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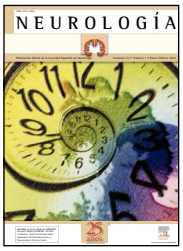

ORIGINAL ARTICLE

Influence of APOE gene polymorphisms on interferon-beta treatment response in multiple sclerosis☆

A.L. Guerrero,* M.A. Tejero, F. Gutiérrez, J. Martín-Polo, F. Iglesias, E. Laherran, J.I. Martín-Serradilla, S. Merino

Sección de Neurología, Complejo Asistencial de Palencia, Palencia, Spain

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Abstract
Objective: Clinical trials with interferon beta in relapsing remitting multiple sclerosis (RRMS) have demonstrated a reduction in the relapse rate. Nevertheless, not all patients respond to this treatment, although there is no consensus regarding the definition of response to therapy. The reasons for this failure are not known but genetic factors probably influence this, as has been previously shown with Interleukin 10 or Interferon gamma polymorphisms.

The role of apolipoprotein E (APOE) gene in MS has been investigated and does not appear to increase risk for MS or influence disease severity. Interestingly APOE variation influences response to cholinesterase inhibitor treatment in Alzheimer disease or to statins in hypercholesterolemia. This might have future implications for MS.

Material and methods: We retrospectively reviewed 38 RRMS patients (32 females and 6 males) treated with interferon beta (INFbeta) over at least two years. Criteria for treatment were uniform accordingly to an "Advisory Committee for the Treatment of Multiple Sclerosis". We collected data variables including age, age of onset, clinical type or disease duration. Patients were classified, two years after the start of treatment, as responders and non-responders based upon clinical criteria available in the literature, which rely on the presence of relapses, increase of disability, or both. APOE genotype was determined from blood samples using validated polymerase chain reaction methods. Correlation between patient responding status with allele E2 or E4 was tested.

Results: A total of 20 patients (52.6%) received subcutaneous INFbeta1b (Betaferon®), 13 (34.2%) INFbeta1a intramuscular (Avonex®), and 5 (13.2%) subcutaneous INFbeta1a (Rebif®). We found 2 patients (5.2%) heterozygous for the E2 allele and 9 (23.7%) for the E4 allele. No patient was homozygous for E2 or E4. Comparison of patients with and

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*Corresponding author.
E-mail: gueneurol@gmail.com (A.L. Guerrero).

without E2 or E4 allele showed no significant differences in any of the ten therapy response variables assessed.
Conclusion: Findings of a recent meta-analysis have not supported a role for APOE in MS susceptibility or severity. We have not found, in our data, any influence of this gene in the RRMS response to INFbeta. However, larger series would be required to validate these results.

PALABRAS CLAVE
Esclerosis múltiple;
Respuesta al tratamiento;
Farmacogenética;
APOE

Influencia del polimorfismo del gen de la APOE en la respuesta al tratamiento con interferón beta en esclerosis múltiple

Resumen
Objetivos: Los ensayos clínicos llevados a cabo con el interferón beta (INFB) en esclerosis múltiple remitente recidivante (EMRR) han mostrado que reducen la tasa de brotes. Sin embargo, no todos los pacientes responden a este tratamiento, si bien aún no hay un absoluto consenso a propósito de la definición de respuesta al tratamiento. Las razones para este fracaso terapéutico no son conocidas, y probablemente hay factores genéticos implicados, como se ha mostrado con los polimorfismos de los genes que codifican la interleuquina 10 o el interferón gamma. El papel del gen de la apolipoproteína E (APOE) en la EM ha sido investigado en los últimos años y no parece aumentar el riesgo de aparición de la enfermedad ni influir en su severidad. Variaciones en este gen influyen en la respuesta al tratamiento con inhibidores de la colinesterasa en la enfermedad de Alzheimer o a las estatinas en la hipercolesterolemia. Esto podría tener implicaciones futuras en la EM.
Material y métodos: Hemos revisado retrospectivamente 38 pacientes diagnosticados de EMRR (32 mujeres y 6 varones) tratados con INFB durante al menos dos años. Los criterios para llevar a cabo el tratamiento eran uniformes de acuerdo con las indicaciones del Comité asesor para el tratamiento de la EM. Recogimos datos acerca de la edad y tiempo de evolución de la enfermedad. Al cabo de dos años del inicio del tratamiento los pacientes fueron clasificados como respondedores o no-respondedores de acuerdo con los criterios clínicos disponibles, basados en la presencia de brotes, evolución de la discapacidad, o ambos. El genotipo APOE se determinó de muestras sanguíneas utilizando métodos validados de reacción en cadena de la polimerasa. Se estudió la correlación entre la condición de respondedor o no respondedor y la presencia de los alelos E2 o E4.
Resultados: Veinte pacientes (52,6%) recibían INFB1b subcutáneo (Betaferón®), 13 (34,2%) INFB1a intramuscular (Avonex®) y 5 (13,2%) INFB1a subcutáneo (Rebif®). Dos pacientes (5,2%) eran heterocigotos para el alelo E2 y 9 (23,7%) para el alelo E4. Ningún paciente era homocigoto para E2 o E4. La presencia o no de estos alelos no se correlacionó con la respuesta al tratamiento de acuerdo con las 10 variables estudiadas.
Conclusión: Tras los resultados de un metanálisis que no muestran influencia del gen APOE en la susceptibilidad o severidad de la EM, no hemos encontrado en nuestra serie influencia de este gen en la respuesta de pacientes con EMRR al INFB. En cualquier caso, series más extensas son necesarias para validar estos resultados.

Introduction

Interferon-beta (INFB) is one of the treatments of choice in remitting-relapsing multiple sclerosis (RRMS). Clinical trials have shown that the frequency of relapses falls compared to placebo by approximately 30%. In addition, it has been put forward that these drugs may delay the progression of disability. Despite these known benefits, there is a significant percentage of patients who do not respond to treatment or who respond sub-optimally.[1-3]

Nonetheless, it is difficult to establish whether or not a specific patient is responding to treatment and to what degree, and a long time may be needed to obtain evidence of this response; in fact, we still do not have a clear, universally-accepted definition of the absence of response to INFB in RRMS.[4,5] Genetic heterogeneity or certain

environmental factors give rise to variations in the clinical patterns of MS and probably contribute to the differences in the response to treatment among different patients. However, no conclusive advances have been achieved.[6]

A large number of studies have investigated the role of the APOE gene in MS. According to a recent meta-analysis, polymorphisms of the APOE gene do not influence the susceptibility to suffer from MS, its clinical course or its severity.[7] An effect of the APOE genotype has been described in the response to treatment with cholinesterase inhibitors in Alzheimer's disease or to statins in hypercholesterolaemia.[8] This has encouraged us to explore whether or not the presence of E2 or E4 alleles of the APOE gene may influence the response to treatment with INFB in a homogeneous population of patients with RRMS.

Patients and methods

We have retrospectively reviewed 38 patients (32 females and 6 males) monitored for at least 2 years at our Neurology Department as a consequence of RRMS treated with INFB.

Treatment was started following the criteria set out by the Advisory Committee of the Spanish Ministry of Health and Consumer Affairs, which finally approved the use of these drugs; in this way treatment began with any of the three forms of INFB (INFB 1a [Avonex®] 30 mcg intramuscularly once a week, INFB 1a [Rebif®] 22 or 44 mcg subcutaneously 3 times a week, or INFB 1b [Betaferon®] 8 MUI subcutaneously every 48 hours) in patients with a diagnosis of RRMS and an evolution of at least 6 months, over 18 years of age, scoring from 0 to 6.5 on the EDSS scale and with at least two acute relapses of the disease confirmed by a neurologist during the preceding three years. A relapse is defined as "the appearance or re-appearance of one or more symptoms attributable to MS, lasting a minimum of 24 hours, with objective involvement on neurological examination, in the absence of fever, and preceded by neurological stability for at least 30 days".

Data were collected about each patient's gender and the age at onset of the disease. We considered the score on the EDSS scale at the start of the treatment, as well as the frequency of relapses in the two preceding years. During the first two years of treatment, we assessed the evolution of the EDSS scale score, as well as the frequency of relapses. Whenever progression in the disability was determined, it had to be confirmed six months later. In 15 patients, 6 years of follow-up have been completed and we have considered the EDSS scale score at the end of this period.

We defined response to treatment after two years in each of the patients in accordance with 10 response criteria,[4] considering patients to be non-responders in each case if they met any of the following criteria:

1. Criterion A: increase of at least 1 step in the EDSS score.
2. Criterion B: one relapse in 2 years.
3. Criterion C: two or more relapses in 2 years.
4. Criterion D: reduction in the frequency of relapses less than 30% compared to the 2 years prior to treatment.
5. Criterion E: reduction in the frequency of relapses less than 50% compared to the 2 years prior to treatment.
6. Criterion F: absence of any drop in the frequency of relapses compared to the 2 years prior to treatment.
7. Criterion G: presence of criterion A or criterion B.
8. Criterion H: presence of criterion A or criterion E.
9. Criterion I: presence of criteria A and B.
10. Criterion J: presence of criteria A and E.

Table 1 Clinical and demographic characteristics of the 38 patients with relapsing remitting multiple sclerosis

	Mean (median[a]) ± SD	Range
Age at onset of MS (years)	30.2±8.9	16-45
Age at start of treatment (years)	36.9±9.9	18-56
Duration of the illness at start of treatment (years)	11.6±6.4	3-29
EDSS at start of treatment	3.0±1.3	1-5.0
EDSS after 2 years	3.4±1.5	1-6.5
EDSS after 6 years (n: 15)	3.8±1.6	2-7
Frequency of relapses at start of treatment	1.2±0.3	1-2
Number of relapses after 2 years	1.1±1.7	0-10
Frequency of relapses after 2 years	0.5±0.9	0-5

[a]Median for EDSS score. SD: standard deviation.

An increase of 1.5 in the score on the EDSS scale was considered to be one step in the increase of disability if the initial value was 0; an increase of 1.0 was considered to be one step if the initial EDSS score was between 1.0 and 5.0; and only 0.5 was considered to be one step if the initial EDSS score was greater than 5.0.[4]

The APOE genotype was determined in blood samples using validated polymerase chain reaction methods. The influence of the presence of the E2 or E4 alleles was explored in the response to treatment with INFB according to the above criteria, as well as the existence of a score of 6.0 or higher 6 years after the start of treatment in patients who had achieved that follow-up time. The statistical study was carried out using the Chi squared test to study categorical variables and Student's "t" test for quantitative variables. A value of p less than 0.05 was considered to be statistically significant. The investigators were blinded to the data on the polymorphism of the APOE gene when establishing the clinical variables.

Table 2 Distribution of APOE genotypes in our population

APOE	N	%
E3/E3	28	73.7
E4/E3	8	21.1
E4/E2	1	2.6
E3/E2	1	2.6

Table 3 Status as responders or non-responders according to the different definitions and the E4 allele

Definition	Non-responders		Responders	
A	7 (18.4%)	E4: 1	31 (81.6%)	E4: 8
		No E4: 6		No E4: 23
B	23 (60.5%)	E4: 6	15 (39.5%)	E4: 3
		No E4: 17		No E4: 12
C	9 (23.7%)	E4: 1	29 (76.3%)	E4: 8
		No E4: 8		No E4: 21
D	5 (13.2%)	E4: 1	33 (86.8%)	E4: 8
		No E4: 4		No E4: 25
E	7 (18.4%)	E4: 1	31 (81.6%)	E4: 8
		No E4: 6		No E4: 23
F	5 (13.2%)	E4: 1	33 (86.8%)	E4: 8
		No E4: 4		No E4: 25
G	24 (63.2%)	E4: 6	14 (36.8%)	E4: 3
		No E4: 18		No E4: 11
H	11 (28.9%)	E4: 1	27 (71.1%)	E4: 8
		No E4: 10		No E4: 19
I	6 (15.8%)	E4: 1	32 (84.2%)	E4: 8
		No E4: 5		No E4: 24
J	2 (5.3%)	E4: 1	36 (94.7%)	E4: 8
		No E4: 1		No E4: 28
EDSS after 6 years (n: 15)	7 (46.7%)	E4: 1	8 (53.3%)	E4: 2
		No E4: 6		No E4: 6

Results

We included 38 patients treated with INFB; 20 (52.6%) received Betaferon®, 13 (34.2%) Avonex® and 5 (13.2%) Rebif® (four of them 44 mcg and one 22 mcg).

The baseline and treatment-related characteristics of these patients are shown in table 1.

Table 2 shows the distribution of the APOE genotypes. No patient was homozygotic for E2 or E4.

The clinical evolution of patients depending on their status as responders or non-responders is given in table 3. It shows how the fact they present allele E4 was not significantly correlated to the patient's status as responder or non-responder according to the 11 criteria used: the 10 after two years combining reduction in the number of relapses and disability progression, plus disability progression after 6 years in the 15 patients for whom this information was available.

Discussion

There is no agreed definition for the response to treatment with INFB in RRMS. Having available a way to classify individual patients adequately according to their response to treatment would make it easier to take rational therapeutic decisions, enable the design of future clinical trials and allow the performance of studies correlating biological markers, such as for instance genetic polymorphisms, to therapeutic response.[1,5]

To begin with, there is no agreement about the follow-up time needed to asses therapeutic response, as it varies between 1 and 6 years in the studies published to date. Clinically, when it comes to evaluating the response to INFB, it is possible to consider the reduction in the frequency of relapses, the absence of disability progression, or the absence of conversion to the secondary progressive phase of the illness. The number of relapses and disability progression are the two basic clinical phenomena in MS, and both can be taken into account when determining response to immunomodulation treatment. A reduction in the frequency of relapses is the main goal of most clinical trials done with INFB in recent years; however, sensorial relapses or other relapses with complete recovery can be considered qualitatively different from motor or polyregional relapses. In addition, the frequency of relapses may be affected by regression to the mean.[9] Thus, on this point consideration could be given to the functional severity of acute symptoms or residual disability to take therapeutic response into account.[10]

On the other hand, disability progression may be taken into account as a marker for response to treatment. This strategy has a less solid basis in terms of the results of

clinical trials, but is more realistic insofar as disability is, at the end of the day, what influences the patient's performance of everyday activities and its consideration seems to be of particular interest when evaluating the long-term situation.[3,11-3] There is no agreement about the observation time needed to confirm disability progression, or about how to consider other factors that may have an influence such as the presence of depression, fatigue, spasticity or other intercurrent diseases.[10,14] In the same way, it is also important to define a criterion for disability progression that depends on the baseline score on the EDSS scale; in our analysis we have used those proposed by Río et al.[4] Thus, one disability step would be an increase of 1.5 in the EDSS score if the EDSS was initially 0, an increase of 1.0 if the initial EDSS score was between 1.0 and 5.0, or only 0.5 if the EDSS score was greater than 5.0. According to these authors, the treatment response criteria with INFB using disability progression are clinically more relevant than those based only on the frequency of relapses and show a high positive predictive value for significant disability after 6 years from the start of treatment. In this way, two thirds of patients with a score of 6.0 or more after 6 under treatment would have been classified as responders after two years on the basis solely of the number of relapses, and only 3% of patients with increased disability after 2 years presented an EDSS score of less than 6.0 after 6 years.[4,15]

On the basis of all the above, and in view of the difficulty for agreeing a homogeneous model defining treatment response,[16,17] we have decided to apply in the analysis of our series the mixed approach proposed by Río et al.[4] One way of simplifying the reading of our results is to opt for using the treatment response criterion followed in the recent pharmacogenetic study into MS published by Comabella et al,[18] namely the absence of relapses and disability progression, which would correspond to criterion I in our analysis.

We appreciate that the interest of our study would have increased if we had assessed the response patterns using nuclear magnetic resonance (NMR).[19] The retrospective design and the heterogeneity in the number of studies and apparatus used to perform the NMR have not allowed us to conduct this analysis.

As mentioned above, a significant number of patients did not show the expected response to immunomodulation treatment. The causes of this occasional lack of response are not completely understood, but we can envisage that both environmental and genetic factors might contribute to the refractory nature of some patients under treatment with INFB.[10,20] Various clinical and demographic variables such as the duration of the illness, the age at start of treatment, the baseline scores on the EDSS scale or the frequency of relapses prior to the start of treatment may have an impact on this response.[12] Several biological markers have been put forward as possible predictors for response to treatment, such as the expression of the TNF-related apoptosis inducing ligand (TRAIL) or the serum levels of INFgamma, or receptor 1 of soluble tumour necrosis factor (sTNF-R1).[5,12,21-23] The possible influence of polymorphisms on the genes in the promoter of IL-10, INFgamma, glypican 5, sub-unit 1 of the INF receptor *(IFNAR),* or the alleles of the HLA gene on response to treatment with INFB in patients with MS has also been suggested,[13,16,24-29] although this influence, if it exists, will probably be polygenic, with an influence on other systems not only implicated in the immune response.[18]

APOE, encoded in chromosome 19q13 is a ligand for lipid transport, initially recognized as a major determinant in the metabolism of lipoproteins and cardiovascular disease. It has been posited that APOE-dependent lipoprotein uptake may play an important role in the development, maintenance and response to harm in the central nervous system. APOE is expressed in humans as 3 isoforms encoded by alleles E2, E3 and E4. Allele E4 is a risk factor for the age of onset and development of Alzheimer's disease; its influence has also been studied on other neurological conditions such as Parkinson's disease or amyotrophic lateral sclerosis, or its relationship with recovery following ictus or trauma-related damage to the central nervous system.[7]

A large number of studies, some of them very recent,[30-32] have investigated the role of the APOE gene in MS. Thus, it has been suggested that carriers of the E4 allele present a more severe form of the illness, while those with allele E2 present a milder course; some authors have limited this latter influence to only females. However, a meta-analysis carried out on 4,048 patients with MS has not shown any influence by APOE gene polymorphism on disease severity.[7] The results from our group have revealed that the absence of any influence by the APOE genotype on MS severity is also notable when considering the Multiple Sclerosis Severity Score (MSSS),[33] probably the best way to determine severity with a single disability metric.[34]

Genetic factors with an influence on certain stages of the illness's development may play a major role in the different response by patients to certain treatments; thus, pharmacogenetic studies have established that the APOE E4 allele is a predictor for a poor response to treatment with cholinesterase inhibitors in Alzheimer's disease.[26] Furthermore, the polymorphism of the APOE gene may influence the response to statins, which may also have an impact on MS due to the immunomodulation profile shown by these drugs.[35,36] For example, it has been shown that the APOE genotype is a significant predictor for a drop in LDL cholesterol or triglycerides when treating hyperlipidaemic males with atorvastatin.[8]

Conclusion

The results of a recent meta-analysis do not support the possible influence of the APOE gene on susceptibility to MS or its severity.

Although the number of patients in our study is too small to obtain statistical significance, we have not detected any influence by this gene on the response to treatment with INFB in patients with RRMS. In any case, studies with a larger number of patients will have to be carried out.

Conflict of interest

The authors have declared that there is no conflict of interest.

References

1. Fernández Fernández O, Fernández Sánchez VE, Mayorga C, Guerrero Fernández M, León A, Tamayo Toledo JA, et al. Respuesta al interferón beta en la esclerosis múltiple asociada con la discapacidad previa al tratamiento. Rev Neurol. 2006;43:322-9.
2. Tomassini V, Paolillo A, Russo P, Giugni E, Prosperini L, Gasperini C, et al. Predictors of long-term clinical response to interferon beta therapy in relapsing multiple sclerosis. J Neurol. 2006;253:287-93.
3. Caon C. Maximising therapeutic outcomes in patients failing on current therapy. J Neurol Sci. 2009;277:533-6.
4. Río J, Nos C, Tintoré M, Téllez N, Galán I, Pelayo R, et al. Defining the response to interferon-beta in relapsing-remitting multiple sclerosis Patients. Ann Neurol. 2006;59:344-52.
5. Rudick RA, Lee J-C, Simon J, Ransohoff RM, Fisher E. Defining interferon beta response status in multiple sclerosis patients. Ann Neurol. 2004;56:548-55.
6. Stürzebecher S, Wandinger KP, Rosenwald A, Sathyamoorthy M, Tzou A, Mattar P, et al. Expression profiling identifies responder and non-responder phenotypes to interferon-beta in multiple sclerosis. Brain. 2003;126:1419-29.
7. Burwick RM, Ramsay PP, Haines Jl , Hauser SI , Oksenberg JR, Pericak-Vance MA, et al. APOE epsilon variation in multiple sclerosis susceptibility and disease severity. Some answers. Neurology. 2006;66:1373-83.
8. Pedro-Botet J, Schaefer EJ, Bakker-Arkema RG, Black DM, Stein EM, Corella D, et al. Apolipoprotein E genotype affects plasma lipid response to atorvastatin in a gender specific manner. Atherosclerosis. 2001;158:183-93.
9. Pozzilli C, Prosperini L. Clinical markers of therapeutic response to disease modifying drugs. Neurol Sci. 2008;29: S211-3.
10. Cohen BA, Khan O, Jeffery DR, Bashir K, Rizvi SA, Fox EJ, et al. Identifying and treating patients with suboptimal responses. Neurology. 2004;63(Suppl 6):S33-40.
11. Villoslada P, Oksenberg JR, Río J, Montalbán X. Clinical characteristics of responders to interferon therapy for relapsing MS. Neurology. 2004;62:1653.
12. Waubant E, Vukusic S, Gignoux L, Durand-Dubief F, Achiti I, Blanc S, et al. Clinical characteristics of responders to interferon therapy for relapsing MS. Neurology. 2003;61: 184-9.
13. Leyva L, Fernández O, Fedetz M, Blanco E, Fernández VE, Oliver B, et al. IFNAR 1 and IFNAR2 polymorphisms confer susceptibility to multiple sclerosis but not to interferon-beta treatment response. J Neuroimmunol. 2005;163:165-71.
14. Cohen JA, Carter Jl , Kinkel RP, Schwid SR. Therapy of relapsing multiple sclerosis. Treatment approaches for nonresponders. J Neuroimmunol. 1999;98:29-36.
15. Río J, Nos C, Tintoré M, Borrás C, Galán I, Comabella M, et al. Assessment of different treatment failure criteria in a cohort of relapsing-remitting multiple sclerosis Patients treated with interferon beta: implications for clinical trials. Ann Neurol. 2002;52:400-6.
16. Rudick RA. Measuring the impact of therapeutic intervention. Thinking beyond traditional outcomes. Neurology. 2010;74(Suppl 3):S1-2.
17. Cutter G. Evidence of treatment benefit: is seeing believing or obfuscation by statistics. Mult Scler. 2009;15:1251-2.
18. Comabella M, Craig DW, Morcillo-Suárez C, Río J, Navarro A, Fernández M, et al. Genome-wide Scan of 500,000 Single-Nucleotide Polymorphisms Among Responders and Nonresponders to Interferon Beta Therapy in Multiple Sclerosis. Arch Neurol. 2009;66:972-8.
19. Chiu AW, Richert N, Ehrmantraut M, Ohayon J, Gupta S, Bomboi G, et al. Heterogeneity in response to Interferon Beta in patients with Multiple Sclerosis. A 3-year monthly imaging study. Arch Neurol. 2009;66:39-43.
20. Martínez A, De las Heras V, Mas Fontao A, Bartolomé M, De la Concha EG, Urcelay E, et al. An IFNG polymorphism is associated with interferon-beta response in spanish MS Patients. J Neuroimmunol. 2006;173:196-9.
21. Gilli F, Marnetto F, Caldano M, Sala A, Malucchi S, Capobianco M, et al. Biological markers of interferon-beta therapy: comparison among interferon-stimulated genes MxA, TRAIL and XAF-1. Mult Scler. 2006;12:47-57.
22. Laske C, Oschmann P, Tofighi J, Kühne BS, Diehl H, Brezenger T, et al. Prognostic value of soluble tumor necrosis factor receptors 1 and 2 in multiple sclerosis patients treated with interferon beta 1b. Eur Neurol. 2001;46:210-4.
23. Wandinger K-P, Lünemann JD, Wengert O, Bellmann-Strobl J, Aktas O, Weber A, et al. TNF-related apoptosis inducing ligand (TRAIL) as a potential response marker for interferon-beta treatment in multiple sclerosis. Lancet. 2003;361:2036-43.
24. Walsh EC, Guschwan-Mcmahon S, Daly MJ, Hafler DA, Rioux JD. Genetic analysis of multiple sclerosis. J Autoimmunity. 2003;21:111-6.
25. Wergeland S, Beiske A, Nyland H, Hovdal H, Jensen D, Larsen JP, et al. IL-10 promoter haplotype influence on interferon treatment response in multiple sclerosis. Eur J Neurol. 2005;12:171-5.
26. Villoslada P, Barcellos LF, Río J, Begovich AB, Tintore M, Sastre-Garriga J, et al. The HLA locus and multiple sclerosis in Spain. Role in disease susceptibility, clinical course and response to interferon-beta. J Neuroimmunol. 2002;130:194-201.
27. Sriram U, Barcellos LF, Villoslada P, Río J, Baranzini SE, Caillier S, et al. Pharmacogenomic analysis of interferon receptor polymorphisms in multiple sclerosis. Genes and Immunity. 2003;4:147-52.
28. Fernández O, Fernández V, Mayorga C, Guerrero M, León A, Tamayo JA, et al. HLA class II and response to interferon-beta in multiple sclerosis. Acta Neurol Scand. 2005;112:391-4.
29. Cenit MDC, Blanco-Kelly F, De las Heras V, Bartolomé M, De la Concha EG, Urcelay E, et al. Glypican 5 is an interferon-beta response gene: a replication study. Mult Scler. 2009;15:913-7.
30. Corona T, Guerrero-Camacho JL, Alonso-Vilatela ME, Flores-Rivera JJ. Ausencia de relación entre los genotipos de apolipoproteína E y la gravedad de la esclerosis múltiple en pacientes mexicanos. Rev Neurol. 2010;50:19-22.
31. Portaccio E, Zipoli V, Goretti B, Hakiki B, Nacmias B, Siracusa G, et al. Apolipoprotein E epsilon 4 allele is not associated with disease course and severity in multiple sclerosis. Acta Neurol Scand. 2009;120:439-41.
32. Van der Walt A, Stankovich J, Bahlo M, Taylor BV, Van der Mei IAF, Foote SJ, et al. Apolipoprotein genotype does not influence MS severity, cognition, or brain atrophy. Neurology. 2009;73:1018-25.
33. Guerrero AL, Laherrán E, Gutiérrez F, Martín-Polo J, Alcázar C, Peralta J, et al. Apolipoprotein E genotype does not associate with disease severity measured by Multiple Sclerosis Severity Score. Acta Neurol Scand. 2008;117:21-5.
34. Roxburgh RH, Seaman SR, Masterman T, Hensiek AE, Sawcer SJ, Vukusic S, et al. Multiple Sclerosis Severity Score. Neurology. 2005;64:1144-51.
35. Neuhaus O, Stüve O, Zamvil SS, Hartung H-P. Are statins a treatment option for multiple sclerosis?. Lancet Neurol. 2004;3:369-71.
36. Paul F, Waiczies S, Wuerfel J, Bellmann-Strobl J, Dörr J, Waiczies H, et al. Oral high-dose atorvastatin treatment in relapsing-remitting multiple sclerosis. PLoS ONE. 2008; 3:e1928.