Article

Alzheimer’s Disease Risk Variant rs3865444 in the CD33 Gene: A Possible Role in Susceptibility to Multiple Sclerosis

Juraj Javor 1,*, Mária Bucová 1, Vladimíra Ďurmanová 1, Dominika Radošinská 1, Zuzana Párnická 1, Daniel Čierny 2, Egon Kurča 3, Daniela Copíková-Cudráková 4, Karin Gmitterová 5 and Ivana Shawkatová 1

Abstract: Polymorphisms in genes encoding receptors that modulate the activity of microglia and macrophages are attractive candidates for participation in genetic susceptibility to multiple sclerosis (MS). The aims of the study were to (1) investigate the association between Alzheimer’s disease-linked variant rs3865444:C>A in the CD33 gene and MS risk, (2) assess the effect of the strongest MS risk allele HLA-DRB1*15:01 on this association, and (3) analyze the correlation of rs3865444 with selected clinical phenotypes, i.e., age of onset and disease severity. CD33 rs3865444 was genotyped in a cohort of 579 patients and 1145 controls and its association with MS risk and clinical phenotypes was analyzed by logistic and linear regression analysis, respectively. Statistical evaluation revealed that rs3865444 reduces the risk of MS in the HLA-DRB1*15:01-positive subpopulation but not in the cohort negative for HLA-DRB1*15:01. A significant antagonistic epistasis between rs3865444 and HLA-DRB1*15:01 alleles in the context of MS risk was detected by the interaction synergy factor analysis. Comparison of allele and genotype distribution between relapsing-remitting MS, secondary progressive MS, and control groups revealed that rs3865444 C to A substitution may also be associated with a decreased risk of transition of MS to its secondary progressive form, irrespective of the HLA-DRB1*15:01 carrier status. On the other hand, no correlation could be found between rs3865444 and the age of disease onset or MS severity score. Future studies are required to shed more light on the role of CD33 in MS pathogenesis.

Keywords: association; CD33; multiple sclerosis; polymorphism; rs3865444; severity; susceptibility

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by multifocal chronic inflammation, loss of oligodendrocytes, gliosis, and demyelination that leads to neuro-axonal degeneration and progressive neurological disability [1]. The exact cause of MS remains unknown, but complex interactions between genetic and environmental factors are at play [2]. Genome-wide association studies (GWAS) have greatly improved our understanding of MS pathogenesis by identifying numerous small-effect risk loci that form the genetic architecture of the disease [3]. The most recent and largest GWAS to date discovered 233 robustly associated susceptibility variants, which together with additional suggestive loci, explain up to 48% of the overall heritability of MS.
Additional analyses observed enrichment for MS susceptibility loci in different cells of the peripheral and brain-resident immune systems, including B and T cells, natural killer cells, myeloid cells, and microglia, emphasizing the role of both the adaptive and innate arms of immunity in MS development [4].

MS has been traditionally considered to develop as a result of autoreactive T and B cells migrating from the periphery and targeting myelin antigens in the CNS [2,5]. Recent studies have begun to highlight the key role of microglia and monocyte-derived macrophages (MDM), which are abundant in demyelinating lesions in the brain and the spinal cord [6,7]. These cells exhibit dynamic phenotypic plasticity and, depending on context and their activation state, may have both beneficial and detrimental roles in MS pathogenesis [5,8–10]. Their neurotoxic action is linked to the production of pro-inflammatory cytokines, reactive oxygen and nitrogen species, and the presentation of myelin-derived antigens to T cells, ultimately contributing to demyelination and axonal loss. The neuroprotective effects include removal of myelin debris and apoptotic cells by phagocytosis, release of anti-inflammatory molecules, and stimulation of the proliferation and growth of oligodendrocytes and their progenitor cells, all of which are essential for resolving inflammation, promoting remyelination, and preventing axonal degeneration [9,11–13]. Dysregulation of microglial/macrophage activation with a shift towards the neurotoxic phenotype can significantly disrupt the remyelination process that takes place in the early phases of MS, thereby contributing to disease development and progression [9,14,15].

The phagocytic uptake and clearance of protein aggregates and debris are under the control of several different membrane receptors, including the scavenger and pattern-recognition receptors, such as CD36, triggering receptor expressed on myeloid cells 2 (TREM2), sialic acid-binding immunoglobulin-type lectins (Siglecs), and others [8,16–18]. Their gene polymorphisms are therefore attractive candidates for participation in genetic susceptibility to MS. Recently, an association between the single nucleotide polymorphism (SNP) rs3865444:C>A in the CD33 gene and MS was reported in the Greek population [19]. Interestingly, rs3865444 is also one of the top-ranked Alzheimer’s disease (AD) risk variants [20–23], indicating that some individual neuroinflammatory mechanisms are shared by different neurological diseases such as MS and AD [15]. CD33 is located on chromosome 19q13.41 and encodes a 67-kDa transmembrane receptor CD33, also known as Siglec-3, expressed mainly on cells of the myeloid lineage, including granulocytes, microglia, monocytes, macrophages, and dendritic cells [24]. Its extracellular ligand-binding Ig V-set domain recognizes sialic acid-containing glycoproteins and glycolipids on the surface of mammalian cells, triggering inhibitory signaling involved in the regulation of myeloid cell function and activities [24–26]. The rs3865444 SNP located upstream of the CD33 transcription start site is indirectly associated with increased production of alternatively spliced CD33 isoform (CD33m, D2-CD33) lacking the Ig V-set domain at the expense of the full-length CD33M [27–35], resulting in alterations of microglial and macrophage functions [36–39].

Independent replication remains an important tool for understanding the role of genetic risk factors across different populations and ethnicities. Given that the role of CD33 rs3865444 in MS susceptibility was reported only in a single paper [19], we decided to perform a case-control study to evaluate the impact of this SNP on MS risk in the Slovak population and further examine whether its association with the disease is affected by the HLA-DRB1*15:01 allele as the single strongest MS genetic risk factor [40,41]. In addition to susceptibility, genetic determinants may also influence various clinical phenotypes [3]. Therefore, we also analyzed the effect of rs3865444 on the age of MS onset and disease severity.

2. Materials and Methods
2.1. Study Subjects

A total of 1724 Slovak Caucasian subjects were recruited for the purposes of an ongoing study of MS risk factors. The MS patient group consisted of 579 unrelated individuals
(403 females and 176 males) recruited at the neurology departments of university hospitals in Bratislava and Martin, Slovakia. The diagnosis of MS was based on the 2010 revised McDonald criteria [42], and only patients with a relapse-onset form of the disease, namely relapsing-remitting (RR) or secondary progressive (SP) MS, were included in the study. The age of onset (AOO) was defined by the first episode of neurological dysfunction suggestive of CNS demyelinating disease. The degree of patients’ neurological disability at the time of examination was determined using Kurtzke’s Expanded Disability Status Scale (EDSS) [43], which was subsequently used to assess the Multiple Sclerosis Severity Score (MSSS) as a measure of the rate of disability accumulation and disease severity. For this purpose, a table with global MSSS generated from 9892 European patients was employed, and the score of each individual patient was simply ascertained by finding the column corresponding to the patient’s EDSS and the row corresponding to the number of years since the onset of MS [44]. The control group comprised 1145 unrelated adults (717 females and 428 males) without a personal or family history of MS and other common autoimmune and neurological diseases. The basic demographic and clinical characteristics of patients and controls are summarized in Table 1.

### Table 1. Demographic and clinical characteristics of multiple sclerosis (MS) patients and controls.

| Parameter                  | Controls (n = 1145) | MS Total (n = 579) | p-Value | MS Females (n = 403) | MS Males (n = 176) | p-Value |
|----------------------------|---------------------|-------------------|---------|----------------------|-------------------|---------|
| Age (years)                | 51.86 ± 21.09       | 41.61 ± 10.51     | <0.0001 | 41.84 ± 10.46        | 41.08 ± 10.62     | 0.26    |
| Age of onset (years)       | -                   | 29.68 ± 9.78      | -       | 29.63 ± 9.60         | 29.80 ± 10.21     | 0.87    |
| Sex (females/males)        | 717/428             | 403/176           | 0.0041  | -                    | -                 | -       |
| MS course (RR/SP)          | -                   | 511/68            | -       | 358/45               | 153/23            | 0.51    |
| MS duration (years)        | -                   | 11.91 ± 7.13      | -       | 12.19 ± 7.11         | 11.26 ± 7.15      | 0.059   |
| EDSS                       | -                   | 3.56 ± 1.56       | -       | 3.56 ± 1.47          | 3.58 ± 1.76       | 0.71    |
| MSSS                       | -                   | 4.41 ± 2.09       | -       | 4.33 ± 1.97          | 4.60 ± 2.32       | 0.19    |
| HLA-DRB1*15:01 positivity  | 230 (20.09%)        | 298 (51.47%)      | <0.0001 | 214 (53.10%)         | 84 (47.73%)       | 0.23    |

Data are shown as the mean with standard deviation or as number (%). Significant p-values are shown in bold.

EDSS: Expanded Disability Status Scale; MS: multiple sclerosis; MSSS: Multiple Sclerosis Severity Score; RR: relapsing-remitting; SD: standard deviation; SP: secondary progressive.

Written informed consent for the enrolment in the study and for personal data management was obtained from all study participants. The investigations were carried out in accordance with the International Ethical Guidelines and the World Medical Association Declaration of Helsinki. The study was approved by the Independent Ethical Committee of the University Hospital Bratislava and the Faculty of Medicine, Comenius University in Bratislava.

### 2.2. Genotyping

Genomic DNA was extracted from EDTA-treated blood samples using the standard phenol-chloroform method. Genotyping of CD33 rs386544 as well as of the specific HLA-DRB1*15:01-tagging SNP rs3135388 [45,46] was performed by the polymerase chain reaction-restriction fragment length polymorphism method according to previously published protocols [47,48]. For quality control, 10% of samples were randomly selected and genotyped in duplicate, and several cases of each genotype were confirmed by direct DNA sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit and Applied Biosystems 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA, USA). The reproducibility of the results was 100%.

### 2.3. Statistical Analysis

Differences in categorical variables (sex, MS type, HLA-DRB1*15:01 carrier status) between the study groups were evaluated by the χ² test, whereas continuous variables (age, AOO, disease duration, EDSS, MSSS) were compared by the Welch’s corrected t-test (for normally distributed data) or Mann–Whitney test (for nonparametric data). Positive
HLA-DRB1*15:01 carrier status was defined as the presence of at least one copy of rs3135388 T allele tagging the HLA-DRB1*15:01 allele. The statistical power of our case-control study was calculated using the online web tool Genetic Association Study (GAS) Power Calculator (https://csg.sph.umich.edu/abecasis/gas_power_calculator/index.html, accessed on 13 July 2022). CD33 rs3865444 genotypes were tested for possible departure from Hardy–Weinberg equilibrium (HWE) by the \( \chi^2 \) goodness-of-fit test with 1 degree of freedom. The association between rs3865444 and MS risk was examined by the logistic regression analysis adjusted for age, sex, and HLA-DRB1*15:01 carrier status as possible confounding covariates. \( p \), odds ratio (OR), and 95% confidence interval (CI) values were computed for the effects of alleles or genotypes in allelic, codominant, dominant, recessive, over-dominant, and log-additive inheritance models. Regression analysis and synergy factor (SF) measurement were used to assess the significance and size of the interaction between CD33 rs3865444 A and HLA-DRB1*15:01 alleles, as previously described [49]. SF is defined as the ratio of the observed OR for both factors combined (OR\(_1\) × OR\(_2\)) to the predicted OR assuming independent effects of each factor (OR\(_1\)). The correlation of CD33 rs3865444 genotypes with AOO and MSSS was tested using the linear regression analysis. \( p \)-values < 0.05 obtained in the above mentioned statistical tests were considered statistically significant. The analyses were performed with the InStat statistical software package (GraphPad Software, Inc., San Diego, CA, USA) and the SNPStats web software available at http://bioinfo.iconcologia.net/SNPstats, accessed on 14 April 2022 [50].

3. Results

3.1. Characteristics of Study Subjects

A total of 579 patients diagnosed with MS were enrolled in this study, including 403 (69.6%) females and 176 (30.4%) males. The mean age was 41.6 years, the mean age of disease onset was 29.7 years, and the mean duration of MS was 11.9 years. The comparison of basic clinical parameters did not reveal any significant differences between male and female MS patients (Table 1). The control group comprised 1145 unrelated individuals with a mean age of 51.9 years, out of whom 717 (62.6%) were females and 428 (37.4%) were males. As shown in Table 1, the mean age of controls was significantly higher (\( p < 0.0001 \)) and their female-to-male ratio was lower (\( p = 0.0041 \)) compared to MS patients. Consistent with our previous observations [51,52], carriers of at least one copy of the major MS risk allele HLA-DRB1*15:01 were significantly overrepresented in the MS group compared to controls (51.5% vs. 20.1%; \( p < 0.0001 \)). As shown in Supplementary Table S1, one copy of the HLA-DRB1*15:01 allele was associated with almost 4-fold increased odds of MS (OR = 3.77; 95% CI = 2.98–4.76; \( p < 0.0001 \)) while subjects homozygous for the allele had 17-fold increased odds of developing the disease (OR = 17.01; 95% CI = 7.73–37.39; \( p < 0.0001 \)). Due to their different distribution between the patient and control groups, HLA-DRB1*15:01 carrier status, age, and sex were included in CD33 rs3865444 association analyses as possible confounding factors.

3.2. Association of CD33 rs3865444 with MS Risk

The statistical power of our case-control study was estimated using the online GAS Power Calculator. With 579 cases and 1145 controls, and assuming the MS prevalence of 0.1%, minor allele frequency of 31%, and an additive model, the power of our study to detect an association at a significance level of 0.05 was 88% for a relative risk equal to 1.3 but only 22% for a relative risk of 1.1. The genotype distribution of CD33 rs3865444 did not show any significant departure from HWE in MS patients (\( \chi^2 = 0.78, p = 0.38 \)) or controls (\( \chi^2 = 0.62, p = 0.43 \)). Analysis of rs3865444 allele frequencies in study cohorts revealed no significant difference between MS patients and controls (allele A: 30.4% vs. 31.1%; \( p = 0.68 \); OR = 0.97; 95% CI = 0.83–1.13). In line with this finding, logistic regression analysis adjusted for the HLA-DRB1*15:01 carrier status, age, and sex did not find an association of CD33 rs3865444 with MS risk in any of the genetic models (Table 2).
Table 2. Association between CD33 rs3865444 and MS in the whole population.

| Allele/Genotype | MS (n = 579) | Controls (n = 1145) | Genetic Model | Logistic Regression Analysis |
|-----------------|-------------|---------------------|---------------|-----------------------------|
|                 |             |                     |               | p-Value  | OR (95% CI)          |
| C               | 806 (69.60%)| 1578 (68.91%)       | Allele contrast (A vs. C) | 0.68    | 0.97 (0.83–1.13)    |
| A               | 352 (30.40%)| 712 (31.09%)        | Codominant (CA vs. CC) | 0.45    | 0.91 (0.73–1.15)    |
| CC              | 285 (49.22%)| 538 (46.99%)        | Dominant (AA + CA vs. CC) | 0.52    | 0.93 (0.75–1.16)    |
| CA              | 236 (40.76%)| 502 (43.84%)        | Recessive (AA vs. CA + CC) | 0.83    | 1.04 (0.72–1.50)    |
| AA              | 58 (10.02%) | 105 (9.17%)         | Over-dominant (CA vs. CC + AA) | 0.43    | 0.91 (0.73–1.14)    |

Allele and genotype counts are presented with frequencies in parentheses. p, OR, and 95% CI values for genotype comparisons were adjusted for age, sex, and HLA-DRB1*15:01 carrier status. CI: confidence interval; MS: multiple sclerosis; OR: odds ratio.

To explore the possible effect of the HLA-DRB1*15:01 allele on the association between rs3865444 and MS, we next examined allele and genotype distribution in cohorts stratified according to the HLA-DRB1*15:01 carrier status. Logistic regression analysis revealed an association of the rs3865444:C>A substitution with a reduced risk of MS in the HLA-DRB1*15:01-positive subpopulation under codominant (p = 0.028, OR = 0.64, 95% CI = 0.44–0.95), dominant (p = 0.040, OR = 0.68, 95% CI = 0.47–0.98), and over-dominant models (p = 0.031, OR = 0.66, 95% CI = 0.46–0.96), while no significant effect of rs3865444 on MS risk was found in the subpopulation negative for the HLA-DRB1*15:01 allele (Table 3).

Table 3. Association between CD33 rs3865444 and MS in cohorts stratified according to the HLA-DRB1*15:01 carrier status.

| Allele/Genotype | MS | Controls | Genetic Model | Logistic Regression Analysis |
|-----------------|----|----------|---------------|-----------------------------|
|                 |    |          |               | p-Value  | OR (95% CI)          |
| HLA-DRB1*15:01-positive cohort |   |          |               |          |                      |
| C               | 424 (71.14%)| 308 (66.96%)| Allele contrast (A vs. C) | 0.14    | 0.82 (0.63–1.07)    |
| A               | 172 (28.86%)| 152 (33.04%)| Codominant (CA vs. CC) | 0.028   | 0.64 (0.44–0.95)    |
| CC              | 154 (51.68%)| 99 (34.04%) | Dominant (AA + CA vs. CC) | 0.62    | 0.85 (0.44–1.64)    |
| CA              | 116 (38.32%)| 110 (35.83%)| Recessive (AA vs. CA + CC) | 0.89    | 1.04 (0.55–1.97)    |
| AA              | 28 (9.39%) | 21 (9.13%) | Over-dominant (CA vs. CC + AA) | 0.031   | 0.66 (0.46–0.96)    |

| HLA-DRB1*15:01-negative cohort |   |          |               |          |                      |
| C               | 382 (67.97%)| 1270 (69.41%)| Allele contrast (A vs. C) | 0.52    | 1.07 (0.87–1.31)    |
| A               | 180 (32.03%)| 560 (30.60%)| Codominant (CA vs. CC) | 0.50    | 1.10 (0.82–1.47)    |
| CC              | 131 (46.62%)| 439 (47.98%) | Dominant (AA + CA vs. CC) | 0.63    | 1.10 (0.69–1.77)    |
| CA              | 120 (42.70%)| 392 (42.84%) | Recessive (AA vs. CA + CC) | 0.49    | 1.10 (0.84–1.45)    |
| AA              | 30 (10.68%) | 84 (9.18%) | Over-dominant (CA vs. CC + AA) | 0.58    | 1.08 (0.82–1.43)    |

Allele and genotype counts are presented with frequencies in parentheses. p, OR, and 95% CI values for genotype comparisons were adjusted for age and sex. Significant p-values are shown in bold. CI: confidence interval; MS: multiple sclerosis; OR: odds ratio.

To further evaluate the statistical epistasis between CD33 rs3865444 A and HLA-DRB1*15:01 alleles, we performed an interaction SF analysis and assessed the risk of developing MS in subjects carrying either one of these traits or both when compared to subjects negative for both alleles. As shown in Table 4, the observed combined effect size of the two alleles (OR = 3.67) was lower than the predicted joint OR assuming independent effects of both rs3865444 A and HLA-DRB1*15:01 (OR = 5.93). As a result, the calculated SF value of 0.62 significantly deviated from 1 (p = 0.032), suggesting that CD33 rs3865444 A and HLA-DRB1*15:01 alleles displayed an antagonistic statistical interaction in the context of
MS risk. On the other hand, no such interaction was observed between the CD33 rs3865444 A allele and sex \((p = 0.68)\).

### Table 4. Statistical interaction between CD33 rs3865444 A and HLA-DRB1*15:01 alleles.

| CD33 rs3865444 A | HLA-DRB1*15:01 | MS \((n = 579)\) | Controls \((n = 1145)\) | Logistic Regression Analysis | SF \((p\text{-Value})\) |
|------------------|----------------|-----------------|--------------------------|-----------------------------|-------------------|
|                  |                |                 |                          |                             |                   |
| − −              | −              | 131 (22.63%)    | 439 (38.34%)             | reference                   | 0.62 (0.032)      |
| + −              | −              | 150 (25.91%)    | 476 (41.57%)             | 0.49                        | 1.10 (0.84–1.45)  |
| − +              | +              | 154 (26.60%)    | 99 (8.65%)               | \(<0.0001\)                 | 5.39 (3.87–7.52)  |
| + +              | +              | 144 (24.87%)    | 131 (11.44%)             | \(<0.0001\)                 | 3.67 (2.68–5.03)  |

The "−" sign denotes no copies of the allele, while the "+" sign denotes the presence of at least one copy of the allele. Allele counts are presented with frequencies in parentheses. \(p\), OR, and 95% CI values were adjusted for age and sex. Significant \(p\)-values are shown in bold. SF was calculated as the ratio of the observed OR for both factors combined \((3.67)\) to the predicted OR assuming independent effects of each factor \((1.10 \times 5.39 = 5.93)\).

Next, we compared the CD33 rs3865444 allele and genotype distribution between RR-MS, SP-MS, and control groups. As shown in Table 5, carriers of the minor A allele were significantly underrepresented in the SP-MS group compared to both the RR-MS (dominant model: \(p = 0.0023\), OR = 0.41, 95% CI = 0.23–0.74) and control groups (dominant model: \(p = 0.0003\), OR = 0.38, 95% CI = 0.22–0.65), suggesting that rs3865444 C to A substitution is associated with a decreased risk of developing the secondary progressive form of the disease. Interestingly, this protective effect was observed in both HLA-DRB1*15:01-positive and negative cohorts (Supplementary Table S2).

### Table 5. Comparison of CD33 rs3865444 allele and genotype distribution between RR-MS, SP-MS, and control groups.

| A/G     | SP-MS \((n = 511)\) | RR-MS \((n = 68)\) | GM | RR-MS vs. C | SP-MS vs. C | SP-MS vs. RR-MS |
|---------|---------------------|-------------------|----|-------------|-------------|-----------------|
| C       | 696 (68.10%)        | 110 (80.88%)      | AC | 0.64        | 0.0032      | 0.0023          |
| A       | 326 (31.90%)        | 26 (19.12%)       | CD1| 0.78        | 0.0005      | 0.0072          |
| CC      | 239 (46.77%)        | 46 (67.65%)       | D  | 0.67        | 0.0003      | 0.0023          |
| CA      | 218 (42.66%)        | 18 (26.47%)       | R  | 0.59        | 0.0016      | 0.0022          |
| AA      | 54 (10.57%)         | 4 (5.88%)         | OD | 0.92        | 0.0007      | 0.0028          |

Allele and genotype counts are presented with frequencies in parentheses. \(p\), OR, and 95% CI values for genotype comparisons were adjusted for age, sex, and HLA-DRB1*15:01 carrier status. AC: allele contrast model (A vs. C); A/G: allele/genotype; C: controls; CD1: codominant model (CA vs. CC); CD2: codominant model (AA vs. CC); CI: confidence interval; D: dominant model (AA + CA vs. CC); GM: genetic model; LA: log-additive model; OD: over-dominant model (CA vs. CC + AA); OR: odds ratio; R: recessive model (AA vs. CA + CC); RR-MS: relapsing-remitting multiple sclerosis; SP-MS: secondary progressive multiple sclerosis. Significant \(p\)-values are shown in bold.

### 3.3. Association of CD33 rs3865444 with Clinical Phenotypes

Linear regression analysis employed to evaluate the relationship between CD33 rs3865444 and selected clinical parameters revealed no significant association of the polymorphism with AOO \((p = 0.88)\) or MSSS \((p = 0.57)\) in the whole patient group. Following the stratification according to the HLA carrier status, we could observe a tendency towards a later MS onset in rs3865444 AA homozygotes within the HLA-DRB1*15:01-positive patient cohort, while the opposite trend was found in the HLA-DRB1*15:01-negative cohort. However, neither of these findings reached the level of statistical significance (Table 6).
Table 6. Association of CD33 rs3865444 with MS phenotypes.

| Phenotype | Patient Group | Genotypes | Best Model | p-Value |
|-----------|---------------|-----------|------------|---------|
|           |               | CC        | CA         | AA      |         |
|           | Whole         | 29.68 ± 9.66 | 29.62 ± 9.88 | 29.88 ± 10.09 | dominant | 0.88 * |
|           | AOO +        | 28.60 ± 8.72 | 28.22 ± 9.22 | 30.54 ± 11.15 | recessive | 0.27 † |
|           | AOO −        | 30.96 ± 10.56 | 30.98 ± 10.34 | 29.27 ± 9.14 | recessive | 0.36 † |
|           | Whole MSSS   | 4.43 ± 2.12 | 4.43 ± 2.10 | 4.27 ± 1.93 | recessive | 0.57 † |
|           | MSSS +       | 4.51 ± 1.99 | 4.45 ± 2.10 | 4.32 ± 1.95 | recessive | 0.41 ‡ |
|           | MSSS −       | 4.34 ± 2.26 | 4.41 ± 2.11 | 4.23 ± 1.94 | dominant | 0.72 $ |

Data are shown as the mean with standard deviation. The “−” sign denotes no copies of the HLA allele, while “+” sign denotes the presence of at least one copy of the allele. Linear regression analysis was adjusted for: * sex and HLA-DRB1*15:01 carrier status; † sex; ‡ AOO, sex, and HLA-DRB1*15:01 carrier status; $ AAO and sex. AOO: age of onset; MSSS: Multiple Sclerosis Severity Score.

4. Discussion

MS is a multifactorial disorder characterized by chronic inflammation and demyelination, which is followed by remyelination attempts to aid in tissue repair and regeneration [2,7]. The phagocytic uptake and removal of myelin debris by CNS-resident microglia and MDM are particularly important for CNS repair as debris accumulation promotes inflammation, impairs axonal regeneration, arrests the differentiation of oligodendrocyte precursor cells, and inhibits remyelination [14,17,53,54]. Deficient clearance of myelin debris may result in the inability to resolve the inflammatory process and promote remyelination, thereby heightening the susceptibility to MS [14,55]. Multiple membrane receptors have been implicated in controlling microglial/macrophage cell functions and phagocytic activity in the brain, including the Siglec family member CD33 [24]. As an immunomodulatory receptor, CD33 participates in homeostatic cellular mechanisms by suppressing innate immune cells following the recognition of specific glycosylation patterns that act as “self-associated molecular patterns” (SAMP). Upon binding of extracellular or cell-surface sialylated glycosphingolipids or glycoproteins to its amino-terminal Ig V-set domain, CD33 triggers inhibitory signals through a cytosolic immunoreceptor tyrosine-based inhibitory motif (ITIM) and an ITIM-like sequence, effectively reducing microglial phagocytic capacity [24,25].

Recently, an association of the AD-linked variant CD33 rs3865444:C>A with a reduced risk of MS was reported in a Greek case-control study [19], suggesting a shared role for CD33 in the pathogenesis of both diseases. In the current study, we aimed to validate this association in the Central European Slovak population and further examine the impact of rs3865444 on the age of disease onset and MS severity. Our initial analysis revealed no significant differences in allele or genotype frequencies between MS cases and controls, indicating that rs3865444 does not confer risk or protection for MS. This is in agreement with the largest GWAS study to date, which did not identify any CD33 SNP as significantly associated with MS [4]. Possible reasons for the observed discrepancy between the studies may include relatively limited sample sizes and statistical power in both our study and that of Šiokas et al. [19], inter-population variation in rs3865444 allele frequencies, differences in linkage disequilibrium between the studied SNP and other functional CD33 variants, differences in MS/control selection criteria, and others [56]. Another explanation could be the modifying effects of other genetic factors. Case-control association studies have traditionally analyzed individual contributions of candidate gene variants to disease risk, thereby neglecting interactive effects between genetic variants, which may be larger or lower than the main effects at the individual loci or even exist without a significant effect of either of them [57]. Given that the HLA-DRB1*15:01 allele is the single strongest genetic risk factor for MS, it is possible that it modulates the expression of MS risk variants via epistasis, making them precluded in GWAS and other studies. Therefore, we were interested in whether the association of CD33 rs3865444 with MS is affected by this HLA allele, which encodes the major histocompatibility complex class II antigen-presenting molecule HLA-
DR15. Analysis in cohorts stratified according to the HLA carrier status indeed revealed a protective effect of rs3865444:C>A substitution in a subpopulation of individuals carrying HLA-DRB1*15:01, while no significant association was found in subjects negative for this HLA allele. In line with the results of the Greek study [19], the protective effect was attributable to the CA genotype, while CC appeared to be the risk-increasing genotype. Furthermore, the interaction SF analysis confirmed a significant statistical epistasis between CD33 rs3865444 A and HLA-DRB1*15:01 alleles, suggesting that they display an antagonistic interaction in the context of MS risk. On the other hand, no interaction could be found between rs3865444 and sex, indicating that this CD33 variant exerts similar effects on MS risk in both sexes. In addition to contributing to the risk of developing the disease, rs3865444 may also affect the course of MS. The results of the present study indicate that the minor A allele is associated with a decreased risk of transition of MS to its secondary progressive form, irrespective of the HLA-DRB1*15:01 carrier status. Furthermore, phenotype-genotype analyses in our MS patients showed no evidence for a significant correlation between rs3865444 and the age of disease onset or MSSS. Altogether, these results suggest that the CD33 polymorphism might play distinct roles in the disease initiation, progression, and its severity.

The rs3865444:C>A SNP is located 372 bp upstream of the CD33 transcription start site and is indirectly, via linkage disequilibrium with the rs12459419 SNP in exon 2, linked to alterations in splicing efficiency and an enhanced rate of exon 2 skipping. As a result, the protective minor A allele is associated with increased production of the short CD33m isoform lacking the exon 2-encoded ligand-binding Ig V-set domain at the expense of the functional full-length CD33M [27–35]. While the wild-type CD33M isoform was shown to repress microglial proliferation, migration, and phagocytosis of cargo [29,36,38], the alternatively spliced CD33m has the opposite effect and is associated with increased microglial and macrophage function, i.e., enhanced proliferation, phagocytosis, and clearance of protein aggregates, cells, or debris [37–39]. The effects of CD33 and its gene polymorphisms on microglia/macrophage-mediated phagocytosis have been most extensively studied in AD, a disease characterized by a failure to clear extracellular amyloid-β (Aβ) peptides from the brain [24]. Here, CD33 activity negatively correlates with the uptake of Aβ and promotes amyloid pathology [28,29]. CD33 rs3865444 A allele likely protects from AD by enhancing Aβ clearance and thus decreasing Aβ plaque burden [25,28,29]. These findings suggest that Aβ plaque can dodge microglia-mediated clearance with the help of sialic acid–CD33 interaction [26].

At the moment, we can only speculate that a similar CD33-related mechanism is also at play in MS pathogenesis. Several recent findings may provide support for our hypothesis. As already mentioned, efficient clearance of myelin debris is important to promote remyelination and inhibit inflammation [54]. CD33 may be among the membrane receptors regulating this process, as CD33M expression was shown to negatively correlate with the uptake of different types of cargo, including myelin [36]. As an ITIM-driven immunoreceptor, CD33 is involved in the modulation of cellular functions by counteracting the activatory signaling induced by various immunoreceptor tyrosine-based activation motif (ITAM) receptors, including TREM2 [58]. The phospholipid-sensing receptor TREM2 is highly expressed by microglia and macrophages in active MS lesions and was shown to promote their survival, proliferation, and phagocytic activity in response to demyelination [16,59,60]. Recent findings indicate that TREM2 activation increases myelin debris uptake and degradation as well as the formation of mature oligodendrocytes from precursor cells, resulting in accelerated clearance of debris by microglia and enhanced remyelination and axonal integrity [16]. It is tempting to suggest that lack of inhibitory signaling in the protective CD33m isoform due to loss of its ligand-binding capacity would unblock TREM2 signaling and lead to enhanced phagocytosis and degradation of myelin debris, a key step to allowing remyelination and resolution of inflammation. Recently, an alternative hypothesis for the protective effect of rs3865444 A allele in AD was proposed, suggesting that its association with enhanced phagocytic activity does not stem from decreased expression of functional
inhibitory CD33M, but rather due to CD33m being a gain-of-function variant [25]. Here, the loss of the Ig V-set domain would allow CD33m to cluster in a novel conformation and constitutively act as an ITAM to activate microglial function [25]. A follow-up study did provide support for this hypothesis by showing that CD33m has a preference for intracellular location and its association with enhanced phagocytosis is dependent on its cytoplasmic signaling motifs and not due to loss of ligand binding [37]. However, it remains to be elucidated if this mechanism also applies to MS.

Besides its strengths, this study also has several limitations. First, the sample sizes and statistical power of the study were relatively limited, and thus additional analyses in independent populations are warranted to validate our findings. Second, the study focused only on the best known CD33 polymorphism, thus omitting other variants within or in close proximity of the CD33 gene which could also contribute to MS risk. Third, although we found a significant statistical epistasis between rs3865444 A and HLA-DRB1*15:01 alleles, we cannot exclude the possibility that other genetic variants also have effects on the association of rs3865444 with MS risk. Fourth, we used MSSS to assess the severity of MS in the current study. This approach, although traditionally used, is not entirely optimal as MSSS may not be a stable measure of long-term outcome and relies on a single EDSS measurement that is prone to inaccuracy [61]. Therefore, future studies should consider survival analysis of long-term disability data.

5. Conclusions

This study provides additional evidence for the possible role of the CD33 rs3865444:C>A SNP as one of the genetic driving forces involved in MS development. Interestingly, the protective effect of the C to A substitution on susceptibility to MS seems to be limited to a subpopulation positive for the major MS risk allele HLA-DRB1*15:01. Furthermore, rs3865444 may also reduce the risk of developing the secondary progressive form of MS, but it does not seem to have an individual impact on the age of disease onset or its severity. Additional studies are required to shed more light on the antagonistic epistasis between CD33 rs3865444 A and HLA-DRB1*15:01 alleles and fully understand the role of CD33 in MS pathogenesis. Therapeutic strategies targeting CD33 through small molecules, monoclonal antibodies or other approaches are already being considered for the treatment of AD [24] and could potentially provide perspective for MS as well.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life12071094/s1, Table S1: Association between HLA-DRB1*15:01 allele and MS; Table S2: Comparison of CD33 rs3865444 allele and genotype distribution between RR-MS, SP-MS, and control groups after stratification according to the HLA-DRB1*15:01 carrier status.

Author Contributions: Conceptualization, J.J., M.B. and I.S.; formal analysis, J.J., M.B. and V.D.; investigation, J.J., V.D., D.R. and Z.P.; data curation and retrieval, J.J., Z.P., D.Č., E.K., D.Č.-C. and K.G.; resources, D.Č., E.K., D.Č.-C., K.G. and I.S.; writing—original draft preparation, J.J.; writing—review and editing, M.B., V.D., D.R. and I.S.; supervision and funding acquisition, I.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research and APC were funded by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences, grant number VEGA/1/0738/20.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Medicine, Comenius University in Bratislava and University Hospital (Reference number 1/0240/16, 21 March 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.
Acknowledgments: The authors wish to express their gratitude to all participants in this study and thank B. Mišović Faragová and Z. Nürnberger for their technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Vila, A.; Casabella, A.M.; Baig, M.; Bidot, C.J.; Jones, B.C. Multiple sclerosis: Enigmatic factors and new controversies. Clin. Case Rep. Rev. 2016, 2, 1–9. [CrossRef] [PubMed]
2. Dendrou, C.A.; Fugger, L.; Friese, M.A. Immunopathology of multiple sclerosis. Nat. Rev. Immunol. 2015, 15, 545–558. [CrossRef] [PubMed]
3. Kim, W.; Patsopoulos, N.A. Genetics and functional genomics of multiple sclerosis. Semin. Immunopathol. 2022, 44, 63–79. [CrossRef] [PubMed]
4. International Multiple Sclerosis Genetics Consortium. Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. Science 2019, 365, eaav7188. [CrossRef] [PubMed]
5. Haase, S.; Linker, R.A. Inflammation in multiple sclerosis. Ther. Adv. Neurol. Disord. 2019, 14, 17562864211007687. [CrossRef] [PubMed]
6. Zia, S.; Rawji, K.S.; Michaels, N.J.; Burr, M.; Kerr, B.J.; Healy, L.M.; Plemel, J.R. Microglia diversity in health and multiple sclerosis. Front. Immunol. 2020, 11, 588021. [CrossRef]
7. Boillée, S. Local and remote interactions between macrophages and microglia in neurological conditions. Curr. Opin. Immunol. 2022, 74, 118–124. [CrossRef]
8. Siew, J.J.; Chern, Y. Microglial lectins in health and neurological diseases. Front. Mol. Neurosci. 2018, 11, 158. [CrossRef]
9. Walsh, A.D.; Nguyen, L.T.; Binder, M.D. miRNAs in microglia: Important players in multiple sclerosis pathology. ASN Neuro. 2021, 13, 175969142089812. [CrossRef]
10. Schirmer, L.; Schafer, D.P.; Bartels, T.; Rowitch, D.H.; Calabresi, P.A. Diversity and function of glial cell types in multiple sclerosis. Front. Mol. Neurosci. 2021, 42, 228–247. [CrossRef]
11. Guerrero, B.L.; Sicotte, N.L. Microglia in multiple sclerosis: Friend or foe? Front. Immunol. 2020, 11, 374. [CrossRef]
12. Calahorra, L.; Camacho-Toledano, C.; Serrano-Regal, M.P.; Ortega, M.C.; Clemente, D. Regulatory cells in multiple sclerosis: From blood to brain. Biomedicines 2022, 10, 335. [CrossRef]
13. Spiteri, A.G.; Wishart, C.L.; Pamphlett, R.; Locatelli, G.; King, N.J.C. Microglia and monocytes in inflammatory CNS disease: Integrating phenotype and function. Acta Neuropathol. 2022, 143, 179–224. [CrossRef] [PubMed]
14. Lampron, A.; Larochelle, A.; Laflamme, N.; Préfontaine, P.; Plante, M.M.; Sánchez, M.G.; Yong, V.W.; Stys, P.K.; Tremblay, M.-É.; Rivest, S. Inefficient clearance of myelin debris by microglia impairs remyelinating processes. J. Exp. Med. 2015, 212, 481–495. [CrossRef] [PubMed]
15. Rossi, B.; Santos-Lima, B.; Terrabuio, E.; Zenaro, E.; Constantin, G. Common peripheral immunity mechanisms in multiple sclerosis and Alzheimer’s disease. Front. Immunol. 2021, 12, 639369. [CrossRef]
16. Cignarella, F.; Filipello, F.; Bollman, B.; Cantoni, C.; Locca, A.; Mikesell, R.; Manis, M.; Ibrahim, A.; Deng, L.; Benitez, B.A.; et al. TREM2 activation on microglia promotes myelin debris clearance and remyelination in a model of multiple sclerosis. Acta Neuropathol. 2020, 140, 513–534. [CrossRef]
17. Grajchen, E.; Wouters, E.; van de Haterd, B.; Haidar, M.; Hardonnière, K.; Dierckx, T.; Van Broeckhoven, J.; Erens, C.; Hendriks, S.; Kerdine-Römer, S.; et al. CD36-mediated uptake of myelin debris by microglia reduces neuroinflammation. J. Neuroinflamm. 2017, 10, 224. [CrossRef]
18. Puigdellivol, M.; Allendorf, D.H.; Brown, G.C. Sialylation and galectin-3 in microglia-mediated neuroinflammation and neurodegeneration. Front. Cell. Neurosci. 2020, 14, 162. [CrossRef]
19. Siokas, V.; Tsouris, Z.; Aloizou, A.M.; Bakirtzis, C.; Liampas, I.; Koutsis, G.; Anagnostouli, M.; Bogdanos, D.P.; Grigoriadis, N.; Hadjigeorgiou, G.M.; et al. Multiple sclerosis: Shall we target CD33? Rep. Genet. 2020, 11, 1334. [CrossRef]
20. Hollingworth, P.; Harold, D.; Sims, R.; Gerrish, A.; Lambert, J.C.; Carrasquillo, M.M.; Abraham, R.; Hamshere, M.L.; Pahwa, J.S.; Moskvina, V.; et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer’s disease. Nature 2011, 43, 429–435. [CrossRef]
21. Naj, A.C.; Jun, G.; Beecham, G.W.; Wang, L.S.; Vardarajan, B.N.; Buros, J.; Gallins, P.J.; Buxbaum, J.D.; Jarvik, G.P.; Crane, P.K.; et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer’s disease. Nat. Genet. 2011, 43, 436–441. [CrossRef] [PubMed]
22. Jansen, I.E.; Savage, J.E.; Watanebe, K.; Bryois, J.; Williams, D.M.; Steinberg, S.; Sealock, J.; Karlsson, I.K.; Hägg, S.; Athanasiu, L.; et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer’s disease risk. Nat. Genet. 2019, 51, 404–413. [CrossRef]
23. Wightman, D.P.; Jansen, I.E.; Savage, J.E.; Shadrin, A.A.; Bahrami, S.; Holland, D.; Rongye, A.; Bérte, S.; Winsvold, B.S.; Drange, O.K.; et al. A genome-wide association study with 1,126,563 individuals identifies new risk loci for Alzheimer’s disease. Nat. Genet. 2021, 53, 1276–1282. [CrossRef] [PubMed]
24. Zhao, L. CD33 in Alzheimer’s disease—Biologie, pathogенезис, and therapeutics: A mini-review. Gerontology 2019, 65, 323–331. [CrossRef]
25. Estus, S.; Shaw, B.C.; Devanney, N.; Katsumata, Y.; Press, E.E.; Fardo, D.W. Evaluation of CD33 as a genetic risk factor for Alzheimer’s disease. *Acta Neuropathol.* 2019, 138, 187–199. [CrossRef] [PubMed]

26. Rawal, P.; Zhou, L. Sialometabolism in brain health and Alzheimer’s disease. *Front. Neurosci.* 2021, 15, 648617. [CrossRef]

27. Pérez-Oliva, A.B.; Martínez-Esparza, M.; Vicente-Fernández, J.J.; Corral-San Miguel, R.; García-Peñaarrubia, P.; Hernández-Caselles, T. Epitope mapping, expression and post-translational modifications of two isoforms of CD33 (CD33M and CD33n) on lymphoid and myeloid human cells. *Glycobiology* 2021, 21, 757–770. [CrossRef]

28. Bradshaw, E.M.; Chibnik, L.B.; Keenan, B.T.; Ottoboni, L.; Raj, T.; Tang, A.; Rosenkrantz, L.L.; Imboywa, S.; Lee, M.; Von Korff, A.; et al. CD33 Alzheimer’s disease locus: Altered monocyte function and amyloid biology. *Nat. Neurosci.* 2013, 16, 848–850. [CrossRef]

29. Malik, M.; Simpson, J.F.; Parikh, S.; Wilfred, B.R.; Fardo, D.W.; Nelson, P.T.; Estus, S. CD33 Alzheimer’s risk-altering polymorphism, CD33 expression, and exon 2 splicing. *J. Neurosci.* 2013, 33, 13320–13325. [CrossRef]

30. Malik, M.; Bhattacherjee, A.; Rodrigues, E.; Jung, J.; Luzentales-Simpson, M.; Enterina, J.R.; Galleguillos, D.; St. Laurent, C.D.; McCord, K.A.; Bains, A.; Sarkar, S.; et al. The CD33 short isoform is a gain-of-function variant that enhances Aβ1-42 phagocytosis in microglia. *Neuron* 2021, 78, 1094–1109. [CrossRef] [PubMed]

31. Raj, T.; Ryan, K.J.; Replogle, J.M.; Chibnik, L.B.; Rosenkrantz, L.; Tang, A.; Rothamel, K.; Stranger, B.E.; Bennett, D.A.; Evans, D.A.; et al. Increased inclusion of exon 2 implicates the Ig V-set domain in Alzheimer’s disease susceptibility. *Hum. Mol. Genet.* 2014, 23, 2729–2736. [CrossRef] [PubMed]

32. Malik, M.; Chiles, J.; 3rd; Xi, H.S.; Medway, C.; Simpson, J.; Potluri, S.; Howard, D.; Liang, Y.; Paumi, C.M.; Mukherjee, S.; et al. Genetics of CD33 in Alzheimer’s disease and acute myeloid leukemia. *Hum. Mol. Genet.* 2015, 24, 3557–3570. [CrossRef] [PubMed]

33. Walker, D.G.; Whetzel, A.M.; Serrano, G.; Sue, L.I.; Beach, T.G.; Lue, L.F. Association of CD33 polymorphism rs3865444 with Alzheimer’s disease pathogenesis and CD33 expression in human cerebral cortex. *Neuropsychobiology* 2015, 63, 571–582. [CrossRef]

34. Lamba, J.K.; Chauhan, L.; Lesinski, A.N.; Mullin, K.; Hooi, B.; Choi, S.H.; Hyman, B.T.; Tanzi, R.E. Alzheimer’s disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron* 2013, 78, 631–643. [CrossRef]

35. Malik, M.; Bhattacherjee, A.; Rodrigues, E.; Jung, J.; Luzentales-Simpson, M.; Enterina, J.R.; Galleguillos, D.; St. Laurent, C.D.; McCord, K.A.; Bains, A.; Sarkar, S.; et al. CD33 Alzheimer’s disease locus: Altered monocyte function and amyloid biology. *Nat. Neurosci.* 2013, 16, 848–850. [CrossRef]

36. Bhattacherjee, A.; Rodrigues, E.; Jung, J.; Luzentales-Simpson, M.; Enterina, J.R.; Galleguillos, D.; St. Laurent, C.D.; Nakhaei-Nejad, M.; Fuchsberger, F.F.; Streith, L.; et al. Repression of phagocytosis by human CD33 is not conserved with mouse CD33. *Commun. Biol.* 2019, 2, 450. [CrossRef] [PubMed]

37. Griciuc, A.; Serrano-Pozo, A.; Parrado, A.R.; Zia, S.; Ho, M.; Eskandari-Sedighi, G.; St. Laurent, C.D.; McCord, K.A.; Bains, A.; Sidhu, G.; McHenry, A.; et al. A human microglia-like cellular model for assessing the effects of neurodegenerative gene variants. *Sci. Transl. Med.* 2017, 9, eaai7635. [CrossRef] [PubMed]

38. Butler, C.A.; Thornton, P.; Brown, G.C. CD33M inhibits microglial phagocytosis, migration and proliferation, but the Alzheimer’s disease protective variant CD33M stimulates phagocytosis and proliferation, and inhibits adhesion. *J. Neurochem.* 2021, 158, 297–310. [CrossRef]

39. Wißfeld, J.; Nozaki, I.; Mathews, M.; Raschka, T.; Ebeling, C.; Hornung, V.; Brüstle, O.; Neumann, H. Deletion of Alzheimer’s disease-associated CD33 results in an inflammatory human microglia phenotype. *Glia* 2021, 69, 1393–1412. [CrossRef]

40. Schmidt, H.; Williamson, D.; Ashley-Koch, A. HLA-DR15 haplotype and multiple sclerosis: A HuGE review. *Am. J. Epidemiol.* 2007, 165, 1097–1268. [CrossRef] [PubMed]

41. Ryan, K.J.; White, C.C.; Patel, K.; Xu, J.; Renz-Oliva, A.B.; Martínez-Esparza, M.; Vendrell, J.; Corral-San Miguel, R.; García-Peñaarrubia, P.; Hernández-Caselles, T. Epitope mapping, expression and post-translational modifications of two isoforms of CD33 (CD33M and CD33n) on lymphoid and myeloid human cells. *Glycobiology* 2021, 21, 757–770. [CrossRef]

42. 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. Diagnosis criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann. Neurol.* 2011, 69, 1097–1109. [CrossRef] [PubMed]

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

44. Roxburgh, R.H.; Seaman, S.R.; Masterman, T.; Hensiek, A.E.; Sawyer, S.J.; Vukusic, S.; Achiti, I.; Confavreux, C.; Coustans, M.; le Page, E.; et al. Multiple sclerosis severity score: Using disability and disease duration to rate disease severity. *Neurology* 2005, 64, 1144–1151. [CrossRef] [PubMed]

45. de Bakker, P.I.; McVean, G.; Sabeti, P.C.; Miretti, M.M.; Green, T.; Marchini, J.; Ke, X.; Monsuur, A.J.; Whittaker, P.; Delgado, M.; et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat. Genet.* 2006, 38, 1166–1172. [CrossRef] [PubMed]

46. Hafler, D.A.; Compston, A.; Sawyer, S.; Lander, E.S.; Daly, M.J.; De Jager, P.L.; de Bakker, P.I.; Gabriel, S.B.; Mirel, D.B.; International Multiple Sclerosis Genetics Consortium; et al. Risk alleles for multiple sclerosis identified by a genomewide study. *N. Engl. J. Med.* 2007, 357, 851–862. [CrossRef]

47. Javor, J.; Úrmanová, V.; Párnická, Z.; Minárík, G.; Králová, M.; Pečenáň, J.; Vaščeková, B.; Režnáková, V.; Štuvovský, S.; Gmitterová, K.; et al. Association of CD33 rs3865444:C > A polymorphism with a reduced risk of late-onset Alzheimer’s disease in Slovaks is limited to subjects carrying the APOE ε4 allele. *Int. J. Immunogenet.* 2020, 47, 397–405. [CrossRef]
48. Benešová, Y.; Vašků, A.; Štourač, P.; Hladíková, M.; Fiala, A.; Bednářík, J. Association of HLA-DRB1*1501 tagging rs3135388 gene polymorphism with multiple sclerosis. *J. Neuroimmunol.* 2013, 255, 92–96. [CrossRef]

49. Cortina-Borja, M.; Smith, A.D.; Combarros, O.; Lehmann, D.J. The synergy factor: A statistic to measure interactions in complex diseases. *BMC Res. Notes* 2009, 2, 105. [CrossRef]

50. Solé, X.; Guinó, E.; Valls, J.; Iniesta, R.; Moreno, V. SNPStats: A web tool for the analysis of association studies. *Bioinformatics* 2006, 22, 1928–1929. [CrossRef]

51. Michalík, J.; Čierny, D.; Kantorová, E.; Kantárová, D.; Juraj, J.; Pánická, Z.; Kurča, E.; Dobrota, D.; Lehotský, J. The association of HLA-DRB1 and HLA-DQB1 alleles with genetic susceptibility to multiple sclerosis in the Slovak population. *Neurol. Res.* 2015, 37, 1060–1067. [CrossRef] [PubMed]

52. Javor, J.; Shawkatová, I.; Ďurmanová, V.; Pánická, Z.; Čierny, D.; Michalík, J.; Čopíková-Cudráková, D.; Smahová, B.; Gmíterová, K.; Peterajová, L.; et al. TNFRSF1A polymorphisms and their role in multiple sclerosis susceptibility and severity in the Slovak population. *Int. J. Immunogenet.* 2018, 45, 257–265. [CrossRef] [PubMed]

53. Kotter, M.R.; Li, W.W.; Zhao, C.; Franklin, R.J. Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. *J. Neurosci.* 2006, 26, 328–332. [CrossRef] [PubMed]

54. Franklin, R.J.; Kotter, M.R. The biology of CNS remyelination: The key to therapeutic advances. *J. Neurol.* 2008, 255 (Suppl. S1), 19–25. [CrossRef]

55. Grajchen, E.; Hendriks, J.J.A.; Bogie, J.F.J. The physiology of foamy phagocytes in multiple sclerosis. *Acta Neuropathol. Commun.* 2018, 6, 124. [CrossRef] [PubMed]

56. Lin, P.I.; Vance, J.M.; Pericak-Vance, M.A.; Martin, E.R. No gene is an island: The flip-flop phenomenon. *Am. J. Hum. Genet.* 2007, 80, 531–538. [CrossRef]

57. Combarros, O.; Cortina-Borja, M.; Smith, A.D.; Lehmann, D.J. Epistasis in sporadic Alzheimer’s disease. *Neurobiol. Aging* 2009, 30, 1333–1349. [CrossRef]

58. Griciuc, A.; Patel, S.; Federico, A.N.; Choi, S.H.; Innes, B.J.; Oram, M.K.; Cereghetti, G.; McGinty, D.; Anselmo, A.; Sadreyev, R.I.; et al. TREM2 acts downstream of CD33 in modulating microglial pathology in Alzheimer’s disease. *Neuron* 2019, 103, 820–835.e7. [CrossRef]

59. Cantoni, C.; Bollman, B.; Licastro, D.; Xie, M.; Mikesell, R.; Schmidt, R.; Yuee, C.M.; Galimberti, D.; Olivecrona, G.; Klein, R.S.; et al. TREM2 regulates microglial cell activation in response to demyelination in vivo. *Acta Neuropathol.* 2015, 129, 429–447. [CrossRef]

60. Poliani, P.L.; Wang, Y.; Fontana, E.; Robinette, M.L.; Yamanishi, Y.; Gilfillan, S.; Colonna, M. TREM2 sustains microglial expansion during aging and response to demyelination. *J. Clin. Investig.* 2015, 125, 2161–2170. [CrossRef]

61. Hrastelj, J.; Robertson, N. Genetics of disease severity in multiple sclerosis, Alzheimer’s disease, and Huntington’s disease: Rejuvenating genome-wide association studies. *J. Neurol.* 2017, 264, 2040–2042. [CrossRef] [PubMed]