**The Australian Multi-centre Study of Environment and Immune Function**

**(The Ausimmune Study)**

**Summary of findings**

**Background**

The Ausimmune Study was a multi-centre case-control study in Australia, running from 2003-2007 in Brisbane, Newcastle, Geelong and the Western Districts of Victoria, and Tasmania. The study aimed to examine:

1. How environmental factors influence immune diseases
2. How immune disorders vary by latitude across Australia

The Ausimmune Study recruited people with recent onset of symptoms similar to those seen in early multiple sclerosis (‘cases’ with a first demyelinating event (FDE)) and also people of the same age and sex, and living in the same study region, who did not have such symptoms (‘controls’). By comparing the collected data between the cases and the controls, we have been able to highlight factors that increase the likelihood of being a case, i.e. having symptoms that may presage later onset of multiple sclerosis. As part of the Ausimmune Study, many of the cases were interviewed again, 2-3 years after their first participation, to check their health.

**Some findings from the Ausimmune Study:**

1. **The incidence of a first demyelinating event varies by latitude in Australia.**

**Figure**: The figure shows the age-standardised incidence of FDE per 100,000 population in the Ausimmune Study centres. The dotted lines are lines of latitude South

The incidence of FDE (new cases per 100,000 population) increased over 4-fold from Brisbane to Hobart. Furthermore, FDE was much more common in women than men in all locations, but nearly 7 times higher in Brisbane, compared to 2.5-times higher in Tasmania. <https://www.ncbi.nlm.nih.gov/pubmed/20167594>.

This pattern is nearly identical to that observed in data from 1981.

1. **Low sun exposure and low vitamin D were associated with increased risk of FDE.**

The graph on the left shows that with higher vitamin D (measured as the blood concentration of 25-hydroxyvitamin D, 25(OH)D), there is a reduced odds (or risk) of being a case, i.e. having an FDE. Furthermore, higher past sun exposure (graph on the right shows sun exposure in the last 3 years, but this was also true for lifetime sun exposure) was associated with a lower risk of FDE. <https://www.ncbi.nlm.nih.gov/pubmed/21300969>

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(the odds ratio is a measure of the risk – an odds ratio greater than one signifies increased risk; below one, reduced risk. The graphs show that higher 25(OH)D level and higher sun exposure are associated with reduced risk (odds ratio less than 1) of FDE.

1. **Sun exposure, vitamin D and disease course after a diagnosis of multiple sclerosis**

Data from the Ausimmune Study were used to show that people with a first CNS demyelinating event (cases) who reported higher sun exposure at entry to the Ausimmune Study were less likely to develop multiple sclerosis, and had fewer relapses after a diagnosis of multiple sclerosis, compared to people with lower sun exposure. Vitamin D status was not associated with risk of developing multiple sclerosis or frequency of relapses. <https://www.ncbi.nlm.nih.gov/pubmed/29449827>

1. **A healthy diet is associated with lower risk of having a first clinical diagnosis of central nervous system (CNS) demyelination.**

Most studies focus on the dietary intake of a specific component of diet, e.g. fat. Here we found that an overall ‘healthy’ diet – high in poultry, fish, eggs, vegetables, and legumes) was associated with a lower risk of CNS demyelination. Thus following healthy eating guidelines may reduce the risk of developing CNS demyelination and multiple sclerosis. <https://www.ncbi.nlm.nih.gov/pubmed/30084751>

1. **Higher intake of omega-3 fatty acids, particularly from fish, was associated with a lower risk of a first clinical diagnosis of CNS demyelination**

In addition to our work on overall dietary pattern (‘healthy diet’) we have also looked at specific elements of the diet. In this paper, we found that higher intake of omega-3 fatty acids, particularly those from fish, was associated with a substantially lower risk of a first clinical diagnosis of CNS demyelination (a nearly 50% reduction in risk for each additional g/day of omega-3). Other studies have found somewhat similar results, although some studies have found no effect. This may be to do with the different ways that diet is assessed in different research studies. In the Ausimmune Study we used one of the most reliable ways of measuring diet in large epidemiological studies. <https://www.ncbi.nlm.nih.gov/pubmed/26362904>

1. **Smoking and other lifestyle factors can alter the risk of developing CNS demyelination**

Data from the Ausimmune Study showed that a history of cigarette smoking was associated with an almost 2-fold increase in the risk of developing CNS demyelination. There was no change in risk for marijuana smoking, although there may have been an earlier age of onset in marijuana smokers, compared to those who did not smoke marijuana. We found no evidence of change in risk in association with alcohol intake, blood pressure, or obesity (at least at the time of the interview). <https://www.ncbi.nlm.nih.gov/pubmed/23670542>; <https://www.ncbi.nlm.nih.gov/pubmed/29937751>

1. **Biomarkers of multiple sclerosis and CNS demyelination**

Of great interest in research in multiple sclerosis, is finding some chemical in blood that can confirm the diagnosis. At the moment, diagnosis is made on the basis of a person’s symptoms, and changes on the MRI scan – and sometimes it turns out to be wrong, even several years after someone has been told they have multiple sclerosis. Using blood samples from the Ausimmune Study, and comparing blood chemicals from cases vs. controls, we found that cases had higher levels of a chemical called neurofilament heavy chain. This may be released from nerve cells when they are damaged, as occurs in multiple sclerosis. Other research studies are now pursuing the measurement of both neurofilament light and heavy chain, to see if levels of this in blood can help to make a definitive diagnosis of multiple sclerosis. <https://www.ncbi.nlm.nih.gov/pubmed/24639436>

1. **Blood evidence of past infection with chicken pox or shingles (caused by the Varicella zoster virus) was not associated with risk of a first clinical diagnosis of CNS demyelination.**

Some previous studies have suggested that past infection with chicken pox alters the risk of developing multiple sclerosis. By comparing the levels of antibodies and evidence of the virus in the blood of cases and controls from the Ausimmune Study, we showed that there was no evidence of alteration in risk of a first clinical diagnosis of CNS demyelination. <https://www.ncbi.nlm.nih.gov/pubmed/23765786>

1. **Epstein Barr virus infection and interaction with genotype**

We asked participants in the Ausimmune Study whether they recalled having had glandular fever, and then tested blood samples for antibodies to Epstein Barr Virus that causes that illness. We also tested the blood samples provided by participants in the Ausimmune Study to see if there was evidence of DNA from Epstein Barr Virus circulating in the blood. We found that a past history of glandular fever was associated with an increased risk of having a first clinical diagnosis of CNS demyelination, and higher levels of antibodies were similarly associated with increased risk. We did not find an association with circulating DNA from Epstein Barr Virus, suggesting that there was not active infection with this virus (only that there had been past infection). <https://www.ncbi.nlm.nih.gov/pubmed/21753179>

By combining data on past infection with Epstein Barr virus and risk genotype from the Ausimmune Study with that from several older studies, we showed that the combination of past history of glandular fever (infectious mononucleosis) and having the risk genotype for multiple sclerosis greatly increased the risk of being diagnosis with CNS demyelination – over 7-fold increase in risk. Bsed on the results of this study and others, it has been suggested that eliminating infection with Epstein Barr Virus could lead to the elimination of multiple sclerosis. <https://www.ncbi.nlm.nih.gov/pubmed/23413297>

1. **Greater number of pregnancies resulting in a live baby was associated with reduced risk of CNS demyelination in women.**

The timing of the Ausimmune Study in early 2000, and the recruitment of participants aged 18-59 years, meant that our women participants spanned a demographic change in the size of families and the number of children women were having. This provided rich data to examine whether having a full term pregnancy altered the risk of developing CNS demyelination. Pregnancy requires an alteration in the immune system so that it is more ‘tolerant’, for example of the developing foetus. We found that the greater the number of offspring, the lower the risk of developing CNS demyelination. This was a very strong effect – a 50% reduction for each additional birth. This may be evidence that each additional pregnancy ‘trains’ the immune system to be more tolerant. However, it is important to note that this result has not been consistently found in other studies, possibly because they have not had the same range of family size that was evident in the Ausimmune Study. This result is important, not because we should advise women to have more children, but because it points to possible pathways whereby the over-reactive immune system that causes multiple sclerosis can be made more tolerant. <https://www.ncbi.nlm.nih.gov/pubmed/22402857>

1. **Hygiene hypothesis and risk of a first clinical diagnosis of CNS demyelination**

The incidence of immune-mediated diseases like multiple sclerosis has been increasing too rapidly over the last 50 years for changes in genetic factors to be the cause. One hypothesis, called the hygiene hypothesis, is that our increasingly hygienic environments mean that the immune system of the infant and child is not being ‘trained’ to be tolerant to external influences, e.g. viral infections. That is, it overacts and there is ‘bystander’ damage that causes immune diseases. Using data from the Ausimmune Study, we showed that having any younger siblings was associated with a reduced risk of CNS demyelination (a 30% reduction for each additional sibling). One explanation for this finding is that younger siblings bring home all sorts of infections that the older sibling is thus exposed to, and this trains the immune system to be more tolerant. Our results provide some support for the hygiene hypothesis. <https://www.ncbi.nlm.nih.gov/pubmed/23600835>

1. **Occupational exposure to livestock is associated with reduced risk of CNS demyelination in women**

This study was again focusing on the hygiene hypothesis, that early exposure to pets and farm animals might train the immune system and reduce the risk of immune disorders. However, we found that women who had been exposed for 10 or more years to livestock had nearly a 3-fold increase in the risk of a first clinical diagnosis of CNS demyelination (or 6 or more years of farming, a 2-fold increase in risk). We did not find the same thing for men, but this may be because we had far fewer men in the Ausimmune Study. <https://www.ncbi.nlm.nih.gov/pubmed/23585328>

1. **Variation in MRI protocols**

The Ausimmune Study collected data from participants in four regions of Australia, and from many different notifying neurologists. Participants had MRI scans from over 20 different MRI scanners and using many different MRI protocols. By careful analysis of the MRI data from cases in the Ausimmune Study we showed that this made comparison of the MRIs from different regions (and even within regions, but from different scans) extremely difficult. This led to a call for a standardised MRI protocol where CNS demyelination was suspected; our follow-up at 2-3 years established a standardised MRI protocol which is still being used nearly 15 years later in the AusLong Study. <https://www.ncbi.nlm.nih.gov/pubmed/23210577>

**What have the findings from the Ausimmune Study meant for MS research in Australia?**

1. **The PrevANZ Study – testing whether vitamin D supplementation can reduce the risk of developing multiple sclerosis after a first demyelinating event**

The findings of the Ausimmune Study led directly to the establishment of a large clinical trial in Australia, funded by MS Research Australia, to test whether vitamin D supplementation, at different doses, could reduce the risk of progression to multiple sclerosis, compared to a placebo. This trial has now been recruiting participants across Australia and New Zealand for several years. We are hoping to reach the required number of participants in 2019, and results should be available in 2020. This study is unique, and its results will be very important to doctors, in knowing whether, and at what dose, they should prescribe vitamin D to people at high risk of developing multiple sclerosis.

1. **The PhoCIS Study – testing whether UVB phototherapy can reduce the risk of developing multiple sclerosis after a first demyelinating event**

In the analysis described in point 2 above, the Ausimmune Study was the first study in the world to show that higher levels of sun exposure were associated with reduced risk of immune-based CNS demyelination in humans. Following from those results, and using protocols for participant recruitment that were the same as those for the PrevANZ Study, this small Perth-based study recruited 20 participants with a FDE whose MRIs showed that they were at very high risk of developing multiple sclerosis. Half of the participants received UVB phototherapy (like a very carefully controlled sunbed) using a protocol that is used in the treatment of people with psoriasis. At the end of 12 months, all of those who did not receive phototherapy and 70% of those who did receive phototherapy had developed multiple sclerosis. This could represent a benefit of phototherapy in reducing the risk of developing multiple sclerosis, but the study was too small to say for sure. A larger study is now being planned in Scotland, where the incidence of multiple sclerosis is much higher. <https://www.ncbi.nlm.nih.gov/pubmed/29780610>

1. **Development of trials for dietary interventions, and guidelines for people with MS about diet**

As a result of the several studies on diet and risk of multiple sclerosis, and other work that is still underway, MS Research Australia has convened several workshops to better define and provide guidelines about modifiable lifestyles for people with multiple sclerosis. This work is ongoing, as is the planning for a number of intervention studies to see whether changes in diet or lifestyle can alter symptoms and/or disease progression in people with multiple sclerosis. <https://msra.org.au/news/modifiable-lifestyle-factors-ms/>

1. **MS Sunshine Study, validating the findings of the Ausimmune Study in a multi-ethnic study in the USA; and the AusLong Study uncovering the determinants of progression and disability in people with multiple sclerosis**

The Ausimmune Study has been very successful in demonstrating risk factors for a first clinical diagnosis of CNS demyelination. Many of the case participants in the Ausimmune Study have opted to continue being followed by the study team, now out to nearly 15 years, in the AusLong Study. This has also been a rich source of data and evidence about what drives disease progression and possible ways to control that. The results from the AusLong Study will be added to this summary, as they become available.

The MS Sunshine Study ran in California from 2011 to 2015 and used many of the protocols from the Ausimmune Study, so that our results would be comparable. We are working with the study team from the MS Sunshine to analyse data on diet and lifestyle factors. One very important result is that the MS Sunshine Study found a similar protective effect of higher levels of sun exposure (reducing the risk of a FDE) as was seen in the Ausimmune Study, and this was apparent in US Whites, Hispanics, and Blacks. But the protective effect of higher 25(OH)D levels (vitamin D status) was apparent only in US Whites. This casts some doubt on the causal role of vitamin D – it may be that the 25(OH)D level at study entry is simply a good measure of a person’s sun exposure and it is this that is important, rather than their vitamin D per se. More work is required to understand the role of vitamin D, including through intervention studies like the PrevANZ Study. https://www.ncbi.nlm.nih.gov/pubmed/29495467