper-limb tremor in MS.

Dissecting the "killer module" and its role in Multiple Sclerosis

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Background: Multiple Sclerosis (MS) is a complex genetic disease associated with both environmental and genetic risk factors. In most cases, the risk factors' individual associations with MS are so weak that any meaningful understanding of the disease will require the identification of molecular pathways that contribute to MS liability.

Objective: To identify molecular pathways associated with MS in five lymphocyte populations, NK cells, monocytes, B cells, and CD4 and CD8 T cells, and then functionally test their role using a mouse model of disease, Experimental Autoimmune Encephalomyelitis (EAE).

Design Methods: We generated a weighted gene co-expression network based on 712 pooled Affymetrix Human Gene 1.0 ST array analyses of magnetic bead sorted lymphocytes from 67 relapsing/remitting MS patients and 102 Healthy unaffected individuals (HC). For each leukocyte population, the strength of differential expression between patients and HC was assessed, and each transcript was tested across the network to identify modules of interest which were tested in vivo and in vitro to elucidate their role in MS.

Results: We identified a major networked module of genes involved in cell-mediated cytotoxicity that is downregulated in the monocytes and NK cells of MS patients. Three genes were elucidated that, when modulated in vitro, affected expression across the entire module. Furthermore, their targeted deletion resulted in more severe EAE.

Conclusions: While there is strong data identifying NK cell deficiencies in MS patients, and key roles for monocytes in myelin and axon destruction and autoantigen presentation, this research identifies a new role for monocytes in immunomodulation by cytotoxicity. Further dissection of this "killer module" should elucidate its role in MS.