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HLA-DRB1* Allele Frequencies in Pediatric, Adolescent and Adult-Onset Multiple Sclerosis Patients, in a Hellenic Sample. Evidence for New and Established Associations

Maria C Anagnostouli[1,2]*, Argyro Manouseli[1,3], Artemios K Artemiadis[1], Serafeim Katsavos[1], Constantina Fillipopoulou[1,3], Sotirios Youroukos[1], Spyros Efthimiopoulos[3] and Ilias Doxiadis[4]

[1]*Immunogenetics Laboratory, 1st Department of Neurology, Aeginition Hospital, Medical School of National and Kapodistrian University of Athens, Athens, Greece*

[2]*1st Department of Neurology, Aeginition Hospital, Medical School of National and Kapodistrian University of Athens, Athens, Greece*

[3]*Department of Biology, Division of Animal and Human Physiology of National and Kapodistrian University of Athens, Athens, Greece*

[4]*Department of Immunohaematology and Blood Transfusion, University Hospital, Leiden, The Netherlands*

*Corresponding author: Maria C Anagnostouli, Assistant Professor of Neurology and Director of Immunogenetics Laboratory, 1st Department of Neurology, Aeginition Hospital, National and Kapodistrian University of Athens, 72-74 Vas. Sophias Avenue, 11528, *Greece*, Tel: +30 210 728 9165; Fax: +30 210 685 2913; E-Mail: managnost@med.uoa.gr

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Abstract

Studies in many populations consistently have showed that the human leukocyte antigens (HLA) and especially the DRB1*15 allele has by far the strongest genetic association with multiple sclerosis (MS). The aim of this study was to investigate the role of HLA-DRB1* alleles in MS risk/resistance and onset. A sample of 165 Hellenic MS patients (18 with pediatric-, 24 with adolescent- and 123 with adult-onset MS) and 107 healthy volunteers were examined with molecular techniques. Comparisons were made according to the Benjamini-Yekutieli method for p value correction. Both adult-onset MS patients and early-onset MS patients (age at onset below 20 years-old) had a significantly higher frequency of the DRB1*15 allele and a significantly lower frequency of the DRB1*11 allele compared to controls. For the early-onset vs. healthy group comparison, subgroup analyses revealed that both the pediatric- and the adolescent-onset MS groups contributed to the aforementioned DRB1*15 significant difference, while the DRB1*11 difference was ascribed solely to the adolescent-MS onset vs. healthy group comparison. Within MS patients comparisons revealed that early-onset MS patients had a tendency for higher DRB1*03 allele and a lower DRB1*16 allele frequency frequencies compared to adult-onset MS patients, although both non-significant. Notably, pediatric-onset MS patients showed complete absence of the DRB1*16 allele, along with a non-significant tendency for higher DRB1*15 allele frequency relative to the adult-onset group. Finally, the adolescent-onset MS group was presented with a lower DRB1*11 allele frequencies compared both to the pediatric- and the adult-onset MS group. Our findings confirm previous studies on the role of HLA-DRB1* in MS. New findings that need to be confirmed by further studies are the pathogenetic role of DRB1*03 for early-onset MS and the putative protective role of the DRB1*16 allele in the pediatric-onset MS.

Keywords Multiple sclerosis; HLA; Genetics; Immunogenetics; Pediatric; Adolescence; Hellenic population

Introduction

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system (CNS) of unknown origin. Both environmental and genetic factors have been implicated in the pathogenesis of the disease leading to the well-known consideration of MS as a complex multi-factorial disease entity [1]. Concerning genetics, linkage studies in many populations consistently have showed that the human leukocyte antigens (HLA), products of the Major Histocompatibility Complex (MHC) on chromosome 6p21.3 are linked to MS [1,2]. MHC represents a cluster of highly polymorphic genes, including the HLA-Class I, II and III genes that encode proteins which serve antigen presentation to T-lymphocytes. Different auto antigens have different binding capacities to HLA molecule, explaining ostensibly the predisposing role of HLA in autoimmune diseases.

Historically, HLA-A and -B (HLA class I) alleles were the first to be associated with adult-onset MS, with HLA class II (such as HLA-DR2 and DQw6) identified in later studies [3,4]. In a recent collaborative European study, DRB1*1501 (split of DR2) had the strongest association with MS, along with DRB1*0301 and DRB1*1301, while HLA-A*0201 has been found to confer protection against MS [5]. Studies in other populations reveal different candidate alleles such as DPA1*02, DPB1*05, DRB1*0403 and DRB1*08 [6-9].

Notably, there is corroborating evidence outlining the prominent role of HLA-DRB1* in adult-onset MS. First of all, HLA-DRB1 has been found to be regulated by vitamin D, which is associated with the risk for MS [10]. The presence of DRB1*15 has also been associated with more disability, more severe spinal cord pathology, positive oligoclonal bands, more potent humoral responses to Epstein-Barr virus, female susceptibility to the disease and earlier onset [11-15]. On the other hand, in some populations, DRB1*01, DRB1*07, DRB1*11 and DRB1*14 appeared to provide protection from the disease [16].

Patients with early-onset MS (before 18 years old) account for the 3.5- 5 % of the general pool of the MS patients [17]. DRB1*15 association with childhood and or earlier onset of MS has been

attested by studies [18,19]. Moreover, DRB1*04 combined with DRB1*15 has been linked with earlier onset of the disease but it may delay age at onset when combined with DRB1*0801 [20]. In a Korean population, close linkage of DRB3*02, DRB1*13 and DQB1*03 was also associated with the risk of childhood MS [21]. Although the scarcity of MS during adolescence could be attributed to the anti-inflammatory and/or neuroprotective role of both androgens and estrogens which are found increased during this age-period, the interplay between HLA and these hormones and its consequences on autoimmunity have been poorly studied in MS [22,23].

In a previous study presenting a three generation Hellenic familial case of bipolar disorder in which there was comorbidity with MS, by Bozikas et al. [24] it was the first time that a sample of 87 Hellenic MS patients had been genotyped for HLA in our laboratory and the increased frequency of DRB1*1501 was found and presented, compared to healthy controls. In contrary, in this study, it was also showed that the members of this three generation family (of both juvenile and adulthood patients) carried the DRB1*16 allele [24]. In the second HLA Hellenic study by Kouri et al. [25] DRB1*1501, DQB1*0602 and DQA1*0102 alleles were more frequent among patients than controls, with the first two detected more frequently in patients with initial symptoms from the brainstem or the cerebellum [25]. The present study expands literature on HLA, by investigating the influence of HLA-DRB1* alleles on disease risk/resistance and age at onset in a Hellenic sample of MS patients, for the first time, with both early-onset (pediatric and adolescent) and adulthood MS, using healthy controls.

Materials and Methods

Subjects

A group of 165 Hellenic MS patients, diagnosed according to the McDonald criteria, was studied at the Immunogenetics Laboratory of the 1st Department of Neurology, of Athens National University, between 2002 and 2012 [26]. According to the age at disease onset, three subcategories were recognized: 1. Two with early-onset MS: a. eighteen (8 males, 10 females, median EDSS: 3.25, EDSS range: 1.5-7) with pediatric-onset MS (below 16 years old) (median age: 14, range: 9-15 years old), b. twenty-four (7 males, 17 females, median EDSS: 3.5, EDSS range: 1-7) with adolescent-onset (between 16 and 19 years old) (median age: 17) and 2. One hundred and twenty-three (46 males, 77 females, median EDSS: 3, EDSS range: 0-8) with adult-onset MS (above 19 years old) (median age: 37.5, range: 21-69 years old). One hundred and seven (48 males and 59 females) Hellenic healthy volunteers, with no history of autoimmune or inflammatory disease were selected from our laboratory records. Each individual gave his/her informed consent and hospital's ethical committee approved this study.

HLA genotyping

HLA genotyping was performed at the *Immunogenetics* Laboratory of the 1st Department of Neurology in *Aeginition* Hospital. High molecular weight DNA was extracted from peripheral blood samples (8mL peripheral blood in sodium citrate, ACD Vacutainer®). HLA class II (DRB1*) frequencies were determined by molecular techniques, in all subjects, for all the specificities included in the HLA Nomenclature of 2012 (we present only the first four numbers of each allele) [27]. After DNA extraction (QIAGEN Maxi Kit), the polymorphic second exon of the HLA-DRB1*gene was amplified and the products were genotyped using PCR-SSO analysis for class II (Bio-Rad-Elpha, High resolution). The technique we have used to perform HLA-DRB1* genotyping was an SSO (Sequence-Specific Oligonucleotide), which results in final absorptions of an Elisa format, which cannot be stored in another way, except for an Elisa sheet. The results of the absorptions are put in an electronic system especially created for this reason (Bio-Rad-Elpha), which finally converts the positive Elisa positions (higher absorptions than the reference) to HLA-DRB1* alleles. We have stored all the Elisa-sheets in our personal computer, along with the electronic results for HLA-DRB1* for all our samples [28].

Statistical analyses

Frequencies of the DRB1* alleles were compared across groups using two-sample chi-square test. Groups according to disease onset were: before 19 years old (≤ 19) or early-onset MS, after 19 years old (>19) or adult-onset MS, before 16 years old (≤ 15) or pediatric-onset MS and 16-19 years old or adolescent-onset MS. P value correction was made according to the Benjamini-Yekutieli method (or B-Y) according to the formula $p(B\text{-}Y)= a/(\Sigma 1/i)$ where i denotes the number of comparisons and a=0.05. As such, the level of significance for 11 comparisons (corresponding to 11 alleles) was 0.017 and for 12 comparisons 0.016. This method has been proposed to be less conservative than the Bonferroni especially in case of multiple comparisons, reducing type II error [29]. Statistical analyses were done using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

Results

Tables 1,2,3 and 4 represent comparisons of MS patients with early-onset disease, pediatric-onset MS, adolescent-onset MS and adult-onset MS versus healthy individuals. Patients with early-onset MS had significantly higher frequencies of the DRB1*15 allele [OR 2.653, (1.219-5.774), p=0.016] and significantly lower of the DRB1*11 allele [OR 0.448, (0.236-0.853), p=0.014]. There was also a non-statistical significant tendency for higher DRB1*03 allele frequency in MS patients of this age. The DRB1*16 allele was found significantly absent in the pediatric group (p=0.011), along with statistically non-significant tendencies for higher frequency of both the DRB1*03 and the DRB1*15 allele. DRB1*11 allele was significantly lower in the adolescent group [OR 0.204, (0.07-0.591), p=0.01], along with statistically non-significant tendencies for higher frequency of both the DRB1*03 and the DRB1*15 allele. Adult-onset MS (above 20 years old) was characterized by significantly higher DRB1*15 allele frequency [OR=2.653, (1.423-4.946), p=0.003] and significantly lower DRB1*11 allele frequency [OR=0.462, (0.297-0.718), p=0.001].

HLA-DRB1*	Early-onset Multiple Sclerosis (84 alleles)			Healthy Individuals (214 alleles)			Fisher Exact test			
	Positive	Negative	%	Positive	Negative	%	OR	Lower Limit	Upper Limit	p value
DRB1*01	5	79	6	12	202	6	1.065	0.364	3.122	1
DRB1*03	15	69	18	20	194	9	2.109	1.023	4.348	0.047
DRB1*04	10	74	12	17	197	8	1.566	0.686	3.575	0.369
DRB1*07	3	81	4	7	207	3	1.095	0.276	4.339	1
DRB1*08	0	0	0	5	209	2	NE	NE	NE	0.327
DRB1*10	2	82	2	6	208	3	0.846	0.167	4.275	1
DRB1*11	14	70	17	66	148	31	0.448	0.236	0.853	0.014*
DRB1*12	1	83	1	5	209	2	0.504	0.058	4.376	1
DRB1*13	7	77	8	21	193	1	0.835	0.341	2.045	0.827
DRB1*14	5	79	6	11	203	5	1.168	0.393	3.469	0.779
DRB1*15	14	70	17	15	199	7	2.653	1.219	5.774	0.016*
DRB1*16	8	76	10	29	185	14	0.672	0.294	1.535	0.436
P(B-Y) = 0.016 (12 comparisons); *p<0.016; NE= not estimated										

Table 1: HLA-DRB1* frequencies and comparisons between patients with early-onset multiple sclerosis (N=42) and healthy individuals (N=107).

HLA-DRB1*	Pediatric-onset Multiple Sclerosis (36 alleles)			Healthy Individuals (214 alleles)			Fisher Exact test			
	Positive	Negative	%	Positive	Negative	%	OR	Lower Limit	Upper Limit	p value
DRB1*01	1	35	3	12	202	6	0.481	0.061	3.816	0.699
DRB1*03	7	29	19	20	194	9	2.341	0.91	6.024	0.083
DRB1*04	5	31	14	17	197	8	1.869	0.643	5.431	0.334
DRB1*07	2	34	6	7	207	3	1.739	0.347	8.727	0.621
DRB1*08	0	0	0	5	209	2	NE	NE	NE	1
DRB1*10	1	35	3	6	208	3	0.99	0.116	8.478	1
DRB1*11	10	26	28	66	148	31	0.862	0.393	1.891	0.845
DRB1*12	0	36	0	5	209	2	NE	NE	NE	1
DRB1*13	3	33	8	21	193	10	0.835	0.236	2.96	1
DRB1*14	1	35	3	11	203	5	0.527	0.066	4.213	1
DRB1*15	6	30	17	15	199	7	2.653	0.955	7.37	0.095
DRB1*16	0	36	0	29	185	14	NE	NE	NE	0.011*
P(B-Y) = 0.016 (12 comparisons); *p<0.016; NE= not estimated										

Table 2: HLA-DRB1* frequencies and comparisons between patients with pediatric-onset multiple sclerosis (N=18) and healthy individuals (N=107).

HLA-DRB1*	Adolescent-onset Multiple Sclerosis (48 alleles)			Healthy Individuals (214 alleles)			Fisher Exact test			
	Positive	Negative	%	Positive	Negative	%	OR	Lower Limit	Upper Limit	p value
DRB1*01	4	44	8	12	202	6	1.53	0.471	4.968	0.504
DRB1*03	8	40	17	20	194	9	1.94	0.798	4.714	0.192
DRB1*04	5	43	10	17	197	8	1.347	0.471	3.852	0.568
DRB1*07	1	47	2	7	207	3	0.629	0.076	5.237	1
DRB1*08	0	0	0	5	209	2	NE	NE	NE	0.588
DRB1*10	1	47	2	6	208	3	0.738	0.087	6.272	1
DRB1*11	4	44	8	66	148	31	0.204	0.07	0.591	0.001*
DRB1*12	1	47	2	5	209	2	0.889	0.102	7.791	1
DRB1*13	4	44	8	21	193	10	0.835	0.273	2.556	1
DRB1*14	4	44	8	11	203	5	1.678	0.51	5.514	0.488
DRB1*15	8	40	17	15	199	7	2.653	1.054	6.677	0.046
DRB1*16	8	40	17	29	185	14	1.276	0.543	2.997	0.646
P(B-Y) = 0.016 (12 comparisons); * p<0.016; NE= not estimated										

Table 3: HLA-DRB1* frequencies and comparisons between patients with adolescent-onset multiple sclerosis (N=24) and healthy individuals (N=107).

HLA-DRB1*	Adult-onset Multiple Sclerosis (246 alleles)			Healthy Individuals (214 alleles)			Fisher Exact test			
	Positive	Negative	%	Positive	Negative	%	OR	Lower Limit	Upper Limit	p value
DRB1*01	19	227	7	12	202	6	1.409	0.667	2.974	0.474
DRB1*03	22	224	9	20	194	9	0.953	0.505	1.798	1
DRB1*04	31	215	13	17	197	8	1.671	0.897	3.113	0.14
DRB1*07	10	236	4	7	207	3	1.253	0.469	3.351	0.84
DRB1*08	0	0	0	5	209	2	NE	NE	NE	0.05
DRB1*10	7	239	3	6	208	3	1.015	0.336	3.069	1
DRB1*11	42	204	17	66	148	31	0.462	0.297	0.718	0.001*
DRB1*12	6	240	2	5	209	2	1.045	0.314	3.474	1
DRB1*13	16	230	7	21	193	10	0.639	0.325	1.259	0.259
DRB1*14	12	234	5	11	203	5	0.946	0.409	2.191	1
DRB1*15	41	205	17	15	199	7	2.653	1.423	4.946	0.003*
DRB1*16	40	206	16	29	185	14	1.239	0.738	2.079	0.496
P(B-Y) = 0.016 (12 comparisons); *p<0.016; NE= not estimated										

Table 4: HLA-DRB1* frequencies and comparisons between patients with adult-onset multiple sclerosis (N=123) and healthy individuals (N=107).

HLA-DRB1* 1	Early-onset Multiple Sclerosis (84 alleles)			Adult-onset Multiple Sclerosis (246 alleles)			Fisher Exact test			
	Positive	Negative	%	Positive	Negative	%	OR	Lower Limit	Upper Limit	p value
DRB1*01	5	79	6	19	227	8	0.756	0.273	2.092	0.808
DRB1*03	15	69	18	22	224	9	2.213	1.089	4.5	0.043
DRB1*04	10	74	12	31	215	13	0.937	0.438	2.004	1
DRB1*07	3	81	4	10	236	4	0.874	0.235	3.255	1
DRB1*10	2	82	2	7	239	3	0.833	0.17	4.089	1
DRB1*11	14	70	17	42	204	17	0.971	0.501	1.885	1
DRB1*12	1	83	1	6	240	2	0.482	0.057	4.062	0.683
DRB1*13	7	77	8	16	230	7	1.307	0.518	3.295	0.62
DRB1*14	5	79	6	12	234	5	1.234	0.422	3.612	0.775
DRB1*15	14	70	17	41	205	17	1	0.514	1.944	1
DRB1*16	8	76	1	40	206	16	0.542	0.243	1.211	0.153
P(B-Y) = 0.017 (11 comparisons)										

Table 5: HLA-DRB1* frequencies and comparisons between patients with multiple sclerosis early-onset (N=42) and adult-onset multiple sclerosis (N=123).

HLA-DRB1*	Pediatric-onset Multiple Sclerosis (36 alleles)			Adult-onset Multiple Sclerosis (246 alleles)			Fisher Exact test			
	Positive	Negative	%	Positive	Negative	%	OR	Lower Limit	Upper Limit	p value
DRB1*01	1	35	3	19	227	8	0.341	0.044	2.631	0.487
DRB1*03	7	29	19	22	224	9	2.458	0.965	6.256	0.073
DRB1*04	5	31	14	31	215	13	1.119	0.405	3.092	0.791
DRB1*07	2	34	6	10	236	4	1.388	0.292	6.608	0.655
DRB1*10	1	35	3	7	239	3	0.976	0.116	8.169	1
DRB1*11	10	26	28	42	204	17	1.868	0.838	4.163	0.164
DRB1*12	0	36	0	6	240	2	NE	NE	NE	1
DRB1*13	3	33	8	16	230	7	1.307	0.361	4.728	0.719
DRB1*14	1	35	3	12	234	5	0.557	0.07	4.418	1
DRB1*15	6	30	17	41	205	17	1	0.391	2.556	1
DRB1*16	0	36	0	20	206	16	NE	NE	NE	0.004*
P(B-Y) = 0.017 (11 comparisons); *p<0.017; NE= not estimated										

Table 6: HLA-DRB1* frequencies and comparisons between patients with pediatric-onset (N=18) and adult-onset multiple sclerosis (N=123).

HLA-DRB1*	Pediatric-onset Multiple Sclerosis (36 alleles)			Adolescent-Onset Multiple Sclerosis (48 alleles)			Fisher Exact test			
	Positive	Negative	%	Positive	Negative	%	OR	Lower Limit	Upper Limit	p value
DRB1*01	1	35	3	4	44	8	0.314	0.034	2.94	0.386
DRB1*03	7	29	19	8	40	17	1.207	0.393	3.704	0.779
DRB1*04	5	31	14	5	43	10	1.387	0.37	5.207	0.738
DRB1*07	2	34	6	1	47	2	2.765	0.241	31.741	0.574
DRB1*10	1	35	3	1	47	2	1.343	0.081	22.219	1
DRB1*11	10	26	28	4	44	8	4.231	1.204	14.868	0.036
DRB1*12	0	36	0	1	47	2	NE	NE	NE	1
DRB1*13	3	33	8	4	44	8	1	0.209	4.776	1
DRB1*14	1	35	3	4	44	8	0.314	0.034	2.94	0.386
DRB1*15	6	30	17	8	40	17	1	0.314	3.188	1
DRB1*16	0	36	0	8	40	17	NE	NE	NE	0.009*
P(B-Y) = 0.017 (11 comparisons) ; *p<0.017; NE= not estimated										

Table 7: HLA-DRB1* frequencies and comparisons between patients with pediatric-onset (N=18) and adolescent-onset multiple sclerosis (N=23).

HLA-DRB1*	Adolescent-onset Multiple Sclerosis (48 alleles)			Adult-Onset Multiple Sclerosis (246 alleles)			Fisher Exact Test			
	Positive	Negative	%	Positive	Negative	%	OR	Lower Limit	Upper Limit	p value
DRB1*01	4	44	8	19	227	7	1.086	0.352	3.347	0.776
DRB1*03	8	40	17	22	224	9	2.036	0.848	4.892	0.119
DRB1*04	5	43	10	31	215	13	0.806	0.297	2.192	0.812
DRB1*07	1	47	2	10	236	4	0.502	0.063	4.017	1
DRB1*10	1	47	2	7	239	3	0.726	0.087	6.043	1
DRB1*11	4	44	8	42	204	17	0.442	0.151	1.295	0.19
DRB1*12	1	47	2	6	240	2	0.851	0.1	7.233	1
DRB1*13	4	44	8	16	230	7	1.307	0.417	4.095	0.753
DRB1*14	4	44	8	12	234	5	1.773	0.547	5.749	0.308
DRB1*15	8	40	17	41	205	17	1	0.436	2.293	1
DRB1*16	8	40	17	40	206	16	1.03	0.449	2.365	1
P(B-Y) = 0.017 (11 comparisons)										

Table 8: HLA-DRB1* frequencies and comparisons between patients with adolescent-onset (N=24) and adult-onset multiple sclerosis (N=123).

The allele frequencies of HLA-DRB1* in patients with early- and adult-onset disease are shown in Table 5. Two-sample chi-square revealed that the DRB1*03 allele was more frequent in patients with early disease onset [OR=2.213, (1.089-4.5), p=0.043) but did not reach statistical significance according to the B-Y criterion. A tendency for lower DRB1*16 allele frequency was also noted in the early-onset group.

The allele frequencies of HLA-DRB1* in patients with pediatric disease onset and adult-onset MS are shown in Table 6. Patients with adult-onset disease onset had a significant increased frequency of DRB1*16 allele versus patients with pediatric onset (p=0.004). The

latter also showed a non-statistical significant tendency for higher DRB1*03 and DRB1*11 allele frequencies. Patients with adolescent-onset MS showed increased frequency of the DRB1*16 allele than the pediatric group (p=0.009) and a non-statistical significant tendency for lower DRB1*11 allele (Table 7). However, the adolescent group showed non-significant difference with the adult group, although a tendency for higher DRB1*03 allele frequency and lower DRB1*11 allele frequency was noted (Table 8).

Discussion

To our knowledge, this is the first study aiming at revealing the HLA-DRB1* allelic distribution in early-onset (both pediatric and adolescent) and adult-onset MS, in the Hellenic population. Our findings are in part new and in part standard, concerning previous results of HLA frequencies in Hellenic MS patients and HLA-genotyping in various other populations.

Our study has showed that the DRB1*15 allele was significantly more frequent in MS patients than healthy controls, irrespective of age at disease onset. A protective effect of HLA-DRB1*11 allele was warranted, since MS patients showed decreased frequencies of this allele, irrespective of age at disease onset. Also, a protective effect of the DRB1*16 allele can be deduced by the lower frequency of this allele in the pediatric-onset group. A tendency for higher DRB1*03 allele frequency was also noted for MS patients with early-onset disease onset versus healthy controls. In respect of MS age at onset, the DRB1*16 allele was found less prevalent in the pediatric group, than the adolescent-onset and adult-onset group, with a tendency of all MS patients with early-onset disease to present with lower DRB1*16 allele frequencies. All patients with early-onset MS had a tendency for higher DRB1*03 frequency versus the adult-onset group, while the pediatric-onset group had a non-significant higher frequency of DRB1*11 allele than the adolescent-onset group.

Our results are consistent with previous studies showing greater susceptibility to MS in patients carrying the HLA-DRB1*15 allele [4-8]. Also, the protective effect of the HLA-DRB1*11 allele has been also confirmed in previous studies [30,31]. However, our study did not reproduce the association between the presence of HLA-DRB1*15 and earlier age of MS onset [18,19]. We have detected two studies with similar negative findings concerning early-onset MS [32,33]. One possible explanation for this discrepancy could be the parent of origin effect on age of MS onset and DRB1*15. In specific, one study has showed that maternally transmitted DRB1*15 promotes a lower age of MS onset [34]. In our research, we have no information about the genotypes of the patients' parents. A second reason could be that given the association between vitamin D levels and DRB1*15 expression, we hypothesize that differences in MS age at onset according to the presence of DRB1*15 are more prominent in populations with low or graded sun exposure [10]. Since in Hellas sun exposure is particularly high throughout the year, expression of DRB1*15 could be more homogenously distributed among the population and confers no risk for earlier disease onset, or larger samples are needed to detect HLA-alleles frequency discrepancies, if they truly exist.

The protective role of DRB1*16 for early onset of the disease was, to our knowledge, a new finding that must be verified in future studies. However, this finding must be interpreted with caution since DRB1*16 was totally absent in the pediatric group with only 18 patients examined (36 alleles), while no statistical differences or tendencies for this allele were noted between the adolescent-onset group and the adult-onset group. Interestingly, X-linked adrenoleukodystrophy, which occurs during childhood and resembles MS, has been associated with DRB1*16 [35].

Interestingly, DRB1*03 allele was more prevalent in early-onset MS, however, the difference did not reach statistical significance according to our B-Y statistical criterion for p value correction. This allele has been previously associated with MS susceptibility and with the presence of Ig Moligoclonal band within the cerebrospinal fluid of these patients [36]. In a meta-analysis DRB1*03 was identified as "probably the only risk factor for MS besides DRB1*15 and a common genetic foundation for autoimmune disease" at least for adult-onset MS [37]. DRB1*03 allele does not seem to affect clinical disease severity, cognition or cerebral atrophy, although in one study it was associated with better MS prognosis [38]. Interestingly, DRB1*03 allele has been strongly associated with neuromyelitis optica (NMO), a humoral-mediated autoimmune disease entity associated with the presence of anti-aquaporin antibodies [39]. According to these findings of the presumed better MS prognosis and NMO association, the relation of DRB1*03 allele with early-onset MS could likely account for different pathogenetic mechanisms in this group of patients, most probably favoring humoral rather than cellular immune responses. In a certain population with certain genetic/immunogenetic background, the difference of HLA-distribution among age groups shows that the proposed immune differentiation for early-onset MS could be attributed to the diverse sex hormone profile during this age-period, which warrants further study by future animal and/or human research.

We have to admit that the sample size of MS patients with age at onset below 18 years old is low, thus decreasing the power of the study. Although, we could argue that early-onset MS consists only the 3-5 % of MS patients, thus our sample is satisfactory representative of the MS population [17]. However, for p value correction, we have adopted a less conservative method than the Bonferroni, thus minimizing type II error. Finally, due to our small sample we have not examined epistatic mechanisms in this study.

In conclusion, our findings demonstrate the role of HLA-DRB1* in MS susceptibility/protectivity and age at onset. New findings in this study are the putative predisposing role of DRB1*03 allele and the protective role of the DRB1*16 allele for early-onset MS. It is necessary that our findings would be confirmed by future studies, especially in Hellas, where evidence is scarce. Studies in different countries, could further elucidate the interplay between environmental (e.g. sun exposure) and genetic factors in MS. To our opinion, delineating the role of HLA in MS could foster future individualized prognostic making and therapeutic choices that will change disease course and optimize quality of life of MS patients.

Conflict of Interest Statement

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