Month of birth, HLA-DRB1*15 locus and risk of multiple sclerosis in offspring

Cristina Guijarro-Castro, Elena Sánchez-Zapardiel, Delicias Muñoz, Óscar Fernández, Laura Leyva, M. José Castro-Panete, Carmen Picón-Muñoz, Marta Talise, Ana Martínez-Feito, Estela Paz-Artal

Introduction. A relationship among April births, HLA-DRB1*15:01 genotype and risk of multiple sclerosis (MS) has been described. We aim to determine this association in our cohort of Spanish MS patients.

Subjects and methods. We genotyped HLA-DRB1*15:01 allele in 326 MS patients and 226 controls (non-neurological disease patients) by SSP-PCR and compared month of birth with local births during the same period.

Results. MS patients carrying HLA-DRB1*15 allele were more frequently born in December (10.3% HLA-DRB1*15+ vs. 3.8% HLA-DRB1*15–; $p = 0.019$). Controls carrying HLA-DRB1*15 allele were less frequently born in December than non-carrier controls (0% HLA-DRB1*15+ vs. 10.3% HLA-DRB1*15–; $p = 0.028$). Thus, December was confirmed as the common month of birth for HLA-DRB1*15-non-carrier controls and MS HLA-DRB1*15-carrier patients.

Conclusions. Month of birth, HLA-DRB1 genotype and risk of MS are associated. In Spain, this association was found in December, supporting the potential interaction of a seasonal risk factor in winter, inside/close to HLA-DRB1*15 locus, during pregnancy or after birth.

Key words. December. HLA-DRB1*15. Month of birth. Multiple sclerosis. Spain.

Introduction

Multiple sclerosis (MS) is an autoimmune disease with genetic susceptibility [1-3]. HLA-DRB1*15:01 genotype is a genetic marker associated with a three-fold increased risk of developing MS in Western Caucasians [4,5].

Studies investigating the risk of MS regarding month of birth have been carried out in several cohorts, but neither size, nor breed, nor statistical methods were similar [6,7]. It has been published that in the Northern Hemisphere countries there are more MS patients born in May and less often in November (contrary to what happens in Australia) and this pattern is related to maternal exposure to environmental ultraviolet (UV) radiation during the first trimester of pregnancy, adjusted for month or place of birth [6-11]. Other studies have found different months of birth associated with risk of MS [12-14].

In our previous studies, conducted in 11 Spanish hospitals and over 4,886 MS patients [15], it has been shown that Spanish MS patients are more frequently born in January and less often in February. Spending the first trimester of pregnancy during June, July and August seems to protect (patients are less frequently born in February), but this would not explain the highest number of births in January. These studies demonstrate that the month of birth could be used as an indicator for a critical prenatal period.

On the other hand, vitamin D may modify HLA-DR expression and antigen presentation. The biological effects of vitamin D depend on vitamin D receptors (VDR). These receptors modify the transcription of vitamin D-dependent genes through their binding to vitamin D-response elements (VDRE) in the promoter region of the gene. Ramagopalan et al [16] studied MS susceptibility loci to prove whether they could be regulated by vitamin D. Thereby, a single VDRE was identified within the promoter region of HLA-DRB1 gene. This VDRE was highly conserved in HLA-DRB1*15 allele, as reflected by the absence of mutations on over 600 studied chromosomes; by contrast, it was less conserved among HLA-DRB1 haplotypes non-related to MS [17]. Functional assays demonstrated that VDRE was able to modify gene expression, so HLA-DRB1*15 locus could be sensitive to vitamin D [5]. VDRE variants located in other HLA-DRB1 genotypes non-associated with MS did not respond to vitamin D. For instance, HLA-DRB1*04, *07 and *09 genes, which are not associated with MS, have a non-functioning VDRE [18].

Therefore, the following hypothesis has been established: vitamin D deficiency during childhood

Neurology Department; Hospital Xeral Cies; Vigo, Pontevedra (D. Muñoz). Neurology Department; Hospital Regional Universitario Carlos Haya; Málaga (O. Fernández, L. Leyva). Faculty of Medicine; Universidad Complutense de Madrid (C. Guijarro-Castro, C. Picón-Muñoz, E. Paz-Artal). Neurology Department (C. Guijarro-Castro); Immunology Department (E. Sánchez-Zapardiel, M.J. Castro-Panete, M. Talise, A. Martínez-Feito, E. Paz-Artal); Hospital Universitario 12 de Octubre; Madrid, Spain.

Corresponding author:
Elena Sánchez Zapardiel MD. Immunology Department. Hospital Universitario 12 de Octubre. Avda. Córdoba, s/n. E-28041 Madrid.

Fax:
+34 914 695 775

E-mail:
eszapardiel@gmail.com

Accepted:
31.05.16.

How to cite this paper:
Guijarro-Castro C, Sánchez-Zapardiel E, Muñoz D, Fernández O, Leyva L, Castro-Panete MJ, et al. Month of birth, HLA-DRB1*15 locus and risk of multiple sclerosis in offspring. Rev Neurol 2016; 63: 201-5.

Versión española disponible en www.neurologia.com

alters HLA-DRB1 gene expression in the thymus, with a loss of central tolerance and an increment of autoimmunity disorders in later stages.

HLA-DRB1*15 allele is more common within female MS patients than male MS patients, which would support the fact that, especially in women, high levels of vitamin D are associated with low frequency of MS [18]. Furthermore, MS association with HLA-DRB1*15 locus is more frequent among Caucasian population than African or Asian individuals. Apparently, the decrease in vitamin D levels within general population due to changes in habits (such as sunscreen use, obesity or decreased outdoor activity) has increased the frequency of HLA-DRB1*15 allele in MS patients.

The timing when this gene-environment interaction takes place is also crucial. MS patients carrying HLA-DRB1*15 allele were more frequently born in April (p = 0.004) compared to non-carrier patients. HLA-DRB1*15-positive MS patients were less frequently born in November, compared to HLA-DRB1*15-negative MS patients. These differences were not observed in controls or non-affected relatives [19].

This supports the theory that, during pregnancy or near post-partum, there must be an interaction between a seasonal risk factor and a locus near HLA-DRB1*15 allele.

The aim of this study is to determine whether there is an association between month of birth and the presence of HLA-DRB1*15:01 allele in our cohort of 326 MS patients.

Subjects and methods

This is an observational study performed at University Hospital 12 de Octubre (Madrid) and Regional University Hospital Carlos Haya (Málaga). Both hospitals serve a population of approximately 750,000 each and have a Multiple Sclerosis Units with 25 years of existence.

We randomly collected 100 blood samples from MS patients at University Hospital 12 de Octubre and 226 blood samples from MS patients at Regional University Hospital Carlos Haya, in order of arrival to the Multiple Sclerosis Office. Control group was obtained from a database of 217 transplant recipients elaborated by the Department of Immunology at University Hospital 12 de Octubre. This study was approved by both Hospital Ethics Committees. Patients were informed and they signed the corresponding Informed-Consent Forms (ICF).

Inclusion criteria: patients' signature on ICF, adult relapsing-remitting and secondary progressive MS patients diagnosed on the basis of the criteria set up by Polman [20], and not meeting any exclusion criteria

Exclusion criteria: inability to perform any of the sections of the survey, and not meeting any inclusion criteria

Demographic variables, type of disease (relapsing-remitting and secondary progressive MS), patient's month of birth, onset age and level in the Expanded Disability Status Scale (EDSS) at illness onset (out of relapses) data were recorded.

In order to obtain MS patient and control births, we compared month of birth in our sample with local births during the same period (National Statistics Institute, INE) [21].

HLA-DRB1*15 genotyping

326 MS patients and 217 non-neurological disease patients were genotyped for HLA-DRB1 locus by Dot Blot Reverse (INNO-LiPA, Innogenetics), following the manufacturer's instructions. Specific patterns for each allele were read by scanners and interpreted by the software (LIRAS ® for LiPA HLA v6.00). To determine the subtype of HLA-DRB1*15 alleles, we used Micro SSP ® Allele Specific HLA Class II Typing Tray – DRB1*15 (One Lambda, Inc).

Statistical analysis

We performed statistical association tests using laboratory (HLA-DRB1 genotypes) and clinical variables (gender, age and month of birth, basal EDSS) obtained in basic studies and trials, respectively. We compared basal EDSS score between patients with at least one HLA-DRB1*15 allele and patients who did not carry this allele, analyzing their respective disease evolution. The sample size calculation was not performed.

Qualitative variables were described as frequencies and they were compared using Pearson's chi-squared test. Quantitative variables were described as mean, standard deviation and confidence intervals 95%, and they were compared with Student's t-test. When results did not follow a normal distribution, they were compared with non-parametric Mann-Whitney test, and they were described as median and percentiles from 25 to 75. We compared more than 2 groups of quantitative variables with analysis of variance (ANOVA) or Kruskal-Wallis test.

We studied the association between the month of birth and the frequency of HLA-DRB1*15 by

comparing allele frequencies in each month with allele frequencies in all the other months, using a χ^2 test of independence 2 × 2.

Results

We genotyped 326 MS patients and 217 non-neurological disease patients (transplanted patients) matched for age and sex. From our cohort of 326 MS patients, 248 (73%) individuals were female and 78 (27%) were males (mean age: 35 ± 0.5 years; range: 18-66). Mean disease duration was 9.9 ± 0.3 years (range: 0.6-19) and mean EDSS score was 2.3 ± 0.6 (range: 0-5.5). We found 56 different genotypes in MS patients. HLA-DRB1*15 allele was present in 116 MS patients (HLA-DRB1*15+), whereas 210 patients were non-carriers (HLA-DRB1*15–). The HLA-DR15 allele was more frequent in patients (35%) than in controls (17%) ($p < 0.001$).

The most frequent genotypes among MS patients (Table I) were DR7/DR15, DR1/DR3 and DR15/– ($p = 0.0068$, $p = 0.0228$ and $p = 0.0539$) and in controls DR11/– and DR11/DR13 ($p = 0.0153$ and $p = 0.0255$).

Unlike previous studies, DR15+ patients had a lower EDSS (2.3) than non-carriers (2.6). Furthermore, DR15+ patients spent a longer period without disabilities (15 ± 9 years vs. 12 ± 9 years; $p = 0.0043$). No differences in gender or age of onset were found, unlike a previous study in which the authors reported more MS female patients carrying HLA-DRB1*15:01 [22].

We found that 90% of MS patients in Madrid were born between 1948 and 1984 and 90% MS patients in Málaga were born between 1946 and 1982. In our previous study [15], we compared the month of birth of general population with the month of birth of 4,886 MS patients and found that MS patients had a statistically significant predisposition to be born in January, according to the data from INE.

There were no differences in the distribution of the month of birth between MS and control samples, compared with the frequency of births in the general population.

We showed that MS patients carrying HLA-DRB1*15 allele were more frequently born in December than non-carrier patients (10.3% HLA-DRB1*15+ vs. 3.8% HLA-DRB1*15–; $p = 0.019$; Table II). On the other hand, healthy controls carrying HLA-DRB1*15 allele were less frequently born in December than non-carrier controls (0% HLA-DRB1*15+ vs. 10.3% HLA-DRB1*15–; $p = 0.028$; Table I). Generally, we found that within HLA-DRB1*15-carrier group, December was the most frequent month of birth among MS patients, compared to healthy controls (10.3% MS patients vs. 0% healthy controls; $p = 0.036$; Table II).

Table I. Frequency distribution of genotypes showing significant differences between MS patients ($n = 326$) and non-MS patients ($n = 350$).

	MS patients	Controls	*p*
Genotype *01*15	4%	1%	0.0228
Genotype *03*–	5%	2%	0.0539
Genotype *07*15	8%	3%	0.0068
Genotype *11*–	0.3%	3%	0.0153
Genotype *11*13	1%	4%	0.0255

Discussion

In our study, we demonstrate that month of birth, HLA-DRB1*15 genotype and risk of MS are associated. In Spain, this association has been found in December, whereas in Northern European countries it has been described in April. Furthermore, this result cannot be only explained by maternal vitamin D deficiency during the first trimester of pregnancy, as described in Northern European countries for patients born in April. The highest number of births in December among Spanish MS patients carrying HLA-DRB1*15 allele compared to non-carriers and controls would mean the interaction of a seasonal factor in winter, not only sun radiation.

The main limitation in our study is the number of MS patients born in each month, which may be responsible for the lack of observed differences in some months, although our previous study [15] showed that Spanish MS patients are more frequently born in January (winter). To our knowledge, this is the only study in Mediterranean countries reporting this association.

Epidemiological studies describe the importance of an adequate supply of vitamin D in early stages. In Spain, the highest number of births in December among patients with HLA-DRB1*15 compared to non-carriers and non-neurological disease controls would not mean less solar radiation during April, May and June. This could be explained if there were an interaction between a seasonal risk factor in

Table II. Number and percentage of births along months, depending on the presence of HLA-DRB1*15 allele.

	Patients			Controls			HLA-DRB15+		
	HLA-DRB15+	HLA-DRB15–	*p*	HLA-DRB15+	HLA-DRB15–	*p*	Patients	Controls	*p*
January	7 (6.6%)	17 (8.1%)	0.4952	4 (10.3%)	12 (6.7%)	0.4468	7 (6.6%)	4 (10.3%)	0.3744
February	8 (6.9%)	14 (6.7%)	0.9369	5 (12.8%)	21 (11.8%)	0.8586	8 (6.9%)	5 (12.8%)	0.2483
March	4 (3.4%)	18 (8.6%)	0.0775	3 (7.7%)	13 (7.3%)	0.9329	4 (3.4%)	3 (7.7%)	0.2695
April	12 (10.3%)	22 (10.5%)	0.9704	3 (7.7%)	13 (7.3%)	0.9329	12 (10.3%)	3 (7.7%)	0.6279
May	10 (8.6%)	18 (8.6%)	0.9879	1 (2.6%)	13 (7.3%)	0.2752	10 (8.6%)	1 (2.6%)	0.2026
June	9 (7.8%)	19 (9.0%)	0.6909	5 (12.8%)	16 (9.0%)	0.4635	9 (7.8%)	5 (12.8%)	0.3401
July	5 (4.3%)	16 (7.6%)	0.2440	3 (7.7%)	10 (5.6%)	0.6210	5 (4.3%)	3 (7.7%)	0.4089
August	12 (10.3%)	13 (6.2%)	0.1772	3 (7.7%)	16 (9.0%)	0.7953	12 (10.3%)	3 (7.7%)	0.6279
September	11 (9.5%)	21 (10.0%)	0.8805	3 (7.7%)	15 (8.4%)	0.8802	11 (9.5%)	3 (7.7%)	0.7358
October	13 (11.2%)	21 (10.0%)	0.7328	5 (12.8%)	21 (11.8%)	0.8586	13 (11.2%)	5 (12.8%)	0.7855
November	13 (11.2%)	23 (11.0%)	0.9440	4 (10.3%)	8 (4.5%)	0.1539	13 (11.2%)	4 (10.3%)	0.8695
December	12 (10.3%)	8 (3.8%)	0.0186	0	20 (11.2%)	0.0280	12 (10.3%)	0	0.0365

winter inside the HLA-DRB1*15 locus or close to it, during pregnancy or after birth.

Vitamin D deficiency and Epstein-Barr virus (EBV) may interact to increase risk of MS. A high proportion of genes related to EBNA-3 viral protein are regulated by vitamin D, so EBV enhances vitamin D deficiency by blocking the effects of vitamin D [23]. Statistical association between the prevalence of infectious mononucleosis and UV radiation could explain 72% of prevalence variations in England [24]. The association between MS and EBV is greater when patients undergo primary infection as adults. Recently [25], Wergeland et al reported that EBNA-1 IgG serum levels are affected by genetic and environmental factors, increasing risk of MS. Although the exact mechanism of action of vitamin D is still unknown, it is recommended to prevent its deficit. It is also suggested to avoid smoking and to facilitate EBV infection during childhood [26].

In our study, we demonstrate that month of birth, HLA-DRB1*15 genotype and risk of MS are associated. In Spain, this association has been found in December, whereas in Northern European countries it has been described in April. Furthermore, it cannot be explained only by maternal vitamin D deficiency during the first trimester of pregnancy, as occurs in patients born in April in Northern European countries. The highest number of births in December in Spanish MS patients carrying HLA-DRB1*15 allele would mean the interaction of a seasonal factor in winter, not only the sun radiation.

We concluded that the interaction, during pregnancy or after birth, of a seasonal risk factor in winter, inside HLA-DRB1*15 gene or close to it, may influence the development of MS in HLA-DRB1*15 carrier patients. Epidemiological studies recommend the prevention of vitamin D deficiency in MS patients.

References

1. Ascherio A, Munger KL, Simon KC. Vitamin D and multiple sclerosis. Lancet Neurol 2010; 9: 599-612.
2. Simon KC, Munger KL, Xing Y, Ascherio A. Polymorphisms in vitamin D metabolism related genes and risk of multiple sclerosis. Mult Scler 2010; 16: 133-8.
3. Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene). Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. Nat Genet 2009; 41: 824-8.
4. Torkildsen O, Knappskog PM, Nyland HI, Myhr KM. Vitamin D-dependent rickets as a possible risk factor for multiple sclerosis. Arch Neurol 2008; 65: 809-11.

5. Handunnetthi L, Ramagopalan SV, Ebers GC. Multiple sclerosis, vitamin D and HLA-DRB1*15. Neurology 2010; 74: 1905-10.
6. Willer CJ, Dyment DA, Sadovnick AD, Rothwell PM, Murray TJ, Ebers CG. Timing of birth and risk of multiple sclerosis: population based study. BMJ 2005; 530: 120-5.
7. Staples J, Ponsonby AL, Lynette L. Low maternal exposure to ultraviolet radiation in pregnancy, month of birth, and risk of multiple sclerosis in offspring: longitudinal analysis. BMJ 2010; 340: 1640-50.
8. Torrey EF, Miller J, Rawlings R, Yolken RH .Seasonal birth patterns of neurological disorders. Neuroepidemiology 2000; 19: 177-85.
9. Rothwell PM, Charlton D. High incidence and prevalence of multiple sclerosis in south east Scotland: evidence of a genetic predisposition. J Neurol Neurosurg Psychiatry 1998; 64: 730-5.
10. Torrey EF, Miller J, Rawlings R, Yolken RH. Seasonality of births in schizophrenia and bipolar disorder: a review of the literature. Schizophr Res 1997; 28: 1-38.
11. Kurtzke JF. Some contributions of the Department of Veterans Affairs to the epidemiology of multiple sclerosis. Mult Scler 2008; 14: 1007-12
12. Vukusic S, Van Bockstael V, Gosselin S, Confavreux C. Regional variations in the prevalence of multiple sclerosis in French farmers. J Neurol Neurosurg Psychiatry 2007; 78: 707-9.
13. Taylor BV, Richardson A, Mason DF, Willoughby A, Abenthy D, Sabel C. Prevalence of multiple sclerosis in New Zealand. Mult Scler 2008; 14 (Suppl): S202-10.
14. Salter AR, Cofield SS, Vollmer T, Cutter GR. Timing of birth in United States-Born MS population. Mult Scler 2010; 16 (Suppl): S210-1.
15. Guijarro-Castro C, Muñoz-García D, Bonaventura-Ibars I, Miralles-Martínez A, Aladro-Benito Y, Martínez-Ginés ML, et al. Month of birth in multiple sclerosis in Spain. Mult Scler 2011; 17: 360.
16. Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton SM, Dyment DA, et al. Expression of the multiple sclerosis-associated MHC class II allele HLA-DBR1*1501 is regulated by vitamin D. PLoS Genet 2009; 5: e1000369.
17. Kragt J, Van Amerongen B, Killestein J, Dijkstra C, Uitdehaag B, Polman CH, et al. Higher levels of 25-hydroxyvitamin D are associated with a lower incidence of multiple sclerosis only in women. Mult Scler 2009; 15: 9-15.
18. Yetley EA. Assessing the vitamin D status of the US population. Am J Clin Nutr 2008; 88: S558-64.
19 Ramagopalan, SV, Link J, Byrnes JK, Dyment DA, Giovannoni G, Hintzen RQ, et al. HLA-DRB1 and month of birth in multiple sclerosis. Neurology 2009; 73: 2107-11.
20. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kapposi L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald criteria.' Ann Neurol 2005; 58: 840-6.
21. Instituto Nacional de Estadística. Censo de nacimientos. URL: http://www.ine.es/jaxi/menu.do?type=pcaxis&path=/t20/e304/&file=inebase. [03.02.2012].
22. Irizar H, Muñoz-Culla M, Zuriarrain O, Goyenechea E, Castillo-Triviño T, Prada A, et al. HLA-DRB1*15: 01 and multiple sclerosis: a female association? Mult Scler 2012; 18: 569-77.
23. Disanto G, Meir U, Giovannoni G, Ramagopalan SV. Vitamin D: a link between Epstein-Barr virus and multiple sclerosis development? Expert Rev Neurother 2011; 11: 1221-4
24. Ramagopalan SV, Handel AE, Giovannoni G, Rutherford Siegel S, Ebers GC, Chaplin G. Relationship of UV exposure to prevalence of multiple sclerosis in England. Neurology 2011; 76: 1410-4.
25. Wergeland S, Myhr KM, Løken-Amsrud KI, Beiske AG, Bjerve KS, Hovdal H, et al. Vitamin D, HLA-DRB1 and Epstein-Barr virus antibody levels in a prospective cohort of multiple sclerosis patients. Eur J Neurol 2016; 23: 1064-70.
26. Simon KC, Munger KL, Ascherio A. XVI European Charcot Foundation lecture: nutrition and environment, can MS be prevented? J Neurol Sci 2011; 311: 1-8.

Mes de nacimiento, HLA-DRB1 y riesgo de esclerosis múltiple en la descendencia

Introducción. El haplotipo HLA-DRB1*1501 es el marcador genético que se ha asociado con un riesgo tres veces mayor de padecer esclerosis múltiple (EM) en caucásicos occidentales. Recientemente se ha sabido que hay una asociación entre el mes de nacimiento en abril, el genotipo HLA-DRB1 y el riesgo de EM en países del norte de Europa y Canadá. Esto apoya la teoría de que debe haber una interacción entre un factor de riesgo estacional con un *locus* cercano al HLA-DRB1*15 durante la gestación o cerca del posparto.

Sujetos y métodos. Se realizó el genotipado de la presencia y subtipo de HLA-DRB1*1501 en 326 pacientes de dos centros españoles y en 226 controles sin patología neurológica. Se compararon los meses de nacimiento de la muestra de pacientes con los nacimientos mensuales locales en los mismos períodos.

Resultados. Comparados los pacientes con EM que eran portadores del alelo HLA-DRB1*15 (10,3%) frente a los pacientes no portadores (3,8%), significativamente más pacientes nacían en diciembre (p = 0,0185). También se confirmaba el mismo mes de nacimiento de diciembre entre sanos portadores frente a no portadores de HLA-DRB1*15 y entre pacientes portadores de HLA-DRB1*15 frente a sanos.

Conclusiones. El mes de nacimiento, el genotipo HLA-DRB1*15 y el riesgo de presentar EM están asociados. A diferencia de los resultados obtenidos en países del norte de Europa, donde esta asociación se ha encontrado en el mes de abril, en España es en diciembre. Se demuestra la interacción de un factor de riesgo estacional en invierno en el *locus* HLA-DRB1*15 o cercano a éste durante la gestación o tras el nacimiento.

Palabras clave. Diciembre. Esclerosis múltiple. España. HLA-DRB1*15. Mes de nacimiento.