*Article*

# HLA-DPB1*03 as Risk Allele and HLA-DPB1*04 as Protective Allele for Both Early- and Adult-Onset Multiple Sclerosis in a Hellenic Cohort

**Maria Anagnostouli** [1,2,*], **Artemios Artemiadis** [2,3], **Maria Gontika** [2], **Charalampos Skarlis** [2], **Nikolaos Markoglou** [2], **Serafeim Katsavos** [2], **Konstantinos Kilindireas** [4], **Ilias Doxiadis** [5] and **Leonidas Stefanis** [6]

[1] Faculty of Neurology in Demyelinating Disease Unit & Director of Immunogenetics Laboratory, 1st Department of Neurology, Medical School, National and Kapodistrian University of Athens, NKUA, Aeginition Hospital, 115 28 Athens, Greece

[2] Immunogenetics Laboratory, 1st Department of Neurology, Medical School, National and Kapodistrian University of Athens, NKUA, Aeginition Hospital, 115 28 Athens, Greece; kmwartem@yahoo.com (A.A.); mary212009@windowslive.com (M.G.); charskarlis@med.uoa.gr (C.S.); nmarkoglou@yahoo.gr (N.M.); serafkatsavos@gmail.com (S.K.)

[3] Medical School, University of Cyprus, Nicosia 1678, Cyprus

[4] Demyelinating Diseases Unit, 1st Department of Neurology, Medical School, National and Kapodistrian University of Athens, NKUA, Aeginition Hospital, 115 28 Athens, Greece; kildrcost@med.uoa.gr

[5] Transplantation Immunological Laboratory, Institute of Transfusion Medicine, University Hospital Leipzig, 04103 Leipzig, Germany; ilidox@planet.nl

[6] 1st Department of Neurology, Medical School, National and Kapodistrian University of Athens, NKUA, Aeginition Hospital, 115 28 Athens, Greece; lstefanis@med.uoa.gr

* Correspondence: managnost@med.uoa.gr

check for updates

**Abstract: Background**: Human Leucocyte Antigens (HLA) represent the genetic loci most strongly linked to Multiple Sclerosis (MS). Apart from *HLA-DR* and *HLA–DQ*, *HLA-DP* alleles have been previously studied regarding their role in MS pathogenesis, but to a much lesser extent. Our objective was to investigate the risk/resistance influence of *HLA-DPB1* alleles in Hellenic patients with early- and adult-onset MS (EOMS/AOMS), and possible associations with the *HLA-DRB1*15:01* risk allele. **Methods**: One hundred MS-patients (28 EOMS, 72 AOMS) fulfilling the McDonald-2010 criteria were enrolled. HLA genotyping was performed with standard low-resolution Sequence-Specific Oligonucleotide techniques. Demographics, clinical and laboratory data were statistically processed using well-defined parametric and nonparametric methods and the SPSSv22.0 software. **Results**: No significant *HLA-DPB1* differences were found between EOMS and AOMS patients for 23 distinct *HLA-DPB1* and 12 *HLA-DRB1* alleles. The *HLA-DPB1*03* allele frequency was found to be significantly increased, and the *HLA-DPB1*02* allele frequency significantly decreased, in AOMS patients compared to controls. The *HLA-DPB1*04* allele was to be found significantly decreased in AOMS and EOMS patients compared to controls. **Conclusions**: Our study supports the previously reported risk susceptibility role of the *HLA-DPB1*03* allele in AOMS among Caucasians. Additionally, we report for the first time a protective role of the *HLA-DPB1*04* allele among Hellenic patients with both EOMS and AOMS.

**Keywords:** Multiple Sclerosis; early-onset; adult-onset; Human Leucocyte Antigens; immunogenetics; clinical phenotype; clinical outcome; therapeutics

## 1. Introduction

Multiple sclerosis (MS) is considered a complex, multifactorial disease entity, as both environmental and genetic factors have been implicated in its pathogenesis [1]. The Major Histocompatibility Complex (MHC) represents a cluster of highly polymorphic genes, including mainly the Human Leukocyte Antigens (HLA) system, namely Class I (A, B, C) and II (DR, DQ, DP) genes, and genes encoding for some other immune factors, like complement components, Bf, C2, C4 and TNF, in Class III and IV loci [2]. HLA molecules mediate antigen presentation to T-lymphocytes, playing a crucial role in immune response and affecting all clinical and neuroimaging characteristics and response to treatment in MS [3,4]. Linkage studies in various populations have consistently demonstrated that the MHC and its polymorphisms represent the genetic locus most strongly linked to MS [3–5], and that the MHC class II (*HLA-DR, HLA-DQ, HLA-DP*) region is the susceptibility complex that accounts for the majority of familial clustering in MS [6].

The MHC class II linkage to MS differs in various populations, with the highest association conferred by the *HLA-DRB1*15:01/HLA-DQB1*06:02* haplotype, present in Caucasians [5]. In 2011, in a collaborative European study, the *HLA-DRB1*15:01* allele exhibited the strongest association with MS, along with the *HLA-DRB1* 03:01* and *HLA-DRB1*13:01* alleles [7], although *DRB1*15:01* was recently found to be hypomethylated and predominantly expressed in monocytes among carriers of *DRB1*15:01*, suggesting putative therapeutic strategies targeting methylation-mediated regulation of this major risk gene [8].

Recent studies have further established the role of *HLA-DRB1*15:01* in early-onset (pediatric and adolescent) MS (EOMS), which accounts for 3–5% of all MS cases, while the role of *HLA-DRB1*04* and *HLA-DRB1*03* remains to be clarified [9–11].

Apart from the well examined *HLA-DR* and *HLA-DQ* genes, other class II genes and their products, *HLA-DP* alleles, have been previously studied regarding their role in MS pathogenesis. One of the earliest studies regarding *HLA-DP* genotyping was performed three decades ago using a small sample of 45 Swedish patients with MS in comparison with 166 Danish controls [12]. Since then, few studies have been published on the role of the *HLA- DPB1* locus concerning genetic risk in adult-onset MS (AOMS), either in Asian [13–16] or European populations [17–21], and no such studies have been performed on EOMS. In 2013, Patsopoulos et al. used single nucleotide polymorphisms (SNP) data from genome-wide studies and tested classical alleles and polymorphisms in eight classical HLA genes in 5091 AOMS cases and 9595 controls [22]. Among a total of 11 identified statistically independent effects, they confirmed a possible association of *HLA-DPB1*03:01*, and also highlighted a more statistically significant effect at amino acid position 65 in the peptide binding groove of *HLA-DPB1** [22]. So far, *HLA-DPB1** alleles have been mainly correlated with neuromyelitis optica spectrum disorders (NMOSD) in Asian but not Caucasian populations [23], while a series of studies suggest a possible role in other autoimmune disorders as well, including juvenile idiopathic arthritis [24], type I diabetes [25] and atopic myelitis in Japanese [26].

The present study attempts to expand the existing data on HLA and MS by investigating the influence of *HLA-DPB1** alleles on disease risk and resistance in a Hellenic sample of 100 patients of both EOMS and AOMS, using healthy controls (HC) for comparisons, given the pre-existing difference in *HLA-DRB1* allele frequencies in EOMS and AOMS in our ethnic group [11] and the total absence of information on *HLA-DPB1* genotyping in the Hellenic MS population.

Additionally, we examined, the putative positive or negative association between the well-defined *HLA-DRB1*15:01* allele and the various *HLA-DPB1** alleles, given the extensive epistatic mechanisms that exist in HLA loci, as clearly illustrated in previous reports [12,19,27,28].

## 2. Materials and Methods

### 2.1. Patients

One hundred patients with MS (62 females, 38 males, mean age 36.9 ± 11.4 years old) were selected, fulfilling the McDonald criteria for MS diagnosis [29]. These patients were enrolled from the outpatient clinic at the Neurology Department of the Aeginition University Hospital (Athens, Greece) after providing written informed consent. The study received ethical approval by the Hospital's Ethics Committee (ethic approval code number: 117/2-4-13), as it was found consistent with the Declaration of Helsinki. At the time this study, 42 patients had the relapsing-remitting type of the disease (RRMS), and 10 patients were identified with primary progressive MS (PPMS), while the rest had the secondary progressive type (SPMS). For all patients, the mean age of disease onset was 27.8 ± 10.8 years old, the mean disease duration was 100.9 ± 80.4 months and the median Expanded Disability Status Scale (EDSS) was 3.0 (range:1.0–8.0) [30]. There were two MS onset groups; 28 in the ≤19 years old or early onset MS (EOMS) group and 72 in the >19 years old or adult onset MS (AOMS) group. Valid Magnetic Resonance Imaging (MRI) and cerebrospinal fluid (CSF) (i.e., presence of oligoclonal bands and IgG index calculation) assessments were available 60 (60%) of the patients. Missing data for MRIs were attributed to the lack of recent MRI scans. With regards to CSF, some but not all patients had been subjected to CSF analysis, since this was not a prerequisite for the MS diagnosis, according to the revised 2010 McDonald criteria [29]. All patients provided informed consent for participation and publication.

### 2.2. HLA-DPB1* and HLA-DRB1* 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 (8 mL peripheral blood in sodium citrate, ACD Vacutainer® tube) using the DNA extraction, Maxi Kit (QIAGEN, Venlo, the Netherlands) as per manufacturer's guidelines in the commercial kit. HLA class II (*HLA-DRB1* and *HLA-DPB1*) frequencies were determined by molecular techniques for all the specificities included in the HLA Nomenclature of 2012 (we present only the first two or four digits of each allele, for low or high resolution respectively) [31]. *HLA-DRB1* genotyping had been previously performed, using a PCR-SSO (Polymerase-Chain-Reaction, PCR, Sequence-Specific Oligonucleotide, SSO) technique (Elpha Bio-Rad, High resolution), as described elsewhere [11]. *HLA-DPB1* genotyping was performed using a different PCR-SSO technique, based on a method that depends on reverse hybridization (Line Probe Assay, INNO-LiPA, Low Resolution, Innogenetics, Fujirebio, Europe) according to the manufacturer's protocol.

### 2.3. Statistical Analyses

The Hardy-Weinberg proportions (HWP) and linkage disequilibrium for *HLA-DPB1*, *HLA-DRB1* haplotypes were ascertained using the PyPoP software [32]. An Ewens-Watterson (EW) homozygosity test for neutrality was also performed. Calculation of the normalized deviate of the homozygosity (i.e., Fnd) was done, with positive and negative values implying directional and balancing selection, respectively. *HLA-DPB1*  genotype frequency in patients with MS was compared with that reported in a previous study of Hellenic HC by using multiple binomial tests [33].

Separate analyses were performed in the EOMS and AOMS groups using the same expected genotype frequencies of the healthy controls [33]. A Fisher's exact test for categorical and Mann-Whitney U test for numerical variables were performed to allow us to make group comparisons. Mantel-Haenszel statistics were used to ascertain the role of MS groups in the association between *HLA-DPB1* genotypes and categorical clinical parameters. In *HLA-DPB1* genotype-related tests (except those for clinical parameters), $p$ value correction was made according to the Benjamini–Yekutieli method (or B–Y) based on the following formula: $p$ (B–Y) = a/($\Sigma 1/i$), where i denotes the number of comparisons and

a = 0.05 [34,35]. Statistical analyses were performed using the SPSS v22.0 software (Armonk, NY, USA: IBM Corp).

## 3. Results

### 3.1. HWP and Linkage Disequilibrium of the Study's Sample

Twenty-three distinct *HLA-DPB1* alleles were identified (total alleles: 200). There were 28 homozygote and 72 heterozygote patients with MS. There were no deviations from the HWP (homozygotes: 29.71 expected, F(1) = 0.1, $p$ = 0.754, heterozygotes: 70.29 expected, F(1) = 0.04, $p$ = 0.838). The most common haplotypes were *HLA-DPB1*04/DPB1*04* (27%), followed by *HLA-DPB1*02/DPB1*04* (13%), *HLA-DPB1*03/DPB1*04* (11%) and *HLA-DPB1*10/DPB1*04* (6%). The EW homozygosity test of neutrality was found to be significantly positive (i.e., Fnd = 3.79, $p$ = 0.992, i.e., over the limit 0.975), denoting a directional selection of the *HLA-DPB1*04* allele.

Twelve distinct *HLA-DRB1* alleles were identified (total alleles: 174) in 87 out of the 100 patients. There were 11 (12.6%) homozygote and 76 (87.3%) heterozygote patients. There were no deviations from the HWP (homozygotes: 10.84 expected, F(1) = 0, $p$ = 0.962, heterozygotes: 76.16 expected, F(1) = 0, $p$ = 0.986). The most common allele was *HLA-DRB1*11* (20.1%), followed by *HLA-DRB1*16* (15.5%), *HLA-DRB1*15* (13.2%), *HLA-DRB1*04* (12.1%) and *HLA-DRB1*13* (10.4%). The most common, but still of low frequency (4.6%), genotype was *HLA-DRB1*11/DRB1*16*. The EW homozygosity test of neutrality was found to be significantly negative (i.e., Fnd = −1.41, $p$ = 0.0033, i.e., lower the limit 0.05), indicating a balancing selection.

The delta distance for the *HLA-DPB1* and *HLA-DRB1* haplotypes was 0.00938 ($p$ = 0.303), denoting linkage equilibrium. This did not change when age of MS onset was taken into account (EOMS: delta 0.0128, $p$ = 0.954, AOMS: delta 0.0133, $p$ = 0.351). The most common (i.e., over 5%) *HLA-DPB1/HLA-DRB1* haplotypes were *HLA-DPB1*04/HLA-DRB1*11* (10.8%), *HLA-DPB1*04/HLA-DRB1*16* (7.7%), *HLA-DPB1*04/HLA-DRB1*04* (7%), *HLA-DPB1*02/HLA-DRB1*11* (6.6%) and *HLA-DPB1*04/HLA-DRB1*03* (5.5%).

### 3.2. Nongenetic Comparisons between Age of Onset Groups

Table 1 presents the main characteristics of the two MS groups. Patients with EOMS were significantly younger and had longer disease duration compared to AOMS, which primarily reflects the blood sampling timing, and has no specific clinical significance. Of most importance, patients with EOMS had significantly higher IgG indexes compared to AOMS. It should be noted that this difference reflects 60 out of the 100 patients with MS of this study, since, as mentioned in the methods section, no CSF testing was available for 40 patients.

**Table 1.** Nongenetic Comparisons Between Age of Multiple Sclerosis (MS) Onset Groups.

| Characteristics | EOMS | AOMS | Sig [1] |
|---|---|---|---|
| Females | 19/28 (67.9%) | 43/72 (59.7%) | 0.499 |
| Age (years old) | 29.9 ± 9.8 | 39.8 ± 10.8 | 0.001 * |
| Duration of MS (months) | 148.1 ± 105.4 | 81.3 ± 69.1 | 0.011 * |
| EDSS | 3.1 ± 1.7 | 3.3 ± 1.6 | 0.414 |
| Primary Progressive | 3/28 (10.7%) | 7/72 (9.7%) | 0.917 |
| Relapses since onset | 5.2 ± 6.1 | 3.7 ± 2.5 | 0.393 |
| IgG index [2] | 1.3 ± 0.7 | 0.8 ± 0.4 | 0.004 * |
| Presence of OCBs [2] | 13/15 (86.7%) | 31/45 (68.9%) | 0.312 |
| Subcortical lesions [2] | 12/17 (70.6%) | 27/43 (62.8%) | 0.765 |
| Periventricular lesions [2] | 16/17 (94.1%) | 42/43 (97.7%) | 0.49 |
| Infratentorial lesions [2] | 12/17 (70.6%) | 30/43 (69.8%) | 1.000 |
| Spinal cord lesions [2] | 12/14 (85.7%) | 29/31 (93.5%) | 0.578 |

Numbers represent means ± standard deviation and absolute (%) frequencies; 1 Fisher exact test for categorical and Mann-Whitney U test for numerical characteristics. OCBs: Oligoclonal Bands, Sig.: significance. 2 Valid MRI and cerebrospinal fluid assessments were available for 60 (66%) and 60 (60%) patients, respectively. * $p \leq 0.05$.

### 3.3. HLA-DPB1 Allele Comparisons between the Age of Onset Groups

No significant *HLA-DPB1* allele differences were found between patients with EOMS and AOMS (Table 2). However, there were significantly fewer *HLA-DPB1*04*-positive patients in the EOMS group compared to HC (64.3% vs. 92.7%). The *HLA-DPB1*03* allele was found to be significantly increased in patients with AOMS compared to HC (23.6% vs. 13.4%). On the other hand, *HLA-DPB1*02* and *HLA-DPB1*04* were found to be significantly decreased ($p < 0.001$) in patients with AOMS compared to HC (22.2% vs. 36.6% and 79.2% vs.92.7%).

A total of 21 out of 87 patients (24.1%) were positive for the *HLA-DRB1*15* allele, which is significantly higher than the expected 11.4% allele frequency in HC ($p < 0.001$), confirming the well-established role of this allele in MS pathogenesis [33]. *HLA-DRB1*15* allele positivity was 20.8% (5/24) for EOMS and 25.4% (16/63) for AOMS ($p = 0.783$).

Table 3 presents the *HLA-DRB1*15* allele frequency among the different *HLA-DPB1** alleles. Only statistically significant associations are presented. The *HLA-DRB1*15* allele was statistically significantly absent among *HLA-DPB1*03* positive patients ($p = 0.001$) and among *HLA-DPB1*03* positive AOMS ($p = 0.003$), whereas it was significantly increased among *HLA-DPB1*04* ($p = 0.048$), *HLA-DPB1*14* ($p = 0.008$) -positive genotype patients. Finally, the *HLA-DRB1*15* allele was positive in the two *HLA-DPB1*14* positive patients with EOMS ($p = 0.036$).

In the 60 patients with available CSF examination, those with the *HLA-DPB1*02* allele had significantly higher IgG indexes than those who were negative for *HLA-DPB1*02* (mean 1.22 ± 0.70 vs. 0.75 ± 0.39, respectively, $p = 0.02$), irrespective of age of MS onset. There were no other significant associations between the *HLA-DPB1* or *HLA-DRB1* alleles (i.e., presence or not of each *HLA-DPB1** allele and *HLA-DRB1*15* allele) and gender, type of MS, MRI or CSF assessments (data not shown). Patients with AOMS who were positive for *HLA-DPB1*02* had significantly fewer relapses since onset than *HLA-DPB1*02* negative patients with AOMS (2.7 ± 2.5 vs. 3.9 ± 2.4, $p = 0.033$), corroborating the protective role of *HLA-DPB1*02* phenotype, as reported above (Figure 1). No other MS group effects on the HLA and clinical parameter associations were found.
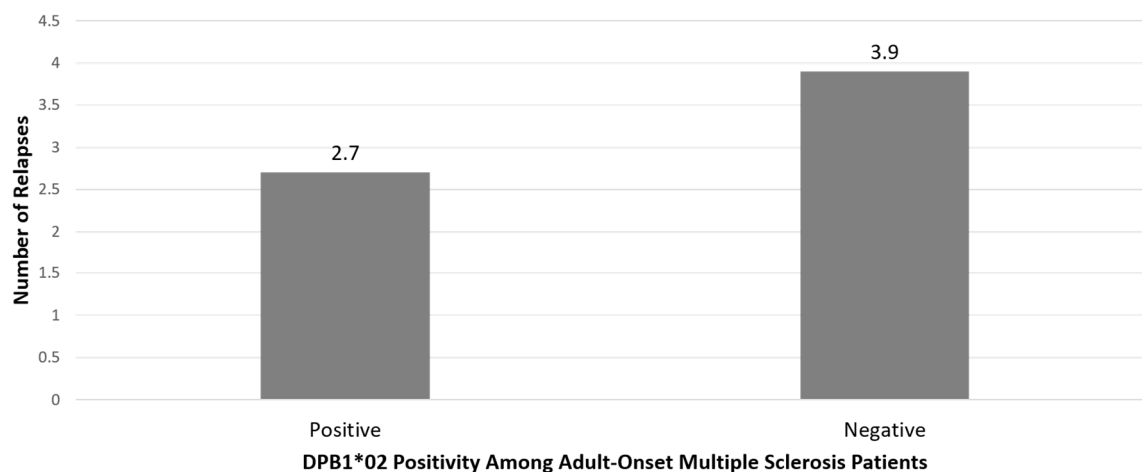


**Figure 1.** Number of relapses in adult-onset Multiple Sclerosis Patients with regards to HLA-*DPB1*02* genotype. Positive patients had significantly fewer relapses than negative ($p = 0.033$).

Table 2. *HLA-DPB1* Allele Frequencies Among Different Age of Multiple Sclerosis (MS) Onset Groups of Patients.

| | Early MS | Adult MS | HCs | Early vs. Adult MS | Early MS vs. HCs | Adult MS vs. HCs |
|---|---|---|---|---|---|---|
| | (N = 28) | (N = 72) | (N = 246) | Sig [1] | Sig [2] | Sig [2] |
| HLA-DPB1*01 | 3.6 | 4.2 | 4.5 | 0.85 (0.09–8.55) 1.000 | 0.79 (0.1–6.37) 0.500 | 0.92 (0.25–3.42) 0.500 |
| HLA-DPB1*02 | 39.3 | 22.2 | 36.6 | 2.27 (0.89–5.80) 0.131 | 1.12 (0.5–2.5) 0.461 | 0.5 (0.23–0.91) 0.008 ** |
| HLA-DPB1*03 | 17.9 | 23.6 | 13.4 | 0.7 (0.23–2.13) 0.602 | 1.4 (0.50–3.95) 0.339 | 2.0 (1.04–3.84) 0.009 ** |
| HLA-DPB1*04 | 64.3 | 79.2 | 92.7 | 0.47 (0.18–1.24) 0.132 | 0.14 (0.06–0.35) <0.001 ** | 0.3 (0.14–0.63) <0.001 ** |
| HLA-DPB1*05 | 3.6 | 2.8 | 2.4 | 1.3 (0.11–14.89) 1.000 | 1.48 (0.17–12.77) 0.500 | 1.14 (0.23–5.79) 0.500 |
| HLA-DPB1*06 | 0 | 1.4 | 0.8 | - 1.000 | - 0.500 | 1.72 (0.15–19.23) 0.500 |
| HLA-DPB1*09 | 0 | 1.4 | 2.8 | - 1.000 | - 0.372 | 0.48 (0.06-3.97) 0.356 |
| HLA-DPB1*10 | 10.7 | 9.7 | 4.9 | 1.11 (0.27–4.65) 1.000 | 2.34 (0.62–8.85) 0.162 | 2.1 (0.8–5.55) 0.052 |
| HLA-DPB1*11 | 0 | 1.4 | - | - 1.000 | - | - |
| HLA-DPB1*13 | 3.6 | 2.8 | 6.1 | 1.3 (0.11–14.89) 1.000 | 0.57 (0.07–4.49) 0.435 | 0.44 (0.1–1.97) 0.176 |
| HLA-DPB1*14 | 7.1 | 6.9 | 3.7 | 1 (0.18–5.48) 1.000 | 2.03 (0.42–9.88) 0.321 | 1.97 (0.64–6.06) 0.126 |
| HLA-DPB1*15 | 0 | 4.2 | 2.0 | - 0.557 | - 0.468 | 2.1 (0.49–8.99) 0.186 |
| HLA-DPB1*19 | 0 | 1.4 | 0.4 | - 1.000 | - 0.500 | 3.45 (0.21–55.87) 0.346 |
| HLA-DPB1*22 | 3.6 | 0 | - | - 0.280 | - | - |
| HLA-DPB1*23 | 3.6 | 0 | 2.4 | - 0.280 | 1.48 (0.17–12.77) 0.500 | - 0.172 |

**Table 2.** *Cont.*

| | Early MS | Adult MS | HCs | Early vs. Adult MS | Early MS vs. HCs | Adult MS vs. HCs |
|---|---|---|---|---|---|---|
| | (N = 28) | (N = 72) | (N = 246) | Sig [1] | Sig [2] | Sig [2] |
| *HLA-DPB1*32* | 3.6 | 0 | 0.8 | - 0.280 | 4.52 (0.4–51.49) 0.279 | - 0.460 |
| *HLA-DPB1*33* | 3.6 | 2.8 | 0.4 | 1.3 (0.11–14.89) 1.000 | 9.07 (0.55–149.24) 0.123 | 7.0 (0.63–78.34) 0.130 |
| *HLA-DPB1*34* | 0 | 1.4 | - | - 1.000 | - | - |
| *HLA-DPB1*35* | 0 | 4.2 | 0.8 | - 0.557 | - 0.500 | 10.65 (1.09–104.03) 0.005 ** |
| *HLA-DPB1*38* | 3.6 | 0 | - | - 0.280 | - | - |
| *HLA-DPB1*46* | 3.6 | 0 | - | - 0.280 | - | - |
| *HLA-DPB1*50* | 0 | 1.4 | 0.4 | - 1.000 | - 0.500 | 3.45 (0.21–55.87) 0.346 |
| *HLA-DPB1*56* | 0 | 1.4 | - | - 1.000 | - | - |

Numbers represent frequencies (%). 1 Fishers's exact test (23 comparisons) 2 Binomial tests (17 comparisons). ** $p \leq 0.015$ (17 comparisons) or $p \leq 0.013$ (23 comparisons), according to the Benjamini–Yekutieli method for 17 comparisons, HCs: Healthy Control

**Table 3.** Significant DRB1*15 Positivity Differences among *HLA-DPB1* Alleles and Age of Onset Groups in Multiple Sclerosis (MS) Patients.

| Total Sample of MS Patients | | |
|---|---|---|
| *HLA-DPB1* * Genotype | *HLA-DRB1*15* Positive | Sig [1] |
| *HLA-DPB1*03* | 0/22 (0%) | 0.001 * |
| *HLA-DPB1*04* | 19/63 (30.2%) | 0.048 * |
| *HLA-DPB1*14* | 5/7 (71.4%) | 0.008 * |
| **Adult-Onset MS** | | |
| *HLA-DPB1* * Genotype | *HLA-DRB1*15* Positive | Sig [1] |
| *HLA-DPB1*03* | 0/17 (0%) | 0.003 * |
| **Early-Onset MS** | | |
| *HLA-DPB1* * Genotype | *HLA-DRB1*15* Positive | Sig [1] |
| *HLA-DPB1*14* | 2/2 (100%) | 0.036 * |

Values represent observed frequencies (%) of *HLA-DRB1*15* allele positivity among the different *HLA-DPB1* genotypes. 1 Fisher exact tests. * $p \leq 0.05$.

## 4. Discussion

HLA-immunogenetics is an old but still rapidly expanding field in MS pathogenesis. In order to keep abreast of rapid developments in this field, we investigated the role of the *HLA-DP* locus in MS pathophysiology. We genotyped 100 Hellenic patients with MS for *HLA-DR* and *HLA-DP* alleles, as described above, which is a rather small sample and the main limitation of this study. *HLA-DPB1* genotyping was performed for the first time on a Hellenic MS population and in patients with EOMS, which is the core novelty of our research, albeit on a small sample (28 patients); however, we highlight again that EOMS is a rare disease entity and represents only the 3–5% of all MS patients in Caucasian populations.

In our study, we replicated the well-established predominance of the *HLA-DRB1*15* genotype in Hellenic patients with MS compared to HC, independently of age at disease onset [11].

No statistically significant *HLA-DPB1* allele differences were found between patients with EOMS and AOMS. All statistically significant differences were investigated in the AOMS group, except for the *HLA-DPB1*04* allele, which is lower in EOMS and AOMS, compared to the HC group at a high statistical level ($p < 0.001$, Table 2), suggesting a possible protective role in the Hellenic population. This is in contrast with an early study in 1988 [12] where the frequencies of DPw4 were 93.3% in patients with MS and 72.3% in controls (relative risk, $R^2 = 5.4$, $p = 0.0014$). Nevertheless, we have to mention that in this early study, the HLA-DNA typing was carried out on a small sample of 45 patients with MS and 63 controls of different ethnic European groups (Swedish and Danish), using the Restriction Fragment Length Polymorphism (RFLP) technique for *HLA-DP* and *HLA-DR* genes. In this same study, the *HLA-DR2* antigen was present in 75.5% of patients and in 33.7% of the controls ($R^2 = 6.1$, $p$ less than $10(-6)$). *HLA-DPw4* was not associated (i.e., was not in linkage disequilibrium) with *HLA-DR2* in patients or controls. Thus, the researchers concluded that in MS, the associations with *HLA-DP* and *HLA-DR* are independent of each other, but the combined presence of *HLA-DPw4* (cellularly defined) and *HLA-DR2* represented a significantly higher risk than either antigen alone, indicating that synergism between *HLA-DP* and *HLA-DR* gene products may play a role in the genetic susceptibility to MS. On the other hand, a recent study on celiac disease showed that the *HLA-DPB1*04:01* allele protects genetically-susceptible children from celiac disease [36], a fact that is in line with our results, concerning children and adults with MS, while in another study in 2015, another *HLA-DPB1*04* allele, namely *HLA-DPB1*04:02*, conferred a strong protective effect against narcolepsy [37]. Finally, the worldwide risk *HLA-DRB1*15* allele in MS, in Caucasians, was found to be significantly increased among *HLA-DPB1*04* positive patients with MS ($p = 0.048$) in our sample.

In another early study in France in 1991, it was found that the distribution of *HLA-DPB1* alleles was not significantly different in patients with MS and controls [20]. Nowadays, it is perfectly clear

that the *HLA-DP*03* allele is associated with MS and epitope spreading in MS [17,22], and in this study, we observed the risk susceptibility of this allele in our Hellenic MS sample, at a highly significant level ($p < 0.009$, Table 2).

Regarding AOMS, the *HLA-DPB1*03* allele could be a risk factor for the disease, as it was found to be significantly increased in patients with AOMS compared to HC. The percentage of *HLA-DPB1*03* positive patients with EOMS was higher than HC (17.9% vs. 13.4%), although at a nonstatistically significant level. The *HLA-DRB1*15* allele was absent among *HLA-DPB1*03* positive patients. This cannot be attributed to linkage disequilibrium, as this was tested. Linkage disequilibrium for the *HLA-DR* and *HLA-DP* genes was excluded in previous studies as well [12]. Since *HLA-DPB1*03* was found to be increased in AOMS, it may constitute a risk factor; this genotype may exert its risk factor effect only in the absence of *HLA-DRB1*15*, at least in AOMS. Moreover, the *HLA-DRB1*15* allele was found to be significantly increased among *HLA-DPB1*04*-positive patients, suggesting that *HLA-DPB1*04* exerts a protective effect only in the absence of *HLA-DRB1*15*. Despite the relatively small sample size in our study, these findings suggest that epistatic mechanisms between Class II *HLA-DR* and *HLA-DP* alleles may play a role in disease pathogenesis and risk of disease occurrence. This conclusion is in line with the results of Dekker et al. [19] who observed that in patients with MS who lacked *HLA-DQB1*06:02* allele, the *HLA-DPB1*03:01* allele frequency was significantly ($p = 0.006$) increased (50.0%) compared with *HLA-DQB1*06:02*-negative controls (9.1%). In parallel, in 2009, Lincoln et al. highlighted the role of epistasis between *HLA-DRB1*15* and *HLA-DQA1*01:02* alleles. More specifically, they proved that *HLA-DQA1*01:02*, which shows no primary MS association, increases disease risk when combined with *HLA-DRB1*15:01*, through transepistatic interactions [27]. Of note is the fact that the presented slight *HLA-DPB1* allele differences between AOMS and EOMS could also reflect the different clinical course of these two groups, given that patients with older age at onset are known to be more at risk of having secondary-progressive disease. For instance, predicting the onset of secondary-progressive multiple sclerosis is accomplished using genetic and nongenetic factors, with the *HLA-A*02:01* allele conferring a decreased risk for MS and also contributing to decreased hazards for SPMS [38].

Another finding in our study that is worthy of mention is the possible protective role of the *HLA-DPB1*02* allele in AOMS. *HLA-DPB1*02* was found to be significantly decreased in AOMS, while those who were *HLA-DPB1*02* positive had, in general, fewer relapses since onset compared to *HLA-DPB1*02*-negative patients with AOMS, corroborating the protective role of the *HLA-DPB1*02* allele reported above (Figure 1).

The *HLA-DPB1*35* allele was found to be significantly increased in patients with AOMS compared to HC, while increased prevalence of the *HLA- DRB1*15* allele in *HLA-DPB1*14*-positive patients with MS and patients with EOMS was also noted. Nevertheless, their possible genetic risk should be interpreted with caution, due to the very low frequency of these alleles.

At this point, we have to mention that the *HLA-DPB1*04* allele is the most frequent in the Hellenic population (92.7%), followed by *HLA-DP*02* (36.6%) and *HLA-DP*03* (13.4%) [30]. Additionally, according to our results, the most common *HLA-DPB1*-haplotypes in Hellenic patients with MS were *HLA-DPB1*04/DPB1*04* (27%), followed by *HLA-DPB1*02/DPB1*04* (13%), *HLA-DPB1*03/DPB1*04* (11%) and *HLA-DPB1*10/DP*04* (6%). Thus, the emerging protective role of the *HLA-DP*04* allele is in parallel with the *HLA-DR*11* allele, which is the most common in the Hellenic population, and the protective *HLA-DRB1* allele in Hellenic patients with MS [11].

The role of *HLA-DPB1* alleles has been studied in a range of other autoimmune diseases, especially NMOSD [23]. More specifically, *HLA-DPB1*05:01*, which is extremely rare in Caucasian populations, has the strongest association with opticospinal MS and anti-AQP4 seropositivity in Asian populations, while *HLA-DPB1*03* possibly offers genetic protection against the disease [23]. Moreover, *HLA-DPB1*02:01* has been associated with oligoarticular and rheumatoid factor-negative polyarticular juvenile idiopathic arthritis and childhood-onset diabetes type I in the Japanese population [24,25].

Therapeutic interventions in MS are sometimes difficult, because the patient's symptoms at the initial stages are not clearly suggestive of a definite demyelinating syndrome, especially in children. Furthermore, sometimes the neuroradiological (MRI) aspects and blood antibody tests are not helpful. In these situations, having a marker or a combination of markers that supports the differential diagnosis is of crucial importance, and has a direct impact on therapeutic decision making. The *HLA-DR* alleles, and especially the *HLA-DR\*15* allele, are the most robust genetic markers for almost every clinical or paraclinical aspect of the disease [4] in Caucasians and for the therapeutic response to different Disease Modified Treatments (DMTs) [4]. Nowadays, the expansion and overlap of various demyelinating diseases, namely MS, NMOSD, ADEM (Acute Disseminating Encephalomyelitis), MOG-Demyelinating (Myelin Oligodendrocyte Glycoprotein-Demyelinating) disease, Optic Neuritis, etc., make the need for specific biomarkers more urgent than ever before, as noted in our previous critical review [39] and in this works of other researchers [40].

Apart from a genetic association with MS and other demyelinating diseases, *HLA-DP* molecules play a key role in MS pathogenesis and progression, as described many years ago [17,41].

Additionally, in our Hellenic cohort, the *HLA-DP* alleles seemed to play an independent role in patients with MS (risk/protective), apart from the *HLA-DR* alleles, a fact that has to be confirmed in larger cohorts in the future. This could pave the way for the usage of these alleles in patient stratification (carriers and noncarriers)—as already happens with various HLA-DRB1 alleles and especially with the *HLA-DRB1\*15* allele [4,42]—for many MS characteristics and therapy responses in different DMTs in Caucasian populations [4,42].

Altogether, clarification of *HLA-DP* allele associations with both EOMS and AOMS is needed in every ethnic group to get a better idea of clinical features and MS phenotypes and disease progression, and as a form of future putative data for better therapeutics.

## 5. Conclusions-Limitations

In conclusion, our study supports the previously reported risk susceptibility role of the *HLA-DPB1\*03* allele in AOMS in many Caucasian populations. Additionally, we report, for the first time in the international literature, the protective role of the *HLA-DPB1\*04* allele for patients with both EOMS and AOMS, and the putative protective role of the *HLA-DPB1\*02* allele in patients with AOMS in our sample. Another finding that is worthy of mention is the total absence of the well-established *HLA-DRB1\*15* allele among patients having the most statistically frequent *HLA-DPB1\*03* allele in our cohort.

A limitation of our study was the relatively small sample size (28 patients with EOMS and 72 patients with AOMS). Indeed, observed small effect sizes for DPB1 alleles from the different group comparisons were the following: EOMS vs. AOMS 7%, EOMS vs. HCs 21.9% and AOMS vs. HCs 25.8%. However, this is a first attempt towards clarifying the role of the *HLA-DPB1* alleles in MS in a Hellenic AOMS and EOMS cohort. Moreover, the small study sample did not allow us to conduct multivariable analyses, which would more readily reveal confounding effects in our analyses.

These novel data could also contribute to personalized MS-therapeutics in the near future, taking into account the rapid expansion of our knowledge of multiple sclerosis and other distinct demyelinating diseases in many ethnic groups.

**Author Contributions:** Conceptualization, methodology, patients' curating, manuscript's drafting, manuscript's proofreading, M.A., statistical analyses, manuscript's drafting, manuscript's proofreading, A.A., patients' curating, bibliography manuscript's drafting, M.G., project administration, bibliography, C.S., data curating, N.M., S.K., critical review of the manuscript, K.K., L.S., statistical suggestions, validation, critical review of the manuscript, I.D. All authors have read and agreed to the published version of the manuscript

**Availability of Data and Material:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

## References

1. Ramagopalan, S.V.; Dyment, D.A.; Ebers, G.C. Genetic epidemiology: The use of old and new tools for multiple sclerosis. *Trends Neurosci.* **2008**, *31*, 645–652. [CrossRef]

2. Gruen, J.R.; Weissman, S.M. Human MHC Class III and IV Genes and Disease Associations. *Front. Biosci.* **2001**, *6*, D960–D972. [CrossRef] [PubMed]

3. Katsavos, S.; Anagnostouli, M. Biomarkers in Multiple Sclerosis: An Up-to-Date Overview. *Mult. Scler. Int.* **2013**, *2013*, 340508. [CrossRef] [PubMed]

4. Stamatelos, P.; Anagnostouli, M. HLA-Genotype in Multiple Sclerosis: The Role in Disease onset, Clinical Course, Cognitive Status and Response to Treatment: A Clear Step Towards Personalized Therapeutics. *Immunogenet* **2017**, *2*, 116.

5. Goodin, D.; Khankhanian, P.; Gourraud, P.A.; Vince, N. Highly conserved extended haplotypes of the major histocompatibility complex and their relationship to multiple sclerosis susceptibility. *PLoS ONE* **2018**, *13*, e0190043. [CrossRef] [PubMed]

6. Bozikas, V.P.; Anagnostouli, M.C.; Petrikis, P.; Sitzoglou, C.; Phokas, C.; Tsakanikas, C.; Karavatos, A. Familial bipolar disorder and multiple sclerosis: A three-generation HLA family study. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2003**, *27*, 835–839. [CrossRef]

7. International Multiple Sclerosis Genetics Consortium; Wellcome Trust Case Control Consortium 2; Sawcer, S.; Hellenthal, G.; Pirinen, M.; Spencer, C.C.; Patsopoulos, N.A.; Moutsianas, L.; Dilthey, A.; Su, Z.; et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* **2011**, *476*, 214–219.

8. Kular, L.; Liu, Y.; Ruhrmann, S.; Zheleznyakova, G.; Marabita, F.; Gomez-Cabrero, D.; James, T.; Ewing, E.; Lindén, M.; Górnikiewicz, B.; et al. DNA Methylation as a Mediator of HLA-DRB1*15:01 and a Protective Variant in Multiple Sclerosis. *Nat. Commun.* **2018**, *9*, 2397. [CrossRef]

9. Gianfrancesco, M.; Stridh, P.; Shao, X.; Rhead, B.; Graves, J.S.; Chitnis, T.; Waldman, A.; Lotze, T.; Schreiner, T.; Belman, A.; et al. Genetic risk factors for pediatric–onset multiple sclerosis. *Mult. Scler.* **2018**, *24*, 1825–1834. [CrossRef]

10. Venkateswaran, S.; Banwell, B. Pediatric multiple sclerosis. *Neurologist* **2010**, *16*, 92–105. [CrossRef]

11. Anagnostouli, M.C.; Manouseli, A.; Artemiadis, A.; Katsavos, S.; Fillipopoulou, C.; Youroukos, S.; Efthimiopoulos, S.; Doxiadis, I. HLA-DRB1* Allele Frequencies in Pediatric, Adolescent and Adult-Onset Multiple Sclerosis Patients, in a Hellenic Sample. Evidence for New and Established Associations. *J. Mult. Scler.* **2014**, *1*, 104. [CrossRef]

12. Odum, N.; Hyldic-Nielsenn, J.J.; Morling, N.; Sandberg-Wollheim, M.; Platz, P.; Svejgaard, A. HLA-DP antigens are involved in the susceptibility to multiple sclerosis. *Tissue Antigens* **1988**, *31*, 235–237. [CrossRef] [PubMed]

13. Wu, X.M.; Wang, C.; Zhang, K.N.; Lin, A.Y.; Kira, J.; Hu, G.Z.; Qu, X.H.; Xiong, Y.Q.; Cao, W.F.; Gong, L.Y. Association of susceptibility to multiple sclerosis in Southern Han Chinese with HLA-DRB1, -DPB1 alleles and DRB1-DPB1 haplotypes: Distinct from other populations. *Mult. Scler.* **2009**, *15*, 1422–1430. [CrossRef]

14. Fukazawa, T.; Yamasaki, K.; Ito, H.; Kikuchi, S.; Minohara, M.; Horiuchi, I.; Tsukishima, E.; Sasaki, H.; Hamada, T.; Nishimura, Y.; et al. Both the HLA-DPB1 and -DRB1 alleles correlate with risk for multiple sclerosis in Japanese: Clinical phenotypes and gender as important factors. *Tissue Antigens* **2000**, *55*, 199–205. [CrossRef] [PubMed]

15. Fukazawa, T.; Kikuchi, S.; Miyagishi, R.; Miyazaki, Y.; Yabe, I.; Hamada, T.; Sasaki, H. HLA-DPB1*0501 is not uniquely associated with opticospinal multiple sclerosis in Japanese patients. Important role of DPB1*0301. *Mult. Scler.* **2006**, *12*, 19–23. [CrossRef] [PubMed]

16. Yoshimura, S.; Isobe, N.; Yonekawa, T.; Matsushita, T.; Masaki, K.; Sato, S.; Kawano, Y.; Yamamoto, K.; Kira, J.; South Japan Multiple Sclerosis Genetics Consortium. Genetic and infectious profiles of Japanese multiple sclerosis patients. *PLoS ONE* **2012**, *7*, e48592. [CrossRef]

17. Yu, M.; Kinkel, R.P.; Weinstock-Guttman, B.; Cook, D.J.; Tuohy, V.K. HLADP: A class II restriction molecule involved in epitope spreading during the development of multiple sclerosis. *Hum. Immunol.* **1998**, *59*, 15–24. [CrossRef]

18. Field, J.; Browning, S.R.; Johnson, L.J.; Danoy, P.; Varney, M.D.; Tait, B.D.; Gandhi, K.S.; Charlesworth, J.C.; Heard, R.N.; Australia and New Zealand Multiple Sclerosis Genetics Consortium; et al. A polymorphism in the HLA-DPB1 gene is associated with susceptibility to multiple sclerosis. *PLoS ONE* **2010**, *5*, e13454. [CrossRef]

19. Dekker, J.W.; Easteal, S.; Jakobsen, I.B.; Gao, X.; Stewart, G.J.; Buhler, M.M.; Hawkins, B.R.; Higgins, D.A.; Yu, Y.L.; Serjeantson, S.W. HLA-DPB1 alleles correlate with risk for multiple sclerosis in Caucasoid and Cantonese patients lacking the high-risk DQB1*0602 allele. *Tissue Antigens* **1993**, *41*, 31–36. [CrossRef]

20. Roth, M.P.; Coppin, H.; Descoins, P.; Ruidavets, J.B.; Cambon-Thomsen, A.; Clanet, M. HLA-DPB1 gene polymorphism and multiple sclerosis: A large case-control study in the southwest of France. *J. Neuroimmunol.* **1991**, *34*, 215–222. [CrossRef]

21. Marrosu, M.G.; Cocco, E.; Costa, G.; Murru, M.R.; Mancosu, C.; Murru, R.; Lai, M.; Sardu, C.; Contu, P. Interaction of loci within the HLA region influences multiple sclerosis course in the Sardinian population. *J. Neurol.* **2006**, *253*, 208–213. [CrossRef] [PubMed]

22. Patsopoulos, N.A.; Barcellos, L.F.; Hintzen, R.Q.; Schaefer, C.; van Duijn, C.M.; Noble, J.A.; Raj, T.; IMSGC; ANZgene; Gourraud, P.A.; et al. Fine-mapping the genetic association of the major histocompatibility complex in multiple sclerosis: HLA and non-HLA effects. *PLoS Genet.* **2013**, *9*, e1003926.

23. Gontika, M.P.; Anagnostouli, M.C. Human leukocyte antigens immunogenetics of neuromyelitis optica or Devic's disease and the impact on the immunopathogenesis, diagnosis and treatment: A critical review. *Neuroimmunol. Neuroinflamm.* **2014**, *1*, 44–50. [CrossRef]

24. Hersh, A.O.; Prahalad, S. Immunogenetics of juvenile idiopathic arthritis: A comprehensive review. *J. Autoimmun.* **2015**, *64*, 113–124. [CrossRef] [PubMed]

25. Nishimaki, K.; Kawamura, T.; Inada, H.; Yagawa, K.; Nose, Y.; Nabeya, N.; Isshiki, G.; Tatsumi, N.; Niihira, S. HLA DPB1*0201 gene confers disease susceptibility in Japanese with childhood onset type I diabetes, independent of HLA-DR and DQ genotypes. *Diabetes Res. Clin. Pract.* **2000**, *47*, 49–55. [CrossRef]

26. Sato, S.; Isobe, N.; Yoshimura, S.; Kanamori, Y.; Masaki, K.; Matsushita, T.; Kira, J. HLA-DPB1*0201 is associated with susceptibility to atopic myelitis in Japanese. *J. Neuroimmunol.* **2012**, *251*, 110–113. [CrossRef] [PubMed]

27. Lincoln, M.R.; Ramagopalan, S.V.; Chao, M.J.; Herrera, B.M.; Deluca, G.C.; Orton, S.M.; Dyment, D.A.; Sadovnick, A.D.; Ebers, G.C. Epistasis among HLA-DRB1, HLA-DQA1, and HLA-DQB1 loci determines multiple sclerosis susceptibility. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 7542–7547. [CrossRef] [PubMed]

28. Moutsianas, L.; Jostins, L.; Beecham, A.H.; Dilthey, A.T.; Xifara, D.K.; Ban, M.; Shah, T.S.; Patsopoulos, N.A.; Alfredsson, L.; Anderson, C.A.; et al. Class II HLA interactions modulate genetic risk for multiple sclerosis. *Nat. Genet.* **2015**, *47*, 1107–1113.

29. Polman, C.H.; Reingold, S.C.; Banwell, B.; Clanet, M.; Cohen, J.A.; Filippi, M.; Fujihara, K.; Havrdova, E.; Hutchinson, M.; Kappos, L.; et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann. Neurol.* **2011**, *69*, 292–302. [CrossRef]

30. Kurtzke, J.F. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology* **1983**, *33*, 1444–1452. [CrossRef]

31. Marsh, S.G. Nomenclature for factors of the HLA system, update June 2012. *Tissue Antigens* **2012**, *80*, 289–293. [CrossRef]

32. Lancaster, A.K.; Single, R.M.; Solberg, O.D.; Nelson, M.P.; Thomson, G. PyPop update—A software pipeline for large-scale multilocus population genomics. *Tissue Antigens* **2007**, *69* (Suppl. 1), 192–197. [CrossRef]

33. Papassavas, E.C.; Spyropoulou-Vlachou, M.; Papassavas, A.C.; Schipper, R.F.; Doxiadis, I.N.; Stavropoulos-Giokas, C. MHC class I and class II phenotype, gene, and haplotype frequencies in Greeks using molecular typing data. *Hum. Immunol.* **2000**, *61*, 615–623. [CrossRef]

34. Benjamini, Y.; Drai, D.; Elmer, G.; Kafkafi, N.; Golani, I. Controlling the false discovery rate in behavior genetics research. *Behav. Brain Res.* **2001**, *125*, 279–284. [CrossRef]

35. Narum, S.R. Beyond Bonferroni: Less conservative analyses for conservation genetics. *Conserv. Genet.* **2006**, *7*, 783–787. [CrossRef]

36. Hadley, D.; Hagopian, W.; Liu, E.; She, J.X.; Simell, O.; Akolkar, B.; Ziegler, A.G.; Rewers, M.; Krischer, J.P.; Chen, W.M.; et al. HLA-DPB1*04:01 Protects Genetically Susceptible Children from Celiac Disease Autoimmunity in the TEDDY Study. *Am. J. Gastroenterol.* **2015**, *110*, 915–920. [CrossRef]

37. Ollila, H.M.; Ravel, J.M.; Han, F.; Faraco, J.; Lin, L.; Zheng, X.; Plazzi, G.; Dauvilliers, Y.; Pizza, F.; Hong, S.C.; et al. HLA-DPB1 and HLA class I confer risk of and protection from narcolepsy. *Am. J. Hum. Genet.* **2015**, *96*, 852. [CrossRef]

38. Misicka, E.; Sept, C.; Briggs, F.B.S. Predicting onset of secondary-progressive multiple sclerosis using genetic and non-genetic factors. *J. Neurol.* **2020**. [CrossRef]

39. Gontika, M.P.; Anagnostouli, M.C. Anti-Myelin Oligodendrocyte Glycoprotein and Human Leukocyte Antigens as Markers in Pediatric and Adolescent Multiple Sclerosis: On Diagnosis, Clinical Phenotypes, and Therapeutic Responses. *Mult. Scler. Int.* **2018**, *2018*, 8487471. [CrossRef]

40. Uher, T.; McComb, M.; Galkin, S.; Srpova, B.; Oechtering, J.; Barro, C.; Tyblova, M.; Bergsland, N.; Krasensky, J.; Dwyer, M.; et al. Neurofilament levels are associated with blood-brain barrier integrity, lymphocyte extravasation, and risk factors following the first demyelinating event in multiple sclerosis. *Mult. Scler.* **2020**. [CrossRef]

41. Warabi, Y.; Matsumoto, Y.; Hayashi, H. Interferon beta1b exacerbates multiple sclerosis with severe optic nerve and spinal cord demyelination. *J. Neurol. Sci.* **2007**, *252*, 57–61. [CrossRef]

42. Werneck, L.C.; Lorenzoni, P.J.; Kay, C.S.K.; Scola, R.H. Multiple sclerosis: Disease modifying therapy and the human leukocyte antigen. *Arq. Neuropsiquiatr.* **2018**, *76*, 697–704. [CrossRef] [PubMed]