



**HAL**  
open science

## Multiple sclerosis prevalence in Ireland: relationship to vitamin D status and HLA genotype.

Roisin Lonergan, Katie Kinsella, Patricia Fitzpatrick, Jennifer Brady, Barbara Murray, Richard Hagan, Ciaran Dunne, Marguerite Duggan, Sinead Jordan, Malachi Mckenna, et al.

### ► To cite this version:

Roisin Lonergan, Katie Kinsella, Patricia Fitzpatrick, Jennifer Brady, Barbara Murray, et al.. Multiple sclerosis prevalence in Ireland: relationship to vitamin D status and HLA genotype.. *Journal of Neurology, Neurosurgery and Psychiatry*, 2011, 82 (3), pp.317. 10.1136/jnnp.2010.220988 . hal-00609402

**HAL Id: hal-00609402**

**<https://hal.science/hal-00609402>**

Submitted on 19 Jul 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Multiple sclerosis prevalence in Ireland: relationship to vitamin D status and HLA genotype.**

**R Lonergan[1], K Kinsella[1], P Fitzpatrick[2], J Brady[3], B Murray[3], C Dunne[4], R Hagan[4], M Duggan[1], S Jordan[1], M McKenna[3], M Hutchinson[1], N Tubridy[1]**

**<sup>1</sup>Department of Neurology, St. Vincent's University Hospital and University College Dublin.**

**<sup>2</sup>School of Public Health and Population Science, University College Dublin**

**<sup>3</sup>Department of Metabolism, St. Vincent's University Hospital**

**<sup>4</sup>National Histocompatibility and Immunogenetics Reference Laboratory, National Blood Centre, Dublin 8**

**Corresponding author: Roisin Lonergan, Department of Neurology, St.**

**Vincent's University Hospital, Dublin 4**

**[roisin.lonergan@st-vincents.ie](mailto:roisin.lonergan@st-vincents.ie)**

**Telephone: 00353-87-6646797**

**Word count: 3,369 in main body of text**

**1 Figure (black and white)**

**4 Tables**

## 46 References

**Keywords:** Multiple Sclerosis prevalence, epidemiology, 25(OH) D, vitamin D, latitudinal variation, genetics, HLA

## **Introduction**

Numerous studies support the hypothesis that multiple sclerosis (MS) is a multifactorial autoimmune disease arising from a complex interplay between genetic and environmental factors. The HLA- DRB1\*1501 allele appears to be the predominant risk allele in northern European populations[1] and the HLA DRB1\*1501-DQB1\*0602 haplotype confers greatest susceptibility to MS in Ireland[2]. More recently, linkage to other candidate genes has been demonstrated, including IL2-Ra, IL-7 RA[1], EVI5 (ecotropic viral integration site 5)[3] and IFNAR[4].

Many environmental factors have been implicated in the aetiology of MS, and may interact with genetic susceptibility. The role of the environment is supported by the distinctive geographical distribution of MS[5-7], with the greatest incidences recorded at high latitudes north and south of the equator.[8-10] It is proposed that the latitudinal variation in MS prevalence is inversely related to sunshine exposure and associated vitamin D production.[3] The hypothesis regarding the environmental contribution of vitamin D is supported by migration studies, which demonstrate an association between place of early residence and MS.[11,12] Geospatial analysis in North America and continental USA showed a strong negative correlation between MS distribution and ultraviolet B (UV-B) exposure[14]. During winter at high latitudes sun exposure does not generate any vitamin D, thus vitamin D insufficiency is common.[14] Lower 25(OH)D levels during relapses, in addition to blunted

parathyroid hormone (PTH) response[15], suggest that activated vitamin D may have immunomodulatory effects and influence disease activity[16]. Activated vitamin D inhibits CD4+T cell and MBP-specific IL-17-secreting T cell proliferation.[17]

Clinically this is supported by seasonal fluctuation in the number of relapses[18] and gadolinium-enhancing lesions on MRI.[19,20 ]

Few studies on the therapeutic effects of vitamin D in RRMS have been performed.

There is no international consensus on optimal 25(OH)D levels or recommended daily intake, and the precise timing of vitamin D action, whether a discrete period or ongoing, remains unknown.

Recent reports suggest that MS prevalence is increasing worldwide.[3,21,22] The variation of prevalence with latitude was previously demonstrated in Ireland[23]which at 53° North lies in a high-risk MS zone where there is minimal skin production of vitamin D between late October and late March due to the attenuation of UV-B rays. Based on previous Irish studies, we know that genetic factors contribute to MS susceptibility [2], but vitamin D may influence risk. We hypothesized that: 1) the latitudinal gradient in MS prevalence persists in Ireland and 2) greater MS prevalence at higher latitudes relates to lower 25(OH)D levels compared to southern areas and 3) serum 25(OH)D is lower in patients with MS than in healthy controls.

Our aims were to 1) compare the prevalence of MS in three areas in Ireland (two rural areas: counties Donegal (northern latitude) and Wexford (southern latitude), and one urban area in southeast Dublin city (intermediate latitude), 2) determine differences in serum 25(OH)D and PTH levels between: (a) MS patients in the three regions and (b) MS patients and healthy control subjects, and 3) examine the frequency of HLA DRB1\*15 in people with MS in northern and southern counties.

## **Patients and methods**

Patients of Irish nationality with MS in each area (Donegal, latitude 54°8' - 55°43', Wexford latitude 52°20' - 52°44', and South Dublin, latitude 53°17') were identified from multiple sources. The ascertainment sources included neurologists and MS nurse specialists, general practitioners, hospital physicians, Public Health Nurses, the MS Society, pharmacies, Cheshire homes and respite facilities. Control participants, frequency-matched for age and sex, were recruited in each of the three areas. The prevalence date was established as December 31<sup>st</sup>, 2007. Informed consent was obtained from each participant. For patients with MS, their MS subtype was recorded[24] and the expanded disability status scale (EDSS) was calculated.[25] The diagnosis of MS was based on the revised McDonald criteria[26], and, where necessary, was confirmed by referring to patients' medical notes. Control participants were recruited in each of the three areas and included spouses and relatives of people with MS in addition to unrelated volunteers. Data from the most recent National Census [27] were used to calculate prevalence. Blood samples from patients and controls were taken in winter months from December to March for serum 25(OH)D and serum PTH measurement, and for DNA extraction. A questionnaire regarding environmental factors including past and current vitamin D supplementation and sun exposure was completed.[28] The Ethics Committee at St. Vincent's University Hospital approved the study.

## **Serum 25(OH)D and serum PTH analysis**

Serum 25(OH)D was measured by a competitive radioimmunoassay (Immunodiagnostic Systems Limited, Boldon, Tyne & Wear, UK). Inter-assay coefficients of variations (CVs) are 6.2% and 7.7% at concentrations of 28.8 nmol/L and 105.4 nmol/L. The intra-assay CVs are 3.0% and 2.7% at concentrations of 28.9 nmol/L and 73.9 nmol/L. Serum 25(OH)D levels of <50 nmol/L were considered to be vitamin D insufficient, and levels below 25 nmol/L were considered to be deficient.[29, 30] Serum intact PTH was measured by electrochemiluminescent immunoassay on the Elecsys 1010 platform (Roche Diagnostics, Basel, Switzerland). Intra-assay CV was <5.8% and inter-assay CV was <7.1%.

---

#### HLA typing

Genomic DNA was isolated from EDTA-anticoagulated peripheral blood samples using the GenoM-6 (Genovision, Vienna, Austria) DNA isolation and purification system. Low resolution HLA-DRB1/3/4/5 typing was performed using the polymerase chain reaction sequence-specific oligonucleotide probe assay (Invitrogen-Dynal Biotech, Bromborough, UK). HLA types were determined using Dynal RELI-SCAN and RELI SSO PMP software.

#### Statistical analysis

SAS version 9 (SAS Institute Inc., Cary, NC, USA) was used. The Z test compared rates of MS in two areas, and the Chi square test was used for comparison of rates in three areas. Win PEPI[31] was used for comparison of rates. For comparison of means and proportions, the student's t test, analysis of variance and chi square test were employed, and Pearson's correlation coefficient was calculated for correlation. Age standardized rates were calculated using direct standardization, with the national

population from the most recent census as the standard population.[27] For all estimates, 95% confidence intervals were calculated.

## **Results**

### **Prevalence**

In total, 632 MS patients were identified in the three regions: 329 in Donegal, 173 in Wexford and 130 in South Dublin (Table 1). MS prevalence on December 31<sup>st</sup>, 2007 was: Donegal 290.3/10<sup>5</sup>, (95% CI; 238.7 – 255.5), Wexford 144.8/10<sup>5</sup>, (95% CI; 138.3 – 151.9), southeast Dublin 127.8/10<sup>5</sup>, (95% CI; 131.6 – 146.7). Prevalence was significantly higher in Donegal than Wexford (p <0.0001). Although prevalence in Wexford was higher than in South Dublin this did not reach significance (p =0.72).

Age standardised MS prevalence was: Donegal – all MS patients 257.8/10<sup>5</sup> (95% CI 253.2-262.4), male MS patients 157.6/10<sup>5</sup> (95% CI 152.7-162.6), female MS patients 358.3/10<sup>5</sup> (95% CI 350.3- 366.5); Wexford- all MS patients 152.0/10<sup>5</sup> (95% CI 148.4-155.6), male MS patients 119.0/10<sup>5</sup> (95% CI 114.8-123.4), female MS patients 176.0/10<sup>5</sup> (95% CI 170.4-181.7); southeast Dublin- all MS patients 123.2/10<sup>5</sup> (95% CI 120.0-126.4), male MS patients 84.2/10<sup>5</sup> (95% CI 80.6-87.9), female MS patients 160.6/10<sup>5</sup> (95% CI 155.2-166.1). The 3 counties were significantly different from each other when comparing age standardized prevalence in all MS patients (p <0.001), males with MS (p <0.001) and females with MS (p <0.001).



In Donegal, MS prevalence had increased significantly since 2001; prevalence was  $184.6/10^5$  in 2001, (95%CI 162.0-209.5) and  $290.3/10^5$  in 2007, (95%CI 238.7-255.5) ( $p=0.001$ ). There was a non-significant increase in prevalence in Wexford; prevalence was  $120.7/10^5$  in 2001, (95%CI 100.6 to 143.8) and  $144.8/10^5$  in 2007, (95%CI 138.3 – 151.9) ( $p= 0.117$ ). Prevalence was lowest in southeast Dublin ( $130.0/10^5$  in 2007) but we did not have previous data for comparison (Table 2). We could not compare age-standardized prevalence rates over time because this data was not included in the 2001 prevalence study.

Overall, the female to male ratio in MS patients was 2.0:1. In Wexford, the ratio was 1.5:1 (2.2 in 2001), in Donegal 2.1:1 (2.48 in 2001) and in southeast Dublin 2.6:1 (no earlier data).

### 25(OH)D and PTH levels

25(OH)D and PTH levels were determined in 329 MS patients (61.3% of all ascertained patients), and 226 healthy control subjects (Table 3).

The mean level of serum 25(OH) D was similar in MS patients (38.6 nmol/L, SD 22.5) and controls (36.4 nmol/L, SD 16.4 ( $p > 0.05$ )). Mean 25(OH)D levels in patients in Donegal were 36.9 nmol/L (range: 13- 97 nmol/L), in Wexford 39.7 nmol/L (15-114 nmol/L) and in southeast Dublin 50.7 nmol/L (13-161 nmol/L). Mean levels were significantly higher in southeast Dublin, the area with lowest MS prevalence, than in Donegal ( $p < 0.0001$ ) and Wexford ( $p < 0.0001$ ).

There was no significant difference between mean 25(OH)D levels in patients in Donegal and Wexford (Donegal 36.9 nmol/L and Wexford 39.7 nmol/L,  $p = 0.284$ ).

The proportion of MS patients from all three study areas with vitamin D deficiency ( $< 25$  nmol/L) (28.3%) was significantly higher than the proportion of control subjects (19.2%) ( $p = 0.004$ ).

There was a significant inverse correlation between 25(OH)D and PTH levels in MS patients ( $r = -0.17$ ;  $p < 0.002$ ) and in controls ( $r = -0.24$ ;  $p < 0.0003$ ). Vitamin D deficiency ( $< 25$  nmol/L) was observed in 93 patients (28.3%) with a mean of 20.3 nmol/L (range 13.3- 24.6) and a mean PTH of 44.8 ng/L (range 16.7- 86.3). There was a non-significant inverse correlation between 25(OH)D and PTH in this group of patients ( $p = 0.28$ ). Vitamin D deficiency ( $< 25$  nmol/L) was observed in 44 control subjects (19.2%) with a mean of 20.8 nmol/L (range 14.3- 24.6 nmol/L) and a mean PTH of 48.3ng/L (range 5.0- 131.5 ng/L). A significant inverse correlation between 25(OH)D and PTH in the control subjects with 25(OH)D  $< 25$  nmol/L ( $r = -0.47$ ;  $p = 0.0013$ ) indicated a normal response to vitamin D deficiency.

Overall, significantly more patients (18.2%) than controls (11.5%) were taking vitamin D supplementation of >800IU/day (800IU- 1500IU/day) ( $p = 0.04$ ), but there was no significant difference in proportions of patients (5.7%) and controls (8.6%) taking vitamin D supplementation of 1,300IU-1,500IU/day ( $p > 0.05$ ). None of the cohort took >1,500IU vitamin D supplementation/day.

We stratified serum 25(OH)D results, looking separately at patients and controls not taking vitamin D supplementation. Mean serum vitamin D level was similar in patients (38.4 nmol/L) and controls (37.7 nmol/L) after stratification ( $p > 0.05$ ). There was no significant difference in mean 25(OH)D levels between patients (41.0 nmol/L) and controls (38.4 nmol/L) who were taking up to 800IU vitamin D/day ( $p > 0.05$ ). The difference was significant only in those with very low levels ('deficiency' =  $\leq 25$  nmol/L), as before, with a greater proportion of MS patients having very low levels ( $p = 0.004$ ).

Mean serum 25(OH)D levels overall (patients and controls) were significantly lower in those taking no supplements or <800IU vitamin D/day (38.1 nmol/L) than in those taking >800IU/day (53.1 nmol/L) ( $p < 0.001$ ). Mean serum 25(OH)D were also significantly lower in those taking no supplements or <1000IU vitamin D/day (39.9 nmol/L) than in those taking 1,000-1,500IU/day (47.9 nmol/L) ( $p = 0.03$ ). Vitamin D supplement data are displayed in table 4.

Although we did not observe significant relationships between serum 25(OH)D levels and age at MS onset ( $p = 0.26$ ) or the presence of relapse in past 2 years ( $p = 0.7$ ), our

data were underpowered to address questions regarding these factors, or to comment on any association between MS risk and season of birth, early sun exposure (two or more hours of daily summer sun exposure in childhood ( $p= 0.21$ ) or vitamin D supplementation (current or up to age 15,  $p= 0.76$ ).

#### Frequency of HLADRB1\*15 allele

The HLADRB1\*15 allele was present in 62.9% of MS patients, compared with 35.2% of control subjects ( $p < 0.01$ ). The frequency was significantly higher in Donegal patients (69%) compared with South Dublin patients (52%) ( $p < 0.04$ ). Homozygosity for the HLADRB1\*1501-DQB1\*0602 haplotype occurred significantly more frequently in Donegal compared with Wexford ( $p = 0.032$ ).

## **Discussion**

In keeping with recent epidemiological studies of MS[3,21-23]we have observed an increase in the prevalence of MS nationally between 2001 and 2007, with a significant increase in the most northerly region of Donegal. We confirmed the latitudinal gradient found in 2001[23], with a significantly greater MS prevalence in the Northwest compared with the Southeast. This latitudinal gradient has been attributed to genetic factors, based on ancestral differences in each area (Celtic in the Northwest and Anglo-Norman in the Southeast), and the heterogeneity in distribution of HLA class II associations in (specifically the HLA DRB1\*1501– DQB1\*0602 haplotype) between these areas. The ethnic make-up of the study cohort was relatively homogenous. Although Dublin county has experienced significant migration over the past decade, the particular area studied is not an area of marked migration, and population ethnicity was comparable with both Donegal and Wexford. Increased prevalence may be influenced by numerous factors in addition to increased incidence, but our case ascertainment and diagnostic techniques have not changed greatly since the last prevalence study in 2001.[23]Incidence could not be calculated due to inability to ascertain year of (MS) onset data in all identified cases. These particular cases were identified from at least two sources, but exact timing of MS onset was not clearly recorded, and patients declined interview.

Our study hypothesis is based on the idea that serum vitamin D levels in adulthood, after MS has presented, are relevant, or at least reflect what has happened previously, a limitation imposed by the nature of our study. Evidence from monozygotic (MZ) twin studies in MS[32] supports the hypothesis that MS is strongly influenced by environmental factors in genetically susceptible individuals, that vitamin D levels are genetically regulated, and that MS itself is not the cause of low vitamin D (e.g. limited sun exposure imposed by indoor lifestyle). Adoptee [33] step-sibling[34] and half-sibling studies[35] indicate that the environmental component is ubiquitous, rather than limited to the shared family environment. However, the exact timing of vitamin D action, and whether adult levels reflect those in the past, is as yet unknown. Chronic low concentrations, seasonal fluctuation, or acute effects may all increase risk, and this limits our ability to draw clear conclusions. Insufficient pre- and perinatal vitamin D levels may imprint on functional characteristics of various tissues, increasing risk of adult onset MS, by interacting with other genetic susceptibility factors[36] and adult exposures (including hypovitaminosis D during adult life).

MS displays a month-of-birth effect, with an excess of patients being born in spring and a deficit in winter; the effect is most pronounced in familial cases[37], implicating interactions between genes and environment related to climate. A recent Scottish study[38] showed a marked difference (50%) in MS risk between being born in April versus November, and this month-of-birth association implicates interaction of a seasonal risk factor with loci at or near HLA-DRB1, the region exerting the single strongest genetic effect in MS, during gestation or shortly after birth.[39] Our study was not sufficiently powered to address month of birth. The nature of this

timing of birth effect remains unknown, but maternal vitamin D deficiency is one plausible candidate.[37, 40, 36]

Although monozygotic twins (MZ) share a common genotype, epigenetic differences may cause phenotypic discordance (including disease susceptibility). Examination of differences in DNA methylation and histone acetylation in a large cohort of monozygotic twins[41] showed that, although epigenetically indistinguishable during early years of life, marked differences in content and genomic distribution of 5-methylcytosine DNA and histone acetylation in older twins affected their gene-expression portrait. The divergence of epigenetic modification patterns with age may be influenced by intrinsic and extrinsic factors such as smoking and diet, and epigenetic drift may occur with aging itself. Fraga et al showed that approximately one third of twins studied displayed epigenetic differences in DNA methylation and histone modification, distributed throughout their genomes and impacting on gene expression. These markers were more distinct in older twins with different lifestyles, highlighting the role of environmental factors in modifying a common genotype to a different phenotype (e.g. discordant MS onset in MZ twins). Environmental modulation of genetic information, by altering the pattern of epigenetic modifications, supports the environment (vitamin D)- genetic interaction findings in our study, and suggests that adult exposures, including vitamin D levels, may play a role in both MS risk and disease modification, even if the critical exposure period began prenatally.

Despite our hypothesis that the prevalence gradient would relate to lower 25(OH)D in MS patients, 25(OH)D levels between the north (Donegal) and south (Wexford) were not significantly different and did not differ from control subjects: winter levels were

low in all participants. It is important to remember that optimal levels remain undefined (and may be much higher than current laboratory reference ranges, e.g.  $\geq 100$  nmol/L)[42]. Our view of sufficiency is arbitrary, and we cannot assume that all individuals require the same levels. However, mean winter levels in our Irish cohort were low overall ( $< 50$  nmol/L) and significantly more MS patients than controls had very low levels ( $< 25$  nmol/L). As previously described by others[15], there was a blunted PTH response only in MS patients with vitamin D deficiency ( $< 25$  nmol/L), but its meaning is unclear. Only a small percentage took  $> 1,000$  IU vitamin D3 daily; maximum intake was  $< 1,500$  IU, but it is likely that at least 4,000 IU daily are required to achieve levels of 100 nmol/L[43]. Despite differences in solar radiation (UV-B) exposure between areas; the northwest of Ireland experiences on average 319,875 joules/cm<sup>2</sup> solar radiation annually, compared with 331,683 joules/cm<sup>2</sup> solar radiation in the southeast[44]; it is probable that the latitudinal range in a small country such as Ireland is insufficient to demonstrate such an effect. Higher 25(OH)D in southeast Dublin may be genetically regulated, or relate to more widespread use of vitamin D supplements (39%) compared with Wexford (24%), and greater availability and consumption of fortified milk in Dublin.[45] Differences in socio-economic group distribution between these areas may influence supplementation practices.[27] However, in 52% of patients and 55% of controls taking vitamin D3 supplements the dose of  $\leq 400$  IU/day was unlikely to alter serum levels significantly.

The HLA DRB1\*15 allele, associated with MS susceptibility, was significantly overrepresented in MS patients compared with controls, and HLA-DRB1\*15 frequency was significantly lower in southeast Dublin compared with areas of higher MS prevalence. Active vitamin D (1, 25-dihydroxyvitamin D) binds the intranuclear



vitamin D receptor (VDR), whose gene locates to chromosome 12q13.1. The resulting complex binds the retinoid X receptor (RXR), resulting in a VDR-RXR complex, which then binds the vitamin D response element (VDRE).[46] Recently, a VDRE was found on HLA DRB1\*1501, the major MS susceptibility allele.[47] Insufficient HLA DRB1\*1501 expression - in the setting of low vitamin D and suboptimal binding to the VDRE, with poor up regulation of HLA DRB1\*1501 - could affect central deletion of auto-reactive T cells. Thus people possessing HLA DRB1\*1501 may be at high risk of auto-immunity (MS) in the setting of vitamin D deficiency and this combination may explain the high prevalence of MS in the Irish population. The latitudinal variation in MS in Ireland is not explained by 25(OH)D levels alone, but most likely the interaction with genetic susceptibility. In a gene- environment interaction, lacking either component can prevent clinical expression of disease, leaving one factor apparently unrelated to risk when both are required. Therefore, similar overall serum 25(OH)D levels in patients and controls does not outrule the role of low vitamin D in MS pathogenesis in our cohort.

In addition to the previously discussed limitation imposed by the nature of our study (i.e. hypothesis based on the idea that serum vitamin D levels in adulthood are relevant in MS), we acknowledge that inclusion of spouses as controls is not always ideal, as they are usually in the same environment as affected cases, and may modify their risk by taking vitamin D supplements more readily. However, we found that significantly more patients (18.2%) than controls (11.5%) were taking vitamin D supplements ( $\geq 800$ IU) ( $p = 0.04$ ), despite this potential spousal influence. Spousal controls were included because they were more willing to give samples than people without affected family or partners.

In conclusion, although unproven, one might hypothesise that combined high frequency of HLADRB1\*15 and widespread vitamin D insufficiency may explain the high, increasing MS prevalence in the Irish population. Vitamin D may only be one factor in a common mechanism by which environment interacts, but it is a potentially modifiable risk. Vitamin D insufficiency is common throughout the population, an important public health observation. The MS prevalence gradient in Ireland is not explained by variation in 25(OH)D levels alone, but differences in frequency of the MS risk allele HLADRB1\*1501 and risk haplotype HLADRB1\*1501-DQB1\*0602 combined with low serum vitamin D levels probably influence latitudinal variation.

### **Acknowledgements**

The authors would like to sincerely thank the consultant neurologists at AMNCH, Tallaght, St. James' Hospital, Beaumont Hospital and Mater Misericordiae hospitals in Dublin, Sligo General Hospital and the Western Regional Hospital, Limerick and Waterford Regional Hospital, and also the general practitioners in each region, for granting access to data of MS patients in our study areas, and also Dr. Bannan, Consultant Physician at Letterkenny Hospital and Dr. Quigley Consultant Physician at Wexford General Hospital, who allowed us to use their clinic rooms for weekend research clinics. We are also grateful to MS Ireland for much help during the study, and for ongoing support. We also thank the patients with MS and the control volunteers who agreed to participate in this study.

## References (46)

- 1 Peltonen L. Old suspects found guilty—the first genome profile of multiple sclerosis. *N Engl J Med* 2007; 357:927–9.
- 2 Dunne C, McGuigan C, Crowley J, Hagan R, Rooney G, Kelleher J, Hutchinson M, Lawlor E. Human leucocyte antigen class II polymorphism in Irish patients with multiple sclerosis. *Tissue Antigens* 2006; 68:257-262.
- 3 Ebers GC. Environmental factors and multiple sclerosis. *Lancet Neurol* 2008; 7:268-77.
- 4 Gilli F, Valentino P, Caldano M, Granien L, Capobianco M, Malucchi S, Sla A, Marnetto F, Bertolotto A. Expression and regulation of IFN $\alpha/\beta$  receptor in IFN $\beta$ -treated patients with multiple sclerosis. *Neurology* 2008; 71:1940-1947.
- 5 Acheson ED, Bachrach CA, Wright FM. Some comments on the relationship of the distribution of multiple sclerosis to latitude, solar radiation, and other variables. *Acta Psychiatr Scand* 1960 Suppl 35:132-147.
- 6 Kurtzke JF, Beebe GW, Norman JE et al. Epidemiology of multiple sclerosis in U.S. veterans: 1. Race, sex, and geographic distribution. *Neurology* 1979-35.
- 7 Miller DH, Hammond SR, McLeod JG, Purdie G, Skegg DC. Multiple sclerosis in Australia and New Zealand: are the determinants genetic or environmental? *J Neurol Neurosurg Psychiatry*. 1990 October; 53 (10):903–905.
- 8 Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis, part I: the role of infection. *Ann Neurol* 2007; 61:288-99.
- 9 Pugliatti M, Sotgiu S, Rosati G (2002). The worldwide prevalence of multiple sclerosis. *Clin Neurol Neurosurg* 104:182-191.

- 10 Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* 2006;296:2832-2838.
- 11 Dean G, Elian M. Age at immigration to England of Asian and Caribbean immigrants and the risk of developing multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1997; 63:565-568.
- 12 Hammond SR, English DR, McLeod JG. The age-range of risk of developing multiple sclerosis: evidence from a migrant population in Australia. *Brain* 2000; 123 (Pt 5):968-74.
- 13 Beretich and Beretich. Explaining multiple sclerosis prevalence by ultraviolet exposure: a geospatial analysis. *Mul Scler.* 2009;15:891-898.
- 14 McKenna MJ. Differences in vitamin D status between countries in young adults and the elderly. *Am J Med* 1992; 93:69-77.
- 15 Soilu-Hanninen M, Laaksonen M, Laitinen I, Eralinna JP, Lilius EM, Mononen I. A longitudinal study of serum 25-hydroxyvitamin D and intact PTH levels indicate the importance of vitamin D and calcium homeostasis regulation in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2008; 79:153-157.
- 16 Hayes CE, Nashold FE, Spach KM, Pedersen LB: The immunological function of the vitamin D endocrine system. *Mol Cell Biol* 2003; 49:277-300.
- 17 Correale J, Ysraelit MC, Gaitan MI. Immunomodulatory effects of vitamin D in multiple sclerosis. *Brain* 2009;132; 1146-1160.
- 18 The Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene). Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. *Nature Genetics* 2009; 41:824 – 828.

- 19 Auer DP, Schumann EM, Kumpfel T, Gossel C, Trenkwalder C: Seasonal fluctuations of gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis. *Ann Neurol* 2000; 47:276-277.
- 20 Embry AF, Snowdon LR, Vieth R. Vitamin D and seasonal fluctuations of gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis. *Ann Neurol* 2000; 48:271-2.
- 21 Alvaro A, Hernán MA. Temporal trends in the incidence of multiple sclerosis. A systematic review. *Neurology* 2008; 71:129-135.
- 22 Gray OM, McDonnell GV, Hawkins SA. Factors in the rising prevalence of multiple sclerosis in the north-east of Ireland. *Mult Scler* 2008; 14:880-886.
- 23 McGuigan C, McCarthy A, Quigley C, Bannan L, Hawkins SA, Hutchinson M. Latitudinal variation in the prevalence of multiple sclerosis in Ireland, an effect of genetic diversity. *J Neurol Neurosurg Psychiatry* 2004; 75:572-576.
- 24 Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis *Neurology* 1996; 46:907-911.
- 25 Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983; 33:1444-52.
- 26 Polman CH, Reingold SC, Edan GE, Filippi M, Hartung H-P, Kapos L, Lublin F, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic Criteria for Multiple Sclerosis: 2005 Revisions to the "McDonald Criteria". *Ann Neurol* 2005; 58:840-846.
- 27 Central Statistics Office Ireland- 2006.

- 28 Van der Mei IAF, Ponsonby A-L, Dwyer T, Blizzard L, Simmons R, Taylor BV, Butzkueven H, Kilpatrick T. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. *BMJ* 2003; 327:316.
- 29 McKenna MJ, Freaney R. Secondary hyperparathyroidism in the elderly: means to defining hypovitaminosis D. *Osteoporos Int* 1998; **8** (Suppl 2):S3-S6.
- 30 Holick M. Vitamin D deficiency. *N Engl J Med* 2007; 357:266-81.
- 31 Abramson, JH. WINPEPI (PEPI-for-Windows): computer programs for epidemiologists. *Epidemiologic Perspectives & Innovations* 2004; 1:6.
- 32 Sarah-Michelle Orton, Andrew P Morris, Blanca M Herrera, Sreeram V Ramagopalan, Matthew R Lincoln, Michael J Chao, Reinhold Vieth, A Dessa Sadovnick, and George C Ebers. Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. *Am J Clin Nutr* 2008; 88:441-7.
- 33 Ebers GC, Sadovnick AD, Risch NJ. A genetic basis for familial aggregation in multiple sclerosis. Canadian Collaborative Study Group. *Nature* 1995; 377:150-1.
- 34 Dyment DA, Yee IM, Ebers GC, Sadovnick AD. Multiple sclerosis in stepsiblings: recurrence risk and ascertainment. *J Neurol Neurosurg Psychiatry* 2006; 77:258-9.
- 35 Ebers GC, Sadovnick AD, Dyment DA, Yee IM, Willer CJ, Risch N. Parent-of-origin effect in multiple sclerosis: observations in half-siblings. *Lancet* 2004; 363:1773-4.
- 36 Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton S-M, Dyment DA, DeLuca GC, Herrera BM, Chao MJ, Sadovnick AD, Ebers GC, Knight JC. Expression of the Multiple Sclerosis-Associated MHC Class II Allele *HLA-DRB1\*1501* Is Regulated by Vitamin D. *PLoS Genet* 2009; 5:1-6.

- 37 Willer CJ, Dymment DA, Sadovnick AD, Rothwell PM, Murray TJ, Ebers GC. Timing of birth and risk of multiple sclerosis: population based study. *BMJ* 2005; 330:120.
- 38 Hannah K. Bayes Christopher J. Weir Colin O’Leary. Timing of Birth and Risk of Multiple Sclerosis in the Scottish Population. *Eur Neurol* 2010; 63:36–40.
39. S. V. Ramagopalan, J. Link, J. K. Byrnes, D. A. Dymment, G. Giovannoni, R. Q. Hintzen, E. Sundqvist, I. Kockum, C. Smestad, B. A. Lie, H. F. Harbo, L. Padyukov, L. Alfredsson, T. Olsson, A. D. Sadovnick, J. Hillert and G. C. Ebers. HLA-DRB1 and month of birth in multiple sclerosis. *Neurology* 2009; 73; 2107-2111.
- 40 Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone* 2002; 30:771–777.
- 41 Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Sun D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu Y-Z, Plass C, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. *PNAS* July 26, 2005; vol.102 no. 30: 10604-10609.
- 42 Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006; 84:18 –28.43?
- 43 Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *American Journal of Clinical Nutrition*, Vol. 69, No. 5, 842-856, May 1999.
- 44 MET EIREANN 2009 (Meteorology Ireland).

45 McKenna MJ, Freaney R, Byrne P, McBrinn Y, Murray B, Kelly M, Donne B, O'Brien M. Safety and efficacy of increasing wintertime vitamin D and calcium intake by milk fortification. Q J Med 1995; 88:895-8.

46 Smolders J, Damoiseaux J, Menheere P, Hupperts R. Vitamin D as an immune modulator in multiple sclerosis, a review. J Neuroimmunology 2008; 194:7-17.

**Table 1: Demographic data of MS patients ascertained in each region of Ireland on 31/12/2007**

	<b>Donegal</b>	<b>Wexford</b>	<b>South Dublin</b>	<b>Overall</b>
<b>Mean age (years)</b>	<b>46.7</b>	<b>47.9</b>	<b>49.5</b>	<b>48.0</b>
<b>Range</b>	<b>(21-80)</b>	<b>(20-75)</b>	<b>(27-89)</b>	<b>(20-89)</b>
<b>F: M ratio</b>	<b>2.1:1</b>	<b>1.5:1</b>	<b>2.6:1</b>	<b>2.0:1</b>
<b>MS duration (years)</b>	<b>15.3</b>	<b>14.9</b>	<b>17.2</b>	<b>15.8</b>
<b>Range</b>	<b>(1-54)</b>	<b>(2-49)</b>	<b>(1-53)</b>	<b>(1-54)</b>
<b>Relapsing remitting %</b>	<b>53.3</b>	<b>47.0</b>	<b>53.1</b>	<b>51.1</b>
<b>Secondary progressive %</b>	<b>37.6</b>	<b>43.2</b>	<b>39.2</b>	<b>39.7</b>
<b>Primary progressive %</b>	<b>9.1</b>	<b>9.8</b>	<b>7.7</b>	<b>9.2</b>
<b>Mean age MS onset (yrs)</b>	<b>31.6</b>	<b>32.5</b>	<b>32.8</b>	<b>32.3</b>
<b>Range</b>	<b>(8-64)</b>	<b>(14-54)</b>	<b>(16-61)</b>	<b>(8-64)</b>
<b>Mean EDSS</b>	<b>3.6</b>	<b>3.9</b>	<b>3.6</b>	<b>3.7</b>
<b>Range</b>	<b>(0-9)</b>	<b>(0-9)</b>	<b>(0-9)</b>	<b>(0-9)</b>

**Legend: MS: Multiple Sclerosis, EDSS: Expanded Disability Status Scale,**

**F: Female, M: Male.**



**Table 2: Population and MS prevalence data in Donegal, Wexford and South Dublin in 2001 and 2007**

<b>Region (Latitude)</b>	<b>Census 1996*</b>	<b>MS cases 2001</b>	<b>Census 2006*</b>	<b>MS cases 2007</b>	<b>Age standardized prevalence rates: All MS cases</b>	<b>Age standardized prevalence rates: Male</b>	<b>Age standardized prevalence rates: Female</b>
<b>Donegal (54° 8' - 55°43')</b>	<b>129,994</b>	<b>240 Prevalence: 184.6/10<sup>5</sup> (95% CI: 162.0-209.5)</b>	<b>113,347</b>	<b>329 Prevalence: 290.3/10<sup>5</sup> (95% CI: 238.7-255.5)  p &lt;0.001</b>	<b>257.8/10<sup>5</sup>  253.2-262.4</b>	<b>157.6/10<sup>5</sup>  152.7-162.6</b>	<b>358.3/100,000  350.3-366.5</b>
<b>Wexford (52° 2' - 52°44')</b>	<b>104,372</b>	<b>126 Prevalence: 120.7/10<sup>5</sup> (95% CI: 100.6-143.8)</b>	<b>119,442</b>	<b>173 Prevalence: 144.8/10<sup>5</sup> (95% CI: 138.3-151.9)  p &gt;0.05</b>	<b>152.0/10<sup>5</sup>  148.4-155.6</b>	<b>119.0/10<sup>5</sup>  114.8-123.4</b>	<b>176.0/100,000  170.4-181.7</b>
<b>South Dublin CCA 1 (53° 17')</b>	<b>127,146</b>	<b>N/A</b>	<b>101,721</b>	<b>130 Prevalence: 127.8/10<sup>5</sup> (95% CI: 131.6-146.7)</b>	<b>123.2/10<sup>5</sup>  120.0-126.4</b>	<b>84.2/10<sup>5</sup>  80.6-87.9</b>	<b>160.6/100,000  155.2-166.1</b>

**Legend: \*Central Statistics Office, MS: Multiple Sclerosis, CCA 1: Community Care Area 1 (Health Service Executive)**

**Table 3: Winter serum 25(OH)D levels, winter serum PTH (parathyroid hormone) levels and HLA-DRB1\*1501 frequency in MS patients and control subjects**

	<b>MS overall</b>	<b>Controls overall</b>	<b>MS Donegal</b>	<b>MS Wexford</b>	<b>MS South Dublin</b>
<b>Mean serum 25(OH) D (nmol/L)</b>	<b>38.57</b>	<b>36.41</b>	<b>36.90</b>	<b>39.71</b>	<b>50.74</b>
<b>Range</b>	<b>13.30-161.90</b>	<b>14.30- 97.20</b>	<b>13- 97</b>	<b>15- 114</b>	<b>13- 161</b>
<b>Mean PTH (ng/L)</b>	<b>41.07</b>	<b>42.04</b>	<b>42.93</b>	<b>39.94</b>	<b>38.95</b>
<b>Range</b>	<b>14.90-321.70</b>	<b>5- 131.50</b>	<b>13- 97</b>	<b>18- 90</b>	<b>8- 39</b>
<b>% with HLA-DRB1*1501</b>	<b>62.90%</b>	<b>35.20%</b> (P<0.01)	<b>69%</b>	<b>63%</b>	<b>52%</b>

**Legend- PTH: Parathyroid Hormone, MS: Multiple Sclerosis, HLA: Human Leucocyte Antigen.**

**Table 4: Vitamin D supplement doses taken by MS patients and controls**

<b>Vitamin D3/day</b>	<b>0</b>	<b>400IU</b>	<b>800IU</b>	<b>1,200IU- 1,500IU</b>	<b>Total samples</b>
<b>MS patients (N) (%)</b>	<b>204 (62.0)</b>	<b>65 (19.8)</b>	<b>41 (12.5)</b>	<b>19 (5.7)</b>	<b>329 (100)</b>
<b>Controls (N) (%)</b>	<b>168 (74.3)</b>	<b>32 (14.2)</b>	<b>6 (2.6)</b>	<b>20 (8.9)</b>	<b>226 (100)</b>

**Legend- IU international units; % percentage**

**Of note, standard multivitamins, usually containing only 400 IU/day, are unlikely to alter serum levels of vitamin D.**

**Legend for figure 1**

Figure 1 Map of Ireland demonstrating the 3 areas studied and their latitudes: Donegal county, South Dublin and Wexford county.

Prevalence rate is the figure stated per 100,000 population. The figure in small print below the prevalence rate is the prevalence rate in 2001<sup>23</sup>. There are no previous prevalence data for the South Dublin area.

Licence for Publication:

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in JNNP and any other BMJPGGL products and sublicences such use and exploit all subsidiary rights, as set out in our licence.

Competing Interest: None declared.